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THEME:

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ABG 001

PREDICTION OF FUNCTIONAL AND STRUCTURAL EFFECTS OF SINGLE NUCLEOTIDE POLYMORPHISM ON BONE MORPHOGENIC PROTEIN RECEPTOR 1- IN SILICO

Musa, S. Ibrahim

Department of Animal Science, Faculty of Agriculture, Nasarawa State, University Shabu - Lafia Campus, Nigeria.
Corresponding Author: dribmazara@yahoo.com

ABSTRACT

The objective of this study was to predict the effects of single nucleotide polymorphism on ovine bone morphogenic protein receptor 1 using computational methods. Information was obtained from the data base of national center for biotechnology information for molecular analysis (Accession No. NM_001009431.1). Analysis using Protein Variation Effect Analyzer (PROVEAN) indicate damaging effects of substitution of glutamine for arginine at position 249 of the protein sequence of BMPR1. Effect of the single nucleotide polymorphism (Q249R) was predicted to be deleterious by Mutpred 2 server. The molecular mechanisms of pathogenicity was predicted as gain of helix, altered ordered interface, gain of alosteric site and altered transmembrane protein. It can be concluded that substitution of glutamate for arginine at position 249 of bone morphogenic protein receptor 1 is deleterious to the protein structure. Single nucleotide polymorphism in bone morphogenic protein receptor 1 could be used as a genetic marker for selection to improve reproductive performance in farm animals.

Keywords: *BMPR 1, IN-SILICO, PREDICTION, SNP*

Introduction

Bone morphogenic protein receptor 1 gene encodes BMP receptor family for transforming serine- Threonine kinases (Wu *et al.*, 2019). Genetic variation in bone morphogenic proteins have been implicated in the development of artherisclerosis. Bone morphogenic proteins have been reported to regulate follicular growth and ovulation rate (Knight and Glister, 2003). It has been observed that granulosa cells are the main sites of BMP1 synthesis, indicating that BMP1 is activated and secreted directly into the antrium from the granulosa cell layers (Pappano *et al.*, 2003). Granulosa cells lose their proliferative activity at the terminal stage of ovarian follicular activity, differentiate in estradiol secretary cells and undergo leutinization in response to leutinizing hormone (Manniause *et al.*, 1997). Granulosa cell transformation is under the influence of endocrine paracrine factors such as pituitary gonadotropin and ovarian follicular growth factors. BMPR1 is involve in regulation of prolificacy in small ruminants. The gene is responsible for fecundity and twining rate in sheep and goats (Polley *et al.*, 2009).

Mutations in fecundity genes have been associated with both ovulation rate and litter size of sheep (Souza *et al.*, 2001). The high reproductive performance of Booroola sheep was associated with mutations in BMP receptor 1B gene. Mutations in BMPR have been associated with embryonic mortality, skeletal abnormalities and cardiac defects. BMPR1 is expressed in the ovary from early fetal stage to adulthood (Elizabeth *et al.*, 2010). It has also been observed that BMP activity decreases with follicular growth. This study evaluates the effect of single nucleotide polymorphism in ovine bone morphogenic protein receptor 1.

Materials and Methods

Data on ovine BMPR 1 with accession no: NM_001009431.1 was retrieved from GenBank of National Centre for biotechnology information (NCBI) for molecular analysis. The effects of Single nucleotide polymorphism on

bone morphogenic protein receptor 1 was analyzed using protein variation effect analyzer (PROVEAN). Clustering of BLAST hits is performed by CD-HIT with a parameter of 75% global sequence identity. The top 30 clusters of closely related sequences form the supporting sequence set, which will be used to generate the prediction. A delta alignment score is computed for each supporting sequence. The scores are then averaged within and across clusters to generate the final PROVEAN score. If the PROVEAN score is equal to or below a predefined threshold, the protein variant is predicted to have a deleterious effect. If the PROVEAN score is above the threshold, the variant is predicted to have a neutral effect (Choi *et al.*, 2012). The impact of substitution of glutamine for arginine at position 249 of ovine bone morphogenic protein receptor 1 B was also predicted using MUTPRED 2 server. The Mutpred 2 package integrate genetic and molecular data to predict the pathogenicity of amino acid substitution. It provide general pathogenicity prediction and specific molecular mechanism of pathogenicity (Sherry *et al.*, 2001). MUTPRED 2 is a web application that classify amino acid substitution as pathogenic or benign. It also predict the molecular mechanism of pathogenicity (Mohaz *et al.*, 2010).

Results

The effects of single nucleotide polymorphism on ovine bone morphogenic protein receptor 1 as predicted by Protein Variation Effect Analyzer (PROVEAN) is shown in figure 1. The result reveal a PROVEAN score of –3.846 against cutoff point of –2.5 which indicate damaging effect of substitution of glutamine for arginine at position 249 of the protein sequence of BMPR 1.

PROVEAN Prediction - Job ID: 1681408457814089

Query sequence (fasta)
Supporting sequence set used for prediction
Number of sequences: 356 (fasta, E-values)
Number of clusters: 30
Score thresholds for prediction
(1) Default threshold is -2.5, that is:
-Variants with a score equal to or below -2.5 are considered "deleterious,"
-Variants with a score above -2.5 are considered "neutral."
(2) How to use a more stringent threshold.

Variant	PROVEAN score	Prediction (cutoff= -2.5)
Q249R	-3.846	Deleterious

Figure 1: Effect of single nucleotide polymorphism on BMPR-1 predicted using PROVEAN software

Analysis of the effects of single nucleotide polymorphism using MUTPRED 2 Server is shown in figure 2. Mutpred 2 score of 0.744 indicate damaging effects of substitution of glutamine for arginine at position 249 of BMPR 1 protein sequence. The result also indicate mechanisms of molecular damage to be gain of helix, altered ordered interface, gain of allosteric site and altered transmembrane protein.

ID	Substitution	MutPred2 score	Remarks	Affected PROSITE and ELM Motifs
NM_001009431.1	Q249R	0.744	Predicted conservation scores	ELME000120, ELME000146
Molecular mechanisms with P-values <= 0.05			Probability	P-value
Gain of Helix			0.28	0.02
Altered Ordered interface			0.26	0.01
Gain of Allosteric site at R254			0.26	7.1e-03
Altered Transmembrane protein			0.16	0.01

Figure 2: Prediction of effects of single nucleotide polymorphism on BMPR 1 using MUTPRED 2 Server.

Discussion

Bone morphogenic proteins are members of transforming growth factors that bind to two types of serine- threonine receptors which are required for signal transduction. Actions of the receptors are influenced by protein modifications (Kohei *et al.*,2010). Mutations in bone morphogenic protein receptor 1 (BMPR 1) have been associated with embryonic mortality. BMPR 1 is expressed in the ovary from early fetal stage to adulthood (Elizabeth *et al.*, 2010). It has been observed that BMPR 1 activity decrease with follicular growth. The significant effect of single gene on reproductive performance of livestock animals have been documented, these genes are referred to as fecundity genes (Hanraham *et al.*, 2009). The genes play a significant role in embryogenesis, ovulation and litter size. Single nucleotide polymorphism in BMPR 1 is reported to have additive effects on ovulation rate. The mutation result in loss of reaction capacity of BMP which play a critical role in formation of primordial germ cells in the ovary (Maskar *et al.*, 2016). The damage to the BMP system resulted in increased average ovulation. The change in amino acid sequence result in functional change in the protein and was reported to affect ovulation rate in numerous breeds of sheep. It can be concluded that single nucleotide polymorphism in bone morphogenic protein receptor can be used as a genetic marker for reproductive performance in sheep.

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ABG 002

AN OVERVIEW OF CATTLE GENETIC RESOURCES IN NIGERIA: A REVIEW

Ma'aruf, B.S., Adamu, H., Shuaibu, A., Maigado, A.I., Umar, H.A. and Salihu, A.M.

Department of Animal Science, Faculty of Agriculture, Federal University of Kashere. P.M.B 182 Gombe State.
Corresponding Author: maarufbs@fukashere.edu.ng. +2348065673636

ABSTRACT

The current studies aimed at reviewing the variation in indigenous cattle genetic resources in Nigeria. Cattle maintain a dominant position in animal protein supply in Nigeria and their estimated population is about 15.3 million. Cattle genetic diversity is a critically important resource that provides the raw material for farmers and pastoralists to improve their breeds and strengthen the adaptation of their populations to changing environments. The wide range of production environments as they existed in Nigeria requires an equally diverse range of genetic materials to enable substantial production. Similarly, the compelling need to produce enough food for the growing population and the efforts to increase livestock production in Nigeria through breeding strategies and policies that encourage the replacement of the indigenous stock with those of the temperate region place the indigenous genetic resources at the danger of extinction. There are about 11 enlisted breeds of cattle Ingenious to Nigeria, namely; Bunaji, Rahaji, Sokoto Gudali, Adamawa Gudali, Azawak, Wadara, N'dama, Keteku, Kuri, Muturu and the Biu cattle. The Bunaji are the most widely distributed, while the Biu breed is the least. Most of the breeds are good in beef production, but generally poor in milk, although some few e.g Bunaji and Sokoto Gudali show a potential for dairying. A significant variation exists in terms of birth, weaning and matured weights, genetic composition, qualitative traits and other important parameters among the breeds.

Keywords: *Cattle, breeds, genetic, resources, variation*

Introduction

Genetic diversity is a critically important resource that provides the raw material for farmers and pastoralists to improve their breeds and strengthen the adaptation of their populations to changing environments as well as to shifting market demands In short, genetic diversity provides an essential buffer to a changing world—and an unpredictable future, Dessie and Mwai (2019). Cattle command a prominent position in meat and milk supply in livestock industry in Nigeria. Beef is estimated to supply about 45 percent of total meat consumed in Nigeria, while the next in rank is sheep and goat meat with 35 percent. Our National herd contains an estimated 9.2 million herds of cattle in 1981, (Kubkomawa, 2017), 13.9 Million herds in 1990 (Lawal-Adebowale 2012) and 15.3 in 2010 (Tibi & Aphunu, 2010)

Maintaining genetic diversity is an insurance against future adverse conditions. In Africa, diversity among environments and nutritional standards as well as challenges from multiple infectious agents require diverse breeds and populations. The breeds therefore act as storehouses of genetic variation, which form the basis for selection and may be drawn upon in times of biological stress such as famine, drought or epidemics. The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity (Hanotte and Jianlin, 2005).

The erosion of locally-adapted genetic resources as the case in Nigeria will significantly limit the option and capacity to

cope with changes to production environments and breeding goals. Understanding of farm animal genetic diversity is therefore required to contribute to meeting current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing environments and breeding objectives, (Mukasa, 2014) Indigenous cattle present enhanced hardiness and disease resistance, low nutritional requirements and higher capability of feed utilization. However, their main products remain meat, milk, hides, and manure, (Claire *et al.,* 2017)

Indigenous cattle breeds

Presently, cattle population in Nigeria has been estimated as 15.3 million, there are many breeds of cattle indigenous to Nigeria, the breeds identified include White Fulani, Red Bororo, Sokoto Gudali, Adamawa Gudali, Wadara, Azawak, Muturu, Keteku, Ndama, Kuri and Biu cattle. (Kubkomawa, 2017)

White Fulani or Bunaji

The original habitat of White Fulani breed is in Northern and Southern Nigeria, and the Northeastern part of Cameroon, associated with the Fulani and House lemens; gradually spread to the south of Chad, and western Sudan (where they are called Fellata, and red Fulani) (Glowatzki-Mullis, *et al.,* 1995). Alphonsus *et al.,* 2010 reported that the white Fulani cattle are the most numerous and wide spread of all the Nigerian cattle breeds.

Their estimated population size is 5,118,547 or about 51 % of the national herd (RIM, 1989). They are characterised by White black-eared and medium -horned breed. Most herds have a few black coloured patches on the coat or with blue flecking or red and white with a mortality rate of 15.6%, calving interval is between 388 and 810 days and age at first calving is 40.00–60.00 months. They are more resistant and tolerant to diseases than other Zebu races with an average milk yield of 627 – 1034kg over lactation periods of 194 – 245 days, a weight gain of 880g over 76 days fattening period and conception typically peaks in April-May. The skin is loose and pigmented and the hair is soft with erect ears. Horns are medium to long lyre-shaped and curve outwards and inwards, they have outward tips of the horns in some of these animals with large and well-developed dewlap of many folds. Sheath and navel flap are not large and female udders are developed with medium-sized teats (Buvanendran *et al.,* 1983; Ngere, 1990; Hall, 1991). The White Fulani cattle are, however, important for their genetic predisposition of hardiness, heat tolerance and adaptation to local conditions (Alphonsus *et al.*, 2012).

Sokoto Gudali

Sokoto Gudali stereotypically occurs mainly in the northwest of Nigeria. Sokoto Gudali race are predominantly found in the arid zone north of Sokoto. It is now distributed widely throughout the country. The NNLRS estimated that they represent some 32% of the national herd (Payne & Wilson, 1999; Blench, 1999). The Nigerian National Livestock Resource Survey estimated that the herd has a population of about 4,351,528 (Rege &Tawah, 1999).

They are characterised by being short-horned, short-legged cattle with very short or effectively absent horns. Some have darkened patches around the front half of the body with well-developed dewlap and skin folds. This breed is considered good as fattening stock (Nuru *et al.*, 1981). Weight gain over 76 days fattening was 920g. The udders in the female are well developed with good teats. They are regarded as indigenous dairy breed, mean milk yield 1,144kg over 266 days to 2,633kg over 322 days (Adebambo, Williams & Babara., 1998). The age at first calving was 38–46 months with a 467 days calving interval (Hall, 1991). According to Umar, *et al.,* 2020 Sokoto Gudali is superior to Bunaji (White Fulani) in most of the body and milk production traits. At maturity, the female weighs an average of about 330 kg, while the male weighs about 450 kg. The female produces an average of 1,500 kg of milk per lactation (Payne & Wilson, 1999). Their milk yield at the National Animal Production Research Institute (NAPRI), Shika was higher than that of White Fulani (Alphonsus *et al.*, 2012).

Rahaji or Red Bororo

The Rahaji is one of the largest zebu breeds and is distinguished by its deep burgundy-colored coat, pendulous ears and long, thick horns (Katie & Alistair, 1986; Williamson & Payne, 1990). The Rahaji is adapted to arid and semi-arid regions and rarely goes further south than Kaduna in the wet season, except for the isolated population on the Mambila Plateau in the north-east (Blench, 1993). The Rahaji is one of the largest Zebu breeds and is distinguished by its deep burgundy-coloured coat, pendulous ears and long, thick horns. It is the third most numerous breed of cattle in Nigeria with a population size estimated to be 3,029,541, about 22% of the national herd. The Rahaji is widely distributed in Kano, Daura, Borno and Adamawa areas. (Blench, 1993). The mortality rate is lower than 15.6% for Bunaji with a 7.3% slaughter figures higher than the Bunaji. Their calving interval is 537 – 585 days with the age at first calving ranging from 47.9 to 53.0 months and calving occurs all-year- round but peaks in April (Ngere, 1990; Adebambo *et al.*, 1998).

Adamawa Gudali

Restricted to Adamawa and southern Borno States, the Adamawa Gudali, is owned by both the Kanuri and Fulani pastoralists. It is rare for them to have complete herds of Adamawa Gudali, and often they are mixed with Wadara, Bunaji or Rahaji. Adamawa Gudali is regarded by many farmers as the indigenous race, of the region and they are common in villages (Rege and Tawah, 1999). The Adamawa Gudali resembles the Bunaji in conformation with a medium to large size, medium-length horns, and usually pied, or a white, black, red or brown coat. It has thick, crescent-shaped horns, a pendulous hump, and a short head and muzzle. The mortality rate is lower than 15.6% for Bunaji with a 7.3% slaughter figures higher than the Bunaji. Their calving interval is 537 – 585 days with the age at first calving ranging from 47.9 to 53.0 months and calving occurs all-year- round but peaks in April (Ngere, 1990; Adebambo *et al.*, 1998).

Azawak

The Azawak is said to be native to the Azawak Valley north-east of Nigeria and is distributed along its north-western border. They are generally, found on the Nigerian border in western Sokoto State and also occur in north-western Kwara State and dotted along the northern border of Nigeria from Sokoto to eastern Kano (Blench, 1993). The NNLRS (1990) estimated that they represent just 0.7% of the national herd with a population size of 103,280. A small population of Azawak cattle exists in Nigeria throughout the year, but the majority of the herds are seasonal transhumance. Although Azawak in Niger are commonly described as red, the Azawak that enter Nigeria are usually a light fawn colour, though they can also be white, brown, pied and black, but at times, they are white, brown, pied and black. Azawak considered very well adapted to drought. It is compact and is suitable for beef production and is also used for work. It is routinely milked (Rege & Tawah, 1999). They cannot trek long distances as Rahaji. They calve annually, are more resistant to diseases, eat less, and on a wider variety of vegetation than the Rahaji (Rege &Tawah, 1999). Their calving interval is 426 – 507 days with age at first calving of 37 – 52.2 months and their conception pattern has a single peak, from January to October. The herd structure is 78.2% females and 21.5% males (Ngere, 1990; Hall, 1991). The average liveweight of mature females is about 300 kg and that of males about 390 kg.

Wadara

Blench (1993, as cited in Mukasa, 2014) reports wadara cattle are the indigenous cattle of Borno, that is located in the north-eastern part of the State and are referred to by the Koyam and related pastoralists as our cattle. They are frequently called 'Shuwa' in the literature, after the Shuwa Arabs who also herded them. A related breed with a white coat, the Ambala, is often traded into Nigeria from Chad. Wadara cattle are medium-sized, lightly- built cattle, and are usually dark red, black, pied or brown. They are short-horned and have a small erect hump, representing about 6.6% of the national herd with a population size of 904,731. Wadara are good dairy animals with average milk yield of 1,212kg over 259 days. The age at first calving is 45.4 - 52.4 months and they have an

annual pattern of conception, which peaks in July with a herd structure of 60.4% females and 39.4% males (Olutogun, 1976).

Muturu (West African Dwarf Shorthorn)

The West African dwarf shorthorn or Muturu is small-bodied, and blocky in conformation with short, fine-boned limbs It has a compact body, no hump, a straight back, and a broad head. The face is slightly-dished, and the horns are very short. In south-central Nigeria, the Muturu is generally black, or black and white. Animals on the Jos Plateau are usually black and white but are distinctly larger than lowland animals. There are more variations in the northern populations; brown, red or tawny animals were recorded (Blench, 1993). Milk yield was 421kg over 216 days with 28.3 – 41.8 months mean of age at first calving while their calving intervals were between 13.9 and 26.8 months with their conception pattern peaking in July with a series of high values in the dry season (February – April and December – January). They are trypano-tolerant whose herd structure is 70.5 – 73.2% females and 27.0 – 29.7% males. The progeny mortality rate is 4.7 – 15.6% and lowest with 4.7% for southern savanna (Ngere, 1990; Hall, 1991).

N'Dama

The lineage of the graceful and sturdy N'Dama cattle is one of the oldest on the African continent. The breed is descended from the Humpless Hamitic Longhorns (*Bos taurus*) brought by ancient pastoralists around 8000 BC via the land route from Asia to present-day Egypt. Over millennia, the N'Dama have evolved adapted traits that allow them to flourish in warm and humid regions uninhabitable to other cattle breeds due to the heat stress and infestation of tsetse fly. With their long arcing horns and hardy constitution, the N'Dama are truly emblematic of the valuable genetic heritage of indigenous African cattle that is in danger of disappearing due to neglect and interbreeding. They display a docile temperament that is well suited for work as draught animals, and they are tolerant of trypanosomiasis tick-borne diseases, and skin ailments that seriously affect zebu cattle. N;Dama breed Medium-sized, with no hump, a minimal dewlap, a large head, and long lyre-shaped horns averaging 60 cm in length. The coat is made of short, fine hair, often fawn coloured, though varying from sandy to black. N'Dama cattle are trypanotolerant, resist tick-borne infections, and are well adapted to warm and humid and hot and dry climates. They are used for milk, meat, manure and traction.

Kuri

In Nigeria, Kuri are found not only on the Lake Chad but on its shores and along the Yobe valley, as far west as Gashagar. The breeds along the Komadugu Yobe are crossed with Zebu and are generally referred to as Jetkoram in the literature (Blench, 1993). Kuri are noted for their extremely-variable colours and their ability to thrive in semia-quatic conditions. The nucleus of the Kuri cattle population is within the region of the former Lake Chad, and along its eastern shores (Meghen *et al.,* 2000). The Kuri are large-bodied, humpless, long-horned cattle whose exact historical origin is unknown (Blench, 1993; Meghen *et al.,* 1999). Average age of breeding for females is 86 months; average age at first calving is 49.9 months and their highly adapted to they are natural new environment. Their milk yield is 3-6 litres per day and lactation period is 6-10 months although record of 1,350 litres per lactation exists and the calving interval is 18.7 months, though it can be less (15.8 months). For the herd structure, 63% are females and 37% are males with an overall mortality rate of 27% which is the highest for any Nigerian breed (Ngere, 1990)

Keteku

The distribution and productivity of Keteku cattle have been studied in more detail by Blench *et al.* (1998). The definition of Keteku has become more problematic in recent years with an increasing proportion of Zebu blood in 'Keteku' herds. As Fulani pastoral herds push even further south and increasingly inhabit regions previously restricted to trypano-tolerant stock, more Zebu stock are bought in for village herds. For example, the 'Biu', a Zebu x Savannah Muturu cross found near Biu in southern Borno and described in the literature (Gates, 1952), has effectively

become submerged in the local Zebu gene pool. Thus, application of the name Keteku to an individual animal may reflect as much the owner's cultural background as its actual genetic composition. . In Nigeria, Keteku herds are restricted to a narrow band along the Benin Republic border in the region usually known as 'Borgu' (Kubkomawa *et al.,* 2017)

Biu Breed

Biu cattle breed is a rare breed that is found in Northern part of the country, is restricted to a hilly volcanic area of Borno State, Nigeria. Number not more than 1000-2000 heads (Stetshwarlo and Adebambo, 1992). The breed is observed to have been displaced in the Northern Nigeria by the zebu after the Fulani invasion (ILCA, 1979). Little is known about information this rare breed of cattle.

Needs for Diverse Cattle Genetic Resources

The wide range of production environments as they existed in Nigeria requires an equally diverse range of genetic materials to enable substantial production (Adebambo, 1992). However, most livestock improvement programmes has emphasizes the development of one or more breeds in each species at the expense of the others.

The genetic diversity of the original larger populations is reduced because the sample of genes in the few founder animals is not likely to be representative of the original gene pool. Reduction in genetic diversity has been expressed primarily in terms of loss of breeds and strains. It is postulated that the current rate of extinction of species, breeds and strains is greater now than at any time in the past (Hammond, 1993).

It is estimated that at least 30% to 40% of all animal genetic resources are currently at high risk of extinction (Hammond, 1994). However, adequate records do not exist to enable reliable estimates of either loss rates of the breeds or of domestic animal diversity itself. The existing data on the number of endangered breeds are likely to be underestimates of the magnitude of the problem. The loss of genetic diversity is occurring both within populations and among populations (Mukasa 2014). The factors that diminish genetic diversity within populations are genetic bottlenecks, random genetic drift, inbreeding and human activities. All these four factors are functions of population size (Lacy, 1987; Lande and Barrowclaugh, 1987).

Loss of Cattle Genetic Diversity

According to Hammond (1994), about 50 % of the total variance for several quantitative traits in cattle, sheep and pig breeds is at the between-breed level, the remainder being common to all breeds. Loss of genetic diversity among-populations occurs when historically divergent and isolated populations experience an artificially high rate of gene flow from other populations. Moreover, there are concerns that, if there exist co-adapted groups of genes within each sub-population, merging may result in the break-up of these and the loss of the particular adaptability of each sub-population to its environment (Frankham, 1994). The effect of gene exchange between sub-populations was to increase the variance within groups, decrease the variance between groups and decrease the total variance (Lacy 1987).

Need for Conservation of Cattle Genetic Resources

Food and Agricultural Organization (FAO, 1983) defined need for conservation of animal genetic resources eligible for conservation as those populations with economic potential, scientific use and cultural interest. The Nigerian endangered cattle breeds should be conserved for their potential economic use in the future. These breeds have regional adaptation developed for the Nigerian environment that may also be beneficial in other areas of the world where similar or complementary conditions exist. The zebu cattle breed for instance had been used successfully indiverse regions of the world (Devillard, 1985). The Nigerian cattle breeds are trypanotorelant, unselected for a particular product or traits, that have evolved and valued under challenging environment and should not

be compared with other breeds in improved, modified conditions or under intensive management (Gwaza *et al.,* 2016). There are many examples where growth rate, prolificacy or milk production have been used to illustrate the inferiority of the purebred indigenous stock over that of exotic imported breeds or their crosses (Hodges, 1986). However when survivability of the offspring, fertility and longevity are taken into consideration, the indigenous stocks are often found to be very productive overall.

Conservation of Cattle Genetic Resources

Breed improvement and conservation are optimally achieved when the available genetic resources are characterised and strategies developed to achieve the goals. The value of the indigenous breeds as biological materials relative to their performance that is superiority, versatility, temperament, heterosis complementary expectations in crosses, and fertility, and other special characteristic of adaptation to environmental conditions are profound importance in tropical areas (Manson, 1983). Conservation of domestic animal diversity has been defined as the sum total of all operations involved in the management of animal genetic resources so that the pool of genetic diversity is maintained over time (Hammond, 1993). Genetic diversity is the basis for a species evolutionary flexibility and responsiveness to environmental changes and that loss of genetic diversity will restrict the opportunities available to mould domestic animals to meet unpredictable future changes and requirements (Mukasa 2014). The current unfavorable economic climate and limited financial resources in most African countries especially South of the Sahara, opportunities to alter the livestock production environments to suit the high potential temperate breeds are now on the decline compared to the period of 1940s – 60s (Adebambo, 1992).

Conclusion

It is concluded that, the genetic improvement is rare in some indigenous cattle breeds, whose there genetic resources are untapped, the survival of some of these breeds is being threatened and are therefore in imminent danger of extinction. The gene pool of Biu, N'dama and Keteku cattle breeds of Nigeria have declined, there is need for management of these breeds in Nigeria in order to maintain the genetic variable or tapped their genetic resources for optimum production level for their demand to carter the teeming population base on their genotype conformation performance, with concise and well define breeding plan or objectives sets to meet the gap between improved breeds.

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Table 1: Some of the productive and reproductive traits of indigenous breeds of cattle in Nigeria

PARAMETERS	WF	RB	SKGD	ADG	KURI	AZW	MTR	NDM	KTK	WDR
Birth Weight	23.54-23 kg	15-23 kg	23 kg		22.5 – 25 kg	20 kg	11.14-11.16 kg	19-22 kg		24.94 kg
weaning weight	79.88 kg		86 kg			98 kg	82-92 kg	63.66 kg	131-149 kg	
Maturity Weight of Cow	325 k`g	350 kg	240-355kg	336 kg	365 kg	325 kg	110 kg	270 kg	295 kg	294.8 kg
Maturity Weight of Bull	500 kg	450 kg	495-660kg	352 kg	499 kg	403.7-458.1 kg	147 kg	350 kg	330 kg	362.8 kg
Age at Maturity	44.79-51.79	47.9 – 53.0 mnth	19.0-23.5 month		36 - 48 mnth	37 – 52.2 month	12.25±0.2-14.60±0.67	29-48 mnth	47 mnth	45.4-52.4 mnth
Total Milk Yield	627 – 1034/kg	295.00 lts	1,144/kg	961.60 lts	1255/kgs	1043 lts	421/kg	500 – 600/kg		879.9±261.4
Daily Milk Yield	3.31±0.36	24.40 lts	4.70 lts		6 kg	3.20 lts		2 -3 litres		
lactation Length	208.78±14.993	1,500 kg	244.8 days	216 days	280 days	325 days	216 days	210 days		186.4±27.3
Dry period Length	102±2.5 days									117.7±39.5
Calving interval	400.86±11.869	360 – 450	467 days	431 days	15 – 18 mnth	426 - 507	350 days	653.77 days	365 days	374±46.5

WF= White Fulani, RB= Red Bororo, ADG= Adamawa Gudali, SKG- Sokoto Gudali, AZW= Azawak, MTR= Muturu, NDM=N'Dama, KT= keteku, WDR, Wadara,

ABG 003

HAEMOGLOBIN POLYMORPHISM AND GENOTYPIC FREQUENCIES AMONG GOATS IN SOUTHERN KADUNA

Yayi, J. and Mallam, I.

Department of Animal Science, Kaduna State University, Kafanchan Campus.
Corresponding Author: kyuukyayi@gmail.com +2348129235498

Abstract

This study was conducted to determine the haemoglobin polymorphism of goats in four Local Government Areas of Southern Kaduna: Jema'a, Kaura, Jaba, and Kachia. The total numbers of goats sampled were 100, with 25 in each Local Government Area. Blood samples (5ml) each were collected and put into heparinized test tubes from the 100 smallholder goats in the four Local Government Areas. The blood samples were collected and were taken for electrophoresis to determine haemoglobin types and its frequencies in indigenous smallholder goats of Southern Kaduna. The electrophoretic test showed the presence of two co-dominant alleles HbA and HbB which produced two haemoglobin phenotypes: Homozygote HbAA and Heterozygote HbAB. HbBB was not detected in this study. The results obtained showed that the genotype with the highest frequency is the AA genotype across three local governments (68%, 60%, and 84%), for Jema'a, Kaura and Jaba Local Government Areas respectively, except Kachia Local Government where AB had the highest genotypic frequency of 68%. The Chi-square test showed high significant differences between observed and expected genotype frequencies. Thus, indicating that the flock were not in Hardy-Weinberg's equilibrium. Therefore, during introduction of goats in a novel environment or selection of goats for breeding purposes, haemoglobin types need to be put into consideration.

Keywords: *Haemoglobin, Frequency, Goats, Southern Kaduna, and Chi-square*

Introduction

Goats are the most populated ruminant livestock, totalling about 53.8 million (FAOSTAT, 2008). Goats are predominantly owned by rural households and ownership is spread across all human age groups and sexes (Akpa, 2000). They are a valuable source of animal protein for humans when their meat or milk is consumed. Goats form the most important group of milk producing animals after dairy cattle in both temperate and tropical agriculture (Bogoro *et al.*, 1999). Goat breeding is a very old tradition among indigenous farmers; no purposeful breeding programme has ever been organized for the species in Nigeria. Goat breeds have been previously evaluated for genetic variation based on morphological, physiological, pathological, productive, reproductive, and behavioural features (Salako *et al.*, 2007; Pourlis and Christodoulous 2008). Advances in the field of biotechnology have led to the introduction of techniques such as routine electrophoresis employed for the detection of polymorphism at protein and enzyme loci as well as other serological and immunogenetic procedures for measurement of variation (Salako *et al.*, 2007). Therefore, the polymorphic variants of different proteins, enzymes, and mineral element or blood group factors represent a more accurate procedure for measurement of genetic variation in goat species. One of the important blood proteins is haemoglobin because of its biochemical, biophysical, and physiological properties, having relevance to selection

phenomenon in animals (Menrad *et al.*, 2002). The objectives of this study, therefore, were to assess haemoglobin forms, the frequencies of the haemoglobin variants, and the phenotypic ratio for two co-dominant alleles of goats found in Southern Kaduna.

Materials and Methods

Study area

The study was carried out in four Local Government Areas of Southern Kaduna State. The four local government areas were Kachia, Jaba, Jema'a, and Kaura local government area. Kaduna state lies on latitude $10^0$20'North of the equator and longitude $7^0$45'E of the Greenwich Meridian (Climate-Data.org/AM OP/OpenStreetMap).

The Animals and their Management

The breeds of goat present are not pure breeds; they are mostly crosses between different breeds, that is, the West African dwarf goat (WAD), red Sokoto goat, and Sahelian goat. This results in goats having physical features which are attributed to two or more breeds. The goats were managed under the semi-intensive system and water was provided *ad libitum*. The goats were also provided with household kitchen waste such as yam peels, potato peels, maize by-products, and left-over meals in addition to grass. There is no specific health service provided to the goats, however, there are animal health personnel in the locality that can come to the aid of the goats when health problem arises.

Sampling Technique

The Local Governments Areas were randomly selected (Saran, 2018) and it includes Kachia, Jaba, Jema'a, and Kaura. During data collection, the farmers were sampled using systematic sampling technique (Saran, 2018), that is, farmers were selected from different locations within the Local Government Area to ensure efficient coverage of the sample space.

Blood Samples Collection

Blood samples (5ml) were collected from each of the 100 goats in the four Local Government Areas. Twenty-five (25) blood samples were collected from each of the four Local Government Areas. The tools used for collection were cotton wool and methylated spirit for cleaning the area in which blood were drawn; needle and syringe were used to collect 5ml of blood from the jugular vein; EDTA bottles were used for storing blood samples and preventing it from coagulating. During collection, the goat was restrained; the goat was put in between the legs of a person who then holds it by locking his knees around the goat, the head of the goat is held and tilted to an angle which exposes the site where the jugular vein is located, and then the needle is inserted and 5ml of blood is drawn into the syringe. The blood samples were taken to National Veterinary Research Institute (NVRI), Jos after collection for the determination of genotype.

Blood Preparation

About 2ml of blood from each sample was placed into a clean test tube, 5ml of cold saline was added and the obtained diluents were centrifuged at 4000 rpm for 10-15 minutes. The supernatant was discarded, and the sample rewashed three times. The sediment was re-suspended using about 2ml of cold distilled water. When the lysate was separated after standing, it was stored at refrigeration temperature pending electrophoresis.

Electrophoresis

The red cell lysate was impregnated on a cellulose acetate paper with a control placed on the electrophoresis tank using forceps and subjected to electrophoresis according to the standard procedure described by Smithies (1955) that was later modified by Boyer and Hiner (1963). The tank was powered with the lead closed and samples allowed to separate for about 10–15 minutes. By electrophoresis, the haemoglobin fractions were separated. The identification of the haemoglobin types in goats sampled was achieved in accordance with the migration speed

of the light spots on the electrophoretic substratum, detected from the start line towards the cathodal zone. The haemoglobin polymorphism was pointed out by detection of three migration zones:

Statistical analysis

Two alleles (A and B) were detected; the haemoglobin genotype and genotypic frequencies were estimated as follows:

Genotype frequency AA = No. of AA/ No. sampled x 100/1

Genotype frequency AB = No. of AB/ No. sampled x 100/1

Genotype frequency BB = No. of BB/ No. sampled x 100/1.

The genotypic frequencies were determined, and Chi-square (x^2) was used to test goodness of fit, that is, to compare observed data and expected data to see if there was a significant difference, and to check whether the distribution of the goats' haemoglobin types is within Hardy Weinberg's equilibrium. The expected phenotypic ratio for two co-dominant alleles (HbA and HbB) is 1:2:1 corresponding to HbAA, HbAB, and HbBB respectively.

$$X^2 = \frac{(O-E)2}{E}$$

Results and Discussion

Table 1 shows the genotypic frequencies of the indigenous goats in the sampled local government areas. From the results obtained two haemoglobin genotypes were detected, homozygote (AA) and heterozygote (AB). No homozygote BB was detected in this study. The results obtained showed that the genotype with the highest frequency is the AA genotype across all local governments (68%, 60%, and 84%) for Jema'a, Kaura and Jaba local government areas, respectively except Kachia local government where AB has the highest genotypic frequency of 68%. The AB genotype had the next highest frequency across the local governments (32%, 40%, and 16%) for Jema'a, Kaura, and Jaba, local government area respectively, except in Kachia local government where it had the highest frequency of 68%. The BB genotype was not detected, that is, genotypic frequency of 0%. The observed phenotypic ratio for the two co-dominant alleles in this study is 3:2:0 corresponding to HbAA, HbAB, and HbBB respectively.

The result of this study is similar to the work of Agaviezor *et al.* (2013) in which they recorded significantly higher frequency of AA genotype (75%) over AB genotype (21.67%) and BB genotype (3.33%) of indigenous goat breeds living in areas of dense vegetation. However, no BB genotype was found in this study. The report of Buvanendran *et al.* (1981) did not find the BB phenotype in Red Sokoto breeds. Similarly, Johnson *et al.* (2002) reported that the BB genotype is not widely distributed, and this suggests that the AA genotype may have selective advantages against helminthic infections as reported by Agaviezor *et al.* (2013). They also suggested that this trend of prevalence decreases from the more humid, high altitude, rain forest breeds of goat to the dryer, low altitude region of the Sahel zone. The same trend has also been reported in Yankasa Sheep (Tella *et al.,* 2000).

Table 2 shows the phenotypic ratio for two co-dominant alleles and the Chi-square analysis of the local government areas sampled. From the table, the calculated Chi-square value X^2 (79.260) is greater than the table X^2 value (11.345) at 1% level which shows that there is a high significant ($P<0.01$) difference between the observed and the expected frequencies of haemoglobin and alleles. This means, the population was not in Hardy Weinberg equilibrium. This suggests that there was a significant change in the gene frequencies probably due to differential mortality in goats possessing different genotypes; goats with the HbA allele had a greater chance of survival as environmental effects seems to favour the fixing of the HbA allele.

Conclusion and Recommendations

Two types of haemoglobin genotypes were found in this study, HbAA and HbAB. The genotype with the highest frequency is the AA genotype.

The observed phenotypic ratio for the two co-dominant alleles in this study was 3:2:0

corresponding to HbAA, HbAB, and HbBB respectively.

The Chi-square test revealed that the goat population were not in Hardy Weinberg's equilibrium.

Environmental factors should always be assessed before goats are being introduced, so that goats with the most suitable genotype can be selected.

Table 1: Genotypic frequencies of the indigenous goats in the sampled local government areas

Local Govt	N	AA%	AB%	BB%	Total
Jema'a	25	68.00	32.00	0.00	100.00
Kaura	25	60.00	40.00	0.00	100.00
Jaba	25	84.00	16.00	0.00	100.00
Kachia	25	32.00	68.00	0.00	100.00
Total	100	61.00	39.00	0.00	100.00

Table 2: The expected and observed phenotypic ratio for two co-dominant alleles and the chi-square analysis of the data obtained

Genotype	Ratio (O)	Ratio (E)	Observed (O)	Expected (E)	$(O-E)^2$	$\frac{(O-E)2}{E}$
AA	3	1	61	25	1296.0	51.840
AB	2	2	39	50	121.0	2.420
BB	0	1	0	25	625.0	25.000
Total			100.0	100.0		X^2 =79.260

DF	5%	1%
3	7.815	11.345

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ABG 004

HERITABILITY ESTIMATE, HAEMOGLOBIN AND ALBUMIN GENOTYPING OF NIGERIAN INDIGENOUS PIGEON IN LAFIA AND ITS ENVIRONS.

Gambo, D., [1]Ajibike, A. B., Imam A. A., Hussaini, Y. I., [2]Ubu, I., Alhassaan I. D. and [3]Kosshak, A. S.

Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Nigeria.
[1]Department of Animal Production Technology, Faculty of Animal and Fisheries Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo State
[2]Department of Animal Breeding and Physiology, College of Animal Science, University of Agriculture, Makurdi, Benue State.
[3]Department of Agricultural Technology, Federal College of Land Resources Technology, Kuru, Plateau State, Nigeria.
Author for correspondence: Email: gambodauda@nsuk.edu.ng gambodauda21@gmail.com
Contact: 08062347830, 08080243132

ABSTRACT

The study was conducted using a base population from which the samples used undergone random mating and had not been selected to determine the heritability estimates of some body and external egg characteristics as well as to determine the genotypes and genes frequency of the haemoglobin and albumin of Nigerian Indigenous Pigeon. Data were collected on body linear measurements, external and internal egg quality characteristics and blood proteins (haemoglobin and albumin). Blood samples were collected from 40 apparently healthy birds for electrophoresis. Data obtained for body and external characteristics were analysed using SPSS statistical software while the blood parameters were analyzed using chi-square and standard formula for determining gene and genotype frequency. Result obtained for heritability estimate indicated that, egg traits generally has moderate to high heritability ranging from a value of 0.26±0.06 for egg weight to 0.42±0.06 for egg width. For the body measurement, shank length demonstrated the highest heritability (0.65±0.07) while body length had the least heritability value (0.15±0.06). The chi-square analysis showed no significant variation between the observed and the expected frequencies of haemoglobin but was very significant (P<0.01) for albumin. For the genotyping, majority of the genotypes were homozygous AA for hemoglobin and heterozygous AB for albumin. Two alleles A and B and three genotypes AA, AB and BB were observed for both haemoglobin and albumin. The genotypic frequencies of Hb AA, AB and BB were 0.85, 0.15 and 0.00 for haemoglobin and 0.00, 0.70 and 0.30 for albumin while the allelic gene frequency for A and B were 0.925 and 0.075 for haemoglobin as well as 0.35 and 0.65 for albumin. The prevalence of these genes at their respective locus is suggestive of their importance to the adaptability and survival of these pigeon in their natural harsh tropical environment. This could be used for future studies involving the use of more blood protein markers with a larger sample size. This may pave way for marker-assisted selection in the genetic improvement of indigenous Pigeon. Also, from the heritability values, it could be concluded that improvement of pigeons for performance in these traits could be done easily using individual or mass selection method.

Keywords: *Body Linear Measurements, Gene frequency, Genotype, Genotypic frequency, Egg qualities. Polymorphism,*

Introduction

Poultry, particularly chickens, have been recognized as an important genetic resource among the avian species (Olowofeso *et al.,* 2005). Genetic diversity, a product of interaction between environment and genetic effects, of indigenous livestock species in developing countries are valuable attributes for production,

adaptation and resistance of the indigenous animals to endemic diseases (reference). . This interaction has led to differentiation of morphological, physiological and productive traits vital to all production systems providing selection criteria for breed improvement as well as adaptation to changing environmental circumstances (Ceriotti *et al.,* 2003). Genetic resources of indigenous breeds would be required for creating more variations of desired economically important trait in order to meet the current production needs and future food security (Ajibike *et al.,* 2017). The unique values of domestic chicken's genes for egg and meat production, disease resistance, hardiness and adaptation to local environment has broaden their use as a genetic resource base for breeding of improved commercial birds (Gwaza *et al.,* 2015). The objective of the study was to comparatively investigated variation in the reproductive performance traits of Nigerian Tiv and Fulani chicken ecotypes.

Materials and Methods

Study location

The experiment was carried out at the Livestock Teaching and Research Farm of the Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi, Nasarawa State. Nasarawa State falls within the Southern Guinea Savannah Zone of Nigeria. Lafia lies between latitude 8°29'38.0" N and longitude 8°30'55.2" E. It has a climate typical of the tropical zone because of its location, with temperature ranging from 20 °C in October to 36 °C in March and rainfall varies from 13.73 - 14.00 cm (NIMET, 2008).

Experimental animal and management

Stratified random sampling technique was used to assemble a total of 100 hens - comprising 50 Tiv and 50 Fulani ecotypes, and 10 breeding cocks comprising 5 per ecotype (5 cocks per ecotypes were kept as reserves in case of mortality) from five different localities for each ecotype (Tiv ecotype was purchased from Uikpan, Daudu, Kadarko, Yelwata and Cohor in Benue and Nasarawa States while Fulani ecotype was purchased from Lafia, Akurba, Adogi, Asakio and Namu in Nasarawa and Plateau States), and used for the experiment.

The birds were housed according to ecotype and location of purchase, and were allowed two weeks for acclimatization and quarantine. During this period, the birds were dusted against ecto-parasites, dewormed and vaccinated against Newcastle disease using Lasota® while anti-stress (vitalyte), antibiotics and coccidiostat were orally administered against possible disease outbreak.

A mating ratio of 1:10 (i.e. 1 cock to 10 hens) was used, and free mating was allowed. Fertile eggs for hatching were collected after four weeks of laying in order to obtain higher fertility and hatchability. The birds were fed breeder diet containing 18% Crude protein and 2700 Kcal/Kg metabolizable energy.

Eggs for hatching were collected twice a day for 5 days, labeled according to location and ecotype, and held in egg crates under room temperature with good ventilation. At the end of 5 days of egg collection, the eggs were transported to the hatchery for incubation and hatching, and were set for pedigree hatching in an automatic electric incubator at weekly internal for four consecutive weeks (four batches).

Data collection

The following parameters were measured based on ecotype, batch of hatch and location:

Fertility: candling was done on the seventh day from first day of incubation to determine fertile eggs. Fertility was determined based on total eggs set.

$$\% \text{ fertility} = \frac{Number\ of\ fertile\ eggs}{Total\ egg\ set} \times \frac{100}{1}$$

Embryonic mortality: This is the fertilized embryo that died before hatching. Embryonic mortality was measured at two levels, early and late mortality. For accurate assessment of the early embryonic mortality, candling was carried out again at the 14th day of incubation. After hatching, all the hatched chicks were taken out of the incubator; the unhatched eggs were broken

open on the hatch day under bright sunlight to identify late embryonic mortality.

% early embryonic mortality

$$= \frac{Number\ of\ dead\ embryo\ at\ 14\ days}{Total\ number\ of\ fertile\ eggs} \times \frac{100}{1}$$

% late embryonic mortality =

$$\frac{Number\ of\ dead\ embryo\ after\ hatching}{Total\ number\ of\ fertile\ eggs} \times \frac{100}{1}$$

Hatchability: This was expressed on the basis of fertile eggs and total eggs set (reproductive capacity).

$$\% \text{ hatchability} = \frac{Number\ of\ hatced\ chicks}{Total\ fertile\ eggs} \times \frac{100}{1}$$

Reproductive capacity =

$$\frac{Number\ of\ hatched\ chicks}{Total\ egg\ set} \times \frac{100}{1}$$

Data Analysis

Data collected were arranged in a Completely Randomized Block Design (CRBD), and analyzed using multivariate analysis of SPSS statistical software ver. 21 (SPSS, 2011). Variables recorded in percentages were first transformed using arc sine transformation before being subjected to analysis. The statistical model used was:

$Y_{ijkl} = \mu + S_i + D_j + E_k + SD_{ij} + SE_{ik} + DE_{jk} + SDE_{ijk} + E_{ijkl}$

Where:

Y_{ijkl} = measure of the j^{th} progeny of the i^{th} ecotype;

μ = population mean;

S_i = effect of ecotype (i = 1 and 2);

D_j = effect of batch of hatch (j = 1, 2, 3 and 4);

E_k = effect of location (k = 1 - 5);

SD_{ij} = interactive effect between ecotype and batch of hatch;

SE_{ik} = interactive effect between ecotype and location;

DE_{jk} = interactive effect between batch of hatch and location;

SDE_{ijk} = interactive effect between ecotype, batch of hatch and location;

E_{ijkl} = random error with mean zero and variance that of the population

Results

Effect of ecotype and batch of hatch on reproductive performance

The effect of ecotype on reproductive performance of the Tiv and the Fulani local chicken ecotypes are presented in Table 1. The effect of ecotype was not significantly (p>0.05) different between the Tiv and the Fulani ecotypes for all the reproductive parameters measured. However, batch of hatch had significant (p<0.05) effect on early and late embryonic mortalities, hatchability and reproductive capacity in both the Tiv and the Fulani ecotypes as well as on fertility in the Tiv ecotype.

Batches 1, 2 and 3 significantly differed in fertility (95.01±2.54%, 94.70±2.64% and 92.81±4.06% respectively) from batch 4 in the Tiv ecotype. Batch 3 had significantly (p<0.05) lowest values for early and late embryonic mortalities (1.87±1.15% and 6.65±2.36% respectively) while batch 2 and 3 had significantly (p<0.05) highest value for hatchability (88.48±4.07% and 91.48±2.75% respectively) and reproductive capacity (83.44±2.26% and 84.84±3.66% respectively) in the Tiv ecotype. In the Fulani ecotype, batch 3 had had significantly (p<0.05) lowest value for early embryonic mortality (1.33±1.33%) while batch 1 showed had significantly (p<0.05) least value (5.40±2.37%) for late embryonic mortality. The highest hatchability value (86.36±2.51%) and reproductive capacity (78.72±2.46%) was observed in batch 2 in the Fulani ecotype.

Table 1: Effect of ecotype and batch of hatch on reproductive performance (%) of Tiv and Fulani chicken ecotype

	Fer	Eem	Lem	Hatch	Repc
Ecotype					
Tiv	92.15±1.46	8.66±1.75	11.92±1.89	79.67±3.06	73.79±3.14
Fulani	90.63±2.06	11.53±2.38	11.41±2.08	77.08±2.64	70.01±3.01
LOS	NS	NS	NS	NS	NS
Batch Tiv					
1	95.01±2.54^{a}	13.37±2.29^{b}	10.55±2.79^{a}	76.08±3.32^{b}	72.29±3.68^{b}
2	94.70±2.64^{a}	2.54±1.57^{a}	9.97±4.75^{a}	88.48±4.07^{a}	83.44±2.26^{a}
3	92.81±3.52^{ab}	1.87±1.15^{a}	6.65±2.36^{a}	91.48±2.75^{a}	84.84±3.66^{a}
4	86.08±1.39^{b}	16.85±2.39^{b}	20.51±2.23^{b}	62.64±3.50^{c}	54.60±2.92^{c}
LOS	*	*	*	*	*
Batch Fulani					
1	91.67±5.27	21.93±5.47^{c}	5.40±2.37^{a}	72.67±6.52^{ab}	65.78±4.99^{ab}
2	91.40±3.53	7.63±2.66^{ab}	6.01±2.75^{a}	86.36±2.51^{a}	78.72±2.46^{a}
3	92.48±4.06	1.33±1.33^{a}	17.61±5.04^{b}	81.05±4.93^{ab}	75.68±7.25^{ab}
4	86.96±4.37	15.21±2.93^{bc}	16.62±3.26^{b}	68.25±3.11^{b}	59.84±5.57^{b}
LOS	NS	*	*	*	*

INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM, late embryonic mortality, HATCH = hatchability, REPC = reproductive capacity, LOS = level of significant, NS = non significant

Effect of location on reproductive performance

The effect of location on the reproductive performance of the two chicken ecotypes is shown in Table 2. It was observed that location had no significant effect (p>0.05) on the reproductive parameters of the two chicken ecotypes.

Interactive effects of ecotype, batch of hatch, and locations on Reproductive Performance

The mean squares result of the effect of ecotype, batch of hatch, location and their interactions on reproductive performance is shown in Table 3. Ecotype, location and interactions between ecotype by batch of hatch, ecotype by location and batch of hatch by location had no significant (p>0.05) effect on fertility, early and late embryonic mortalities, hatchability and reproductive capacity.

Batch of hatch had significant (p<0.05) effect on early and late embryonic mortalities, hatchability and reproductive capacity. However, batch of hatch had no significant (p>0.05) effect on infertility and fertility.

Interactions between ecotype × batch × location was significant (p<0.05) on infertility, early and late embryonic mortalities. The interactions between ecotype × batch × location had no significant (p>0.05) effect on fertility, hatchability and reproductive capacity. The coefficients of determinant (R^2) values were 0.147, 0.418, 0.297, 0.395 and 0.499 for fertility, early and late embryonic mortalities, hatchability and reproductive capacity respectively.

Table 2: Effect of Location on Reproductive Performance (%) of the Tiv and the Fulani Chicken Ecotypes

	Fer	Eem	Lem	Hatch	Repc
Tiv ecotype					
1	92.83±4.25	9.31±2.33	13.92±3.82	76.78±6.05	71.32±6.75
2	93.54±3.01	7.17±2.59	12.28±3.17	80.55±4.29	75.64±5.95
3	92.40±4.41	8.71±5.53	12.25±6.06	80.29±7.92	74.24±7.74
4	89.70±3.75	9.98±4.94	8.60±2.77	81.45±6.53	74.64±7.71
5	92.29±2.14	8.14±5.29	12.57±6.22	79.29±11.45	73.13±10.38
LOS	NS	NS	NS	NS	NS
Fulani ecotype					
1	84.35±5.59	5.56±3.21	14.97±4.56	79.48±4.09	67.15±5.92
2	91.17±3.28	18.17±8.96	10.35±4.77	71.48±8.88	64.64±6.79
3	95.31±4.69	13.23±4.00	12.27±2.18	74.60±2.74	71.35±5.44
4	88.26±5.30	14.59±5.24	12.02±6.95	73.40±5.33	65.40±7.27
5	94.05±3.95	6.09±2.15	7.44±5.45	86.48±6.41	81.49±7.68
LOS	NS	NS	NS	NS	NS

INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM, late embryonic mortality, HATCH = hatchability, REPC = reproductive capacity, LOS = level of significant, NS = non significant, Tiv ecotype: location 1-5 = Uikpan, Daudu, Kadarko, Yelwata and Cohor, Fulani ecotype: location 1-5 = Lafia, Akurba, Adogi, Asakio and Namu

Summary Statistics and Coefficient of Variations of Reproductive Traits

The percentage coefficient of variations (Table 4) showed variability in reproductive traits between locations of the two ecotypes. Early embryonic mortality had the highest values of CV while the CV for fertility had the least values in both the Tiv and the Fulani ecotypes. In the Tiv ecotype, birds from Uikpan and Kadarko (locations 1 and 3) had CV values of 118.49% and 115.89% percent in infertility. CV of 127.08% and 129.94% was obtained in early embryonic mortality in birds from Kadarko and Cohor (locations 3 and 5 respectively) in the Tiv ecotype. In the Fulani ecotype, birds from Namu (location 5) had CV of 132.62% for infertility while birds from Lafia (location 1) had CV of 115.47% for early embryonic mortality. Birds from Asakio and Namu (locations 4 and 5) had CV of 115.65% and 146.45% respectively for late embryonic mortality.

Table 3: Mean square values of the effect of ecotype, batch of hatch, location and their interactions on reproductive performance (%) of Tiv and Fulani chicken ecotypes

Traits	Sources of variation	Df	Mean square	R^2
Fertility	Ecotype	1	23.23^{ns}	0.147
	Batch	3	106.10^{ns}	
	Location	4	47.63^{ns}	
	Ecotype*Batch	3	11.42^{ns}	
	Ecotype *Location	4	39.79^{ns}	
	Batch*Location	12	72.29^{ns}	
	Ecotype*Batch*Location	12	426.58^{ns}	
	Error	200	53.49	
Early embryonic mortality	Ecotype	1	82.226^{ns}	0.418
	Batch	3	631.87***	
	Location	4	56.293^{ns}	
	Ecotype*Batch	3	57.70^{ns}	
	Ecotype *Location	4	70.00^{ns}	
	Batch*Location	12	21.52^{ns}	
	Ecotype*Batch*Location	12	238.81***	
	Error	200	50.65	
Late embryonic mortality	Ecotype	1	2.621^{ns}	0.297
	Batch	3	249.83*	
	Location	4	25.591^{ns}	
	Ecotype*Batch	3	147.12^{ns}	
	Ecotype *Location	4	20.78^{ns}	
	Batch*Location	12	88.64^{ns}	
	Ecotype*Batch*Location	12	276.46***	
	Error	200	54.00	
Hatchability	Ecotype	1	66.93^{ns}	0.395
	Batch	3	1091.17***	
	Location	4	55.44^{ns}	
	Ecotype*Batch	3	107.97^{ns}	
	Ecotype *Location	4	102.57^{ns}	
	Batch*Location	12	69.03^{ns}	

	Ecotype*Batch*Location	12	571.85^{ns}	
	Error	200	97.24	
Reproductive capacity	Ecotype	1	143.26^{ns}	0.499
	Batch	3	1259.43***	
	Location	4	87.54^{ns}	
	Ecotype*Batch	3	98.89^{ns}	
	Ecotype *Location	4	115.15^{ns}	
	Batch*Location	12	109.10^{ns}	
	Ecotype*Batch*Location	12	490.67^{ns}	
	Error	200	94.09	

Df = degree of freedom, *= ($p<0.05$), ** = ($p<0.01$), *** = ($p<0.001$), ns=Not significant and R^2 =Adjusted values

Table 4: Statistics summary of the Reproductive Performance (%) of two Nigerian Chicken Ecotypes

TR	L	B	Tiv Ecotype							Fulani Ecotype						
			MI	MA	RA	ME	SD	SE	CV	MI	MA	RA	ME	SD	SE	CV
	1	4	83.33	100.00	16.67	92.83	8.49	4.25	9.15	75.00	100.00	25.00	84.35	11.19	5.59	13.27
	2	4	86.29	100.00	13.71	93.53	6.01	3.01	6.43	85.71	100.00	14.29	91.17	6.57	3.29	7.20
FER	3	4	83.88	100.00	16.12	92.40	8.81	4.41	9.54	81.25	100.00	18.75	95.31	9.38	4.69	9.83
	4	4	82.24	100.00	17.76	89.70	7.50	3.75	8.36	75.00	100.00	25.00	88.26	10.59	5.30	12.00
	5	4	87.50	96.67	9.17	92.29	4.27	2.14	4.63	83.33	100.00	16.67	94.05	7.90	3.95	8.40
	1	4	5.00	13.64	8.64	9.31	4.67	2.33	50.14	0.00	11.11	11.11	5.56	6.41	3.21	115.47
	2	4	0.00	12.00	12.00	7.17	5.17	2.59	72.19	0.00	40.00	40.00	18.17	17.92	8.96	98.60
EEM	3	4	0.00	23.08	23.08	8.71	11.07	5.53	127.08	6.67	23.53	16.86	13.23	7.99	4.00	60.43
	4	4	0.00	22.22	22.22	9.98	9.87	4.94	98.96	0.00	25.00	25.00	14.59	10.49	5.24	71.90
	5	4	0.00	22.22	22.22	8.14	10.58	5.29	129.94	0.00	10.00	10.00	6.09	4.29	2.15	70.48
	1	4	5.55	22.73	17.18	13.92	7.64	3.82	54.89	9.09	28.57	19.48	14.97	9.12	4.56	60.90
	2	4	4.76	20.00	15.24	12.28	6.34	3.17	51.61	0.00	23.08	23.08	10.35	9.55	4.77	92.21
LEM	3	4	0.00	27.73	27.73	12.25	12.11	6.06	98.89	5.88	15.58	9.70	12.27	4.36	2.18	35.52
	4	4	4.35	16.67	12.32	8.60	5.53	2.77	64.35	0.00	25.00	25.00	12.02	13.90	6.95	115.65
	5	4	0.00	27.78	27.78	12.57	12.44	6.22	99.00	0.00	23.08	23.08	7.44	10.89	5.45	146.45
HAT	1	4	63.64	88.89	25.25	76.78	12.09	6.05	15.75	71.43	90.91	19.48	79.48	8.19	4.09	10.31

	2	4	68.00	86.36	18.36	80.55	8.57	4.29	10.64	50.00	92.31	42.31	71.48	17.78	8.89	24.87
	3	4	61.54	100.00	38.46	80.29	15.85	7.92	19.74	69.23	80.00	10.77	74.60	5.47	2.74	7.34
	4	4	70.00	94.12	24.12	81.45	13.06	6.53	16.03	58.33	83.33	25.00	73.40	10.66	5.33	14.52
	5	4	50.00	100.00	50.00	79.29	22.90	11.45	28.88	69.23	100.00	30.77	86.48	12.82	6.41	14.82
	1	4	56.00	88.89	32.89	71.32	13.50	6.75	18.93	55.56	77.78	22.22	67.15	11.84	5.92	17.63
	2	4	58.62	86.36	27.74	75.64	11.90	5.95	15.73	50.00	80.00	30.00	64.64	13.57	6.79	21.00
RCP	3	4	51.61	85.71	34.10	74.24	15.48	7.74	20.85	56.25	80.00	23.75	71.35	10.89	5.44	15.26
	4	4	61.29	91.30	30.01	74.64	15.42	7.71	20.66	43.75	75.00	31.25	65.40	14.53	7.27	22.22
	5	4	45.00	90.00	45.00	73.13	20.75	10.38	28.38	64.29	100.00	35.71	81.49	15.36	7.68	18.84

TR = traits, L = locations, B = batch of hatch, MI = minimum value, MA = maximum value, RA = range, ME = mean, SD = standard deviation, SE = standard error of the mean, CV = coefficient of variation, INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM = late embryonic mortality, HAT = hatchability and RCP = reproductive capacity, Tiv ecotype: location 1-5 = Uikpan, Daudu, Kadarko, Yelwata and Cohor, Fulani ecotype: location 1-5 = Lafia, Akurba, Adogi, Asakio and Namu

Discussion

The observed variations within and between Tiv and Fulani ecotypes for fertility, embryonic mortality, chick mortality and hatchability as obtained in this study had been reported earlier by Gwaza *et al.* (2015). The observed percent fertility value in both chicken ecotypes strongly agreed with the range of 83.0 - 92.7% for Bangladesh local chickens as reported by Islam and Nishibori (2009) and 79.65±0.45-86.65±0.07% reported by Amao (2017) for Nigerian Nacked neck chickens. Gwaza *et al.* (2015) reported a slightly lower percent fertility of 70.62±3.70 and 77.75±6.28 % for Fulani and Tiv chicken ecotype respectively.

Early embryonic mortality obtained for Tiv and Fulani chicken ecotypes were similar to 14.90% reported by Gwaza *et al.* (2015) while late embryonic mortalities are lower than 14.90% reported by Gwaza *et al.* (2015). The observed similarity could be due to the facts that the birds were of the same ecotype. Percentage Hatchability observed for both chicken ecotype fell within the range of 72- 93.1% and 85.07±8.90% reported by Ajayi *et al.* (2008) and Amao (2017) respectively while Islam and Nishibori (2009) reported a lower and wider range of 52.4-87.0% for indigenous full feathered chickens.

Fulani chicken ecotype had higher reproductive capacity (hatchability based on total egg set) value range of 59.84 - 78.72% while Tiv ecotype recorded 54.60 - 84.84% which fell within the reported values of of 58.00, 55.14, 61.31 and 56.90% by Farooq *et al.* (2001), Kurshid *et al.* (2004), Daikwo (2011) and Gambo *et al.* (2014)for Japanese quail respectively. Reproductive capacity is of more practical importance to farmer than hatchability (reference). Genetic variations in hatchability of fertile eggs may arose from the hen due to quality of laid eggs which affects the development of embryo to chicks during incubation as well as emergence of chicks from the egg at hatching (Anang *et al.,* 2001; Wolc *et al.,* 2009). The observed high fertility, hatchability and reproductive capacity for both chicken ecotypes could be due to better fertility, and thus, suggested high prolific which could be utilized efficiently in meat and egg production enterprise. However, the higher values demonstrated by the Tiv ecotype compared to the Fulani ecotype for fertility, hatchability and reproductive capacity could be that the levels of inbreeding among the population of the Tiv local chicken ecotype were low compared to that of the Fulani chicken ecotype. The significant effect of batch of hatch indicated diversity of alleles that was sampled at segregation, independent assortment and recombination of genes

Conclusion

It can be concluded that there is no much variation in the reproductive performance of the two Nigerian chicken ecotypes studied, despite observed high percentage fertility, hatchability and reproductive capacity coupled with low early and late embryonic mortalities. Therefore, the high reproductive performance of both chicken ecotypes should be genetically exploited for commercial hatching and sales/distribution of local day-old chicks as well as for planning of breeding and conservation strategy.

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ABG 005

SHORT-TERM EVALUATION OF PULLETS REPRODUCTIVE TRAITS IN SHIKABROWN® PARENTS' LINES

[1]Olutunmogun, A.K., [2]Kabir, M., [1]Nwagu, B.I. and [2]Yahaya, H.K.

[1]National Animal Production Research Institute, Ahmadu Bello University (NAPRI/ABU) Shika-Zaria, Kaduna State, Nigeria
[2]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
Corresponding Author: olutunmogunkayode@yahoo.com +234 7039338987.

Abstract

The study was carried out to evaluate the short-term reproductive traits of pullets from ShikaBrown® parents' lines. The two lines are the sire (SG-98) and dam lines (SS-98) bred for egg weight and egg numbers respectively. Pure mating design was adopted to produced progenies of SG x SG and SS x SS. Data were collected and analyzed for bodyweight (day-old chick weight, 20, 24, 40weeks bodyweight and bodyweight at first egg), and reproductive traits (age at first egg, egg weight at first egg, egg weight at 40weeks and egg number). The results on bodyweight showed that the dam line had a significant ($p<0.05$) high day-old bodyweight over the sire line (32.18g vs 29.87g) while the sire line had a significant ($p<0.05$) better bodyweight at 20, 24 and 40weeks respectively. Also, the bodyweight at first egg had a significantly ($p<0.05$) difference with the sire line being better. The results on the reproductive traits showed that the dam line had a significant ($p<0.05$) difference over the sire line in age at first egg (160 vs 171days) and egg number (77.5 vs 73.8) while the sire line had a significant ($p<0.05$) difference on egg weight at 40weeks (44.03g vs 41.39g). However, both lines showed no significant ($p>0.05$) difference in egg weight at first egg. It is therefore concluded that the lines are still distinct from each other for which they were selected however, selection be aimed at increasing egg size as to enable their hybrid layers perform better or the two lines be crossed with similar exotic genetic genes that has better reproductive performance so has to increase their performances.

Keywords: *Bodyweight, Reproductive traits, Pullets, ShikaBrown® Chicken.*

Introduction

In Nigeria, egg production is a major means to increase the quality and quantity of protein requirement for her growing population (Ogbu *et al.*, 2015). Achievements have been obtained through genetic research on egg laying chicken carried out by the National Animal Production Research Institute (NAPRI) between the mid-80s to 2000. The breeding programme at NAPRI apply reproductive traits of age at first egg, body weights at first egg, 20, 24 and 40weeks, egg number (EN) and egg weights (EW) to the improvement of parent lines and the production of layer hybrids. The egg production of the individual bird is predisposed to a number of laying traits characteristics which includes clutch/sequence number, clutch/sequence length, the rate of lay, oviposition time, oviposition interval, lag time, frequency of pauses (pause number), number of pause days (pause length or size), tendency to go broody and length of the broody period (Eltayeb *et al.*, 2010; Al-Nedawi *et al.*, 2008). In parental lines, results obtained from these economic traits will predict the progeny abilities when crossed (crossbreeding) to obtain their hybrids or more

intensive selection on the primary traits on the parental lines (Szwaczkowski *et al.*, 2003). The aim of this study was to evaluation the short-term reproductive traits of pullets from parent lines of ShikaBrown® chicken.

Materials and Methods

Experimental location

The experiment was conducted at the Poultry Breeding Unit of the Poultry Research Programme farm, National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika, Zaria, Kaduna State, Nigeria. Shika is geographically situated in the Northern Guinea Savannah zone between latitude 11º 12' 42" N and longitude 7º 33'14" E at an altitude of 640m above sea level (Ovimaps, 2017). The climate is characterized by well-defined dry and wet seasons which are divided into cold-dry season (November–February), the hot-dry season (late February - April) and the wet season (early May – October). Temperature during the harmattan season ranges from 14ºC to 30ºC and 21 to 36ºC during the hot season. Relative humidity varies from approximately 21% during the harmattan to 37% during the hot season. The average temperature and humidity during the wet season are 24.8ºC and 77% respectively (IAR, 2016).

Foundation birds and management

The foundation flock used for the study comprises of two lines of ShikaBrown® parent chickens i.e. sire line (golden) and dam line (silvery). A comprehensive description is as described in Table 1.

Table 1: Description of the two lines of ShikaBrown® parents

Lines	Morphological Features			Purpose
	Comb type	Plumage	Shank/Beak	
Sire	Single	Gold/Brown	Brown/Brown	Body and egg weight
Dam	Single	Silvery/White	Yellow/Yellow	Egg number

288 chickens from each of the parental lines (SG-98 and SS-98) comprising of 32 ♂ and 256 ♀ totaling 576 chicken where used as parents to generate the experimental chickens. The chickens were randomly selected for the research work from a random-bred population maintained on the Poultry Research Farm of the Poultry Research Programme in National Animal Production Research Institute Shika-Zaria. The birds were put into a mating ratio scheme of 1 cock: 8 hens which forms the foundation flock for the experiment. The birds were exposed to natural day light and were fed breeder mash and water was provided *ad libitum*.

Experimental birds and Management

The chicks hatched from each line were wing tagged with an industrial galvanized aluminum tags at the web of the wing at day-old. The day-old chicks were brooded in a separate pen according to line. At 16weeks, the pullets were transferred to individual laying cages according to their lines to determine their reproductive performance. The chickens were fed with formulated diet which supplied metabolizable energy of 2652.45, 2400.18 and 2520Kcal/ kg and crude protein of 20.99, 16.08 and 18.05% at chick, grower and breeder phases respectively. Clean water was supplied *ad-libitum*. Medication and vaccination were done as at when due.

Data collection and design

Data were collected and analyzed for bodyweight (day-old chick weight, 20, 24, 40weeks bodyweight and bodyweight at first egg), and reproductive traits (age at first egg, egg weight at first egg, egg weight at 40weeks and egg number). The body weights were taken using a Camry model sensitive weighing scale (model EK5350).

A completely randomized design (CRD) was used for the study.

Statistical analysis

Data collected were subjected to analysis of variance using Statistical Analysis System (SAS) for windows (SAS 2002). Where significant differences were observed, the differences between means were separated using Duncan New Multiple Range Test (DMRT year).

The statistical model used was:

$Y_{ij} = \mu + L_i + e_{ij}$

Where Y_{ij} = observation of the j^{th} random error of the i^{th} lines, μ = the overall common mean, L_i = the effect of i^{th} lines (i = 1, 2) and e_{ijk}= random error

Results and Discussion

The egg production traits and body weight of the two parent lines of ShikaBrown® chicken is as presented in Table 2. The results showed that most traits had significant ($p<0.05$) differences with the sire line being superior in traits of 20weeks body weight, body weight at first egg, 24weeks body weight, 40weeks body weight and egg weight at 40weeks. However, day-old weight and age at first egg were significant ($p<0.05$) in the dam line over the sire line. Information on the body weight at hatch both for exotic and indigenous laying birds are very limited (Khawaja *et al*., 2012) due to its small magnitude and the fragility of the neonates. Balami *et al.* (2018) reported a non-significant difference in day-old weight between the two line in a mating ratio study where the dam line recorded 29.83 to 31.75g and the sire had 30.25 to 32.83g. The result on day-old body weight is contrary to reports of Adedeji *et al.* (2008) who stated that in modern breeding, the sire line has significant genetic effect to body weight and egg weight. However, observations on other body weights presents that the sire line performed superior as it was genetically engineered to be. This is in accordance with the findings of Amao (2017) who reported superior body weight at sexual maturity in pure genotype of Fulani ecotype chicken followed by crosses between Rhode Island Red and Fulani ecotype and Fulani ecotype and Rhode Island Red. Although the report was not in line with that of pure Rhode Island Red crosses for body weight which was for lower. Meanwhile, the reports on the reproductive trait of age at first egg agrees with Adedeji *et al.* (2008) that parental dam line are genetically design for laying trait performance as observed with the dam line coming to lay earlier than sire line. Udeh *et al.* (2013) reported variations in this trait in three parent line strains with the exotic 1 having 49.4g, exotic 2 had 41.76g and the local strain having 33.98g. The report on parental exotic 2 is similar to that of this present study however this egg size is relatively small in size. This could be attributed to similar genetic make-up or environment. Egg weight at 40weeks correlate the purpose for which each of the lines where selected for which also validates the earlier report of Adedeji *et al.* (2008) that sire lines are selected for weights which indirectly selects for egg weight. The result on egg number of this study (dam =77.5eggs vs sire =73.8eggs) though lower agrees with the work of Oni *et al.* (2003) who obtained 82.4eggs and 78.4eggs for the dam and sire lines respectively.

Conclusion

It can therefore be concluded that the lines are still distinct from each other for which they were selected however, selection be aimed at increasing egg size to enable their hybrid layers perform better or the two lines be crossed with similar exotic genetic genes that has better reproductive performance so has to increase their performances.

Table 2: Egg production traits and body weight of two lines of ShikaBrown® parents' chicken

Traits	Sire line	Dam line
DO body weight (g)	29.87±14.09[b]	32.18±14.06[a]
20weeks body weight (g)	1886±13.09[a]	1624±12.89[b]
Body weight at first egg (g)	1879±13.01[a]	1628±12.00[b]
24weeks body weight (g)	2017±30.22[a]	1784±30.20[b]
Age at first egg (days)	171±4.21[b]	160±4.28[a]
Egg weight at first egg (g)	40.55±1.65[ns]	41.02±1.70[ns]
40weeks body weight (g)	1907.5±25.11[a]	1831.33±24.87[b]
Egg weight at 40weeks (g)	47.39±3.52[a]	44.03±3.18[b]
Egg number	73.8±1.17[b]	77. 5±1.29[a]

[ab] = Means with different superscript within the same row differ significantly ($p<0.05$), DO = Day-old, ns = not-significant

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ABG 006

MORPHOLOGICAL TRAITS OF CAMEL (*CAMELUS DROMEDARIUS*) IN NORTH-WESTERN NIGERIA

Bello, M.O., Ojo, O.A., Kabir, M. and Akinsola, O.M.

Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria.
National Agriculture Extension and Research Liaison Service, Ahmadu Bello University, Zaria.
Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria.
Department of Theriogenology and Production, Faculty of Veterinary Medcine, University of Jos, Jos.

Corresponding author: Princessmty2@gmail.com +2347039622888, +2348052138536

Abstract

This study examined the morphological traits of Camels located in Jigawa, Kastina,Sokoto and Zamfara States of North-Western. One hundred and twenty-four Camels were used for the study, of which 83 males and 41 females were randomly sampled. The morphological traits observed were: Hair type (long-rough; short-rough; long-smooth and short-smooth), Coat colour (Black; Brown; Red/white; Red and White) and Eye colour (Black; Brown and White). Data analysis was done using General Linear Model procedure of Statistical Analytical System (SAS) 0.9 (2004). Results obtained revealed significant variations across the Morphological traits. It can be concluded that exploited morphological variations existed across locations for improvement of indigenous Camel population in Nigeria.

Keywords: *Age, Camel, Location, Morphological, Sex and Strain*

Introduction

The world's Camel population is estimated at 19 million with 80% found in Africa (FAO, 2010). According to FAOSTAT (2013), the total population of dromedary in Nigeria is 20,500 heads of Camels. These animals are found in the Northern states of Nigeria. Camels play important role in the economy of these states. The Northern States of Nigeria where *Camelus dromedarius* (one hump Camel) are found include: Borno, Yobe, Jigawa, Kano, Katsina, Kebbi, Sokoto and Zamfara (James-Rugu and Jidayi, 2004; Mohammed and Hoffman, 2006). According to Timothy *et al.* (2015) 80% of the total population of Camels in Nigeria are found in Sokoto State, Katsina State, Kano State and Borno State which cover a combine area of 70, 714 km^2 and are desert gate ways with important Camel trade. They can lose up to about 30% of their body weight by loss of water and they can replenish this loss rapidly by drinking large volume of water quickly at the next opportunity (Porter *et al.,* 2016). During drought periods, they are able to utilize thorny and spine plants for feeding that other animals will not be able to utilize and they can continue to produce milk and meat for human consumption under drought circumstances that would lead to the death of other livestock breeds (Porter *et al.*, 2016). These information's are required for the design of appropriate selection and breeding strategy for utilization and improvement of the potential of Camel genetic resources.

Materials and methods

Description of Sokoto State:

Sokoto state is located in Sudan savanna zone in the extreme North-Western Nigeria. It lies between longitude 4°8′E and 6° 54′ E and latitude 12° N and 13° 58′ E (Mamman *et al.*,

2000). The mean annual rainfall is 750mm and potential evapotranspiration rates have been reported to be 162cm (Mamman *et al.*, 2000). The annual mean temperature is 34.9°C with highest temperature recorded in April (41.0°C) and the minimum temperature (13.2°C) occurring in January (Climate-data, 2017).

Katsina State:
Kastina State covers an area of 23,938sq km, is located between latitudes 11°8′ and 13°22′N and longitudes 6°52′E and 9°20′E (Ovimap, 2017).
Jigawa State:
Jigawa State is situated in the North Western part of the country between latitudes 11.00°N to 13.00°N and longitudes 8.00°E to 10.15°E (Ovimap, 2017).
Zamfara State:
Zamfara State has general elevation of land which ranges from 244m to 366m above sea level (Ovimap, 2017). The Latitude and Longitude of Zamfara State is 12.1844°N and 6.2376°E respectively. 12.1844°N Latitude and 6.2376°E Longitude can be mapped to closest address of Zamfara, Nigeria (Ovimap, 2017).
Sampling size and sample structure
Simple random sampling method was used to sample Camels using morphological or morphometric traits within a given state. Ten (10) Camels were sampled from 3 local government areas in each State, making a total of thirty (30) Camels per state for Kastina and Zamfara States. For Sokoto and Jigawa States, a total of thirty-two (32) Camels were sampled in each State. The total sample size for the four (4) States was 124 Camels. The local government areas are; Maigatari, Sule Tankarkar and Kaugama in Jigawa state; Jibia, Maiaduwa and Daura in Katsina state; Gada, Kware and Yabo in Sokoto state and Bakura, Talata Mafara and Kaura Namoda in Zamfara state. The sample size for age group were: pre-weaned (5), young (57) and Adult (56) making a total of 118 Camels. The morphological and morphometric traits were determined using visual observation and body linear measurement techniques, respectively.
Data Collection and Statistical analysis
The morphological characters considered on each animal using ICAR (2012) include the following;
Hair Type: This was observed and categorized into short-smooth, short-rough, long-smooth and long-rough (woolly) hair types.
Eye colour: This was observed and categorized as black, brown and white.
Coat colour: This was observed and categorized as red, black, white, brown, brown/white and red/white.

Statistical analysis

Data on morphological traits were analyzed using frequency counts and Chi Square test. General Linear Model (GLM) procedure of SAS 0.9 (2004) was used.
Statistical Model: Model used as shown below:
$Y_{ijklm} = \mu + S_i + A_j + L_k + V_l + \varepsilon_{ijklm}$
Where; Y_{ijklm} = observation on each trait; μ = population mean; S_i = Fixed effect of the i^{th} sex (i=2; male and female); A_j = Effect of the j^{th} age group (j=3; pre-weaned, young and adult); L_k = Effect of k^{th} location (k=4; Kastina, Jigawa, Sokoto and Zamfara states); V_l = Fixed effect of l^{th} strain (l=5; Baki, Ja, Mahari, Fari and Ruwan-kasa); ε_{ijklm}= residual error, which is assumed to be identically independent and normally distributed with zero mean and constant variance (iind, 0 σ^2).
Phenotypic frequency analysis for morphological traits
The distribution of the measured qualitative traits were assessed for significance using Chi-square analysis and computed based on pooled data within strain and sex using the frequency procedure of JMP statistical package.

$$\text{Chi-square } (x^2) = \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

Table 1: Morphological distribution of Camels in some North-Western States of Nigeria.

Traits	N	Variations	% frequency	Confidence level %	χ^2 – value	P-value
Coat colour	124	-	100		620.00	0.0001**
	24	Black	19.35	13-27		
	29	Brown	23.39	17-32		
	1	Brown and white	0.81	0.1-4		
	45	Red	36.29	28-45		
	3	Red and white	2.42	0.8-7		
	22	White	17.74	12-25		
Hair type	124	-	100		372.00	0.0001**
	27	Long rough	27.77	15-30		
	28	Long smooth	22.58	16-31		
	14	Short rough	11.29	7-18		
	55	Short smooth	44.35	36-53		
Eye colour	124	-	100		248.00	0.0001**
	64	Black	51.61	43-60		
	53	Brown	42.74	34-52		
	7	White	5.65	3-11		

N= sample size, %= percent, χ^2 = chi-square, **P<0.01

Table 2: Distribution of morphological traits among Camel strain in some North-Western States of Nigeria

		Strain (%)							
Traits	N	Baki	Ruwan-kasa	Fari	Ja	Mahari	Others	χ^2 value	P-value
Hair type	124	-	-	-	-	-	-	25.44	0.04**
Long rough	27	33.33	3.70	11.11	48.15	3.70	0.00		
Long smooth	28	17.86	21.43	17.86	32.14	7.14	3.57		
Short rough	14	21.43	35.71	0.00	42.86	0.00	0.00		
Short smooth	55	12.73	30.91	25.45	30.91	0.00	0.00		
Eye colour	124	-	-		-	-	-	29.93	0.0009**
Black	64	14.06	28.13	15.63	42.19	0.00	0.00		
Brown	53	24.53	18.87	18.87	33.96	3.77	0.00		
White	7	28.57	14.29	28.57	0.00	14.29	14.29		

N= sample size, χ^2 = chi-square and **P<0.01.

Table 3: Effect of Sex on morphological traits of Camel in some North-Western States of Nigeria

Traits	Sex (%)		χ^2-value	p-value
	Male (n = 83)	Female (n = 41)		
Eye colour			3.53	0.17
Black	56.0	44.0		
Brown	37.0	54.0		
White	7.0	2.0		
Hair type				
Long rough	20.0	2.0	1.72	0.63
Long smooth	22.0	24.0		
Short rough	10.0	15.0		
Short smooth	48.0	37.0		
Coat colour				
Black	17.0	24.0	1.44	0.92
Brown	24.0	22.0		
Brown-white	1.0	0.0		
Red	37.0	34.0		
Red-white	2.0	2.0		
White	18.0	17.0		

n= sample size, χ^2 = chi-square, $P<0.05$.

Table 4: Effect of location on morphological traits of Camel in some selected North-Western States of Nigeria

Traits	Location (%)				χ^2	p-value
	Jigawa (n=32)	Kastina (n = 30)	Sokoto (n = 32)	Zamfara (n = 30)		
Eye colour						
Black	56.0	40.0	44.0	67.0	11.36	0.08
Brown	41.0	60.0	47.0	23.0		
White	3.0	0.00	9.0	10.0		
Hair type						
Long rough	22.0	20.0	25.0	20.0	9.71	0.37
Long smooth	22.0	20.0	25.0	23.0		
Short rough	19.0	7.0	0.0	20.0		
Short smooth	37.0	53.0	50.0	37.0		
Coat colour						
Black	16.0	17.0	16.0	30.0	18.54	0.24
Brown	28.0	27.0	22.0	17.0		
Brown-white	3.0	0.0	0.0	0.0		
Red	5.0	33.0	28.0	33.0		
Red-white	0.0	0.0	6.0	3.0		
White	3.0	23.0	28.0	17.0		

n= sample size, χ^2 = chi-square and $P<0.05$.

Table 5: Effect of age on morphological traits of Camel in some selected North-Western States of Nigeria

Traits	Age (%)			χ^2	p-value
	Pre-weaned (<1years) n=5	Young (1-6 years) n= 57	Adult (>6 years) n=56		
Eye colour					
Black	60.0	53.0	48.0	2.41	0.66
Brown	40.0	39.0	48.0		
White	0.0	8.0	4.0		
Hair type					
Long rough	60.0	33.0	9.0	25.31	0.0003**
Long smooth	40.0	26.0	20.0		
Short rough	0.0	16.0	9.0		
Short smooth	0.0	25.0	62.0		
Coat colour					
Black	20.0	23.0	16.0	5.35	0.87
Brown	20.0	23.0	23.0		
Brown-white	0.0	2.0	0.0		
Red	40.0	37.0	36.0		
Red-white	0.0	0.0	5.0		
White	20.0	15.0	20.0		

n= sample size, χ^2 = chi-square, **P<0.01

Results

Characterization of camels using morphological traits

Table 1 shows the distribution of some observed morphological characteristics in Camels. The most common coat colour was red (36.29%) with colours like brown-white and red-white in rear occurrence (0.81% and 2.42% respectively). Four hair types were observed with short-smooth hair type (44.35%) being the most popular. The occurrence of short-rough, long-smooth and long-rough was in the range of 11%-28%. Majority (51.61%) of the Camels had black eyes followed by brown (42.74%) while white eye colour rarely occurred (5.65%).

However, the reliability of the distribution of measured characteristics differed from one characteristics to the other. There was a high level of reliability for red coat colour (28-45%), distribution of brown having an intermediate occurrence of (17-32%); while red-white had rare occurrence of (0.8-7%) similarly brown-white had a very rare occurrence (0.1-4%) for Camels in Jigawa, Kastina, Sokoto and Zamfara States. Short-smooth hair type had high level of reliability with the occurrence of (36-53%); with long-rough and long-smooth having an intermediate occurrence of (15-30% and 16-31% respectively). The eye colour of Camels in some selected State of North-Western Nigeria would more often than not, turn out to be black (43-60%); with brown eye colour showing an intermediate occurrence (34-52%) while white eye colour was rare (3-11%).

Distribution of Qualitative traits of Camels as influenced by Strain, Sex, Location and Age

Strain distribution of qualitative traits of Camels is reported in Table 2. Significant differences ($P<0.05$) occurred between the populations of Camels for some observed morphological traits in some selected States of North-Western Nigeria. Most of the Camels (55) had short-smooth hair type. It was observed that both Ruwan-kasa strain and Ja strain of Camels have (30.91%) of short-smooth hair while Mahari strain had (0.00%) of it. Majority (64) of the Camels had black eye colour of which (42.19%) was recorded in red Camels while the least was also recorded in Mahari strain (0.00%) of Camels.

Distribution of qualitative traits of Camels based on sex is shown in Table 3. There were no significant differences ($P>0.05$) among all the qualitative traits. Table 4 shows the distribution of qualitative traits of Camel on the basis of location. From the results, there were no significant difference ($P>0.05$) among all the qualitative traits.

Table 5 gave the age distribution of Camels for qualitative traits. There were no significant differences ($P>0.05$) among all the qualitative traits with the exception of hair type. Long-rough hair type favoured (60%) of the pre-weaned, followed by the young (33%). The adult had more Short-smooth hair type (62%) followed by long-smooth hair type (20%).

Discussion

Distribution of qualitative traits of Camels as influence by strain, sex, age and location

The red coloured Camels were found to be predominant among the Camel population while brown-white and red-white coloured Camels were fewer in number. This agrees with the finding of Akin and Bode (2018) which stated that red constitute the largest population of Camels and are almost evenly distributed among small holder farmers. The general pattern for coat colour, hair type and eye colour showed high variations which is at congruent with the study of Berhanu *et al.* (2016) who observed significant variation among six qualitative traits (coat color pattern, coat color type, hair type, ear orientation, face profile and lip shape) in Oromia Regional State of Ethiopia. This implied that the propensity towards smooth hair structure could be an advantage as it provides a medium for conventional heat loss from the animal body surface from the tropical environment. This is supported by the assertion that hair structures have an important role to play in the

adaptability of animals to different ecological zones (Banerji, 1984). However, the degree of tolerance or susceptibility of local Camel to the stressful environment due to variation in phenotypic characteristics is a subject of interest in this study. Since colour plays an important role in the absorption and reflection of ultraviolet radiation, Black Camels may be more susceptible to heat stress under intense solar radiation. Camels with white phenotypic characteristics on the other hand may be more tolerant under same conditions.

Morphological Characterization of Camels and Factors affecting them

Though the frequencies of some coat colours were small in studied population, the current study demonstrated that the studied Camel populations have a wide range of coat colours. In general, when appraised visually, the Camel from the four locations were different, this might have resulted from differences in geographical locations. Hair type, eye colour and coat colour were observed to be significantly different ($P<0.05$) among the sampled population. In general, the Nigerian indigenous Camel is known to be hardy and quite adapted to the local environment (Kamal, (2008); Meiloud *et al.* (2011)). Selection of better performing animals to be parents of the future generation is the basic tool for animal improvement. Thus, the diversity in phenotypic characteristics in the population of Camel studied presents opportunity for selection. Such selection will be useful in the improvement of the Camel if one or more of the observed characteristics were positively linked to traits of economic importance.

Conclusion

This study has shown that Camels in Kastina, Jigawa, Sokoto and Zamfara States were mostly red coloured (28-45 %), black eyed (43-60 %), short-smooth haired (36-53 %). Most of the long-rough hair type was Ja strain of Camel (48.15 %). Zamfara State had the highest (30%) number of Baki strain of Camels while Jigawa had the lowest (5%) number of Ja and Fari Camels. Significant variation existed across the strains of Camel for morphological traits, this suggest that they could be used for improvement of Camels for production in some selected North-Western State of Nigeria.

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NIGERIAN SUDAN SAVANNA: AN ANCESTRAL ABODE FOR WILD POPULATIONS OF *Drosophila melanogaster*

[1]Abdulazeez, R., [3]Iyiola-Tunji, A.O., [1]Shehu, D.M. and [1,2]Ndams, I.S.

[1]Department of Zoology, Ahmadu Bello University Zaria, Nigeria
[2]Africa Centre of Excellence for Tropical Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University Zaria
[3]National Agricultural Extension Research and Liaison Services, Ahmadu Bello University Zaria, Nigeria

Corresponding author: rashidat.int@gmail.com; rabdulazeez@abu.edu.ng +2348085001701; +2347033701917

Abstract

To have insights into the natural evolutionary forces that characterize Drosophila melanogaster in Nigeria, the level of genetic diversity of wild populations of D. melanogaster were inferred using six microsatellite markers. Drosophila melanogaster was collected from thirty vegetable markets in fifteen States located in eight ecological zones of Nigeria. The fruit flies were pooled into groups of seven males and Genomic DNA was extracted using the Zymo Tissue/Insect miniprep kit. Multiplex PCRs were run in thirty (30) cycles. The PCR products were amplified on 2% agarose gel and scored using Molecular Imager Lab™ Software of BIO-RAD. Three of the markers used were highly polymorphic. The highest genetic diversity was observed in the Sudan savanna (He = 0.500), indicating the possibility of the zone being the ancestral home of D. melanogaster in Nigeria. The highest genetic distance was estimated between Northern Guinea Savanna and rainforest (2.690) suggesting the absence of shared-similar alleles between these populations. D. melanogaster populations are genetically diverse, not related and exhibit a high level of inbreeding. The level of genetic diversity obtained in this study indicated Sudan savanna as the ancestral home of D. melanogaster in Nigeria.

Keywords: *Pomace fruit flies, Genetic diversity, Microsatellite markers, Ecological zones*

Introduction

Interaction of an organism with other organisms and with their environment forms a complex chain, which shapes the organism's biology, drives speciation and evolution. The ability of a population to evolve and cope with environmental challenges, new diseases, pest epidemics, long-term viability and persistence depends on its genetic diversity (Caliskan, 2012; Saini and Parkashyadav, 2013). The major goal of evolutionary biology is to understand the natural forces such as natural selection, mutation, migration, genetic drift, inbreeding and anthropogenic forces such as habitat fragmentation via urbanization, environmental pollution, that shape the patterns of genetic diversity within and among populations and species (Geisbert and Jahrling, 2004). To have precise evolutionary history and distribution of genetic diversity for a species, it is paramount to study large populations of the species within a wide geographical area and with considerations to the impact of ecological variables (Verspoor and Haddrill, 2011).

Drosophila melanogaster is considered the best biological model for studies in genetics, evolution, population biology since the pioneered study by H.T. Morgan in 1909 due to its short generation time, high fecundity, ease in rearing and relatively simple genetics

(Guruprasad and Padmaja, 2016). Several studies have tried to provide a detailed evolutionary history of the fruit flies based on either few populations as representatives of a continent or from inbred lines, which already have certain degrees of mutation (Andolfatto and Przeworski, 2000; Kauer *et al*., 2002; Glinka *et al*., 2003; Ometto *et al*., 2005; Li and Stephan, 2006; Pool and Aquadro, 2006; Nunes et al., 2008; Verspoor and Haddrill, 2011).

Although the fly is speculated to have originated from sub-Saharan Africa, more precisely, East Africa (Kauer *et al*., 2002; Gibert *et al*., 2004; Pool and Aquadro, 2006; Schlötterer *et al*., 2006; Verspoor and Haddrill, 2011; Bykov *et al*., 2019), relatively little is known about the genetic diversity and demographic history of *D. melanogaster* on a broad scale for each of the Sub-Saharan African countries. This study, therefore, characterized the genetic diversity of *Drosophila melanogaster* based on the ecological zones of Nigeria. Nigeria has a diverse geography, with a climate ranging from arid to humid equatorial and comprises eight ecological zones (Ajayi *et al.,* 2019).

Materials and Methods

Population sampling

Drosophila melanogaster (fruit flies) were collected using bottle baited trap method (modified from Pavkovic-Lucic and Kekic, 2014) between September 2018 to May 2019 from eight ecological zones of Nigeria. The collection was done from thirty (30) vegetable markets located within fifteen (15) states across the ecological zones (Figure 1). Following the taxonomic identification of Markow and O'Grady (2006), males were preserved in 1.5ml Eppendorf tubes containing 70% ethanol, transported to the lab on an ice pack and stored at -20°C until Nucleic acids extraction (Machado *et al*., 2003).

Microsatellite Genotyping

Genomic DNA from one hundred and five (105) preserved male flies pooled into groups of seven (7) individuals per state was extracted using the Quick-DNA™ Tissue/Insect Miniprep Kit (Zymo Research, Catalog No. D6016), according to the manufacturer's protocol. Genotyping of *D. melanogaster* was carried out using six (6) species-specific microsatellites primers (Table 1). Multiplex PCR and Simple Sequence Repeat Protocol were carried out in a volume of 20 µL containing 5µL of genomic DNA as a template, 2.5 µL of Taq buffer, 2.0 µL of $MgCl_2$ (NEB B9014S), 0.5 µL of dNTPs (NEB N0447S), 0.25 µL of each primer (Inqaba Biotec) and 0.5 µL of hot-start *Taq* polymerase (400 units/ µL, NewEnglandBiolabs.inc). PCR was done with a typical cycling profile of 30 cycles with pre-denaturation at 94˚C for 5 minutes, denaturation at 94˚C for 1 minute, annealing at 62˚C for 1 minute, extension at 72˚C for 30 seconds and a final extension at 72˚C for 5 minutes. (Schlötterer *et al*., 2006; Verspoor and Haddrill, 2011). Amplification for all samples was checked by running on a 2% Agarose gel prepared in 1xTBE buffer stained with Ethidium bromide (2µL / 200mL) for visualizing the DNA bands.

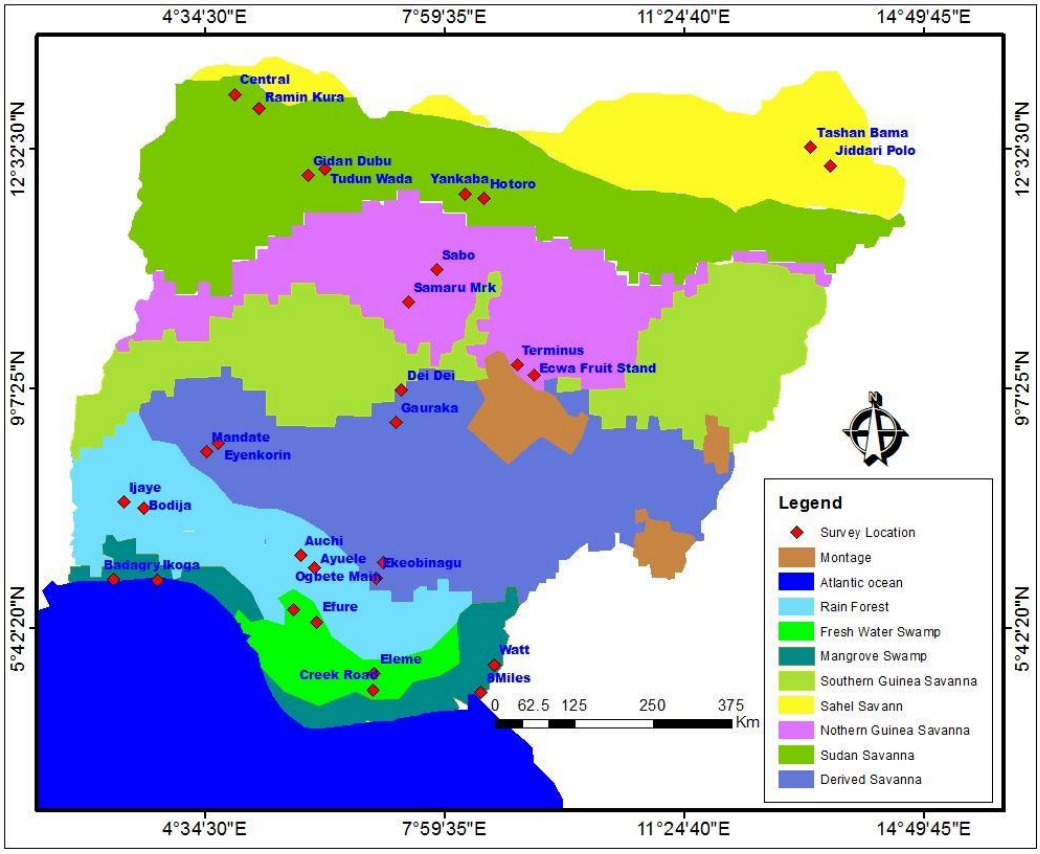

Figure 1: Geographical distribution of sampled populations across eight ecological zones of Nigeria

Table 1. Microsatellite Loci Screened for Genotyping *D. melanogaster*

Locus	Flybase ID (FBgn)	Chromo-some No.	Functions	Base size	Annealing Temp(℃)	GC (%)	Forward primer (5' - 3') Reverse primer (3' - 5')
DM30	0001297	3R	Protects sensory neurons from degeneration Circadian rhythm	784	60.0; 60.0	55; 60	GTCCTCGCACTCGTTTTCTC CCAAACTTGTGACGTCATGG
DROACS2	0004170	X	Involves in sex determination Peripheral Nervous System development	498	60.0; 60.0	50;45	GGTCGAGGACCTCACAAAAA TTCGTTGTCCAGATGATCCA
DMEHAB	0000564	3R	Regulation of eclosion Chitin based cuticle	407	60.0; 60.1	50; 45	GTATACGCCGCATGGTCTTT CCGAATCGAACTGAAATCGT
Ref(2)P	0003231	2L	Implicated in Sigma multiplication Necessary for male fertility	151	60.0; 60.0	50; 55	ATTGGGTTCCGCTACAAGTG ATCCTGGACCTGTGAACCAG
DROEXO2	0003345	X	Embryo development Cell proliferation	306	60.0; 60.0	50; 50	AGGGTTAGAATCGCCACCTT AATCCCTACACAGCCAAACG
DMRHOa	0004635	3L	Learning or memory Gland morphogenesis	641	60.0; 60.0	45; 45	GGCTTTTCCTTCGCTTTTCT CCGCCCCCTATATCATTTTT

Source: (Abdulazeez *et al*., 2019; Harr and Schlötterer, 2000; Irvin *et al*., 1998; Schug *et al*., 1998). Primers were designed based on release 6.07 of the *D. melanogaster* genome (http://www.flybase.org) and PRIMER 3 OUTPUT (http://www-genome.wi.mit.edu/genome-software/other/primer3.html)

The bands were analyzed and scored using the Image Lab™6.1 software. Measures of genetic diversity were calculated using GenAlex version 6.501 (Peakall and Smouse, 2012) and Cervus 3.0.7 (Marshall, 2014). Population structure and level of admixture were analysed using the Bayesian model-based program *STRUCTURE* 2.3.4. (Novembre, 2016). Both Burn-in and MCMC (Markov Chain Monte Carlo) of 100,000, 20 iterations as suggested by (Gilbert *et al.*, 2012) and K values of 1-8 were used. The best model of K was then selected using the *STRUCTURE HARVESTER* (Evanno *et al.*, 2005). A phylogenetic tree using the UPGMA algorithm was constructed using the MEGA X software (Kumar *et al.*, 2018).

Results and Discussion

Diversity indices of the selected SSR loci

The polymorphism and gene diversity indices of six selected microsatellites loci are presented in Table 2. The markers detected a total of 35 alleles in the 15 pooled populations of *D. melanogaster*. Of the six SSR loci, REF(2)P, DROEXO2 and DMEHAB were highly informative (PIC ≥ 0.5) while DROACS2, DMRHOa and DM30 were the least informative (PIC < 0.25). This suggests that DROACS2, DMRHOa and DM30 are monomorphic markers in these populations. DROEXO2 showed the highest gene diversity, which indicates the suitability of this locus in population studies of *Drosophila melanogaster* in any geographical area. The number of alleles (0.00-15.00) in this study was higher than the report of Verspoor and Haddrill (2011), for a worldwide sample of *D. melanogaster* using nine (9) microsatellite markers (2.22- 9.11).

Genetic diversity

Table 3 shows the genetic diversity indices of *D. melanogaster* for the eight ecological zones of Nigeria. Although none of the populations deviated from Hardy-Weinberg Equilibrium ($p < 0.01$), most of the studied populations except in montane vegetation, had observed heterozygosity which was less than the expected heterozygosity (Ho < He), this indicates non-random mating and deficiency in heterozygosity. The decrease in heterozygosity could be due to genetic drift and positive assortative non-random mating as explained by (Samah *et al.*, 2016). This finding is contrary to the report of Abdulazeez *et al.* (2019) for Nigerian Savanna populations of *D. melanogaster* but somewhat similar to the report of Singh and Singh (2010) who reported inbreeding in natural populations of *D. anannasae* in India. High genetic diversity is said to be a function of population size and age as larger and older populations tend to have and maintain higher levels of genetic diversity compared to a recently colonized habitat (Rampersad, 2013). This suggests that Sudan Savanna is the largest and ancestral home of *D. melanogaster* while montane vegetation is a recently colonized environment for the vinegar fruit flies in Nigeria.

The ecological zones had varying percentages of polymorphism. The differences in polymorphism across the ecological zones and frequency of allelic loci, might be as a result of low effective population sizes or repeated severe bottlenecks as explained by (Schmack *et al.*, 2019). Krimbas and Powell (1992) stated that variations in polymorphism could be due to the stability and favourability of a locale to the Drosophila species, which further suggests Sudan savanna to be a favourable habitat for *D. melanogaster* in Nigeria.

The mean *Ho* (0.060) and *He* (0.265) in this study is lower than the mean *Ho* (1.000) and *He* (0.500) for Savanna populations of *D. melanogaster* in Nigeria inferred from seven microsatellite markers as reported by Abdulazeez *et al.* (2019). The mean *He* was also lower to the report of (Verspoor and Haddrill, 2011) for *D. melanogaster* in various countries. The researchers reported a mean *He* of 0.30 in Morocco, 0.35 in the UK, 0.37 in France, 0.42 in the USA and 0.59 in Ghana using nine microsatellite markers. The values of *He* obtained from this study (0.083 - 0.500) were however higher than those for Indian populations of *D. anannasae* (*He* = 0.15 - 0.45) using cosmopolitan inversion as a marker (Singh and Singh, 2010). The number and type of

microsatellite loci and genetic markers could be the reason for the differences in genetic diversity indices observed in these studies.

Table 2: Diversity Indices of the SSR Loci Selected

SSR loci	Na	Ho	He	PIC
REF(2)P	15.000	1.000	0.214	0.885
DROEXO2	12.000	1.000	0.500	0.863
DMEHAB	8.000	1.000	0.286	0.844
DROACS2	0.000	0.000	0.083	0.000
DMRHOa	0.000	0.000	0.232	0.000
DM30	0.000	0.000	0.167	0.000
Mean	5.833	0.500	0.464	0.432

Na (number of alleles); Ho (observed heterozygosity);
He (expected heterozygosity);
PIC (polymorphic information content)

Table 3: Genetic Diversity in Natural Populations of *D. melanogaster* Across Ecological Zones of Nigeria

Zones	Na	Ne	Np	I	Ho	He	PP (%)	F	HWE
SWM	1.167	1.167	0.167	0.231	0.000	0.167	33.33	1.000	0.052
FWM	1.333	1.333	0.211	0.347	0.000	0.250	50.00	1.000	0.079
RAF	1.333	1.333	0.211	0.347	0.000	0.250	50.00	1.000	0.130
SSG	1.500	1.500	0.211	0.347	0.000	0.250	50.00	1.000	0.157
NGS	1.833	1.778	0.211	0.520	0.083	0.354	66.67	0.800	0.184
SUS	2.000	2.000	0.258	0.693	0.167	0.500	100.0	0.667	0.201
SAS	1.833	1.778	0.211	0.520	0.083	0.354	66.67	0.800	0.052
MON	1.000	1.000	0.167	0.000	0.000	0.000	0.000	0.000	0.052
Mean	1.500	1.486	0.206	0.375	0.042	0.266	52.08	0.856	
S.E	±0.13	±0.12	±0.01	±0.07	±0.02	±0.05	±10.1	±0.12	

SWM (Saltwater mangrove); FWM (Freshwater mangrove); RAF (Rain forest); SGS (Southern Guinea savanna); NGS (Northern Guinea savanna); SUS (Sudan savanna); SAS (Sahel savanna); MON (Montane); Na (number of alleles); Ne (effective number of alleles); Np (number of private alleles; I (Shannon index); Ho (observed heterozygosity); He (expected heterozygosity); PP (percentage polymorphism); F (fixation); HWE (Hardy-Weinberg Equilibrium) level of significance at $p<0.01$

Estimates of how related the populations of *D. melanogaster*

(Table 3) showed that Northern Guinea savanna and rainforest populations are highly genetically distant populations, implying that the alleles in these zones are completely different from each other, while freshwater mangrove versus Sahel savanna; montane vegetation versus freshwater mangrove, rain forest and Sudan savanna were closely genetically related, suggesting recent common ancestors and an indication of a higher number of similar alleles in the populations. The divergence (mean D = 1.600) observed in these populations could be due to the varying ecological conditions, as each population tries to adapt to its locality. This report is in agreement with the work of Abdulazeez *et al.* (2019) as they also reported genetic divergence in natural populations of *D. melanogaster* from savanna ecological zones of Nigeria (mean D= 1.320). The UPGMA dendrogram based on Nei's genetic distance revealed three clusters with Northern Guinea savanna being a monoclade, which seems to have diverged earlier than other populations of *D.* melanogaster based on the horizontal length (0.78) as shown in Figure 2.

Table 4. Genetic Distance Estimates among Populations of *D. melanogaster*

	SWM	FWM	RAF	SGS	NGS	SUS	SAS	MON
SWM	0000							
FWM	1.607	0000						
RAF	0.760	1.540	0000					
SGS	0.886	1.155	1.378	0000				
NGS	2.064	0.493	2.690	1.312	0000			
SUS	1.242	0.953	2.562	1.301	1.109	0000		
SAS	1.840	--------	2.284	2.122	2.048	1.920	0.000	
MON	2.282	--------	-------	1.648	2.266	-------	0.657	0.000
Mean	1.360							
S.E	±0.15							

SWM (Saltwater mangrove); FWM (Freshwater mangrove); RAF (Rain forest); SGS (Southern Guinea savanna); NGS (Northern Guinea savanna); SUS (Sudan savanna); SAS (Sahel savanna); MON (Montane)

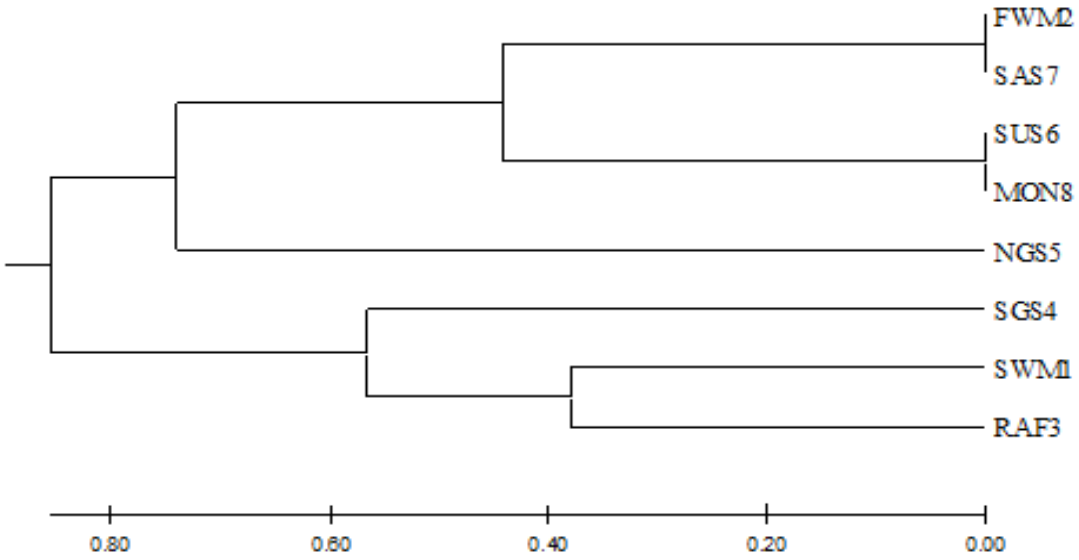

Figure 2: Unweighted population distance tree of *D. melanogaster* populations generated by UPGMA algorithm of the genetic distance matrix
SWM (Saltwater mangrove); FWM (Freshwater mangrove); RAF (Rain forest); SGS (Southern Guinea savanna); NGS (Northern Guinea savanna); SUS (Sudan savanna); SAS (Sahel savanna); MON (Montane)

Conclusion

Some of the markers used were highly polymorphic and hence can be reliably used in genetic studies of the vinegar fruit flies. Although none of the populations deviated from Hardy-Weinberg Equilibrium, there was a deficiency of heterozygosity implying the occurrence of inbreeding in the populations. Low to moderate genetic diversity were estimated in the studied populations while Sudan savanna had the highest genetic diversity, hence regarded as the ancestral population of *D. melanogaster* in Nigeria. Most of the studied populations had a recent common ancestor but the overall genetic distance revealed that the populations were not genetically related. Investigations on the effects of settlement-type and altitudes on genetic variation of *D. melanogaster* should be conducted.

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ABG 008

DIETARY INCLUSION OF *OCIMUM GRATICIMUM* LEAF MEAL AFFECTS INTERLEUKIN1β GENE EXPRESSION IN CHICKEN SPLEEN AT 28 AND 56DAYS

Ufuoma G. Sorhue[1], Raphael E. Onainor[1], Adimabua Moemeka1, Irikefe-Ekeke Peterson[2]

[1]Department of Agricultural Education, Delta State College of Education Mosogar
[2]Department of Agricultural Education, College of Education Warri

Corresponding author: gtsorhue@yahoo.com

ABSTRACT

This study was conducted to examine the effects of replacing in-feed antibiotics with Ocimum graticimum (OG) on expression of interleukin1β gene in two chicken strains. A total of 150chickens (75 of each strain) were randomly allotted into five dietary treatments at fifteen birds per treatment. Birds were fed diet containing varying levels of Ocimum graticimum leaf meal. Treatment one had 0% OG, while treatment two, treatment three, treatment four and treatment five had 0.5% OG, 1.00% OG, 1.5% OG and 2% OG respectively. Feed and water was provided adlibitum throughout the feeding trial. Three birds were slaughtered from each treatment at day 28 and 56, while liver samples were collected and stored using RNAlater in a -20°c freezer prior to RNA extraction. RNA cleanup was done using the RNA cleanup kit (Qiagen) while the quality and concentration was measured using a Nanodrop. Real-time qPCR was performed in 40cycles using the PowerUp SYBR Green reagent and analyzed using the ΔΔCt method. Gene expression data were subjected to analysis of variance in a completely randomized design. Interleukin1β was up-regulated in the spleen but the expression patterns decreased with increased inclusion rate of the test ingredients at 28 and 56 days in both chicken strains. The expression of IL1β in the Arbor-acre Cobb strains were significantly different (P<0.05). Ocimim graticimum leaf meal shows promise in the regulation of inflammation in chickens and can be used to efficiently replace antibiotics

Keywords: *(Inflammation, Gene Expression, Interleukin1β, Scent leaf, Cytokine)*

Introduction

Any direct or indirect damage of intestinal epithelial cells may cause a breakdown in gut barrier and consequentially disruption of normal mucosal immune balance that can potentially lead to uncontrolled chronic intestinal and systemic inflammation (Schulzke et al 2009). When inflammation occurs, cells have to recruit other cells to local sites by secreting inflammatory cytokines and chemokines, but prolonged inflammation may lead to unnecessary energy expenditure (Huang and Lee 2018). Phytochemicals have been reported to decrease the expression of pro-inflammatory cytokines IL1-B, IL6 and TNF and increase anti-inflammatory cytokines IL-10 in LPS-activated cells (Ghareeb et al 2013). Interlukin1, 6 and tumor necreotic factor alpha (IL1B, IL6 & TNFalpha) are three known pro-inflammatory cytokines expressed in monocytes and macrophages after invasion of pathogens are identified (Huang and Lee 2018, Ghareeb et al 2013). These cytokines mediates metabolic changes which enhance the immune response and disease resistance thereby stunting growth and performance (Huang and Lee 2018, Ghareeb

et al 2013). Reports indicated a strong correlation between the levels of IL1B and the amount of intestinal inflammation in chickens, making IL1B the chief cytokine responsible for the initiation and multiplication of the inflammatory responses (Brisbin et al 2010). In chickens, the spleen act as both reservoir and activation site for leukocytes and therefore an analysis of the mRNA levels in the spleen can be reliable in ascertaining systemic immune function (Redmond et al 2010). Compared with synthetic antibiotics or inorganic chemicals, plant-derived products are natural, less toxic than antibiotics, and typically residue free. Scent leaf *(Ocimum gratissimum L)* is a good example of Herbs and spices; report has shown that they possess antimicrobial, antioxidative, anti-inflammatory, as well as immuno-modulatory properties (Sofowora, 1993). There is however, inadequate research information especially on the effect of *Ocimum gratissimum* on the immunomodulation in the chicken. This study was therefore designed to examine the role of scent leaf meal in the expression of interleukin1B in chicken spleen.

Materials and Methods

Experimental Site, Birds and Sample harvest

The study shall be carried out at the Poultry Unit of the Teaching and Research Farms of Delta State College of Education Mosogar. One Hundred and Fifty (150) day old unsexed and healthy commercial broiler chicks, including 75 Cobb 500 and 75 Arbor Acre strains were sourced from Zartech Farms, Ibadan, Nigeria. The birds were raised on deep-litter system, and fed for a period of eight weeks (56 days). Feed and water were provided ad-libitum throughout the experimental period. Scent leaves were harvested from a farm at Oki, Agbor, in Ika South Local government Area, Delta State, Nigeria. The leaves were air dried while still showing greenish coloration. The dried Scent leaves were hammer milled to obtain a final leaf meal. Five (5) Experimental broiler starter and finisher diets were formulated, and scent leaf meal was incorporated at the rate of 0.00%, 0.50%, 1.00%, 1.50% and 2.00% dietary levels in five (5) treatments as Treatment 1, Treatment 2, Treatment 3, Treatment 4 and Treatment 5 respectively. The birds were randomly allotted into five dietary treatment groups per strain. Each strain had 15 birds per group. Each group was divided into three (3) replicates of five (5) birds per strain. The birds in group I (T1) were given normal medications for broilers, while birds in groups II (T2), III (T3), IV (T4) and V (T5) had Scent leaf meal included in their feed at the rate of 0.50%, 1.00%, 1.50% and 2.00% respectively. three birds from each of the treatment groups (one from each replicate, one from each strain)was slaughtered at 4weeks and at the end of the feeding trial (56 days) to examine the mRNA expression patterns of genes regulating immune responses in Chicken. The spleen was collected from the experimental birds and stored in eppendorf tubes with the aid of RNALater solution in a freezer prior to RNA extraction.

Statistical Analyses

Data collected was subjected to two-way analysis of variance (ANOVA) using the Graphpad Prism software, USA, while differences between strains were compared using paired t-test.

RNA Concentration, Reverse Transcription andReal-time polymerase chain reaction

The concentration and purity of the isolated RNA was assessed by a spectrophotometer (Nanodrop). This was followed by reverse transcription (the conversion of RNA to cDNA). The 20μL reverse transcription reaction system shall be comprised of the following: 2 μg of RNA, 1 μL of Oligo (dT) 15 Primer, 1μL of random primers, 10 μL of nuclease-free water, 1 μLof GoScript TM Reverse Transcriptase, 1.6 μL of nuclease freewater, 0.4 μL of Recombinant RNasin Ribonuclease Inhibitor, 4 μL of GoScript TM 5× reaction buffer, 2 μL ofMgCl2 (25 mM), and 1 μL of PCR Nucleotide Mix. The reaction procedure shall be conducted under the following conditions: denaturation for 5 min at 70°C, annealing for 5min at 25°C, extending annealing for 60 min at 42°C, inactivated reverse transcriptase for 15 min at 70°C, and then

storage at 4°C. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed to determine the expression IL1B. RT-qPCR was done using PowerUp SYBR Green reagent. The β-actin gene was used as the house keeping gene. The relative expression of interleukin 1(IL1β) was calculated using the $2^{-\Delta\Delta ct}$ method.

Results and Discussion

Figures 1, 2, 3, and 4, shows the expression patterns of interleukin1β in the spleen of chickens fed varying levels of Ocimim graticimum leaf meal at 28days and 56days in two chicken strains. Interleukin1β was up-regulated in the spleen but the expression patterns decreased with increased inclusion rate of the test ingredients at 28 and 56 days in both chicken strains. The expression of IL1β in the Arbor-acre strain was significantly different ($P<0.05$) for the control and treated groups, but treated groups did not differ significantly ($P<0.05$) at 56days, while T1 and T2 significantly ($P<0.05$) differed from T5; T3, T4 and T5 did not significantly differ ($P>0.05$) from each other at 28days, while in Cobb500, T1 significantly differed ($P<0.05$) from T2, but not T3, T4 and T5 at 28days and 56days respectively. Although some phenolic compounds such as terpenes have been reported to have anti-inflammatory activities, lack of sufficient antioxidants to eliminate reactive oxygen species (ROS) can trigger inflammation due to oxidative damage (Fang et al 2008, Siana et al 2013, Huang and Lee 2018). Phytochemicals have also been reported to decrease the expression of pro-inflammatory cytokines IL1-B, IL6 and TNF and increase anti-inflammatory cytokines IL-10 in LPS-activated cells (Ghareeb et al 2013), which is in tandem with this study as increased inclusion of dietary Ocimum gratisimum consistently reduced expression of interleukin 1B in the spleen of the two chicken strains studied. This study is also in agreement with reports that Scent leaf *(Ocimum gratissimum L)* possess antimicrobial, antioxidative, anti-inflammatory, as well as immuno-modulatory properties (Sofowora, 1993). The anti-inflammatory role of Scent leaf was evident in the reduction of IL1B that happens to be the chief pro-inflammatory cytokine in cells (Brisbin et al 2010). When inflammation occurs, cells have to recruit other cells to local sites by secreting inflammatory cytokines and chemokines, but prolonged inflammation may lead to unnecessary energy expenditure and as such, the alleviation and prevention of over inflammation and the return of immune status to normal condition is vital to livestock production (Huang and Lee 2018). Since one of the biological effect of IL-1β is to stimulate inflammation by activating the immune system in an acute phase response (Wigley and Kaiser 2003), scent leaf meal can be used to moderate inflammation in chickens by reducing the effect of pro-inflammatory cytokines that causes weight loss and stunted growth occasioned by excessive inflammation.

Conclusion

This study confirms the anti-inflammatory role of phytochemicals and Ocimum graticimum in particular. These further shows that the test ingredient can be used to replace antibiotics, stabilize immune system, and prevent weight loss occasioned by excessive inflammation in chickens during infections.

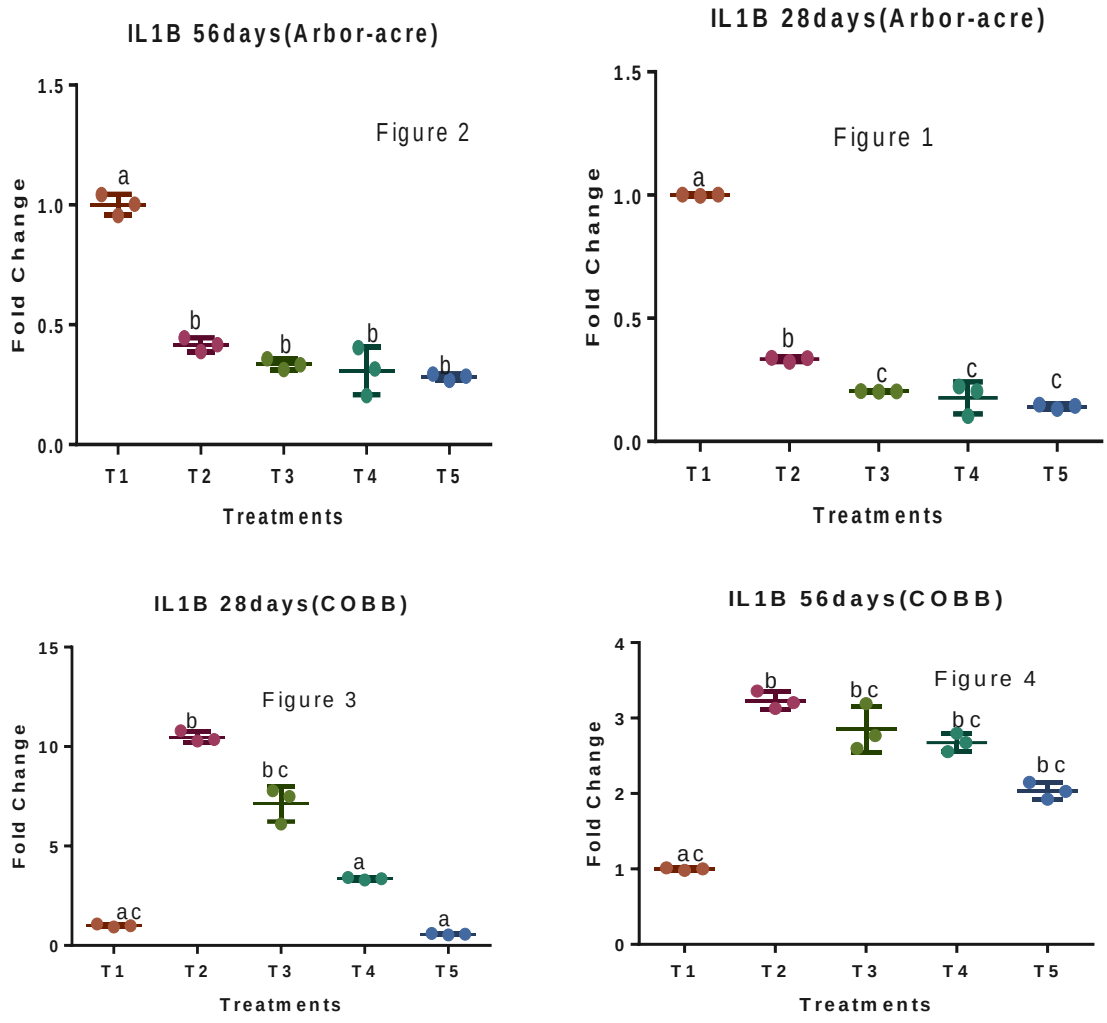

Figure 1, 2, 3 and 4 above shows the relative expression of Interleukin 1B (IL1B) in the spleen of two chicken strains at 28 and 56days of age. abc; treatments carrying different superscripts are significantly different (P<0.05).

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ABG 009

EFFECTS OF SOME FACTORS AFFECTING OPTIMUM REPRODUCTIVE CAPACITY OF LOCAL BREEDS OF SHEEP IN NIGERIA

D. Zahraddeen[1*], N. M. Lemu[2], P. P. Barje[3], I. S. R. Butswat[4], and N. A. Jama'a[5]

[1]Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria,
[2]Kaduna State Agricultural Development Agency, Kaduna, Nigeria,
[3]National Animal Production Research Institute, Shika, Ahmadu Bello University, Zaria, Nigeria,
[4]National Open University of Nigeria, Abuja,
[5]Department of Animal Production, Abubakar Tafawa Balewa University, Bauchi, Nigeria

*Corresponding author e-mail: zdzariya@yahoo.com; Tel: +2348024078638

Abstract

This study was conducted to investigate some of the factors affecting optimum reproductive capacity of the indigenous breeds of sheep in Nigeria. A total of 767 sheep of different breeds were investigated. The reproductive indices considered were birth/weaning weights, litter size, parity, mortality, reproductive problems/disorders, body condition score (BCS), as well as growth traits. The results showed that litter size, parity and BCS had significant (P< 0.05) effects on birth/weaning weights, mortality rates and growth traits of the sheep breeds studied. Similarly, the rearing method/system significantly (P<0.05) influenced other reproductive traits such as birth/weaning weights, mortality, growth performance of lambs. However, the major reproductive problems/disorders in the ewes were dystocia (30.94%), retained placenta (16.91%), mastitis (15.83), pregnancy toxaemia (11.51%), uterine prolapse (6.48%) and vaginal prolapse (3.24%). In the rams, the incidence of reproductive problems included cryptorchidism (1.08%), orchitis (2.87%) and scrotal dermatophilosis (1.79%), among others. This study concludes that the four breeds of sheep (Balami, Yankasa, Uda and West African Dwarf sheep) and their crosses exhibited varied genetic make-up and potentials. However, the large number of sheep farmers practicing the extensive production system might be responsible for the low reproductive performance of this species in the country. It is, therefore, recommended that significant improvement could be achieved through enhanced management practices of these animals.

Introduction

Nigeria has a large population of small ruminants consisting of about 52.4 million goats and 33 million sheep. About 70% of these are found in the northern region of the country [1]. Nutrition in terms of seasonality in quality and quantity of feed supply is considered as one of the major constraints to the small ruminants and possibly limiting productivity [2]. Small ruminants managed under the smallholder husbandry system associated with bad management experienced colossal rate of occurrence of reproductive problems, which relegated them to reduced reproductive performance levels [3]. This study was, therefore, designed to investigate some of the factors limiting efficient reproductive performance of the local breeds of sheep in Nigeria.

Materials and Methods

The study area is located in the Northern part of Kaduna State, a part of Northern Guinea Savannah ecological zone of Nigeria. The area is located between latitude 8°45' and 11°35' North and longitude 7°33' and 8°44' East at an altitude between 469 and 1,632 meters above sea level[4].

The study used the four (4) indigenous breeds of sheep in the country. These breeds were Balami (BA), Uda (UD), Yankasa (YK), West African Dwarf (WD) sheep and some of their crosses. These breeds were studied under three management systems (extensive, semi-intensive and intensive). A total of 767 sheep were used in the study. Data were collected on both growth and reproductive traits. Body weights were taken using a hanging spring scale. Birth weight was taken at birth. Average daily weight gain was determined as the difference between weights taken at ten days intervals starting with weight at birth over a period of 120 days. Weaning weight was taken between 90 to 120 days post-partum, and for those lambs weaned at more than 90 days; their weight were corrected to 90 day basis using the formulae reported by [5]. BCS was determined as per the method of [6]. Mortality rate was computed based on the number died in a flock. The parameters were recorded as influenced by sex, BCS, parity and liter size in a breed. The data collected was subjected to statistical analysis of variance, using the General Linear Model (GLM) [7]. Significant means were separated using the Duncan's Multiple Range Test.

Results and Discussion

Data on growth and reproductive traits are presented in Tables 1 to 3. Table 1 presents data on birth weight of various breeds of sheep. The results showed that BA x UD crosses had higher birth weight of 3.17kg vs 2.97kg for rams and ewes, respectively. BA x UD cross also had higher average daily weight gain (90.53g vs 89.20 g for rams and ewes, respectively), weaning weight (12.05 kg vs 10.93 kg for rams and ewes, respectively) and adult body weight (32.32 kg vs 29.79 kg for rams and ewes, respectively). The body weight obtained in this study was higher than the value report by [8] but lower than that of [5]. The result was similar to that of [9] which showed significant genotype effect on lamb birth weight at various age groups. Significantly ($P<0.05$) higher birth weight was observed on litter size 1 (single birth) and the birth weight decreased as litter size increased (multiple birth), this is in agreement with the findings of [9]. Higher birth weight was observed at BCS 2, parity 5 and at litter size 1 for all the genotypes. UD, WD sheep and YK x WD cross had higher birth weight at single birth compared to other genotypes.

Table 1: Effect of genotype and sex on body weights of local breeds of sheep in Nigeria

Parameters	Sex	BA	UD	YK	WD	BAxUD	BAxYK	UDxYK	YKxWD	SEM	LOS
BW (kg)	M	2.84^{c}	2.73^{d}	2.17^{f}	2.18^{f}	3.17^{a}	2.84^{c}	2.91^{b}	2.47^{e}	0.04	*
	F	2.82^{b}	2.68^{bc}	2.01^{d}	1.99^{d}	2.97^{a}	2.75^{b}	2.79^{b}	2.29^{c}	0.04	*
ADG (g)	M	91.68^{a}	85.29^{b}	68.67^{d}	54.69^{e}	90.53^{a}	86.04^{b}	80.51^{c}	69.94^{d}	1.21	*
	F	89.02^{a}	83.75^{b}	67.37^{d}	56.01^{e}	89.20^{a}	84.03^{b}	79.42^{c}	68.87^{d}	1.14	*
WW (kg)	M	11.61^{b}	11.17^{c}	8.94^{ef}	7.36^{f}	12.05^{a}	9.20^{e}	10.56^{d}	9.47^{e}	0.17	*
	F	10.91^{a}	10.60^{b}	8.41^{e}	7.11^{f}	10.93^{a}	8.78^{d}	10.17^{c}	8.95^{d}	0.16	*
BWT(kg)	M	32.99^{a}	31.46^{a}	26.85^{abc}	19.35^{b}	32.32^{a}	33.15^{a}	28.97^{ab}	27.75^{ab}	0.65	*
	F	30.77^{a}	29.92^{ab}	25.40^{c}	21.19^{d}	29.79^{ab}	31.94^{a}	27.83^{b}	25.72^{c}	0.59	*

Table 2: Effects of management practice and sex on growth traits of local breeds of sheep in Nigeria

Parameter	Sex	Intensive	Extensive	Semi-intensive	SEM	LOS
BW (kg)	M	2.53[b]	2.67[a]	2.39[c]	0.04	*
	F	2.40[b]	2.60[a]	2.29[c]	0.05	*
ADG (g)	M	92.04[a]	85.80[b]	85.00[b]	1.10	*
	F	89.90[a]	83.93[b]	84.26[b]	1.04	*
WW (kg)	M	11.68[a]	9.98[b]	9.02[c]	0.21	*
	F	10.96[a]	9.71[b]	8.65[c]	0.18	*
BWT (kg)	M	32.68[a]	28.62[b]	30.88[a]	0.92	*
	F	30.12[a]	28.20[ab]	29.62[a]	0.81	*

BW- Birth weight, ADG- Average daily weight gain, WW-Weaning weight, BWT-Body weight, M- Male, F- Female, SEM-Standard error mean, LOS-Level of significance, * - Significant, a,b,c : Values within the same row with different superscripts are significantly different (P < 0.05)

Significant (P < 0.05) effect of BCS, parity, litter size and sex was observed in the weaning weight of the genotypes (Table 2). This agreed with the reports of [10, 11] that parity and litter size influenced birth weight of lambs. The weaning weight of lambs increased as parity advanced and decreased as the litter size increased, while the effect of BCS and litter size on the weaning weight was as well significant (P<0.05) for some genotypes and sexes. BCS was significant (P<0.05) in all the genotypes except in BA, UD and UD x YK rams. There were significant (P<0.05) differences in litter size among WD sheep, BA x YK and YK x WD crosses. Highest weaning weight was observed at BCS 2, parity 5 and litter size 1. The highest weaning weight (11.95kg) was in male BA x UD and least value (6.06 kg) was in WD ewes at litter size 4. However, the weaning weight was lower than 13.55kg reported by [6] but higher than 8.98kg reported by [12] for WD sheep. The present investigation agrees with the findings of [12] that weaning weights increased as the parity advanced. The reason for the lower weaning weight might have been due to the effects of nutrition and climate, considering that most of the affected genotypes were aliens to the area under study, probably due to differences in ecological niche of these breeds within the country. There were significant (P<0.05) differences in birth and weaning weights at different management practices in sheep (Table 3). Sheep managed under the intensive system had higher performance than in other systems. This was followed by the semi-intensive and lowest in sheep managed under the extensive system.

The results also revealed that mortality rates increased as BCS and litter size increased, and a decrease in mortality as the parity advanced. This was similarly reported by [13]. The highest mortality rate (50%) was in YK x WD crosses at parity 1 and the least mortality (21.74%) was in UD x YK crosses at parity 5. This was in agreement with the reports of [5] that YK x WD crosses had the highest mortality rates among the sheep genotypes. Litter size per ewe was lowest in the northern genotypes (YK, BA and UD).

Table 3: Prevalence of some reproductive problems/disorders in local breeds of sheep as reported in the veterinary clinics

Reproductive problems/disorders	Frequency	Incidence rate
Cryptochidism	3	1.08
Orchitis	8	2.87
Phimosis	1	0.36
Scrotal dermatophilosis	5	1.79
Vulvoviginitis	3	1.08
Abortion	8	2.87
Pregnancy toxaemia	32	11.51
Dystocia	86	30.94
Still birth	6	2.16
Vaginal prolapse	9	3.24
Uterine prolapse	17	6.48
Retained placenta	48	16.91
Parturient paresis	8	2.88
Mastitis	44	15.83
Total	**278**	**100.0**

n = Sample size (767)

Data on reproductive disorders in the sheep breeds (Table 3) showed that dystocia, pregnancy toxaemia, retained placenta and mastitis were the major reproductive disorders among ewes. This agrees with the findings of [14, 15] from their studies in other locations of the country. Even though, the overall prevalence rate of these disorders was (1.58%) in sheep was relatively low compared to 4.07% reported by [16] and 9.6% by [9] from other locations. The reason for these differences might not be unconnected with prevailing environmental conditions and other management practices. The result also agrees with the findings of [14] that reproductive disorders were more in ewes than rams. The major reproductive disorders in ewes were dystocia (30.94%), retained placenta (16.91%) and pregnancy toxemia (11.51%). The incidence of mastitis was (15.83%), abortion and stillbirth were rarely reported to the clinics except when it proceeded with complications. This might be the reason why there were low records of 2.87% for abortion, which was lower than the value reported by [15]. Incidences of other disorders observed in the ewes were parturient paresis and prolapses, while vulvovaginitis and scrotal dermatophilosis were also reported. About 2.16% of rams had orchitis. pizzle rot, urolithiasis and paraphimosis cases were not recorded while 0.36% incidence of phimosis, 1.08 % of cryptochidism were also recorded.

Conclusion

This study concludes that the four breeds of sheep in Nigeria exhibited varied genetic make-up and potentials. The large number of farmers practicing the extensive system of production might have been responsible for the low reproductive performance of these animals in the country. It is suggested that significant improvement could be achieved through improved management practices of the animals in order to exhibit their optimum reproductive capacity.

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ABG 010

DISTRIBUTIONS OF QUALITATIVE TRAITS AMONG SOKOTO GUDALI CATTLE BREED IN SOME SELECTED STATES

*[1]John, P.A., [1]Kabir, M., [2]Iyiola-Tunji, A.O. and [1]Adedibu, I.I.

[1]Department of Animal Science, Ahmadu Bello University, Zaria
[2]National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria

*Corresponding author: johnpaulapagu@gmail.com

Abstract

This study was conducted to determine the distributions of coat colour pattern among Sokoto Gudali breed of cattle. A multistage (three stages) approach was used to select the breed of cattle from the States with large population of agro-pastoralists that rear Sokoto Gudali cattle. Four States were selected. A Local Government Area (LGA) per State was purposively selected which saw cattle been sampled from Lamurde, Shongom, Wurno and Ardokola from Adamawa, Gombe, Sokoto and Taraba, respectively.Snowball method was used to sample 264 Sokoto Gudali cattle from agro-pastoralist in many communities of the LGAs. Qualitative data on coat colour patterns were classed into solid white (WT), solid pale yellow (PY) and patched white/black (WT/BL), patched white/pale yellow (WT/PY) and patched pale yellow/black (PY/BL).Data collected were subjected to Chi-Square analysis to determine the significance of distributions of coat colour patterns across the age groups of the animals. Results obtained showed that animals with patched coat colour pattern in weaner 64, young 56, adult 40, males 84 and females 56 were preponderant over the ones with solid coat colour pattern. Animals with the highest number of white with black patched were recorded from Gombe (72) and Adamawa State (62) respectively. Links between coat colour patterns and productivity of Sokoto Gudali cattle should be explored using conventional animal breeding techniques and high-end molecular technologies.

INTRODUCTION

Sokoto Gudali is stereotypically occurs mainly in the North-West of Nigeria, but in reality, it is distributed widely throughout the country (Williamson and Payne, 1990; Payne and Wilson, 1999). The Sokoto Gudali is a uniform cream, light grey or dun, the dewlap and skin fold are highly developed and horns are almost absent. The breed has multiple coat colours although the most common one is black and white coating with light underside. They have deeper body than White Funali breed and resemble East African Boran and the Sudanese Kenana (Rege and Tawah 1999). It is similar in conformation, size and origin to the large East African short horn zebu. The head is long and wide between the eyes and across the forehead, with a straight or slightly convex facial profile. Ears are long, large and convex, sometimes pendulus. The Sokoto Gudali is the strain found in Nigeria, Northern Benin, Ghana and Mali (Tawah and Rege, 1996). The Gudali are principally found in Nigeria, Cameroon and Central African Republic, but a small population also inhibits Ghana. About 90% of the Sokoto Gudali are owned and managed by Fulani and house pastoralists and transhumant herders (Ngere, 1985), who feed their cattle on communally owned grazing lands and browse especially in the dry season (Tawah and Rege, 1996). They are known for their hardiness in the arid Northerly environments. The breed is not at a risk of disappearance, (RIM., 1992; DAD-IS, 2005). This study aimed at determining the distribution of coat colour pattern of Sokoto Gudali breed in some selected States in Nigeria.

Materials and Methods

Study was conducted to determine the distribution of coat colour pattern among weaner, young and adult Sokoto Gudali cattle

breed in some selected States in Nigeria. A multistage (three stages) approach was used to select the breed of cattle from the States with large population of agro-pastoralists that rear Sokoto Gudali cattle. Four States were selected. A Local Government Area (LGA) per State was purposively selected which saw cattle been sampled from Lamurde, Shongom, Wurno and Ardokola from Adamawa, Gombe, Sokoto and Taraba, respectively. Snowball method was used to sample 264 Sokoto Gudali cattle from agro-pastoralist in many communities of the LGAs. Qualitative data on coat colour patterns were classed into solid white (WT), solid pale yellow (PY) and patched white/black (WT/BL), patched white/pale yellow (WT/PY) and patched pale yellow/black (PY/BL). The distribution of qualitative traits on the body of the animals was done using visual observation. Data collected were subjected to Chi-Square analysis to determine the significance of distributions of coat colour patterns across the age groups of the animalsand computed using frequency procedure of SPSS (2015) version 16.

Results and Discussion

The distributions of qualitative traits among weaner, young and adult cattle are presented in Table 1. Patched (64) weaner cattle were preponderant compared to the solid (24) coat colour pattern. Similar trend was observed in young cattle with coat colour type variant in patched (56) while the solid coat colour pattern recorded the least (32) in young cattle with an intermittent occurrence for patched (40) and solid (48) in adult cattle. Coat colour showed significant differences ($P<0.01$) for pale Yellow/black, White, White/black and White/Pale Yellow. The proportions of adult (50), young (48) and weaner (48) cattle with White/black were higher compared to other coat colours. Animals with pooled horn nature were higher in weaner (51) with an equal number of occurrence in young (40) and adult cattle (40). Short/dropy horn nature were prevalent in young (17) and weaner cattle (13). The weaner (24) and young (24) cattle recorded very short horn nature compared to adult cattle (29). Cattle with pendulous ear nature recorded high value in weaner (50) Sokoto Gudali cattle compared to the young (40) and adult (40) while those with short ear nature recorded high value in young (48) and adult (48) compared to weaner (33). Significant differences ($P<0.01$) were observed for weaner, young and adult cattle. Developed dewlap (16), small dewlap (48) and well developed dewlap (24) occurred at an equal between young and adult cattle while the weaner cattle recorded the least (7).The result of this study is similar with the report of (RIM, 1994) who reported grey or cream (white with black patched) in Sokoto Gudali cattle.

The distributions of qualitative traits between male and female are shown on Table 2.Significant differences ($P<0.01$) among coat colour type variant. White/black coat colourwas the most preponderant in females (75) cattle compared to their male counterpart (72) while those with White colour were higher in males (52) compared to the females (46). Dewlap in males and females occurred at an intermittent occurrence with males being developed (36) than their female counterpart. Thedewlap and skin folds are well developed and horns almost absent in males but little longer in females (Blench, 1999).

The distributions of qualitative traits among cattle based on location are shown on Table 3. Significant differences ($P<0.01$) for coat colour patterns of the animals across the States. Cattle with high number of coat colour pattern were recorded from Taraba (72) while Gombe (40) and Sokoto (48) occurred at an intermittent occurrence. Gombe (72) recorded the highest number of cattle with White/black coat colour compared to Adamawa (62) while the least were recorded in Taraba State (12). Significant differences ($P<0.01$) also existed for horn nature of the animals. Cattle with pooled horn nature from Taraba (72) and Sokoto (48) were preponderant compared to other horn nature with the exception of cattle that had very short horn nature from Adamawa State. Pendulous ear

nature also recorded high value from Taraba (72) and Adamawa State (48) except for animals that had short ear nature from Adamawa (72)and Gombe (57). Sokoto State recorded cattle with developed dewlap (48) compared to Taraba (23) while those with high number of occurrence of small dewlap were observed from Gombe (72) and Taraba State (49) respectively. Cattle with sub-concave head profile were recorded from Sokoto (42) while those with straight head profile from Adamawa (72), Gombe (72) and Taraba State (72) occurred at an equal occurrence with the least from Sokoto State (6). The results of this study is also similar with the findings of RIM.(1992) who reported well developed dewlap in Sokoto Gudali cattle.

Table 1. Distributions of qualitative traits among weaner, young and adult cattle

Traits	Weaner	Young	Adult	DF	Chi-square	P value
Coat colour pattern						
PT	64	56	40	5	14.22	0.001
SD	24	32	48			
Coat colour						
PY/BL	10	0	0	11	40.65	0.000
WT	30	40	28			
WT/BL	48	48	51			
WT/PY	0	0	9			
Eye colour						
BE	88	88	88	0	0	NS
Horn nature						
PL	51	40	40	11	39.60	0.000
S/DR	13	17	0			
SH	0	7	19			
VS	24	24	29			
Ear nature						
PD	50	40	40	8	15.03	0.005
S/ER	5	0	0			
SE	33	48	48			
Dewlap						
DV	39	16	16	8	25.93	0.000
SM	42	48	48			
WD	7	24	24			
Head profile						
SC	16	16	10	5	2.04	0.361
ST	72	72	78			

PT: Patched, SD: Solid, PY/BL: Pale yellow/black, WT: White, WT/BL: White/black, BE: Black eye, PL: Pooled, S/DR: Short/dropy, SH: Short, VS: Very short, PD: Pendulous, S/ER: Short/erect, SE: Short ear, DV: Developed, SM: Small, WD: Well developed, SC: Sub-concave, ST: Straight, LOS: Level of significance.

Table 2.Distributions of qualitative traits between male and female cattle

Traits	Males	Females	DF	Chi-square	P value
Coat colour pattern					
PT	84	76	3	1.02	0.314
SD	48	56			
Coat colour					
PY/BL	8	2	7	13.03	0.005
WT	52	46			
WT/BL	72	75			
WT/PY	0	9			
Eye colour					
BE	132	132	0	0	NS
Horn nature					
PL	71	60	7	1.94	0.586
S/DR	13	17			
SH	12	14			
VS	36	12			
Ear nature					
PD	70	60	5	1.60	0.450
S/ER	2	3			
SE	60	69			
Dewlap					
DV	36	35	5	1.67	0.558
SM	72	66			
WD	24	31			
Head profile					
SC	24	18	3	1.02	0.313
ST	108	132			

PT: Patched, SD: Solid, PY/BL: Pale yellow/black, WT: White, WT/BL: White/black, BE: Black eye, PL: Pooled, S/DR: Short/dropy, SH: Short, VS: Very short, PD: Pendulous, S/ER: Short/erect, SE: Short ear, DV: Developed, SM: Small, WD: Well developed, SC: Sub-concave, ST: Straight, LOS: Level of significance.

Table 3: Distributions of qualitative traits among cattle based on location

Traits	Adamawa	Gombe	Taraba	Sokoto	DF	Chi-square	P value
Coat colour pattern							
PT	0	40	72	48	7	1.90	0.000
SD	72	32	0	0			
Coat colour							
PY/BL	0	0	0	10	15	2.72	0.000
WT	0	0	60	38			
WT/BL	63	72	12	0			
WT/PY	9	0	0	0			
Eye colour							
BE	72	72	72	48	0	0	NS
Horn nature							
PL	0	11	72	48	15	4.35	0.000
S/DR	0	30	0	0			
SH	0	26	0	0			
VS	72	5	0	0			
Ear nature							
PD	0	10	72	48	11	2.41	0.000
S/ER	0	5	0	0			
SE	72	57	0	0			
Dewlap							
DV	0	0	23	48	11	3.53	0.000
SM	17	72	49	0			
WD	55	0	0	0			
Head profile							
SC	0	0	0	42	7	2.25	0.000
ST	72	72	72	6			

PT: Patched, SD: Solid, PY/BL: Pale yellow/black, WT: White, WT/BL: White/black, BE: Black eye, PL: Pooled, S/DR: Short/dropy, SH: Short, VS: Very short, PD: Pendulous, S/ER: Short/erect, SE: Short ear, DV: Developed, SM: Small, WD: Well developed, SC: Sub-concave, ST: Straight, LOS: Level of significance

Conclusion and Recommendations

Animals with White and black patch coat colour were preponderant compared to other distributions of coat colour patterns in the studied populations. Animals with patchedcoat colour pattern were preponderant over the ones with solid coat colour pattern.

Links between coat colour patterns and productivity of Sokoto Gudali cattle should be explored using conventional animal breeding techniques and high-end molecular technologies.

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ABG 011

THE INFLUENCE OF *Moringa oleifera* SEED MEAL ON THE MILT COUNT OF *Heterobranchus bidorsalis* BROOD STOCK FED AT GRADED LEVELS.

Imgbian, T.D[1], Kigbu, A.A[2] and Mmere,C. P[3].

[1,2]Department of Aquaculture and Fisheries Management, Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi
Corresponding Author: awashila@gmail.com +2348025663457

Abstract

The experiment was conducted to assess the influence of Moringa oleifera seed meal as feed additive on the milt quality of Heterobranchus bidorsalis brood stock at the Department of fisheries, Nasarawa state University, Keffi. A total of 20 pieces of male brood stocks with weight ranging from 0.5kg to 0.6kg were divided into four treatments. The inclusion of Moringa oleifera seed meal was 0 for control (T!), 0.2, 0.4 and 0.6kg for T2, T3 andT4, respectively. The brood fish were fed for 49 days between 6.30-8.00am in the morning and 6.30-8.00pm in the evening while water was changed twice weekly to remove unconsumed feeds. The mean milt count of T3 (40.00=00±1.73) was significantly different (p<0.05) higher than those of the other treatments including the control. The highest mean weight and length of milt sac was in the 0.4kg (T3) inclusion and differed (p<0.05) significantly from other treatments. The proximate analysis of the formulated feed showed significant (<0.05) difference in treatment T4 which had the highest value of crude protein content and crude fibre content when compared to the other treatments. Hence the inclusion of Moringa oleifera seed meal up to 0.4kg is recommended in the diet of Heterobranchus bidorsalis male brood stock for increased milt cell production for improved fertilization rate and hatchability.

Introduction

Heterobranchus bidorsalis is one of the two main genera of the African mud catfish (*Clarias* and *Heterobranchus*) widely cultured in Africa, Asia and Europe (Adewolu and Adoti, 2010; Kori-Siakpere *et al*., 2006). This is due to their outstanding culture characteristics such as ability to withstand unfavourable environmental conditions, efficient in utilizing various types of locally formulated fish feed, resistance to diseases, high economic potential and simple techniques in the propagation of their fingerlings (Fagbenro and Sydenham, 1988; Dada and Olarewaju, 1996) This high demand of the clariids resulted in generally high prices which serve as an added inducement to would-be fish farmers or groups interested in commercial fish culture .Studies on *Heterobranchus* species in Nigeria focused on stock and chromosome manipulations, performances of intraspecific hybrids and growth performances at different dietary compositions in indoor and outdoor concrete tanks (Madu *et al*., 1991, 1993; Aluko and Aluko, 1998; Aluko *et al*., 1999, 2000; Madu and Aluko, 1999;Ndimele, 2011). It has been reported to have fast growth and weight of about 30kg has been reported in Nigerian wild water(Reed *et al* 1967).Considerable energy is normally wasted on the wild hunting of its fingerlings to stock ponds as a result of inadequate milt when the females are ready to spawn at the on-set of breeding season. The use of Malian traps and other fish gears which are not selective and therefore destructive to wild juveniles of both *Heterobranchus bidorsalis* and other fishes that are supposed to grow and replenish our wild waters has become a serious issue of concern. Artificial spawning which is

seen as the only solution to the problem of seed scarcity has been faced with the challenge of the good knowledge of the reproductive biology of the fish. According to Cabrita *et al* (2009), stress associated with captivity and the absence of appropriate environmental signals create reproductive problems in fishes. The use of plant seed and other products of high vitamins and minerals has been found to improve stress situation which in-turn improved the reproductive and overall well being of organisms. *Moringa oleifera*, a member of the family *Moringaceae*, is a fast-growing plant widely available in the tropics and subtropics with great economic potentials for the food and medical industry (Foidl *et al*, 2001). The seeds are a rich source of oil such as palmitic acid (9.3%), steric acid (7.4%), behenic acid (8.6%) and 65.7% oleic acid. It is also a rich source of vitamins, minerals and macronutrients (Shah *et al*, 1982; Makkar and Becker, 1997). The presence of adequate levels of essential amino acids and the low levels of anti nutrients indicates their high nutritional quality (Makkar and Becker, 1997c). According to Chatepa and Mbewe (2018), the seed consist of 96.86±0.30% dry matter, 28.54±0.41% crude protein, 5.37±0.11% ash, 7.90±0.27% crude fibre and 23.27±0.65% carbohydrate. No work has been done on the influence of *moringa oleifera* seed meal on the milt count of *Heterobranchus bidorsalis,* this cherished fish to fish marketers, farmers and consumers despite its outstanding values among which are high crude fiber content, high unsaturated fatty acids as well as high crude protein content. *Heterobranchus bidorsalis* seed production has been facing serious challenges in terms of fry survival rate during the early stages of life due to poor milt quality. This makes breeding and culture of *Heterobranchus bidorsalis* more difficult for many hatchery operators in Nigeria and beyond. Therefore finding a solution to the problem of raising the fish in captivity with high milt quality will be a major breakthrough to the successful hatchery operation. The objective of the study is to determine the influence of *Moringa oleifera* seed meal on the milt cell count of *Heterobranchus bidorsalis* brood fish raised in Lafia-Nigeria.

Materials and Methods

Experimental Site

The experiment was carried out at the Fisheries Unit of the Experimental Farm of the Department of Aquaculture and Fisheries Management of the Faculty of Agriculture, Nasarawa State University Keffi, Shabu- Lafia Campus. Lafia is located on latitude 8° 35'N, longitude 8° 32'E, altitude 181.53m above sea level with a mean temperature of 34°C, relative humidity of 40-86% and average day light of 9-12h (NIMET, 2011).

Test ingredients and experimental diet

The fish feed ingredients and the *Moringa oleifera* seed were obtained from the central market in Lafia and from other neighboring markets. The dried seed were sun dried and grounded into fine powder with a grinding machine.

Procurement of Experimental Fish

Twenty (20) healthy male*Heterobranchus bidorsalis*were obtained from Korlem fisheries (Nigeria) limited, Karu –Nasarawa state, Nigeria. The experimental fish were acclimatized to experimental condition in an outdoor concrete tank in the study area for 14 days. The fish were starved for 24 hours to maintain uniform stomach condition and to induce their appetite prior to the use of experimental diets.

Formulation of experimental diet

Fish feed of 35% crude protein was formulated for brood stock catfish using the following ingredients which includes fishmeal(8kg), soybean meal(6kg), groundnut cake(2kg), flour(2kg), maize flour(5kg), wheat offal(4kg), maize offal(4.6kg), vitamin premix(0.1kg), lysine(0.1kg), methionine(0.1kg), salt(0.1kg). The experimental diet comprised of four different treatmentsout of which the powdered *moringa oleifera* seed meal was incorporated into three treatments at 0.2kg, 0.4kg and 0.6kg. The required quantity of the test ingredients was weighed using a sensitive scale during the feed preparation.

Table 1: Composition of experimental diet. (Dietary treatment/8kg/DM)

Ingredients/*M.oleifera* (kg)	0.0	0.2	0.4	0.6
Fish meal	26.95	26.75	26.55	26.35
Soya bean meal	22.85	22.85	22.85	22.85
Groundnut cake	10.24	10.24	10.24	10.24
Wheat offal	9.50	9.50	9.50	9.50
Maize	11.84	11.84	11.84	11.84
Maize offal	6.01	6.01	6.01	6.01
Flour	4.61	4.61	4.61	4.61
Vitamin premix	2	2	2	2
Methionine	2	2	2	2
Lysine	2	2	2	2
Salt	2	2	2	2
	100	100	100	100

Experimental set up

The experiment consisted of four (4) treatments. The fish were randomly distributed into four (4) concrete tanks measuring 1.5m×1.5m×1.5m. The tanks were labeled as treatment 1, 2, 3, and 4 respectively. The fish were fed with the formulated diet for seven (7) weeks so as to ensure full maturation of the gametes. The fish were sacrificed in order to remove the milt, after which the length and weigh of the milt in each treatment was recorded.

Statistical Analysis

Data collected was subjected to one way analysis of variance (ANOVA). The level of significance of means from each treatment was determined using post Hoc test.The results obtained were presented using bar charts.

Results and Discussion

Table 2 shows the proximate analysis of the experimental diet with crude protein of 31.56 for T1, 32.25 for T2, 33.97 forT3 and 35.31 for T4. The crude fibre content of 2.96 was recorded for T1, 2.78 for T2, 3.07 for T3 and 3.24 for T4. The moisture content for T1 was8.05, while that of T2, T3 and T4 had 5.55, 7.55 and 5.40 respectively. The fat content ranged between 15.09 to 23.47 while the Ash content ranged between 14.65 to 21.25. The NFE ranged between 17.93 to 24.67.

Table 2: Proximate analysis of experimental diet

Treatments	T1(0.0kg)	T2(0.2kg)	T3(0.4kg)	T4(0.6kg)
Crude protein	31.56	32.25	33.97	35.31
Crude fiber	2.96	2.78	3.07	3.24
Moisture	8.05	5.55	7.55	5.40
Fat	15.09	18.70	17.99	23.47
Ash	21.25	16.05	17.43	14.65
NFE	21.09	24.67	19.99	17.93

Table 3 shows the mean weight value of the milt sac, length of milt sac and milt cell count of *Heterobranchus bidorsalis* fed *Moringa oleifera* seed meal at graded levels. The mean weight of the milt sac was highest in treatment 3 with mean value of 0.55±0.29. Treatment 3 in the mean length of the milt sac recorded 5.33±0.29; the milt cell count also recorded 40.00±1.73 in treatment 3

Table 3:Mean weight of milt sac, length of milt sac and milt cell count of *Heterobranchus bidorsalis* brood stock fed *moringa oleifera* seed meal at graded levels.

Treatments(kg)	T_1(0.0)	T_2(0.2)	T_3(0.4)	T_4(0.6)
Weight(g)	0.35±0.13	0.32±0.85	0.55±0.29	0.44±0.75
Length(cm)	5.16±0.23	5.09±0.09	5.33±0.18	5.05±0.94
Milt cell count(10^6/L)	34.00±1.15	38.00±0.58	40.00±1.73	37.00±1.15

gure 1 represents the mean weight of milt sac of*Heterobranchusbidorsal*

The mean difference is significant at the (p< 0.05) level

Figure 1 represents the graph of themean weight of the milt sac of *Heterobranchus bidorsalis* brood stock feed *Moringa oleifera* seed meal at graded levels with T3 having the highest mean weight of 0.55±0.29, T4 had 0.44±0.75, while T1 and T2 had 0.35±0.13 and 0.32±0.85 respectively.

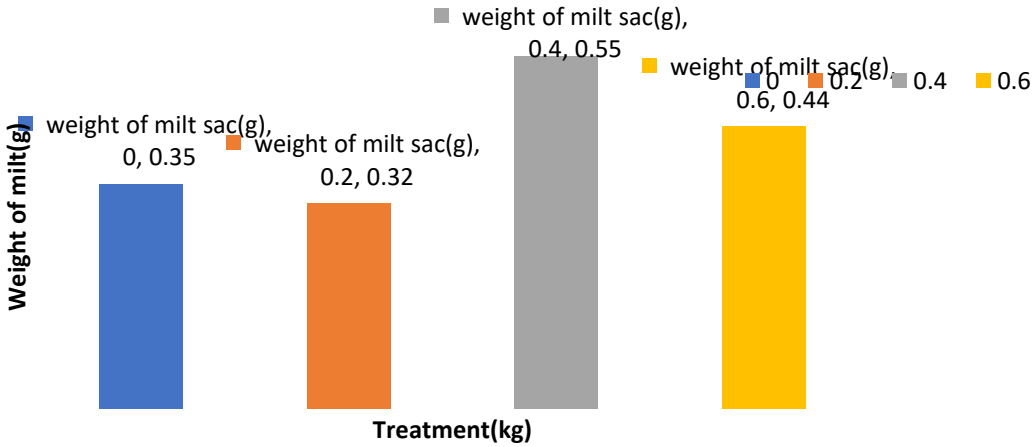

Fig. 1:Mean weight of milt sac of *Heterobranchus bidorsalis* brood stock fed graded level of *Moringa oleifera* seed meal.

Figure 2 shows the graph of the length of the milt sac of *Heterobranchus bidorsalis* brood stock fed graded levels of *Moringa oleifera* seed meal. Treatment 3 recorded the highest mean sac length of 5.33±0.18, treatment 1, 2 and 4 recorded 5.16±0.23, 5.09±0.09 and 5.05±0.94 respectively.

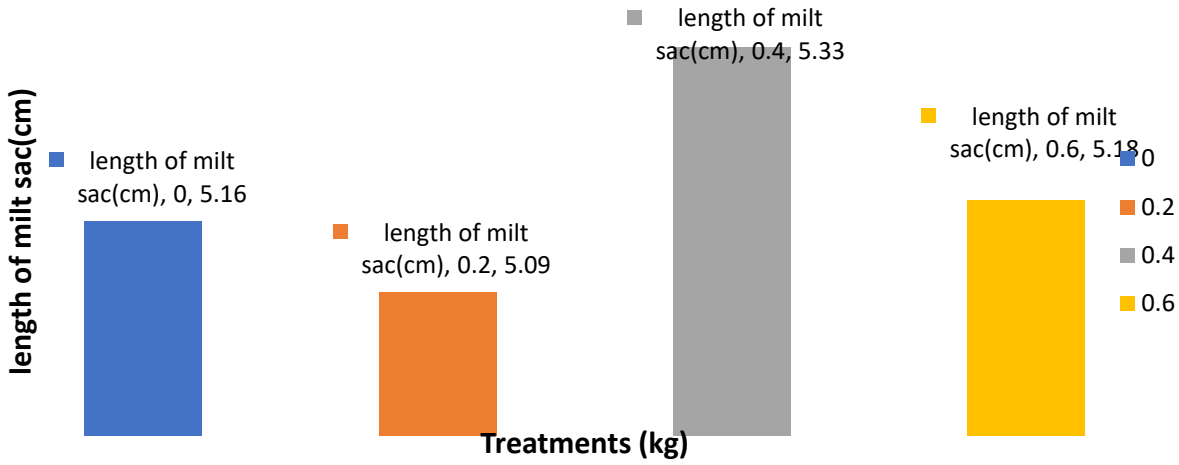

Fig. 2: Mean length of milt sac of *Heterobranchus bidorsalis* brood stock fed graded levels of*Moringa oleifera* seed meal.

Figure 3 represents the mean milt cell count of *Heterobranchus bidorsalis* brood stock fed *Moringaoleifera* seed meal at graded levels, with T3 having the highest milt cell count of 40.00±1.73 at 0.4kg inclusion level of *Moringa oleifera* seed meal. T_2 recorded 38.00±0.58, while T1 and T4 recorded 34.00±1.15 and 37.00±1.15 respectively.

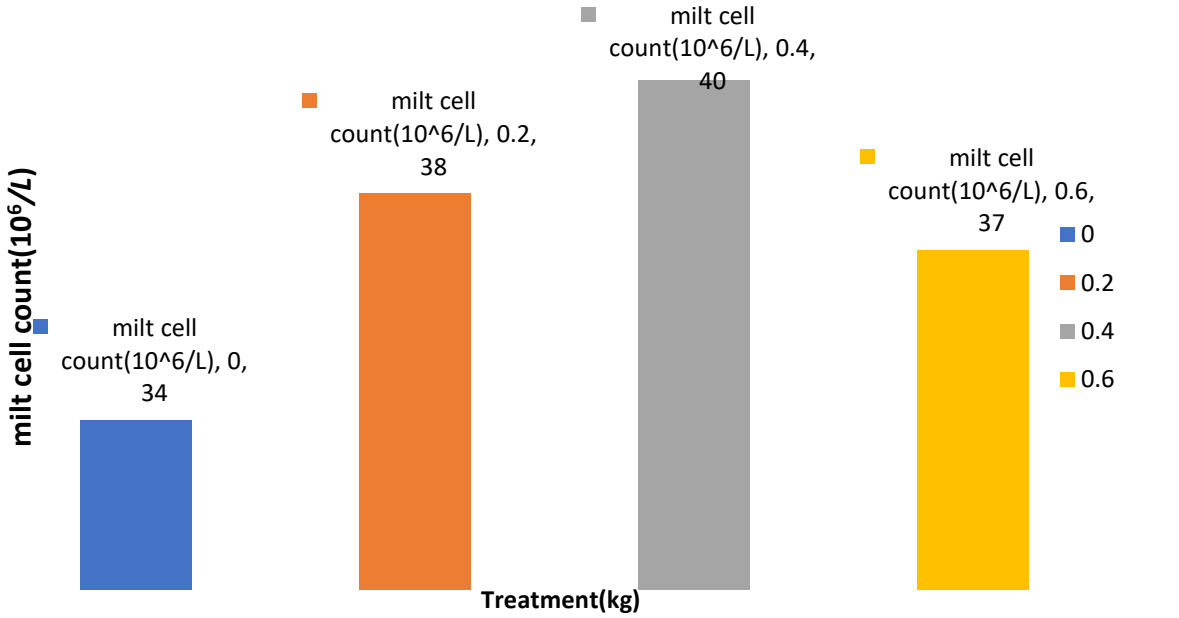

Fig. 3: Mean milt cell count of *Heterobranchus bidorsalis* fed graded levels of *moringa oleifera* seed meal

The high content of crude protein and fat in the proximate composition of the feed in treatment 4 is as a result of higher inclusion level of *moringa oleifera* seed meal in the diet. This is similar to the report of Ndabiganegeser and Narasiah (1998), Foild *et al.* (2001), Chatepa and Mbewe (2018) and Compare *et al* (2011) who reported that *moringa oleifera* seed are a good source of protein, fats, and crude fibre. The anti nutritional factors content in *moringa oleifera* seed meal (MSM) were rather high compared to some other plant based non-conventional feed ingredient like African bread fruit (*Treculia africana*) (Osabor *et al.* 2009).

The milt count has increased in treatment 3 (40.00±1.73) compared to 38.00±0.58 in treatment 2. This might be as a result of increase in the fibre content of *Moringa oleifera* seed meal as it was increased from 0.2kg to 0.4kg which may have increased the digestibility. The high crude fibre is likely to have reduced the tendency of fats deposition in the visceral which may have increased milt formation. Also the anti oxidant attributes of *Moringa oleifera* seed meal as highlighted by (Foidl *et al*, 2001), ,palmitic acid and fatty acids may have improved the milt count of the fish in treatment 3.this is an indication of high milt quality as regard fry production as highlighted by Coban *et al*(2011). At higher inclusion of *Moringa oleifera* seed meal in treatment 4 (0.6kg), the interaction of vitamins, minerals and fat may have had adverse implication on the milt cell count of the fish hence the decrease in the milt cell count in treatment 4.The research showed that there was a higher increase in the mean weight of milt sac (0.55±0.18), mean length of milt sac (5.33±0.18) and mean milt cell count(40.00±1.73) in treatment 3 from table 3 which had 0.4kg inclusion level of *Moringa oleifera* seed meal when compared to the other treatments.It was also observed during the course of the experimental set up that the body weight (growth) of the fish increased as a result of the addition of *moringa oleifera* seed meal in the diet. Therefore the inclusion of *Moringa oleifera* seed meal in diet of *Heterobranchus bidorsalis* at the level of (0.4kg/8kg) level has increased the milt cell count of the fish and iproved the breeding value of the fish.

Conclusion

In conclusion, the inclusion of *moringa oleifera* seed meal in the diet of *Heterobranchus bidorsalis* brood stock at a moderate level (0.4kg) has increased the milt cell count of the fish as well as body weight (growth).

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EFFECT OF ECOTYPE, BATCH OF HATCH, LOCATION ON REPRODUCTIVE PERFORMANCE OF TWO NIGERIAN LOCAL CHICKEN ECOTYPES

Gambo, D., Momoh[1], O. M., Gwaza [1], D. S., Ajibike[2], A. B., and Alokoson[3], J. I.

Department of Animal Science, Faculty of Agriculture, Nasarawa State University Keffi, Nasarawa State
[1]Department of Animal Breeding and Physiology, College of Animal Science, University of Agriculture, Makurdi, Benue State.
[2]Department of Animal Production Technology, Faculty of Animal and Fisheries Technology, Oyo State College of Agriculture and Technology, Igboora, Oyo State
[3]Livestock Unit, Nasarawa Agricultural Development Programme, Nasarawa State.

Corresponding Author: gambodauda@nsuk.edu.ng, gambodauda21@gmail.com +2348062347830

Abstract

This study was undertaken to investigate and compare the reproductive performance of two Nigerian chicken ecotypes - Tiv and Fulani chicken was. A total of 110 birds were purchased (comprising of 10 hens and 1 cock per location from five randomly selected locations for each chicken ecotype) as base population, and were housed according to their ecotype and location of purchase. A mating ratio of 1 cock to 10 hens was applied, and fertile eggs were collected for hatching after 4 weeks of laying. Data were collected on fertility, early and late embryonic mortality, hatchability and reproductive capacity, and were subjected to multivariate analysis using the SPSS statistical package. The result indicated significant effect ($p<0.05$) of batch of hatch on reproductive characteristics. Batches 2 and 3 demonstrated significantly higher fertility, hatchability and reproductive capacity. Ecotype and location had no significant effects ($p>0.05$) on reproductive parameters. The interactions between ecotype × batch × location was significant ($p<0.05$) on fertility, early and late embryonic mortalities. The coefficients of determinant (R^2) values for fertility, early and late embryonic mortalities, hatchability and reproductive capacity was 0.147, 0.418, 0.297, 0.395 and 0.499 respectively. From the findings of this research, it can be recommended that high reproductive performance of Tiv and Fulani chicken ecotype should be exploited genetically for commercial hatching and sales/distribution of local day-old chicks.

Keywords: *Fertility, Fulani ecotype, hatchability, interactions, Tiv ecotype and reproductive capacity*

Introduction

Poultry, particularly chickens, have been recognized as an important genetic resource among the avian species (Olowofeso *et al.,* 2005). Genetic diversity, a product of interaction between environment and genetic effects, of indigenous livestock species in developing countries are valuable attributes for production, adaptation and resistance of the indigenous animals to endemic diseases (reference). . This interaction has led to differentiation of morphological, physiological and productive traits vital to all production systems providing selection criteria for breed improvement as well as adaptation to changing environmental circumstances (Ceriotti et al., 2003). Genetic resources of indigenous breeds would be required for creating more variations of desired economically important trait in order to meet the current production needs and future food security (Ajibike *et al.,* 2017). The unique values

of domestic chicken's genes for egg and meat production, disease resistance, hardiness and adaptation to local environment has broaden their use as a genetic resource base for breeding of improved commercial birds (Gwaza *et al.,* 2015). The objective of the study was to comparatively investigated variation in the reproductive performance traits of Nigerian Tiv and Fulani chicken ecotypes.

Materials and Methods

Study location

The experiment was carried out at the Livestock Teaching and Research Farm of the Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi, Nasarawa State. Nasarawa State falls within the Southern Guinea Savannah Zone of Nigeria. Lafia lies between latitude $8^0$29'38.0" N and longitude $8^0$30'55.2" E. It has a climate typical of the tropical zone because of its location, with temperature ranging from 20 ^{0}C in October to 36 ^{0}C in March and rainfall varies from 13.73 - 14.00 cm (NIMET, 2008).

Experimental animal and management

Stratified random sampling technique was used to assemble a total of 100 hens - comprising 50 Tiv and 50 Fulani ecotypes, and 10 breeding cocks comprising 5 per ecotype (5 cocks per ecotypes were kept as reserves in case of mortality) from five different localities for each ecotype (Tiv ecotype was purchased from Uikpan, Daudu, Kadarko, Yelwata and Cohor in Benue and Nasarawa States while Fulani ecotype was purchased from Lafia, Akurba, Adogi, Asakio and Namu in Nasarawa and Plateau States), and used for the experiment.

The birds were housed according to ecotype and location of purchase, and were allowed two weeks for acclimatization and quarantine. During this period, the birds were dusted against ecto-parasites, dewormed and vaccinated against Newcastle disease using Lasota® while anti-stress (vitalyte), antibiotics and coccidiostat were orally administered against possible disease outbreak.

A mating ratio of 1:10 (i.e. 1 cock to 10 hens) was used, and free mating was allowed. Fertile eggs for hatching were collected after four weeks of laying in order to obtain higher fertility and hatchability. The birds were fed breeder diet containing 18% Crude protein and 2700 Kcal/Kg metabolizable energy.

Eggs for hatching were collected twice a day for 5 days, labeled according to location and ecotype, and held in egg crates under room temperature with good ventilation. At the end of 5 days of egg collection, the eggs were transported to the hatchery for incubation and hatching, and were set for pedigree hatching in an automatic electric incubator at weekly internal for four consecutive weeks (four batches).

Data Collection

The following parameters were measured based on ecotype, batch of hatch and location:

Fertility: candling was done on the seventh day from first day of incubation to determine fertile eggs. Fertility was determined based on total eggs set.

$$\% \text{ fertility} = \frac{Number\ of\ fertile\ eggs}{Total\ egg\ set} \times \frac{100}{1}$$

Embryonic mortality: This is the fertilized embryo that died before hatching. Embryonic mortality was measured at two levels, early and late mortality. For accurate assessment of the early embryonic mortality, candling was carried out again at the 14th day of incubation. After hatching, all the hatched chicks were taken out of the incubator; the unhatched eggs were broken open on the hatch day under bright sunlight to identify late embryonic mortality.

$$\% \text{ early embryonic mortality} = \frac{Number\ of\ dead\ embryo\ at\ 14\ days}{Total\ number\ of\ fertile\ eggs} \times \frac{100}{1}$$

$$\% \text{ late embryonic mortality} = \frac{Number\ of\ dead\ embryo\ after\ hatching}{Total\ number\ of\ fertile\ eggs} \times \frac{100}{1}$$

Hatchability: This was expressed on the basis of fertile eggs and total eggs set (reproductive capacity).

$$\% \text{ hatchability} = \frac{Number\ of\ hatced\ chicks}{Total\ fertile\ eggs} \times \frac{100}{1}$$

$$\text{Reproductive capacity} = \frac{\textit{Number of hatched chicks}}{\textit{Total egg set}} \times \frac{100}{1}$$

Data Analysis

Data collected were arranged in a Completely Randomized Block Design (CRBD), and analyzed using multivariate analysis of SPSS statistical software ver. 21 (SPSS, 2011). Variables recorded in percentages were first transformed using arc sine transformation before being subjected to analysis. The statistical model used was:

$Y_{ijkl} = \mu + S_i + D_j + E_k + SD_{ij} + SE_{ik} + DE_{jk} + SDE_{ijk} + E_{ijkl}$

Where:

Y_{ijkl} = measure of the j^{th} progeny of the i^{th} ecotype;

μ = population mean;

S_i = effect of ecotype (i = 1 and 2);

D_j = effect of batch of hatch (j = 1, 2, 3 and 4);

E_k = effect of location (k = 1 - 5);

SD_{ij} = interactive effect between ecotype and batch of hatch;

SE_{ik} = interactive effect between ecotype and location;

DE_{jk} = interactive effect between batch of hatch and location;

SDE_{ijk} = interactive effect between ecotype, batch of hatch and location;

E_{ijkl} = random error with mean zero and variance that of the population

Results

Effect of ecotype and batch of hatch on reproductive performance

The effect of ecotype on reproductive performance of the Tiv and the Fulani local chicken ecotypes are presented in Table 1. The effect of ecotype was not significantly (p>0.05) different between the Tiv and the Fulani ecotypes for all the reproductive parameters measured. However, batch of hatch had significant (p<0.05) effect on early and late embryonic mortalities, hatchability and reproductive capacity in both the Tiv and the Fulani ecotypes as well as on fertility in the Tiv ecotype.

Batches 1, 2 and 3 significantly differed in fertility (95.01±2.54%, 94.70±2.64% and 92.81±4.06% respectively) from batch 4 in the Tiv ecotype. Batch 3 had significantly (p<0.05) lowest values for early and late embryonic mortalities (1.87±1.15% and 6.65±2.36% respectively) while batch 2 and 3 had significantly (p<0.05) highest value for hatchability (88.48±4.07% and 91.48±2.75% respectively) and reproductive capacity (83.44±2.26% and 84.84±3.66% respectively) in the Tiv ecotype. In the Fulani ecotype, batch 3 had had significantly (p<0.05) lowest value for early embryonic mortality (1.33±1.33%) while batch 1 showed had significantly (p<0.05) least value (5.40±2.37%) for late embryonic mortality. The highest hatchability value (86.36±2.51%) and reproductive capacity (78.72±2.46%) was observed in batch 2 in the Fulani ecotype.

Table 1: Effect of ecotype and batch of hatch on reproductive performance (%) of Tiv and Fulani chicken ecotype

	Fer	Eem	Lem	Hatch	Repc
Ecotype					
Tiv	92.15±1.46	8.66±1.75	11.92±1.89	79.67±3.06	73.79±3.14
Fulani	90.63±2.06	11.53±2.38	11.41±2.08	77.08±2.64	70.01±3.01
LOS	NS	NS	NS	NS	NS
Batch Tiv					
1	95.01±2.54^{a}	13.37±2.29^{b}	10.55±2.79^{a}	76.08±3.32^{b}	72.29±3.68^{b}
2	94.70±2.64^{a}	2.54±1.57^{a}	9.97±4.75^{a}	88.48±4.07^{a}	83.44±2.26^{a}
3	92.81±3.52^{ab}	1.87±1.15^{a}	6.65±2.36^{a}	91.48±2.75^{a}	84.84±3.66^{a}
4	86.08±1.39^{b}	16.85±2.39^{b}	20.51±2.23^{b}	62.64±3.50^{c}	54.60±2.92^{c}
LOS	*	*	*	*	*
Batch Fulani					
1	91.67±5.27	21.93±5.47^{c}	5.40±2.37^{a}	72.67±6.52^{ab}	65.78±4.99^{ab}
2	91.40±3.53	7.63±2.66^{ab}	6.01±2.75^{a}	86.36±2.51^{a}	78.72±2.46^{a}
3	92.48±4.06	1.33±1.33^{a}	17.61±5.04^{b}	81.05±4.93^{ab}	75.68±7.25^{ab}
4	86.96±4.37	15.21±2.93^{bc}	16.62±3.26^{b}	68.25±3.11^{b}	59.84±5.57^{b}
LOS	NS	*	*	*	*

INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM, late embryonic mortality, HATCH = hatchability, REPC = reproductive capacity, LOS = level of significant, NS = non significant

Effect of location on reproductive performance

The effect of location on the reproductive performance of the two chicken ecotypes is shown in Table 2. It was observed that location had no significant effect (p>0.05) on the reproductive parameters of the two chicken ecotypes.

Table 2: Effect of Location on Reproductive Performance (%) of the Tiv and the Fulani Chicken Ecotypes

	Fer	Eem	Lem	Hatch	Repc
Tiv ecotype					
1	92.83±4.25	9.31±2.33	13.92±3.82	76.78±6.05	71.32±6.75
2	93.54±3.01	7.17±2.59	12.28±3.17	80.55±4.29	75.64±5.95
3	92.40±4.41	8.71±5.53	12.25±6.06	80.29±7.92	74.24±7.74
4	89.70±3.75	9.98±4.94	8.60±2.77	81.45±6.53	74.64±7.71
5	92.29±2.14	8.14±5.29	12.57±6.22	79.29±11.45	73.13±10.38
LOS	NS	NS	NS	NS	NS
Fulani ecotype					
1	84.35±5.59	5.56±3.21	14.97±4.56	79.48±4.09	67.15±5.92
2	91.17±3.28	18.17±8.96	10.35±4.77	71.48±8.88	64.64±6.79
3	95.31±4.69	13.23±4.00	12.27±2.18	74.60±2.74	71.35±5.44
4	88.26±5.30	14.59±5.24	12.02±6.95	73.40±5.33	65.40±7.27
5	94.05±3.95	6.09±2.15	7.44±5.45	86.48±6.41	81.49±7.68
LOS	NS	NS	NS	NS	NS

INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM, late embryonic mortality, HATCH = hatchability, REPC = reproductive capacity, LOS = level of significant, NS = non significant, Tiv ecotype: location 1-5 = Uikpan, Daudu, Kadarko, Yelwata and Cohor, Fulani ecotype: location 1-5 = Lafia, Akurba, Adogi, Asakio and Namu

Interactive effects of ecotype, batch of hatch, and locations on Reproductive Performance

The mean squares result of the effect of ecotype, batch of hatch, location and their interactions on reproductive performance is shown in Table 3. Ecotype, location and interactions between ecotype by batch of hatch, ecotype by location and batch of hatch by location had no significant (p>0.05) effect on fertility, early and late embryonic mortalities, hatchability and reproductive capacity.

Batch of hatch had significant (p<0.05) effect on early and late embryonic mortalities, hatchability and reproductive capacity. However, batch of hatch had no significant (p>0.05) effect on infertility and fertility.

Interactions between ecotype × batch × location was significant (p<0.05) on infertility, early and late embryonic mortalities. The interactions between ecotype × batch × location had no significant (p>0.05) effect on fertility, hatchability and reproductive capacity. The coefficients of determinant (R^2) values were 0.147, 0.418, 0.297, 0.395 and 0.499 for fertility, early and late embryonic mortalities, hatchability and reproductive capacity respectively.

Table 3: Mean square values of the effect of ecotype, batch of hatch, location and their interactions on reproductive performance (%) of Tiv and Fulani chicken ecotypes

Traits	Sources of variation	Df	Mean square	R^2
Fertility	Ecotype	1	23.23^{ns}	0.147
	Batch	3	106.10^{ns}	
	Location	4	47.63^{ns}	
	Ecotype*Batch	3	11.42^{ns}	
	Ecotype *Location	4	39.79^{ns}	
	Batch*Location	12	72.29^{ns}	
	Ecotype*Batch*Location	12	426.58^{ns}	
	Error	200	53.49	
Early embryonic mortality	Ecotype	1	82.226^{ns}	0.418
	Batch	3	631.87***	
	Location	4	56.293^{ns}	
	Ecotype*Batch	3	57.70^{ns}	
	Ecotype *Location	4	70.00^{ns}	
	Batch*Location	12	21.52^{ns}	
	Ecotype*Batch*Location	12	238.81***	
	Error	200	50.65	
Late embryonic mortality	Ecotype	1	2.621^{ns}	0.297
	Batch	3	249.83*	
	Location	4	25.591^{ns}	
	Ecotype*Batch	3	147.12^{ns}	
	Ecotype *Location	4	20.78^{ns}	
	Batch*Location	12	88.64^{ns}	
	Ecotype*Batch*Location	12	276.46***	
	Error	200	54.00	
Hatchability	Ecotype	1	66.93^{ns}	0.395
	Batch	3	1091.17***	
	Location	4	55.44^{ns}	
	Ecotype*Batch	3	107.97^{ns}	
	Ecotype *Location	4	102.57^{ns}	
	Batch*Location	12	69.03^{ns}	
	Ecotype*Batch*Location	12	571.85^{ns}	
	Error	200	97.24	
Reproductive capacity	Ecotype	1	143.26^{ns}	0.499
	Batch	3	1259.43***	
	Location	4	87.54^{ns}	
	Ecotype*Batch	3	98.89^{ns}	
	Ecotype *Location	4	115.15^{ns}	
	Batch*Location	12	109.10^{ns}	
	Ecotype*Batch*Location	12	490.67^{ns}	
	Error	200	94.09	

Df = degree of freedom, *= ($p<0.05$), ** = ($p<0.01$), *** = ($p<0.001$), ns=Not significant and R^2 =Adjusted values

Summary Statistics and Coefficient of Variations of Reproductive Traits

The percentage coefficient of variations (Table 4) showed variability in reproductive traits between locations of the two ecotypes. Early embryonic mortality had the highest values of CV while the CV for fertility had the least values in both the Tiv and the Fulani ecotypes. In the Tiv ecotype, birds from Uikpan and Kadarko (locations 1 and 3) had CV values of 118.49% and 115.89% percent in infertility. CV of 127.08% and 129.94% was obtained in early embryonic mortality in birds from Kadarko and Cohor (locations 3 and 5 respectively) in the Tiv ecotype. In the Fulani ecotype, birds from Namu (location 5) had CV of 132.62% for infertility while birds from Lafia (location 1) had CV of 115.47% for early embryonic mortality. Birds from Asakio and Namu (locations 4 and 5) had CV of 115.65% and 146.45% respectively for late embryonic mortality.

Table 4: Statistics summary of the Reproductive Performance (%) of two Nigerian Chicken Ecotypes

TR	L	B	Tiv Ecotype							Fulani Ecotype						
			MI	MA	RA	ME	SD	SE	CV	MI	MA	RA	ME	SD	SE	CV
	1	4	83.33	100.00	16.67	92.83	8.49	4.25	9.15	75.00	100.00	25.00	84.35	11.19	5.59	13.27
	2	4	86.29	100.00	13.71	93.53	6.01	3.01	6.43	85.71	100.00	14.29	91.17	6.57	3.29	7.20
FER	3	4	83.88	100.00	16.12	92.40	8.81	4.41	9.54	81.25	100.00	18.75	95.31	9.38	4.69	9.83
	4	4	82.24	100.00	17.76	89.70	7.50	3.75	8.36	75.00	100.00	25.00	88.26	10.59	5.30	12.00
	5	4	87.50	96.67	9.17	92.29	4.27	2.14	4.63	83.33	100.00	16.67	94.05	7.90	3.95	8.40
	1	4	5.00	13.64	8.64	9.31	4.67	2.33	50.14	0.00	11.11	11.11	5.56	6.41	3.21	115.47
	2	4	0.00	12.00	12.00	7.17	5.17	2.59	72.19	0.00	40.00	40.00	18.17	17.92	8.96	98.60
EEM	3	4	0.00	23.08	23.08	8.71	11.07	5.53	127.08	6.67	23.53	16.86	13.23	7.99	4.00	60.43
	4	4	0.00	22.22	22.22	9.98	9.87	4.94	98.96	0.00	25.00	25.00	14.59	10.49	5.24	71.90
	5	4	0.00	22.22	22.22	8.14	10.58	5.29	129.94	0.00	10.00	10.00	6.09	4.29	2.15	70.48
	1	4	5.55	22.73	17.18	13.92	7.64	3.82	54.89	9.09	28.57	19.48	14.97	9.12	4.56	60.90
	2	4	4.76	20.00	15.24	12.28	6.34	3.17	51.61	0.00	23.08	23.08	10.35	9.55	4.77	92.21
LEM	3	4	0.00	27.73	27.73	12.25	12.11	6.06	98.89	5.88	15.58	9.70	12.27	4.36	2.18	35.52
	4	4	4.35	16.67	12.32	8.60	5.53	2.77	64.35	0.00	25.00	25.00	12.02	13.90	6.95	115.65
	5	4	0.00	27.78	27.78	12.57	12.44	6.22	99.00	0.00	23.08	23.08	7.44	10.89	5.45	146.45
	1	4	63.64	88.89	25.25	76.78	12.09	6.05	15.75	71.43	90.91	19.48	79.48	8.19	4.09	10.31
	2	4	68.00	86.36	18.36	80.55	8.57	4.29	10.64	50.00	92.31	42.31	71.48	17.78	8.89	24.87
HAT	3	4	61.54	100.00	38.46	80.29	15.85	7.92	19.74	69.23	80.00	10.77	74.60	5.47	2.74	7.34
	4	4	70.00	94.12	24.12	81.45	13.06	6.53	16.03	58.33	83.33	25.00	73.40	10.66	5.33	14.52
	5	4	50.00	100.00	50.00	79.29	22.90	11.45	28.88	69.23	100.00	30.77	86.48	12.82	6.41	14.82
	1	4	56.00	88.89	32.89	71.32	13.50	6.75	18.93	55.56	77.78	22.22	67.15	11.84	5.92	17.63
	2	4	58.62	86.36	27.74	75.64	11.90	5.95	15.73	50.00	80.00	30.00	64.64	13.57	6.79	21.00
RCP	3	4	51.61	85.71	34.10	74.24	15.48	7.74	20.85	56.25	80.00	23.75	71.35	10.89	5.44	15.26
	4	4	61.29	91.30	30.01	74.64	15.42	7.71	20.66	43.75	75.00	31.25	65.40	14.53	7.27	22.22
	5	4	45.00	90.00	45.00	73.13	20.75	10.38	28.38	64.29	100.00	35.71	81.49	15.36	7.68	18.84

TR = traits, L = locations, B = batch of hatch, MI = minimum value, MA = maximum value, RA = range, ME = mean, SD = standard deviation, SE = standard error of the mean, CV = coefficient of variation, INF = infertility, FER = fertility, EEM = early embryonic mortality, LEM = late embryonic mortality, HAT = hatchability and RCP = reproductive capacity, Tiv ecotype: location 1-5 = Uikpan, Daudu, Kadarko, Yelwata and Cohor, Fulani ecotype: location 1-5 = Lafia, Akurba, Adogi, Asakio and Namu

Discussion

The observed variations within and between Tiv and Fulani ecotypes for fertility, embryonic mortality, chick mortality and hatchability as obtained in this study had been reported earlier by Gwaza *et al.* (2015). The observed percent fertility value in both chicken ecotypes strongly agreed with the range of 83.0 - 92.7% for Bangladesh local chickens as reported by Islam and Nishibori (2009) and 79.65±0.45-86.65±0.07% reported by Amao (2017) for Nigerian Nacked neck chickens. Gwaza *et al.* (2015) reported a slightly lower percent fertility of 70.62±3.70 and 77.75±6.28 % for Fulani and Tiv chicken ecotype respectively.

Early embryonic mortality obtained for Tiv and Fulani chicken ecotypes were similar to 14.90% reported by Gwaza *et al.* (2015) while late embryonic mortalities are lower than 14.90% reported by Gwaza *et al.* (2015). The observed similarity could be due to the facts that the birds were of the same ecotype. Percentage Hatchability observed for both chicken ecotype fell within the range of 72- 93.1% and 85.07±8.90% reported by Ajayi *et al.* (2008) and Amao (2017) respectively while Islam and Nishibori (2009) reported a lower and wider range of 52.4-87.0% for indigenous full feathered chickens.

Fulani chicken ecotype had higher reproductive capacity (hatchability based on total egg set) value range of 59.84 - 78.72% while Tiv ecotype recorded 54.60 - 84.84% which fell within the reported values of of 58.00, 55.14, 61.31 and 56.90% by Farooq *et al.* (2001), Kurshid *et al.* (2004), Daikwo (2011) and Gambo *et al.* (2014)for Japanese quail respectively. Reproductive capacity is of more practical importance to farmer than hatchability (reference). Genetic variations in hatchability of fertile eggs may arose from the hen due to quality of laid eggs which affects the development of embryo to chicks during incubation as well as emergence of chicks from the egg at hatching (Anang *et al.,* 2001; Wolc *et al.,* 2009). The observed high fertility, hatchability and reproductive capacity for both chicken ecotypes could be due to better fertility, and thus, suggested high prolific which could be utilized efficiently in meat and egg production enterprise. However, the higher values demonstrated by the Tiv ecotype compared to the Fulani ecotype for fertility, hatchability and reproductive capacity could be that the levels of inbreeding among the population of the Tiv local chicken ecotype were low compared to that of the Fulani chicken ecotype. The significant effect of batch of hatch indicated diversity of alleles that was sampled at segregation, independent assortment and recombination of genes

Conclusion

It can be concluded that there is no much variation in the reproductive performance of the two Nigerian chicken ecotypes studied, despite observed high percentage fertility, hatchability and reproductive capacity coupled with low early and late embryonic mortalities. Therefore, the high reproductive performance of both chicken ecotypes should be genetically exploited for commercial hatching and sales/distribution of local day-old chicks as well as for planning of breeding and conservation strategy.

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ABG 013

PERFORMANCE CHARACTERISTICS AND PREDICTION OF BODY WEIGHT (g) USING LINEAR BODY MEASUREMENTS (cm) IN THREE *STRAINS* OF BROILER CHICKEN REARED IN THE SEMI-ARID ZONE OF NIGERIA

[1]Ahmed, I.A., [3]Kabir, M., [2]Adeyinka, I.A., [3]Musa, R.A., [4]Idris, D.Y.

[1]Department of Animal Production Technology, Audu Bako College of Agriculture, Dambatta, P.M.B. 3159, Kano State, Nigeria[3]Department of Animal Science, Ahmadu Bello University, Samaru, Zaria
[2]National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria
[3]Department of Remedial and General Studies, Audu Bako College of Agriculture, Dambatta, P.M.B. 3159, Kano State, Nigeria
[4]Department of Animal Production and Health Technology, Federal College of Agricultural Produce Technology, Kano, P.M.B. 3032, Kano State, Nigeria
*Corresponding Author: iliyasunadada@gmail.com *+2348065532319*

ABSTRACT

A study was conducted to evaluate performance characteristics of three strains of broiler chicken at 8 weeks of age and predict their body weight using linear body measurements. A total of three hundred and fifteen (315) day-old broiler chick strains comprising NAPRI-X, Marshal and Ross (105 each) were used in a Completely Randomized Design (CRD) replicated seven (7) times with fifteen (15) birds per pen. The traits recorded were weight gain (g), total feed intake (g), feed conversion ratio, mortality (%) and some linear body measurements (Initial and Final Body Weights in grams, Neck Length (NEL), Back Length (BKL), Thigh Length (THL), Shank Length (SHL), Breast Weight (BRW), Body Length (BDL) and Wing Length (WNL) all in.centimtres. Prediction equations relating body weight to linear body measurements at 8 weeks of age were established for the three strains of broilers using Multiple Regression Method. The results indicated a highly significant ($p<0.001$) difference among the three strains in the initial and final body weights at 8 weeks of age. Marshal broiler was heavier at 8 weeks of age compared to NAPRI-X and Ross. However, at 8 weeks Marshal attained an average body weight of 1, 870.69g the values of which were higher compared to its counterpart (1, 530.58g NAPRI-X and 1, 588.69g Ross) respectively. The regression analysis was highly significant ($p<0.001$) in all the strains. The coefficient of determination (R2) was more than 50% in all the strains indicating that the prediction equations obtained in this study could be used to predict body weight of broilers.

Keywords: *Broiler Strains, Performance Characteristics, Linear Body Measurement, Prediction Equations, Body Weight*

Introduction

In Nigeria, poultry contributes significantly in supply of animal protein to the populace (Ojedapo, 2013). The poultry population was put at 114.3 million comprising of 82.4 million chickens (11% of which was commercially raised) and 31.9 million other poultry which include pigeons, ducks, guinea fowls and turkeys (RIM, 1992). Chicken production is increasing due to increased product output per animal, high feed conversion efficiency, improved fertility, hatchability, growth rate, egg yield and meat quality (Ojedapo, 2013). Poultry keeping requires less land, and most of the poultry species are more prolific than other species of livestock (Ojedapo, 2013). Poultry breeders have tried to establish the relationships that exist between body weight and physical characteristics (body conformation) such as body length, shank length, thigh length, breast girth and keel length as this information reflects on the feed efficiency as well as performance of the broiler

birds. Interrelationships among body measurements can be applied speedily in selection and breeding. Besides, this will help the breeders to organize the breeding program in order to achieve an optimum combination for maximum economic return (Okon *et al.*, 1997). Breeders of meat-type chicken have become interested in adult body weight; the trend being towards a big-bodied chicken at early age in order to attract better price at marketing (Malik *et al.*, 1997). Body weight is regarded as a function of frame work or size of the animal and its condition. An increase in body weight is highly correlated with feed consumption when selecting for rapid growth under *ad-libitum* feeding, indicating that more energy is available for growth over the maintenance requirement of chickens. Body weights are shown to be influenced by maternal effect or dominance effects or both, up to maturity as indicated by consistently higher heritability estimates from dam variance components as opposed to those from sire components. Adeyinka *et al.* (2004) reported moderate heritability for body weights for naked neck broilers at various ages, but observed high heritability estimates for body weight at 56 days of age and finally suggested that selection for body weight at this age will improve body weight in subsequent generations. Linear body measurements otherwise called conformation traits are important parameters in predicting body weight and this has been observed by commercial breeders and producers. In places where scales are not available as is the case in most rural African communities (Nesamvuni *et al.,* 2000), linear body measurements such as shank length, drum stick length, and wing length can be used in a predictive equation to predict body weight in broilers (Akanno *et al., 2007)*

Materials and Methods

Experimental Site:

The experiment was conducted at the Poultry Unit of Teaching and Research Farm, Department of Animal Health and Husbandry, Audu Bako College of Agriculture, Dambatta, Kano State. The College is located between latitude 12o 20.260´ North-East of the equator and longitude 8o 31.567´ East of the Greenwich Meridian (Ahmed, 2014). The College possesses s tropical climate with annual rainfall in Dambatta was 600mm which lasts for four months (between May and September) and the mean annual temperature is 38oC with highest temperature occurring in April (41^0C) and lowest in January (30^0C) (Abdulrashid *et al.,* 2012). The relative humidity ranged from 22 to 52% as recorded by (KNARDA, 2011).

Experimental Birds, Design and Management

A total of 315 day old broiler chicks comprising 105 each of NAPRI-X, Marshal and Ross strains replicated 7 times with 15 birds per pen in a Randomized Completely Design (CRD) were used in this study. The experiment lasted 8 weeks. Each strain was identified by wing tag, randomized and allotted to pens in a brooder house with floor covered using wood shavings which was kept dry throughout the experimental period. The birds were brooded with the aid of kerosene stoves and charcoal as heat source and reared on deep litter from day old to 8 weeks of age. All the chicks were fed *ad libitum* with a broiler starter feed containing 23.75% Crude Protein (CP) and 3,038.64 MEKcal/kg up to 8 weeks of age in accordance with NRC (1994) nutrient standard for broiler birds. Fresh, cool drinking water was also given *ad libitum.* Vaccination and other routine medication were carried out as at and when due.

Data Collection:

During the period of 8 weeks, records were kept on Body weight (BDW), this was measured on weekly basis using digital electronic weighing balance of 3,000g capacity. Feed intake was also recorded on daily basis in grams. Feed conversion ratio was calculated as feed intake divided by weight gain and mortality was evaluated as the number of birds that died divided by total number of birds started with at the initial stage of the experiment multiplied by 100. The linear body measurements Neck Length (NEL), Back Length (BKL), Thigh Length (THL), Shank Length (SHL), Breast Width (BRW), Body Length (BDL), and Wing Length (WNL) were

measured on weekly basis using a tape rule in centimetres.

Data analysis

The data collected on each strain were subjected to General Linear Model (GLM) procedure of Analysis of Variance (ANOVA) in a Completely Randomized Design using Statistical Package for Social Science SPSS (2011) version 20. Significant differences among means were separated using Duncan's Multiple Range Test procedure (Duncan, 1955). The following statistical model was used in the analysis:

$Yij = \mu + Ci + eij$

Where,

Yij = Observation (Body Weight, Feed Intake, Feed Conversion Ratio and Mortality) made on the jth individual belonging to the ith strain of broilers.

μ = Overall estimation of the population mean

Ci = Effect of ith strain of broiler (i=1, 2 and 3)

eij= Random error associated with each measurement

Multiple regression models were fitted to determine prediction equations for the three strains of broilers at 8 weeks of age.

Results and Discussion

Table 1 presents mean performance values for the three strains of broiler chicken at 8 weeks of age. Significant differences ($P<0.001$) was observed among the strains for growth performance. Marshal strain had the highest mean values for Initial Body Weight (IBW), Final Body Weight (FBW), Daily Weight Gain (DWG), Total Weight Gain (TWG), Daily Feed Intake (DFI) and Total Feed Intake (TFI). However, no significant ($P>0.05$) differences were observed between strains in FCR and Mortality (MTLTY). The findings revealed that Marshal Strain was superior when compared with NAPRI-X and Ross strains. The significant ($P<0.01$) higher values for body weights (initial and final) for Marshal over NAPRI-X and Ross strains were not in agreement with the findings of Amao *et al.* (2011) who reported that body weight of Ross showed significantly ($P<0.05$) higher values than Anak and Marshal strains. These differences in body weights and other growth traits in this experiment could be attributed to the genetic merit of Marshal over other strains in the semi-arid zone of Nigeria. Moreover, daily weight gain, total weight gain, daily feed intake and total feed intake were significantly ($P<0.05$) different among the three strains. The ability with which Marshal strain consumed higher feed at this phase to put higher body weight and other growth traits could be attributed to its higher genetic make-up over other strains in the semi-arid zone of Nigeria. The findings are inconsistent with the results of Amao *et al.* (2011) who revealed that feed intake and weight gain were significantly ($P<0.05$) higher in Ross strain than Anak and Marshal strains in the derived savannah environment of Nigeria. However, Marshal strain has the least FCR than NAPR-X and Ross strains, respectively. This indicates better utilization of feed by Marshal as they had the least FCR, since the lower the feed conversion ratio (FCR) the better the diet. This was in line with the report of Amao *et al.* (2009). Doma, (1998) reported that the lower the FCR the better the diet in monogastric animals. In the present study, better FCR was recorded for Marshal Strain while Amao *et al.* (2011) recorded better FCR in Ross strain. These differences could be due to differences genetics and or environmental factors. Regression equation relating body weight to body linear measurements with their accuracy of prediction (R2) values for the three strains of broilers are shown in table 2. It was observed that the regression analysis were highly significant ($p<0.001$) in all the groups and the coefficient of determination (R2) values more than 50% in all the strains. This suggests that the body traits are very good predictors for body weight in broilers. Therefore, in rural areas where scale is not available, any of these body measurements could be used to predict body weight of broiler chicken. A similar observation was reported by Akanno, *et al.* (2007).

Table 1: Comparative growth performance for the three broiler strains at 8 weeks of age

Traits	Treatments NAPRI-X	Marshal	Ross	SEM	LOS
Initial Body Weight (g/bird)	446.28b	548.01a		472.13b	12.26 ***
Final Body Weight (g/bird)	1530.58b	1870.69a	1588.69b	45.87	**
Daily Weight Gain (g/bird)	38.72b	47.24a	39.88b	1.25	**
Total Weight Gain (g/bird)	1084.29b	1322.69a	1116.56b	35.07	**
Daily Feed Intake (g/bird)	109.96c	125.85a	116.86b	1.91	***
Total Feed Intake (g/bird)	3078.92b	3523.67a	3229.18b	52.35	***
Feed Conversion Ratio	2.89	2.67	3.01	0.08	NS
Mortality (%)	3.00	2.57	2.86	0.24	NS

(g) = grams, LOS = Level of significance, SEM = Standard error of mean, *** = very highly significant (P = < 0.001), ** = highly significant (P = < 0.01), NS = Not significant.

Table 2: Regression equation relating body weight (g) and linear body measurements (cm) at 8 weeks of age for three strains of broiler chicken

Strain type	Prediction equations	R^2	S.E.	Sig
NAPRI-X	BW = - 676.68 + 82.96NEL – 27.71BKL -12.30THL + 33.49SHL + 15.77BRW + 18.46BDL + 28. 00WNL	98.0	91.28	***
Marshal	BW= -980.00+ 69.62NEL – 47.00BKL + 39.13THL + 24.42SHL – 43.67BRW + 125.23BDL -19.06WNL	97.6	81.53	***
Ross	BW= -1089.94+ 16.67NEL + 56.44BKL + 26.76THL + 47.54SHL – 6.23BRW + 50.92BDL – 42.45WNL	93.4	117.98	***

NAPRI-X = A synthetic broiler breed of National Animal Production Research Institute, BW = Body weight, NEL = Neck length, BKL = Back length, THL = Thigh length, SHL = Shank length, BRW = Breast width, BDL =Body length, WNL =Wing length, R2 = Coefficient of determination, S.E. = Standard error, *** = very highly significant (p<0.001), (g) = gram and (cm) = centimetre.

Conclusion and Recommendations

It was concluded that Marshal was significantly ($p<0.001$) superior to Ross and NAPRI-X broiler strains in all the growth traits and also has the least FCR than NAPR-X and Ross strains, respectively. This indicates better utilization of feed by Marshal as they had the least FCR, since the lower the feed conversion ratio (FCR) the better the diet; even though there is no significant difference with respect to FCR and mortality. The results of Multiple Regression Analysis showed that it was possible to predict body weight of broiler using linear body measurements. It is recommended that traits that were significantly correlated (IBW, FBW, DWG, TWG, DFI, TFI, FCR, NEL, BKL, THL, BDL, SHL and WNL) should be given priority in selection due to their high tendency of influencing body weight of the strain. It is also recommended that broiler farmers in this part of the country should concentrate on Marshal Strain due to its fast growth rate and low feed intake for profitable venture.

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ABG 014

RELATIONSHIP OF BODY WEIGHT AND LINEAR BODY MEASUREMENTS IN RED SOKOTO BUCKS AND DOES REARED IN SEMI-ARID REGION OF NIGERIA

[1]Ahmed, I.A., [3]Kabir, M., [2]Adeyinka, I.A., [3]Musa, R.A., [4]Idris, D.Y., [1]Galadima, N.M.

[1]Department of Animal Production Technology, Audu Bako College of Agriculture, Dambatta, P.M.B. 3159, Kano State, Nigeria
[3]Department of Animal Science, Ahmadu Bello University, Samaru, Zaria
[2]National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria
[3]Department of Remedial and General Studies, Audu Bako College of Agriculture, Dambatta, P.M.B. 3159, Kano State, Nigeria
[4]Department of Animal Production and Health Technology, Federal College of Agricultural Produce Technology, Kano, P.M.B. 3032, Kano State, Nigeria
*Corresponding author: iliyasunadada@gmail.com +2348065532319

Abstract

The study examines body linear measurements of Red Sokoto Goats (Bucks and Does). The study was carried out in a weekly Market at Dambatta local Government Area of Kano State, Nigeria. Data were collected on 200 goats of averagely the same age group (1-2 years). Data were collected for body weight in kilograms (Kg) and linear body measurements in centimeters (cm) comprising of Top Line (TOP), Hight at Withers (HTW), Heart Girth (HG), Face Length (FCL), Head Girth (HEG), Body Weight (BDW) and Stomach Girth (STG). Data were analyzed using an independent t-test for the difference in body weight and linear body measurements between bucks and does. The results showed that a significant difference ($p<0.01 – 0.05$) was found in TOP (t (198) = -6.04, p= .000), HG (t (198) = -2.96, p= .003), BDW (t (198) = -2.52, p= .012) and STG (t (198) = -4.46, p= .000) respectively, indicating that the linear body measurements vary between males and females Red Sokoto Goats. However, descriptive statistics also showed that Does were higher in traits like TOP (m = 79.93 and 71.42; SD = 9.49 and 8.98), HG (m = 62.30 and 58.21; SD = 8.67 and 9.99); BDW (m = 19.66 and 17.31; SD = 5.41 and 7.57) and STG (m = 74.58 and 68.50; SD = 8.98 and 9.10). However, no significant difference was found in HTW (t (198) = -1.89, p= .060), FCL (t (198) = .017, p= .986) and HEG (t (198) = -876, p= .382) in the two sexes. Descriptive statistics were reported as indicating no significant difference between males and females Red Sokoto Goats in HTW traits (m = 51.55 and 53.12; SD = 5.12 and 5.67); FCL (m = 19.86 and 19.84; SD = 6.55 and 6.66) and HEG (m = 27.64 and 28.96; SD = 10.94 and 9.66). It was concluded that female Red Sokoto Goat has better body linear measurements than the males. It is therefore recommended that the information on differences between body weight and linear body measurements of goats can be used for estimation of size and shape of goats suitable for breeding and fattening.

Keywords: *Red Sokoto Goats, Bucks, Does, Body weight, Linear Body Measurements.*

Introduction

Nigeria is blessed with a number of Indigenous breeds of goats, which comprised of West African Dwarf (WAD) goat, Sahel/desert goat-(West African Long-Legged) and Sokoto Red/Maradi (Aina and Oppong, 2011). The distribution of goat breeds in the country showed that the West African Dwarf (WAD) goat is common to Southern Nigeria while the Sahel or desert goat and Sokoto Red are common to the Northern region of the country (Febusoro, 2006). Characterization of goats breeds were made basically on their origin, body size, body length and other linear body measurements (Umar *et al.,* 2018). Nigerian goats are classified into large, medium and small breeds respectively based on height at withers (Davendra and Mcleroy, 1982; RIM, 1992). Goat is a multi functional animal and plays a significant role in the economy and nutrition of landless, small and marginal farmers in the country (Fida, *et al.,* 2006). The authors further reported that Goat rearing is an enterprise which has been practiced by a large section of population in rural areas. Goats can efficiently survive on available shrubs and trees in adverse harsh environment in low fertile lands where no other crops can be grown. They contribute to livestock industry in terms of milk, meat, skin and hair. The relationship between linear body measurements, such as body length, heart girth, and height at withers, and body weight is found to be positive in goats and could be used as selection criteria for the goats (Khan *et al.,* 2006). The information on the relationship between body weight and linear body measurements of goats is important for the estimation of size and shape of goats suitable for breeding and slaughter (Nor, *et al.,* 2011). The objectives of this study was to determine the relationship of body weight and linear body measurements of Male and female Red Sokoto goats in the semi-Arid region of Nigeria.

Materials and Methods

Experimental Animals

The data used for this study were collected on 65 male (Bucks) and 135 female (Does) Red Sokoto goats aged 1-2 years at Dambatta weekly market Kano State, Nigeria. The animals were brought to market for sale by individuals; permission was obtained for data collection through the traditional head of the market (*Sarkin Kasuwa*). The ages of the animals were determined using rostral dentition. Weights of the animals were taken using hanging scale in grams while linear body measurements were obtained using tailor's tape in centimeters.

Body measurements

Body weight and linear body measurements were taken in male and female Red Sokoto goats to determine differences in body conformation. Body weight (BDW) and linear body measurements were determined using hanging weighing scale (100kg) and tailor's tape respectively throughout the market day. Top line (TOP) that is Body length was measured as the distance from the point of the shoulder (dorsal spine of scapular) to the posterior edge of the pin bones (tuber Ischia) using measuring tape; Height at withers (HAW) was recorded from the bottom of the front foot (phalanges) to the highest point of withers between the shoulders by using one meter ruler. Heart girth (HG) or circumference of chest was measured as the circumference of the body immediately behind the shoulder blades in a vertical plane perpendicular to the long axis of the body and measured by using a girth tape in centimeter; Head Girth (HEG) was measured by taking measurement of the circumference of the head. Face Length (FL) was taken from occipital protuberance to the mouth. These measurements were taken in centimeters; each goat was adequately restrained in the natural position as much as possible. The above measurements were taken on each animal as described by Brown *et al.,* (1983).

Statistical Analysis

The collected data were subjected to descriptive statistics that is Percentage, Means and Standard deviation using Statistical Package for Social Science SPSS (2011) version 20.

Results and Discussion

An independent t-test was computed for the difference in linear body measurements: TOP, HTW, HG, FCL, HEG, BDW and STG between male and female Red Sokoto goats. The results show that a significant difference was found in TOP (t (198) = -6.04, p= .000), HG (t (198) = -2.96, p= .003), BDW (t (198) = -2.52, p= .012) and STG (t (198) = -4.46, p= .000) respectively, indicating that the traits vary between male and female Red Sokoto Goats. However, descriptive statistics show that female Red Sokoto goats are higher in TOP (Body length) (m = 79.93 and 71.42; SD = 9.49 and 8.98), HG (m = 62.30 and 58.21; SD = 8.67 and 9.99); BDW (m = 19.66 and 17.31; SD = 5.41 and 7.57) in kg and STG (m = 74.58 and 68.50; SD = 8.98 and 9.10) in cm than its males counterpart. However, no significant difference was found in HTW (t (198) = -1.89, p= .060), FCL (t (198) = .017, p= .986) and HEG (t (198) = -876, p= .382). Descriptive statistics are reported as follows indicating no significant difference between male and female Red Sokoto Goats in HTW traits (m = 51.55 and 53.12; SD = 5.12 and 5.67); FCL (m = 19.86 and 19.84; SD = 6.55 and 6.66) and HEG (m = 27.64 and 28.96; SD = 10.94 and 9.66).

The results of this experiment show significant difference in near body measurements between male and female Red Sokoto goats. This agreed with the report of Fida *et al.,* 2006 who reported that significant difference existed between male and female goats with respect to body weight and linear body measurements. However, descriptive statistics show that female Red Sokoto goats are higher in TOP (Body length) (m = 79.93 and 71.42; SD = 9.49 and 8.98), HG (m = 62.30 and 58.21; SD = 8.67 and 9.99); BDW (m = 19.66 and 17.31; SD = 5.41 and 7.57) in kg and STG (m = 74.58 and 68.50; SD = 8.98 and 9.10) in cm than its males counterpart. This report was not in agreement with the work of Fida *et al.,* (2006) who reported that males were higher in mean body weight (BDW) (18.69±1.81, 14.50±1.19) in kg, Body length (BDL) (59.60±0.74, 58.70±0.84), Height at withers (HTW) (68.25±1.42, 66.50±1.18) and Heart girth (HG) (59.10±0.86, 57.60±0.95) in cm respectively. The variations between male and female Red Sokoto goats in these traits could be due to environment, breed differences and factors affecting these measurements which are known to be sex, nutrition, type of birth, and environment (Hassan and Ciroma, 1990).

Mean, SD, t and p Value for Body Linear Measurements in Red Sokoto Goat.

Conclusion and Recommendations

Based on the findings of the study, it was concluded that female Red Sokoto goats were significantly vary from male counterpart in body measurements ($p<0.01 – 0.05$) in Semi-Arid region of Nigeria. This indicates that female goats have better performance and conformation traits than its counterparts in the experimental site.

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Mean, SD, t and p Value for Body Linear Measurements in Red Sokoto Goat

Traits	Sex	N	Mean	Std. Dev.	t	p
TOP	Male	65	71.4154	8.98417	-6.04	.000
	Female	135	79.9259	9.48654		
HTW	Male	65	51.5538	5.12357	-1.89	.060
	Female	135	53.1259	5.67454		
HG	Male	65	58.2154	9.99608	-2.96	.003
	Female	135	62.3037	8.67427		
FCL	Male	65	19.8615	6.55476	.017	.986
	Female	135	19.8444	6.66565		
HEG	Male	65	27.6462	10.54519	-.876	.382
	Female	135	28.9630	9.66098		
BDW	Male	65	17.3077	7.57241	-2.52	.012
	Female	135	19.6667	5.41694		
STG	Male	65	68.5077	9.10893	-4.46	.000
	Female	135	74.5852	8.98040		

SD = Standard deviation, P <0.05, N = number of observation, Std. Dev. = Standard deviation, TOP = Top line, HTW = Height at withers, Head girth, FCL = Face length, HEG = Head girth, BDW = Body weight and STG = Stomach girth.

ABG 015

PHYLOGENETIC STUDIES OF SOME CATFISH SPECIES FROM RIVER GALMA, ZARIA, KADUNA STATE, NIGERIA

[1]Yashim, E.Y*, [1]Aken'Ova T. O. L., [2]Auta, J., [3]Adeyinka, I. A., .[4]Atawodi, S. E., [4]Fodeke, H. and [1]Oniye, S.J.

[1] Department of Zoology, Ahmadu Bello University, Zaria
[2] Department of Biology, Ahmadu Bello University, Zaria
[3]National Animal Production Research Institute, Ahmadu Bello University, Zaria
[4] Department of Biochemisty, Ahmadu Bello University.
Corresponding Author: nmana2007@yahoo.com

ABSTRACT

The findings on phylogenetic analyses of twelve species of catfish from River Galma, Zaria, belonging to six families: Clariide, Schilbeidae, Mochokidae, Bagridae, Claroteidae and Malapteruridae are presented in this report. Following DNA extraction and amplification of cytochrome b gene L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' by Polymerase Chain Reaction (Applied biosystems GeneAmp PCR system 2700) from fish tissues in appropriate reaction mix and conditions with the manufacturer's guideline (BIONEER Corporation, Daejeon, South Korea) adhered too, the target Cytochrome b gene (585bp) was amplified in all 12 catfish species and phylogeny revealed two monophyletic groups with six species each in clusters. Clarias gariepinus, C. anguillaris, C galmaensis, Heterobranchus bidorsalis B. bajad, Malapterurus electricus and Synodontis melanoptera, S. schall in one group while Schilbe isidori, H. longifilis, B. docmac and A. occidentalis clustered together, suggesting a close relationship within members of each cluster. It is recommended that these species can be utilized in assessing the relationship of catfishes in taxonomic studies alongside their morphometrics.

Keywords: *Phylogeny Catfish Cytochrome b Gene*

Introduction

Catfishes (Order Siluriformes) are a diverse group of fish representing more than 3,000 species, 478 genera and 36 families (Ferraris and de Pinna, 1999; Nelson, 2006). Catfish species are of growing interest to the processing industry and consumers because of their biological suitability (hardiness) and palatability. However, available data on the nutritional qualities of these species are insufficient to comprehensively evaluate their nutritional significance (Usydus *et al.*, 2011).

Molecular species identification techniques hold the potential for rapid and accurate assessment of proper labelling (Wong *et al.*, 2011). Existing phylogenetic hypotheses on the intra-relationships of catfishes are not completely resolved and based almost entirely on morphological data. Constructing phylogenomic data sets for large numbers of taxa is quite challenging. Most attempts to use this approach have been based either on few available complete sequence data for relatively few taxa (Rokas *et al.*, 2003). In addition, the discovery of the phylogenetic relationships among family-level and higher catfish groups are essential for completing the taxonomy and a predictive classification of siluriforms. Due to worldwide distribution and diversity of freshwater catfishes, they are of great interest to ecologists and evolutionary biologists. Siluriforms are key subjects in biogeography on all scales from regional to global view (Lundberg *et al.*, 2000). It is also necessary to gather and document data on the composition of fish in order to make the best use of it as food source (Massresha *et al.*, 2017).

Mitochondrial gene cytochrome *b* (cyt *b*) has been used widely to infer phylogenetic relationships within and among taxonomic categories of fishes ranging from populations to classes (Briolay *et al*., 1998; Reed *et al*., 2002)

Materials and Methods

Species of catfish belonging to six families were obtained from River Galma, Zaria, Kaduna State. River Galma (Latitude 10° 27'N, 11° 24'N; Longitude 7°23'E and 8° 45'E) is one of the main tributaries of River Kaduna, it has its headwaters near the north western edge of the Jos Plateau and falls near the Magami village into Kaduna plains. The main tributaries of River Galma are Shika River in the middle course and the Rivers Kinkiba and Likarbu in its lower course. The Galma reservoir popularly called Zaria dam was constructed across the river in 1975 (Samuel *et al*., 2015).

Five to ten (5-10) fish samples of each of the twelve (12) species, of the six (6) catfish families were bought live from fishermen at the fish landing points along River Galma and conveyed in hard plastic containers containing water to the laboratory. The fish were identified using taxonomic guides, descriptions and publications of Teugels (1986); Idodo-Umeh (2003); Aken'Ova (2007); Olaosebikan and Raji (2013), Lewis (1974) and Froese and Pauly (2017). Fish samples were properly washed with running tap water and labelled for analyses.

Fish families and representative species used for the study were:

Clariidae:

Clarias species: *Clarias gariepinus* (Bürchell, 1822), *Clarias anguillaris* (Linnaeus, 1758) *Clarias galmaensis* Aken'Ova, 2007

Heterobranchus species: *Heterobranchus bidorsalis* Geoffroy Saint-Hilaire, 1809 *Heterobranchus longifilis* Valenciennes, 1840

Bagridae: *Bagrus bajad* (Forskall, 1775), *B. docmac* (Forskall, 1775)

Mochokidae: *Synodontis schall* Bloch & Schneider, 1801, *S. melanoptera* Boulenger, 1902

Malapteruridae: *Malapterurus electricus* Gmelin, 1789

Schilbeidae: *Schilbe isidori* Valenciennes, 1840

Claroteidae: *Auchenoglanis occidentalis* Valenciennes, 1840

Muscles of each species were cut, preserved in labeled sample bags for vitamin and molecular analysis

Molecular Analysis

The molecular analysis was achieved through a DNA extraction and amplification of cytochrome b gene conducted by Polymerase Chain Reaction.

DNA extraction

AccuPrep®Genomic DNA extraction kit (K-3032) for the extraction of DNA from animal tissue was used (BIONEER Corporation, Daejeon, South Korea) following the manufacturers guideline.

Procedure

Fish samples (muscle and fin) were homogenized (25-50mg) with a mortar and pestle, and placed in a clean 1.5ml tube and 200 µl of TL buffer added; 20 µl of Proteinase K and 10 µl RNase A were added and the mixture by vortexed. The mixture was incubated at 60°C for 1hour until the the tissue is completely lysed. 200 µl of GB buffer was added and vortexed. The mixture was incubated at 60°C for 10 min (using a Thermolyne Type 17600 Dribath). 400 µl of absolute ethanol was added and mixed by pipetting. The lysate was carefully transferred into the upper reservoir of binding column tube (fitted in a collection tube) without wetting the rim. The tube was closed and centrifuged at 8,000rpm for 1min (using an Eppendorf centrifuge 5415C). The solution was discarded from the collection tube and the collection tube was reused. 500 µl of WA1 buffer was added without wetting the rim, the tube was closed and centrifuged for 1 min. The solution from the collection tube was discarded and the tube was reused. 500 µl of W2 buffer was added without wetting the rim, closed and centrifuged at 8,000 rpm for 1min. The solution from the collection tube was discarded and the tube was reused. The tube was centrifuged once more at 13,000 rpm for 1 min to completely remove the ethanol and checked that there is no droplet clinging to the bottom of the binding tube. The binding column tube was transferred to a new 1.5 ml tube for elution, 50 µl of EA buffer was added onto binding column tube kept for

atleast 1 min at room temperature (15-25°C), then centrifuge at 8,000 rpm for 1 min to elute.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) was acheived using an Applied biosystems GeneAmp PCR system (2700). The PCR was performed in a total volume of 20µl (Table 3.1) using a Hotstart premix (AccuPower® Dual-HotStart™ RT-PCR PreMix, BIONEER Corporation, Daejeon, South Korea). Cytochrome *b* gene (585 bp) was amplified using primers L15267, 5'-AAT GAC TTG AAG AAC CAC CGT-3' and H15891, 5'-GTT TGA TCC CGT TTC GTG TA-3' (Briolay *et al*., 1998; Nwafili and Gao, 2007) for 7 min at 72°C. The PCR cycling conditions were 5 min initial denaturation at 94°C and 35 cycles of 30s at 94°C for denaturation, 30s at 56°C for annealing, 30s at 72°C for extension, and a final extension at 72°C for 7 min. Amplicons were loaded on 0.8-1.5% agarose gel and photographed under UV light. Gels were assigned binary scores; DARWin (6.0.01) software was employed for the phylogenetic analysis.

Phylogenetic Analyses

The PCR amplification of cytochrome b gene was positive in all the fish species (Plate 1). All 1).

samples showed the amplicons at the same level revealing the close relationship between them as cytochrome b gene, which is a marker for inferring phylogeneny which showed positive amplification as species similarity and close relationship is infered. The phylogentic tree also divided the species into two clades with six species clustering together. *Clarias gariepinus*, *C. anguillaris*, *C galmaensis* clustered together alongside *H. bidorsalis* and *B. bajad*, *M. electricus* have a closer relationship these species with neighbor joining in the dendogram, much more in a closer relationship with *Clarias* species. On the other had *Synodontis melanoptera*, *S. schall* and *Schilbe isidori* clustered closely together with *H. longifilis* and *B. docmac* similarly closely connected alongside *Auchenoglanis occidentalis*. Similarities index of 0.5 (95%) shows a high silimarity between the fish species following the transforming multiallelic banding patterns at each locus into the corresponding homozygous or heterozygous states, a simple mismatch coefficient that most suitablely measure dissimilarity between banding patterns of closely related haploid forms (Figure

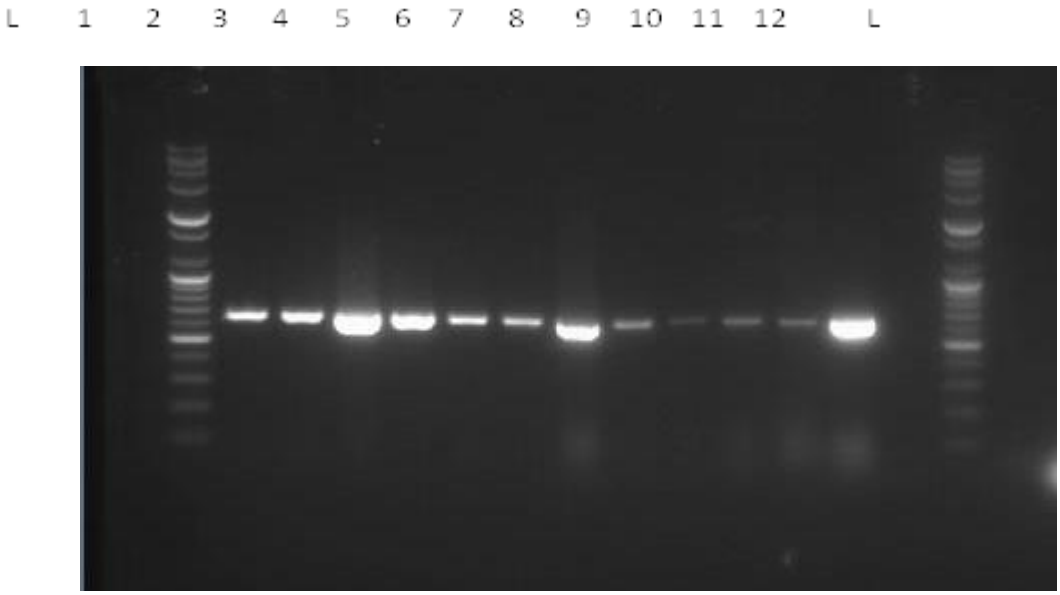

Plate I: Polymerase Chain Reaction (PCR) of Cytochrome b gene isolated from the catfish species
Key: 1 = *Clarias gariepinus*, 2 = *C. anguillaris*, 3 = *C galmaensis*, 4 = *Heterobranchus bidorsalis*, 5 = *H. longifilis*, 6 = *Bagrus docmac*, 7 = *B. bajad*, 8 = *Synodontis melanoptera*, 9 = *S. schall*, 10 = *Schilbe isidori*, 11 = *Auchenoglanis occidentalis*, 12 = *Malapterurus electricus*; Ladder=585bp

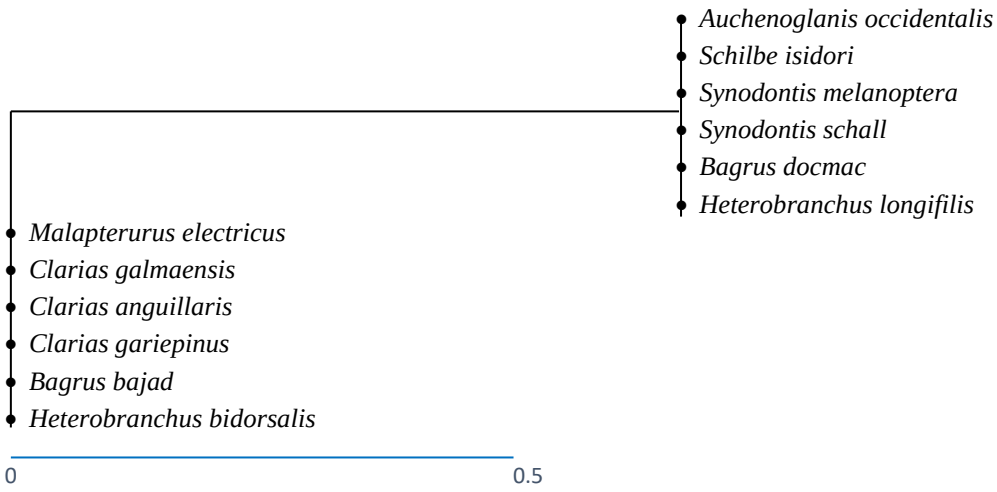

Figure 1: Dendrogram of the catfish species investigated

Discusion

The present study shows that the amplicons at the same level, though at different intensity, suggest the close relationship between the species. Mitochondrial gene cytochrome *b* (cyt *b*) has been used widely to infer phylogenetic relationships within and among taxonomic categories of fishes ranging from populations to classes (Briolay *et al*., 1998; Reed *et al*., 2002). The dendogram divided the species in two groups with six species clustering points at the similarity index coefficient. The lowest level of genetic variability in this study validates the results of the morphological data. Vigliotta (2008) showed that *Chiloglanis* is possibly a paraphyletic assemblage, while *Synodontis* must include *S. membranous* and *S. batensoda* (formerly placed in *Hemisynodontis* and *Brachysynodontis* respectively) clarified the phylogenetic relationship among the mochokid genera and that the reciprocal monophyly of *Atopochilus* and *Euchilichthys* are questionable at best; all remaining genera are maintained as valid and monophyletic, reflecting their fresh water origin. Therefore, in this study *Clarias gariepinus*, *C. anguillaris*, *C galmaensis* clustered together alongside *Heterobranchus bidorsalis* and *B. bajad*, *Malapterurus electricus* are considered to have a closer relationship with these species, more so with *Clarias* species. The relationship between *Clarias galmaensis*, *C. gariepinus* and *C. anguillaris* is very close, genetically, corroborating monophyly and validating of their placement in the genera *Clarias*. Howes (1985) suggested a sister group relationship between the Malapteruridae and Schilbeidae using the activating gene sequences, *rag1* and *rag2* assembled. This is slightly different from this study where the electric catfish *Malapterurus elctricus* is oberved to be closely related to *Clarias* species, due to difference in targeted gene. A sample of African *Clarias* catfishes from the Senegal River was studied using morphometry, allozyme variation, microsatellites and RFLPs of mitochondrial DNA whose report confirms the close relationship between C. *gariepinus* and *C. anguillaris* (Agnese *et al*., 2005). A combination of morphological and molecular analysis gives a clearer picture of the taxonomy of the species. In this study, *Synodontis melanoptera*, *S. schall* and *Schilbe isidori* clustered closely with *H. longifilis* and *Bagrus docmac* similarly connected with neighbour *Auchenoglanis occidentalis* Claroteidae that is split into its monophyletic subfamilies, Claroteinae and Auchenoglanidinae by insertion of African schilbids as sister to claroteines (Sullivan *et al*., 2006) was also observed in this study. Of the 35 nominal living catfish families in this study 31 are represented by two or more species and of these, 25 families are supported as monophyletic; the monophyly of these species implies a common freshwater origin, shared histories or similar ecological conditions. Discrepancies between molecular and morphological data do exist in some genera, for example the genus *Cathorops* this could imply a morphological convergence which explains a tendency of unrelated animals to evolve superficially similar characteristics under similar environmental conditions (Tenorio-Colin *et al*., 2010).

Conclusions

Catfishes from River Galma are seperated into two clades with six species each: *Clarias gariepinus*, *C. anguillaris*, *C galmaensis*, *Heterobranchus bidorsalis* *B. bajad*, *Malapterurus electricus* and *Synodontis melanoptera*, *S. schall* in one group and *Schilbe isidori*, *H. longifilis*, *B. docmac* and *A. occidentalis* clustering together, suggesting a close relationship within members of each group.

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ABG 016

SEXUAL DIMORPHISM OF MORPHOSTRUCTURAL TRAITS IN INDIGENOUS AND EXOTIC BOER GOATS USING MULTIVARIATE ANALYSIS IN SEMI-ARID, NIGERIA

Muhammad, H. A[1*]., Umar, Z.H[1]. and Umar, U. A[2].

[1*]Department of Animal Science, Faculty of Agriculture, Bayero University, Kano, P.M.B 3011 Kano, Nigeria
[2]National Animal Production and Research Institute (NAPRI), Zaria, Kaduna State.
Corresponding Author: hamuhammad.asc@buk.edu.ng; **+2348066241225**

ABSTRACT

The aim of the study is to determine the variation between morphometric traits among indigenous and exotic Boer goats in semi-arid, Nigeria. A total of 100 goats consisting of Kano Brown, Sahel and Boer goats from Bayero University Teaching and Research Farm, Kano State, Gujungu market, Jigawa State and Bellmari farm, Kano State, respectively were used for the study. The animals were within 5 years of age determined through dentition. Live body weight (kg) of animals was taken using weighing scale and morphometric traits were measured with measuring tape in cm. The parameters measured include body weight (BW), body length (BL), heart girth (HG), neck length (NL), cannon length (CL), head length (HL), head circumference (HC), ear length (EL), hip height (HH), height at wither (HW), udder length (UL), udder width (UW), udder circumference (UC), gonadal length (GL), gonadal width (GW) and gonadal circumference (GC). Data were analyzed using JMP pro 14.0 (SAS, 2018). The result of standardized Canonical coefficient of the discriminant analysis among male goats shows HG, NL, HC, GL and HH contributed to first canonical variable while BW, BL, HG and HH contributed to the second canonical variable. In females, the Standardized Canonical coefficient shows that BW, BL, EL and HC contributed to first canonical variables, BL, NL and EL contributed to the second canonical variable while BW and BL contributed to third canonical variables. Multivariate statistics for the differences between males and females of all breeds shows highly significant difference (P<0.001) in the four multivariate tests (Wilks' Lambda, Pillai' Trace, Hotelling-Lawley Trace and Roy' Max Root). In conclusion, this study insight that Boer goats are superior to other indigenous goat breeds in many of the quantitative traits, thus, Boer male goats can be selected using BW, BL, HG and EL traits and Boer female can be selected using BW, BL, NL and HC traits for the improvement of indigenous breeds. Further studies at molecular level with large data set is therefore recommended to ascertain the findings.

Keywords: *Boer, canonical, indigenous, multivariate, morphometric*

Introduction

Goats (*Capra hircus)* are animals belonging to the family Bovidae, Order *(Ruminantia)*, hollow-horned ruminant mammals. They are believed to first appear in Miocene approximately 20 Million years ago and represent the most diverse group of living ungulates, constituting more than 300 fossil taxa and approximately 140 living species, including sheep, cattle, antelopes and gazelles (Hassanin & Douzery, 1999; Fernandze & Vrba, 2005).

Morphometric or linear body measurement is the quantitative analysis of form, a concept that encompasses size and shape. Morphological measurements are very important method used to evaluate and assess the characteristics of various breeds of animals. These measurements can help to provide the basic information on suitability of

the animals towards their selection (Nesamvuni, Mulaudzi, Ramanyimi, and Taylor, 2000; Mwacharo, Okeyo and Kamande, 2006; Martins *et al.*, 2009 & Yakubu, 2010). Measurements of phenotypic traits can be used as selection tool for growth. It also enables the breeder to recognize early maturing animals of

different size (Nesamvuni *et al.*, 2000). Morphometric characteristics are essential in breed identification and classification. Gizaw, Van Arendonk, Komen, Windig, and Hanotte (2007) stated that morphostructural description of traits remained essential component of characterization that can be used to physically identify, describe and recognize a breed. It also helps in the classification of livestock breeds. The knowledge of morphometric traits aids in appropriate breeding design, feeding and health management (Thiruvenkadan, 2005).

Materials and Methods

Experimental Location

The study was conducted at Small Ruminant Unit of Teaching and Research farm, Bayero University, Bellmari Farm at Bebeji Local Government in Kano State and Gujungu market at Taura Local Government of Jigawa State. A total of 100 goats comprising of three breeds of goats were randomly selected and used for this study. The breeds include; twelve (12) Kano Brown (KB), thirty (30) Sahel (S), fifty (50) exotic Boer (B) and eight (8) crosses between the indigenous breeds. The animals were within 5years of age.

The Teaching and Research farm of Bayero University, Kano lies between 11°59.022' N and 008° 25.415'. E (GPS coordinates). It is located at Ungogo Local government area of Kano State which falls in the semi-arid zone of Nigeria at an altitude of 460m above sea level (Olofin, 1987). The animals are managed under semi-intensive system of management whereby they are allowed to graze in the morning and provided with supplementary feed in the afternoon. There are three breeds of goats on the farm which include Sahel, Kano Brown, West African Dwarf (WAD) goats and with their crosses.

Bellmari Farm is located at Bebeji Local Government Area of Kano State. Bebeji Local Government is located 45 km Southwest of the State, it lies between 11°40'3.65'N and 8°15'43.20 E. The farm has four sections; agronomy, fish farm complex, maintenance and

livestock sections. In the livestock sections, goats are the main small ruminant reared, and Boer goat is the only breed reared. Semi-intensive system of management is practiced in the farm.

Gujungu market is a weekly market that takes places every Sunday, it is located at Taura Local Government with GPS coordinates of 12^{0}21.811'N 009^{0}34.788 E. Animals from different areas surrounding the States are brought to the market for sale. The local government area is bordered by Ringim local government to the West, to the North by Garki and Gagarawa local governments, to east by Kaugama while to South by Miga and Jahun local governments.

Data Collection

Live body weight (BW) of animals was measured using weighing scale in Kg while morphometric traits were measured using measuring tape in cm after physically restraining the animals as previously described for goats by Hassan and Ciroma (1992). The traits measured include; body length (BL), heart girth (HG), height at wither (HW), ear length (EL), neck length (NL), cannon length (CL), head length (HL), hip height (HH), head circumference (HC), udder length (UL), udder width (UW), udder circumference (UC), gonadal length (GL), gonadal width (GW), gonadal circumference (GC) and tail length (TL)

Statistical Analysis.

Data was recorded in Excel sheets and multivariate analysis of discriminant canonical was used to analyze the data using JMP pro 14.0 Statistical Analysis System (SAS, 2018). Canonical discriminant analysis is a multivariate technique used to identify the combination of variables that clearly separate the genetic groups. This analysis is a multivariate technique that describes the relationship between two variable sets by calculating the linear combinations that are

maximally correlated (Tabachnick & Fidell, 2001). Variables that best fitted the model were selected using C(p)

statistic, Alkaike's Information Criteria (AIC), Bayesian Information Criteria (BIC), R^2 (R-square) and MSE (Mean square of error).

Results and Discussion

Table 1 and 2 showed the multivariate statistics of male and female goats respectively. Result of the multivariate statistics showed highly significant ($P<0.001$) in all the four multivariate tests (Wilks' Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Max Root). This is the same with the findings of Netsanet Tadelle and Kefelegn (2017) which shows that there is a highly significant difference in the four multivariate test for Central Highland and Woyto-Guji goats breeds of Ethiopia. Yunusa, Salako and Oladejo (2013) also reported the same finding for indigenous sheep of Nigeria. Canonical correlation analysis (CCA) proposed by Hotelling in 1935 (Thompson, 1984) is a technique for describing the relationship between two variable sets by calculating linear combinations that are maximally correlated. Also, Canonical correlation analysis has the ability to deal with two variable sets simultaneously and to produce both structural and spatial meanings (Bilgin *et al.,* 2003). Since CCA permits the researchers to examine the effect of multiple predictor variables on multiple criterion variables, the body measurements and the live weight variables can be utilized simultaneously for better meat production in livestock. Table 3 and 4 showed the standardized canonical discriminant function coefficients of male and female Goats. Standardized canonical coefficient shows the distinctive traits among two indigenous breeds of goats in Nigeria (Kano Brown and Sahel) and Boer goats of both males and females. In the males, it shows that Boer goat is distinguished from the indigenous breeds by its body weight, body length, ear length and heart girth. This implies that body weight, body length, ear length and hear girth are the most important traits for selection in breeding of the goat. Neck length is the most distinctive features that differentiates Sahel goats from the other breeds. Similar research was done by Sanni *et al.* (2018) in establishing phenotypic standards for Nigerian goats and their Southern African Kalahari goat counterpart, where it reveals that Sahel goats are distinctly classified from other goats based on their wither height, the Kalahari red goat have wider chest depth and longer body length than West African Dwarf goat (WAD).

Conclusion

It can be concluded from the result of this study that, Boer male goats have higher body weight, body length, heart girth and ear lengths than the Kano Brown and Sahel goats. The female Boer goats have higher body weight, body length, neck length and head circumference than the female of other breeds. It also showed that both sexes of Sahel goats have longer neck than the Kano Brown and Boer goats. It is therefore recommended that Boer goats can be cross bred with our indigenous goats to improve their body weight and other growth parameters.

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Table 1: Multivariate Statistics of Male Goats

Statistics	Value	Approx.F	Num DF	Den DF	Prob >F
Wiks' Lambda	0.07	9.65	16	48	<0.001*
Pillai' Trace	1.35	6.55	16	50	<0.001*
Hotelling-Lawley Trace	9.48	13.8	16	35	<0.001*
Roy' Max Root	8.64	26.99	8	25	<0.001*

Num DF: Numerator Degree of Freedom Den Df: Denominator Degree of Freedom

Table 2: Multivariate Statistics of Female Goats

Statistics	Value	Approx.F	NumDF	Den Df	Prob>F
Wilks' Lambda	0.07	13.33	18	158	< 0.001*
Pillai's Trace	1.44	8.91	18	174	< 0.001*
Hotelling-Lawley	6.08	18.58	18	106	< 0.001*
Roy's Max Root	4.89	47.24	6	58	< 0.001*

Num DF: Numerator Degree of Freedom Den Df: Denominator Degree of Freedom

Table 3: Standardized Canonical Discriminant Function Coefficient of Male Goats

Variable	F1 (CAN1)	F2 (CAN2)
Body Weight (kg)	-0.47	0.45
Body Length (cm)	-0.58	0.16
Heart Girth (cm)	0.16	1.50
Neck Length (cm)	0.64	-0.10
Ear Length (cm)	-1.60	-0.48
Head Circumference	1.04	-0.7
Gonadal Length (cm)	1.54	-2.54
Hip Height (cm)	0.50	14.5

CAN= Canonical F= Function coefficient

Table 4: Standardized Canonical Discriminant Function Coefficient of Female Goats

Variable	F1 (CAN1)	F2 (CAN2)	F3 (CAN3)
Body Weight (kg)	0.49	-0.07	0.26
Body Length (cm)	0.35	0.25	0.85
Neck Length (cm)	-0.03	0.86	-0.02
Ear Length (cm)	0.80	0.22	-0.72
Head Circumference	0.25	-0.8	0.12

CAN= Canonical F= Function coefficient

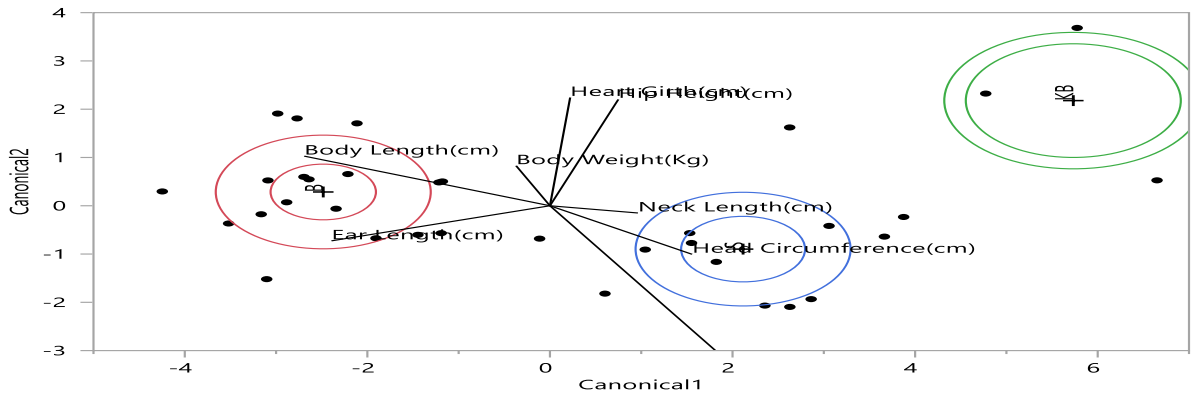

Figure 1: Discriminant Canonical Analysis of Male Goats

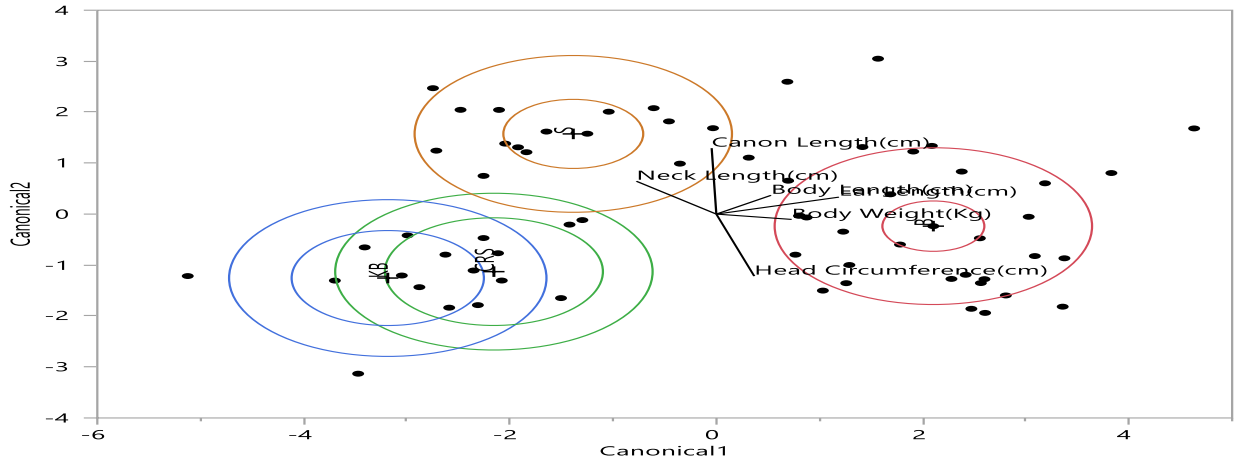

Figure 2: Discriminant Canonical Analysis of Female

ABG 018

INHERITANCE OF WATTLE AND ITS RELATIONSHIP WITH MILK PRODUCTION IN RED SOKOTO GOATS

[1]Abubakar, M.Y., [2]Kabir, M. and [3]Orunmuyi, M.

1. College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road, Kaduna State, Nigeria
2.Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria
3.Department of Animal Production and Health, Federal University, Oye Ekiti State, Nigeria.
Corresponding Author address: mukhtary2@gmail.com **Phone Number:** 08176136003

ABSTRACT

Data from 166 Red Sokoto goats consisting of 6 adult bucks and 60 adult does and 100 kids/progeny comprising 47 males and 53 females were used to study the inheritance of wattle and its relationship with milk production, in Red Sokoto goats. The data were collected over a 3 years period (December, 2015 to December, 2018) at the Teaching and Research Farm of the Department of Animal Production Technology, College of Agriculture and Animal Science, Division of Agricultural Colleges, Ahmadu Bello University, Mando Road, Kaduna. The data were analysed using the General Linear Model (GLM) SAS. The results revealed that from the mating of wattled and non-wattled Red Sokoto bucks and does; 32 progeny had wattle and 35 had none. The mating in which neither of the parents had wattle, all the 33 progeny were without wattle. The mean value for wattle and non-wattle were 3.92 ± 0.540 and 4.08 ± 0.526 respectively and did not differ significantly ($p > 0.05$). Genotypic and gene frequencies were determined using the Hardy-Weinberg principles, $(P + q)^2 = 1$ where p and q are frequency of the dominant and recessive alleles respectively. The appearance of wattle was bilateral in all the cases examined in this study. The gene frequency for the absence of wattle in the Red Sokoto kids in this study was 0.82. The genotypic frequencies were 0.67, 0.30 and 0.03 respectively for homozygous recessive (ww), heterozygous (Ww) and homozygous dominant individuals. Of the 100 Red Sokoto kids examined in this study, 68% had no wattle compared with 32% with wattle giving a ratio of 2:1. The ratio of male: female with wattle was 1.0: 1.3 respectively. The milk yield characteristics measured were average daily yield (ADY), Initial yield (IY), Peak yield (PY), total yield (TY), Last test day yield (LTDY), peak days (PD) and Lactation length (LL). The mean value for ADY, IY, PY, TY, LTDY, PD and LL were 330.45 ± 15.70g, 430.28 ± 11.80g, 559 ± 12.7g, 55.60 ± 1.19kg, 110.02 ± 1.94g, 32.05 ± 0.70 days, and 113.19 ± 0.52 days respectively. Udder traits measured were udder height (UH), udder circumference (UC), teat length (TL), teat circumference (TC) and Distance between teat (DT). Wattle does produced significantly ($p < 0.01$) higher milk yield (59.80kg) compared to non-watttled does (48.08kg). There were significant ($P<0.01$) effects of wattle on udder morphological traits with wattled does recording higher values compared to non-wattled does. It is recommended from the outcome of this research that livestock keepers and breeders should breed does and bucks with wattle to increase milk production, produce heavier body weight and sizes kids for increase productivity of their herds.

Keywords: *Wattled, non-wattled, milk yield, Red sokoto goats*

Introduction

In African continent, the largest goat population exists in Nigeria, Sudan and Kenya (Skapetas and Bampidi, 2016). Nigeria has 53.8 million goats (FAOSTAT, 2008). The Red Sokoto goats contribute to 70% of the country's goat population (Osuhor *et al.,* 2002). Despite large numbers and importance of small ruminant in developing countries, information on sustainable genetic improvement programme is scarce, especially for adopted indigenous breeds (Kosgey *et al.,* 2006, Devendra and Lung, 2012).

Wattle represents the congenital thumb-shaped appendages on the ventral throat and was common in domestic goats (Reber *et al.,* 2015). The presence of wattle is controlled by autosomal dominant gene (W) with complete or

incomplete penetrance (Gasu *et al.,* 1970). Many production traits are correlated with presence or absence of wattle. Gasu *et al.,* (1970) observed in Sardinian breed of sheep that ewes with wattle produced more milk than ewes without wattle; and the lambing rate were higher in ewes with wattle than in ewes without them. Shongjia *et al.,* (1992) linked possession of wattle with reproductive traits such as higher prolificacy, higher milk yield, higher litter size and conception rate in Saanen, GuanLang and Crossbred goats.

Goat milk plays a significant role in the nutrition and well-being of human population. There are about 921 million gats worldwide (FAOSTAT, 2011). In 2010, goat milk production in the world was greater than 12,000 million litres, from which Asia produced 53.3%, Africa 23%, Europe 20% and 3.7% in the American continent (Cesar, 2011). Goat milk is a valuable source of protein, fat, calcium, iron, phosphorus, magnesium and vitamins, particularly Vitamin A (Hazirah *et al.,* 2018).

There are virtually scarce information on how the wattle trait is inherited form parents to offsprings and its relationship with milk production in Red sokoto goats. This study was therefore carried out to determine how wattle trait is inherited from parent of offsprings and its relationship with milk production in Red sokoto goats.

Materials and Methods

The study was conducted at the Teaching and Research Farm of the Department of Animal Production Technology, College of Agriculture and Animal Science, DAC/ABU, Mando Road, Kaduna. The College is on Longitude $7^0$25' – $26^0$41'E (www.google.com). The rainfall in this area varies between 1000cm and 1500mm per annum and rainy season lasts for 150 – 200 days and dry season stars from October to early April. The mean annual temperature was about 34^0C with hottest months being from March to April (40^0C) and the coolest period (13^0C), which were between December and January (NIMET, 2015).

Animals and their Management

Forty Red Sokoto does in their first and second parities and four Red Sokoto bucks aged between 1 to 2 years were randomly selected to constitute the initial stock for this research work, comprising twenty wattled does versus two wattled bucks and twenty non-wattled does versus two non-wattled bucks. The goats were purchased from the Fulani herdsmen that reared them in Kaduna and Kano States. The goats were housed in a well-ventilated pens according to their groupings. They were identified using ear tags and were kept under semi-intensive management system. The animals were released for grazing in batches or groups. The animals were supplemented with maize offals, cowpea husks and groundnut haulms which gave 87.6% dry matter, 7.9% ash, 17% crude protein, 2.3% ether extract and 12.6% crude fibre. Mineral lick was also provided, water was given ad-libtum. All the animals were de-wormed and vaccinated at the beginning and during the period of the research. The experiment lasted for thirty-six months (December, 2015-December, 2018).

Mating Plan

The does were grouped into four pens; one wattled Red Sokoto buck was mated to ten wattled Red Sokoto does. The other pen contained one non-wattled Red Sokoto buck mated to ten non-wattled Red Sokoto does, the third group ; one non-wattled Red Sokoto buck mated to ten wattled Red Sokoto does and lastly one wattled Red Sokoto buck to ten non-wattled Red Sokoto does. Mating lasted for 30 days. During the mating period, supplementary feeding were provided to the does in the form of groundnut haulm, maize offals, threshed bean pods '*kowa*'. These served as '*flushing*' strategy. The offsprings obtained from the above matings were mated. Wattled female offsprings were mated to wattled buck. The non-wattled female offsprings were mated to non-wattled buck.

Data Collection

Pattern of Inheritance for Wattle

Observation was made on the appearance of wattle on the first and second generation offsprings out of the four breeding groups. The result of the parent mating were observed in the first generation offsprings, while that of the first generation offsprings were observed in the second generation offspring.

The frequency of the recessive gene alleles were estimated using Hardy Weinberg equilibrium (Falconer and Makey, 1996) as shown below.

$$q = \sqrt{\frac{m}{M}}$$

where q = frequency of recessive gene (w)
m = observed number of animals exhibiting the particular recessive traits.
M = total number of animals sampled.

Milk Production and Evaluation

Milk production was monitored on both parents and offsprings populations for three years (2015-2018). After parturition, the kids were allowed to suckle their dams for seven days to enable them receive colustrum before the milking of the does commences. The does were hand milked for 120 days. Milking was done twice daily; in the morning and evening and milking was also carried out twice in a week (Wednesdays and Saturdays). On the night preceeding the milking day, the kids were separated from their dams and rejoined only after the evening milking. During the period of separation, the kids were bottle fed with goats' milk to avoid starvation. The does were milked by gently palpating the udder until milk letdown. Milking continues until milk 'letdown'. Milking continues until the two halves of the udder were dried. The milk samples collected from the does were measured using a scale and values recorded in gram per day. The following determination were made; initial yield, peak yield, days at peak, total yield (A per Akpa *et al.,* 2012).

- Initial yield:- This is the first week average daily yield estimated as the average of 2 days yield of the week.
- Peak yield:- is the highest recorded test day yield within the sampling period.
- Peak day:- the first day, out of the recorded test days that had the highest yield.
- Days at peak:- these are days within the peak production after which it declined.

Weekly total = $\frac{W + S}{2} \times 7$

Where W = Wednesdays, S = Saturdays.

Total yield $= \sum_{l}^{n} Xij = X_i + X_2 + - X_n$

X_i = number of weeks in 120 days of lactation.

Average daily yield $\frac{\sum Xi}{N}$

Where N = number of days of lactation

Udder Morphology

The udder morphological characteristics of the does were taken once a week (Saturday) prior to the milking of the does in the morning. The following udder morphological traits were measured:

- Udder height:- the udder external features that were measured from rear attachment of the udder to the point where it blends with the body.
- Udder Circumference:- this is measured as the widest point of the udder around it.
- Teat length:- this is measured as the distance from the upper part of the teat, where it hangs perpendicular from the udder to the top of the teat.
- Teat circumference:- this is measured as the wildest point around the teat.
- Distance between two teat:- This is measured as the space between the base of the two teats (Williams *et al.,* 2014).

Statistical Analysis

The data for this study were analyzed by the General Linear Model (GLM) Procedure of (SAS, 2012). Significant differences were separated using Duncan's Multiple Range Test (Duncan, 1955). The data were analyzed using the following model:

$Yij = \mu + W_i + M_j + E_{ij}$

Where Y_{ij} = any observation
μ = the overall mean
W_i = effect of Wattle
M_j = effect of Milk yield
E_{ij} = the random error term

Result and Discussion

Inheritance of Wattle

The phenotypes of progeny of mating between different combinations of wattled and non-wattled Red Sokoto goats are presented in Table 1, group a, one wattled Red Sokoto buck was mated to 10 wattled Red Sokoto does, the progeny produced were 5 wattled Red Sokoto (males) and 7 wattled Red Sokoto (female) kids, while 4 non-wattled (males) and 6 non-wattled female were also obtained in this group (a pure breeding mating).

In group b, 1 non-wattled Red Sokoto buck was mated to 10 non – wattled Red Sokoto does; the offspring obtained from this pure breeding mating were all non-wattled (10 males and 11 females) progeny.

In group c, mating between 1 non-wattled Red Sokoto buck and 10 wattled Red Sokoto does (cross breeding) produced 5 wattled (males) and 3 watled (females) progeny and 6 non-wattled (males) and 5 non-wattled (females) progeny. The mating of 1 wattled buck to 10 non wattled Red Sokoto does in group d produced 2 wattled (males) and 4 wattled (females) offspring and 5 non-wattled (males) and 4 non-wattled (females). A total of 73 offspring (35 males and 38 females) were obtained in this parent mating of wattled and non-wattled Red Sokoto goats.

The phenotype of progeny from mating of F_1 generation between wattle and non-wattled Red Sokoto goats are presented in Table 2. In group 1, 1 wattled Red Sokoto buck was mated to 10 wattled Red Sokoto does, the offspring produced were 4 wattled (males) and 6 wattled (female) Red Sokoto kids. The progeny obtained in mating between 1 non-wattled Red Sokoto buck and 10 non-watled Red Sokoto does (Group II) were 17 all non-wattled Red Sokoto goats (8 males and 9 females).

The mating between wattled buck to wattled does in this study produced wattled and non-wattled progeny. This indicates that the parents were heterozygotes for wattle appearance. Mating between wattled buck and non-wattled does; and between non-wattled buck and wattled does in this study produced wattled and non-wattled progeny. This is in agreement with the report that inheritance of wattle is controlled by autosomal dominant gene; (Gasu *et al.,* 1970, Lauvergene *et al.,* (1987) and Reber *et al.,* (2015). The within mating of non-wattled bucks and non-watled does in this study produced all non-wattled progeny. This indicates that the parents might be pure breeding lines. This is in agreement with the report of Hermiz *et al.,* (2016) in Shami goats, Osinowo *et al.,*(1990) in Yankasa Sheep and Gasu *et al.* (1970)in Sardinian breed of sheep. , Russel (2002) reported that crosses that always produced progenies with the same parental phenotype must have been between homozygous recessive or dominant.

Effect of Wattle on Milk Production and Udder Characteristics

The mean $\pm$ S.E. for lactation performance of wattled and non-watled Red Sokoto goats are shown in Table 3. In this study, wattle was highly significant ($p < 0.01$) affected ADY, IY, PY and TY. There were also significant ($p < 0.05$) effect of wattle on LTDY, PD and LL. The ADY, IY, PY, TY, LDY, PD and LL for wattled and non-watled does were 402.38g, 503.10g, 681.43g, 59.95kg, 80.95g, 33.40days 117.07 days and 339.07g, 328.33g, 457.67g, 48.08kg, 77.33g, 31.09days, 113.17 days respectively.

These are in agreement with the reports of Ijomanta (2012) in Red Sokoto goats who reported that wattled does significantly produced more milk (277.8mls/day) compared to non-wattled does (250.8mls/day); Williams *et al.* (2015) reported in West African Dwarf (WAD) goats that wattled animals produce significantly higher milk yield (297.72g/day) compared to their non-wattled counterparts (255.11g/day)

and Hermiz *et al.,* (2016) in Shami goat who reported that does with wattle produced more milk (280.920kg) than those without wattle (225.475kg). Shongjia *et al.,* (1992) observed in Saanen does with wattle yielded higher milk than does without wattle. The higher value for wattle does compared to the non-wattted does obtained in this study is in line with the report of Gasu *et al.,* (1970), in Sardinian breed of sheep that ewes with wattle produced more milk than ewes without wattle.

The udder morphological traits for wattle and non-wattled Red Sokoto goats are shown in Table 4. Wattle and non-wattled condition significantly ($p < 0.01$) affected UH, UC ($p < 0.05$), TL, TC, and DT. Wattle does were observed to record higher values for all the traits measured, compared to non-wattled does. This might be probably due to the fact that possession of wattle was linked with higher production capacity (Shongjia *et al.,* 1992).

Conclusion

- It was concluded that the inheritance of wattle in Red Sokoto goats is controlled by an autosomal dominant gene (W) and from the ratio of males to females progeny that were examined in this study, the inheritance was not related to sex.
- The wattled does produced higher milk yield during the lactation period as well as higher twinning rate compared to non-wattled does.
- It was also seen that wattled does were superior in udder morphological traits examined in the present study; with wattled does recording higher values compared to non-wattled does.

Recommendation

It is recommended from the outcome of this research that livestock keepers and breeders should breed does and bucks with wattle to increase milk production.

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Table 1: Phenotype of progeny from mating between different combinations of wattled and non wattled Red Sokoto goats.

Sire		Dam		Progeny			
Phenotype	Gen.	Phe.	Gen.	Phe.	M	F	Total
a) 1 WT ♂	Ww	10WT ♀	Ww	WT	5	7	12
				NWT	4	6	10
b) 1 NWT ♂	Ww	10 Nwt ♀	Ww	All - NWT	8	9	17
c) 1 NWT ♂	Ww	10 WT ♀	Ww	WT	5	3	8
				NWT	6	5	11
d) 1 WT ♂	Ww	10NWT ♀	ww	WT	2	4	6
				NWT	5	4	9
TOTAL					35 :	38	73

Phe: Phenotype, Gen=genotype, WT= wattled, NWT= non wattled , M= male , F= female

Table 2: Phenotype of Progeny from matings of F_1 generations between wattled and non-wattled Red Sokoto goats.

Sire		Dam		Progeny			
Phenotype	Gen.	Phe.	Gen.	Phe.	M	F	Total
a) 1 WT ♂	Ww	10WT ♀	Ww	WT	4	6	10
b) 1 NWT ♂	ww	10 Nwt ♀	Ww	All - NWT	8	9	17
TOTAL					12	15	27

*Phe: Phenotype, Gen=genotype, WT= wattled, NWT= non wattled , M= male , F= female

Table 3: Mean Values of Lactation Performance of Wattled and Non-wattled Red Sokoto Goats.

Characteristics	Wattled	Non-wattled	LOS	S.E.M
ADY(g)	402.38[a]	339.07[b]	**	9.30
IY(g)	503.10[a]	328.33[b]	**	9.11
PY(g)	681.43[a]	457.67[b]	**	9.80
TY(kg)	59.80[a]	48.08[b]	**	1.11
LDY(g)	80.95[a]	77.33[b]	*	1.96
PD(days)	33.40[a]	31.09[b]	*	0.70
LL(days)	117.07[a]	113.17[b]	*	0.52

ADY=average daily yield, IY = Initial yield, PY=peak yield, TY= total yield, LDY = last day yield, PD=peak days, LL = lactation length, LOS= Level of significance, S.E.M=Standard error mean.

ABG 019

Comparative Genomics Analysis of Distribution Of Growth Hormone, Insulin-Like Growth Factor 1 And Myostatin Gene In Chicken, Rabbit And Sheep Genome

Abdulrahman M.* and Kabir M.**

*National Animal Production Research Institute, Shika, Ahmadu Bello University, Zaria, Kaduna, Nigeria. +2348065654946
**Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Kaduna, Nigeria. +2348035926820

ABSTRACT

The gene sequences of Growth hormone (GH), Insulin-like Growth Factor 1 (IGF-1) and Myostatin (MSTN) were downloaded from National Centre for Biotechnology Information (NCBI) database, in FASTA format, using respective accession numbers (Table 1) of the genes in the GenBank to access the necessary gene information. They were subjected to on-line BLAST-like alignment tool (BLAT), and analysed for gene number in a genome, DNA strand, sequences identity percentage range and number of chromosomes the genes are distributed by submitting the genes sequences in FASTA format to the tool. Results indicated that higher body weight and size of sheep might have been due to, high gene number of GH (232), the number of chromosomes GH gene distributed in sheep genome was higher than that in rabbit and chicken, and most are found on opposite strand. Rabbit and sheep IGF-1 has higher number, which might be the reason for higher body weight at maturity compare to chicken. It is concluded that; the number of GH gene of sheep is higher than that of chicken GH, than rabbit GH but the number IGF-1 gene of rabbit and sheep are higher than that of chicken IGF-1 gene, while the number of rabbit myostatin is higher than that of chicken and sheep Myostatin. The number of genes in chicken, rabbit and sheep genomes plays a key role in establishing effective gene function. It is recommended that; Chicken, rabbit and sheep growth could be improved through increasing the number of their GH or IGF-1 gene by transgene techniques.

Keywords: *Bioinformatics, BLAT, Genes, Sequences, Similarities, Variations.*

Introduction

Bioinformatics is the science of storing, extracting, organizing, analysing, interpreting and utilizing information from biological sequences and molecules (Khalid, 2010). Bioinformatics is often defined as the application of computational techniques to understand and organize the information associated with biological macro-molecules (Luscombe*et al.,* 2001). It has been mainly fuelled by advances in DNA sequencing and mapping techniques (Khalid, 2010). A biological database is a collection of information, or data from a biological system, stored in a computer readable format. Sharing of data between scientists accelerates the speed of discoveries and has the potential to greatly advance a scientific field as a whole (this is known as the Fourth Paradigm of Data-Driven Scientific Discovery (Hey *et al.*, 2009).

The primary goal of bioinformatics is to increase the understanding of biological processes (Khalid, 2010).DNA Sequencing is a technique/method by which the exact order of nucleotides within a DNA molecule is determined (Mayor *et al*., 2000). Comparative data analysis provides the opportunity to determine what is shared and what is unique to each species (Mayor *et al*., 2000).

Growth in animals is controlled by a complex system, in which the somatotropic axis plays a key role. The genes that operate in the somato-tropic axis are responsible for the postnatal growth, mainly GH that acts on the growth of bones and muscles mediated by IGF-1 (Sellier, 2000). The growth hormone (GH) and insulin-like growth factor 1 (IGF-1) genes are candidates for growth in bovine, since they play a key role in growth regulation and development (Hossner*et al*., 1997; Tuggle and Trenkle, 1996). Effects of GH on growth are observed in several tissues, including bone, muscle and adipose tissue. These effects result from both direct action of GH on the partition of nutrients and cellular multiplication and IGF-1-mediated action stimulating cell proliferation and metabolic processes associated to protein deposition (Boyd and Bauman, 1989). IGF-1 stimulates protein metabolism and is important for the function of some organs, being considered a factor of cellular proliferation and differentiation (Andrea *et al*., 2005).

Myostatin (MSTN) is a negative regulator of the muscle growth factor, which belongs to the transforming growth factor beta superfamily (McPherron*et al*., 1997). It is able to negatively control the growth of muscle cells by inhibiting the transcriptional activity of MyoD family members. Its expression is negatively correlated with muscle weight (Weber *et al*., 2005). Mutations in the myostatin gene have also been shown to cause double muscling in humans and other species (Clop *et al*., 2006). These findings suggest that strategies for inhibiting myostatin function may be applied to improve animal growth. Cattle with mutations of the MSTN gene-conserved Ribbon bases exhibit the advantage of strong muscle in increase birth weight, and obvious double-hip muscle characteristics (Casas *et al*., 1999).

Description of problem

There is need to identify other means of genetic improvement of farm animals, as most of the animals has undergone intensive artificial selection and breeding forfast rate in growth and body size to maturity, final body size at maturity, and body conformation at maturity. These three species are among the common available farm animals that shared same genes for growth but varied for the mentioned traits of interest.

Objectives of the Study

To determine the distribution of GH, IGF-1 and MSTN genes and their chromosomal and DNA strand contained in the genome of chicken, rabbit and sheep.

MATERIALS AND METHODS

Research Centre/Location

This Comparative genomic research study was conducted at A.B.U. ICT/Digital Centre, Animal Science Department, Faculty of Agriculture, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

BLAT Analysis of UCSC Genome Browser Computational Tool

For Custom comparison to whole genomes; University of California at Santa Cruz (UCSC) Genome Browser output was analysed using on-line BLAST-like alignment tool (BLAT)for gene number in a genome, DNA strand, sequences identity percentage range and number of chromosomes the genes are distributed by submitting the genes sequences in FASTA format to the tool, in FASTA format, using respective accession numbers (Table 1) of the genes in the GenBank to access the necessary gene information. For Chicken, rabbit and sheep whole genome comparison of the GH, IGF-1 and MSTN gene sequence according to the procedure of Kent *et al.* (2002). The different scoring system for the percent identity range of 50 – 100% level of conservation. The number of GH, IGF-1 and MSTN genes all over the genome, number of chromosomes in which the genes are distributed, and the type and number of DNA strand (input or opposite strand), in which the genes are found in each of whole chicken, rabbit and sheep genome by submitting the genes respective sequences in FASTA format to the tool (http://genome.ucsc.edu/).

RESULTS AND DISCUSION

BLAT Analysis result using Genome Browser

Growth Hormone (GH) Gene

The copies of chicken GH gene predicted within chicken genome was 201, distributed over the chicken specie, which might have been as a result of paralog process that might have been through mutation, deletions or insertions in some of the regulatory elements and regions. These mutations might have led to significant variation in the gene sequences and also vary a little in function and might have also led to the different identity percentage (58 – 100), distributed over 29 different chromosomes at different locations but on the two different DNA strand, having 102 copies on the input strand and 99 copies on the opposite strand, which might be to function through different pathways as the orientations of

the genes on different strands might change (Table 2). The input strand seems to per-take most in establishing some of the functions, as the percentage of the chicken GH genes are higher in this strand. The chicken GH gene large number compare to the body size might be one of the reasons behind IGF-1 gene small number since the chicken GH gene abundance in the genome, the effect is almost in every cell.

Rabbit GH gene predicted in rabbit genome was 11 copies only, which might also be as a result of paralog process that was also distributed only within 9 different chromosomes in the rabbit specie, which the gene effect might cover few locations and with 7 copies on input strand and 4 copies opposite strand though with higher identity percentage of the copies (94 – 100), which is an indication of few single nucleotide polymorphisms or mutations between the rabbit GH genes found in the rabbit genome, this was also an indication of how close they are in function. The input strand also seems to per-take most in establishing some of the functions, as the percentage of the rabbit GH genes are higher in this strand.

The sheep GH gene numbers predicted in sheep genome was 232 copies, which is expected given the large body size of sheep species at birth and at maturity, but in comparison with chicken GH gene of 102 number and body size, it is an indication that the GH gene is not the sole determinant of the large body size of sheep species, this might explain the role of large size of IGF-1 gene in sheep and the role of the opposite DNA strand in which most of the sheep GH genes are found might explain the large body size pathways of sheep species at birth and at maturity (112 copies on input strand and 120 copies on opposite strand), all with high identity percentage of (71 – 100) distributed all over a wide range of 46 different chromosomes in the sheep genome. Duplications of pieces of a genomic sequence can occur during replication, sometimes leading to the copying of an entire gene. In principle these errors are a random process, and whether the result is fixed in a descendent lineage depends on the frequency of the mutation and natural selection (Dutilh and Keşmir, 2016).

Insulin-Like Growth Factor-1 (IGF-1) Gene

The chicken IGF-1 gene predicted in chicken genome was 209 copies, which vary little in size with chicken GH gene with only (8 copies), almost a 1:1 mapping ratio between the genes and the DNA strands (105 copies on input strand and 104 copies on opposite strand) with high identity percentage (85 – 100), found among 22 different chromosomes in the chicken genome. This might explain the reason for the final small body size of chicken because chicken GH gene is not complemented by the chicken IGF-1 genes

compared to rabbit and sheep. So the chicken GH gene effect is not multiple in folds by the chicken IGF-1 gene. The chicken IGF-1 gene is also distributed over small range of different chromosomes compared to the chicken GH gene, which might also limit the spread of the effect of the chicken GH gene. Transgenic mice had been used to study the expressed increased insulin-like growth factor-I (IGF-1), it was observed that they too grew larger than normal (Mathews *et al.,* 1988). When the expressed transgenes encodes a growth promoting hormone (growth hormone or growth hormone releasing factor), transgenic mice were reported to grow up to twice their normal size (Palmiter*et al.,* 1983; Hammer *et al.,* 1985a).

The rabbit IGF-1 gene information could not be display due to large size of the rabbit IGF-1 gene and large size of the gene in the genome data available on the website for the gene. The sheep IGF-1 gene information could not be retrieved due to large size of the sheep IGF-1 gene and large size of the gene in the genome data available on the website for the gene.

Myostatin (MSTN) Gene

The chicken Myostatin gene predicted in chicken genome was only 3 copies (Table 2), which might explain more on the negative effect of myostatin gene on fast growth and body weight, which might have retarded the growth to the normal chicken specie at high level.If the chicken GH gene regulate fast growth through the input strand, might also explain the one copy of the chicken myostatin gene on input strand to reduce the effect of myostatin gene and the two copies of chicken myostatin gene on the opposite strand to reduce the matured body size and weight, with identity percentage of (81 – 100) found only in 3 different chromosomes may be to also further reduce the effect of the myostatin gene since the chicken IGF-1 gene is also small in size.

The rabbit myostatin gene predicted in rabbit genome is 5 copies, higher than that of chicken and sheep species, which might be as a result of large size of rabbit IGF-1 gene that multiply the effect of small size of rabbit GH gene. The 2 copies of rabbit myostatin gene on the input DNA strand might be a pathway to reduce excessive fast growth from birth to maturity of rabbit species while the 3 rabbit myostatin gene copies on opposite strand might be the pathway to reduce the final body weight at maturity of rabbit species. The identity percentage of the rabbit myostatin gene copies (78 – 100) found in 5 different chromosomes may be to further increase the effect of the rabbit myostatin gene compared to chicken and sheep myostatin genes.

The copies of sheep myostatin gene predicted in sheep genome was 3, with 2 copies on input strand might explain the slow growth rate from birth to maturity of myostatin gene of sheep

species compared to rabbit and chicken species and 1 copy on opposite strand might also explain the less effect of myostatin gene on the large body weight and size of sheep species compared to rabbit and chicken species, at identity percentage of (80 – 100) found in 2 different chromosomes. This might also indicate the route of action of myostatin gene on growth might be through supressing IGF-1 gene effect. In the muscle tissue of high growth line broilers, it has been reported that a positive relationship between muscle IGF-1, mRNA levels, which determine paracrine IGF-1 levels, and post hatch muscle growth (Duclos, 2005).

CONCLUSION

The number of GH gene of sheep (232) is higher in size than that of GH gene of chicken (201) and GH of rabbit (11) but the number of IGF-1 gene of rabbit and sheep are higher in size than that of chicken IGF-1 gene, while the number of rabbit myostatin (5) is higher than that of chicken (3) and sheep (3) Myostatin, and the number of genes (GH, IGF-1, MSTN) in a chicken, rabbit and sheep genomes plays a key role in establishing effective gene function.

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Table 1: Accession numbers of GH, IGF-1 and Myostatin Genes of Chicken, Rabbit and Sheep.

Specie	GH gene Accession	IGF-1 gene Accession	Myostatin gene Accession
Chicken	NC_006114.4	NC_006088.4	NC_006094.4
Rabbit	NC_030827.1	NC_013672.1	NC_013675.1
Sheep	NC_019468.2	NC_019460.2	NC_019459.2

Source: GenBank, National Center for Biotechnology Information (NCBI) database

Table 2: BLAT analysis result using genome browser

Gene	No. of Gene in Genome	DNA strand	Identity % range	No. of Chromosomes
Chicken				
GH	201	102+, 99-	58 – 100	29
IGF-1	209	105+,104-	85 - 100	22
Myostatin	3	1+,2-	81 – 100	3
Rabbit				
GH	11	7+,4-	94 – 10	9
IGF-1	Not available on this website due to large size			
Myostatin	5	2+,3-	78 – 100	5
Sheep				
GH	232	112+,120-	71 – 100	46
IGF-1	Not available on this website due to large size			
Myostatin	3	2+,1-	80 – 100	2

+ : input strand; - : opposite strand

SUB-THEME: CROP GENETICS AND BREEDING

CGBPB 001

Callus Induction Using Embryonic Explant of Red Sorghum (*Sorghum bicolor* (L) Moench)

Hassan, L.B., Usman, I.S., Katung, M.D., Bugaje, S.M, Ahmed, H.O.and Sami, R.S.

Department of Plant Science, Ahmadu Bello University, Zaria, Kaduna State
Corresponding Author: Hassan Bolanle Lateefat, haslat2000@yahoo.com +2347034707849

Abstract

This work was designed to study the effect of different concentrations of 2, 4-D alone or in combination with kinetin on callus induction in red sorghum using embryonic axes of immature embryo as explant in vitro. Murashige and Skoog (MS) basal medium was fortified with different concentrations of 2, 4-D (0.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/L) alone or in combination with 0.5mg/L Kinetin (KN) for callus induction. Data were collected on percentage callus formation, callus fresh weight and nature of callus induced. Highly significant difference was observed for all the traits measured among the treatment tested. Whitish compact calli were obtained at one week of inoculation most of which changed to yellowish globular calli after three weeks of cultured. Media fortified with 2 mg/L 2,4-D in combination with 0.5mg/L KN rec\orded the highest percentage callus formation (92%) but not significantly different from what was obtained from the media fortified with 3.5 mg/L 2,4-D alone (80%) with better callus growth of 1.48g. Suitability of 2, 4-D alone or in combination with kinetin on callus induction in red sorghum using embryonic axes as explant was observed in this study. Further study is recommended to determine the suitability of this calli for shoot formation, genetic transformation as well as flavonoids production in red sorghum

Keywords: *Sickle cell anaemia, Red sorghum, in vitro, callus induction, Flavonoids*

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench is one of the most important grain crops grown globally for food security. It ranks fifth after wheat, maize, rice, and barley (FAO, 2011) worldwide and second after maize in Sub-Saharan Africa (Zidenga, 2004, Mofokeng *et al.,* 2017). The four leading sorghum producers in Africa are Nigeria, Ethiopia, Burkina Faso and Niger. About 74% of sorghum in Africa is used for food (Acquaah, 2012). Although the production varies widely among countries, sorghum remains an important food constituent in the diet of many rural people (Mofokeng *et al*., 2017).

Apart from food value, sorghum varieties are known to have antioxidant activities because they are very rich in phenolic acids, as well as polyphenols, especially 3-deoxyanthocyanidins, such as luteolinidin and apigeninidin. (Dykes, 2006 and Devi *et al*., 2011). Apigeninidin is an important bioactive compound in sorghum with anti-anaemic, anti-cancer, antioxidant well as anti-sickling properties. Sorghum is a unique crop among major cereals because it adapts well to environmental extremes, notably drought and heat. These attributes make sorghum the logical grain to support human and animal populations in areas with extreme heat and low rainfall (Howe *et al.* 2006). Sorghum being an important crop with food, feed and medicinal values, there is need to improve this crop through biotechnological intervention using tissue culture technique as well as genetic transformation

Plant tissue culture is a technique or collection of techniques used to maintain and grow plant cells, tissues or organs under sterile conditions on a artificially prepared nutrient medium under a very clean environmental condition. Plant tissue

culture made it possible to produce a large number plant in a short period of time (Barewa, 2018). Also with the use of tissue culture technique secondary metabolites can be produced from medicinal plants at any time of the year irrespective of weather and climatic condition.

In vitro callus induction is required in the production of secondary metabolites and genetic transformation in sorghum. Protocols had been established for *in vitro* plant regeneration of sorghum from different type of explants like immature embryos, immature inflorescences and shoot tips or apices and mature embryo (Indra *et al*., 2009). This work was carried out in order to determine the effect of 2,4–D alone or in combination with kinetin on callus formation using only embryonic axes of immature embryo as explant

Materials and Methods

Experimental site

This experiment was carried out at Biotechnology Laboratory in Department of Plant Science Institute for Agricultural Research (IAR) and Multi user Laboratory in the Department of Chemistry, Ahmadu Bello University, Samaru, Zaria. (110 111N, 070 381, 680 m above sea level) Samaru –Zaria is situated in the northern guinea savannah ecological zone of Nigeria. It has a mean annual rainfall of about 1045mm; rainfall is well distributed over the growing season of about 130-190 days between May and October

Planting Materials

'Karan dafi' seeds collected from farmers' fields in Katsina State. The seeds were planted in the green house in order to obtain immature seeds. The immature seeds of sorghum were harvested from main spikes of caryopsis at 17 days after anthesis. The immature seeds harvested for use as immature embryo were kept moist at 4°C until embryonic axes were excised as described by (Sudhakhar *et al*., 2008)

Surface sterilization

Immature seeds were surface sterilized by dipping in 95% ethanol (v/v) for 2 minutes followed by treatment with 20% commercial bleach (containing 3.5% Sodium hypochlorite) with 2 drops of tween 20 for 20 minutes (Mackinnon *et al*, 1986). Afterwards, the seeds were rinsed three (3) times with sterile distilled water and embryonic axes were aseptically excised from embryo using sterilized forceps and surgical blade

Preparation of Media for Callus Induction

The excised embryonic axes were used for callus induction on modified semi solid Murashige and Skoog basal medium (Murashige and Skoog, 1962) plus sucrose 30g/L. The MS basal media was supplemented with varying concentration of 2, 4-D (0.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mg/L) alone or in combination with 0.5mg/L Kinetin (KN) for callus induction. The pH of the media was adjusted to 5.8 using 0.5 M HCl or 0.5 M NaOH, agar (0.8%) was used as gelling agent and the media was heated to boiling for proper mixing. The sterilization of the media was done in an autoclave at 121°C for 30 minutes and media was dispensed into petri-dishes.

Callus Induction

The embryonic axes of immature embryo were placed on the media with their axes in contact with the media for callus induction, five (5) embryos were cultivated in each petri dish (11mm diameter) and five (5) petri dishes were used per treatment for callus induction using completely randomized design. The cultures were incubated in the darkness for callus proliferation at 25°C±2°C. Activated charcoal (1%) was used to control phenolic secretion (Sudhakar *et al*., 2008).

Data Collection

Data were collected on the following variables

Percentage callus formation: This was calculated on the basis of number of explants that formed calli and number of explants inoculated

$$\% \text{ Callus formation} = \frac{\text{Number of explant that formed calli}}{} \times 100$$

Number of explants inoculated

Callus fresh weight (g): After three weeks of inoculation, the callus fresh weight of five randomly selected calli was measured in gram (g) with the help of a digital balance (Ohausas, AR3130, China)

Nature of callus : After one and three weeks of inoculation, nature of calli induced was observed and recorded

Results and Discussion

Outgrowth of embryonic axes was observed third day after inoculation when they were cultured in callus induction media. Callus induction started from the radicle portion of the embryonic axes and grew upwardly towards the plumule. The result of this study showed highly significant difference ($p< 0.01$) for percentage callus formation among the different concentrations of 2, 4-D alone or in combination with kinetin used. The highest callus formation was obtained from the media fortified with 2.0 mg/L 2, 4-D +0.5 followed by the media fortified with 2.0 mg/L 2, 4-D (88%) and this was not significantly different from was obtained from the media fortified with 3.5mg/L 2,4-D that recorded highest callus fresh weight (1.48g) but significantly different from what was obtained from the media supplemented with 1.5 mg/L 2,4-D (60%) (Table1.0). The highest percentage callus formation observed in the media fortified with 2.0 mg/L 2, 4-D in combination with 0.5 mg/L KN implies that this hormonal combination is suitable for callus formation in sorghum. This agrees with findings of Sudhakar *et al* (2008) who reported the highest percentage callus formation in the media fortified with 2 mg/L 2,4-D+ 0.5 mg/L KN. The highest callus fresh weight observed in the media supplemented with 3.5 mg/L 2,4-D+ 0.5 mg/L KN implies concentrations above 2 mg/L 2,4-D promote better callus growth rate in sorghum. Similar finding was reported by Zhao et al (2010). No callus formation was observed in hormone free media and media fortified with 0.5 mg/L KN. This implies that 2,4-D is paramount for callus formation in sorghum. This agrees with findings of Bi *et al.* (2008) who reported that auxin such as 2,4-D is important for callus formation in cereals.

Differences in nature of calli overtime were observed in this study shown in table 2.0, whitish compact calli with no browning was observed after one week of inoculation. The calli remained whitish in color up to two weeks of inoculation and changed to globular yellowish calli after three weeks of inoculation for most of the treatment tested. The media fortified with 2.0 mg/L and 2.5 mg/L 2, 4-D and 0.5 mg/L KN retained their compactness up to the three weeks after inoculation. The change in color of the calli observed after three weeks of inoculation shows in stability in the quality of calli over time. Pigment production observed in some calli obtained may be attributed to the nature of crop subjected to callus induction which is rich in phenolic compounds such as flavonoids. The red pigmentation observed in some calli induced agreed with findings of Mackinnon (1986) who reported production of red calli in sweet sorghum

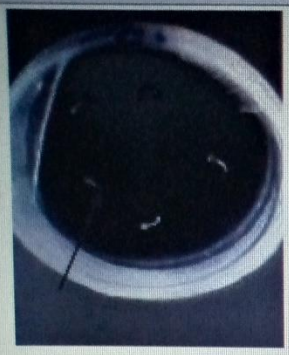
Table 1.0: Effect of PGRs on callus formation and fresh weight in red sorghum

PGRs		% Callus formation	Callus fresh weight (g)
2,4-D (mg/L)	Kinetin (mg/L)		
0.0	0.0	0.00^{c}	0.00^{f}
1.5	0.0	60.00^{b}	0.33^{cf}
2.0	0.0	88.00 ^{a}	0.50^{cde}
2.5	0.0	72.00^{ab}	0.47^{cde}
3.0	0.0	80..00 ^{a}	0.80^{c}
3.5	0.0	80.00 ^{a}	1.48^{a}
4.0	0.0	80.00 ^{a}	0.61^{cde}
0.0	0.5	0.00c	0.00^{f}
1.5	0.5	84.00^{a}	0.35^{de}
2.0	0.5	92.00 ^{a}	1.14^{b}
2.5	0.5	84.00 ^{a}	0.76^{c}
3.0	0.5	84.00 ^{a}	0.71^{dc}
3.5	0.5	80.00 ^{a}	0.66^{cde}
4.0	0.5	84.00 ^{a}	0.56^{cde}
CV		2.90	42.12
SE		0.04	0.56

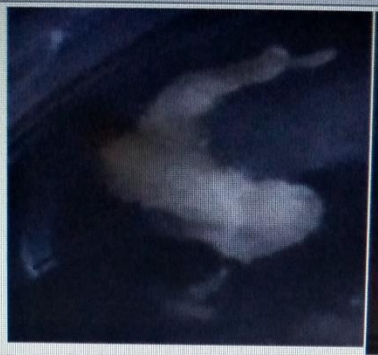
Means followed by the same letter(s) within a column are not significantly different at 5% level of significance using DMRT. EBA = Embryonic axes, PGR = Plant Growth Regulator,

Table 2.0: Nature of callus induced at one week and three weeks of inoculation using embryonic axes

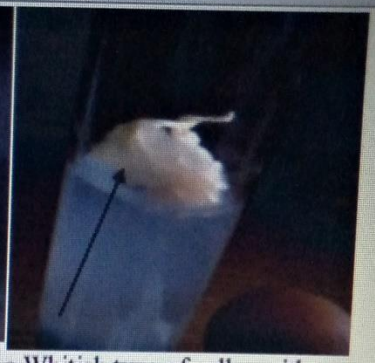
PGRs		Nature of Callus formation at one week of age	Nature of Callus formation at three weeks of age
2,4-D	Kinetin		
0.0	0.0	No callus formation	No callus formation
1.5	0.0	Whitish compact callus	Globular yellowish callus with and without pigment
2.0	0.0	Whitish compact callus	Globular yellowish callus with pigment
2.5	0.0	Whitish compact callus	Globular yellowish callus with pigment and whitish compact callus
3.0	0.0	Whitish compact callus	Globular yellowish callus with and without pigment
3.5	0.0	Whitish compact callus	Globular yellowish callus
4.0	0.0	Whitish compact callus Globular yellowish callus	Globular yellowish callus
0.0	0.5	No callus formation	No callus formation
1.5	0.5	Whitish compact callus	Globular yellowish callus
2.0	0.5	Whitish compact callus	Globular yellowish callus with pigment and whitish compact callus with and without pigment
2.5	0.5	Whitish compact callus whitish compact callus with and without pigment	globular yellowish callus with pigment
3.0	0.5	Whitish compact callus	Globular yellowish callus with and without pigment
3.5	0.5	Whitish compact callus	Globular yellowish callus with and without pigment
4.0	0.5	Whitish compact callus	Globular yellowish callus with and without pigment



a. Outgrowth of embryonic axes 3 days after inoculation

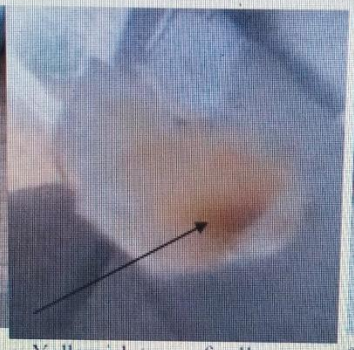


b. Whitish type of callus 1 week after inoculation



c. Whitish type of callus with no pigment after 3 weeks of inoculation

d. Whitish type of callus with pigment after 3 weeks of inoculation



e. Yellowish type of callus

f. Globular yellowish callus after 3 weeks of inoculation

g. Reddish type of callus

h. Control A (Hormone free

i. Control B (0.5KN)

Conclusions

Suitability of embryonic axes for callus induction using 2, 4-D alone or in combination with kinetin was observed in this study. Media fortified with 2mg/L 2,4-D+0.5mg/L KN is optimum for percentage callus formation. Further study is recommended to determine the suitability of this calli for shoot formation, genetic transformation as well as flavonoids production in red sorghum

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CGBPB 002

EVALUATION OF COTTON LINES AND VARIETIES FOR AGRONOMIC PERFORMANCE AND FIBRE QUALITY

Ari, A.S., *Yahaya, A.I.[1] and Mohammed, M.S.

Department of Plant Science, Institute for Agricultural Research Samaru Ahmadu Bello University, Zaria-Nigeria.
[1]Corresponding author's email: abdulyahyu@gmail.com

Abstract

Seed cotton yield in Nigeria has been declining since the 80s despite breeding efforts. In order to address this decline, twenty six cotton genotypes were evaluated for seed cotton yield at Institute for Agricultural Research (IAR) Farm Samaru, Northern guinea savannah ecological zone of Nigeria during 2017 cropping season. The experimental design used was randomized complete block design (RCBD) with three replications. Analysis of variance revealed highly significant differences (P<0.01) for the plant height, number of sympodial branches per plant, number of bolls per plant, boll weight, seed cotton yield per plant and seed cotton yield per hectare while, significant differences (P<0.05) were obtained for number of monopodial branches and lint index. Seed cotton yield per hectare had the highest genotypic coefficient of variation. High, and low broad-sense heritability estimates were obtained for number of bolls per plant, and seed index respectively. Correlation analysis revealed that plant height, number of sympodial branches, number of bolls per plant and boll weight were significant and positively correlated with seed cotton yield while, significant and negative correlation were observed between days to 50% flowering and monopodial branches, seed index with number of bolls per plant respectively. From rank summation index genotypes EZL5, MULT/ADU 14, N1, MCL2, LST14 and SAMCOT 10 obtained from Nigeria, USA, Cameroon and Egypt were the best genotypes based on the selected traits. These genotypes are, therefore, considered the best potential parents that could be used in hybridization programmes for the improvement of seed cotton yield. Although, the study provided information about the level of variability within a small subset at Samaru, a comprehensive study across multi-locations is recommended to fully explore the potentials of the genotypes.

Keywords: Cotton, Varieties, Lines, Fibre, Quality

Introduction

Cotton or white gold is the most important fibre crop on the planet earth. Cotton is used in the textile industry, as the starting point of the production chain. Nigeria is one of the leading cotton producing countries in West Africa. Cotton, until recently was fifth among the major cash crops of Nigeria (Ado *et al.* 2008). With a potential land cover of about 650,000 ha, only a third is exploited currently to produce about 150,000-300,000 tons of seed cotton by an estimated 250,000 farmers (FMARD, 2011). Insect infestation causes serious yield loss, reduction in seed and malformation of the plant parts. Yield losses of up to 50 % have been reported on cotton fields in the northwest ecological zone of Nigeria (Yahaya, 2008). As a result of damage caused by insect pests , decline in yield of IAR released cotton varieties has been a major concern to breeders in recent time, hence the need to evaluate some cotton promising lines and varieties to assess their performances with respect to their agronomic traits. The outcome of this work will help to identify promising cotton lines and varieties with better agronomic performance and equally assess the association between seed cotton yield and yield components. Correlation estimates are important in any breeding programme. Correlation coefficient gives the association of

one character with another. Hence, improvement in one trait may be helpful in subsequent improvement in related trait. The objectives of this research work were to; to evaluate some promising cotton lines and varieties for agronomic performance and, to determine the association between seed cotton yield and yield components.

Materials and Methods

The experimental materials for this study consisted of 26 cotton genotypes obtained from the cotton breeding unit of IAR during the 2016 cropping season. The list of the genotypes and their origin is presented in Table 1.

Evaluation was carried out during the 2017 cropping season at the Institute for Agricultural Research (IAR) Farm Samaru. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Four to six seeds were sown per stand which were later thinned to two plants per stand 3-4 weeks after sowing (WAS). Three supplementary hoe weeding were done with the first at 3-4 WAS and the second and the third at 6 and 9 WAS, respectively. A compound fertilizer (NPK) was applied at 4 WAS with the second application at 8 WAS at a rate of 50-kg N., 25 kg- P_2O_5, and 20-kg K_2O O per hectare. Each genotype was sown in one row of 5m length at inter and intra row spacing of 90cm and 45 cm, respectively. All the six commercial cotton varieties namely; SAMCOT 8, SAMCOT 9, SAMCOT 10, SAMCOT 11, SAMCOT 12, and SAMCOT 13 were used as checks. All other agronomic packages as recommended for cotton production by CDC (2007) were adhered to.

Table 1: Names and Origin of Genetic materials utilized in the study

S/N	NAME	ORIGIN
1.	N1	Cameroun
2.	N2	Cameroun
3.	ILO 3	India
4.	MULT/ADU 15	USA
5.	MCL 2	Egypt
6.	LST 20	Nigeria
7.	LST 6	Nigeria
8.	EZL 4	Nigeria
9.	LST 14	Nigeria
10.	LST 19	Nigeria
11	GH3	Nigeria
12.	LST 4	Nigeria
13.	NZL 1	Nigeria
14.	LST 7	Nigeria
15.	EZL 5	Nigeria
16.	MULT/ADU 1	USA
17.	ILO 2	India
18.	MULT/ADU 14	USA
19.	SCL 1	Egypt
20.	GH 2	Nigeria
21.	SAMCOT 8	Nigeria
22.	SAMCOT 9	Nigeria
23.	SAMCOT 10	Nigeria
24.	SAMCOT 11	Nigeria

25.	SAMCOT 12	Nigeria
26.	SAMCOT 13	Nigeria

Results and Discussion

The existence of significant and highly significant differences in the traits studied suggests the presence of considerable genetic variability among the genotypes depicting the possibility of improvement by selection. On the other hand, the existence of insignificant differences for some of the morphological traits suggests lack of ample genetic variability among the genotypes. The extent of variability as measured by PCV and GCV also gives information regarding the relative amount of variation. The highest coefficients of both phenotypic 760.1 and genotypic 638.7 coefficients of variation showed by SCY (kg) to SCY (g) 360.5 and 302.9 and NBP 194.9 and 169.8 (Table xy)indicate the extent of genetic diversity among the materials studied and their potential use for improvement through hybridization and selection. These results are in agreement with the findings of Sumathi and Nadarajan (1996), Jagtab and Mehetre (1998), and Rao and Reddy (2001).

The high differences between PCV and GCV indicate the influence of the environment on the expression of the traits thereby restricting the scope for their improvement through selection (Table xyz). Low values of GCV for SI, LI and DFF indicate a narrow range of variability for these traits thereby restricting the scope for selection. These results are in agreement with the findings of Girase and Mehetre (2002).

The presence of moderate to high broad-sense heritability for SCY (g), BW, NBP, PH, SB, NPP, SCY (kg) and MB (Table xyz), indicates less influence of the environment on the traits and so there is good scope for the improvement of these traits through direct selection. On the other hand, low broad-sense estimates for DFF and SI are indicative that the environment in which the plants were tested had large effects on the measured traits. It could also be as a result of the narrow genetic base of the materials. This result is in tandem with the findings of Basbeg and Gencer (2004).

Generally, the range observed in all the means of the agronomic traits also indicates presence of moderate variability among the genotypes. Best genotypes could be selected for each trait as no single genotype possesses all the desirable traits, hybridization can give a desirable improved variety. The results from the rank summation index indicated that genotypes EZL 5, MULT/ADU 14, N1, MCL2, LST 14 and SAMCOT 10 (Table xyz), which were obtained from Nigeria, USA, Cameroon and Egypt were the best genotypes for the selected traits. These genotypes are therefore considered the best potential parents that could be used in hybridization programMEs for the improvement of seed cotton yield.

Before launching any breeding programme, information regarding association among various traits is a prerequisite as it provides the opportunity for the selection of genotypes having desirable traits simultaneously (Ali et al., 2009). This result indicates that selection of plants with a good number of sympodial branches, plant height, number of bolls per plant and boll weight will indirectly result in high cotton seed yield obtainable from a plant. The positive correlation among these yield contributing traits suggested that these characters are important for direct selection of high yielding genotypes. This result is in tandem with the findings of Farooq et al., (2013) who reported positive correlation of yield with yield contributing traits in cotton. The significant positive association between NBP and PH indicates that selecting taller plants might be accompanied with an appreciable increase in the number of bolls. These results corroborate the findings of Abdullahi et al (2016) who found that with increase in plant height, number of bolls also increased. Similarly, the significant positive correlation between SB and PH indicates that seed cotton yield may be improved through increase in sympodial branches and vice-versa. These results are in agreement with the findings

of Fatih et al. (2005) who reported positive correlation of PH with SB. The results suggest that NBP, PH, SB and BW are important yield contributing traits and selection based on these traits would be effective in seed cotton yield improvement.

Table 2: Mean performance of 26 cotton genotypes for eleven a gronomic traits evaluated at Samaru in 2017.

GENOTYPES	NPP	DFF	PH(cm)	SB	MB	NBP	BW(g)	SCY(g)	SI	LI	SCY(kg)
EZL 4	9.33	68.67	120.73	17.13	1.53	52.10	49.23	150.91	2.22	1.57	670.71
EZL 5	8.33	70.33	109.60	16.70	2.03	98.07	45.72	241.31	2.30	1.61	1072.47
GH 2	12.33	68.67	97.03	15.27	2.79	45.63	25.47	151.34	2.35	1.37	672.61
GH 3	8.67	70.33	111.07	16.67	1.27	49.23	46.66	162.08	2.36	1.32	720.37
ILO 2	10.33	68.67	85.87	13.47	1.30	78.17	20.46	137.58	2.00	1.25	611.47
ILO 3	9.00	70.33	108.27	14.20	1.80	58.40	37.33	113.12	2.24	0.79	502.77
LST 14	9.00	70.33	115.20	19.97	2.23	60.10	42.47	185.48	2.31	1.09	824.36
LST 19	10.00	68.67	99.53	16.50	2.11	51.73	37.47	155.31	2.19	1.19	690.25
LST 20	8.33	68.67	99.70	16.60	2.30	58.30	39.93	170.81	2.29	0.55	759.14
LST 4	11.67	68.67	102.40	14.13	1.50	54.40	45.80	221.49	2.34	0.90	984.41
LST 6	11.00	70.33	105.27	13.60	2.03	61.57	41.67	181.79	2.39	1.21	807.94
LST 7	8.67	70.33	100.60	13.40	3.33	58.33	36.75	150.65	2.39	1.17	669.57
MCL 2	10.00	68.67	113.80	13.77	2.50	69.10	32.09	244.09	2.09	0.86	1084.83
MULT/ADU 1	11.33	70.33	113.27	17.67	1.50	66.27	42.97	166.56	2.21	1.27	740.27
MULT/ADU 14	9.00	68.67	110.17	18.00	2.47	66.70	44.25	202.59	2.35	1.45	900.39
MULT/ADU 15	8.00	72.00	120.83	17.03	1.97	76.93	38.80	157.40	2.29	1.28	699.57
N1	13.33	68.67	124.63	16.60	1.50	82.23	48.45	208.26	2.31	0.74	925.59
N2	9.67	72.00	84.80	12.30	1.43	52.00	32.66	117.11	2.22	0.75	520.47
NZL 1	9.33	72.00	95.83	13.27	2.23	62.10	43.20	152.48	2.25	1.54	677.69
SAMCOT 10	9.67	68.67	104.30	15.87	2.77	61.17	46.15	212.05	2.70	1.19	942.44
SAMCOT 11	12.67	70.33	113.27	18.00	1.97	52.63	34.37	133.72	2.48	1.41	594.31
SAMCOT 12	15.00	70.33	117.07	13.80	1.43	57.33	43.95	142.49	2.86	1.48	633.30
SAMCOT 13	13.33	72.00	75.97	6.20	2.60	60.37	17.39	75.04	2.35	1.35	333.50
SAMCOT 8	10.33	70.33	102.93	14.13	2.47	56.70	40.90	157.01	2.53	1.25	697.84

SAMCOT 9	12.67	67.00	69.53	11.20	1.87	30.73	28.14	128.73	2.75	1.47	572.12
SCL 1	11.33	72.00	72.73	11.93	2.07	36.43	32.01	105.69	2.49	1.24	469.75
MEAN	10.47	69.88	102.86	14.90	2.04	59.87	38.24	162.50	2.36	1.20	722.24
RANGE	8.0-15.00	67.0-72.0	69.53-124.63	6.20-19.97	1.27-3.33	30.73-98.07	17.39-49.23	75.04-244.09	2.0-2.86	0.55-1.61	333.50-1084.83
LSD	3.22	4.22	18.69	4.91	1.10	12.12	8.17	40.87	0.51	0.58	181.66
CV (%)	18.75	3.68	11.08	20.13	33.14	12.34	13.04	15.33	13.31	29.75	15.33

NPP = No. of Plants per Plot, DFF = Days to 50% flowering, PH = Plant height, SB = Sympodial branches, MB = Monopodiall branches, NBP = No. of Bolls per plant, BW = Boll weight, SCY(g) = Seed cotton yield per plant, SI = Seed Index, LI = Lint Index, SCY(kg) = Seed cotton yield per hectare.

Table 3: Mean squares from the analysis of variance for 26 cotton genotypes evaluated at Samaru in 2017

Mean squares												
SOURCE	**DF**	**NPP**	**DFF**	**PH**	**SB**	**MB**	**NBP**	**BW**	**SCY(g)**	**SI**	**LI**	**SCY(kg)**
Replication	2	20.55	0.96	517.87	33.18	0.19	110.76	61.82	624.81	0.05	0.05	12342.06
Genotypes	25	10.29**	5.70	657.17**	23.47**	0.82*	573.11**	212.37**	5095.41**	0.10	0.23*	100650.13**
Error	50	3.85	6.62	129.98	8.99	0.45	54.67	24.87	621.16	0.09	0.12	12270.01

NPP=No. of Plants per Plot, DFF=Days to 50% flowering, PH=Plant height, SB=Sympodial branches, MB=Monopodial branches, NBP=No. of Bolls per plant, BW=Boll weight, SCY(g)=Seed cotton yield per plant, SI=Seed Index, LI=Lint Index, SCY(kg)=Seed cotton yield per hectare,

Table 4: Estimates of error variance, genotypic, phenotypic coefficient of variation and broad sense heritability in cotton genotypes evaluated at Samaru in 2017

TRAITS	MEAN	σ_e^2	σ_g^2	σ_p^2	GCV	PCV	h_B^2
NPP	10.474	3.85	2.14	5.99	45.20	75.62	35.73
DFF	69.885	6.62	-0.3	6.32	0.00	30.07	-4.75
PH	102.862	129.98	175.72	305.7	130.70	172.39	57.48
SB	14.9	8.99	4.82	13.81	56.88	96.27	34.90
MB	2.038	0.45	0.12	0.57	24.27	52.89	21.05
NBP	59.874	54.67	172.8	227.47	169.88	194.91	75.97
BW	38.242	24.87	62.49	87.36	127.83	151.14	71.53
SCY(g)	162.503	621.1	1491.4	2112.5	302.95	360.55	70.60
SI	2.356	0.098	0.003	0.101	3.57	20.70	2.97
LI	1.204	0.128	0.036	0.164	17.29	36.91	21.95
SCY(kg)	722.236	12270	29460	41730	638.67	760.12	70.60

NPP=No. of Plants per Plot, DFF=Days to 50% flowering, PH=Plant height, SB=Sympodial branches, MB=Monopodial branches, NBP=No. of Bolls per plant, BW=Boll weight, SCY(g)=Seed cotton yield per plant, SI=Seed Index, LI=Lint Index, SCY(kg)=Seed cotton yield per hectare, σ_e^2=error variance, σ_g^2=genotypic variance, σ_p^2=phenotypic variance, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation, h_B^2=broad sense heritability.

Table 5: Rank Summation Index for 4 traits in 26 cotton genotypes evaluated at Samaru in 2017

GENOTYPES	SB	NBP	SCY(g)	SCY(kg)	RSI	RANK
EZL 5	7	1	2	2	12	1
MULT/ADU14	2	6	6	6	20	2
N1	10	2	5	5	22	3
MCL 2	18	5	1	1	25	4
LST 14	1	12	7	7	27	5
SAMCOT 10	12	10	4	4	30	6
MULT/ADU1	4	7	10	10	31	7
MULT/ADU15	6	4	12	12	34	8
LST 4	15	18	3	3	39	9
LST 20	9	15	9	9	42	10
LST 6	19	9	8	8	44	11
GH 3	8	23	11	11	53	12
EZL 4	5	20	17	17	59	13
SAMCOT 8	16	17	13	13	59	14
NZL 1	22	8	15	15	60	15
LST 19	11	22	14	14	61	16
ILO 2	20	3	20	20	63	17
SAMCOT 11	3	19	21	21	64	18
GH 2	13	24	16	16	69	19
LST 7	21	14	18	18	71	20
SAMCOT 12	17	16	19	19	71	21
ILO 3	14	13	24	24	75	22

SAMCOT 13	26	11	26	26	89	23
N2	23	21	23	23	90	24
SAMCOT 9	25	26	22	22	95	25
SCL 1	24	25	25	25	99	26

Table 6: Correlation coefficients between seed cotton yield and yield components in cotton genotypes evaluated at Samaru in 2017

Pearson Correlation Coefficients											
	NPP	**DFF**	**PH**	**SB**	**MB**	**NBP**	**BW**	**SCY(g)**	**SI**	**LI**	**SCY(kg)**
No. of Plants per Plot	1.000										
Days to 50% Flowering	-0.114	1.000									
Plant Height	-0.186	-0.089	1.000								
Sympodial Branches	-0.393	-0.254	0.749**	1.000							
Monopodiall Branches	-0.194	0.006	-0.151	-0.135	1.000						
No. of Bolls per Plant	-0.267	0.091	0.495*	0.278	-0.056	1.000					
Boll Weight	-0.286	-0.080	0.709**	0.642**	-0.214	0.238	1.000				
Seed Cotton yield per Plant	-0.269	-0.430*	0.562**	0.532**	0.080	0.533**	0.583**	1.000			
Seed Index	0.472**	-0.063	-0.122	-0.156	0.142	-0.415*	0.147	-0.123	1.000		
Lint Index	0.134	0.099	-0.046	-0.007	0.054	-0.034	-0.001	-0.133	0.306	1.000	
Seed Cotton yield/ha	-0.269	-0.430*	0.562**	0.532**	0.080	0.533**	0.583**	1.000	-0.123	-0.133	1.000

NPP = No. of Plants per Plot, DFF = Days to 50% flowering, PH = Plant height, SB = Sympodial branches, MB = Monopodial branches, NBP = No. of Bolls per plant, BW = Boll weight, SCY(g) = Seed cotton yield per plant, SI = Seed Index, LI = Lint Index, SCY(kg) = Seed cotton yield per hectare, σ_e^2 = error variance, σ_g^2 = genotypic variance, σ_p^2 = phenotypic variance, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, h_B^2 = broad sense heritability.

CONCLUSIONS

Results from this study revealed significant differences ($p < 0.05$ and 0.01) for NPP, PH, SB, NBP, BW, SCY(g), SCY(kg), MB and LI while non-significant differences were observed for the other agronomic traits . Mean performance showed considerable differences for all the traits, each character exhibited considerable range of its expression. The phenotypic variances were consistently higher than the genotypic variances for all the traits. Similarly, PCV was greater than the corresponding GCV for all the traits studied. The magnitudes of phenotypic variance suggest that improvement is possible through hybridization and selection in these traits. From rank summation index, genotypes EZL 5, MULT/ADU 14, N1, MCL2, LST 14 and SAMCOT 10 obtained from Nigeria, USA, Cameroon and Egypt were the best materials for the selected traits. These genotypes are therefore considered the best potential parent that could be used in hybridization programmes for the improvement of seed cotton yield. Highly significant and positive correlations were observed between seed cotton yield and some yield components, indicating that selection of plants with good SB, PH, NBP and BW can effectively be utilized to improve seed cotton yield.

Based on the results of the current work, important morphological traits like greater seed cotton yield potential, plant height, number of sympodial branches, number of bolls per plant and boll weight . served as a selection criterion to produce promising cotton genotypes. Genotypes EZL 5, MULT/ADU 14, N1, MCL 2, LST 14 and SAMCOT 10 obtained from Nigeria, USA, Cameroon and Egypt were the best genotypes under consideration that could be used in hybridization programmes for the improvement of seed cotton yield.

These results suggest that sympodial branches, plant height, number of bolls and boll weight are important yield contributing traits and selection based on these traits would be effective in seed cotton yield improvement.

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CGBPB 003

FLORAL BIOLOGY AND IDENTIFICATION OF THE VARIOUS GUM PRODUCING *ACACIAS* FOR ENSURING QUALITY GUM ARABIC.

Fakuta, N. M[1]., Ojiekpon, I. F[1]., Abubakar, M[1]., Jonah P. M[2]., Bichi, M[3]., Aboki I. Y[1]., Amao, A. O[4].

[1]Rubber Research Institute of Nigeria, Gum Arabic Sub-Station, Gashua, Yobe State, Nigeria
[2]Department of Crop Science, Adamawa State University, PMB 25, Mubi, Nigeria.
[3]Department of Plant Science, Faculty of Agriculture, Institute for Agriculture Research, Ahmadu Bello University, Zaria, Nigeria.
[4]Forestry Research Institute of Nigeria, Jericho, Hills Ibadan, Oyo State, Nigeria.

ABSTRACT

Identification of floral and morphological characteristics of the various gum producing Acacias in Nigeria will ensure good quality gum arabic in the market which will boost revenue from export. Gum arabic in Nigeria is harvested from a number of tree species of Acacia either by natural exudation or after tapping. The quality of gum from Acacia is very dependent on the botanical source of planting materials. The most traded gum is obtained either from Senegalia senegal, Acacia seyal and Acacia laeta. Therefore, understanding of the flower and morphological characteristics of the Acacia species in plantation and in the wild by farmers will ensure the production of quality gum in Nigeria.

Keywords: *Acacia species, gum arabic, identification, flower, seed source, morphology*

Introduction

The *Acacia* is a large genus of shrubs and trees belonging to the family Fabaceae (Dorthe, 2000). *Acacias* are present in all terrestrial habitats, including rainforests, woodlands, grasslands, coastal dunes, deserts and alpine settings. In Africa, Howe (1949) reported that *Acacia*s are widely distributed in the drier parts of tropical Africa, from Nigeria, Senegal, Sudan and Mauritania in the west to Eritrea, Ethiopia in the north-east and central and southern Africa (basically the Sahel Savannah and desert belt of the continent). It is the largest genus of flowering plants with almost 1,000 species. However, not all *Acacias* produce gum of economic importance.

Senegalia senegal [L.] Britton popularly known as "Gum arabic" produces gum of economic value. This species of *Acacia* has four varieties (Brenan, 1959, 1970, 1983; Cossalter, 1991) and is naturally adapted to the hot, dry and barren regions of Africa particularly in areas along the southern borders of Sahara desert including Nigeria (Chikamai and Odera, 2002). The crop is well adapted to the Sudan and Sahelian vegetation of Africa. The evidence from genetic variability found among *S. senegal* as well as its localised adaptation and distribution in Africa is a probably a pointer to its centre of origin and/or diversity in Africa (Aghughu, 2001). Recently scientists have shown an increasing interest on the floral and reproductive biology of *Acacias* (Diallo, 1997) for identification and selection of the best seed source. The quality of gum from *Acacia* is very dependent on the botanical source of planting materials. Thus, identifying the floral and morphological characteristics of *S. senegal* varieties by farmers in plantations and wild

will ensure the production of quality gum in Nigeria which will boost revenue from export.

Gum arabic tree species

Gum arabic in Nigeria is harvested from a number of tree species of *Acacia* either by natural exudation or after tapping. However, the most traded gum is obtained either from *S. senegal* (Dakwara), *A. seyal* (Faryn Kaya) *and Acacia laeta* (Zindi) (FAO, 2007). *S. senegal* family has four varieties namely *S. senegal* variety *senegal*, *S. senegal* variety *kerensis, S. senegal* variety *leiorhachis and S. senegal* variety *rostrata* (Brenan, 1983). In Nigeria gum from the three *Acacias* has different trade names at the International market. The gum from *S. senegal* is adjudge to be the best, i.e., "Grade one" and attract a premium prize at the international market while the gum from *A. seyal and laeta* is usually referred to as "grade two". However, there are other plant species other than *Acacia* from which gum has been collected for local use or minor shipments for export. These are *Combretum nigrican, Boswellia neglecta, B. papyifera* etc (Mokwunye and Aghughu, 2010).

Floral biology of *Acacia species*

This is the area of ecological research that studies the evolutionary factors that patterns the structures, behaviour and physiological aspects involved in the flowering of plants. The biological function of a flower is to effect reproduction in the plant. Flowers are usually borne on elongated inflorescences (groups of flower heads on a floral shoot), which produce nectar and are typically protogynous with the styles protruding above the stamen in open flowers. The protogynous flowering habit of *Acacia* makes it a highly cross-pollinated crop. The inflorescence axis opens from the base to the tips of inflorescences in 2-3 days with individual flowers consisting of a narrow corolla tube occupied by many stamens (Graham *et al.,* 1998). *Acacia* pollen usually presented in the form of polyads (compound pollen grains) with eight polyads per anther. Pollen release (dehiscence) is influenced by daily pattern of microclimate over sites where a critical low relative humidity causes anther dehiscence (Graham *et al.,* 1998). The *Acacia* flower opened in mid to late morning (900 – 1100 hours) and anther dehiscence followed immediately, giving peak pollen availability during the middle of the day (1200 - 1300) at a relative humidity of about 50 – 60% which is evidenced by the influx of daytime active flower pollinators (insect) of *Acacia* tree during this period. The stigma is cup shaped 25-30 µm deep. The bowed appearance of the stigma constitutes the receptive portion. The stigma becomes receptive two hours prior to anther dehiscence and continues to remain so for a period of 8-12 hours (Tandon *et al*., 2001). Pollination is by insect and the specie self-incompatibility (Tandon *et al*., 2010) might just be the reason for low fruits set.

In Nigeria flower starts from June-July in Sahel ecology and the peak occurs around August while in Sudan ecology flower starts from April-May and the month of July accounts for the peak flower incidence. Fruits mature between the ends of November to middle of December irrespective of the ecology. Good seed year are relatively irregular.

For most of the plantations, different *Acacia species* are found growing resulting from the source material from which the plantation was established. Harvesting gum from such a plantation may be met with the following challenges.

All trees will not produce gum when tapped.

Such plantation will have a mixture of gum due to the different *Acacia species* growing in the plantation and

The gum from such a plantation will be under priced at both local and international market.

However, with the understanding of the flower and morphological characteristics of the *Acacia species*, differentiation is possible so that even in previously established plantations, selective tapping of the right species can be done. Even in the event that all trees are tapped, sorting of gum can be done during collection to avoid missing all grades of gum.

Description of gum arabic producing trees

Based on their floral and morphological characteristics the following varieties and species are presented:
Senegalia senegal varieties are the most widely distributed of the *Acacia species* and the most important and best quality source of gum arabic. They are deciduous small to medium sized thorny tree up to 15 m high, flat to round crown, yellowish-brown to purplish black bark, with a stem which is irregular in form and often highly branched. In contrast with other members of the *A. species,* it has characteristic sets of prickles on the branches, usually in threes with the middle one hooked downward and the lateral ones curved upward. The bark ranged from papery to not papery or peeling. Leaves are pinnate. The inflorescence is usually longer than the leaves. It is spicate, 2 –12 cm long, pedunculate. The flowers are white or cream with purplish-green calyx. The pods dehiscent, yellowish to brown, flat, papery oblong to acute round 2 – 19 x 2– 4 cm. The seeds are sub circular, olive brown in colour, 8–12 mm in diameter (Orwa *et al.,* 2009). Three varieties of *S. senegal* are found in Nigeria (Ojiekpon, *et al*., 2015). These are:

S. senegal variety *senegal* is recognized as a tree with a flat or rounded crown and rough non-papery and non-peeling bark. The under bark is dark pink in colour. Trees are tall with mostly single stem. Leaves are bipinnate, small, greenish-grey, with 5 pairs of pinnae and leaflets 10 - 12 pairs. Pods are 8 – 10 cm long with 4 - 5 seeds per pod. Seasonal flowers with purplish-green calyx (Boer, 2002) and flowers emerged before the leaves are fully formed.

S. senegal variety *kerensis* - grows as a single or several-stemmed shrub with lateral branches forming near the base and with smooth yellowish-brown peeling bark on the stem. The under bark is cream-white in colour. Leaves are bipinnate and broader than the other two varieties, greenish-grey with 2-3 pair of pinnae and leaflets in 3-5 pair Pods are apical shape, 6 - 7cm long with 2-3 seeds per pods. Though seasonal flowers emerged about the same time with the leaves, variety *kerensis* produce scanty flowers that are about fifty to sixty day after variety *senegal and leiorhachis* emerged. The calyx is purplish-green in colour.

S. senegal variety *leiorhachis* - Trees are short, starting with a branched bushy base then thinning out to two slender whippy erect tall stems with open rounded crown. The barks are thin, yellowish papery and peeling with red colour under bark. Young branchlets and inflorescence axis are glabrous (without hair). Leaves are small greenish-grey, with 3- 4 pair of pinnae and 8 - 10 leaflets pairs. Pods are apical to acute round shape, 7 - 8 cm long with mostly 4 seeds per pod. Seasonal flowers emerged at the same time with leaves and the calyx is purplish-green in colour.

Acacia seyal is a small, slender tree, reaching 6 -15 m in height, with a stem diameter up to 60 cm and an adult tree develops a characteristic umbrella-shaped canopy. Bark is usually smooth, pale green to greenish yellow covered with powdery coating. Twig with many small reddish gland and paired axillary thorns up to 8 cm long, narrow and straight, sharp ended and grey in colour. Leaves are dark green, with 4 - 12 pair of pinnae having each 10 - 22 pair of leaflets. Rachis is up to 8cm long. Flowers clustered by 2-3 with bright conspicuous yellow globose head about 1.5cm in diameter on 3cm long penducle starting from leaves axis. Pods hanging, slightly curved, dehiscent, light brown when matured 10-15cm long by 1 cm wide at the bottom, containing 6 -10 seed each (Houerou, 1977a). There are two varieties of *A. seyal* that produces grade two gum arabic.

A. seyal var. *seyal*, it is pale green to grey in colour and rusty red. The bark is greyish black in colour in older trees.
A. seyal var. *fistula*, the bark is white or greenish yellow, large, straight spines occur on the branches, and smaller curved thorns are present near the tips of the branches.

Acacia laeta is a shrub or small tree which is distinct by its leaves; these are bipinnate with 3-5 pair of fairly large pinnate and 2-5 pair pinnae, the leaflets being clearly separate from each other and symmetric. Thorns in pairs of recurved axillary prickles, with sometimes a third prickle recurved forward; when the latter is missing it is replaced by a leaf. Flowers are very fragrant and white-cream in colour set out in 3-8cm long spikes, pedunculated and disposed in triplet. The pods are apiculate (ending short sharp point), a character that permit easy differentiation between the *laeta* and *senegal* (Orwa *et al.,* 2009).

Conclusion

Gum arabic in Nigeria is harvested from a number of tree species of *Acacia* either by natural exudation or after tapping. However, the most traded gum is obtained either from *A. senegal, A. seyal and Acacia laeta.* For most of the plantations, different *Acacia species* are found growing resulting from the source material from which the plantation was established. However, with the understanding of the flower and morphological characteristics of the *Acacia species*, differentiation is possible so that even in previously established plantations, selective tapping of the right species can be done. Even in the event that all trees are tapped, sorting of gum can be done during collection to avoid missing all grades of gum.

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CGBPB 004

INDUCED-GROWTH AND YIELD RESPONSES TO THREE DIFFERENT CONCENTRATIONS OF COLCHICINE ON SOME SELECTED TRAITS IN EGGPLANT (*Solanum melongena L.*)

Aminu, Y., Bichi, B.S., Tijjani, I. U. and Hassan, F. S.

Biology Department, College of Education and Preliminary studies, Kano State.
Corresponding Author: aminuyahayafagge@gmail.com+2347035347997

ABSTRACT

The effect of Colchicine-induced mutation on the growth and yield of two varieties of Eggplant was investigated. It was aimed at inducing variability that could be exploited in the improvement of some quality traits in Egg plants. Three different treatments of Colchicine were applied on to the two Egg plant varieties (White Eggplant and Green Apple). The seeds of the two varieties were treated with four different concentrations of Colchicine (0.1mM, 1.0 mM, 2.0 mM and control-0.0 mM). The result obtained revealed a highly significant difference (P≤0.01) in the effects of Colchicine on germination percentages, plant heights, survival rates and number of fruits. Significant improvement (P≤0.05) was also recorded on fruit diameter. Similarly, highly significant differences (P≤0.01) were found between the treatments in all the selected traits except on the number of fruits, where no significant differences exist. Furthermore, significant differences exist in the varietal response to the treatments except in the percentage germinations. More so, significant differences were found in the traits between the seasons except germination percent one week after planting (1 WAP), fruit number, and fruit diameter. The results showed that Colchicine improved important quality traits of tomatoes. It was deduced that the variety Green Apple eggplant responds significantly to Colchicine. It was also concluded that the mutant eggplant could be grown during the rainy season. It was concluded that 0.1 mM concentration of Colchicine improved some important quality traits of eggplant that could be utilized for further improvement of eggplant crop.

Keywords: *Colchicine, White eggplant, Green apple eggplant, Germination percentage, Plant height*

Introduction

The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits which limit their productivity or enhance their quality. The increasing demand of eggplants has gone along with the rapid growth of population. This is due to the increasing awareness toward the benefit of vegetables in fulfilling the nutrient of the family (Jumini and Marliah, 2009). According to Sowinska and Krygier (2001), eggplants contain low calorie and high nutrients. Gandhi and Sundari (2012) described that eggplant can also be utilized as medicine to reduce cholesterol in blood and it is suitable as a diet for regulation of hypertension. Owing to high nutrient content of the eggplant, it is presumed that

the demand of eggplant will increase, so that the production should be increased as well (Sowinska and Krygier, 2013).

Eggplant production has decreased for the last 3 years. In 2013, the national production of eggplants experienced a 509,380 ton reduction from 519, 481 ton in 2011 (Directorate General of Horticulture, 2014). One of the main causes of such reduction is the decreasing fertility and organic matters of the soil (Ullah *et al.*, 2008). According to Waseem *et al.* (2013), long term use of inorganic fertilizer reduces physical, chemical, and biological traits. It also reduces organic matter content in the soil thereby affecting efficiency of nutrient absorption. Excessive application of inorganic fertilizers would contaminate environment and the food yield that may harm human health (Jagatheeswari, 2013).

Therefore, some efforts are required to fulfill apart of nutrients and improve the physical, chemical, and biological traits of the so throug h the application of organic fertilizers. The demand of nutrients for the eggplant could not be fulfilled completely through the application of organic fertilizers. Sutanto (2002) described that the only application of organic fertilizer might reduce the production, whereas the use of chemical fertilizers without organic fertilizers could damage the environment.

Efforts to increase vegetable production will keep relying on the use of outer input, including organic and chemical fertilizers. These fertilizers are used to fulfill the required nutrients, particularly for the superior varieties, which are responsive to fertilizer (Suwandi, 2009).

Colchicine is a mutagen that prevents formati on of microtubules and which is usually used for doubling the chromosome number. Thus, it is routinely utilized in polyploid plant formation. Colchicine effectively functions as a "mitotic poison," leading to noticeable mutagenic effects. Many reports highlight th e mutagenic effects of colchicine on plant performance (Balkanjieva, 1980; Castro *et al.*, 2003). Colc hicine has been used to induce useful mutations in several economic ornamental plant species, such as *Datura, Portulaca, Petu nia, Allium,* and *Cucurbita.*

The resulting mutants generally produce larger inflorescences, fruits,and pollen grains, and s horter stems, (Pickens et al., 2006). Apart fro m the phenotypic traits, the mutagenic effects can be assessed more pr ecisely using molecular markers. Molecular markers are considered essential tools in detecting genetic diversity among plant species (deOliveira *et al.*, 1996). However, little information is available for using mutation in the improvement of certain plants of economic interests in Nigeria. The main objective of this research is to discover the mutagenic effects of Colchicine in some selected traits on two varieties of *Solanum melongena.*

Materials and Methods

Study Site

The research was conducted in the Green House of the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University Zaria (Lat 11^0 12^1N, Long 7^0,37^1E, Alt 550-700 m above sea level) (Anonymous, 2014).

Sources of the Seeds

Seeds of two varieties of cultivated Eggplant (*White and Green apple varieties*) were collected from the Institute for Agricultural Research (I.A.R), Ahmadu Bello University Zaria, Nigeria.

Treatment and Experimental Design

The seeds of two Egg plant varieties (*White and Green apple varieties*) were treated with four different concentrations of Colchicine (0.1mM, 1.0mM, 2.0mM and control-0.0mM)

via pre-soaking for four hours as described by Asmahan (1993). The controls were pre-soaked in distilled water. The treated plants were washed in running water for one hour and allowed to dry under room temperature for 24 hours. The seeds were then sown in polythene bags arranged in a Completely Randomized Design (CRD) with three replications and grown during the 2013 rainy season. Data were obtained on germination percentages, number of fruits/plant, diameter of the fruits, survival rate and plant height.

Data Analysis

All the data obtained were analyzed using Analysis of Variance. Where significant, means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The result of the mean effects of different concentrations of Colchicine on the selected traits of Eggplant is shown in Table 1.

Table 1: Effects of Colchicine Concentrations on the Selected Traits of Two Egg plant Varieties

Concentrations (mM)	GP % (1 WAP)	GP% (2 WAP)	SR (%)	HM(cm)	NF	FD(cm)
0.0	29.30^{d}	40.54^{d}	29.65^{d}	31.27^{d}	2.03^{d}	0.17^{c}
0.1	77.61^{a}	80.27^{a}	67.59^{a}	51.05^{a}	6.35^{a}	0.34^{a}
1.0	59.67^{b}	69.21^{b}	54.16^{b}	43.09^{b}	4.12^{b}	0.26b^{a}
2.0	44.50^{c}	57.06^{c}	42.13^{c}	38.44^{c}	2.92^{c}	0.23^{b}
Mean	52.77	59.52	48.38	40.96	3.85	0.25

N.B:*Means within the columns with the same letter(s)are not significantly different($P \leq 0.05$)
Key: GP- Germination Percentage, SR- Survival Rate, HM- Height at Maturity, NF- Number of Fruits, FD- Fruit Diameter

The result showed that 77.61% of the mutants treated with 0.1mM concentration germinated after one week of planting; and the germination rate decreased to 44.50% among those treated with 2.0 mM. Similarly, 80.27% of the mutants treated with 0.1mM germinated after two weeks of planting while 57% of those treated with 2.0mM germinated after two weeks of planting. More so, the mutants treated with 0.1mM concentrations attained the highest mean heights at maturity stage with 31.81 cm and 51.05 cm respectively. Similarly, 67.59% of the mutants treated with 1.0mM concentration survived to maturity stage while 42.13% of those treated with 2.0mM survived to maturity stage. Furthermore, the mutants treated with 0.1mM concentration produced the highest number of fruits (6 fruits/plant) with a mean weight of 16.16g. More so, the mutants treated with 0.1 mM concentration produced larger fruits that are 0.34 cm in diameter.

The distinct differences observed in most of the quantitative and qualitative traits among the Colchicine induced mutants of eggplant evaluated showed significant improvements in the selected traits. Although there were few traits with no significant differences in responses to the applied treatments. In the present investigation, the increase in number of fruits per plant due to colchicine treatments is also in conformity with the work of Adamu and Aliyu (2007) who reported increased growth and yield parameters of tomato due to Colchicine treatments. Similarly, it has been reported by Kumar *et al.* (2009) that chemical mutagens induce physiological damages (injury), gene mutations and chromosomal mutations in the organisms in M_1 generation (which can be measured by seed germination, survival reduction [lethality], plant height reduction (due to injury), fertility reduction or sterility (reduction in pod and seed formation). This also agrees with the findings of Deepalakshmi (2000) and Thanga Hamavathy (2002) who independently reported similar effects of mutagens in black gram and Kumar *et al.* (2009) in cowpea. Similar result was also reported by Nura *et al.* (2013) on the effect of chemical mutagen in improving the number of fruits and size of sesame leaves.

More so, the improvement in the quality traits of tomato due to Colchicine treatments is in conformity to the work of Adamu *et al.* (2002) when groundnut was treated with gamma rays and Sheeba *et al.* (2005) when gamma rays and EMS were used to treat *Sesanum indicum* L. where seed germination, survival, plant height and pollen fertility were reduced significantly with an increase in dosage levels of both mutagens. However, in contrast, Sasi *et al.* (2005) showed that all plant mutant types registered lower yields compared to their parents in the study of the effects of diethyl sulphate and EMS on Okra (*Abelmoschus esculentum* (L.) var. MDU-1).

The increase in fruit quality on fruit number due to induced mutagenesis by Colchicine signifies the vital role played by the mutagen in improving the quality traits of Eggplant.

Conclusion

It was concluded that there is significant difference in the effects of various concentrations of colchicine on the selected quality traits of eggplant. The effect of the mutagen is significant in inducing variability that could be exploited in the improvement of highly economic crops like eggplant. Lower concentration of Colchicine (0.1 mM) was found to be more effective in improving the quality traits of Eggplant. It was also concluded that the mutant Egg plant can be grown all year round in the rainy season.

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CGBPB 005

VARIABILITY, HERITABILITY AND GENETIC ADVANCE OF YIELD AND RELATED CHARACTERS IN OKRA

David, Grace S., Iwo, G. A., Ittah, Macauley A. and Funyowei, Felicia D.

Department of Crop Science, University of Calabar, Calabar, Nigeria.
Corresponding Author: graceukoha@gmail.com +2348066292593

ABSTRACT

Okra (Abelmoschus esculentus (L.) Moench) is an important vegetable crop grown in various countries of the world. Exploiting the genetic variability is important for the continuous improvement of this crop. A field experiment was carried out in Calabar in 2016 to assess the variability, heritability and genetic advance of yield and related characters in selected genotypes of okra. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data collected on both morphological and reproductive traits were subjected to analysis of variance which was used to partition the gross (phenotypic) variability into the components due to genetic (hereditary) and non-genetic (environmental) factors and to estimate the magnitude of these. The results obtained from this study showed considerable variability among the genotypes for plant height at all sampling periods except 4 weeks after planting (WAP) and number of days to 50% flowering. Phenotypic coefficient of variation ranged from 4.78 – 24.05 and genetic advance (GA as % of mean) ranged from 8.35 – 36.67 with the lowest value recorded in number of days to 50 % flowering and the highest in plant height at 6 WAP. The highest heritability of 85% and 77% were recorded in number of days to 50% flowering and plant height at 8 WAP respectively, this was closely followed by plant height at 6 WAP with heritability value of 74 %. This shows that selection is possible for these traits and could be exploited for further breeding studies.

Keywords: *Okra, variability, heritability, trait, selection.*

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop that is grown in the tropical and sub-tropical regions of the world (Schippers, 2000). India ranked the highest in okra production with 6,126,000 tonnes, Nigeria comes second with 1,978,286 tonnes yearly production and Sudan third. Total okra production worldwide is 9,872,826 tonnes (FAOSTAT, 2020). Okra is of economic importance because the fruits, buds, flowers are often eaten. It is also medicinal and the leaves can serve as feed to animals (Siesmonsa and Kouame, 2004). Genotypes show variability in various characters such as yield, number of days to first flowering, number of pods per plant and height (Jagan *et al*., 2013). Heritability and genetic advance are suitable measures for accessing the genetic portion of total variability and this aids selection for various characters. Adeoluwa and Kehinde (2011) in their study on 35 accessions of okra observed a wide variability for all characters except leaf and petal colour. Phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) thus revealing the effect of environmental factors.

High PCV and GCV were observed for pod yield per plant and peduncle length respectively. Likewise, Nwangburuka *et al.,* (2012) reported high GCV broad sense heritability and genetic advance for plant height, fresh pod length and width, number of branches and pod weight per plant.

Okra is an important vegetable crop in Nigeria and West Africa and to meet the increasing demand for this crop, it is important to evaluate various genotypes also, assessing variability for continuous improvement of this crop. The objective of this study was to assess the genetic variability in some okra genotypes and identify traits that will provide basis for selection.

Materials and Methods

The experiment was carried conducted at Agricultural Development Project (ADP), Calabar, Cross River State from April – August 2016. Calabar has a bimodal annual rainfall of about 2000–3500mm and temperature ranging from 27 – 35 ^{o}C. The experiment was laid out in a randomized complete block design (RCBD) with three replications. A total of 17 genotypes were used. Seeds were sown at a spacing of 40 x 30 cm giving a plant population of 8,333 plants per hectare. All plots received ring application of NPK 15:15:15 3 weeks after planting (WAP). Weeding was also done manually at 3 and 6 WAP. Pods were harvested every 2 – 3 days at maturity. Data was collected on the following: numbers of days to 50% emergence, plant height, number of leaves, leaf area, leaf area index, number of days to 50 % flowering, number of pods, fresh weight of pods. Data collected was analyzed using genstat 10.3 which was used to partition variability into components due to genetic and non - genetic factors. Variance components (PCV, GCV and error variances) were estimated according to Uguru (1995) using the formula:

$$Vg = \frac{Msg - Mse}{r}$$

$$Vp = \frac{Msg}{r}$$

$$Ve = \frac{Mse}{r}$$

where Msg, Mse and r = mean squares of genotype, error and replication respectively. PCV and GCV and H^2bs were computed according to Allard (1960) as:

$$PCV = \frac{(Vp)^{0.5}}{x} \times 100$$

$$GCV = \frac{(Vg)^{0.5}}{x} \times 100$$

$$H^2bs = \frac{Vg}{Vp} \times 100$$

$$GA = k \times sp \times H^2bs$$

where Vp, Vg and x = phenotypic variance, genotypic variance and grand mean for the trait under consideration.

H^2bs, GA, k and sp= Heritability in broad sense, genetic advance, constant 2.06 at 5 % selection pressure and phenotypic standard deviation respectively.

Results and Discussions

Results showed considerable variation among traits. Mean square genotype was generally higher than mean square error for the traits studied (Table 1). Phenotypic variances were generally higher than the genotypic variances for the characters studied (Table 2). This is due to the environmental influence, as phenotypic variance is a component of both genetic and environmental factors. The highest phenotypic and genotypic variance in the characters considered was observed and recorded for plant height at 12 WAP. PCV

generally ranged from 4.78 -24.05 with the highest PCV for plant height at 6 WAP and the lowest in number of days to 50 % flowering respectively (Table 3). Similarly, the genotypic

coefficient of variability GCV ranged from 4.41 - 20.66 with the highest GCV in plant height at 6 WAP.

Generally, broadsense heritability varied from 66 – 85 % with the lowest value in number of days to 50 % flowering and the highest for plant height at 6 WAP. Also, genetic advance (% of mean) ranged from 8.35 for number of days to 50 % flowering to 36. 64 for plant height at 6 WAP respectively. Genetic variability is useful in selection and overall crop improvement. The significant differences observed in the performance of genotypes for the traits under consideration is an indication of variability which implies that this crop can be improved by selection, hybridization and other breeding methods.

Table 1: Mean squares (genotype and error), variance ratios and grand mean of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	Mean squares			
	Genotype	Error	Variance ratio	Mean
Number of days to 50 % flowering	25.00	3.76	6.65**	60.33
Plant height at 6 WAP	70.72	18.52	3.82*	20.19
Plant height at 8 WAP	81.38	18.89	4.31**	25.49
Plant height 10 WAP	111.48	37.64	2.96*	33.77
Plant height 12 WAP	158.03	49.47	3.19*	39.35

* , ** Significant at 5 % and 1% level respectively

Table 2: Phenotypic (Vp), genotypic (Vg) and error (Ve) variances of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	Vp	Vg	Ve
Number of days to 50 % flowering	8.33	7.08	1.25
Plant height at 6 WAP	23.57	17.40	6.17
Plant height at 8 WAP	27.13	20.83	6.30
Plant height 10 WAP	37.16	24.61	12.55
Plant height 12 WAP	52.68	36.19	16.49

Table 3: Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) broad sense heritability (H^2bs) and genetic advance (GA) of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	PCV	GCV	H^2b (%)	GA	GA (% Mean)
Number of days to 50 % flowering	4.78	4.41	85	5.04	8.35
Plant height at 6 WAP	24.05	20.66	74	7.4	36.67
Plant height at 8 WAP	20.43	17.91	77	8.26	32.41
Plant height 10 WAP	18.05	14.69	66	8.29	24.54
Plant height 12 WAP	18.44	15.29	69	10.32	26.22

This result agrees with Nwangburuka *et al.,* (2011) who reported high variability among some okra genotypes. From this study, the highest PCV and GCV was observed in plant height at 6 WAP and the lowest in number of days to 50 % flowering which is an indication of high variability for these traits. The highest estimate of genetic advance was observed in plant height at 12 WAP while the lowest was observed in number of days to 50 % flowering. Furthermore, there was high heritability estimates for number of days to 50% flowering and plant height at all sampling periods, this suggests that the genotypic factor had greater effect on the phenotypic performance of these traits. Hence, selection based on the phenotypic performances of these characters will be reliable and effective. Similarly, the highest estimate of genetic advance was observed in plant height at 12 WAP while the lowest value was observed in number of days to 50 % flowering. The high GCV observed in the characters suggest very strong inherent association between characters at genetic level. This is similar to the findings of Ibrahim and Hussein (2006) on roselle (*Hisbiscus sabdariffa).* From this study, variability shows that there are considerable potentials in improvement of this crop. Any trait with high heritability and genetic advance shows the presence of additive genes and the transmissibility of this trait to subsequent generations.

Conclusion

Okra is an important vegetable crop with great potentials for improvement. The variability observed among genotypes from this study is a clear evidence which is also in consonant with reports from other researchers. For further studies, more emphasis should be given to number of days to 50 % flowering and plant height at 6 WAP.

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CGBPB 006

YIELD PERFORMANCE AND STABILITY OF SOME INTRODUCED CASTOR HYBRIDS IN THE SAVANNA AGRO-ECOLOGY OF NIGERIA

Salihu, B. Z.[1*], Ajaye, O. F.[1], Okoh, J.[2], Tanimu, A. M.[3], Ehirim, B. O.[1], Gbadeyan, T.[1], Apuyor, B. O. [1], Kabaraini, M. A.[1], Ishaq, M. N.[1] Salahu, M. S.[1], and Shehu, H.[1]

[1]National Cereals Research Institute Badeggi, Niger State, PMB 8, Bida, Niger State
Corresponding Author: bz.salihu2007@gmail.com +2347067739913

ABSTRACT

Castor (Ricinus communis L.) is an industrial oil crop that is widely recognized as an ideal crop for tropical and subtropical regions. In this research, three introduced castor hybrids (Code 11, Code 22 & Code 33) and two local checks (NCRICAS2 & NCRICAS2) were assessed for their yield performance and stability in Nigeria. The castor genotypes were evaluated during the 2019 raining season at six locations across the Southern and Northern Guinea savanna ecologies of Nigeria. The experimental design was Randomized Complete Block Design and standard agronomic practices for castor were followed. The results revealed significant differences for traits assessed among the genotypes. Average seed yield among the hybrids ranged between 596.58 kg/ha and 1372.49 kg/ha. Average seed yields of the checks ranged between 849.58 kg/ha and 1239.63 kg/ha. The hybrid Code 33 out yielded the two local checks at four locations (i.e Badeggi, Benue, Kebbi & Sokoto) out of the six locations. The check 1 (NCRICAS2) recorded a significantly higher yield (1705.28kg/ha & 1648.15kg/ha) than the hybrid Code 33 (1573.91kg/ha & 867.58kg/ha) at Bacita and Bauchi locations. Besides recording comparable seed yield to the best hybrid (Code 33), the local check 1 (NCRICAS2) matured earlier than the hybrids evaluated. The GGE biplot view revealed good stability and responsiveness for both hybrid Code 33 (G5) and NCRICAS2 (G2). Based on performance per se, the hybrid Code 33 can be introduced to Nigerian farmers. However, cost benefit analysis of the hybrid(s) in comparison to the local varieties is highly recommended.

Keywords: *Castor, Yield, Stability, Hybrid, Nigeria*

Introduction

Castor (*Ricinus communis* L.) is one of the neglected oil crops with high economic values around the world (Gana *et al.*, 2013). Its production contributes notable foreign exchange credits to the economies of many countries, including India, Brazil and China (Ibeagha and Onwualu, 2015). Out of 1,512,761.80 metric ton of world castor production, Nigeria contributes only about 0.26% of the production with local consumption rate of castor oil and its derivatives being estimated at about 300,000 tonnes per year (FAO, 2013; Ibeagha and Onwualu, 2015; Salihu *et al*,. 2014). The potential of Nigeria to exploit this economic opportunity can be harnessed only if there are adequate research attentions, addressing some of the problems affecting castor production in the country. Therefore in this study, (three Farm Tech Biogene) FTB Castor Hybrids and two local checks (NCRICAS2 & NCRICAS1) were assessed for their yield performance and stability at six locations in Nigeria.

Materials and Methods

The castor genetic materials used were three hybrids (Code-11, Code-22 and Code-33) from Farm Tech Biogene Pvt. Ltd., India and two open pollinated local checks (NCRICAS2 & NCRICAS1) from National Cereals Research Institute, Badeggi, Nigeria. The castor genotypes were evaluated during the 2019 raining season at six locations across the Southern and Northern Guinea savanna ecologies of Nigeria. The locations are NCRI Badeggi, Niger State; NCRI Kebbi Station, Kebbi State; University of Agriculture Makurdi, Benue State; NCRI Bacita, Kwara State; Kafin Madaki, Bauchi State and Uthman Danfodio University Sokoto, Sokoto State. The experimental design was Randomized Complete Block Design. The entries were evaluated on a plot size of 9m x 7.5m with inter-row and intra-row spacing of 0.9m and 0.75m respectively. Data were taken on seedling establishment, days to flowering, days to first raceme maturity, height at maturity (cm) and seed yield (kg/ha). Data were subjected to analysis of variance. Stability analysis through Genotype, Genotype by Environment (GGE) Biplot was done following the procedure of Plant Breeding Tools Package (PBTools 1.3).

Results and Discussion

The seed yields of the hybrids and the checks for individual locations are presented in Table 1. Genotype average yield across the locations ranged from 1282.11 kg/ha to 674.07 kg/ha. The hybrid Code 33 out yielded the two local checks at four out of six locations. The check 1 (NCRICAS2) recorded significantly higher yield (1705.28 kg/ha & 1648.15 kg/ha) than the hybrid Code 33 (1573.91 kg/ha & 867.58 kg/ha) at Bacita and Bauchi locations (Table 1). The Check 2 (NCRICAS1) significantly out yielded all the hybrids at Bauchi location (Table 1). The hybrid Code 11, out yielded the two checks at Badeggi, Makurdi and Sokoto. The hybrid Code 22 recorded least yield among the hybrids and it only out yielded the Check 2 (Acc.001) at one location (Sokoto).

The hybrid Code 11 recorded a potential yield of 1509.98 kg/ha at Makurdi. Code 33 and Code 22 had potential yields of 1798.47 kg/ha and 1002.53 kg/ha, at Sokoto (Table 1). Average seed yields of the entries across the locations are presented in Table 2. The hybrid Code 33, Code 22 and Code 11 had average seed yields of 1372.49 kg/ha, 596.58 kg/ha and 1146.47 kg/ha respectively (Table 2). The best hybrid (Code 33) had a non-significantly different average yield to local check 1 (NCRICAS2). However, the hybrid (Code 33) showed 10.72% yield advantage over the local check 1 (NCRICAS2). Hybrid Code 11 and Code 33 had a percentage yield advantage of 34.95% and 61.55% respectively over the local check 2 (NCRICAS1) (Table 2). Hybrid Code 22 recorded lower seed yield than the two checks. Besides recording comparable seed yield to the best hybrid (Code 33), the local check 1 (NCRICAS2) matured earlier than all the hybrids evaluated (Table 2). The GGE biplot view revealed good stability and responsiveness for the hybrid Code 33 (G5) and Check 1 (NCRICAS2) (G2) (Figure 1). The Figure 2 presents What-Won-Where Biplot for the yield performance of the entries. Hybrid Code 33 performed best in location E2 (Badeggi), E4 (Kebbi), E5 (Makurdi) and E6 (Sokoto). The Check 1 (NCRICAS2) performed best in location E1 (Bacita) and E3 (Bauchi). Genotype by environment (GE) interaction is a major problem in the yield stability of a variety because it complicates the process of selecting ideal genotypes with reliable yield performance prediction (Delacy *et al.*, 1996; Yan, 2002). The results presented in this study is similar to that reported by Sakhare *et al.* (2018) on mean performance and stability of castor hybrids in Vidarbha region of Maharashtra state, India.

Conclusions and Recommendations

From the results of the trials, hybrid Code 33 outperformed the local checks in four out of the six locations. However, the average seed yield of the best hybrid (Code 33 = 1372.49 kg/ha) and that of the local check 1 (NCRICAS2 = 1239.63 kg/ha) are comparable and not significantly different. Based on performance per se, the hybrid Code 33 can be introduced to Nigerian farmers. However, cost benefit analysis of the hybrid(s) in comparison to the local varieties is highly recommended.

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Table 1: Mean Values for Seed Yield (Kg/ha) of the introduced Castor Hybrids and Two Local Checks at Six Locations in Nigeria

GENOTYPES	BACITA	BADEGGI	BAUCHI	KEBBI	MAKURDI	SOKOTO
NCRICAS2 (Check 1)	1705.28	884.45	1648.15	1055.56	908.13	1234.51
NCRICAS1 (Check 2)	945.15	858.43	1138.88	314.81	896.09	920.50
Code11	1190.95	1464.04	592.60	666.67	1509.98	1454.57
Code22	843.57	486.42	350.93	185.19	710.85	1002.53
Code33	1573.91	1641.81	867.58	1148.15	1205.04	1798.47
Mean	1251.77	1067.03	919.63	674.07	1046.02	1282.11
SE-Mean	47.03	109.33	39.31	117.27	139.55	91.42
LSD	108.45	252.1	90.81	270.42	321.79	211.00

Note: DF = Days to 50% Flowering, RPP = Racemes per Plant, DM – Days to Maturity, HM = Height at Maturity, SY = Seed Yield, % YA = Percentage Yield Advantage

Table 2: Combined Means for Agronomic Traits of the Introduced Castor Hybrids and Two Local Checks at Six Locations in Nigeria

GENOTYPE	DF	RPP	DM	HM (cm)	SY (Kg/ha)	% YA Over Check 1	% YA Over Check 2
NCRICAS2 (Check 1)	45.53	17.68	93.59	124.01	1239.63		
NCRICAS1 (Check 2)	66.95	11.66	111.11	124.05	849.58		
Code11	54.83	18.19	106.06	139.85	1146.47	-	34.95
Code22	64.33	11.65	116.83	147.91	596.58	-	-
Code33	53.72	13.31	107.06	135.35	1372.49	10.72	61.55
Mean	57.07	14.50	106.93	134.23	1040.95		
S.E. Mean	1.45	1.50	2.52	11.65	90.65		
LSD	3.52	2.71	4.74	NS	209.10		

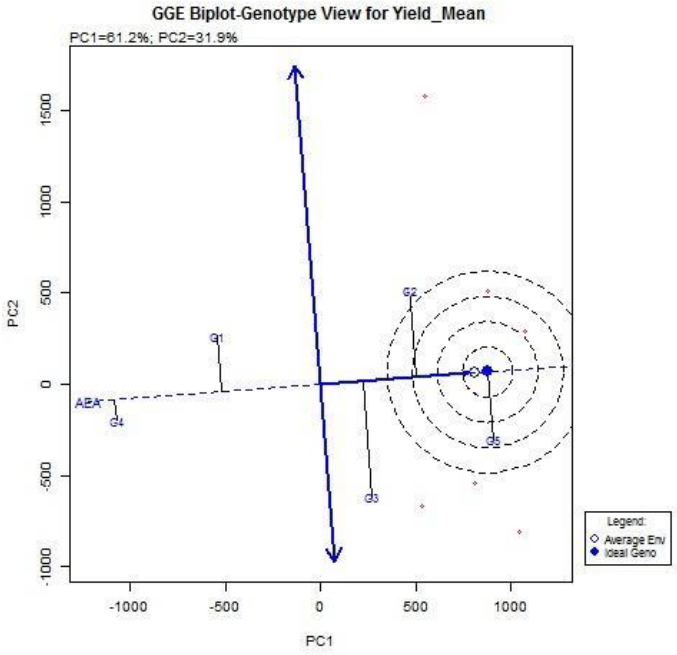

Figure 1: Mean performance and stability of three castor hybrids and two local checks in terms of grain yield as measured by principal components across different locations

Note: NCRICAS1 (Check 2) = G1, NCRICAS2 (Check 1) = G2, Code11 = G3, Code22 = G4, Code33 = G5

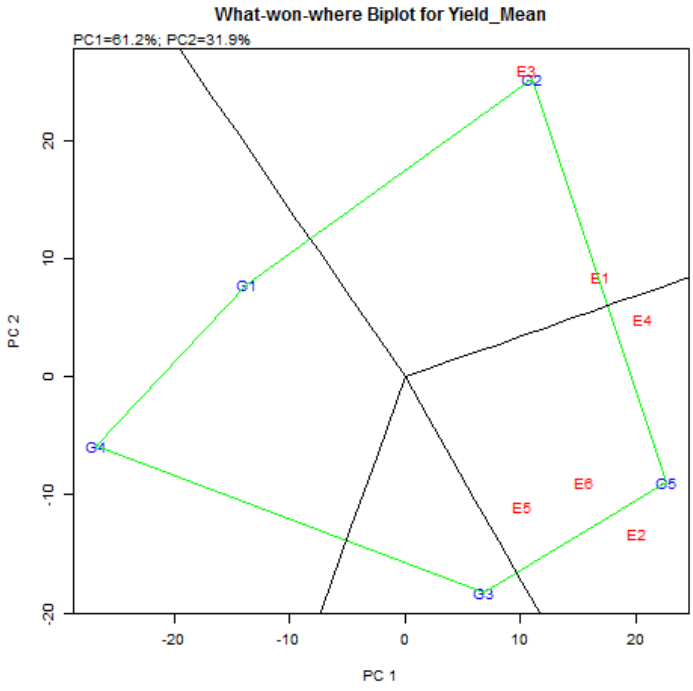

Figure 2: The which-won-where view of the GGE biplot showing which genotypes performed best in which environment

Note: NCRICAS1 (Check 2) = G1, NCRICAS2 (Check 1) = G2, Code11 = G3, Code22 = G4, Code33 = G5, BACITA = E1, BADEGGI = E2, BAUCHI = E3, KEBBI = E4, MAKURDI = E5, SOKOTO = E6

CGBPB 007

GENETIC VARIABILITY FOR ESTIMATED GLYCEMIC INDEX IN COWPEA (*Vigna unguiculata* [L.] Walp)

[1]*Umar, A.A., [2]Ishiyaku, M.F., [2]Umar, M.L. and [2]Usman, I.S.

[1]Department of Crop Production Technology, Audu Bako College of Agriculture, Dambatta
[2]Department of Plant Science, Ahmadu Bello University, Zaria
*Corresponding Author E-mail/Tel: auwaluumaradamu@gmail.com; 07034668835

ABSTRACT

Thirty diverse cowpea genotypes were evaluated with a view to study genetic variability for glycemic index, agronomic and nutritional traits. The field experiment was conducted at Audu Bako College of Agriculture Dambatta during the 2017 rainy season. The experiment was laid out in randomized complete block design with three replications. Proximate analysis was conducted at the Product Development Research Programme of the institute for Agricultural Research Samaru, Zaria. Analysis for total starch and glycemic index were done at the Biotechnology laboratory of the Department of Plant Science, ABU, Zaria. Data were collected from field plants on days to 50% flowering (DFF), days to 95% maturity (DT95%M), grain yield kg ha^{-1}, and fodder yield kg ha^{-1}. Data were recorded on moisture content (%), ash content (%), lipid content (%) , crude protein content (%) , carbohydrate content(%), total starch, crude fibre content (%) , phytate content and estimated glycemic index. Analysis of variance revealed significant differences among the genotypes for all characters except percentage ash content. Nutritional traits such as protein content(%), carbohydrate content(%), total starch and fibre content(%) exhibited large variations. Although there was a wide range of variation (43.3-55.3) in the estimated glycemic index (GI), all the genotypes studied are within the range of 0 - 55 (i.e. low GI food). High estimates of heritability (90.0-97.4) along with genetic advance as per cent of the mean (22.2-102.3) were observed for the characters days to 50% flowering , days to 95% maturity, dry fodder yield kg ha^{-1}, moisture content(%) , lipid content(%), protein content(%), total starch, fibre content(%) and phytate content. This indicates that selection will be effective for improving these traits.

Keywords: *Glycemic index, Cowpea, Variability, Genetic advance.*

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp) is an important leguminous crop grown in Semi-arid tropics covering Africa, Asia, Southern Europe and Central and South America. FAO (2002) reported that cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics. The crop is grown mainly for its grains (Fatokun, 2002). Cowpea grain contains about 53-66% carbohydrate most of which is found in the form of starch (Hoover *et al.*, 2010). It is a major source of protein in the diet of many people in sub-Saharan Africa. According to Kamai *et al.* (2014), cowpea supplies about 40% of the daily protein requirements of most people in Nigeria. The total protein content of its grain ranges from 23% to 32% (Cruz *et al.,* 2014). The crop is also a good source of minerals and vitamins. Adams (1984) and Achuba (2006) reported that, the calcium and iron content of cowpea are higher than that of meat, fish and egg, and

the vitamins- thiamin, riboflavin, niacin (water soluble) and their levels compare with that found in lean meat and fish.

Glycemic index (GI) is a concept introduced to classify foods on the basis of their postprandial blood glucose response (Jenkins *et al.,* 1981). It is usually determined by measuring the effect of 50 g available carbohydrate of a test food on blood glucose when compared with that of a control food, usually glucose or white bread (Englyst *et al.,* 1999). GI of 0 -55 has been classified as low, 56 -70 as medium, and >70 as high (Foster-Powel *et al.,* 2002). The concept of GI has been developed to supplement information available on the chemical composition of foods given in food tables. The FAO/WHO expert suggested the use of GI concept for classifying carbohydrate- rich food to guide choice for maintenance of health and disease control (FAO, 1992). According to Gilbertson *et al.* (2003); Wylie-Rosett *et al.* (2004) and Liu (2006) low postprandial glucose concentration and diets with a low glycemic index are associated with a reduced risk for the development of diabetes mellitus, obesity and cardiovascular disease. Low GI foods raise blood sugar slowly and steadily giving continuous energy, while high GI foods induce a sharp rise in blood glucose, which declines within a short period of time (Ludwig, 2002).

Previous studies indicate that cowpea is a low GI food. However, data on variability and heritability for GI is not available. T herefore, the objective of this study was to estimate genetic variability and heritability for GI , agronomic and nutritional traits in some selected cowpea genotypes.

Materials and Methods

Thirty diverse genotypes collected from the cowpea breeding unit of Institute for Agricultural Research (IAR) Ahmadu Bello University Zaria were used in this study (Table 1). The field experiment was laid out in a randomized complete block design (RCBD) with three replications during the 2017 rainy season at Dambatta. Each plot consisted of four ridges of 5 m long. Three seeds were sown at the spacing of 40 x 75 cm later thinned to two per stand. NPK 15:15:15 fertilizer at the rate of 150 kg ha^{-1} was applied at planting. Manual hoe weeding was done at 3, 6 and 9 weeks after sowing (WAS) to control weeds. One of the recommended insecticide lambda cyhalothrin (Karate 2.5EC) was applied fortnightly with a knapsack sprayer to control flower and pod boring insects starting from vegetative through flowering and podding stages. Observations were recorded on 13 characters namely days to 50% flowering (DFF), days to 95% maturity (DT95%M), grain yield kg ha^{-1}, fodder yield kg ha^{-1}, percentage moisture content, percentage ash content, percentage lipid content, percentage crude protein content , percentage carbohydrate content, total starch, percentage crude fibre content , phytate content and estimated GI . Days to 50% flowering and days to 95% maturity were recorded on a per plot basis. Grain yield kg ha^{-1} was obtained by threshing and weighing of all the plugged pods from each plot and the value obtained was converted to per hectare basis. Fodder yield kg ha^{-1} was obtained by sun-drying stem and leaves from each plot to a constant weight. The weight obtained was converted to per hectare basis. Percentage Moisture, percentage ash, percentage lipid, percentage protein, and percentage fibre were determined according to AOAC (1990). Total carbohydrate was calculated by the difference method (summing the value of moisture, crude protein, ash and crude fat (ether extract) and subtracting the sum from 100) (Pearson 1976). Phytate content was determined according to the method of Wheeler and Ferrel (1971). Total starch and estimated GI were determined by following the method of Goni *et al.,* (1997).

All the data collected were subjected to analysis of variance (ANOVA) while significant means were separated with Duncan's Multiple Range

Test (DMRT) using SAS (2005). Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953). Heritability in a broad sense was estimated as per the formula suggested by Allard (1960) and g enetic advance was estimated as per the formula proposed by Lush (1940) and Johnson *et al.* (1955).

Results and Discussion

The analysis of variance (Table 2) revealed significant differences among the genotypes assessed for all characters measured except percentage ash content suggesting the presence of a good amount of variation. Agronomic characters such as days to 50% flowering, days to 95% maturity, grain yield kg ha^{-1} and dry fodder yield kg ha^{-1} had a wide range of variation suggesting the presence of variability for these characters and an opportunity to select better genotypes to exploit yield in cowpea. Similar results were reported by Sawant (1994) and Khan (2015). Large variability was also observed for most nutritional traits: percentage protein content, percentage carbohydrate content, total starch and percentage fibre content. Although, there is a wide range of variation (43.3 -55.3) in the estimated GI , all the genotypes studied were within the range of 0 - 55 (i.e low GI food).

The total variation present in a population arises due to genotypic and environmental effects. Hence, it is necessary to split the overall variability into its heritable and non-heritable components resorting to estimation of genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). In the present study, estimates of PCV were higher than GCV for all the characters. The PCV and GCV were classified as suggested by Sivasubramanian and Madhavamenon, (1973) into low (0-10%), moderate (10.1-20%) and high (>20%). As per the classification, PCV were high for days to 50% flowering, days to 95% maturity, grain yield kg ha^{-1} and dry fodder yield kg ha^{-1}, percentage fibre content and phytate content (Table 3). Wide variation in days to pod maturity has been reported by Nwosu *et al.* (2013). Percentage m oisture content, lipid content, protein content, ash content and total starch have moderate PCV. While percentage carbohydrate content and estimated GI showed low PCV. Similarly, high GCV was observed for days to 50% flowering, days to 95% maturity, grain yield kg ha^{-1} and dry fodder yield kg ha^{-1}, percentage fibre content and phytate content. While, p ercentage m oisture content, lipid content, protein content and total starch exhibited moderate GCV. Low GCV was observed for percentage a sh content, carbohydrate content and estimated GI .

All the characters studied except percentage ash content recorded high heritability estimates indicating that selection would be effective in improving these traits. High heritability indicates high scope of genetic improvement of these characters through selection. Findings of Karpe *et al.* (2006) and Chaudhari *et al.* (2013) also reported high heritability in the case of protein content. In case of grain yield, high heritability was reported by Guptha, (2010) and Olawale and Bukola (2016).

Genetic advance as percent of the mean was categorized as suggested by Johnson *et al.*, (1955) as low (0-10%), moderate (10.1-20%) and high (>20%). High genetic advance as percent of the mean was observed for all the traits except percentage ash content, carbohydrate content and estimated GI. For percentage carbohydrate content, genetic gain was moderate. While, for percentage ash content and estimated GI genetic gain was low (Table 3). High genetic advance for most of the characters in cowpea indicates that improvement of these characters is possible by the selection. Dry fodder and grain yield kg ha^{-1} had high genetic advance , indicating that gain in dry fodder and grain yield kg ha^{-1} could be expected if judicious selection is exercised. Chaudhari *et al.* (2013) also reported high genetic advance for grain yield per plant.

High estimates of heritability along with genetic advance as per cent of mean for the characters days to 50% flowering , days to 95% maturity, dry fodder yield, kg ha-1, percentage moisture content, percentage lipid content, percentage protein content, total starch, percentage fibre content and phytate content. This indicates that selection will be effective for improving these traits. High heritability in conjunction with high genetic advance in case of seed protein content was reported by Ushakumari *et al*. (2010). In the case of days to 50% flowering and grain yield, Manggoel (2012) reported high estimates of heritability for these traits.

Moderate to high heritability estimates and low to high genetic advance as percent of mean observed for characters; percentage carbohydrate content, percentage ash content and estimated GI indicate the presence of non-additive gene action and influence of environment in the expression of these characters and thus, the selection would be less effective.

Conclusions

High magnitude of the PCV , GCV , heritability and genetic advance was observed for all the agronomic traits studied here. Also nutritional traits such as protein, fibre and lipid content exhibited high heritability and with high genetic gain. This suggests that selection will be effective for improving these traits. Estimated GI showed high heritability with low genetic gain indicating that selection will be less effective in this trait.

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Table 1: Origin and description of cowpea genotypes used in the study

S/n.	Genotypes	Origin	Coat Colour	Coat Texture	Seed Size	Maturity
1.	Babban wake	IAR	White black eye	Rough	Large	Late
2.	Bodeje	IAR	Cream-brown	smooth	small	Early
3.	Borno local	IAR	Brown /pink	Rough	Large	Late
4.	Dan wuri (A)	IAR	White	Rough	medium	Late
5.	Dan misra	IAR	White	Rough	Large	Late
6.	Farin wake	IAR	White	Rough	medium	Early
7.	Jan wake	IAR	Brown	Rough	Large	Late
8.	Jan wake Dan wuri	IAR	Brown	Rough	Medium	Late
9.	Kanannado brown	IAR	Brown	Rough	Large	Late
10.	L P 5	IAR	Dark brown	Smooth	small	Early
11.	Mai bargo	IAR	Brown with darker brown back	Rough	Large	Late
12.	Nabbewela	IAR	Brown with small dark brown spots	Rough	Large	Late
13.	NG/Lo/Bo/11/006/ 11	IAR	Brown	Rough	large	Late
14.	NG/Lo/Bo/11/014/ 11	IAR	White black eye	Rough	Large	Late
15.	NG/Lo/Bo/11/019/ 11	IAR	Brown	Rough	Medium	Late
16.	NG/Lo/Bo/11/023/ 11	IAR	Brown	Rough	medium	Late
17.	Sampea 7	IAR	Brown	rough	Large	Early
18.	Sampea 8	IAR	White /black eye	Rough	Large	Late
19.	Sampea 9	IAR	White	Rough	medium	Late
20.	Sampea 10	IAR	White /black eye	Rough	medium	Early
21.	Sampea 14	IAR	White red eye	Rough	Large	Early
22.	Tvu 15204	IAR	Cream-brown	smooth	medium	Late
23.	TVU 1560	IAR	White	rough	medium	Late
24.	Tvu 15892	IAR	Dark brown with spots	Smooth	medium	Early
25.	Tvu 15107	IAR	Brown	Smooth	medium	Early
26.	Tvu 16393	IAR	Brown	Smooth	Large	Late
27.	Tvu 16575		Pink	Smooth	Small	Early
28.	Tvu 1801	IAR	Dark brown with spots	Smooth	medium	Early
29.	Tvu 8455	IAR	Cream	Smooth	Small	Early
30.	Yarwaja	IAR	White green eye	Rough	Large	Late

Table 2: Means squares for agronomic and nutritional traits and estimated glycemic index of thirty cowpea genotypes evaluated at Dambatta in 2020.

s/n.	Trait	Mean squares		
		Rep df =2	Genotypes df = 29	Error df = 58
1.	Days to 50% flowering	28.00	1413.126**	10.31
2.	Days to 95% maturity	36.15	1375.99**	22.26
3.	Grain yield kg ha^{-1}	838.12	380101.89**	28853.88
4.	Dry fodder yield kg ha^{-1}	12865.76	955967.52**	86343.24
5.	Percentage Moisture content	0.66	4.26**	0.26
6.	Percentage Ash content	0.99	0.51^{ns}	0.32
7.	Percentage Lipid content	0.54	5.85**	0.337
8.	Percentage Protein content	0.10	35.89**	0.39
9.	Percentage Carbohydrate content	0.11	51.72**	1.25
10.	Total starch	0.18	106.76**	0.89
11.	Percentage Fibre content	0.15	1.95**	0.07
12.	Phytate content	0.01	2.29**	0.08
13.	Estimated Glycemic Index	18.96	25.65**	2.85

*Small = <15g/100-seed weight, **Medium =15-19g/100-seed weight, ***Large = >20g/100-seed weight; (Omoigui et al., 2006).

Table 3: Estimates of genetic parameters for agronomic and nutritional traits and estimated glycemic index of cowpea genotypes evaluated at Dambatta in 2020

Characters	Mean	Range		GCV (%)	PCV (%)	h^2b %	GA	GAM %
		Min	Max					
Days to 50% flowering	76.62	43.00	100.00	28.22	28.53	97.84	37.64	49.13
Days to 95% maturity	101.92	70.00	128.00	20.84	21.35	95.30	36.49	35.81
Grain yield kg ha^{-1}	550.60	169.20	1913.30	62.14	69.38	80.02	0.54	99.38
Dry fodder yield kg ha^{-1}	816.15	269.40	2966.70	65.97	75.15	77.05	0.83	102.38
Percentage Moisture content	7.35	5.75	10.48	15.65	17.16	83.68	1.85	25.27
Percentage Ash content	3.34	2.43	4.31	7.49	18.56	16.52	0.18	5.40
Percentage Lipid content	9.47	7.68	12.27	14.36	15.52	84.50	2.18	23.09
Percentage Protein content	24.80	18.98	34.25	13.87	14.11	96.81	5.96	24.05
Percentage Carbohydrate content	52.04	41.51	58.01	7.88	8.17	93.08	6.96	13.38
Total starch	46.49	34.64	56.95	12.78	12.95	97.54	10.33	22.23
Percentage Fibre content	3.00	1.48	5.58	26.33	28.00	89.95	1.33	44.33
Phytate content	3.49	2.10	5.45	24.64	26.07	90.02	1.45	41.40
Estimated Glycemic Index	47.86	43.24	55.28	5.77	6.75	72.73	4.13	8.64

Genetic gain as percentage of mean

CGBPB 008

RICE IMPROVEMENT OPPORTUNITIES USING CRISPR/CAS

Mowobi G. Gabriel[1*], Alhasan A. Usman[1], Osuji Charles[2], Abdulrasheed I. Nafisat[1], Morgan I. Oscar[3].

[1]Institute for Agricultural Research Samaru, Ahmadu Bello University Zaria.
[2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, P.M.B. 186, Garki, Abuja, Nigeria.
[3]Institute for Industrial Research Oshodi, P.M.B. 21023, Ikeja, Lagos, Nigeria.
Correspondence: mowobig@gmail.com

ABSTRACT

The rice (Oryza sativa L.) grain makes up 20% of the world's dietary energy supply and more than three billion people across the globe eat rice daily. Advances in genome editing approaches have opened up possibilities to breed for almost any given desirable trait. However, reduction in rice yield owing to several factors including the environmental issues posing great threat to global food security, climate change and emergence of pests and pathogens has been reported in recent decade. There is still continuous increase in demand to produce more rice and associated cereals to satisfy the increasing growing population which is expected to reach 9.7 billion by 2050. CRISPR/Cas known as Clustered Regularly Interspaced Short Palindromic Repeats /CRISPR-associated protein system is an emerging gene-editing tool in genetic engineering. Recently, it has emerged as an alternative nuclease-based method for efficient and versatile genome engineering in which only the targeting sequence within the Single-Guide RNA (sgRNA) needs to be changed to target different genes. The simplicity of the cloning strategy, high precision in inducing genetic variations and the few limitations on potential target sites make the CRISPR/Cas system very appealing. Hence rice grain quality improvement opportunities will have significant impact on functional rice genome research. Here, we review the application of CRISPR/Cas system in rice quality improvement using different system, site specific translational regulation, precise gene insertion and replacement, base editing system and strategies of multiplex editing.

Keywords: *CRISPR/Cas, Rice, RNA, Genome, Editing.*

Introduction

Recently, traditional breeding methods and it limitations have been superseded by emergence of genome editing technologies, thereby opening up a new era of crop improvement. Engineered site-specific nucleases (SSNs) are involved in genome editing to modify specific genes at desired locations in the genome. The SSNs such as transcriptional activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) make a double-stranded break (DSB) in the target DNA which is subsequently repaired by cell's own natural repair mechanism of homologous recombination (HR) or non-homologous end joining (NHEJ) (Miglani, 2017).

The HR pathway is much more precise in the exchange of homologous sequence leading to gene knock in or gene replacement (Voytas and Gao, 2014; Baltes *et al.,* 2014) while non-homologous end joining NHEJ repair is the error prone pathway which creates random insertions and deletions (Indels) and results in frame shift mutations and targeted gene knockouts (Feng *et al.*, 2013; Bortesi and

Fischer, 2015). Most recently, these base editing technology such as CRISPR/Cas9 has emerged as a new approach which overcomes some of the shortfalls of NHEJ and HR methods and converts one target base into another without the requirement of a DSB or donor template (Komor *et al.*, 2016).

This emergence of genome editing technologies which surmounted the limitations of traditional breeding methods is revolutionizing crop improvement process (Jinek *et al.*, 2012; Deepa *et al.,* 2018).

Grain and yield quality improvement is an important area for rice breeders. This is a multigenic trait that is influenced simultaneously by many factors. Over the past few decades, breeding for hybrids and semi-dwarf cultivars has significantly contributed to the progress achieved in attainment of high yield demands but reduced grain quality. Thus, the need for researchers to pay attention to this reduced grain quality. Gene discovery has been facilitated by the availability of rice genome sequence data which has also enabled targeted mutagenesis and revealed functional aspects of rice grain quality attributes (Fiaz, *et al.,* 2019). Application of molecular markers has led to some successes achieved in the understanding of the genetic mechanisms for better rice grain quality as researchers however opt for novel strategies. Genomic alteration with little or no ethical issues is being presently utilized which includes genome editing technologies (GETs). The emergence of Clustered Regularly Interspaced Short Palindromic Repeats and its associated protein Cas9, known as CRISPR/Cas9 for reverse genetics has opened new avenues of research in the life sciences, including for rice grain quality improvement. Currently, CRISPR/Cas9 technology is widely used by researchers for genome editing to achieve the desired objectives, because of its simple targeting mode of action (Fiaz, *et al.,* 2019). Over the past few years many genes that are related to various aspects of rice grain quality have been successfully edited via this technology. Interestingly, studies on functional genomics at larger scales have become possible because of the availability of GETs (Fiaz, *et al.,* 2019). Several genes have been successfully modified using this system among the agriculturally important crops and some agronomic important traits have also been rapidly generated which indicates its potential applications in both scientific research and plant breeding (Sajid *et al.,* 2019). The wide varieties of applications for this technology include simple non-homologous end joining, homologous recombination, gene replacement, and base editing. Recently, the CRISPR/Cas12a (originally named Cpf1) system was discovered and developed into a genome-editing tool (Zaidi *et al.,* 2017). A feature that further broadens the target sequence space of CRISPR/Cas systems. Studies in rice suggest recently that several Cas12a variants are temperature sensitive and editing efficiencies can be substantially increased when plants are grown at 28 ^{0}C instead of 22 ^{0}C (Malzahn *et al.,* 2019). The frequency of off-target mutations generated by CRISPR/Cas systems has been raised as a concern, even though the specificity of CRISPR/Cas9 in plants appears to be higher than in mammals. Unexpected DSBs have been reported for only a minority of gRNAs, even when whole-genome sequencing has been used to screen for off target mutations (Feng *et al.,* 2014). Along with strategies to increase the lengths of the recognition sequence by engineering an inactive Cas9 enzyme fused to the FokI nuclease domain, several high-fidelity variants of SpCas9 and SaCas9 have also been developed (Minkenberg *et al.,* 2019).

The CRISPR/Cas 9 System: Clustered Regularly Interspaced Short Palindromic Repeats-associated endonuclease Cas9 is the most advanced genome editing tool in plant biology. It consists of a short RNA molecule called guide RNA which is associated with a DNA endonuclease called Cas9 (Weeks *et al.,* 2016). The guide RNA is a two-component system consisting of the crRNA (CRISPR-derived RNA) and tracrRNA (trans-activating RNA). Naturally, crRNA targets the double stranded DNA to be cut, and has

a short region of homology allowing it to bind the tracrRNA. The tracrRNA provides a stem loop structure which associates with Cas9 protein. CRISPR-associated protein 9 (Cas9) is a DNA endonuclease responsible for cutting the invading phage DNA into pieces, which then gets integrated into the CRISPR array as a spacer. In the CRISPR/Cas9-based genome editing system, the crRNA and tracrRNA were engineered into a single guide RNA chimera (sgRNA) that can also direct sequence-specific Cas9 DNA cleavage (Jinek *et al.,* 2012). The protein/RNA complex (Cas9-sgRNA) moves along the DNA strand and makes a double stranded break (DSB) where the sgRNA matches the target DNA sequence (Jinek *et al*., 2014).

The CRISPR/Cas9 and its modified versions have wide applications in animals, plants, yeast, and human as well as in non-human cell lines (Khatodia *et al*., 2016; Miglani, 2017). CRISPR/Cas9 system has been successfully used in major crops and model plants due to its simplicity, adaptability, and high precision (Xu *et al., 2016*). It has produced successful genome editing results in a variety of plants, including wheat, sorghum, maize, Arabidopsis, rice, tobacco and tomato (Jiang *et al.,* 2013). Unlike in animals, direct delivery of RNA into cells is technically difficult in plants. In most cases, the constructs expressing sgRNA and/or Cas9 are co-transformed by Agrobacteria into plant cells to generate a functional CRISPR/Cas9 complex. Although the sgRNA and Cas9 could be delivered by separate vectors, most studies have used the all-in-one constructs which combine the two expression cassettes in one binary vector to increase the efficiency of mutagenesis (Mikami *et al.,* 2015).

Brief Historical Development of the CRISPR/Cas 9 System: The CRISPR/Cas system evolved as an adaptive immune response in bacteria and archaea to defend against invading viral and plasmid DNAs (Bassett *et al.,* 2013). In 1987, a team of Japanese scientist led by Yoshizumi Ishino accidentally discovered, an unusual series of interspersed sequences while analyzing alkaline phosphatase converting enzyme in *E. coli.* It was confirmed experimentally by another team of researchers led by Rodolphe Barrrangou using Streptococcus bacteria found in diary cultures. From their report, these short inter spacing sequences were actually DNA remnants from viruses that have previously attacked bacteria and it provided an immune system for defending the bacteria against subsequent attacks (Cong *et al.,* 2013; Jinek *et al.,* 2012; Niewoehner *et al.,* 2013; Wiedenheft *et al.,* 2012). An internal safety mechanism that ensures that cas 9 doesn't just cut anywhere in the genome known as Photospacer Adjacent Motifs (PAMs) serve as tags and sit adjacent to the target DNA sequence. For the CRISPR/Cas system from S. pyogenes, the 20-bp DNA target must lie immediately 5' of a PAM sequence that matches the canonical form 5'-NGG (Gasiunas *et al.,* 2012). Thus, Cas9 nuclease can be targeted to any DNA sequence of the form 5'-N (20)-NGG simply by changing the first 20-nt guide sequence within the sgRNA. Cas9 has two conserved nuclease domains: an HNH nuclease domain and a RuvC-like nuclease domain. In this system, a synthetic single- guide RNA (sgRNA) binds directly to a 20-nt sequence followed by a 5'-NGG PAM (protospacer adjacent motif) on the target DNA to provide sequence specificity, and a Cas9 nuclease coupled with the sgRNA to induce the site-specific cleavage in the genome. This system consists of a single gene encoding the Cas9 protein and two RNAs, a partially complementary trans-activating crRNA (tracrRNA) and a mature CRISPR RNA (crRNA). The crRNA-tracrRNA hetero duplex can be fused to generate a chimeric sgRNA containing a designed hairpin 18.

Codon-optimised SpCas9 was used to target rice endogenous genes and the mutation frequency was 4.0%±9.4% in transgenic lines and 14.5%±38.0% in protoplasts (Shan *et al.,* 2013). CRISPR/ Cas9 system was used to edit OsMPK5 at three target sites to enhance the disease resistance of rice, it was reported that editing efficiency is lower than the on-target site, off-target mutations exist at a non-target site and mutation rates of the sites was

3%±8% in the rice protoplasts (Xie and Yang 2013).

CRISPR/Cas System In Rice: Gene knock-out mediated by CRISPR/Cas system was developed from the CRISPR/Cas9 system in rice using *Streptococcus pyogenes* Cas9 (SpCas9) following the successful achievement of genome editing in mice and human (Cong *et al.,* 2013; Mali *et al.,* 2013). Codon-optimised SpCas9 was used to target rice endogenous genes, the mutation frequency is 14.5%±38.0% in protoplasts and 4.0%±9.4% in the transgenic lines (Shan *et al.,* 2013). It was reported that off-target mutations exist at a non-target site and the editing efficiency is lower than the on-target site and also that CRISPR/ Cas9 system was used to edit OsMPK5 at three target sites to enhance the disease resistance of rice and the mutation rates of these sites are 3%±8% in rice protoplasts (Xie and Yang, 2013). Xu *et al.* (2017) found that pre-crRNAs with a full-length direct repeat sequence have higher editing efficiencies than mature crRNAs. Codon-optimised LbCpf1 and *Acidaminococcus sp.* BV3L6 Cpf1 (AsCpf1) with matched crRNA expression arrays and targeted multiple rice genome loci, respectively and showed that LbCpf1 can effectively edit the rice genome, whereas AsCpf1 cannot achieve efficient genome editing (Tang *et al.*, 2017; Hu *et al,* 2017). The above reports showed that fragment deletion is the most common type of mutation caused by Cpf1 in rice. Ma *et al.* (2015) reported that homozygous mutations can be found in T0 plants. Cpf1 also can introduce mutations in the rice genome (Wang *et al.,* 2017b; Xu *et al.,* 2017).

Mutagenesis Directed By Oligonucleotide: This is a technique that uses the endogenous HDR pathway to correct the mismatches in rice genome generated by pairing the exogenous oligonucleotide which carries the sequence desired to the near-complementary target site in the rice genome (Julia *et al.,* 2019). In an approach known as oligonucleotide-directed mutagenesis (ODM), 20 to 200 nucleotides in length of mutagenic DNA oligonucleotides have been delivered into some plant cells to introduce point mutations in target genes. The oligonucleotide therefore acts as both a mutagen and a DNA repair template. Mutagenesis can be achieved using standard single-stranded DNA oligonucleotides (ssODNs) but these have a short intracellular half-life, and their efficiency has therefore been improved by stabilizing modifications (Julia *et al.,* 2019). ODM efficiency is generally low and correlates positively with oligonucleotide length, like in the case of ssODNs where increasing the length to 200 nucleotides resulted in precise editing frequencies of up to 0.05% at a transgenic locus in *Arabidopsis thaliana* protoplasts (Sauer *et al.,* 2016). Targeted mutation frequencies could also be enhanced by ODM in combination with nonspecific DSB-inducing reagents such as antibiotics, or sequence-specific nucleases such as TALENs and CRISPR/Cas9 in Arabidopsis and flax (Linumusitatissimum) (Sauer *et al.,* 2016). Chimeraplasts did not increase mutation frequencies more than the level of spontaneous mutations in tobacco (*Nicotianatabacum*) or rapeseed (Brassica napus) (Ruiter *et al.,* 2003). Generated Off-target mutations is made possible by ODM because of the oligonucleotide recombination or off-target mismatch repair, it hasn't been recorded. ODM allows multiplexing theoretically (multiple conversions at several targets within a single gene or the simultaneous conversion of multiple targets in a single cell).

Multiplex Gene-Editing Systems: A system that can simultaneously integrate multiple gRNAs into a single vector based on the isocaudomer technique including two kinds of vectors in this system was also established by Wang *et al.* (2015). The gRNAs generated by intermediate vectors will then be integrated into the pC1300-Cas9 vector for multiplex genome editing to generate the final binary vector. Single gene editing using the CRISPR/Cas9 system in rice has been achieved, although simultaneously targeting multiple genes is required in many cases. Based on Golden Gate ligation or Gibson Assembly, multiplex genome-editing systems have been developed (Xing *et al.,* 2014; Ma *et*

al., 2015). Shen et al (2017a) obtained an eight- mutant rice line using Cas9 protein and gRNAs and methods have been designed that co-expressed Cas9 protein and gRNAs from a single Pol II promoter. Multiple (2±4) gRNA expression cassettes are assembled into a single binary vector in as little as one cloning step to target multiplex genes simultaneously.

Base Editing

Base editing combining CRISPR/ Cas9 and cytidine deaminase or adenine deaminase were applied in rice after first successfully applied in animal cells (Gaudelli *et al.,* 2017). Point mutations and gene replacements modification generated by CRISPR/Cas system remains a serious challenge because of its limited frequency. the successful conversion of cytosine (C)-guanine (G) base pairs to adenine (A)-thymine (T) base pairs in rice using the Base editing system involving the cytidine deaminase enzyme nSpCas9 (Cas9-D10A), APOBEC1 and the uracil glycosylase inhibitor (UGI) have been reported reported (Zong *et al.,* 2017; Lu and Zhu, 2017;). Efficiency of the C to T conversion ranges from 0.39% to 43.48% in OsSBEIIb, OsCDC48 and OsNRT1.1B and InDel mutations are found in the target sites. The conversion of AÂT base pairs to GÂC base pairs is achieved in rice was then subsequently achieved (Hua *et al.,* 2018a; Yan *et al.,* 2018; Li C *et al.,* 2018). In Rice, Base editing was used to expand the scope of the adenine base editor nSaCas9, SaCas9 and SpCas9 variants (Hua *et al.,* 2018a; Hua *et al.,* 2018b) and InDel mutations were not found in this target sites. The wild-type Escherichia coli TadA gene and its mutants are fused together to nSpCas9 for generating several Base editing systems in rice. The efficiency of A to G conversion is up to 62.26% when TadA and TadA*7.10 were fused together to the N-terminus of nSpCas9 (Hua *et al.,* 2018a; Hua *et al.,* 2018b).

Transcriptional Regulation

A new technology was developed in other to achieve transgene- free genome editing. To induce targeted genome modifications, preassembled Cas9 protein-gRNA ribonucleoproteins (RNPs) was directly delivered into rice protoplasts in which it mutation frequencies range from 8.4% to 19.0% (Woo *et al.,* 2015). One of the prevalent transgenic technique used in rice is transgene-free genome editing in rice Agrobacterium-mediated callus infection. This causes random insertions of T-DNA into the genome, which may lead to potential security problems. In using this method, the rice genome can be edited without integration of exogenous DNA and while it is technically challenging to regenerate the edited rice protoplast into plants generally (Lu et al, 2017). The rice plants with an integrated CRISPR/Cas9 system and OsCYP81A6 RNAi are sensitive to bentazone, showing a lethal symptom. The CYP81A6-hpRNAi expression element was used with CRISPR/Cas9 system in other to generate transgene-free rice with expected mutations. This strategy greatly simplifies the screening process of transgene-free rice. Both 35S-CMS2 and REG2-BARNASE expression cassettes is also a strategy developed for efficient screening and enriching of transgene-free plants (He *et al.,* 2018). Transcriptional regulatory elements fused with dCas9 can result in transcriptional inhibition or activation in an organism (Lowder *et al.,* 2018). Li *et al.,* (2017) developed dCas9-TV (dCas9-6TAL-VP128), a dCas9-based transcriptional activation system which exhibits relatively strong transcriptional activation effects compared with the dCas9-VP64 activator in Arabidopsis and rice. Although dCas9-TV system can also work in mammalian cells.

Off-Target Effects of CRISPR/Cas System: Undesired off-target mutations of Cas9 were reported in many studies of rice and many other crops, which are caused by a few nucleotide mismatches when sgRNAs recognize DNA (Tsai *et al.,* 2015; Kleinstiver *et al.,* 2016). Tang *et al.* (2018) assessed off-target effects of Cas9 and Cpf1 by a large-scale whole-genome sequencing (WGS) in rice. It was discovered that only one Cas9 sgRNA results in off-target mutations among 12 Cas9 sgRNAs in T0 lines, which shows a

higher specificity of Cpf1, and the off-target sites could be predicted in silico.

Advantages of CRISPR/Cas9: CRISPR/Cas9 improvement of rice possesses several potential advantages over ZFNs and TALENs. The ZFNs are limited by the range of targetable sequences because of the absence of fingers for all possible DNA triplets. In the CRISPR/Cas system improvement of rice, the only requirement for the target site is the 20-bp target sequence preceding a 5'-NGG PAM. Due to the CRISPR/Cas9 system simplicity, it has superseded others in research applications and has literatures describing the use of this system in many different plant species. The CRISPR/Cas9 system can also be used to target different genes in parallel by providing multiple gRNAs simultaneously, including unrelated genes (Vazquez-Vilar *et al.,* 2016; Xing *et al.,* 2014). The ease of multiplexing with the CRISPR/Cas9 system is an advantage for the generation of knockouts using this dual-gRNA approach (Bortesi *et al.,* 2016). The superiority of CRISPR/Cas9 over others is that Cas9 enzyme does not need to be engineered at the protein level to recognize different targets. It target specificity is given entirely by the spacer region of the gRNA, and the sequence can be modified using standard molecular biology methods (Mali *et al.,* 2013). The CRISPR/Cas9 system is at least efficient, approaching 100% in cereal crops and most species (Zhu *et al.,* 2017; Bortesi *et al.,* 2016). The CRISPR/Cas system provides a straightforward method for rapid gene targeting within 1–2 weeks in protoplasts, and mutated rice plants can be generated within 13–17 weeks (Qiwei *et al.,* 2014). Targeting one gene at two positions increases the overall mutation frequency in the CRISPR/Cas system provides a straightforward method for rapid gene targeting within 1–2 weeks in protoplasts, and mutated rice plants can be generated within 13–17 weeks (Qiwei *et al.,* 2014). Also, it allows recovery of homozygous mutants in one generation (Bortesi *et al.,* 2016).

Limitations

Despite the popularity of CRSPR, the technology has limitations on rice such as off-targets which may occur as a result of targeting homologous sequences in unintended loci 45–47. It is necessary to monitor the genome-wide presence of such target sequences on rice and to avoid selecting target sequences with homology to many other sites in other to minimize off-target effects (Qiwei *et al.,* 2014). Low mutagenesis efficiency and its dependency on in-vitro regeneration protocols for the recovery of stable plant lines is also another limitations (Niaz *et al.,* 2019). Several other issues such as persisted CRISPR activity in subsequent generations, the potential for transferring to its to wild type population, the risk of reversion of edited version to its original phenotype particularly in cross pollinated plant species when released into the environment and the scarcity of validated targets are other potential threat. Also, certain sgRNAs may be low in efficiencies or fail to work because of the chromatin states of unwanted hairpin structures of sgRNA or target loci other unknown factors (Qiwei *et al.,* 2014; Niaz *et al.,*2019).

Conclusions

The emergence of the CRISPR/Cas9 technology has provided not only simple, but also an efficient genome editing platform for rice researchers. The platform provides strong support for functional genomics. Trait improvement and has also shown some promises which will surely be influenced by these new developments in CRISPR/Cas9 technologies. These new developments will most likely help scientists to develop generation of transgene-free edited rice, direct site-specific integration, create rice resistant to diseases, improve tolerance to stress, gene expression regulation, creation of large scale modifications of chromosome structure, edit rice at the multicellular level and epigenome editing of rice. The use of optimization strategies, endogenous promoter and improvement of sgRNA expression, the editing efficiency of the rice CRISPR/ Cas9 system has been greatly improved and this has provided more

powerful genome-editing tools. However, there are some problems for rice genome editing such as the efficient delivery of the CRISPR/Cas9 system without integrating into the rice genome. Also the accurate to knock-in and replace endogenous genes via the HDR repair are relatively limited.

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CGBPB 009

ASSESSMENT OF GENETIC DIVERSITY IN SOME CASTOR BREEDING LINES

Salihu, B. Z.,[1] Tanimu, A. M.[2], Ajaye, O. F.[1], Kabaraini, M. A.[1], Shehu, H.[1], Ishaq, M. N.[1], Salahu, M. S.[1] and Ehirim, B. O.[1]

[1]National Cereals Research Institute Badeggi, Niger State
[2]Agricultural Research Council of Nigeria, Abuja, Nigeria
Corresponding e-mail: *bz.salihu2007@gmail.com* 07067739913

ABSTRACT

Castor (Ricinus communis L.) is an important oil-crop, considered very critical for many industrial applications. In the present study, the genetic diversity in the available castor breeding lines at the National Cereals Research Institute Badeggi was assessed. The lines were evaluated on replicated progeny row plots arranged using a randomized complete block design. The results showed variation in the 8 agronomic traits studied among the entries. Days to first raceme maturity varied from 80 days to 118 days with an average mean of 104 days. The length of the spike was between 15.40 cm and 49.20 cm among the lines. Height of the first raceme ranged between 44.20 cm and 135.4 cm among the lines. The seed yield per plant among the lines ranged from 0.08 to 0.48 (kg/plant). From cluster analysis, six cluster groups with 2 to 20 members were generated. Cluster I was the largest group with 20 cluster members. Cluster III consisted of two early maturing lines (Line9 and Line43) with a range of 96 to 97 days. Populations with high seed yields could be constituted from the cluster I and IV. A cophenetic coefficient of 0.812 was obtained among the clusters. The results revealed optimum genetic distance among the breeding lines evaluated; therefore, they could serve as good parental materials for castor breeding in Nigeria.

Keywords: *Castor, Breeding lines, Genetic diversity, Nigeria*

Introduction

Castor oil plant (*Ricinus communis* L.) is an oil-crop considered very critical for many industrial applications because of its ability to form many important derivatives (Ogunniyi, 2006). The oil, which is extracted from the seed, is used in more than 700 applications, including the applications in pharmaceutical industries, rubber/plastic industries, and lubricants/biodiesel industries (Mutlu and Meier, 2010). In the southern part of Nigeria, a food condiment (*Ogiri*) among the Igbo tribe is produced from castor seeds (Salihu *et al*., 2014). The residual meal of castor seed, after detoxification by boiling, could be used as supplement feed in preparation of broiler finishing diets without any harmful effects (Ani and Okorie, 2009). Also, the meal (autoclaved) could be used in place of the soybean meal in sheep rations (Pompeu, 2009). Organic fertilizer produced from castor meal was reported to have the advantage of high nitrogen content, fast mineralization and

anti-nematode effects (Lima *et al.*, 2011). The leaves, seeds and capsules of castor are used for traditional medicines (Gana, 2015). Concoctions prepared from the leaves and roots are used in the treatment of after birth weakness in babies.

Despite the huge economic benefits of castor, its genetic improvement in Nigeria has not been receiving much attention. Consequently, the seed yield among Nigerian castor farmers is low in comparison with the average yields in other countries (Salihu *et al.*, 2019). Therefore, there is the need for active castor research in order to exploit the potential of the crop for economic growth. With the availability of castor germplasm in some research centres like National Cereals Research Institute Badeggi, there are opportunities to increase the yield level of castor in Nigeria. However, for any successful genetic improvement programme, identification of appropriate parents is very crucial (Shivanna, 2008). Information on genetic diversity of available breeding lines is of high priority. In view of this, the present research was initiated to assess the genetic diversity in the available castor breeding lines at the National Cereals Research Institute Badeggi, Nigeria.

Materials and Methods

Seventy four selected improved breeding lines sourced from the National Cereals Research Institute (NCRI) Badeggi, were evaluated at NCRI castor experimental field at Badeggi during the 2020 raining season. The lines were evaluated on progeny row plots arranged in a randomized complete block design with three replications. The plot size was 1 m x 7 m with a spacing of 75 cm and 1m intra and inter-row respectively. Data were taken on Days to first raceme maturity, number of nodes, number of leaf lobes, leaf length (cm), leaf petiole length (cm), spike length (cm), height to 1st raceme (cm) and yield (kg/ha). The genetic diversity among the lines was assessed using cluster analysis procedure of Statistical Package for Agricultural Research (STAR 4.0.1).

Results and Discussion

Table 1 presents the means of the morphological parameters recorded among the castor lines evaluated. The results revealed variations for all the studied traits among the entries. Days to first raceme maturity varied from 80 days to 118 days with an average mean of 103.95 days. Number of internodes to first raceme ranged between 11 and 21. The length of the spike was between 15.40 cm and 49.20 cm. Height to first raceme varied from 44.20 cm to 135.4 cm. The seed yield per plant recorded among the lines was between 0.08 and 0.48 (kg/plant). Agglomerative cluster dendrogram constructed from morphological data among the lines is presented in Figure 1. The summary statistics for the agronomic performances of the cluster groups is shown in Table 2. Six cluster groups, with 2 to 20 members, were generated (Figure 1 and Table 2). The cophenetic coefficient was 0.812, signifying optimum genetic distance among the lines. Cluster I was the largest group with 20 cluster members. Cluster III comprises two early maturing lines (Line9 and Line43) with a range between 96 to 97 days; however extra-early maturing lines of about 80 to 84 days could be sourced from the cluster V, IV and VI (Table 2). Short height to first raceme could be found among the members of the cluster V and VI. Parents with long spikes could be sourced within the members of cluster III. Populations with high seed yields could be constituted from the cluster group I and IV.

Genetic improvement in any crop can be accomplished only if there is optimum diversity in the breeding populations. The findings from the present study indicate adequate variability for the traits studied. Golakia *et al.* (2015) also documented variability for most of the characters reported

in this study. Assessments of castor genetic diversity and phenotypic variability through several methodologies have been reported by several authors (Allan *et al*., 2008; Rao *et al.,* 2006 and Zheng *et al*., 2010). Anjani and Reddy (2003) reported high divergence among twenty-one castor genotypes for yield and yield attributes. In a study on genetic diversity among nine castor accessions, Costa and Pereira (2006) distinguished two clusters and said that the prominent variability to the diversity among the accessions were contributed by days to flowering, raceme length, plant height and seed oil content. Zhang-Xishun and Yang-Jian (2006) considered forty-six castor germplasm and distinguished four clusters with no geographic clustering pattern.

Conclusion

From the findings of the study, it is shown that the breeding lines evaluated are divergent in nature and they could serve as good parental materials for castor breeding in Nigeria.

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Table 1: Means of 8 Morphological Traits Studied among 74 Castor Lines at Badeggi, Nigeria

Entries	Days to First Raceme Maturity	Number of Internodes to First Raceme	Leaf Length (cm)	Petiole Length (cm)	Spike Length (cm)	Height to First Raceme (cm)	Number of Leaf Lobes	Seed Yield (Kg/plant)
Line71	80.00	14.60	19.40	15.00	21.00	44.20	9.00	0.13
Line58	102.00	14.40	25.20	20.00	26.50	48.20	9.00	0.12
Line63	96.00	13.40	16.40	12.40	25.00	48.60	8.80	0.09
Line54	107.00	16.00	16.20	12.60	19.60	49.60	8.60	0.28
Line64	94.00	14.20	19.20	12.00	25.20	55.40	9.00	0.15
Line61	111.00	16.60	24.00	20.20	39.00	56.20	9.00	0.12
Line56	111.00	16.00	25.40	19.40	34.40	57.40	9.20	0.17
Line69	100.00	16.40	25.00	17.80	37.40	61.80	8.00	0.17
Line51	101.00	15.80	18.60	18.00	17.60	61.80	9.20	0.27
Line43	97.00	11.80	19.60	16.60	39.60	62.40	8.60	0.20
Line33	104.00	14.80	19.40	19.60	19.20	63.40	9.00	0.14
Line19	83.00	14.40	17.00	15.80	26.00	63.60	9.60	0.36
Line29	84.00	17.80	21.40	17.33	19.00	64.00	9.00	0.25
Line62	102.00	13.80	23.60	15.00	32.80	64.60	7.80	0.10
Line72	94.00	13.40	20.00	12.60	26.80	64.80	8.20	0.11
Line73	87.00	12.60	20.80	17.20	25.20	65.40	8.60	0.09
Line65	93.00	15.40	20.20	16.20	37.00	65.60	9.00	0.15
Line50	102.00	18.40	22.40	17.80	18.60	65.60	9.00	0.25
Line57	88.00	15.40	28.60	19.00	49.20	66.00	8.80	0.13
Line59	84.00	14.40	25.00	17.60	22.60	66.60	9.20	0.17
Line70	102.00	14.80	19.40	15.00	32.00	68.60	8.00	0.11
Line55	97.00	14.40	28.60	18.80	30.40	69.20	8.20	0.10
Line39	92.00	14.80	19.60	15.20	21.80	69.60	8.40	0.28
Line28	106.00	19.20	22.00	19.40	19.60	69.80	8.00	0.33
Line45	110.00	15.40	24.00	19.40	25.00	71.00	9.60	0.16
Line38	107.00	14.20	21.80	18.20	17.20	71.20	8.80	0.33
Line52	96.00	15.40	22.40	20.60	27.80	71.60	9.20	0.32
Line48	110.00	18.00	21.00	17.60	28.60	71.60	9.80	0.44
Line11	118.00	17.60	23.20	17.20	35.40	72.20	9.80	0.17
Line53	99.00	16.60	20.00	18.80	19.80	73.20	9.40	0.21
Line23	110.00	16.80	21.80	17.60	23.60	73.40	8.80	0.36
Line3	112.00	14.40	22.00	20.40	30.00	75.20	8.40	0.21
Line35	112.00	14.80	18.60	17.40	28.60	75.20	8.20	0.23
Line60	87.00	15.60	24.00	17.40	30.20	78.20	9.00	0.18
Line46	114.00	19.00	21.20	16.80	19.60	78.60	9.60	0.26
Line68	107.00	13.80	20.40	16.40	35.00	78.80	8.40	0.08
Line37	117.00	17.20	20.80	19.40	17.40	80.80	9.00	0.19
Line32	114.00	15.60	21.60	23.00	24.60	80.80	8.80	0.35
Line36	102.00	14.20	19.80	18.00	18.40	81.40	8.80	0.23

Line5	112.00	17.00	19.00	14.40	15.40	84.40	8.80	0.09
Line22	112.00	17.60	25.40	19.40	19.80	84.80	9.40	0.16
Line1	103.00	19.60	25.40	21.40	32.80	84.80	9.80	0.45
Line13	109.00	15.60	16.20	12.60	26.60	86.20	9.80	0.36
Line41	98.00	13.60	21.00	15.20	32.00	86.80	8.60	0.21
Line44	102.00	15.00	21.60	15.80	28.00	86.80	9.40	0.38
Line34	92.00	13.60	19.20	19.20	27.40	87.20	9.40	0.33
Line17	104.00	15.00	19.80	16.60	25.20	87.60	9.80	0.40
Line10	113.00	18.40	21.80	18.40	25.60	88.60	8.80	0.18
Line31	112.00	16.50	27.20	21.20	20.80	89.60	9.20	0.28
Line30	103.00	17.80	24.20	20.40	20.00	90.20	9.40	0.23
Line47	114.00	16.40	23.40	18.00	17.40	90.40	9.00	0.30
Line40	114.00	19.20	19.00	15.20	24.80	91.40	9.40	0.29
Line74	96.00	17.00	22.60	20.80	37.40	91.80	9.20	0.14
Line14	109.00	15.80	21.00	15.40	33.80	92.60	10.00	0.37
Line67	97.00	16.00	24.40	17.20	29.20	92.80	9.20	0.15
Line4	112.00	17.60	19.00	17.80	19.60	92.80	9.80	0.22
Line2	117.00	15.80	20.60	18.80	17.00	93.00	9.00	0.24
Line26	112.00	16.40	25.40	21.00	27.20	93.60	9.40	0.48
Line49	112.00	19.80	21.80	16.80	30.20	95.60	9.00	0.16
Line66	96.00	11.40	21.20	17.80	41.60	96.60	8.60	0.08
Line9	96.00	12.60	22.60	22.60	29.60	96.80	9.40	0.18
Line16	102.00	13.60	18.80	12.80	28.00	97.60	9.20	0.36
Line12	102.00	14.60	22.20	18.00	24.40	98.00	9.00	0.33
Line27	114.00	21.00	26.60	21.60	30.20	98.20	10.00	0.40
Line20	112.00	17.20	17.60	16.40	23.40	98.40	9.00	0.17
Line25	99.00	14.20	20.00	14.60	30.60	104.40	9.00	0.29
Line24	114.00	18.20	20.80	17.40	30.80	105.40	9.60	0.47
Line15	117.00	14.60	23.40	19.20	19.20	106.40	9.60	0.27
Line18	112.00	16.80	18.40	17.60	29.20	108.20	9.60	0.31
Line7	117.00	17.60	22.00	18.60	18.80	111.00	10.00	0.20
Line42	99.00	15.80	19.40	18.60	21.40	114.60	8.60	0.33
Line21	110.00	19.00	21.40	18.40	22.00	114.80	9.60	0.32
Line8	115.00	18.60	21.00	17.20	19.40	130.60	10.00	0.32
Line6	112.00	12.40	23.60	20.00	27.00	135.40	10.00	0.38
Minimum	80.00	11.00	16.20	12.00	15.40	44.20	7.80	0.08
Maximum	118.00	21.00	28.60	23.00	49.20	135.40	10.00	0.48
Mean	104.00	16.00	21.58	17.64	26.39	81.26	9.09	0.24
STD Mean	4.11	2.00	3.20	4.30	5.42	14.23	1.52	0.02

Table 2: Summary Statistics for Agronomic Performances of the Cluster Groups Generated among 74 Castor Lines

Variable	Cluster I		Cluster II		Cluster III		Cluster IV		Cluster V		Cluster VI	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Days to Flowering	103.00	118.00	99.00	117.00	96.00	97.00	83.00	109.00	80.00	107.00	84.00	111.00
Height to First Raceme	71.00	135.40	61.80	130.60	62.40	96.80	63.60	114.60	44.20	96.60	48.20	92.80
Leaf Length (cm)	18.40	26.60	17.60	27.20	19.60	22.60	16.20	22.40	19.60	41.60	22.60	49.20
Number of leaf lobes	8.20	10.00	8.00	10.00	8.60	9.40	8.40	9.80	8.00	9.00	7.80	9.20
Number of Internodes	12.40	21.00	14.20	19.20	11.80	12.60	13.60	17.80	11.40	16.00	13.80	17.00
Petiole Length (cm)	15.40	23.00	14.40	21.20	16.60	22.60	12.60	20.60	16.20	21.20	22.60	28.60
Spike Length (cm)	17.00	35.40	15.40	24.80	29.60	39.60	19.00	32.00	12.00	17.80	15.00	20.80
Seed Yield (kg/plant)	0.16	0.48	0.09	0.33	0.17	0.20	0.21	0.40	0.08	0.28	0.10	0.18
Number of Members	20		18		2		13		10		11	
Members	Line1 Line2 Line3 Line6 Line10 Line11 Line14 Line15 Line18 Line23 Line24 Line26 Line27 Line32 Line35 Line38 Line45 Line47 Line48 Line49		Line4 Line5 Line7 Line8 Line20 Line21 Line22 Line28 Line30 Line31 Line33 Line36 Line37 Line40 Line46 Line50 Line51 Line53		Line9 Line43		Line12 Line13 Line16 Line17 Line19 Line25 Line29 Line34 Line39 Line41 Line42 Line44 Line52		Line54 Line63 Line64 Line65 Line66 Line68 Line70 Line71 Line72 Line73		Line55 Line56 Line57 Line58 Line59 Line60 Line61 Line62 Line67 Line69 Line74	

COPHENETIC CORRELATION COEFFICIENT = 0.812

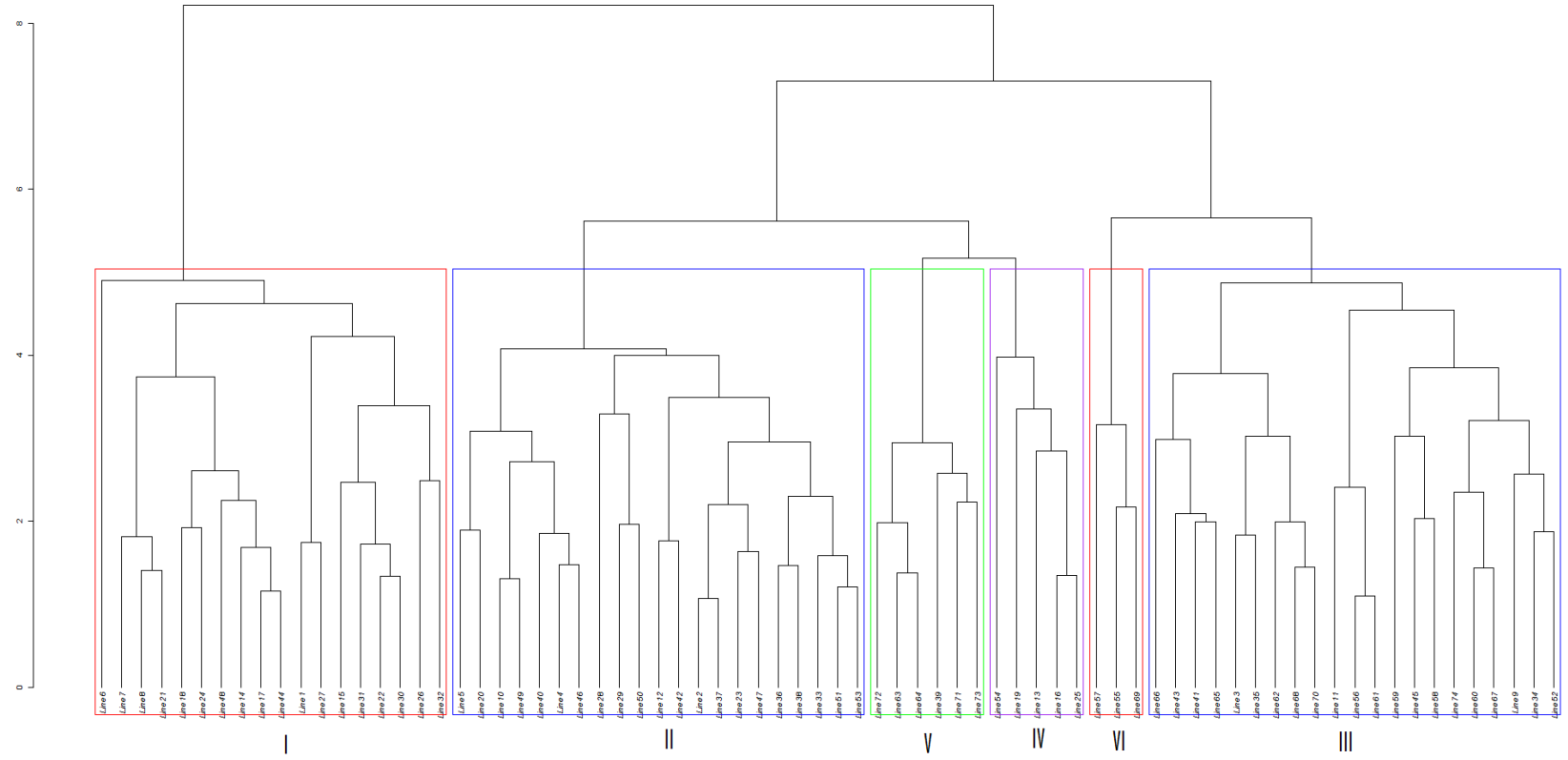

Figure 1: Dendrogram Constructed from 8 Morphological Traits of 74 Castor Breeding Lines at Badeggi, Nigeria

CGBPB 010

EVALUATION OF EFFECTS SODIUM HYPOCLORITE CONCENTRATIONS ON POLLEN VIABILITY AND POLLEN DIAMETER ON JUTE (*Corchorus olitorius)*

[*1] OLAWUYI Peter Olanrewaju, [2] Jumobi, M.,[3] Malgwi, M.M , [4] Opalere,O.T [5] Ogunsola, A. [6] Waja, S. [7] Aji, P.B. [8]. Zakari, B. G. [9] Morris, H.D.& Anjili, S.M.

[1]Department of Biological Sciences, Federal University of Technology, Minna, Niger State
[2] NECO, EDD Biology Unit Minna Niger State.
[3] Department of Plant Sciences, Moddibo Adama University Yola Nigeria
[4] Department of Educational Technology FUT Minna, Niger State.
[5] Department of Crop Production and Soil Science, LAUTECH Ogbomoso Nigeria.
[6]Department of Biological Sciences and Technology, Federal Polytechnic, Mubi Adamawa State.
[7] Department of Biological Sciences, Federal University Wukari, Taraba State.
[8] Department of Plant Sciences, Moddibo Adama University Yola.
[9]Department of Plant Sciences, Moddibo Adama University Yola .
[10]Department of Plant Sciences, Moddibo Adama University Yola.
Corresponding Author: olapeter2013@gmail.com 08061639249

ABSTRACT

Pollen viability and pollen diameter are essential criteria in plant breeding program. This study was carried out to examine the effects of sodium hypochlorite concentrations on pollen parameters of Corchorus olitorius. Seeds of Corchorus olitorius were obtained from the National Institute of Horticulture, (NIHORT) Ibadan and seeds in each case were treated with sodium hypochlorite solutions which range from 25% to 100% for six hours. Fifty seeds in each group were treated and the control was treated with distilled water. All the seeds were germinated and raised to maturity with four replicates in a randomized complete block design (RCBD). The field experiment was conducted at the garden of Centre for Preliminary and Extramural Studies, Federal University of Technology, Minna, Niger State. Pollen viability and diameter were determined using standard procedure. The result revealed significant differences (P<0.05) in pollen parameters among concentrations of sodium hypochlorite tested. The numbers of viable pollen grain (VPG) was weakly correlated (0.347) with concentrations of sodium hypochlorite increases and number of pollen diameter (PD) was strongly correlated (0.938) with concentrations of sodium hypochlorite. A positive correlation was recorded in pollen diameter (0.938). Pollens from all treatment were regular in shape and the cytoplasm stained dark brown in colour were assumed to be fertile and counted as viable while those that appeared diverted irregular in shape with a light pinkish colour or not stained at all were considered non viable. The study revealed that pollens of Corchorus olitorius could be reasonable tools for inducing genetic variability in Corchorus olitorius.

Keywords: *Corchorus olitorius, NIHORT, RCBD, VPG and PD*

Introduction

Corchorus is a genus of about 40-100 species of flowering plants in the family Malvaceae, native to tropical and subtropical regions throughout the world. The plants are usually annual herbs, reaching a height of 4m, un-branched or with side branches. It is an erect woody herb. The leaves are alternate, simple, lanceolate, 5-15 cm long, with an acuminate tip and a finely serrated or lobed margin. The flowers are small (2-3 cm diameter) and yellow, with five petals; the fruit is a many-seeded capsule. The common English names of *Corchorus olitorius* are jute plant and bush okra Jew's Mallow, Bangla Tossa Jute (India), etc. Nigerian names for the crop include ewedu in Yoruba, ahuara in Igbo, malafiya and ayoyo in Hausa (Akoroda, 2008). *Corchorus* leaves are consumed in the cuisines of various countries. The leaves have mucilaginous texture, when cooked and used as sauce. The seed is used as a flavoring and herbal tea is made from the dried leaves. The leaves of *Corchorus* are rich in beta-carotene, Iron, Calcium, and Vitamin C. The plant has an antioxidant activity with a significant α-tocopherol E (Ya Tang *et al.*, 2013). A powder prepared from dried leaves is also used to prepare sauce during the dry season. The immature fruits, called bush okra, are dried and ground to a powder for the preparation of slimy sauce. In East Africa and some parts of Nigeria, *Cochorus*, may be cooked with cowpea, pumpkin, cocoyam leaves, sweet potato, milk and butter, meat, and flavored with peppers and lemon. Thus, the potential for *Corchorus olitorius* in Nigeria were high. This has led to the growing in demand jute for domestic uses, nutritional values and economic importance.

Sodium hypochlorite is mutagenic in nature, poisonous to water organism and plants. It is very toxic and corrosive in nature. Pollen studies are widely used in convectional plant breeding, tissue culture and plant biotechnology. Pollen is used in cultivated plants to increase crop yield which is ultimate goal of most plant breeding program. Mutation breeding had been identified as a viable tool for improvement of crop plant (Girija and Dhanavel, 2013). Pollen viability connotes the ability of pollen to complete post pollination events and to fertilization. The quality of pollens lies in their ability to possess both traits. The extent of pollen viability and diameter in any plant is an indication of the effectiveness of its male parent.

Abejide *et al.*, (2013) stressed that pollination and fertilization in crop are essential for fruit formation. Pollen grains produced serve as one most valuable source of evidence for phylogenetic studies and clarification of higher level relationships (Akhila and Beevy, 2015).

However, despite all the tremendous benefit of jute to world economy, its diversity and uses were under threat in Nigeria due to low yield, lack of improved varieties and low fertilization rate with little or no concern from both government and plant breeders. There is an urgent need breeding strategy to improve high yielding genotypes that could stand the test of time. Attempt had been made to improve on the production rate using various approached such as improvement of crop and development of improved cultivars through hybridization and mutation breeding. Improvement of any crop depends on degree of characterization and variability present in the gene pool of that crop.

Besides, numerous works have been done on diversity of pollens in different mutated crops, such as direct irradiation of matured pollens like in sunflower by Aktas *et al.*, (2018), in cotton by Aslam *et al.*, 2018, Kiwi fruit by Lopes *et al.*, (2020) and in Melon by Ivanova (2020). Sodium hypochlorite has been used to cause variation in pollen viability in crops. In the present study to assessed the effects of sodium hypochlorite concentrations on pollen morphological parameters of *Corchorus olitorius* in respect to viability and diameter.

Materials and Methods

The study was carried out at experimental field, Centre for Preliminary and Extra- mural studies Federal University of Technology, Minna, Niger State in North central Nigeria between July – November 2012. The area is located within longitude $6^{0}33$'E and 9^{0} 37', and

the climate is tropical, with mean annual temperature, relative humidity and rainfall of 30.20^0, 61.00% and 1334mm respectively. The vegetation is a typical Guinea Savannah type consisting mainly of grassland with scattered trees (Olayemi *et al*., 2009).

One Kilogram of *Corchorus olitorius* seeds were obtained from the National Institute of horticulture, (NIHORT) Ibadan, the seeds were kept separately in envelopes and tied in white polythene bags. Healthy seeds were pre-soaked in distilled water by floatation method and treated were with different concentrations (0%, 25%, 50% and 75%) of sodium hypochlorite solution.

Abdullahi's (2015) method was adopted with little modification for the experimental design. The planting of the seeds was done in five-liter size pots filled with rich loamy soil were arranged in a completely randomized block design (CRBD) with ten replicates in each accession. Ten seeds were sown at the depth of 1-2cm for each accession. At two weeks after sowing, the emerging seeds (seedling) were thinned out to two per pot to reduce competition. The pots were placed in open sunlight. These plants were monitored for morphological variables at budding and flowering stages of development.

Pollen Viability Test

Eti *et al.* (2011) and Norton (2010) methods were adopted in the determination of pollen viability and pollen diameter. One gram of Potassium iodide (KI)) the solution was prepared by dissolving iodine (I) and potassium iodide (KI) in 100 ml distilled water. Freshly opened buds were randomly collected from selected plants in the morning hour at 8.00 am. Matured anthers of the flowers were collected and squashed on a microscopic slide. A drop of two percent of iodine was added and covered with a cover slip. The pollen viability counts were made within few minutes under a light microscope after pollens were placed in potassium iodide solution. Afterwards, the slides were mounted on a light microscope for observation and pollens were examined at magnification of X40. The pollen grains that were regular in shape and the cytoplasm stained dark brown were assumed to be fertile and counted as viable, while those that appeared diverted, irregular in shape, and with a light pinkish colour or not stained at all were considered non-viable. Approximately, 300 pollens were counted in each slide. The numbers of pollen grain per flower were determined using a hemacytometer. Pollen viability percentages were calculated for each accession using the formula below:

$$\text{Percentage pollen viability (PPV)} = \frac{\text{Number of viable pollens (NVP)}}{\text{Total number of pollens counted (TNP)}} \times 100$$

Pollen morphology (diameter): Pollen diameter was determined by citing an eyepiece graticule. The diameter of 30 randomly selected pollens from accessions was measured using the microscopic eye piece graticule (Abubakar *et al*., 2015) and recorded in micrometer (µm). The data generate were subject to one way analysis of variance (ANOVA) at $P<0.05$ to test for significant difference among means and Pearson linear correlation.

Result and Discussion

The result obtained showed strong correlation (0.938) exist between in pollen diameter (PD) with the concentrations. The result of anther per flower (APF) and pollen diameter (PD) were highly correlated (0.938) to the concentrations. Strong positive correlation was recorded in PD with the values decreasing as the concentrations increases. However, viable pollen grain (VPG) showed very weak correlation with the viability of pollens in *Corchorus olitorius* not increasing in proportion with the concentration. Also, non viable pollen grain (NVPG) was negatively correlated (-0.358) to the concentration. This indicated that as the concentrations of sodium hypochlorite increases the viability of the pollen grain

decreases. From the result obtained in this study, the pollen parameters showed that as concentrations increases in PPF, APF, PD, VPG and NVPG decrease in Table 1.1.

This indicates that *Corchorus olitorius* which were subjected to higher concentrations of sodium hypochlorite leads to reduction in pollen parameters values. In all the concentrations, 0% (control) and PPF (384952), APF (80.3), PD (0.0559), VPG (90.47) and NVPG (24.30) values were highest while 100% concentrations and PPF (151190.48), APF (30.24), PD (0.0312), VPG (76.07) and NVPG (09.96) were lowest respectively. This result is in agreement with finding of Beadle (2009) who reported that sodium hypochlorite is toxicity in nature. These values indicated that 0% concentrations and its pollen parameter values were highly significant while 100% concentration and its pollen parameter values were not significant on *Corchorus olitorius*, this might be due to toxicity of sodium hypochlorite which leads to reduction in pollen parameters. The significant differences observed in the pollen produced per flower, anther produced per flower and viable pollen grain in the different concentrations (0%, 25%, 50%, 75% and 100%) of sodium hypochlorite solution. This study has revealed that sodium hypochlorite solutions could be used to induce genetic variability in with regards to pollen production of *Corchorus olitorius.* The changes produced by sodium hypochlorite solutions could play in significant role in the crop improvement. The present study corroborates with the findings of Baslam (2008), Arshad *et al.*,(2003) who recorded a similar correlation with Winter rape cultivated chiefly for nutritional and medicinal purposes in India.

This result revealed non significant differences in viable pollen grain (VPG) and non viable grain (NVPG) studied. On contrary with the findings of Falusi (2006), Falusi and Salako (2003a) observed high pollen viability in irradiated sesame cultivars. Similar finding have been previously reported by Abejide *et al.*, (2013), Kumari *et al.*, (2016) and Aritharasutharsan *et al.*, (2019) in *sesamum indicum*. The pollen viability observed in all the treatment was generally high in this study which could be attributed to environmental factors and varietal differences. The fertility of male plants is dependent on its pollen viability and any flowering plants with high pollen viability have great tendencies of producing high seed production.

The significant differences observed in pollen diameter in some concentrations of sodium hypochlorite in this study might be due to the fact that *Corchorus olitorius* require slightly higher concentration of concentration to produce useful variation from the gene controlling important traits can be determined.

In conclusion, this study revealed that effects of sodium hypochlorite solutions on pollen viability had high percentage which could be used to induce genetic variability and improve the pollen on *Corchorus olitorius.* The pollen parameter would be ideal selection criteria for further improvement in *Corchorus olitorius.*

Table 1.1: Pearson's Linear Correlation on Effects of Sodium Hypochlorite in Pollen Parameters of Jute (*Corchorus olitorius)*

Concentrations (%)	PPF	APF	PD	VPG	NVPG
0	384952.38	80.33	0.0365	90.27	24.30
25	299285.71	59.86	0.0559	86.72	15.24
50	211428.57	42.29	0.0355	85.22	13.86
75	172516.25	32.65	0.0315	84.87	10.20
100	151190.48	30.24	0.0312	76.07	09.96
r	0.864	0.938	0.938	0.347	-0.358

KEYS: PPF- pollen produced per flower, APF- anther per flower, PD- pollen diameter, VPG- viable pollen grain and NVPG- non viable pollen grain

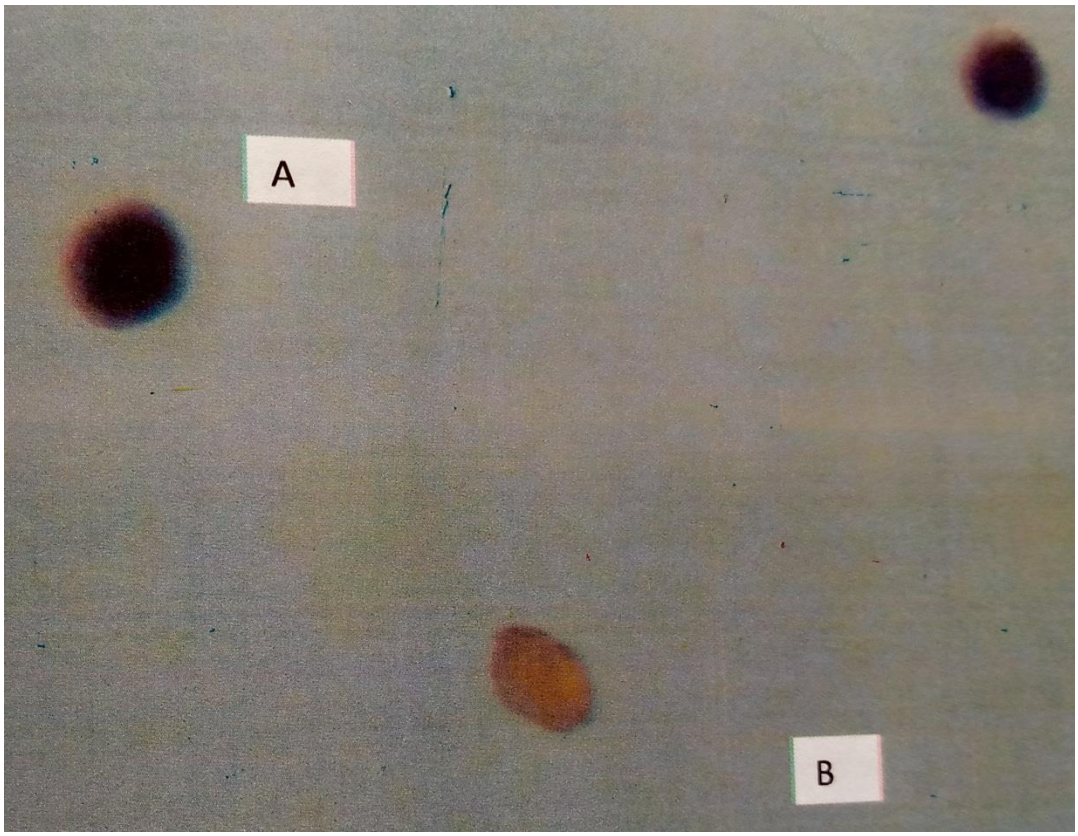

Figure 1: Viable (A) and Non viable pollen grain of *Corchorus olitorius*

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CGBPB 011

DETERMINATION OF EFFECTIVE LETHAL CONCENTRATION AND EFFECT OF ETHYL METHANESULFONATE ON GERMINATION PERCENTAGE AND SEEDLING PARAMETERS IN COWPEA (*VIGNA UNGUICULATA* L. WALP)

***Aliyu, A[1]., Aliyu, R. E[1]., Adamu, A. K[1]., Usman, A[2]., Mohammed, B. S[2]**

[1]Deapartment of Botany, Ahmadu Bello University, Zaria
[2]Deapartment of Plant Science, Institute for Agriculture Research, Ahmadu Bello University, Zaria
*Corresponding author email: aminualiyu43@yahoo.com
Tel: +2348135607081

ABSTRACT

Ethyl methanesulfonate (EMS) is a chemical mutagen used to induce mutations that can be useful in developing new breeding lines with desired attributes. In this study, EMS was used at different concentrations on a popular cowpea variety (SAMPEA 7) to generate mutants. The research was conducted to determine the most efficient lethal concentration and effect of EMS on germination percentage and seedling growth parameters of cowpea. Dry healthy seeds of the variety were treated with eight concentrations of EMS (0, 15, 20, 25, 30, 35, 40, and 45 mM) at four soaking times (2, 4, 6 and 8 h). Treatments were laid in a completely randomized design (CRD) in two replications. Data was collected on germination percentage, survival percentage, shoot length, root length, total seedling length and vigour index. The data were subjected to analysis of variance and LC50 was computed with a simple LC50 tool kit using the percentage germination data. The results revealed wide phenotypic variation in the measured attributes as the concentration and time of treatment increases with the highest being 15 mM concentration at 2 h and the lowest at 45 mM at 8 h. Treatment of cowpea seeds with 40 mM EMS at 8 h was found to be most suitable for inducing mutation and recorded percentage germination of 50%. The current findings may serve as reference concentration and time of treating cowpea with EMS to generate novel mutants for use in genetic improvement of the crop.

Keywords: *Lethal concentration (LC50), Ethyl methanesulfonate (EMS), cowpea, and seedling parameters*

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is an important grain legume of the family fabaceae. The crop is believed to be native to central Africa and is grown in both tropic and subtropic regions of the world (Singh, 2005). Cowpea grains, pods and leaves serve as a source of excellent food and feed for both human and livestock due to their rich protein, and vitamin content (Boukar *et al.*, 2018). It is one of the most prominent food legume cultivated by farmers in sub-Saharan African countries, this is partly due to itsability to survive under limited moisture conditions, where other legume crops can not grow well. (Sanginga *et al.,* 2003).

Genetic variation is the basis for plant breeding programmes (Lydia *et al.*, 2016). Most conventional breeding utilizes natural genetic variation present among germplasm pools (Ceccarelli and Grando, 2007). The natural genetic variation is a result of spontaneous mutation which is very low and insufficient to produce most of the desired agronomic phenotypes. Hence, the need to generate novel phenotypes using artificial mutations through the use of different

mutagens is crucial (Raina, 2018). Induced mutation breeding has been proven to be an effective approach to creating novel genetic variants in a wide range of crops within a relatively short period without distorting the overall genetic constitution of the crop (Khursheed *et al.*, 2016). New variants in crop plants can be created using chemical or physical mutagenic treatments followed by selection of desired and heritable phenotypes (Khursheed *et al.*, 2016. Mutants have been deployed directly as cultivars and or as a source of novel traits during hybridization (Ahloowalia *et al.,* 2004). The mutants obtained are used to overcome yield plateaus and generate desirable traits in crops including cowpea (Ahloowalia *et al.,* 2004). Mutation breeding has resulted in the release of more than 3218 officially-released mutant varieties worldwide (FAO 2014b). Alkylating agents such as EMS account for over 80% of the registered new mutant plant varieties obtained via chemical mutagenesis. One of the most crucial requirements for a successful mutation breeding programme is the selection of an effective and efficient concentration and duration of exposure of the mutagen for mutagenizing the starting material (Chopra, 2005).

Determination of a suitable lethal concentration (a dose at which germination inhibition is 50%) provide an information on the optimum concentration of a mutagen. Optimum concentration is considered a dose that induce a higher rate of mutations with less biological damage. Germination percentage is considered the most effective approach for determining LC50 (Raina, 2018). Furthermore, Tshilenge-Lukanda *et al.* (2012) described that the optimum mutation concentration can be determined by recording the percentage seed germination, epicotyl and hypocotyl lengths, among others. Therefore, this research was conducted to determine the most efficient lethal concentration and effect of the chemical mutagen EMS on germination percentage and seedling parameters in cowpea.

Materials and Methods

Study sites

The research was conducted at the physiology laboratory of the Department of Botany, Ahmadu Bello University, Zaria. Located at Long $07^0$38'E and lat.$11^0$11'N

Sources of materials

Pure dry seeds of a cowpea variety (SAMPEA 7) was obtained from the Cowpea Breeding Unit at the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. The variety was selected for its distinguishing characteristics, such as its preference by farmers and consumers, and good taste. It has a light brown seed coat and rough texture. However, it is **susceptible to scab, bacterial blight, septoria leaf spot, brown blotch, thrips, Maruca pod borer, pod sucking bugs and bruchids.** EMS was obtained from Zayo Sigma Aldech (ZSA) chemical Ltd.

EMS treatment for optimization

Seeds of SAMPEA 7 were treated with eight concentrations of EMS (0 (control), 15, 20, 25, 30, 35, 40, and 45mM) at four soaking times (2, 4, 6 and 8 h). In total, there were 40 treatment combinations (10 concentrations × 4 soaking times). For each treatment, 10 seeds were treated with EMS in 250 ml beaker at room temperature. Treated seeds were then washed three times with distilled water and sown immediately in petri dishes containing two layers of filter paper in a completely randomized design (CRD) in two replications.

Data collection

Germination percentage was evaluated 10 days after treatment (DAT). It was computed using the formula below;

$$Germination\ percentage\ (\%) = \frac{Total\ number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown} \times 100$$

Survival percentage was recorded at 14 DAT and computed with the formula;

$$Survival\ percentage\ (\%) = \frac{\text{Number of plants survive after germination}}{\text{Total number of germinated seeds}} \times 100$$

Shoot length (cm) and root length (cm) of the seedlings from different treatments and time

were assessed on 14 DAT. The total seedling length (cm) was calculated by adding the root length and seedling length. Vigor index was calculated by multiplying germination percentage and total seedling length (cm).

Data analysis

The data obtained for the parameters assessed was analyzed using analysis of variance (ANOVA) and the least significant difference test (LSD) was used to separate the means, when significant mean squares were observed in the ANOVA test.

Results and Discussions

Chemical mutagen concentration causing 50% reduction in germination (known as LC_{50}) are likely the most effective and efficient concentration to induce beneficial mutation on crop plants (Ke *et al.,* 2019). Lethal concentration has also been described as the optimum concentration that causes high frequency of favourable mutations with less damage to the test plant (Rajarajan *et al*., 2016).

The results indicated that 40 mM EMS concentration at 8 hours treatment reduced the germination percentage by 50% (Figure 1). This finding is similar to report of Horn *et al.* (2016) in cowpea genotypes. The LC50 reported in the current study can serve as a baseline for a subsequent concentration and time of treatment that can be used to treat and study a larger population of crops.

It is of utmost importance to consider germination percentage when setting up crop mutagenesis experiment, this is because the viability of seeds indicates the extent of damages caused by mutagen exposure or treatment. The result of the present study indicated significant decrease in germination percentage with increasing concentration and time of treatment, with the 45mM at 8 hours exposure time having the lowest germination percentage (40%). This significant reduction in germination percentage by the highest concentration and time could be due to the damage caused by the mutagen on the seed embryonic cell leading to subsequent death of the embryo before germination. Also, the inhibitory effect of the highest concentration on germination could be due to reduction in seed enzymatic activities which affect the development of plumule and radicle. This agrees with the work of Ke *et al.* (2019) who observed that higher concentration of EMS and treatment time reduce germination percentage of Cauliflower. Such concentration dependent relationship was also reported by Rajarajan *et al.* (2016).

The results indicates decrease in survival percentage (40%) at highest concentration of EMS (45 mM) at 8 hours time (Figure 3). The highest survival percentage (95%) was recorded at the 25 mM concentration and 4 hours . Also, higher survival rates were recorded at lower and intermediate concentration and time, in addition lower survival rates are recorded at higher mutagenic treatments and time. Reduction in the plant survival percentage might be due to the inhibitory effects of EMS on the meristematic tissues of the seed which results chromosomal damages. This contradict the work of Geng *et al.* (2019) who reported that higher doses of mutagen treatment results in higher survival percentage of the crop. Ki *et al.*(2019) reported that reduction in survival percentage with increasing concentration could be due to alteration of protein and promoters for survivability.

The results on shoot length, root length, total seedling length and vigour index on EMS induced mutants showed a significant effect on all the traits as compared to the control (Figure 4, 5 and 6). Highest shoot length (15.75cm), root length (11cm), total seedling length (26.75cm) and vigor index (2530) was recorded at low concentration of 25mM treatment for 2 hours. The least values for the aforementioned traits was observed at highest concentration 45 mM for 4 hours treatment. The reduction in seedling growth with increased concentration of the mutagen could be as a result of effect of mutagen on auxin, ascorbic acid content and physiological injury and biochemical disturbances as reported by Yusuf and Nair, (1974). These results are in accordance with Rajarajan *et al.* (2016).

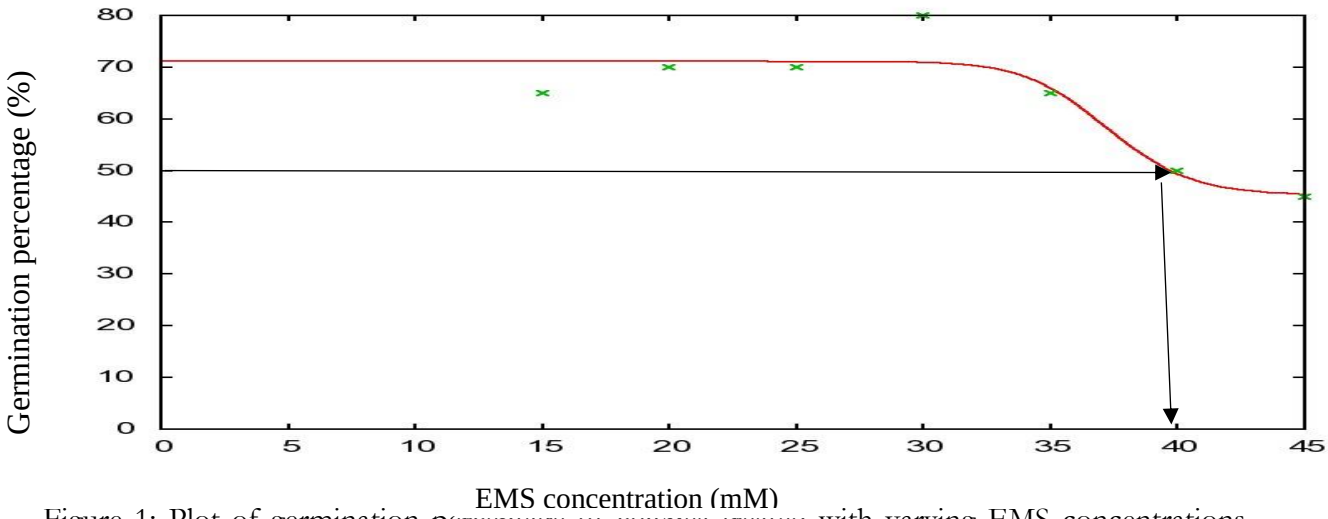

Figure 1: Plot of germination percentage of cowpea treated with varying EMS concentrations showing lethal concentration on SAMPEA 7

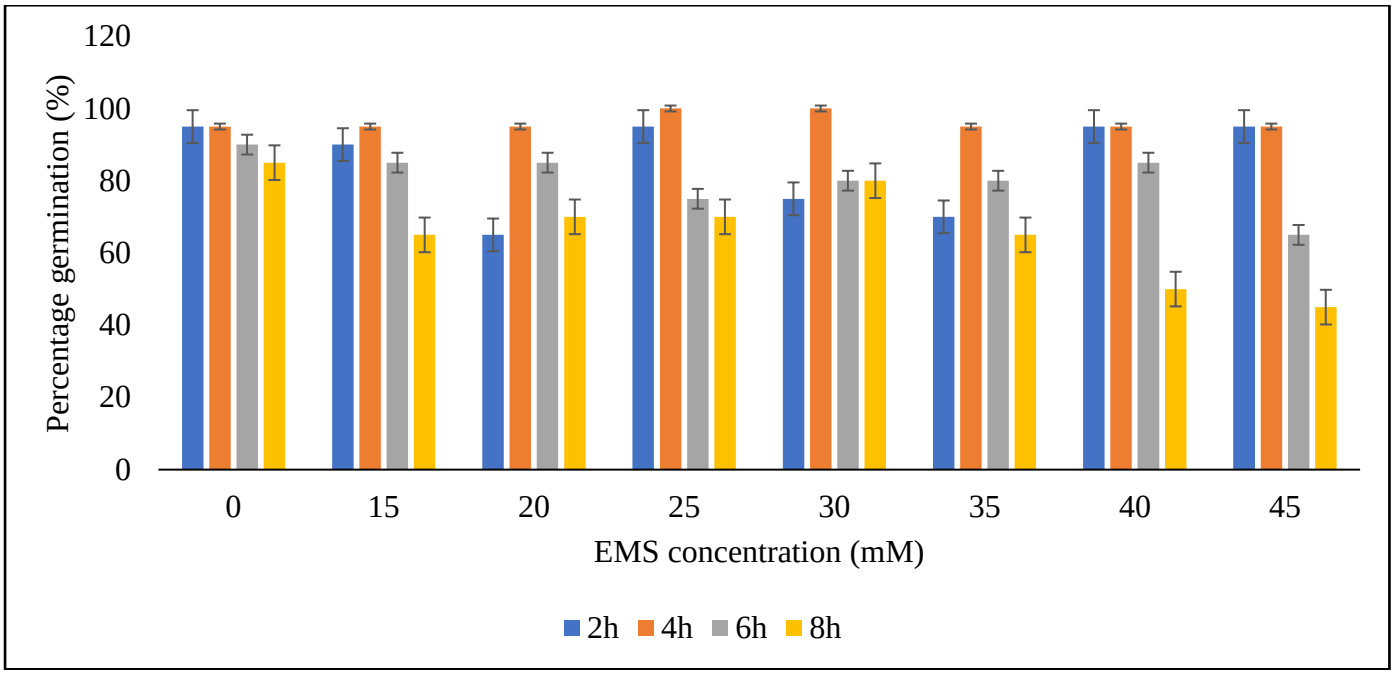

Figure 2: Percentage germination (%) of SAMPEA 7 cowpea treated with different concentrations of EMS at varying time

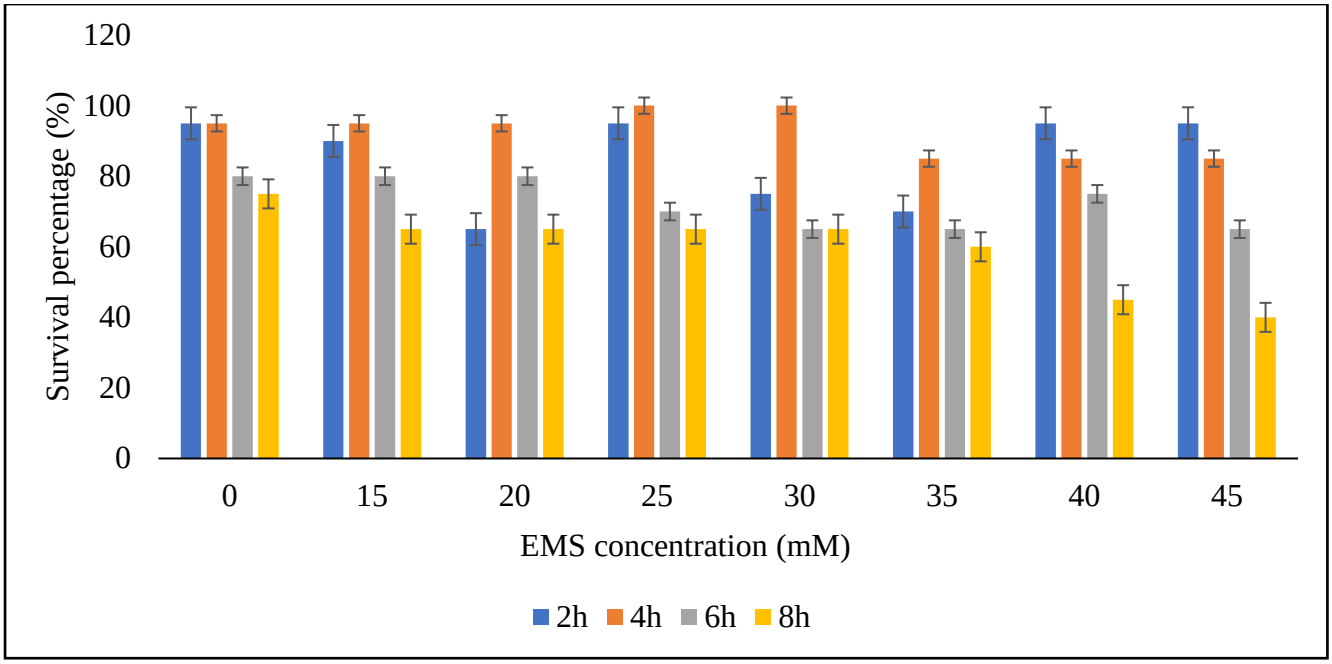

Figure 3: Survival percentage (%) of SAMPEA 7 cowpea treated with different concentration of EMS at varying time

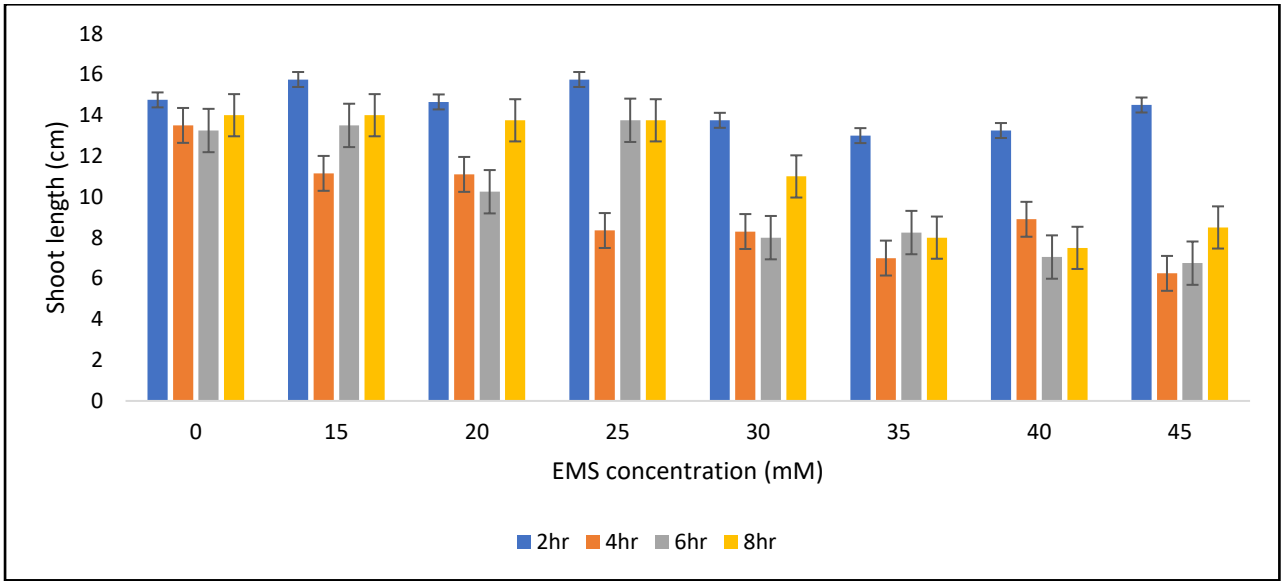

Figure3: Shoot length (cm) of SAMPEA 7 cowpea treated with different concentration of EMS at varying time

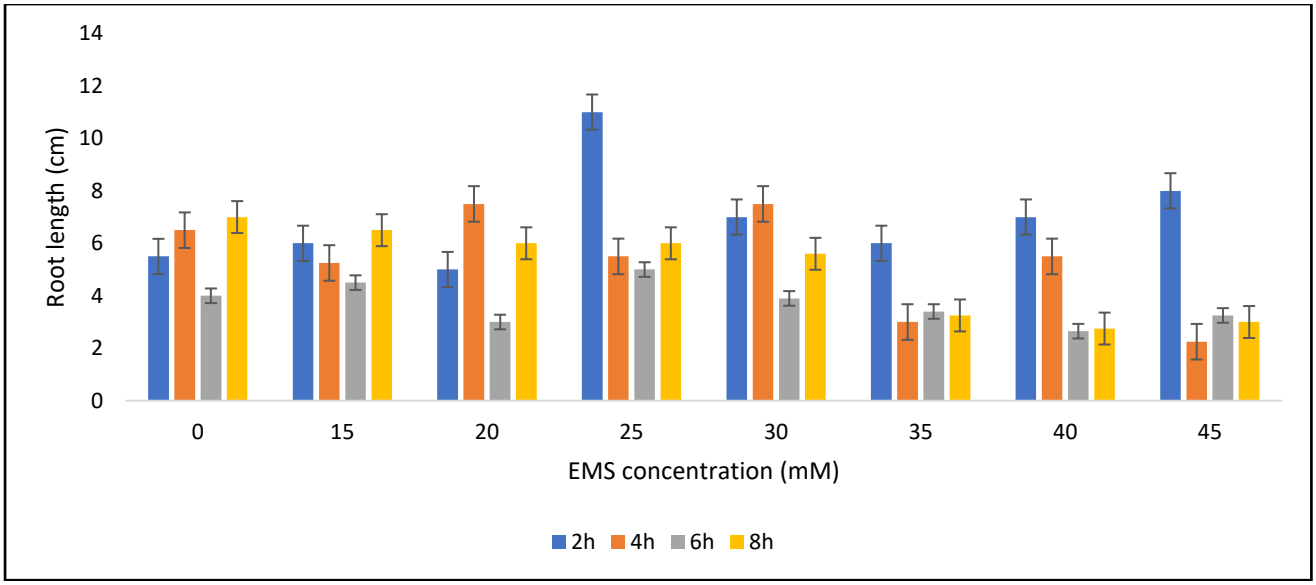

Figure4: Root length (cm) of SAMPEA 7 cowpea treated with different concentration of EMS at varying time

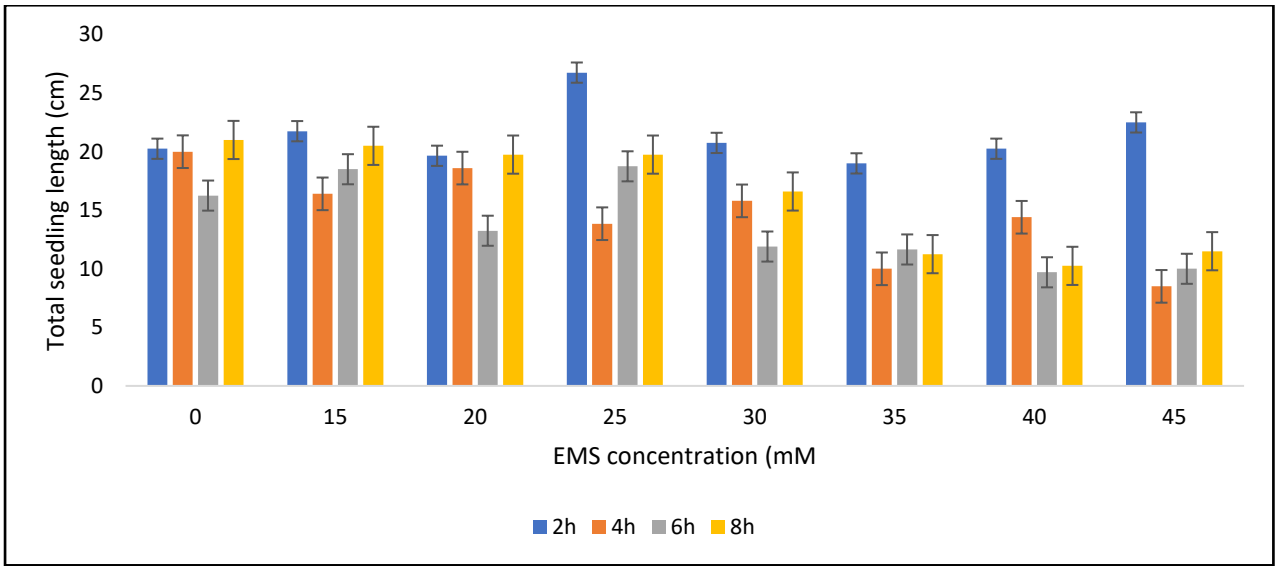

Figure 5: Total seedling length (cm) of SAMPEA 7 cowpea treated with different concentration of EMS at varying time

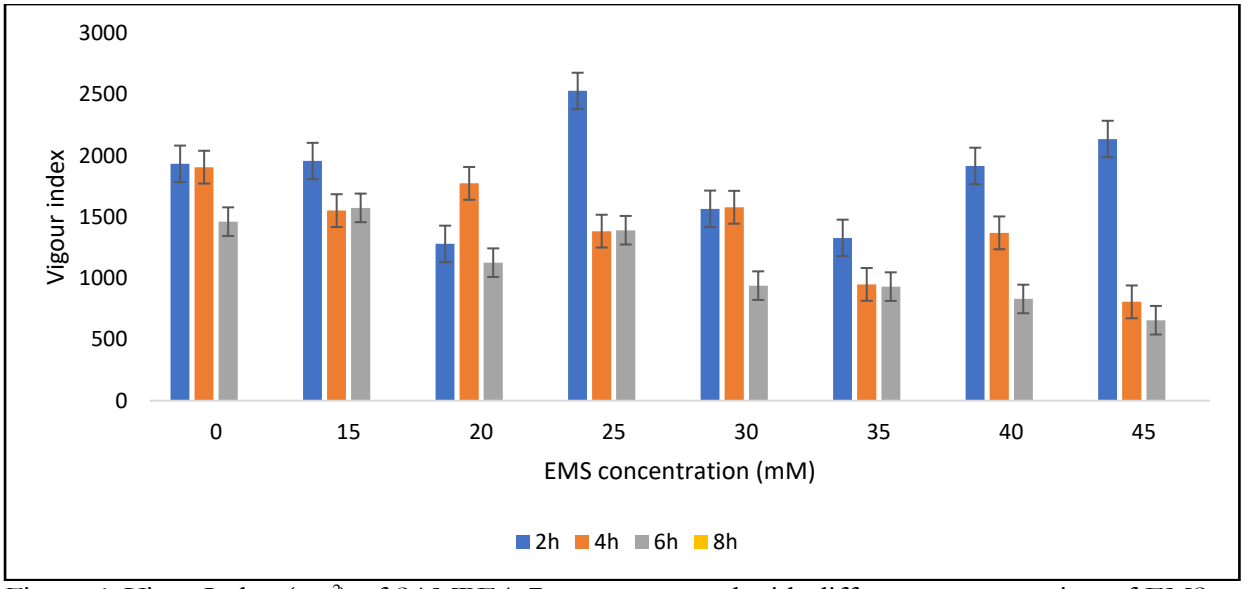

Figure 6: Vigor Index (cm^2) of SAMPEA 7 cowpea treated with different concentration of EMS at varying time

Conclusion and Recommendation

The present study showed that the most efficient lethal concentration for SAMPEA 7 was 40mM at 8 hours of treatment under laboratory assessment of seed germination percentage. Different concentration of EMS at varying time has been shown to enhance the germination percentage, survival percentage and seedling growth of cowpea with the highest recorded at 25mM for 2 hours and 4 hrs. Therefore, the recorded lethal concentration of EMS can be utilized for carrying out a successful broad base mutation breeding programme. In addition, the potential of EMS efficiency and effectiveness in inducing beneficial mutation without disturbing the growth of cowpea has been shown.

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CGBPB 012

GENETIC EVALUATION AND CORRELATION ANALYSIS IN RELATION TO SEEDLING STAGE MORPHOLOGICAL RESPONSE OF MAIZE VARIETIES UNDER DIFFERENT WATER STRESS LEVELS

***Indabo, S. S.[1], Jimoh, S. O.[1], Aliyu, R. E.[2], Muhammad, H. U.[1], Aliyu, A.[2] Bayero, U.,[1] and Adamu, A.[3]**

[1]Department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria.
[2]Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria.
[3]Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria.
*Corresponding author: sadambiologist@gmail.com, +2348130679660

ABSTRACT

Maize is an important food crop that is highly sensitive to abiotic stress conditions. The current study aimed at evaluating genetic parameters and correlation analysis of seedling stage morphological response of maize varieties subjected to water stress levels. The experiment was conducted at the screenhouse of the B otanical garden, Department of Botany, Ahmadu Bello University Zaria, Nigeria. Seeds of six maize (Zea mays L.) varieties were obtained from the seed unit, Institute for Agricultural Research (IAR), Ahmadu Bello University. The experiment was arranged in Completely Randomized Design (CRD) with three replications . Three (3) seeds of each variety were sown in polythene bags and thinned to one seedling after germination. The seedlings were maintained using standard protocols. Water stress was then imposed by withholding irrigation. The experiment was grouped into five treatments; T_0 (control) and T_1 to T_4 where irrigation was withheld for one to four weeks respectively. Data were collected on plant height, stem diameter, leaf length, leaf width and number of leaves per plant before and after stress imposition. Analysis of variance revealed significant differences for all the traits. The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the traits. Highest PCV, GCV and broad sense heritability of 16.6 %, 17.9 % and 93.0 %, respectively were recorded in stem diameter. Highest correlation (r = 0.8) was obtained between stem diameter and leaf width. Therefore, selection for these traits could be promising in maize improvement programmes.

Keywords: *Genetic evaluation, correlation, morphological, maize, stress*

Introduction

Maize (*Zea mays*) belongs to the family *Poaceae* (*graminea*). It is the third most important food crop in Nigeria and worldwide at large (Ali *et al.*, 2011; Kamara *et al.*, 2014). Maize is a valuable commodity that is geographically dispersed and cultivated across Nigeria. It is perhaps the most common staple food in developing countries, providing food for 900 million people earning less than US $2 per day (IITA, 2020). Over the years, maize has become an important crop, taking over acreages from traditional cereal crops such as millet and sorghum. In 2018, about 10.2 million metric tons of maize was produced from 4.8 million hectares in Nigeria, making the country the highest producer in Africa

(Oyekunle and Badu-Apraku, 2013; FAO, 2018).

The world-wide consumption of maize is more than 116 million tons, a testament to the fact that there is a high consumption of maize globally. Despite the importance of maize, its production has been insufficient to meet the demands. The domestic demand for maize in Nigeria stands at 11800 metric tones (USDA, 2020). It is projected that by 2050, the demand for maize in the developing world is expected to double (Agricdemy, 2020). In spite of all efforts, maize production in the African continent is very low compared to developed countries due to many constraints, such as biotic, abiotic, poor agronomic practices, low soil fertility, drought, and unavailability of improved germplasm (Ali *et al*., 2011; Agricdemy, 2020).

Maize is affected by drought at different growth stages in different regions. Plants have developed numerous strategies which enabled them to cope with drought stress. Maize germplasms also have numerous features which enable some accessions to cope with water stress. Direct selection for grain yield under drought in breeding is often not effective due to the complexity of the trait, which is influenced by several component traits. Secondary traits are the morpho-physiological traits which affect yield indirectly, and could assist in the identification of the genotypes that can easily adapt with the stressed environment (Belay, 2018). The intelligent exploitation of maize for genetic analyses requires a detailed knowledge of genetic parameters (Ali *et al*., 2013). Genotypic variability can be exploited efficiently by selection depending upon heritability and the genetic advance of individual traits. Heritability estimates are useful for breeding quantitative traits in terms of selection through prediction of genetic gain in breeding programmes (Belay, 2018). The efficiency of selection can be achieved using estimates of genetic parameters, which are fundamental in plant breeding since they are useful in identifying the nature of gene actions involved in the control of quantitative traits (Vashistha *et al*., 2013). Therefore, this study conducted to assess; xyz

Materials and Methods

Study aArea

The experiment was conducted under the screenhouse at the Botanical g arden, Department of Botany, Ahmadu Bello University, Zaria, Nigeria.

Plant materials

Six (6) different varieties of maize were collected from the seed unit, Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. The varieties include Sammaz 14 (quality protein maize), Sammaz 15 (S triga resistant), Sammaz 17 (quality protein maize), Sammaz 29, Sammaz 51 and Sammaz 53.

Description of the Experiment

Polythene bags filled with top loamy soil were used for the sowing. Three (3) seeds of each variety were sown per bag and thinned to one healthy seedling after germination (Akinwale *et al*., 2016). The experiment was laid out in Completely Randomized Design (CRD) with three replicates. All the agronomic and cultural practices were followed using standard procedures.

Drought i nduction

The seedlings were maintained under normal irrigation for one week after emergence. For drought induction, the experiment was grouped into the following treatments; T_0 = control (continuously irrigated), T_1 (irrigation was withheld for one week), T_2 (irrigation was withheld for two weeks), T_3 (irrigation was withheld for three weeks), and T_4 (irrigation was withheld for four weeks). For each treatment, watering was resumed after the respective drought imposition .

Data collection

Data was collected before and after stress imposition at the vegetative stage of the plant. The following parameters were taken: plant height (cm), stem diameter (mm), leaf length

(cm), length width (indicate unit), and number of leaves/plant.

Data a nalyses

Data obtained on plant height, stem diameter, leaf area, leaf lengths and leaves per plant were subjected to analysis of variance (ANOVA) using SAS software. Genotypic, and phenotypic coefficients of variation (GCV, and PCV) and heritability were estimated using the following formulae of Schmidt *et al.* (2019)

$$GCV = \frac{\sqrt{\sigma g}}{X} \times 100 \qquad PCV = \frac{\sqrt{\sigma p}}{X} \times 100$$

Where: GCV = Genotypic coefficient of variation (%), X = Grand mean of the traits, PCV= Phenotypic coefficient of variation (%), σp = p henotypic variance, σg= g enotypic variance.

Broad sense heritability was calculated as a ratio of the genotypic variances to phenotypic variances. Phenotypic (r_p) and genotypic (r_g) correlation coefficients were calculated as outlined by K won and Torrie (2013).

$$rp = \frac{Mij}{\sqrt{(Mii)(Mjj)}} \quad \text{and} \quad rG = \frac{Cov\ gij}{\sqrt{(Vargi)(Vargj)}}$$

Genetic advance (GA) was calculated using the formula of Ali *et al.* (2013): $GA = \sigma P \times h^2 \times i$

Results and Discussion

The mean values for the morphological parameters are presented in T able 1. There was a significant difference ($p < 0.05$) in the morphological parameters across the treatments. Plant height in the control was higher over the remaining treatments. These suggests the presence of variation among the genotypes. Water stress at the vegetative stage reduced the plant height and also affected other traits that could indirectly affect the yield. Similar findings were reported by Sabiel *et al.* (2014) on genetic variation of plant height and stem diameter traits in maize (*Zea mays* L.) under drought stress at different growth stages. Genetic variability is essential in order to realize response to selection pressure. The genetic parameters are presented in T able 2. The magnitude of phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) in all the traits studied, indicating that environmental influence has played a role in the expression of these traits. GCV values ranged from 10.15% to 16.60% while PCV values ranged from 12.22% to 17.86%. The highest PCV (16.60%) and GCV (17.86%) were recorded in stem diameter. Belay (2018) also reported similar findings. Heritability was highest (92.98%) in stem diameter, indicating high genetic influence on the trait. So, improvement in stem diameter can be made based on phenotypic performance (Belay, 2018).

Table 3 presented the correlation of the agronomic traits. Correlation analysis in plant breeding reveals the relative importance of different plant traits. There was significant positive correlation between plant height and the traits; stem diameter, leaf length and leaf width. Also, there was correlation between leaf length and leaf width . Highest correlation (0.758) was recorded between stem diameter and leaf width. The strong correlation indicated that selection on the basis of these traits may be useful to improve the yield of maize genotypes under stress conditions.

Table 1: Means for the morphological parameters of maize before and after water stress at seedling stage in 2020

GENOTYPES	TREATMENTS	BS PH (cm)	SD (cm)	LL (cm)	NL	LW (cm)	AS PH (cm)	SD (cm)	LL (cm)	NL	LW (cm)
SAMMAZ 14											
	CONTROL (T0)	10.08	1.74	17.80	3.20	1.52	15.80	3.62	50.80	6.00	2.72
	T1	10.08	1.84	20.11	3.40	1.48	17.60	3.26	51.20	5.00	2.40
	T2	7.30	1.74	12.50	3.00	1.46	15.20	2.34	36.60	5.00	1.72
	T3	7.76	1.80	18.76	3.00	1.26	15.50	2.40	41.00	4.00	1.81
	T4	7.40	2.00	19.62	3.40	1.52	16.60	2.34	41.80	5.00	1.74
SAMMAZ 15											
	CONTROL (T0)	6.84	1.74	14.40	2.80	1.54	16.80	3.40	45.60	5.00	2.26
	T1	6.64	1.84	15.00	2.80	1.50	19.48	3.70	51.40	5.90	2.52
	T2	7.50	1.94	16.20	2.80	1.62	17.05	3.45	43.30	4.61	2.46
	T3	6.72	1.98	16.02	3.20	1.64	17.00	3.26	45.80	5.60	2.07
	T4	5.89	1.88	12.98	3.00	1.94	13.20	2.12	27.90	5.00	1.58
SAMMAZ 17											
	CONTROL (T0)	8.18	2.12	21.50	3.60	1.76	20.14	3.40	38.30	5.60	2.52
	T1	6.66	1.78	14.62	3.00	1.46	15.70	2.76	43.70	5.60	1.88
	T2	5.96	1.64	14.87	2.80	1.30	11.80	2.50	27.00	4.00	1.80
	T3	9.70	2.14	22.26	3.60	1.84	13.60	2.16	31.00	4.60	1.88
	T4	7.06	1.98	16.46	3.40	1.44	16.60	2.30	34.40	4.60	1.80
SAMMAZ 29											
	CONTROL (T0)	7.96	1.88	24.92	3.60	1.60	18.40	3.46	45.30	7.60	2.42
	T1	7.94	1.94	22.04	3.60	1.43	17.30	3.00	45.10	6.60	2.50

	T2	7.49	1.76	19.58	3.40	1.60	18.40	3.24	52.20	5.00	2.32
	T3	8.86	1.79	19.34	2.80	1.52	16.00	3.04	42.00	5.00	1.90
	T4	8.00	1.78	16.90	3.00	1.46	11.40	1.94	37.80	4.00	1.58
SAMMAZ 51											
	CONTROL (T0)	7.64	1.84	18.00	3.40	1.58	18.40	3.12	39.80	5.00	2.12
	T1	7.72	1.86	19.84	3.40	1.52	16.00	3.22	37.70	5.00	1.76
	T2	7.20	1.75	13.31	3.00	1.50	19.36	3.27	42.78	5.60	2.40
	T3	7.76	1.72	14.16	2.80	1.42	15.80	3.16	39.28	5.00	2.20
	T4	6.92	1.82	15.18	2.80	1.50	16.30	2.36	33.08	4.00	1.84
SAMMAZ 53											
	CONTROL (T0)	7.10	1.78	16.14	3.200	1.60	16.00	3.30	43.20	5.00	1.80
	T1	8.32	1.92	19.86	3.40	1.50	17.60	3.06	50.50	5.00	2.46
	T2	7.84	1.82	19.00	3.20	1.52	17.75	2.85	34.75	5.00	1.80
	T3	7.64	1.94	22.27	3.25	1.46	15.20	3.20	43.20	5.00	1.86
	T4	8.70	2.12	24.76	3.60	1.72	15.60	2.54	39.40	4.33	1.86

PH = Plant height, LPP = Number of leaf/plant, SD = Stem diameter, LL= Leaf length, LW = Leaf width, T_0 = Control, T_1 = irrigation withheld for one week, T_2 = irrigation withheld for two weeks, T_3 = irrigation withheld for three weeks, T_4 = irrigation withheld for four weeks, BS = Before Stress, AS = After Stress

Table 2: Estimates of genetic components of maize varieties under different water stress at seedling stage in 2020

Traits	GCV (%)	PCV (%)	GV	PV	ECV (%)	EV	GA	H^2 (%)
PH	12.30	12.81	4.06	4.41	1.14	0.04	3.39	92.14
LPP	13.87	17.45	0.50	0.79	10.58	0.29	0.99	63.21
SD	16.60	17.86	0.23	0.27	6.58	0.04	0.85	92.98
LL	15.99	16.72	43.38	47.43	4.89	4.05	11.05	91.46
LW	10.15	12.22	0.04	0.06	6.79	0.02	0.37	83.08

PH = Plant height LPP= Number of leaf/plant SD = Stem diameter LL= Leaf length LW= Leaf width GCV = Genotypic coefficient of variation PCV = phenotypic coefficient of variation ECV = Environmental coefficient of variation EV = Environmental variance GA = Genetic advance H^2 = Broad sense heritability.

Table 3: Correlation matrix of agronomic traits of maize varieties under different water stress conditions evaluated at the seedling stage in 2020

Var.	PH	LPP	SD	LL	LW
PH	1.000				
LPP	0.453	1.000			
SD	0.686**	0.489	1.000		
LL	0.545**	0.425	0.669**	1.000	
LW	0.652**	0.523**	0.758**	0.667**	1.000

PH = Plant height, LPP = Number of leaf/plant, SD = Stem diameter, LL = Leaf length, LW = Leaf width, Var. = variables, ** = Significant at ($P \leq 0.01$)

Conclusions and Recommendations

Genetic variation is important in determining the success of any breeding programme. There was considerable variability present in the materials studied. Therefore, the results will be useful in choosing parents for breeding maize varieties for tolerance to water-stressed environments. Stem diameter correlated with several traits, it is therefore recommended that maize genotypes at vegetative stage can be selected on the basis of stem diameter and plant height; secondary traits which influences yield. This could be used to predict genotypes with high yield.

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GENETIC POLYMORPHISMS AND MATERNAL LINEAGE OF PIGEON PEA [*CAJANUS CAJAN* (L.) MILLSP.] INFERRED FROM *MATK* AND *PEPB REGIONS OF CHLOROPLAST DNA* AND *ITS* REGION OF NUCLEAR DNA

***[1]Udensi, Ogbuagu Ugorji, [2]Efienokwu, Jude Ngozichukwuka*, [3]Achilonu, Conrad , [1]Emeagi, Lasbrey Ikenna, and [4]Thomas, Luka Tentishe**

[1] Plant Genetic Resource Management and Genomics Unit, Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria
[2] Department of Science Laboratory Technology, Delta State Polytechnic, P.M.B 1030, Ogwashi-Uku, Nigeria.
[3] Department of Genetics, Faculty of Natural & Agricultural Sciences, University of the Free State, P.O. BOX 339, Bloemfontein 9300, Free State, Republic of South Africa
[4] Department of Biological Science, Taraba State University, Jalingo, Taraba State, Nigeria
*Author(s) for correspondence: princeudensi@unical.edu.ng; ugorji2012@gmail.com efienokwu.jude@mydspg.edu.ng; judencj@yahoo.co.uk

ABSTRACT

The problems of food and nutrition security in Sub-Saharan African (SSA) countries, especially Nigeria have not been adequately and critically analyzed, despite various approaches employed to addressing these challenges. The precarious environmental and climatic fluctuations have severe impact on global food production (Udensi et al., 2012; Lesk et al., 2016) implying that in order to meet up with the food demand of a rapidly growing world population, yields to important economically crops must be increased exponentially (Tester & Langridge, 2010). Crop breeders have been extremely successful in combining beneficial loci within germlasm, which results in the enhancement of yield of modern agriculture (Mickelbart et al., 2015). Unfortunately, however, the tremendous success of selective breeding has also caused a drastic reduction if genetic diversity in many elite crop germplasm pools thus limiting the potential for future genetic gain (Qian et al., 2017). Understandably, breeding crops that consistently achieve high yields, the environment notwithstanding is a highly challenging (Qian et al., 2017). With the enormous recent advances in DNA sequencing and genotyping technologies, these challenges can be surmounted (Qian et al., 2017).

Keywords:

Introduction

For sustainable food and nutrition security in Nigeria, there has to be urgent need to explore, exploit, develop and improve landraces of crops, especially underutilized legumes, taking into cognizance the ravaging and scourging wind of malnutrition across the African continent due to protein deficiency as well as the rapid genetic erosion of crop species (Naylor et al., 2004; Udensi and Edu, 2014). The importance attached to the leguminous family, especially pigeon pea cannot be over-emphasized in mitigating protein deficiency in the rural population, which is more than 60% of the entire population in most SSA countries, including Nigeria (Udensi *et. al.,* 2017). It has been observed that the economic importance of pigeon pea notwithstanding, there are very little research attention in terms of breeding and improvement as pertains the application of molecular and genomic tools (Varshney *et al.,* 2009; Odeny *et al.,* 2009; Saxena *et al.,* 2009). Undoubtedly, landraces of crops are repository/reservoirs of important economic

traits as they accumulate mutations and recombination, which has placed them at vantage position for use in widening the genetic base through introgression into existing gene pool of crop species.

Effective and efficient determination of species taxonomy and evolutionary pattern recently has been hinged on molecular/sequence data (Udensi et al., 2017), which have been sourced from chloroplast, mitochondrial as well as nuclear genomes. For emphasis, mtDNA is located in the cytoplasm and is involved in supplying cellular energy, signaling cellular differentiations, maintaining as well as controlling cell cycle and growth (McBride et al., 2006) while cpDNA contain organelles of endo-symbiotic origin (Babiychuk et al., 2012), which are active centers for the conversion of solar energy to carbohydrates through photosynthetic process (Daniel et al., 2016). Maturase K (*matK*) and Petite B (*petB*) are chloroplast-encoding genes. *matK* is a chloroplast-encoding gene nested between the 5' and 3' exons of *trnK*, tRNA-lysine in the large single copy region of the chloroplast genome (Steane, 2005; Daniell et al., 2006; Turnmel et al., 2006) while Petite B (*petB*) encodes a subunit of the cytochrome b6/f complex, which is found on the opposite DNA strand 537bp upstream of the *frxC gene* (Huang and Liu, 1992). Internal transcribed spacer (*ITS*) gene is nuclear-based gene, which is located between the 16S – 23S rRNA genes, lying between the small subunit (SSU) rDNA and the large subunit (LSU) rDNA coding regions. It varies between 534 and 561bps. The variations in gene sequences located in these genomes have been very pivotal in deciphering and elucidating species differences at the species level. Evolution and the resultant speciation of plant species is as result of how favourable a mutation process is in the plant.

Genetic polymorphisms are the differences in DNA sequences among species or populations, which includes single nucleotide polymorphisms (SNPs), insertions, deletions as well as recombination. Of interest in this present discourse is the SNPs, which have been used in model and non-model species for genetic diversity and population structure analysis, marker assisted selection and association studies (Frascaroli et al., 2003). SNPs creates different variants or alleles of a particular gene and whose sequences tend to be transmitted unchanged along generation. According to Yu et al. (2009) the informativeness of SNPs depends on species being considered. Sequence variation caused by single nucleotide polymorphisms is the most common source of genetic polymorphisms, which could be due to transition or transversion nucleotide base substitutions and have been reported to cause speciation of plants – *petB* and *ITS* gene sequences (Hao et al., 2010; Jiang et al., 2011), *matK* gene sequences (Zhu et al., 2007). Importantly, it might be difficult to discuss SNPs without mention of haplotype, which is a particular pattern of sequential SNPs found on a chromosome, which tend to be inherited together over time. This has served as disease-gene markers. This becomes imperative owing to the fact that mutations in one copy of a chromosome pair can be masked by normal sequences present on the other copy. SNPs have been reported to have limitations, especially when applied in GWAS (genome-wide association studies) as they provide only biallelic information at any individual locus as well as not adequately representing rare alleles (Maher, 2008), which is a significant fraction of the genetic variance for a given quantitative traits. Additionally, commercial genotyping arrays find it difficult to detect (Wray et al., 2013; Voss-Fels & Snowdon, 2016). An effective approach to overcome the biallelic limitations of SNPs intended to increase the allelic resolution of candidate genomic regions is to employ haplotype, which is the specific combination of jointly inherited nucleotides or markers from polymorphic sites in the same chromosome segment (Stephens et al., 2001; Lu et al., 2010). According to Bernardo (2010), a haplotype is two or more SNP alleles that tend to be inherited as a unit and the construction of haplotype based on empirical markers data is very informative (Korte & Farlow, 2013). According to Jiang

et al. (2015) using haplotype network analyses showed that strong allelic selection in the course of breeding, domestication and polyploidization has narrowed the genetic diversity of three wheat *CWI* genes. Recent study showed that haplotype-based approaches are also useful to disclose relationships between crop characteristics that appear to be unrelated (Qian et al., 2017). Based on the strong genomic structure present in the breeding pools of most crops, the deployment of haplotypes could be a powerful complementary tools to improve accuracy and efficiency of both MAS and GS (Qian et al., 2017). Bevan et al.

(2017) opined that haplotype-related markers can be used to identify lines with novel recombination in chromosomal blocks of interest in order to separate favourable and unfavourable genetic variation.

This paper is poised at detecting SNPs, selection types, haplotypes, and tracing evolutionary lineage of *matK, petB* and *ITS gene* sequences, with the view to deciphering the extent of genetic polymorphisms and understanding evolutionary origin of these sequences, which can be harnessed for gene(s) encoding traits location and identification.

Materials and Methods

Collection of plant materials and genomic DNA extraction

Seeds of pigeon pea *Cajanus cajan* (L.) Millsp.] accessions were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Niger, sub-station. All seed samples were homogenized in liquid nitrogen and stored at – 80°C for DNA extraction. DNA was isolated using DNeasy Plant Mini Kit (QIAGEN, *www.qiagen.com*).

Agarose gel Electrophoresis

A 2% agarose gel was prepared by using electrophoresis grade agarose in 1x Tris, Acetic acid, EDTA (TAE) buffer (pH 8.0) and ethidium bromide (10mg/ml) to a concentration of 15 mg/ml. The PCR product and 6x loading dye solution was mixed and loaded on the agarose gel. A Gene

Primer Design

Integrated DNA Technologies-IDT primer tool (www.eu.idtdna.com) was used to design the primers by computer stimulation. For the determination of the appropriate melting temperature (Tm), GC content as well as any possible formation of Hairpin's for all the primers oligonucleotide properties calculator online was adopted. On delivery of the primers, a concentrated stock solution of 100µM (100pmol/µl) was prepared (dissolving each lyophilized pellet in Tris-EDTA (TE) buffer containing 10mM Tris, pH7.5; 1mM EDTA pH 8.0.

PCR amplification of the Genes

Maturase K (*matK*) (plastid gene), Internal transcribed spacer (*ITS*) (nuclear gene) and peptite B (*petB*) mitochondrial gene were amplified. The PCR reactions with the resulting DNA template were carried out in

6 PCR tubes of forward and reverse primers (10µM) – *matK*: F 5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3';R 3'-CGTACAGTACTTTTGTGTTTACGAG-5'; *petB*: F 5'-CTCACTATTTAGACCTCGCAACC-3' ;

3'-CGCCTGTGACCCAAGTTAAT-5'; *ITS*: F 5'-CCTTATCATTTAGAGGAAGGAG-3'; R 3'-TCCTCCGCTTATTGATATGC-5'.

Gradient PCR was performed in a Thermal Cycler "G-Storm Labtech GS04822" (Labtech International Ltd) using the primers sets.

Ruler of 1kb DNA Ladder (New England's BioLabs® Inc.) was loaded in the first lane as a molecular size marker. The PCR products were separated at 100 Volts for 60mins and visualized on a gel documentation device [Vacutec Syngene G-box (Vacutech)] under UV light.

Sequencing of PCR products

The gradient PCR samples of the DNA fragments with fine bands were cut off and purified with a purification kit (BIoFlux gel extraction kit; Bioer). A cycling sequencing reaction volume was prepared for each template with a BigDye terminator v3.1 kit (Applied Biosystems). The reaction component of Terminator Premix, sequencing primer (3.2μM), dilution buffer, and 2ng purified DNA was added. The reactions were performed in a Thermal Cycler (G-Storm Labtech GS04822) under the following conditions: initial denaturation at 96 °C for 1min., followed by 25 cycles of 96°C for 10sec, 50°C for 5sec, 60°C for 4mins., and stored at 4°C. Each sequencing reaction volume was transferred to a 1.5ml Eppendorf tube and adjusted to a final volume with 1x dH_2O, 125mM EDTA and absolute ethanol. This was followed by 5sec vortex and then precipitated at room temperature for 15mins. The samples were centrifuged at 20000 g for 15mins at 4°C and the supernatant completely aspirated. The pellets were washed with 70% ethanol and centrifuged at 20000 g for 8mins at 4°C. The supernatant was completely aspirated and the pellet air-dried for 20mins. The samples were then stored in the dark at 4°C until analysis using an "Applied Biosystems 3130 Genetic Analyser" (Applied Biosystem).

Bioinformatics and phylogenetic analyses

Chromas software 2.6.4 (www.technelysium.com.au) was used to view and edit the gene sequences in the form of chromatograms. Multiple sequence alignment was done excluding all the gaps using muscle as the default parameter (MEGA version 6.0.6: Tamura et al., 2013). Detection of SNPs was done with Codon Code Aligner software while determination of selection types was performed using MEGA version 6.0.6 (Tamura et al., 2013). NETWORK 4.6.1.1 was used for network analysis of the different haplotypes (Bandelt *et al.*, 1999). Using MEGA software, *matK, petB* and *ITS* gene sequences of other legumes were downloaded from the NCBI database, while the query sequences the 3 sets of genes obtained from pigeon pea were used to trace maternal lineages.

Results and Discussion

Results

DNA polymorphisms detected on *matK*, *petB* and *ITS* gene sequences

We report polymorphic sites detected on the 3 sets of gene sequences to be 617, 363 and 197 and the total mutations leading to the DNA polymorphisms were 1717, 1049 and 435, respectively. 68%, 67% and 49% nucleotide diversity (Pi) were reported for *matK*, *petB* and *ITS* gene sequences while average number of nucleotide differences (K) were 419.918, 241.862 and 99.317. The number of haplotypes (h) for the 3 sets of the genes were 14, 20 and 21 as haplotype/gene diversity (Hd) observed were 0.966±0.003, 0974±0.002 and 0.953±0.004 while variance of haplotype diversity were $1.8x10^{-4}$, $0.6x10^{-4}$ and $3.3x10^{-4}$. However, estimate of recombination for gene were 224, 350 and 27.1 for *matK*, *petB* and *ITS* gene sequences while recombination between adjacent sites were 3.310 $x10^{-1}$, 6.681 $x10^{-1}$ and 9.12 $x10^{-2.}$ Additionally, minimum number of recombination events (Rm) for *matK* and *petB* gene sequences were 3 and 9 as there was no recombination events for ITS gene sequences.

Mutation analysis of SNPs of *matK*, *petB* and *ITS* gene sequences

A total of 1,625SNPs were detected in the 3 sets of gene sequences 697 SNPs were detected on *matK* gene sequences while 608 SNPs were detected on *petB* gene sequences. However, 320 SNPs were detected on *ITS* gene sequences. The prevalence ratio of synonymous to non-synonymous sites was 26: 671 for *matK* gene; 15: 593 for *petB* and 16:304 for *ITS* genes, respectively, giving a total ratio of 57:1468 for the 3 genes. Our result also revealed that the prevalence of the non-synonymous sites were higher than those of the synonymous sites. SNPs that were as a result of transversion mutation were higher than transition type of mutation, which was in the ratio of 641:56 for *matK*, 529:79 for *petB* and 230:90 for *ITS* gene. Also the incidence of nucleotide change from T <

A in all the genes was higher than the others as shown in table 1.

Selection analysis of *matk, petB* and *ITS* gene sequences

Selection analysis revealed that there were more positive selections on the single nucleotide polymorphisms (SNPs) that underwent non-synonymous mutations than those of synonymous mutations, respectively with the trend *matK*>*petB*>*ITS*. This trend was maintained for the negative type of selection (Table 2). The SNP site index where the non-synonymous mutations occurred on *petB* gene sequences of the pigeon pea accessions investigated were; 19, 85,136, 145, 154, 157, 166, 172, 181, 223, 229, 241, 253, 274, 280, 286, 304, 334 as well as 355. For *ITS* gene sequences, non-synonymous mutation occurred in 32SNP sites while in *matK* gene sequences, it occurred in 51SNP sites.

Table 1: Mutation analysis of SNPs of *petB*, *matK* and *ITS* gene sequences

petB S/N	SNP	AA Change	Syn/Non-Syn	Mutation Type	matK S/N	SNP	AA Change	Syn/Non-Syn	Mutation Type	ITS S/N	SNP	AA Change	Syn/Non-Syn	Mutation type
1	1T>A	Xaa/Lys	NS	Transversion	1	1T>A	Tyr1Asm	NS	Transversion	1	1T>C	Ser1Pro	NS	Transition
2	2M>C	Xaa1Ser	NS	Transversion	2	2A>C	Tyr1Ser	NS	Transversion	2	2C>T	Ser1Phe	NS	Transition
3	3A>C	Xaa1Gly	NS	Transversion	3	3T>A	Tyr1Pro	NS	Transversion	3	3C>T	Ser1Ser	S	Transition
4	4A>T	Asn2Tyr	NS	Transversion	4	4T>A	Phe2Ile	NS	Transversion	4	4T>C	Ser2Pro	NS	Transition
5	5A>T	Asn2Ila	NS	Transversion	5	5T>C	Phe2Ser	NS	Transition	5	5C>G	Ser2Stp	NS	Transversion
6	6C>T	Asn2Tyr	NS	Transition	6	6C>A	Phe2Ile	NS	Transversion	6	6C>G	Ser2Pro	NS	Transversion
7	7A>T	Lys3Tyr	NS	Transversion	7	7A>T	Ile3Phe	NS	Transversion	7	7G>C	Ala3Leu	NS	Transversion
8	8A>T	Lys3Ile	NS	Transversion	8	8T>A	Ile3Lys	NS	Transversion	8	8C>T	Ala3Leu	NS	Transition
9	9A>C	Lys3Gly	NS	Transversion	9	9T>G	Ile3Lys	NS	Transversion	9	10T>A	Tyr4Ile	NS	Transversion
10	10A>C	Lys4Asp	NS	Transversion	10	10T>C	Xaa4Leu	NS	Transition	10	11A>T	Tyr4Ile	NS	Transversion
11	11A>T	Lys4Leu	NS	Transversion	11	11K>T	Xaa4Leu	NS	Transversion	11	13T>G	Stp5Asp	NS	Transversion
12	12A>C	Lys4Ile	NS	Transversion	12	12A>C	Xaa4Gly	NS	Transversion	12	14G>A	Tyr6Met	NS	Transition
13	13A>C	Asn5Pro	NS	Transversion	13	13T>A	Stp5Lys	NS	Transversion	13	15A>T	Tyr6Met	NS	Transversion
14	14A>C	Asn5Pro	NS	Transversion	14	14A>C	Stp5Pro	NS	Transversion	14	16T>A	Tyr6Met	NS	Transversion
15	15T>A	Asn5Lys	NS	Transversion	15	15A>T	Stp5Try	NS	Transversion	15	17A>T	Ala7Leu	NS	Transversion
16	16A>T	Lys6STP	NS	Transversion	16	16T>A	Stp6Lys	NS	Transversion	16	18T>G	Ala7Leu	NS	Transversion
17	17A>T	Lys6Phe	NS	Transversion	17	17A>G	Stp6Gly	NS	Transition	17	19G>C	Ala7Lys	NS	Transversion
18	18A>T	Lys6Asn	NS	Transversion	18	18A>T	Stp6Tyr	NS	Transversion	18	20C>T	Stp8Lys	NS	Transition
19	19A>C	Ile7Gln	NS	Transversion	19	19A>T	Ile7Ser	NS	Transversion	19	21T>A	Stp8Arg	NS	Transversion
20	20T>A	Ile7Gln	NS	Transversion	20	20T>C	Ile7Ser	NS	Transition	20	22T>A	Stp8Leu	NS	Transversion
21	21A>T	Ile7Tyr	NS	Transversion	21	21A>T	Ile7Ile	S	Transversion	21	23A>G	Thr9Leu	NS	Transition
22	22M>T	Xan8Pro	NS	Transversion	22	22G>A	Xaa8Met	NS	Transition	22	24A>C	Thr9Leu	NS	Transversion
23	23W>T	Xaa8Ila	NS	Transversion	23	23K>G	Xaa8Gly	NS	Transversion	23	25A>C	Thr9Leu	NS	Transversion
24	24T>A	Xaa8Ile	NS	Transversion	24	24R>G	Xaa8Met	NS	Transversion	24	26C>T	Gln10Ser	NS	Transition
25	25T>A	Cys9Asn	NS	Transversion	25	25T>C	Xaa9Pro	NS	Transition	25	27T>C	Gln10Ser	NS	Transition
26	26G>A	Cys8Cyr	NS	Transition	26	26M>C	Xaa9Pro	NS	Transversion	26	28C>A	Gln10Ser	NS	Transversion
27	27T>A	Cys9Gla	NS	Transversion	27	27W>A	Xaa9Pro	NS	Transversion	27	29A>G	Arg11Gly	NS	Transition
28	28T>A	Tyr10Asn	NS	Transversion	28	28A>G	Lys10Asp	NS	Transition	28	30G>C	Arg11Tyr	NS	Transversion
29	29A>G	Tyr10Trp	NS	Transition	29	29A>G	Lys10Arg	NS	Transition	29	31C>G	Arg11Arg	NS	Transversion
30	30C>T	Try10Val	NS	Transition	30	30R>A	Lys10Arg	NS	Transversion	30	32G>A	Val12Pro	NS	Transition

31	31K>T	Xaa11Stp	NS	Transversion	31	31C>A	Xaa11Met	NS	Transversion	31	33G>C	Val12Stp	S	Transversion
32	32A>T	Xaa11Val	NS	Transversion	32	32K>G	Xaa11Gly	NS	Transversion	32	34G>T	Val12Stp	NS	Transversion
33	33A>T	Xaa11Asp	NS	Transversion	33	33T>G	Xaa11Gly	NS	Transversion	33	35T>A	Ala13Pro	NS	Transversion
34	34W>A	Xaa12Asn	NS	Transversion	34	34T>A	Phe12Arg	NS	Transversion	34	36A>G	Pro14Stp	NS	Transition
35	35A>G	Xaa12Gly	NS	Transition	35	35T>G	Phe12Arg	NS	Transversion	35	37G>C	Ala13Pro	NS	Transversion
36	36R>A	Xaa12Stp	NS	Transversion	36	36T>G	Phe12Leu	NS	Transversion	36	40A>T	Pro14Stp	NS	Transversion
37	37A>T	Lys13Stp	NS	Transversion	37	37T>G	Phe13Asp	NS	Transversion	37	41A>C	Pro14Arg	NS	Transversion
38	38A>T	Lys13Met	NS	Transversion	38	38T>A	Phe13Asp	NS	Transversion	38	42A>C	Pro14Arg	NS	Transversion
39	39A>T	Lys13Cys	NS	Transversion	39	39T>C	Phe13Ser	NS	Transition	39	43C>G	Pro15Gly	NS	Transversion
40	40A>T	Lys14Stp	NS	Transversion	40	40G>T	Val14Leu	NS	Transversion	40	44C>T	Pro15Leu	NS	Transition
41	41A>C	Lys14Pro	NS	Transversion	41	41T>G	Val14Gly	NS	Transversion	41	45T>G	Pro15Leu	NS	Transversion
42	42A>C	Lys14Asm	NS	Transversion	42	42G>C	Val14Phe	NS	Transversion	42	46G>A	Asp16Thr	NS	Transition
43	43A>C	Leu15Pro	NS	Transversion	43	43T>A	Phe15Ile	NS	Transversion	43	47A>C	Asp16Thr	NS	Transversion
44	44A>C	Lys15Pro	NS	Transversion	44	44T>G	Phe15Arg	NS	Transversion	44	48C>G	Asp16Arg	NS	Transversion
45	45A>C	Lys15Asn	NS	Transversion	45	45T>G	Phe15Arg	NS	Transversion	45	49C>T	Leu17Stp	NS	Transition
46	46A>T	Lys15Stp	NS	Transversion	46	46T>C	Phe16Pro	NS	Transition	46	50T>G	Leu17Stp	NS	Transversion
47	47A>G	Lys16Arg	NS	Transition	47	47T>C	Phe16Pro	NS	Transition	47	51G>A	Leu17Stp	NS	Transition
48	48A>T	Lys16Arg	NS	Transversion	48	48T>A	Phe16Ile	NS	Transversion	48	52A>G	Arg18Gly	NS	Transition
49	49A>T	Lys17Tyr	NS	Transversion	49	49T>C	Stp17Gln	NS	Transition	49	53G>T	Arg18Val	NS	Transversion
50	50A>T	Lys17Ile	NS	Transversion	50	50A>C	Stp17Ser	NS	Transversion	50	54G>T	Arg19Gly	NS	Transversion
608	613R>A	Xaa205xaa	S	Transversion	697	697M>A	Arg232Arg	NS	Transversion	320	325M>A	Xaa109xaa	S	Transversion

Table 2: Selection analysis of *matk, petB* and *ITS* genes sequences

Genes	Selection type	d_N	d_S	d_N-d_S	Site index	P-value
	Positive	483.7249	301.0015	182.7234	51	0.016
MatK	Negative	256.7574	354.3607	- 97.6033	31	0.025
	Neutral	0.0000	0.0000	0.0000	0	0.000
petB	Positive	251.7068	169.1666	82.5402	20	0.006
	Negative	115.6781	196.0749	-80.3968	11	0.010
	Neutral	0.0000	0.0000	0.0000	0	0.000
ITS	Positive	193.1568	124.9698	68.1870	32	0.012
	Negative	132.0612	172.6994	-40.6382	20	0.015
	Neutral	0.0000	0.0000	0.0000	0	0.000

matK = Maturase K
petB = Petite B
ITS = Internal transcribed Spacer
d_N (non- synonymous)
d_S (synonymous)

Haplotype variation and median joining network analysis in *matK*, *petB* and *ITS* sequences in pigeon pea accessions

Results of the haplotype variations in *matK*, *petB* and *ITS* gene sequences in the 55 pigeon pea accessions analyzed is presented in table 1. It was observed that in the *matK* gene sequences, a total of 14 haplotypes (Hap-1-Hap-14) were identified in 14 samples, the haplotype frequency was the same in all the samples analyzed (1.81%). A total of 31 haplotypes were identified from the three sets of genes (*matK*, *petB* and *ITS*). Haplotypes 1 – 14 were observed in the *matK* gene sequences while haplotypes 15 – 18 (4) were found in *ITS* gene sequences. However, *petB* gene sequences produced 13 haplotypes (hap – 19 – 31). There were no shared haplotypes in the *matK*. However, *petB* gene sequences had shared haplotypes for Hap-20 (shared by ICP 6974, ICP 69067, ICPL 87119, ICP 7118 and ICP 87), Hap-21 (shared by ICP 12734 and ICP14231) and Hap-30 (shared by ICP 8738 and ICP13555). However, all the observed haplotypes found in the *ITS* gene sequences were shared. Median joining network analysis showed that the haplotypes were clustered into four networks. The network analysis revealed the clustering of the identified haplotypes was gene-specific except Hap-23 which is found in *petB* gene sequence.

Maternal lineage (relationship) of *matK*, *petB,* and *ITS* gene sequences in pigeon pea accessions

There was very high maternal relationship between *matK* gene sequences of *Cajanus scarabaeoides* and *Rhynchosia reniformis* with that of *Cajanus cajan*. However, the *matK* gene sequences of *Flemingia rhodocarpa*, *Paracalyx scariosus* and *Cajanus reticulatus* shared common ancestry origin. The result showed that *Cajanus reticulatus matK* gene sequence did not share the same ancestry origin. Generally, the results revealed that *matK* gene sequences in the selected legumes may have originated from either *Cajanus scarabaeoides* or *Rhynchosia reniformis*.

For *petB* gene sequences, given the limited sequence data in the NCBI database for *petB*, only one sequence was downloaded – MG77261.1 for *Acacia karina* isolate. However, tracing the maternal lineage, it revealed that probably ICP11297 pigeon pea accession may have shared common ancestry origin with *Acacia karina*.

ITS gene sequences of all the pigeon pea accessions used did not share any common ancestry with the other pigeon pea *ITS* gene sequences downloaded from the NCBI database. Although the clustering pattern was very specific as the sequences downloaded were grouped together with no ancestry linkage (MG730709, MG730708 and MG730713; MG730714, MG730712 and MG730711), however, ICEAP850 *ITS* gene sequence seems to share relationship with MG730714, MG730712 and MG730711 and linked with MG730709, MG730708 and MG730713.

Table 3: Haplotype variations in *petB*, *matK* and *ITS* gene sequences in pigeon pea accessions

Haplotypes	Pigeon pea accession	Frequency (%)	Haplotypes	Pigeon pea accession	Frequency (%)
Hap_{-1}	M1F	0.018 (1.81%)	Hap_{-21}	P3F, P25F	0.036 (3.6%)
Hap_{-2}	M2F	0.018 (1.81%)	Hap_{-22}	P8F	0.018 (1.81%)
Hap_{-3}	M6F	0.018 (1.81%)	Hap_{-23}	P9F	0.018 (1.81%)
Hap_{-4}	M7F	0.018 (1.81%)	Hap_{24}	P10F	0.018 (1.81%)
Hap_{-5}	M9F	0.018 (1.81%)	Hap_{-25}	P11F	0.018 (1.81%)
Hap_{-6}	M10F	0.018 (1.81%)	Hap_{-26}	P13F	0.018 (1.81%)
Hap_{-7}	M13F	0.018 (1.81%)	Hap_{-27}	P14F	0.018 (1.81%)
Hap_{-8}	M14F	0.018 (1.81%)	Hap_{-28}	P17F	0.018 (1.81%)
Hap_{-9}	M17F	0.018 (1.81%)	Hap_{-29}	P18F	0.018 (1.81%)
Hap_{-10}	M18F	0.018 (1.81%)	Hap_{-30}	P20F, P21F	0.036 (3.6%)
Hap_{-11}	M20F	0.018 (1.81%)	Hap_{-31}	P23F	0.018 (1.81%)
Hap_{-12}	M21F	0.018 (1.81%)			
Hap_{-13}	M22F	0.018 (1.81%)			
Hap_{-14}	M24F	0.018 (1.81%)			
Hap_{-15}	I1F, I4F, I7F, I13F	0.091 (9.1%)			
Hap_{-16}	I2F, I9F, I16F, I17F, I19F, I20F	0.109 (10.9%)			
Hap_{-17}	I3F, I5F, I8F, I10F, I12F, I23F	0.109 (10.9%)			
Hap_{-18}	I14F, I15F, I21F, I22F, I24F	0.018 (1.81%)			
Hap_{-19}	P1F	0.018 (1.81%)			
Hap_{-20}	P2F, P7F, P12F, P16F, P19F	0.091 (9.1%)			

M= *matK*; I = *ITS*; P = *petB*

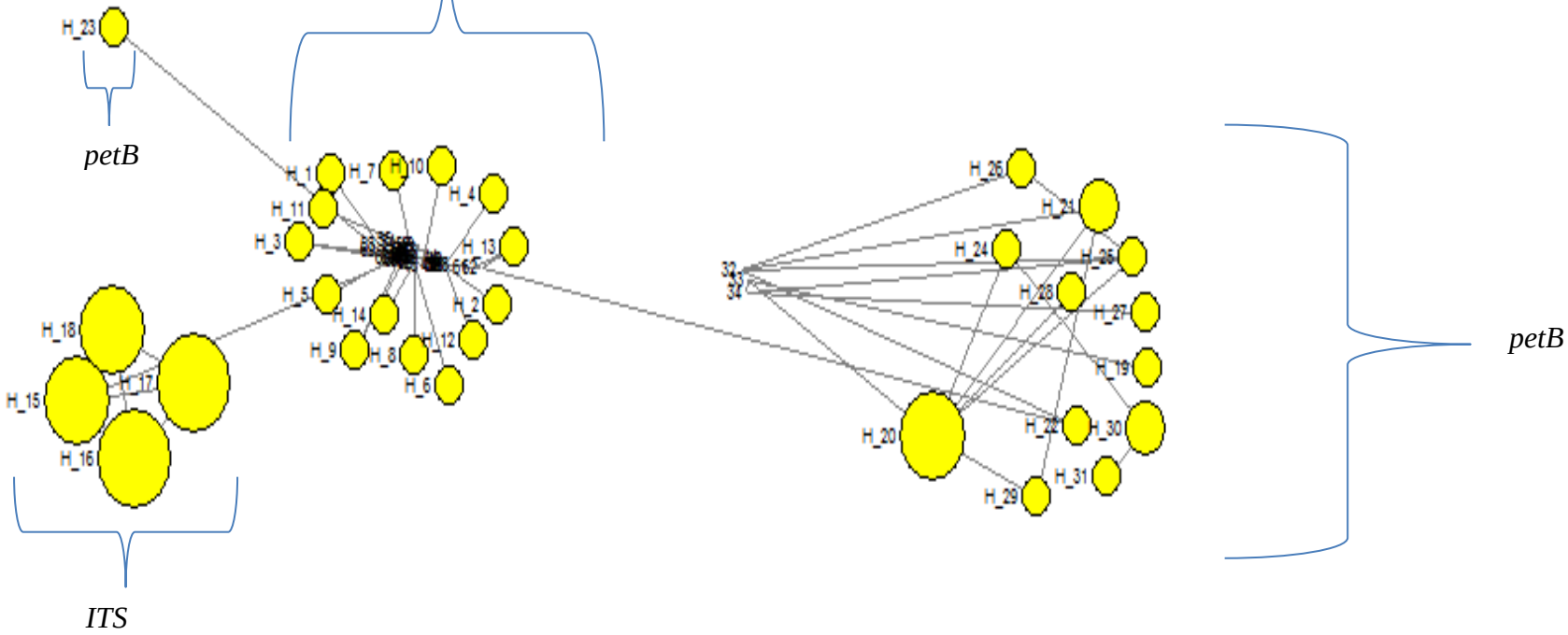

Figure 1: Median joining network analysis of the 31 haplotypes identified on 3 set of gene sequences (*petB*, *matK* and *ITS*) in pigeon pea accessions

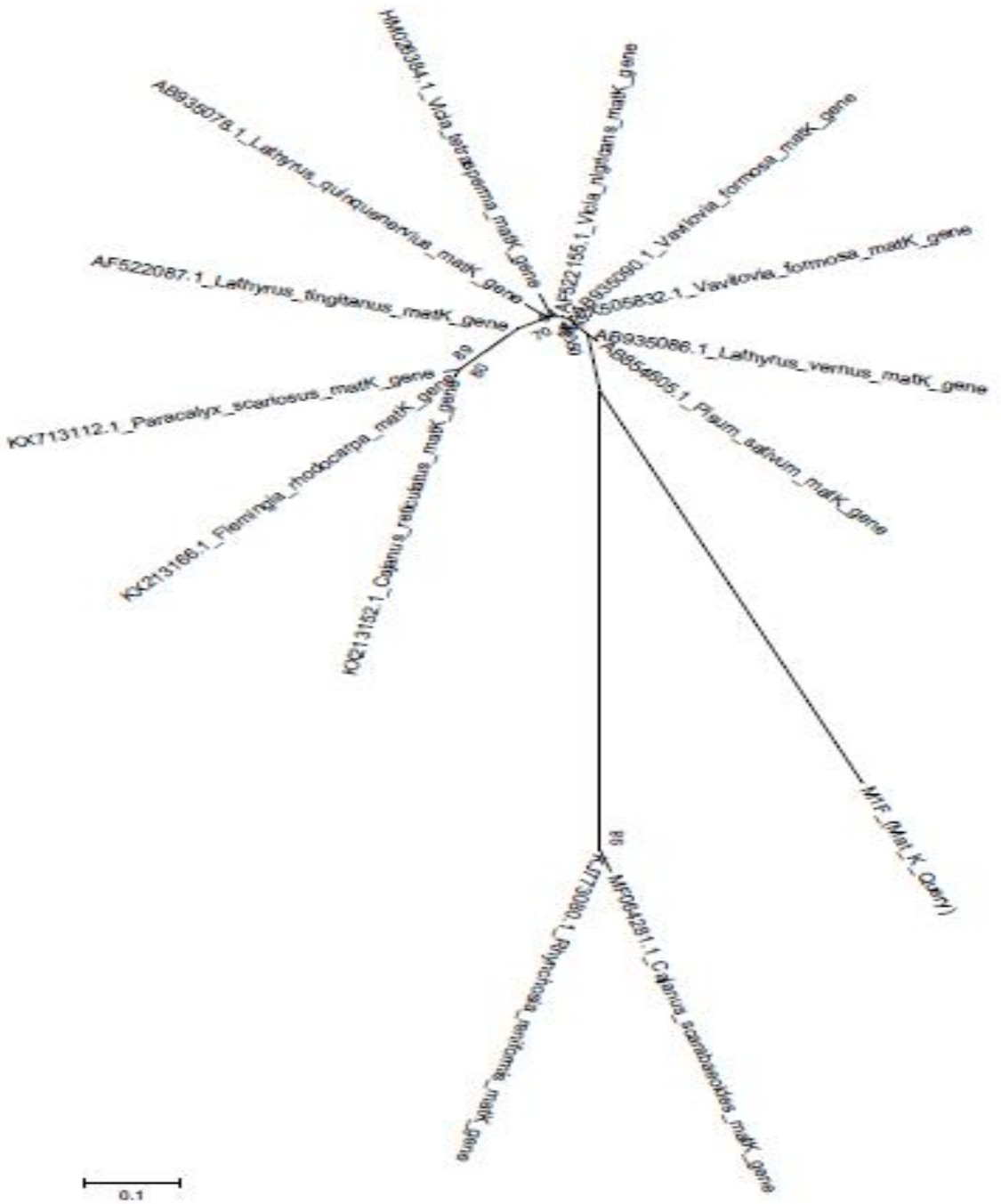

Figure 2: Maternal lineage of *matK* gene sequences in pigeon pea accessions using maximum likelihood method

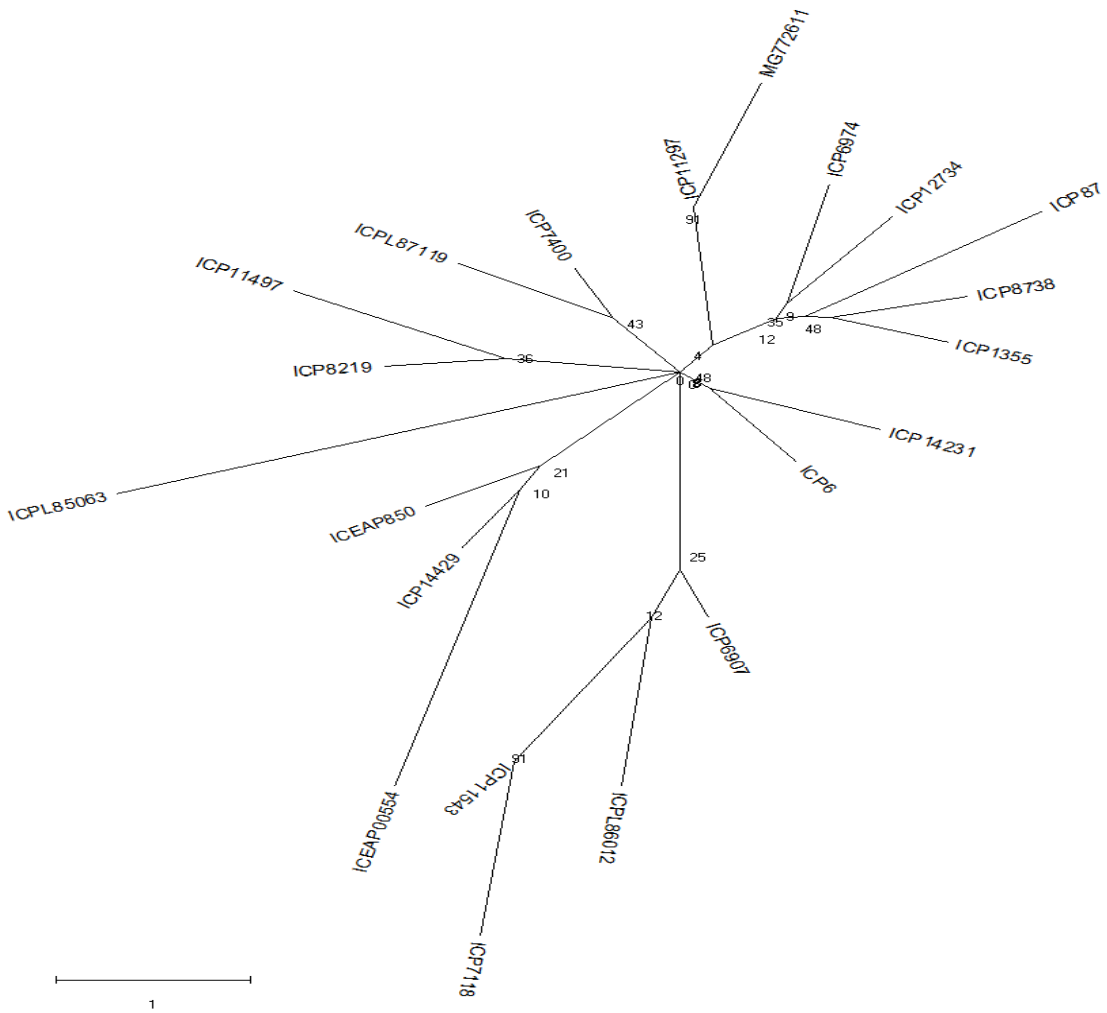

Figure 3: Maternal lineage of *petB* gene sequences in pigeon pea accessions using maximum likelihood method

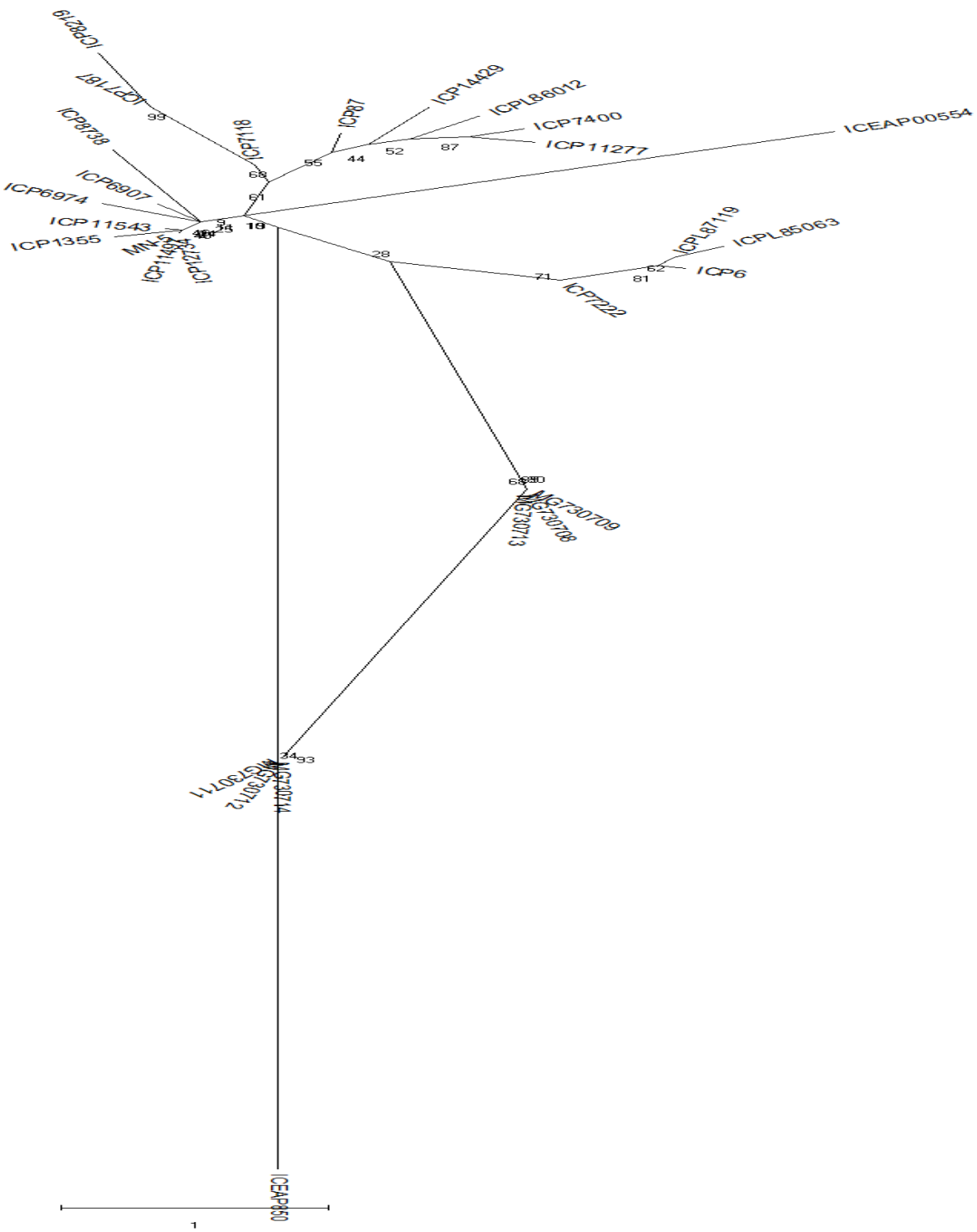

Figure 4: Maternal lineage of *ITS* gene sequences in pigeon pea accessions using maximum likelihood method

Discussion

Mutation whether naturally or artificially induced is the basis of evolution, which leads to organisms' speciation. These mutations occur on gene sequences or chromosomes, which result to changes in the nucleotides of gene sequences. Molecular systematics have greatly relied on data generated from either partial or complete sequenced genes where these mutations occur. Informatively, genomic regions have been deployed in genetic diversity analyses, evolutionary and ecological studies (Duminil et al., 2002), which is based on the extent nad level informativeness, mutation rate, low level of recombination, low base substitution rate as well as high copy number per cell (Abdul et al., 2005; Zhu et al., 2007). Hilu et al. (2014) had reported that these genomic regions differ in their contributions to resolving molecular phylogeny as pertains to informativeness, which by extension is based on the extent of mutations that have occurred in the genes found on these regions. Going by the results reported on DNA polymorphisms in this present paper, showed that *matK* gene comparatively revealed more genetic polymorphism than *petB* and *ITS* genes. As have been previously reported by Hilu et al. (2003) and Muller et al. (2006) that *matK* gene has high nucleotide and amino acids substitution rates, which in our thinking should be the basis for its advantage over other gene sequences (*rbcL* and *trn-F*) (Muller et al., 2003) but with the gene sequences used in the present study – *petB* and *ITS* in revealing DNA polymorphisms. From the results on DNA polymorphisms of the 3 sets of genes used, *ITS* gene sequences revealed very low polymorphisms, which might be attributed to the observation made by Hao and Xiano (2015) that the informativeness of *ITS* gene sequences is better when analyzing genetic differences among subgenera rather than between closely related species. This was quite similar to the position of Cutmore et al. (2010) that pointed out that the conserved nature of *ITS* gene sequences made it better for among species differentiation in genetic analysis. Genetic recombination admittedly, should create new combinations of alleles, which directly may affect the extent of genetic polymorphisms as well as creates differences in the DNA sequences. The results of the recombination per gene and the minimum recombination events on the 3 sets of genes might suggest that there is no functional association between recombination events on the gene sequences and the DNA polymorphisms. The second possibility is that the recombination activities on the gene sequences did not have significant nucleotide changes or substitutions that should have led to increased DNA polymorphisms on respective genes.

SNPs are single base substitution based mutation in DNA and the most common source of genetic polymorphism. It has been reported that the genomic variation resulting from SNPs is responsible for diversity in crop species. The implication is that the more SNPs detected on any particular gene sequences will proportionally lead to increased genetic diversity in the crop species wherein the genes are sequenced. Given that *matK* gene sequences produced the highest number of SNPs (617) followed by *petB* (363), it is imperative to infer that *matK* gene is very informative in deciphering genetic diversity (Hilu *et al.,* 2003; Muller *et al.,* 2006). Importantly, there were more non-synonymous mutations than synonymous on all the 3 gene sets, leading to more positive selection than negative selection. Since non-synonymous mutation could lead to phenotypic change, the implication is that it should be under the influence of positive selection pressure. This might result to the selection of important traits as a result of change on a nucleotide/amino acid. Conversely, it presupposes that the more the number of non-synonymous mutations on the crops' gene sequences that affect a trait, the more should be important traits that will be under positive selection pressure. As have been reported in the present study that there

was more positive selection, implying that selection favours non-synonymous substitutions, which might be beneficial to plant breeders. According to Mortan (1995) and Liang and Hilu (1996), the likelihood of transversion mutation in gene sequences is influenced by the GC content, which is most probably caused by the fact that the GC content varies while the AT content is constant. This notwithstanding, it is also reported that the rate of transversion substitutions could be influenced by factors possibly intrinsic (genetic) or external (environmental). Transversion mutations are considered more reliable mutations. However, Ouicke (1993) opined that there are more transition mutations than transversion mutations in *matK* gene sequences. This however is not the case in the present result as transversion mutations were more frequent than the transition mutations on all the gene sequences investigated.

According to Mackay et al. (2016), Riaz et al. (2017) observed that crop gene banks represent a rich diversity of haplotype variants much broader than in commercial breeding programmes. We report here that 31 haplotypes were detected out of which 7 were shared with varying frequencies. The implication of the shared haplotypes is that there was high minimum recombination events (9) on the *ITS* genes of the pigeon pea accessions. Haplotypes can have larger effects if they combine multiple mutations on a chromosomal regions that effect the trait in the same direction, which increases the power to identify genomic regions for the trait, even if they have small effect. Though the focus of the present study was not to this direction, this could the next line of research on the crop, especially on drought tolerance. Similar to what Bevan et al. (2017) opined that prospective approaches for haplotypes utilization in genomics-based crop improvement through re-sequencing of large ancestral and wild relative populations of a given crop species with the bid to identifying haplotypes with broader range of genetic variation than in present in elite breeding pools.

Important to note is that conserved sequences are very similar or identical in their nucleic acids, proteins, or polysaccharides across species or within different molecules produced by the same organism, implying that they have been maintained by evolution despite speciation. Suggestively, sequence similarity is an evidence of structural, functional and evolutionary relationships. Using *matK* gene sequence obtained from pigeon pea, it revealed that the gene sequence might be maternally be inherited from *C. scarabaeoides* and *R. reniformis*. The implication might be that since *C. scarabaeoides* is a wild species of *C. cajan*, introgression of gene carrying important traits will be possible. For *petB* gene sequence, ICP11297 accession of pigeon pea share seeming relationship with *Acacia karina*, implying evolutionary relatedness.

Conclusively, *matK* gene sequences showing higher polymorphisms in all parameters investigated should be used for genetic diversity analyses, especially in pigeon pea germplasm. The SNPs detected and the unshared haplotypes could be harnessed for breeding programmes. Interestingly also the observed relatedness in the *matK* gene sequence of *C. cajan* and those of *C. scarabaeoides* and *R. reniformis*, which might lead to carrying out a barcoding analyses for further investigation. The sharing of haplotypes reported in *ITS* gene sequences could be very informative.

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SCREENING COTTON (*GOSSYPIUM HIRSUTUM* L.) FOR DROUGHT STRESS

Jaafar, K.S., Abdulmalik, M.M. and *Mohammed, M.S.

Department of Plant Science Ahmadu Bello University Zaria P.M.B 1044, Zaria Kaduna State, Nigeria.
Corresponding Author'sagir007@gmail.com +2348033467262

ABSTRACT

Cotton (Gossypium hirsutum L.) crop is the most important source of natural fibre and an important source of feed, foodstuff and oil. Drought stress severely restricts cotton growth and development, thereby affecting the production and productivity of cotton plant. Identifying drought tolerant cotton genotypes will help in breeding for drought tolerance in cotton. Hence, the present study was undertaken to screen 12 cotton genotypes for drought tolerance. Seeds of cotton were sown under control and stress condition in a randomized complete block design (RCBD) of two replicates. Seedlings were thinned to two plants per pot, two weeks after emergence. Plants were allowed to grow under optimum water regime from sowing to 38 days after emergence (DAE) after which drought was imposed, by withholding water for three weeks for the stress treatment while the control were irrigated regularly. All the necessary agronomic practices were observed. Data were collected on morphological, physiological and yield parameters. A highly significant (P≤0.01) difference was observed for all the parameters analysed. Among the genotypes evaluated CL-07, SAMCOT 8, SAMCOT 11, SAMCOT 9, LINE 18 and LINE 30 recorded the highest values for most of the parameters. Stress x Genotype interaction shows CL-07, SAMCOT 8, SAMCOT 11 and LINE 30 genotypes had the highest values for most of the parameters, where CL-07 recorded the highest plant height and number of bolls (55.56cm and 12), SAMCOT 8 highest root dry weight and chlorophyll content (0.75g and 38.75SPAD value), SAMCOT 11 highest boll weight (38.54g) while LINE 30 recorded highest shoot dry weight and relative water content (9.1g and 88.39%). Stress susceptibility index (SSI) shows that CL-07 and LINE 30 have values less than 1 for the number of bolls (0.60 and 0.75) and boll weight (0.69 and 0.08) respectively. Therefore these genotypes prove to be more tolerant to drought compared to the other genotypes. Conversely, MA-15 consistently recorded the least values of these parameters. Hence, suggesting this genotype to be susceptible to drought.

Keywords: *Cotton, Drought stress, Morphological, Physiological and Yield parameters*

Introduction

Cotton (*Gossypium hirsutum* L.) crop is the most important source of natural fibre, providing half of the global fibre requirement (Pretorius, 2009). Drought stress severely restricts cotton growth and development, by affecting plant height, leaf dry weight, stem dry weight, leaf area index, node number, fibre quality, canopy and root development (Loka *et al.,* 2011). Water availability and quality affect the growth and physiological processes of all plants as water is the primary component of actively growing plants ranging from 70–90% of plant fresh mass (Babu, 2015). Due to its predominant role in plant nutrient transport, chemical and enzymatic reactions, cell expansion and transpiration, water stresses result in anatomical and morphological alterations as well as changes in physiological and biochemical processes affecting functions of the plants (Megha and Mummigatti, 2017).

Identifying drought tolerant cotton genotypes will help in breeding cotton for drought tolerance. Hence, the present study is undertaken to screen 12 cotton genotypes for drought tolerance.

Material and Methods

Treatment comprised of 12 cotton genotypes and two watering regimes (stressed and well watered). These were laid out in randomized completely block design (RCBD) replicated two times. Twenty-litre plastic pots perforated at the base with 15cm radius were filled with 18kg of soil composed of loam soil, sandy soil and cow dung in 2:1:1 ratio. Pots were watered to field capacity before planting. Eight cotton seeds per pot were sown. Seedlings were thinned to two plants per pot, two weeks after emergence. Plants were allowed to grow under optimum water regime from sowing to 38 days after emergence (DAE) after which drought was imposed, by withholding water for three weeks for the stress treatment while the control were water regularly. All the necessary agronomic practices were observed.

Data Collection and Analysis

Data were collected on plant height (cm), shoot dry weight (g), leaf area, root length, root dry weight (g), number of bolls per plant, boll weight (g), number of flower buds, relative water content (%) and chlorophyll content. Plant height was measured from the collar region to the tip. Shoots were separated from the seedlings and were put in paper envelopes and dried in an electric oven at 70°C for 24 hours. After drying, dry shoot weights were recorded by using a weighing balance. Leaf area per plant was measured using a leaf area meter (model LI-3100 C Biosciences). Root length of seedlings were measured from collar region to the tip of the longest root by using a metre rule. Roots were separated from the seedlings and were put in paper envelopes and dried in an electric oven at 70°C for 24 hours. After drying, dry root weights were recorded by using a weighing balance. The number of bolls per plant was counted and recorded. The weight of bolls for each plant was measured and recorded using a weighing balance. Number of flower buds were counted and recorded. Three fully developed leaf samples were taken from each of the pots when the plants were showing symptoms of drought stress. These samples were placed in polythene bags soon after excision and fresh weight was recorded using a weight balance. The leaf samples were placed in distilled water overnight for recording the turgid leaf weight. The samples were oven-dried at 70°C for 2 days and the dry weight was taken.

Relative water content (RWC) was calculated using the following formula:

RWC = [{(Fresh weight – Dry weight)/Turgid weight – Dry weight)}] × 100

Chlorophyll content was measured using a portable chlorophyll meter model. Three leaves were measured per plant and the average taken as the chlorophyll content.

Stress Susceptibility Index (SSI) is a unit minus the ratio of seed cotton yield under full irrigated and deficit irrigated conditions to drought intensity.

SSI= [1- (Ys/Yp)]/DI

Where Ys and Yp are seed cotton yield under deficit irrigated and fully irrigated conditions, respectively and DI is drought intensity index = 1-[(mean Ys of all genotypes in the non-irrigated treatment)/mean Yp of all genotypes in the irrigated treatment)] Fisher and Maurer (1978)

All data recorded were subjected to Analysis of Variance (ANOVA) using the factorial ANOVA procedure of SAS 9.0 (SAS Institute Inc. 2002). Means were separated using Least Significant Difference (LSD).

Results and Discussion

Effect of Water Stress and Genotypes on Morphological, Physiological and Yield Parameters of 12 Cotton Genotypes

Highly significant (P≤0.01) differences were observed for the effect of water stress on all the morphological parameters evaluated (Table 1). Plant height, shoot dry weight, leave area, root length and root dry weight significantly (P≤0.05) recorded the highest values under

control condition, while the least was observed under stress treatment. The longest (61.82cm) plant height was observed in the control, while the least (47.61cm) was observed under stress conditions. The highest (9.18g) dry weights was observed in the control while the least (7.02g) was observed under stress conditions. This was comparable to the findings of Azhar and Rehman (2018) who reported that under water stress condition, plant height is adversely affected in cotton plants. Mvula *et al.* (2018) observed that shoot fresh weight and shoot dry weight were much lower under water-stressed conditions, suggesting that shoot growth was more sensitive to water stress than root growth. Highly significant (P≤0.01) differences were observed for leaf area with the control recording the highest (206.7) leaf area while the least (173.4) was observed under stress conditions. Several authors have reported a decrease in leaf area of cotton genotypes under drought stress (Parida *et al.*, 2007; Noreen *et al.*, 2013). Variation in root length and root dry weights was observed to be highly significant (P≤0.01) with the control recording the higher and longest (21.2cm and 1.51g) values, while the stressed recorded the shortest and least (15.63cm and 0.55g) values respectively. This indicates that as water availability decreases, root length decreases. Luo *et al.* (2016) reported that mild and initial stage of drought stress enhances root length in cotton, but long time water deficit reduce the root activity.

Among the genotypes, highly significant (P≤0.01) differences were also observed for shoot parameters (Table 1). CL-07 and LINE 17 recorded the longest (61.7cm) plant height which was comparable to SAMCOT 8 (59.75cm) while, SAMCOT 8 was statistically similar to MA-1 (58.39cm). CL-07 recorded the highest (252.65) value of leave area among all the genotypes evaluated while, SAMCOT 8 recorded the least (162.81) leave area. Veesar *et al.* (2020) reported genotypic variation for shoot length and leave area in cotton. LINE 30 recorded the longest root length which was comparable to SAMCOT 8 and LINE 17 while, SAMCOT 11 recorded the least (16.15g). SAMCOT 8 recorded a higher root dry weight of 1.8g while LINE 18 recorded the least (0.56g). Root related parameters are more important for drought tolerance. Iftikhar *et al.* (2019) observed that root linked parameters i.e. root length, fresh root weight, dry root weight were contributing more to drought tolerance as compared to the shoot related parameters which were more associated with drought-sensitive behaviour in cotton. Genotypes with higher root dry weight under stress conditions are said to be drought tolerant.

Relative water content and chlorophyll content were highly significantly (P≤0.01) different due to water stress with the control recording the highest (83% and 39.72) value while stressed recorded the least (63% and 34.40) respectively (Table 2). Result from the present study showed that relative water content significantly reduced under water stress. Relative water content indicates the water status of a plant and is among the important indicators of water stress in leaves (Hessini *et al.*, 2009). The reductions in chlorophyll concentrations could be attributed to the increased electrolyte leakage due to leaf senescence (Pirzad *et al.*, 2011). Similarly, the number of bolls, boll weight and the number of flower buds recorded the highest values (4, 11.47g and 56) under control conditions while the stressed treatments recorded the least (3, 8.12g and 4) values, respectively. Sezener *et al.* (2015) observed that the number of boll per plant declined significantly under drought conditions. Highly significant (P≤0.01) differences were observed among the genotypes evaluated for relative water content with LINE 30 recording the highest (92%) which was similar to SAMCOT 8 and CL-07 with 88% and 86% values respectively while SAMCOT 12 recorded the least (53%) relative water content value. Iftikhar *et al.* (2019), observed that drought-tolerant genotypes showed good relative water contents while drought-sensitive genotypes showed low relative water contents in cotton. Genotypic variation for relative water content was also

observed by Parida *et al.* (2007).Similar observations were made for chlorophyll contents where SAMCOT 8 recorded the highest (42.70) value which was similar to SAMCOT 11 (40.98), while SAMCOT 10 recorded the least (32.89). Highly significant (P≤0.01) differences were observed for the number of bolls with CL-07 recording the highest number of bolls which was similar to SAMCOT 8 and SAMCOT 11 with 14, 12 and 12 numbers of bolls, while SAMCOT 12 recorded the least (7). SAMCOT 11, SAMCOT 8 and CL-07 recorded the highest (42.4g, 41.35g and 34.51g) boll weights compared to the other genotypes while SAMCOT 12 recorded the least (19.27g). LINE 17 and LINE 30 recorded the highest (23 and 22) number of flower buds while SAMCOT 9 recorded the least (8) value for the number of flower buds. During flowering and boll-forming stages, cotton biomass, final yield, and yield composition at the harvest stage were significantly lower than those of the control (Jie *et al.*, 2020).

Table 1: Effect of water stress on shoot parameters of 12 cotton genotypes under screen house condition at Samaru in 2018

	Shoot parameters				
Treatments (T)	PH (cm)	SDWT(g)	LA/plant	RDWT (g)	RL (cm)
Control	61.82	9.175	206.7	1.50	21.20
Water stress	47.61	7.021	173.4	0.55	15.63
LSD	2.14	1.30	21.63	0.21	1.80
Genotypes (G)					
SAMCOT 8	59.75	7.375	162.81	1.80	21.25
SAMCOT 9	49.15	6.975	189.68	0.95	17.23
SAMCOT 10	52.46	8.075	174.40	1.05	17.75
SAMCOT 11	56.57	8.225	215.03	1.08	16.15
SAMCOT 12	53.90	8.775	180.13	0.90	16.58
SAMCOT 13	51.60	9.425	191.83	0.93	18.1
CL-07	61.70	9.25	252.65	1.18	18.8
LINE 17	61.66	7.925	197.83	0.75	20.5
LINE 18	54.60	8.85	150.58	0.56	16.68
LINE 30	53.35	9.8	201.12	1.20	23.33
MA-1	58.39	6.4	187.48	0.98	16.78
T x G	**	**	NS	**	NS
CV (%)	3.46	3.28	7.27	13.38	10.1
LSD	5.25	3.18	55.98	0.52	4.40

** Highly significant, NS=Not significant, PH= Plant height, SDWT= Shoot dry weight. LA= Leaf area, RDWT= Root dry weight, RL= Root length

Table 2: Effect of water stress on physiological and yield parameters of 12 genotypes of cotton under screen house condition at Samaru in 2018

Treatments (T)	Physiological and Yield Parameters				
	RWC (%)	CHL (SPAD value)	NB/plant	BWT (g/plant)	NFB/plant
Control	83.26	39.72	11.25	34.4	16.5
Water stress	62.95	34.40	7.5	24.36	11.75
LSD	6.35	1.18	0.38	1.00	0.46
Genotypes (G)					
SAMCOT 8	87.69	42.70	12.00	41.35	18.75
SAMCOT 9	76.63	39.34	7.50	23.78	7.50
SAMCOT 10	71.43	32.89	8.25	26.86	12.00
SAMCOT 11	60.07	40.98	12.00	42.44	17.25
SAMCOT 12	53.12	33.23	6.75	19.27	11.25
SAMCOT 13	70.93	33.73	8.25	30.14	10.50
CL-07	85.65	37.25	13.50	34.51	19.50
LINE 17	74.89	38.55	11.25	23.23	23.25
LINE 18	60.48	37.05	7.50	24.02	9.00
LINE 30	92.24	36.38	10.50	32.34	21.75
MA-1	77.00	33.23	7.50	27.05	9.75
T x G	**	*	*	**	NS
CV (%	3.62	4.11	9.238	3.947	11.72
LSD	15.56	2.90	0.96	2.46	0.83

** Highly significant,*significant, NS=Not significant, RWC=Relative water content, CHL= Chlorophyll content, LA=Leaf area, Number of bolls, BWT= Boll weight and NFB= Number of flower buds

Interaction between Water Stress and Genotypes on Morphological, Physiological and Yield Parameters of Cotton under Screen House Condition

The interaction effect was observed to be highly significant (P≤0.01) on plant height, shoot dry weight, root length and root dry weight. LINE 17 recorded the longest (71.28cm) plant height while MA-15 recorded the shortest (50.58cm) under control conditions (Fig. 1a). CL-07 recorded the longest (55.65cm) plant height while MA-1 recorded the shortest (36.35cm) under stress conditions. SAMCOT 13, LINE 30 and SAMCOT 12 recorded higher shoot dry weights of 11.05g, 10.50g and 10.35g respectively while MA-15 recorded the least (6.70g) shoot dry weight (Fig. 1b). Under stress conditions, CL-07 recorded the highest (8.85g) shoot dry weight while MA-1(5.45g) recorded the least shoot dry weight. Iqbal *et al.* (2011) also reported that fresh and dry shoot weights are adversely affected by drought. Significant stress x genotype interaction was observed for plant height, shoot dry weights and root dry weight. This revealed that the cotton genotypes performed variably over the stress treatments. Significant treatments x genotypes interactions for shoot length and leaf area have been reported in cotton under water stress condition (Veesar *et al.,* 2020). SAMCOT 8 recorded the highest (2.85g) root dry weight while, LINE 18

and MA-15 recorded the least (0.8g) under control conditions (Fig. 1c). SAMCOT 8, SAMCOT 9 and MA-1 recorded the highest root dry weight of 0.75g while MA-15 had the least (0.15g) under stress conditions. The interaction of stress x genotype was significant for root dry weights, but not for root length per plant. On the contrary Veesar *et al.* (2020) reported significant treatment x genotype interactions for root length and number of lateral roots.

CL-07, SAMCOT 8, LINE 30, and SAMCOT 9 recorded the highest relative water content of 97%, 97%, 96% and 92% respectively while, SAMCOT 11 had the least (71%) under control condition (Fig. 2a). LINE 30 (88%) recorded higher relative water content under stress conditions while SAMCOT 12 recorded the least (32%) relative water content. Iftikhar *et al.* (2019), observed that drought-tolerant genotypes showed good relative water contents while drought-sensitive genotypes showed low relative water contents in cotton.

SAMCOT 8, SAMCOT 11 and SAMCOT 9 recorded the highest chlorophyll content of 46.65, 44.05 and 42.50 respectively while MA-1 recorded the least (34.20) under control treatment (Fig. 2b). SAMCOT 8 recorded higher (38.75) chlorophyll content under stress conditions while SAMCOT 10 had the least (28.25) chlorophyll content. Water stress significantly reduced chlorophyll content. Ahmad *et al.* (2020) reported a decrease in chlorophyll content under drought stress in all the cotton varieties evaluated, with the most tolerant genotype maintaining higher chlorophyll content while the most susceptible genotype contains the least chlorophyll content under drought stress.

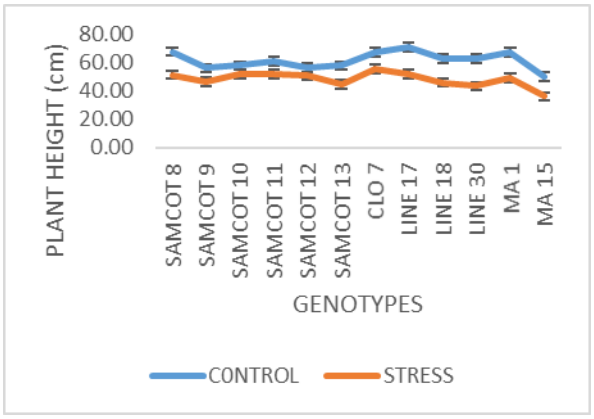
SAMCOT 8, SAMCOT 11 and CL-07 recorded a higher number of bolls of 15 while, SAMCOT 9, SAMCOT 12, SAMCOT 13, LINE 18, MA-1 and MA-15 recorded the least (9) under control condition (Fig. 2c). CL-07 recorded the highest (12) number of bolls while SAMCOT 12 recorded the least (5) under stress conditions. Azhar and Rehman (2018) reported that water stress reduces the number of bolls, weight of bolls, fibre yield and fibre length of cotton. Similarly, Sezener *et al.* (2015) observed that the number of boll per plant declined significantly under drought conditions. Alishah and Ahmadikhah (2009) also reported significant interaction of genotype x irrigation on yield of cotton varieties. On the contrary SAMCOT 11and SAMCOT 8 recorded the highest (46.34g and 45.26g) boll weight respectively while SAMCOT 12 recorded the least (25.27g) under control conditions (Fig. 2d). SAMCOT 11 and SAMCOT 8 recorded the highest boll weight of 38.54g and 37.44g respectively while SAMCOT 9 recorded the least (10.89g) under stress conditions. Dalvi *et al.* (2019) observed genetic variation for the number of bolls, boll weight and yield in cotton under drought.

Stress Susceptibility Index (SSI)

The stress susceptibility index (SSI) for the number of bolls was found to be higher in SAMCOT 12 (1.5) while the least (0.5) was recorded in SAMCOT 13 (Table 3). SAMCOT 9 recorded the highest (2.41) SSI for boll weight while LINE 30 recorded the least SSI (0.08). CL-07 and LINE 30 recorded lower values of SSI for the number of bolls (0.6 and 0.75) and boll weight (0.69 and 0.08) respectively. Stress Susceptibility Index (SSI) is a good indicator to determine drought-tolerant genotypes in plants. Genotypes with SSI less than 1.0 are regarded as water stress-tolerant genotypes while genotypes with SSI greater than 1.0 as susceptible (Gutierrezi *et al.*, 1998). In the present study, CL-07 and LINE 30 had SSI less than 1 for both number of bolls and boll weight which indicates their tolerance to drought. Alishah and Ahmadikhah (2009) identified two drought tolerant varieties of cotton Siokra-324 (0.86) and Tabladila (0.71) based on Stress Tolerance Index.

Table 3: Mean number of bolls and boll weight of 12 cotton genotypes evaluated under stress and control condition at Samaru in 2018

	Number of bolls/plant			Boll weight (g)		
Genotypes	NB C	NB S	SSI NB	BWT C	BWT S	SSI BWT
SAMCOT 8	15.00	9.00	1.20	45.26	37.44	0.59
SAMCOT 9	9.00	6.00	1.00	36.66	10.89	2.41
SAMCOT 10	10.50	6.00	1.25	34.50	19.22	1.52
SAMCOT 11	15.00	9.00	1.20	46.35	38.54	0.58
SAMCOT 12	9.00	4.50	1.50	25.37	13.17	1.65
SAMCOT 13	9.00	7.50	0.50	35.46	24.83	1.03
CL-07	15.00	12.00	0.60	38.39	30.63	0.69
LINE 17	13.50	9.00	0.98	27.50	18.96	1.06
LINE 18	9.00	6.00	1.00	25.71	22.34	0.44
LINE 30	12.00	9.00	0.75	32.78	31.91	0.08
MA -1	9.00	6.00	1.00	31.97	22.13	1.05
MA -15	9.00	6.00	1.00	32.85	22.34	1.09
Mean	11.25	7.50	1.00	34.40	24.36	1.02



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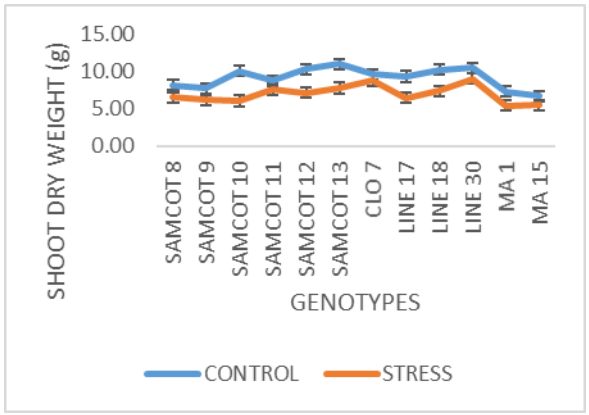
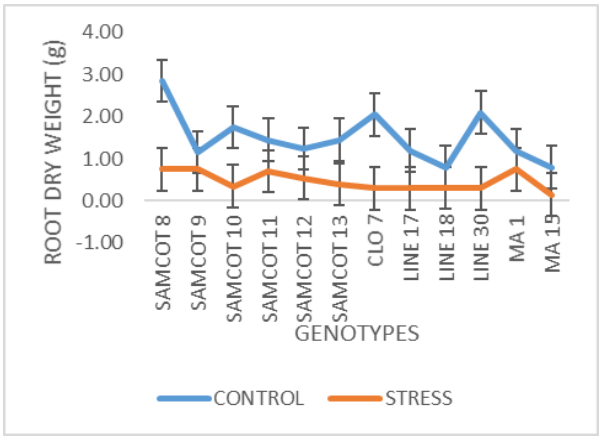


b



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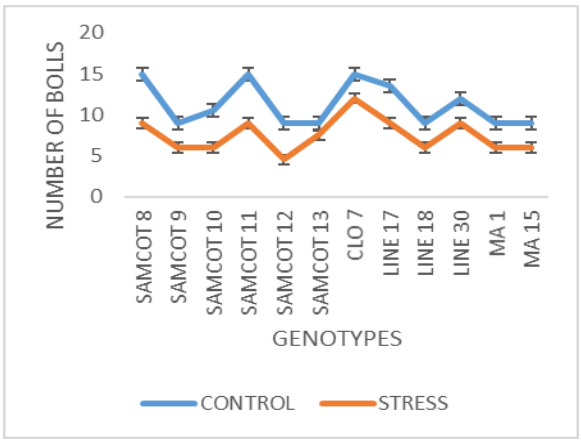

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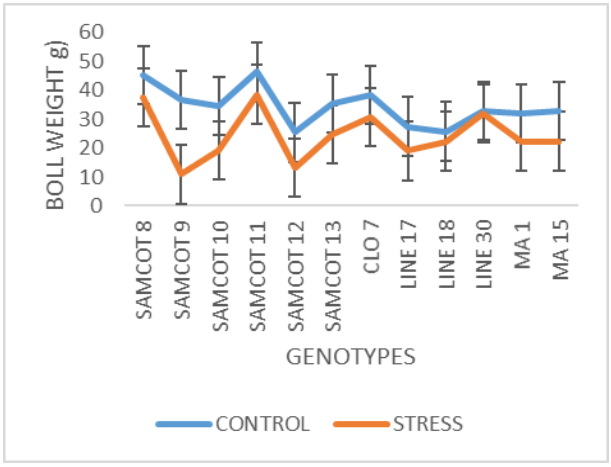

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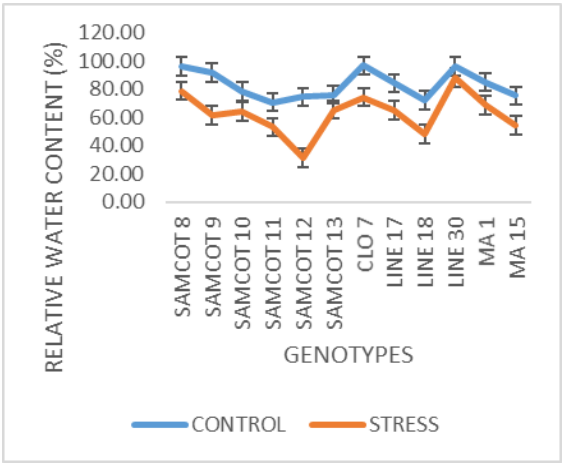

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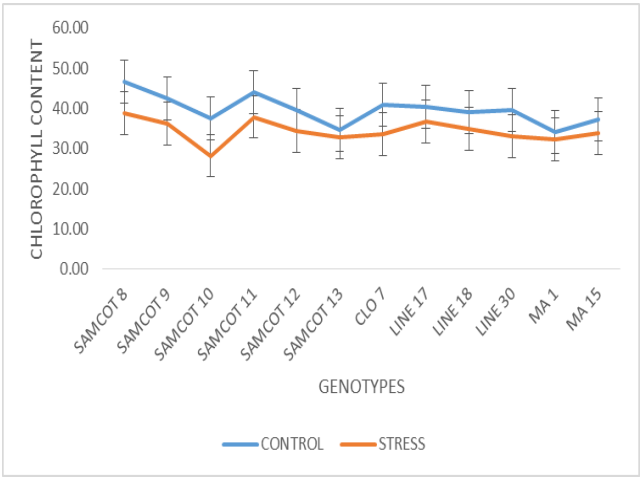

g

Fig. 1: Effect of water stress x Genotype interaction on; a) plant height of cotton (cm) b)shoot dry weight of cotton (cm) c) root dry weight of cotton (g), d) relative water content of cotton (%) and e) chlorophyll contents of cotton, f) number of bolls of cotton and g) boll weight of cotton (g)

Conclusions

Result from this study shows that CL-07, SAMCOT 8, SAMCOT 11 and LINE 30 genotypes had the highest values for most of the parameters evaluated. Therefore these genotypes prove to be more tolerant to drought compared to the other genotypes. Conversely, MA-15 consistently recorded the least values of most of the parameters. Hence, suggesting this genotype to be susceptible to drought.

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APPENDIX

CGBPB 015

GENETIC VARIATION OF EARLY LEAF SPOT DISEASE RESISTANCE AMONG GROUNDNUT GENOTYPES

[*1]Lawan, Z.M., [2]Usman, A., [2]Mohammed, M.S., [2]Umar, M.L., [3]Ajeigbe, H.A., [1]Mohammed, S.G., [1]Shaibu, A.S., [4]Abdullahi W.M., and [2]Ahmed, H.O.

Department of Agronomy, Bayero University, Kano
Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
International Institute of Tropical Agriculture, Kano Station
*Corresponding Author: zainablawanmiko@gmail.com

ABSTRACT

In the semi-arid savannas of West Africa where groundnut is an important component of the farming systems. Stress imposed by early leaf spots impact adversely on the crop's performance, resulting to yield loss of up to 70%. A study was conducted using 23 genotypes in two locations under two treatment conditions (inoculated and uninoculated) using a randomized complete block design with two replications., The study was designed to determine the reaction of different groundnut genotypes to early leaf spot disease. The analysis of variance indicated highly significant difference (P≤0.01) for pod weight, disease scoring and disease incidence at 65 and 90 days after sowing. The result indicated variety ICG 12991 and Samnut 22 had the lowest disease scoring and disease incidence, as well as having higher pod weight. At both locations disease traits had negative correlation of about 40% with pod weight. The study found significant genetic variation among the groundnut genotypes, that can exploited in in improvement of the crop. Further studies are needed to determine the type and the number of genes controlling early leaf spot disease tolerance in groundnuts for enhanced breeding strategies.

Keywords: *Early leaf spot, inoculated, tolerance, selection correlation*

Introduction

Groundnut (*Arachis hypogaea* L. 2n =4x = 40), is one of the most important oilseeds and food crops cultivated in the semi-arid tropics. It is a self-pollinated, tropical annual legume. It originated in South America, and now cultivated in about 115 countries covering temperate and tropics with a total area of about 26.54 million (M) hectares (ha) with a global production of about 43.91 M tons and an average yield of about 1655 kg/ha (FAOSATAT, 2017). The Asian continent ranks first with over 58.3% of world production, followed by the African continent (31.6%), American continent (10.0%) and Oceania (0.1%). The major producing countries are China (16.55 M tons), India (6.56 M tons), Nigeria (3.41 M tons), USA (2.35 M tons) and Sudan with 1.77 M tons (FAOSATAT, 2017). In Africa, groundnut production has grown significantly from year 1990 to 2000 (quantify the growth in terms of percentage increase from 1999 to 2018). This growth is mainly due to increased production in West African countries such as Nigeria, Senegal, Ghana, Burkina Faso and Mali .(Revoredo and Fletcher, 2002). For example Nigeria, the third largest producer in the world, accounted for about a fourth of groundnut production in Africa in 2014 (FAOSATAT, 2017).

Groundnut is a good source of fat, protein and minerals and hence it plays important role in human nutrition. Its seed contains 48–55% oil and 26–28% protein, and is a rich source of dietary fiber, minerals and vitamins (Janila *et al.,* 2013). The haulms and groundnut cake are important sources of animal feed. In addition, groundnut has ability to fix atmospheric nitrogen to the soil to help in the maintenance of soil fertility. The hardiness, plasticity, the multiplicity of uses of groundnut makes it one of the important useful legume crops.

Despite its economic importance, the productivity of groundnut is severely constrained by several biotic and abiotic factors. The yield of groundnut in Africa is very low, around 1 ton/ha, compared to global average yield of about 2 ton/ha (FAOSTAT (2014). Among the major constraints, biotic factors particularly foliar diseases constitute a serious yield limiting challenge in groundnut production. In the semi-arid savannas of West Africa where groundnut is an important component of the farming systems, stress imposed by the incidence of early leaf spots (caused by *Cercospora arachidicola* S. Hori (Berk. and Curt.) Deighton), drought and low soil fertility impact adversely on the crop's performance (Padi, 2008). The leaf spots diseases) are major diseases in peanut growing regions across the world causing yield loss up to 70% and economic losses around US $ 599 million globally (FAO, 2004; Ogwulumba, 2008). Early leaf spots, caused by the fungi *Mycosphaerella arachidis* is a severe disease of groundnuts worldwide. In Africa, they are reported to be major problems in Burkina Faso, Malawi, Mali, Nigeria and Sudan.

Early leaf spot can cause total defoliation and reduce pod and fodder yields to an extent of over 50% thereby resulting in low yield (Waliyar, 1991). Losses due to diseases can be attributed to the high percentage defoliation caused by leaf spot diseases, which thus affect pod filling and subsequent grain yield. Defoliation percentage affects hay quality of vines that are fed to animals (Tsigbey *et al*., 2004). In addition, fallen leaves from infected plants provide organic matter as a food source for other fungi particularly, Sclerotium rolfsii, and this can contribute to inoculums build-up on farms (Lucas *et al*., 1992). Diseases of groundnut reduce yield and quality of grains and increased cost of production wherever the crop is grown (Wynne *et al*., 1992). Although these disease can be controlled by spraying chemicals such as insecticides and fungicides, these increases production costs for farmers by 10% (Coffelt and Porter, 1986) and also pollutes the environment, and are beyond the reach of small farmers, who are the major producers of this crop. Thus, host plant resistance is a more feasible and viable approach. To do this, there is a need to identify genes that confer tolerance to groundnut for these diseases. The objective of the study was to screen groundnut genotypes for resistance to early leaf spot disease.

Materials and Methods

The field evaluation experiment were conducted at two locations; Bayero University Kano Research Farm, Kano ($11^{0}48^{1}$N: $8^{0}25^{1}$E; 600mm-1000mm annual rainfall) situated in the Sudan savanna and Institute for Agricultural Research farm Samaru, Zaria ($11^{0}11^{1}$ N: $7^{0}38^{1}$ E; 686 m asl, 1200 mm annual rainfall) situated in the northern Guinea savannah. The genetic materials used for the study comprise of 23 groundnut genotypes, which were obtained from ICRISAT, Kano station. The genotypes were phenotyped for ELS disease resistance which was carried out during 2019 rainy seasons at B.U.K and I.A.R research farm under artificial infestation. These stations have been known to be a hotspot for ELS. The experiments were being laid out using randomized complete block design with two replications at both locations under two treatment conditions (inoculant and uninoculant). The seeds of the groundnut genotypes were planted in the field at a spacing of 0.75 m between rows and 0.30 m between plants in a row, Plot size of single 4 m row were used and two seeds were planted per hill. Based on the recommendation on manual obtained from ICRISAT the mixture of single superphosphate SSP at 200kg per hectare and NPK 15:15:15 at 100kg per hectare at ratio of

2:1, were apply by method of drilling at 2 to 3 weeks after sowing. Hand weeding were carried out using hoe at 3rd, 8th, and 12th weeks after sowing (WAS) to prevent weed infestation and competition between plants and weeds. Soil mounds were packed around the plants using mould board to enable the plant to peg inside the soils. Artificial inoculation was done to increase the inoculum's potential by spraying the plants at thirty days after sowing with a spore suspension using a sprayer. Inoculation was done between the hours of 5:00 - 6:00 PM in the evenings. Observations on early leaf spot scores at 65 and 90 DAS were recorded on each plant using a 9-point scale as described by (Subrahmanyam et al., 1995). Genotypes were categorized into resistant (≤3), moderate resistance (4–5), susceptible (6–7), and highly susceptible (>7) as described by (Sudini et al., 2015) based on disease severity score. The data collected were subjected to analysis of variance (ANOVA) and the means were compared using LSD at 5% level of probability.

Results and Discussions

Mean Squares

The mean square performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at BUK and Samaru are presented in Table 1 and 2 respectively. For kano, highly significant differences (p≤ 0.01) were observed among the genotypes for disease scoring at 90 DAS and disease incidence at 90 DAS under both inoculated and uninoculated condition, significant differences (p≤ 0.05) were observed among genotypes for pod weight under uninoculated condition. However, for both disease condition, no significant difference were observed among genotype for disease scoring at 65 DAS, disease incidence at 65 DAS and pod weight expect for pod weight under uninoculated condition. For samara, under uninoculated condition, highly significant differences (p≤ 0.01) were observed among the genotypes for disease scoring at 90 DAS and disease incidence at 90 DAS, while significant differences (p≤ 0.05) were observed among genotypes for disease scoring at 90 DAS and disease incidence at 90 DAS under inoculated condition. No significant difference was observed among genotype for other characters studied under both disease conditions. The significant differences among groundnut in the study indicate significant genetic variability within the mentioned traits in each of the location under both disease conditions, thus genetic improvement can be achieved for the characters studied. The amount of genetic improvement that can be achieved among a given set of genotypes depends on the amount of genetic diversity that is present within the population (Falconer and Mackey, 1996). High genetic diversity for the mini core collections for ELS and pod yield has been previously reported in the Savannas of Nigeria (Shaibu *et al.*, 2020a).

Mean performance among the genotypes

The mean performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at BUK and Samaru are presented in Table 3 and 4 respectively. The result showed a considerable variation for different characters. For BUK, under inoculated condition, Disease scoring at 90 DAS has a mean of 4.89 ranging from 2.00 (ICG 12991 and SAMNUT 22) to 7.50 (ICGV-IS-07213). Disease incidence at 90 DAS has a mean of 65.69% ranging from 22.22% (ICG 12991 and SAMNUT 22) to 83.33% (ICGV-IS-07213). Under inoculated condition, Disease scoring at 90 DAS has a mean of 6.09 ranging from 3.00 (ICG 12991 and SAMNUT 22) to 8.00 (ICGV-IS-07213 and SAMNUT 26). Disease incidence at 90 DAS has a mean of 67.63% ranging from 33.33 % (ICG 12991 and SAMNUT 22) to 83.33% (ICGV-IS-07213 and SAMNUT 26). For samara, under inoculated condition, Disease scoring at 90 DAS has a mean of 5.84 ranging from 2.00 (ICG 12991) to 7.50 (ICGV-IS-07213, SAMNUT 24 and 12CS-004). Disease incidence at 90 DAS has a mean of 65.69% ranging from 22.22% (ICG 12991) to 83.33% (ICGV-IS-07213, SAMNUT 24 and 12CS-004). Under inoculated condition, Disease scoring at 90 DAS has a mean of 6.11 ranging

from 2.00 (ICG 12991 and SAMNUT 22) to 7.50 (ICG-10053, SAMNUT 26 and 12CS-004). Disease incidence at 90 DAS has a mean of 66.67% ranging from 33.33% (ICG 12991 and SAMNUT 22) to 88.89% (ICGV-IS-86024). Pod weight has a mean of 482.54kg/ha ranging from 131.48kg/ha (ICG-12991 and SAMNUT 22) to 970.67kg/ha (12CS-004). The significant differences among groundnut in the study indicated the presence of variability in the material used and provide good opportunity for improving groundnut ELS resistance. Among the genotypes, ICG12991 and SAMNUT 22 in the present study showed a moderate resistance to ELS disease incidence with low disease scoring and should be involved in breeding program as a source of resistance to ELS.

Correlation

The estimate of correlation among the traits studied under inoculated and uninoculated conditions evaluated at Kano and Samaru presented in Table 5 and 6 respectively. For Kano, The result showed that disease scoring at 65DAS and 90DAS show no significant correlation with other traits. Pod weight exhibited a negative correlation with disease incidence 65DAS and 90DAS (-0.02) and (-0.19) respectively. While for Samaru, The result showed that disease scoring at 65DAS and 90DAS show no significant correlation with other traits. Pod weight showed a highly significant negative correlation with disease incidence at 90DAS (-034) and also negative correlation with disease incidence at 65DAS (-0.17). Correlation coefficient analysis helps to determine the nature and degree of relationship between two measured characters; it resolves the complex relationship between characters into simple form of association, Channayya, (2009). In this study, a significant negative correlation was observed between pod weight and disease incidence, indicating that increase in disease incidence leads to decrease in pod weight. This result corroborate with the findings of Ait Abd *et al.,* (2010).

Conclusions and Recommendations

The study revealed significant difference among some of the traits studied indicating high genetic variability, thus genetic improvement can be achieved for the characters studied. The study also revealed that the mean performance of ICG12991 and SAMNUT 22 showed a moderate resistance to ELS disease incidence with low disease scoring and incidence, however significant negative correlation was observed between pod weight and disease incidence. Based on the observed results, it can be recommended that selection for ELS resistance is possible using the available materials. Genotype ICG12991 and SAMNUT 22 showing moderate resistance to ELS disease can be selected for breeding of resistance to ELS and further evaluated in more environments can be made.

Table 1: The mean square performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at BUK during 2019 raining season

		INOCULATED					UNINOCULATED				
Source of variation	Df	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)
Genotype	22	0.208	4.921**	306716.373	25.773	607.602**	3.5164*	2844645.23**	88.112*	49.382	3.5164**
Replication	1	0.363	1.454	46409.164	44.893	0.2956	0.400	582580.77	197.530	434.1247	0.400
Error		0.2683	1.2640693	408201.99	33.1355	156.0579	1.242	1348380.96	48.0831	153.346	1.242

* and ** = significant at 0.05 and 0.01 probability levels respectively, DS= disease scoring, DI= disease incidence, PW= pod weight.

Table 2: The mean square performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at Samaru during 2019 raining season

		INOCULATED					UNINOCULATED				
Source of variation	Df	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)
Genotype	22	0.3763	3.842*	387154.71	46.461	474.351*	0.179	3.642**	118020.338	22.128	449.637**
Replication	1	0.029	0.029	1462205.26	3.631	3.631	0.900	0.225	5257.848	111.111	27.777
Error		0.4044	1.654	2143540.77	49.9273	204.248	0.3210	1.3829	206544.1210	39.6361	170.7278

* and ** = significant at 0.05 and 0.01 probability levels respectively, DS= disease scoring, DI= disease incidence, PW= pod weight.

Table 3: The mean performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at Kano during 2019 raining season

		INOCULATED					UNINOCULATED				
S/N	GENOTYPES	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)
1	12CS 004	2.00	4.00	771.96	22.22	44.44	2.00	4.00	183.34	22.22	44.44
2	12CS032	2.00	4.00	827.05	22.22	44.44	3.00	7.00	395.10	33.33	77.77
3	ICG 07839	1.00	3.00	279.23	11.11	33.33	3.00	8.00	551.07	33.33	88.88
4	ICG 10053	2.00	4.00	695.21	22.22	44.44	2.00	6.50	479.94	22.22	72.22
5	ICG 10092	2.50	5.50	1154.13	27.78	61.11	2.50	5.50	581.85	27.78	61.11
6	ICG 111	2.00	6.00	592.58	22.22	66.67	2.50	5.50	949.67	27.78	61.11
7	ICG 114	1.50	3.00	688.34	16.67	33.33	2.00	4.00	1254.86	22.22	44.44
8	ICG 11515	2.00	5.00	611.08	22.22	55.55	2.50	6.00	353.79	27.78	66.67
9	ICG 11855	2.50	4.00	545.31	27.78	44.44	2.00	5.00	1537.33	22.22	55.55
10	ICG 12991	2.00	2.00	826.84	22.22	22.22	2.00	3.00	1813.35	22.22	33.33
11	ICG 1711	2.50	3.50	515.29	27.78	38.89	2.50	5.00	2359.32	27.78	55.55
12	ICGV-IS 07213	2.50	7.50	469.95	27.78	83.33	3.00	7.00	1442.68	33.33	77.77
13	ICGV-IS 14861	2.00	3.50	869.56	22.22	38.89	3.00	6.50	521.88	33.33	72.22
14	ICGV-IS 14876	2.00	5.50	350.25	22.22	61.11	4.00	7.00	320.30	44.44	77.77
15	ICGV-IS 15415	2.00	7.00	385.72	22.22	77.78	3.00	7.50	750.05	33.33	83.33
16	ICGV-IS 86024	2.50	6.00	917.96	27.78	66.67	4.00	7.00	1141.58	44.44	77.77
17	ICGV-IS 94379	2.00	5.00	1949.26	22.22	55.56	2.50	7.00	326.39	27.78	77.78
18	J 11	2.00	6.00	550.15	22.22	66.66	2.00	6.50	729.74	22.22	72.22
19	SAMNUT 22	1.50	2.00	1569.31	16.67	22.22	2.00	3.00	5919.86	22.22	33.33
20	SAMNUT 24	2.00	6.50	535.95	22.22	72.22	3.00	7.50	1171.30	33.33	83.33
21	SAMNUT 25	2.00	6.50	554.38	22.22	72.22	2.50	7.50	1121.94	27.78	83.33
22	SAMNUT 26	2.00	6.50	917.45	27.78	66.66	2.00	8.00	784.36	22.22	88.88
23	Ex-dakar red	2.00	6.50	1281.43	22.22	72.22	3.00	6.00	2680.22	33.33	66.66
	MEAN	2.022	4.891	776.451	22.705	54.104	2.609	6.087	1189.996	28.984	67.629
	SE±	0.074	0.336	83.007	0.849	3.683	0.126	0.311	254.397	1.395	3.451

DS= disease scoring, DI= disease incidence, PW= pod weight.

Table 4: The mean performance of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at Samaru during 2019 raining season

		INOCULATED					UNINOCULATED				
S/N	GENOTYPES	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)	DS65	DS90	PW(kg/ha)	DI65 (%)	DI90 (%)
1	12CS 004	2.50	7.50	249.67	27.78	83.33	2.50	7.50	970.67	27.78	83.33
2	12CS032	3.00	5.0	279.50	33.33	55.55	2.00	7.00	219.89	22.22	77.78
3	ICG 07839	2.00	7.00	99.13	22.22	77.77	1.50	6.50	559.54	16.67	72.22
4	ICG 10053	3.00	5.00	181.70	33.33	77.77	2.00	7.50	342.88	22.22	83.33
5	ICG 10092	2.00	6.50	1035.78	22.22	72.22	2.50	7.00	333.68	27.78	77.78
6	ICG 111	1.50	7.00	524.06	16.67	77.78	2.00	6.50	501.80	22.22	72.22
7	ICG 114	1.50	6.00	459.88	16.67	66.67	3.00	6.00	399.75	33.33	66.67
8	ICG 11515	1.50	5.50	442.00	16.67	61.11	2.00	6.00	439.70	22.22	66.67
9	ICG 11855	2.50	5.50	377.23	27.78	61.11	2.50	6.50	265.85	27.78	66.67
10	ICG 12991	2.00	2.00	1415.38	22.22	22.22	2.50	3.00	829.63	27.78	33.33
11	ICG 1711	2.50	6.00	1248.46	27.78	66.67	2.50	6.00	370.45	27.78	66.67
12	ICGV-IS 07213	2.50	7.50	312.97	27.78	83.33	2.50	3.50	504.56	27.78	38.89
13	ICGV-IS 14861	2.50	5.50	1708.04	27.78	61.11	2.00	7.00	494.81	22.22	77.78
14	ICGV-IS 14876	2.50	5.50	396.50	22.22	61.11	2.00	7.00	356.28	22.22	77.78
15	ICGV-IS 15415	2.00	4.00	370.50	22.22	44.44	2.50	7.00	854.7	27.78	77.78
16	ICGV-IS 86024	2.00	7.00	479.05	22.22	77.78	2.00	8.00	712.73	22.22	88.89
17	ICGV-IS 94379	2.00	5.50	419.69	22.22	61.11	2.50	6.00	147.18	27.78	66.67
18	J 11	2.00	6.50	518.05	22.22	72.22	2.00	6.00	208.00	22.22	66.67
19	SAMNUT 22	1.00	2.50	4285.13	11.11	27.78	2.00	3.00	773.09	22.22	33.33
20	SAMNUT 24	2.50	7.50	631.04	27.78	83.33	2.50	3.50	131.48	27.48	61.11
21	SAMNUT 25	2.00	6.50	485.27	22.22	72.22	2.00	6.50	711.98	22.22	72.22
22	SAMNUT 26	2.00	6.50	529.56	22.22	72.22	2.00	7.50	217.57	22.22	83.33
23	Ex-dakar red	2.00	7.00	296.83	22.22	72.22	2.00	6.00	752.30	22.22	66.67
	Mean	2.130	5.848	728.062	23.430	65.699	2.217	6.109	482.544	24.624	66.667
	SE±	0.104	0.308	189.322	1.135	3.417	0.071	0.312	48.662	0.787	3.822

DS= disease scoring, DI= disease incidence, PW= pod weight.

Table 5: Correlation coefficient of groundnut genotypes for early leaf spot disease and Pod weight under Inoculated and uninoculated conditions evaluated at Kano during 2019 raining season

	DS65	DS90	PW (kg/ha)	DI65 (%)	DI90 (%)
DS65	1.00000				
DS90	0.44233**	1.00000			
PW (kg/ha)	-0.02521	-0.19717	1.00000		
DI65 (%)	1.00000**	0.44233**	-0.02521	1.00000	
DI90 (%)	0.44233**	1.00000**	-0.19717	0.44233**	1.00000

* and ** = significant at 0.05 and 0.01 probability levels respectively, DS= disease scoring, DI= disease incidence, PW= pod weight.

Table 6: Correlation coefficient of groundnut genotypes for early leaf spot disease and Pod weight under inoculated and uninoculated conditions evaluated at Samaru during 2019 raining season

	DS65	DS90	PW (kg/ha)	DI65 (%)	DI90 (%)
DS65	1.00000				
DS90	0.00935	1.00000			
PW (kg/ha)	-0.17467	-0.33523**	1.00000		
DI65 (%)	1.00000**	0.00935	-0.17467	1.00000	
DI90 (%)	0.00935	1.00000**	-0.33523**	0.00935	1.00000

* and ** = significant at 0.05 and 0.01 probability levels respectively, DS= disease scoring, DI= disease incidence, PW= pod weight.

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AGRO-MORPHOLOGICAL DIVERSITY INBRED OF MAIZE (*ZEA MAYS* L.) IN A DIALLEL CROSS

M. Saleh[1*] and A. U. Izge[2]

Department of Crop production, School of Agriculture and Agricultural Technology, Abubakar Tafawa Balewa University Bauchi, Bauchi, Nigeria
Corresponding Author: nafson850@gmail.com +2348035748178

ABSTRACT

Background and Objective: Genetic diversity provides the capacity for plants to meet changing environments. Multivariate analysis is the most popular approach for genetic variability estimation to study patterns of variation and their genetic relationships. The objectives of the study were: (i) To assess the extent of genetic diversity in maize through Principle Component Analysis (PCA), (ii) To assess the relationship between grain yield and yield related traits of maize genotypes. Materials and method: Five maize inbred lines were crossed in a full diallel fashion producing twenty hybrids. The hybrids and the inbred parents were evaluated in a randomized complete block design (RCBD) replicated three times in two locations viz: ATBU Research Farm and in the farmers field in Misau also in Bauchi State in the 2019 rainy season. Results: The PCA revealed that, five vectors accounted for 88.40 % and 90.30 % of the total variability produced by all the traits under study in Bauchi and Misau respectively. The first canonical vector PCI accounted for 38.90 % and 40.80 % percent of the total variability followed by second vector PCII which accounted for 22.30 % and 21.90 % of total variability in Bauchi and Misau respectively. On the other hand, the third vector PCIII accounted for 16.10 % and 18.40 % percent of the total variance while PCIV and PCV accounted for 6.00 %, 5.30 % and 5.20 %, 3.80 % of the total variability in Bauchi and Misau respectively. Days to 50 % tasseling, days to 50 % silking, anthesis silking interval, cob diameter, cob length followed by cob weight/plant, number of ear/plant, kernel weight/plant and yield/plant in that order are the major contributors to the total divergence suggesting their importance in maize improvement. Conclusion: Attention should be given to days to 50 % tasseling, days to 50 % silking, anthesis silking interval, cob diameter, cob length in the improvement of maize by breeders and geneticists and that the traits mentioned positively correlate with yield thus, should be given due consideration in grain yield improvement.

Keywords: *Maize, PCA, diallel, vector, multivariate*

Introduction

Maize (*Zea mays* L.) is an important cereal crop of the family *Poaceae* that belongs to the tribe *Maydeae*. The plant is native to South America. Worldwide maize is the most important cereal food crop after wheat and rice accounting for 9 per cent of the total food grain production. It has occupied a prominent place in Nigerian agriculture as it is widely grown in varied climatic situations throughout the year suggesting its wider adaptability Sandeep *et al*[10] . Nigeria in 2018 grew 4853349 hectare maize and produced about 10.155 million tons of grains, with an average yield of 2092 kgha^{-1} FAOSTAT[4]. It is consumed as food by humans and as a feed for the livestock and poultry. It is also used as basic raw material in numerous industrial products including starch, oil, protein, alcoholic beverages, Food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package, paper industries and so on Avinash and Mishra[3]. Maize has high nutritive value as it contains 72 per cent

starch, 10 per cent protein, 4.80 per cent oil, 8.50 percent fiber, 30 per cent sugar and 1.70 per cent ash Mustafa *et al*[9].

The major objective of any maize breeding programmes is to develop high yielding hybrids than the existing cultivars as hybrids are popular among the farming community for their yield advantage over others. To develop high yielding hybrid s in maize, the development and evaluation of inbreds form major thrust area of plant breeding programmes. Hence, inbred lines developed through sib mating need to be evaluated for their genetic diversity and performance to plan an effective hybrid breeding programme as genetically diverse parents are known to produce high heterotic effects. Evaluation, characterization and classification of genotypes based on estimates of genetic diversity will help to identify diverse parental lines which can be used in hybrid breeding to develop potential hybrids or varieties. Several methods have been reported to decipher the pattern and magnitude of variability such as Mahalanobis D2 analysis, Principal Component Analysis and hierarchical cluster analysis based on Ward's minimum variance method Mounika and Mishra[7]. Multivariate analysis is the most popular approach for genetic variability estimation to study the patterns of variation and their genetic relationships among germplasm collections to enhance their use in crop breeding Ahmed *et al*[1]. The PCA and cluster analysis is better utilized for studying the diversity among the genotypes in various crops. In view of the aforementioned, five inbred lines were used to study the nature and magnitude of genetic divergence for grain yield and its component traits to provide a basis for selection of parents in hybridization programme in maize hybridization program Solanke *et al*[11]. Many researchers have used principal component analysis to assess genetic variability among maize genotypes because it retrieves small numbers of components that account for most of the variations in the data Asare *et al*[2]. The aim of the study is to deduce the trait(s) in maize that should preferentially considered in maize breeding program improvement. The objectives of the study were: (i) To assess the extent of genetic diversity in maize through Principle Component Analysis (PCA), (ii) To assess the relationship between grain yield and yield related traits of maize genotypes.

Materials and Methods

Study area: The nursery study was conducted at the Abubakar Tafawa Balewa University Bauchi Experimental Farm with on latitude 10.31° and longitude 9.85° at an altitude of 628 m (2,060 feet) above sea level in the 2018 rainy season. Five maize inbred lines (OBATANPA 14, SAMAZ 15, SAMAZ 16, SAMAZ 32, and SAMAZ 33) obtained from the International Institute for Tropical Agriculture (IITA) was crossed in a diallel mating design.

Table 1: Inbred lines, their traits and source

S/N	MAIZE INBRED LINE	FEATURES OF THE INBRED LINES	SOURCE OF INBRED LINES
1	OBATANPA14	white kernel, quality protein (QPM) maize, medium maturing (110 days),yield potential 3-4.5 tone/hectare	International Institute for Tropical Agriculture (IITA)
2	SAMAZ15	White normal kernel, tolerant to *Striga hermonthica*, medium maturing(110 days),yield potential 6.9 tone/hectare	International Institute for Tropical Agriculture (IITA)
3	SAMAZ16	White normal kernel, tolerant to *Striga hermonthica*, late maturing(120 days),yield potential 6.4 tone/hectare	International Institute for Tropical Agriculture (IITA)
4	SAMAZ32	Yellow seeded kernel, extra early, drought tolerant, yield potential 3-4 tone/hectare	International Institute for Tropical Agriculture (IITA)
5	SAMAZ33	White seeded kernel, extra early, drought tolerant, quality protein maize (QPM) yield potential 3-4 tone/hectare	International Institute for Tropical Agriculture (IITA)

Maize is a diclinous plant because it has separate male and female flowers (efflorescence) and it is also monoecious crop because it has both the male and female flowers on same plant. The male efflorescence that produce the pollen or male gametic cells are formed at the apex or tip of the plant borne on a structure known as tassel where anthers are borne. The tassel begins to produce pollen immediately it blooms and normally the pollen is dispersed by wind to aid cross pollination. The female efflorescence that produce the kernel are produce at the side of the plant on a structure called the ear which is having long special stigmas on a style called the silk where each silk leads to a single ovary that grows from individual maize kernel.

Controlled crossing was achieved using parchment paper bag, stapler, marker, small knife and small white glossy bags. The inbred lines of SAMAZ 14, SAMAZ 15 and SAMAZ 16 were sown on 26th of June 2018 after proper soil tillage with inter and intra row spacing of 75 cm × 75 cm. The planting pattern were oriented so that particular cross can be obtained in a specific plot having the male intended plants on the outer row with the female intended plants occupied the inner rows. On 10th of July SAMAZ 32 and SAMAZ 33 was stagger planted or sown 15 days after to allow for nicking to take place. The nursery was well spaced to allow for proper observation as well as ease of pollination.

After emergence of the ear and before silk emergence, the ears were covered. Blooming started in tassels near the tip of the central axils and it produces downward. It takes nearly 14 days to complete. The pollen grains become viable for 24 hours when mature. The male efflorescence that produces the pollen was covered with brown envelopes that were well labeled. The opening of the inverted envelopes were then folded and stapled to prevent pollen from falling off. The glossy bag was slipped to cover the entire ear and fixed firmly in-between the ear and the stem. In cases where the silk took too long to emerge the tips of the ear husks were cut to accelerate silk emergence. After making sure of silk emergence, pollens were collected from particular plant tassels that was covered a day before with brown envelope by bending the stem of the plant with covered tassel and gently taping the envelope to drop pollen.

The envelope was gently removed in erect position to prevent pollen from falling. The envelope containing the pollen was the taken to appropriate plant where the pollens are dusted on the silk (receptive stigmas) after the glossy white bag covering the ear was removed. Immediately after dusting the ear was covered with the envelope that conveyed pollen to allow for fertilization to take place without contamination by unknown pollen. After maturing the kernel from each cross were harvested separately where they were manually threshed and stored in a cool dry place. A total of twenty 20 hybrids were produced from five parents which were evaluated the following season.

Evaluation of the trial was conducted in two locations in Sudan savannah zone of Nigeria i.e. Abubakar Tafawa Balewa University Bauchi, Experimental Farm and Misau Farmers Training Field at Kukadi, in 2019 rainy season. The 25 treatments were laid out in a randomized complete block design (RCBD) replicated three times. The seeds were sown manually by hand on the 10th July, 2019 in Bauchi and 17th July 2019 in Misau and after germination were thinned to two plants per hill, at two weeks after sowing. Any hill that did not have plants was gap-filled during the first weeding. The plot consisted of 4 ridges 3 m long and 75 cm apart, interplant spacing on the rows were 50 cm having 7 ridges with a plot length of 3.5 m. Each sub plot had an area of 10.50 m^2.

Ten plants were tagged from internal rows for recording observations for each entry/plot for all the quantitative characters except for days to 50 % tasseling, silking and anthesis silking interval. Mean of the ten plants for each entry in each replication was computed for each character and used for statistical analysis. Observations on the following quantitative characters days to fifty percent tasseling (DFT), days to fifty percent silking (DFS), anthesis silking interval (ASI), number of tessel branch (NTB), number of ear per plant (NEPP), number of days to maturity (NDM), cob weight per plant (CWPP), number of row per ear (NRPE), number of kernel per row (NKPR), cob length (CLT), cob diameter (CDM), hundred seed weight (100SW), kernel weight per ear (KWPE), kernel weight per plant (KWPP) and yield per hectare (YPH) were recorded at the appropriate stages of plant growth.

The set of data collected were subjected to a multivariate analysis specifically principal component analysis (PCA). Principal component analysis (PCA) is a mathematical procedure that transforms a large number of possibly correlated variables into a smaller number of uncorrelated variables called the principal component. To compute for principal component from large different sets of data is simply the Eigen decomposition of covariance or correlation matrix.

Covariance (COV x, y) = $\frac{1}{n}\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})$

Correlation (r_{xy}) = $\frac{\sum_{i=1}^{n}(X_i-\overline{X})(Y_i-\overline{Y})}{\sum_{i=1}^{n}\sqrt{(X_i-\overline{X})^2(Y_i-\overline{Y})^2}}$

Where the entries are fed in a matrix format to produce COVARIANCE MATRIX or CORRELATION MATRIX.

Eigenvalues and Eigenvectors can be computed from the covariance or correlation matrix, state as follows

$A\bar{v} = \lambda\bar{v}$
$A\bar{v} - \lambda I\bar{v} = 0$
$(A - \lambda I)\ \bar{v} = 0$
$\det(A - \lambda I) = 0$

A = is a covariance matrix
or
λ = is the eigenvalue
$\bar{v}$ = is the eigenvector
I = identity matrix
The eigenvalue and the eigenvectors give the foundation of PCA

By matrix method which is the products of a transpose of a matrix and the matrix itself.

Covariance Matrix = $X^T X$

Where

X = is a set of matrix of data

X^T = is the transpose of matrix X

Results and Discussion

The principal component analysis result for the fifteen traits in Bauchi presented in Table 2 were the Eigen values, % variance, % cumulative variance and factor loading of different traits are given. In canonical variant analysis the number of variable is produce to linear function called canonical vector which account for most of the variation produce by these characters. The five vectors accounted for 88.40 % to the total variability produced by all maize genotypes for yield. The result indicated that all the traits showed positive loading on PCI which contributed to 38.9% of the total variation having an Eigen value of 5.8349 with the highest loadings coming from days to 50 % tasseling, anthesis silking interval and cob diameter with values of 0.309, 0.345 and 0.346 respectively. The least contribution came from number of ear/plant with value of 0.155. The PCII which contributed to 22.30 % of the total variability with Eigen value of 3.3421 showed maximum positive loadings on cob weight/plant (0.392), number of ear/plant (0.289), kernel weight/plant (0.390) and yield/ha (0.390), while number of tassel branch (-0.287), number of row/ear (-0.347), number of kernel/row (-0.231) had the higher negative values. The PCIII which contributed to 16.10 % of total variability with an Eigen value of 2.411 showed maximum positive loadings on days to 50 % tasseling, days to 50 % silking and number of ear/plant, with the corresponding values of 0.338, 0.308 and 0.396 respectively with the highest negative values on 100 seed weight (-0.427) and kernel weight/ear (-0.462). The PCIV which contributed to 8.32% of the total variability with an Eigen value of 0.8957 showed maximum positive loadings on cob length (0.819), with the least value of -0.411 from number of tassels/branch. The PCV which contributed to 5.20% of the total variability with an Eigen value of 0.7747 showed maximum positive loadings on number of ear/plant, number of days to maturity, and number of kernels/row with loading factors of 0.409, 0.423 and 0.315 respectively with the least values of -0.486, and -0.394, for 50 % tasseling and days to 50 % silking respectively.

The principal component analysis result for the fifteen traits in Misau in Table 3 indicated that Eigen value, % variance, % cumulative variance and factor loading of different traits. In canonical variant analysis the number of variable was produced to linear function called canonical vector which accounted for most of the variation among traits. Five vectors accounted for 90.30 % of total variability produced by all the genotypes for yield. The result indicated that all the traits showed positive loading on PCI which contributed to 40.80 % of the total variation having an Eigen value of 6.1247 with the highest loadings coming from days to 50 % tasseling, days to 50 % silking, anthesis silking interval, cob length and cob diameter with values of 0.329, 0.321, 0.339 and 0.340 respectively. The least contribution came from number of ear/plant and 100 seed weight with 0.161 and 0.167 respectively. Principal component 2 which contributed to 21.90 % of the total variability with an Eigen value of 3.2907 showed maximum positive loadings on number of ear/plant (0.369), cob weight/plant (0.456) while number of tassel branch (-0.209) and number of row/ear (-0.282) had higher negative values.

Principal component 3 which contributed to 18.4% of total days to 50 % tasseling variability with an Eigen value of 2.7626 showed maximum positive loadings on cob length, kernel/row, and number of row/ear with values of 0.200, 0.251 and 0.195. PCIV on the other hand contributed to 5.30% of the total variability with an Eigen value of 0.7997 had maximum positive loadings on 100 seed weight, number of days to maturity, number of tassel branch and anthesis silking

interval, with corresponding values of 0.235, 0.242, 0.231 and 0.0.247 with the least value of -0.506 from kernels/row. PCV contributed to 3.80 % of total variability with an Eigen value of 0.5722 showing maximum positive loadings on kernel/row and number of tassel branch with values of 0.373 and 0.721 respectively, while the least values are -0.344, -0.258 and -0.207 for cob length, number of days to maturity and days to 50 % silking

Agro-morphological diversity was the outcome of several factors along with a factor geographic diversity, the result of this research showed the same pattern in eigen component as well as factor loadings for the characters between the two locations (Bauchi and Misau), this indicated that the diversity noticed is as a result of genetic diversity. Hence, selection for hybridization should be more based on genetic diversity than geographic diversity. The Principal Component Analysis sorted out the total characters into five main principal components. By PCA, the in-depth analysis for genetic diversity can be made. Principal Components revealed characters viz., days to 50 % tasseling, anthesis silking interval and cob diameter in PC1 and cob weight/plant, number of ear/plant, kernel weight/plant and yield/ha were loaded in PC2, while days to 50 % tasseling, days to 50 % silking and number of ear/plant in PC3, the characters mentioned instantly contributed more towards variability and thus, the characters should be given more attention in selection for hybridization . The contribution of the main characters for variance easily identified by the characters loaded on PC1 as it explained maximum variance are therefore, first and foremost considered in selection for hybridization followed in accordance by characters in the other vectors. In the present study, principal component analysis revealed that days to 50% tasseling, days to 50%silking, Anthesis silking interval, cob diameter, cob weight/plant, number of ear/plant, and kernel weight/plant are the major contributors to the total divergence therefore, the characters easily affects Maize yield positively and thus, the characters can be utilized in breeding programs. The results of principal components analysis corroborated with results obtained by Ahmed *et al*[1], Hafiz *et al*[5] Sandeep *et al*[10] , Solanke *et al*[11] and Worknesh *et al*[12].

All the characters under the study positively correlated with yield in the first principal component this deduce that all the characters under the study are yield related characters. It was observed that days to 50% tasseling, days to 50%silking, Anthesis silking interval, cob diameter, cob weight/plant , number of ear/plant, and kernel weight/plant were identified as the mostly correlated characters with grain yield in all environments this deduce that the characters can be use simultaneously in a Maize breeding program for yield improvement. This finding is in accordance with the result of Ahmed *et al*[1]. Morphoagronomic characters, days to 50% tasseling, days to 50%silking, Anthesis silking interval, cob diameter, cob weight/plant , number of ear/plant, and kernel weight/plant that had high values in the first five components indicated their importance as maize descriptors and could be helpful for differentiation and characterization of maize genotypes. Overall, PCA enable to recognize the most crucial characters viz. according to their importance in breeding program to improve grain yield as follows days to 50% tasseling, days to 50%silking, Anthesis silking interval, cob diameter, cob weight/plant, number of ear/plant, and kernel weight/plant respectively for classifying the variability within the genotypes. This outcome is in accordance to the findings of Khodarahmpour and Hamidi[6], Hafiz *et al*[5], Solanke *et al*[11] *and* Worknesh *et al*[12].

Table 2: Principal component analysis for fifteen traits in Bauchi 2019

Eigen Components (EC)	ECI	ECII	ECIII	ECIV	ECV
Eigenvalue	5.835	3.342	2.411	0.896	0.7 75
Proportion	0.389	0.223	0.161	0.060	0.052
Cumulative	0.389	0.612	0.773	0.832	0.884
Principal Components (PC) Variable	PCI	PCII	PCIII	PCIV	PCV
Days to 50% tasseling	0.275	-0.117	0.338	0.080	-0.486
Days to 50% silking	0.309	-0.141	0.308	0.050	-0.394
Anthesis Silking Interval	0.345	-0.191	0.100	-0.078	0.075
Number of Tassel Branch	0.178	-0.287	0.090	-0.411	-0.107
Number of Ear/Plant	0.155	0.289	0.396	-0.142	0.409
Number of Days to Maturity	0.251	-0.131	0.261	0.133	0.423
Cob Weight/Plant	0.278	0.392	0.025	-0.174	0.047
Number of Row/Ear	0.216	-0.347	-0.231	-0.110	0.259
Number of Kernel/Row	0.216	-0.251	-0.286	0.096	0.315
Cob Length	0.199	0.138	0.037	0.819	0.048
Cob Diameter	0.346	-0.231	-0.072	0.057	0.038
100 Seed Weight	0.255	-0.009	-0.427	-0.044	-0.136
Kernel Weight/Ear	0.208	0.185	-0.462	0.076	-0.232
Kernel Weight/Plant	0.278	0.390	-0.069	-0.152	0.004
Yield/ha	0.278	0.390	-0.069	-0.152	0.004

Table 3: Principal Component Analysis for fifteen traits in Misau 2019

Eigen Components (EC)	ECI	ECII	ECIII	ECIV	ECV
Eigenvalue	6.1247	3.2907	2.7626	0.7997	0.5722
Proportion	0.408	0.219	0.184	0.053	0.038
Cumulative	0.408	0.628	0.812	0.865	0.903
Principal Components (PC) Variable	PCI	PCII	PCIII	PCIV	PCV
Days to 50 % tasseling	0.329	-0.102	-0.262	0.107	-0.131
Days to 50 % silking	0.321	-0.054	-0.273	0.104	-0.207
Anthesis Silking Interval	0.339	-0.129	-0.160	0.247	0.180
Number of Tassel Branch	0.264	-0.209	-0.130	0.231	0.721
Number of Ear/Plant	0.161	0.369	-0.288	-0.387	-0.016
Number of Days to Maturity	0.261	-0.015	-0.284	0.242	-0.258
Cob Weight/Plant	0.222	0.456	0.013	-0.021	0.060
Number of Row/Ear	0.261	-0.282	0.195	-0.326	-0.062
Number of Kernel/Row	0.211	-0.150	0.251	-0.506	0.373
Cob Length	0.317	-0.168	0.200	-0.203	-0.344
Cob Diameter	0.340	-0.164	0.126	-0.071	-0.164
100 Seed Weight	0.167	-0.011	0.478	0.235	-0.119
Kernel Weight/Ear	0.091	0.136	0.494	0.436	0.000
Kernel Weight/Plant	0.218	0.451	0.102	-0.019	0.085
Yield/ha	0.218	0.451	0.102	-0.019	0.085

Figure 1 and 2 reveals the graph of screen plot plotted for Eigen values on vertical axis against principal component number the graph indicated sharp decline in Eigen values from principal components one (PCI) to principal component five (PCV) accounted for 88.40% and 90.30% of the total decline on Bauchi and Misau location respectively on Figure 1 and 2 accordingly. Thus, is sufficiently enough to explain quick and immediate level of change in the diversity of the traits. The vector loadings ranging from PCI – PCV is therefore sufficient and significantly enough to explain the diversity of the different traits. The graph show the magnitude of change in diversity loaded on the various principal components which provided the level of magnitude in the principal component that sufficiently explain the diversity.

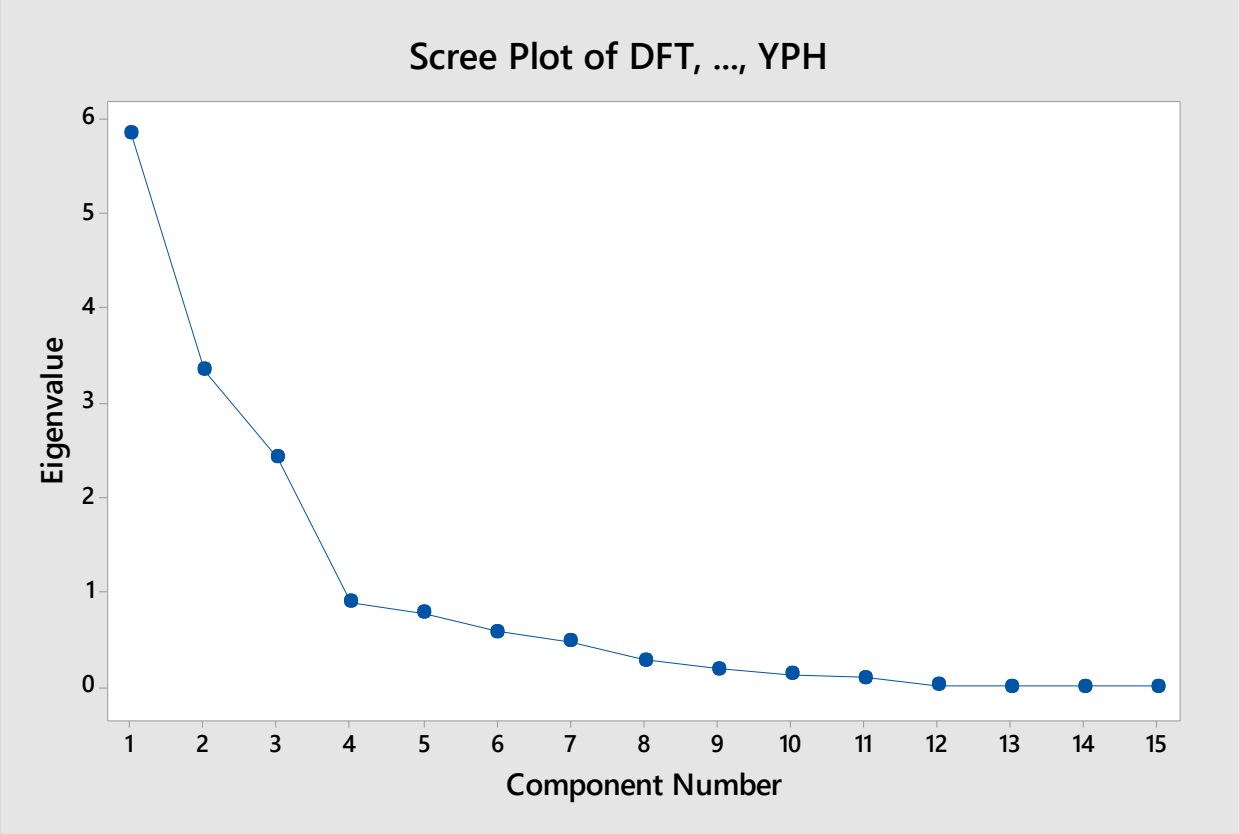

Figure 1: Screen plot for fifteen traits in principal component analysis Bauchi location

DFT = Day to fifty percent tasselling
DFS = Day to fifty percent silking
ASI = Anthesis silking interval
NTB = Number of tassel branch
NEPP = Number of ear per palnt
NDM = Number of days to maturity
CWPP = Cob weight per plant (g)
NRPE = Number pf rows per ear
NKPR = Number of kernel per rows
CLT = Cob length (cm)
CDM = Cob diameter (cm)
100SW = Hundred seed weight (cm)
KWPE = Kernel weight per ear
KWPP= Kernel weight per plant
YHP = Yield per hectare (kg

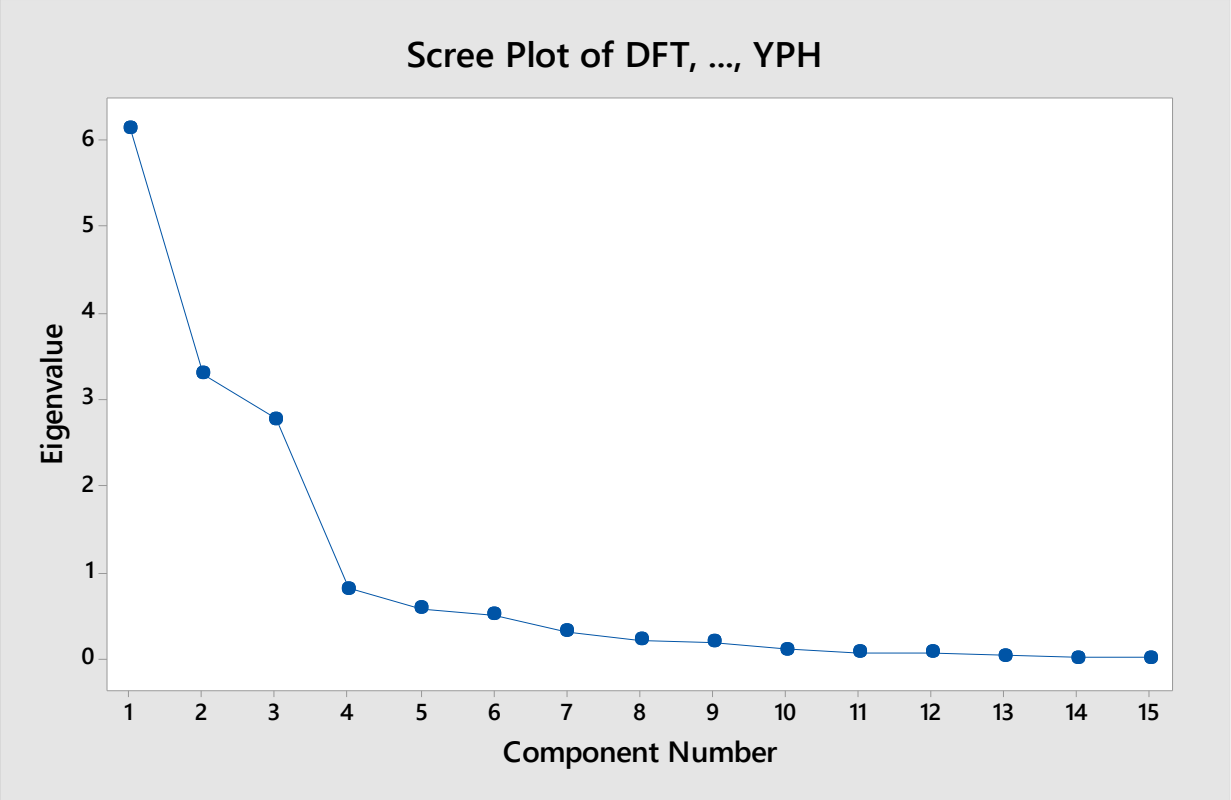

Figure 2: Screen plot for fifteen traits in principal component analysis Bauchi location

DFT = Day to fifty percent tasselling
DFS = Day to fifty percent silking
ASI = Anthesis silking interval
NTB = Number of tassel branch
NEPP = Number of ear per palnt
NDM = Number of days to maturity
CWPP = Cob weight per plant (g)
NRPE = Number pf rows per ear
NKPR = Number of kernel per rows
CLT = Cob length (cm)
CDM = Cob diameter (cm)
100SW = Hundred seed weight (cm)
KWPE = Kernel weight per ear
KWPP= Kernel weight per plant
YHP = Yield per hectare (kg)

Figure 3 and 4 is the graph of factor loadings for the characters under the study with PCII on vertical axis and PCI on horizontal axis indicated the magnitude and direction to which each trait contributed to on either positive or negative variable loadings on PCI and PCII which explain 61.20% and 62.80% of the total magnitude of the diversity loaded by the traits under the study . All the traits under the study loaded on to positive direction on PCI in both the locations (Bauchi and Misau) with the highest magnitude of 38.90% and 40.80% respectively. The graph of plot loadings for the traits identifies the importance of characters number of ear/plant, kernel weight per ear, cob weight per plant, and kernel weight/plant as they load in positive direction on PCI and PCII axis together with yield/hectare in all the locations (Bauchi and Misau). Principal component II on vertical axis indicated negative loadings for all the traits under the study except for number of ear per plant, cob weight per plant, cob length, kernel weight per ear, kernel weight per plant and grain yield the two the locations (Bauchi and Misau). The loading plot graph indicated the magnitude and direction of the various traits under the study thus, revealed the use

fullness of number of ear per plant, cob weight per plant, cob length, kernel weight per ear, kernel weight per plant as preferred traits in yield improvement as they positive correlated with yield on the factor loadings of both PCI and PCII with the highest loadings revealed by kernel weight per ear and kernel weight per plant been the most important traits to be considered by breeders and geneticist in the improvement of grain yield in maize breeding program. The results of principal components analysis corroborated with results obtained by Ahmed *et al*[9], Hafiz *et al*[5], Sandeep *et al*[10] and Solanke *et al*[11].

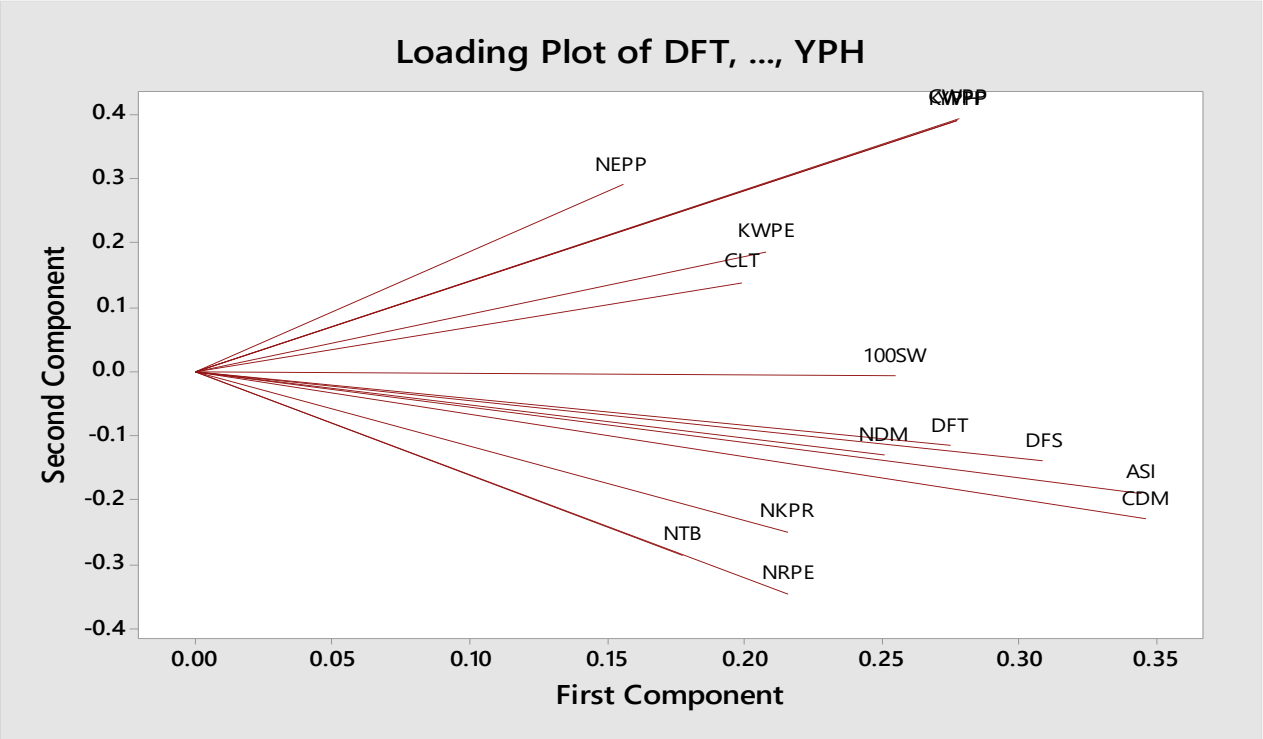

Figure 3: Loading plot for fifteen traits on PCI and PCII Bauchi location

DFT = Day to fifty percent tasselling
DFS = Day to fifty percent silking
ASI = Anthesis silking interval
NTB = Number of tassel branch
NEPP = Number of ear per palnt
NDM = Number of days to maturity
CWPP = Cob weight per plant (g)
NRPE = Number pf rows per ear
NKPR = Number of kernel per rows
CLT = Cob length (cm)
CDM = Cob diameter (cm)
100SW = Hundred seed weight (cm)
KWPE = Kernel weight per ear
KWPP= Kernel weight per plant
YHP = Yield per hectare (kg)

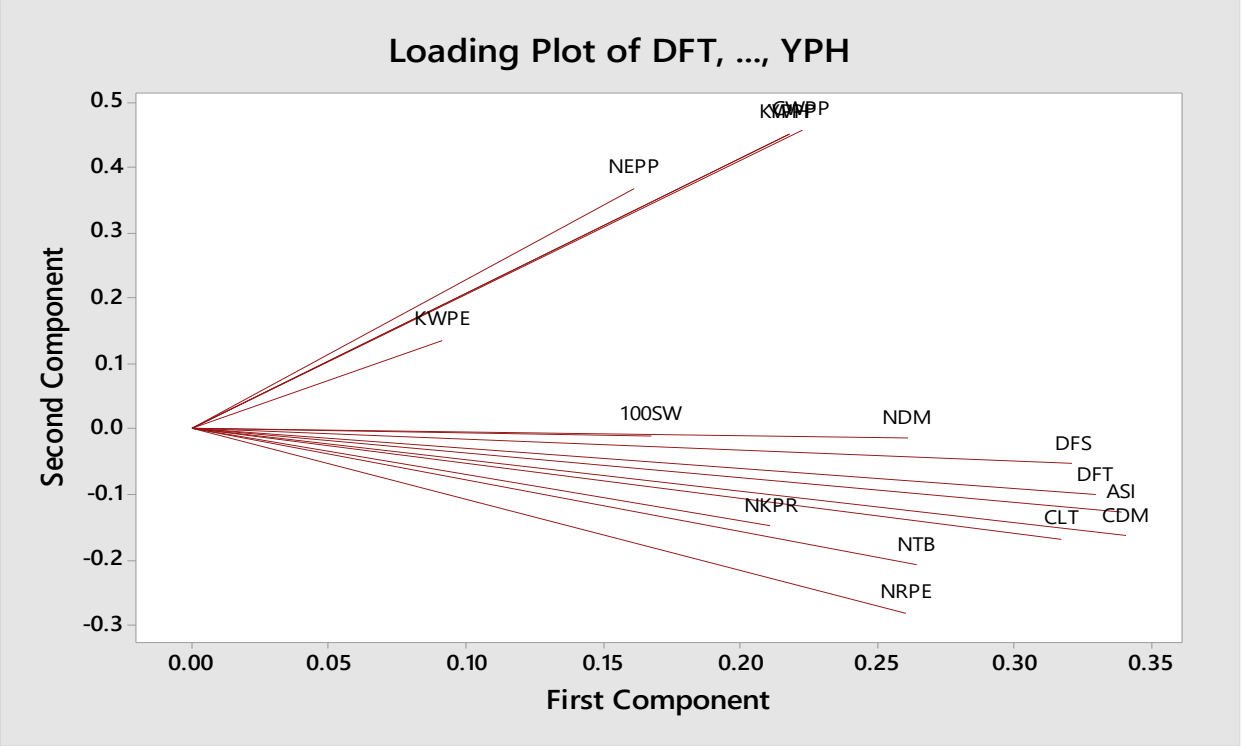

Figure 4: Loading plot for fifteen traits on PCI and PCII Misau location

DFT = Day to fifty percent tasselling
DFS = Day to fifty percent silking
ASI = Anthesis silking interval
NTB = Number of tassel branch
NEPP = Number of ear per palnt
NDM = Number of days to maturity
CWPP = Cob weight per plant (g)
NRPE = Number pf rows per ear
NKPR = Number of kernel per rows
CLT = Cob length (cm)
CDM = Cob diameter (cm)
100SW = Hundred seed weight (cm)
KWPE = Kernel weight per ear
KWPP= Kernel weight per plant
YHP = Yield per hectare (kg)

Conclusion and Recommendations

The results indicated that the characters days to 50% tasseling, days to 50 % silking, anthesis silking interval, cob diameter, cob length showed the maximum positive contribution to the divergence on PCI in both the locations followed by cob weight per plant, number of ear per plant, kernel weight per plant and yield per plant are major contributors to the total divergence on PCII with maximum positive contribution. PCIII had 100 seed weight and kernel weight, ear with the maximum positive contribution suggesting their importance in maize improvement. The traits were also found to positively contribute to diversity as reported by Hafiz *et al*[5], Manoj *et al*[8] and Worknesh *et al*[12].

Positive correlations existed among the traits to up to 38.90 % and 40.80 % in Bauchi and Misau locations respectively. This therefore means that improvement in one of the traits can bring about improvement of other traits and that the traits can be simultaneously improved with 40.80 and 38.90 %t success. Number of ear/plant, cob weight/plant, kernel weight/plant, kernel weight/ear, cob length and yield/ha positively correlate up to 81.20 %.

Significance Statement

This study discovered the importance traits days to 50 % tasseling, days to 50 % silking, anthesis silking interval, cob diameter, cob length can be beneficial for the improvement of grain yield in maize by breeders and geneticists, and that the traits mentioned should be given due consideration in maize breeding program targeted on grain yield. This study will help

breeders and geneticists to uncover the critical traits in maize which contribute positively and most significantly to grain yield of maize in the northern guinea and sudan savanna of Nigeria that many researchers were not able to explore. Thus, a new approach on maize breeding program targeted on grain yield may be arrived at.

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CGBPB 017

MORPHOLGICAL CHARACTERIZATION OF TWO EXOTIC AND TWO INDIGENOUS CULTIVARS OF CUCUMBER (*Cucumis sativus L.*) IN HUMID TROPICAL AGRO-ECOLOGICAL ZONE

L. J. AGAH* and U. AFANGIDEH*

*Department of Crop Science, Faculty of Agriculture, Forestry and Wildlife Resources Management. University of Calabar, PMB 1115, Calabar, Cross River State, Nigeria
*Corresponding Author: l.j.agah@unical.edu.ng; linusagah81@gmail.com+2348069642707

ABSTRACT

Two exotic (Markmore 76 and Tablegreen 72) and two indigenous (Ex – Eket and Ex – Calabar) cultivars of Cucumis sativus were morphologically characterized in 2005 cropping season at the University of Calabar Teaching and Research Farm. A plot size measuring 11.2 m x 14.1 m (157.92 m^2) was laid out in randomized complete blocks design (RCBD) with three replications. Data were collected on morphological traits and analyzed for variance components. There were variation for vine length per plant at six weeks WAP, number of leaves per plant at two and six WAP, number of branches per plant at two, four and six WAP, number of days to 1st flowering initiation, number of days to 50% flowering, number of male flowers and mean fruit number Result showed that most of the agronomic traits showed significant differences between the exotic and local cultivars. The exotic cultivar (Markmore 76) had the highest mean fruit number (8.50 t/ha) than Ex – Eket (3.67 t / ha) and others cultivars. The exotic cultivars fruited earlier than the local cultivars. However, the indigenous cultivars producing the highest number of pollens (7.67) than exotic cultivars (3.33).. The variation observed among the cultivars seems to suggest that there are varietal differences which should be exploited for future breeding work on this crop.

Keywords: *Morphological traits, genotypic variation, variance components*

Introduction

Cucumber *(Cucumis sativus* L.) is a member of the plant family Cucurbitaceae, along with crops like melons, squash and fluted pumpkin. India had been proposed as the center of origin (Renner *et al.,* 2007 and Anonymous, 2012). Cucumber crop is grown in both temperate and tropical regions (Eifediyi and Remison, 2010). It is an important source of minerals and vitamin such as calcium, phosphorus, iron, sodium, potassium vitamins A and C, Riboflavin, Thiamine, Niacin, Ascorbic acid but low in protein and carbohydrate. Some cultivars have high medicinal value like snake gourd Eno- obong (2001), Whitaker and Davis (1996) .

A large number of local lines are cultivated in Nigeria but there is no recommended cultivar. No serious attempts have been made to upgrade the productivity and acceptability of this crop. The productivity of the vegetable can be increased to greater extent through varietal improvement. For developing superior varieties, it is necessary to improve yield components in cucumber. Morphological traits contribute significantly to yield improvement and each of these components adds its own value to the genetic system and is useful in the improvement of yield trait (Ndukauba *et al.,* 2015, Om and Vijay 2016). Characterization of these morphological traits is a mean of identifying

and selecting superior genotypes among the exotic and indigenous cultivars. Fruit yield in cucumber is quantitatively inherited, thus improvement in yield trait requires an indirect approach of selecting yield characteristics that have high response to selection. Genetic studies rely on analysis of statistical tools to measured variations of morphological traits for selection response. The tools include mean, variance, heritability and genetic advance. These tools are not only helpful in evaluating the genetic stability and performance of genotype but it is also a measure to determine the effectiveness of selection for a particular trait in that genotype. . The success of any breeding programme depends greatly on the genetic diversity available in a population (Afangideh *et al.,* 2005; Subramanian and Subbaraman, 2010). The variation among the genotypes performance in Cucurbitaceae has been widely studied by many scholars (Afangideh *et al.,* 2005; Ene *et al;* 2016 , Bernard *et al.,* 2014, Adjoumani *et al.*, 2016, Ajisefinanni, 2004, Agah and Ittah 2018) but systematic work to compare the vegetative and reproductive traits, characterized the performance of exotic and indigenous cultivars for selection response in the humid agro – ecology zone is scanty. This has poses a challenge in cucumber breeding programme in Nigeria, as the plant breeders rely heavily on the variability among genotypes as a mean of identifying, classifying and obtaining germsplam for effective selection. This study is therefore designed to bridge the gap of comparing the morphological traits among the cultivars studied, providing adequate information about the genotypes and make possible recommendation of genotypes for selection response. This research was therefore designed to characterize two exotic and two local cultivars of cucumber based on various vegetative and reproductive parameters and make possible genotypes recommendation the will suit in humid agro – ecological zone.

Materials and Methods

Study Area

The experiment was conducted at the University of Calabar Teaching and research farm, Cross River State, Nigeria. The University of Calabar is located at Latitude $4^{0}56'$ to 17.39'N and longitude 8^{0} 21' 0.37 E.

Planting materials and Experimentation

The seeds of the four varieties of *C. sativus* were obtained from three different places. The exotic varieties (Markmore 76 and Tablegreen 72) was obtained from Cucumber Breeding Station (CBS), United States of America (USA) at North Carolina State University , (North Central Regional plant introduction station) while one of the local variety Ex-Eket was obtained from Eket farmers in Akwa Ibom State and the other local variety, Ex-Calabar was obtained from local farmers in Calabar, Cross River State, Nigeria.

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications and (16) sixteen stand per replicate; the four cucumber varieties formed the four treatments. A plot of land, 11.2 m x 14.1 m (157.92 m^2) was manually cleared, prepared and planted in March 2005. Two seeds were sown per hole at a spacing of 60 cm x 60 cm and seedlings were thinned to one at two weeks after sowing. N. P. K fertilizer (18g N/ha) was incorporated into the soil a day before sowing while N was split applied twice ½ before planting and ½ at 5^{th} weeks after sowing. A spacing of 60 cm x 60 cm to give a plant population of 27,777 plants / ha and a net plot size of 0.6 m x 0.6 m (0.36 m^2) were maintained and set aside for data collection.

Data, based on four tagged plants, were collected on number of leaves per plant at two, four and six weeks after planting (WAP), vine length per plant at two, four and six weeks after planting (WAP), number of branches at two, four and six weeks after planting (WAP), number of days to flowering initiation, number of days to 50 % flowering number, number of male flowers, number of female flowers and mean fruit number.

Data were subjected to analysis of variance (ANOVA) and significant means were separated using L.S.D at 5% probability level,

variance components of vegetative and reproductive trait for both exotic and local cultivars.

Results and Discussions

The result of characterization studies in the cucumber cultivars are presented in Table 1. The data on morphological traits for means value of four cultivars of *C. sativus* is presented in table 1. Characters assessed included the vine length per plant, number of leaves per plant, number of branches per plant, number of days to 1st flowering, number of days to 50 % flowering, number of male flowers, number of female flowers and mean fruit number. The length of vines per plant at two WAP ranged from 4.62 cm in Tablegreen 72 to 6.77 cm in Ex – Eket. There was increased with time in Ex – Eket, producing the longest vines (71 cm) at six WAP. However, there was no significant difference in length of vine among the cultivars except at six weeks. The number of leaves per plant produced at 2 weeks after planting (WAP) ranged from 4.0 in Tablgreen 72 to 7.33 in Ex - Calabar, the number of leaves per plant produced by the cucumber cultivars was not significantly different ($p \geq 0.05$) at four WAP expect two and six WAP, Ex – Eket producing the highest number of leaves (122.33) at six WAP. There was significant difference in the leaf number among the cultivars for two and six WAP. This result agrees with the reports of Adjoumani *et al.*, 2016, Agah and Ittah 2018 that observed no significant difference for length of vine, number of leaves per plant and number of branches per plant for cucumber and watermelon. The number of branches per plant at two, four and six WAP ranged from 2 in Ex – Eket, to 13.67 in Ex – Calabar. The indigenous cultivars producing the highest number of branches 13.67 at six WAP. However, there were varietal similarity in Markmore 76 and Tablegeen 72 for number of branches at two WAP. The result also agrees with the report of Afangideh *et al.,* 2005 that observed significant differences for number of branches in cucumber. The higher number of branches, number of leaves and length of vine observed among the different cultivars could increase the rate of photosynthetic activities in growth stage and yield stages in the crop. This observation is similar to report by Adjoumani *et al.*, 2016 that noted significant difference in indigenous cultivars and exotic cultivars on cucumber for vine length, number of branches, leaves and mean fruits number. This result also agrees with Afangideh *et al.*, 2005 that observed longer vine in exotic cucumber. There could be an indication of genetic similarity among exotic cultivars evaluated for number of branches at two WAP for Matkmore 76 (2.33) and Tablegreen 72 (2.33). There was significant difference ($p \leq 0.05$) in reproductive characters such as number of days to 1st flowering, number of days to 50 % flowering, number of male flowers and mean fruit number.

Markmore 76 had the lowest number of days to 1st flowering initiation (28.67) while Ex – Eket producing the highest number of days to 1st flowering of (58.67). Days to 50% flowering per plant ranged from 36 days in Markmore 76 to 68.33 days in Ex – Calabar that produced flowers earliest to 68.33 days than others cultivars (Table 1). There were varietal differences for number of days to 50 % flowering. This result agrees with Adjoumani, *et al.*, 2016 and Afangideh *et al.*, 2005 that observed significant difference in days to 50 % flowering of cucumber. Number of male flowers ranged from 3.33 in Markmore 76 to 7.67 in Ex – Calabar. The indigenous cultivars producing the highest number of pollens than exotic cultivars. Among the reproductive attributes measured only number of female flowers per plant is not significant among cultivars evaluated. However, there was increased in number of female flowers from 5.94 in Tablegreen 72 to 3.42 in Ex – Eket. The exotic cultivar producing higher number of flowers per plant whiles the local cultivars producing lowest number of female flowers. There were significant differences for mean fruit number among cultivars studied. The exotic cultivar Markmore 76 producing the highest mean fruit number (8.50 t /ha) and Ex – Eket producing the lowest mean fruit number of

(3.67 t /ha). This report was at variance with Adjoumani, *et al.*, 2016 and Afangideh *et al.,* 2005 that observed higher mean fruit number in indigenous cultivars than exotic cultivars. It appears from this study that branching was more profuse in the exotic cultivars and this could be due to apical dominance in local cultivars. Apical dominance is generally associated with vine length in vegetable like pumpkin, muskmelon, watermelon, snake gourd and cucumber. In this study, the local cultivars had longer vines, lower yield than the exotic cultivars. The higher yields of the exotic cultivars could be due to increase number of branches which resulted in more fruits (Table 1). This would agrees with the reports by Adjoumani, *et al.*, and 2016 Afangideh *et al.,* 2005 that noted high positive and significant correlation between fruit number per plant and yield. This could be as a result of superior performance of Markmore 76 is attributed to longer vine, higher number of branches, leaves and yield observed in exotic cultivars.

When comparing L.S.D values with the mean value of the four cultivars, the results indicate that number of leaves at 2wks, number of branches at 2wks, number of branches at 4wks, number of branches at 6wks, number of days to 1st flowering, number of days to 50% flowering, number of male flowers and mean fruit number value were greater than L.S.D values in the four cultivars. Only vine length at 6wks, in Tablegreen and Ex-Calabar, number of leaves at 6wks, in the two exotic an Eket Local had L.S.D value which were greater than the mean values as presented in table 1. This could be an indication that there are enough differences between the cultivars to suggest that hybridization among the exotic and indigenous cultivars and subsequent progeny selection over generation might result in improved cucumber varieties.

Table 1: Means of vegetative and reproductive attributes for exotic and local cultivars of *C.sativus*

A / C	VL 2 WAP	VL 4 WAP	VL 6 WAP	NL 2 WAP	NL 4 WAP	NL 6 WAP	NB 2 WAP	NB 4 WAP	NB 6 WAP	DF	D50% F	MF	FF	MFN
Markmore 76	5.47	50.78	52.11	4.67	17.10	50.00	2.33	9.33	10.67	28.67	36.00	3.33	5.14	8.50
Tablegreen 72	4.62	20.62	17.83	4.00	10.33	17.83	2.33	10.33	11.33	26.33	37.00	3.50	5.94	6.83
Ex- Eket	6.77	55.80	71.00	4.08	11.50	37.52	2.00	6.00	9.00	58.67	66.33	6.00	3.42	3.67
Ex - Calabar	6.67	31.50	35.33	7.33	24.33	122.33	3.00	8.67	13.67	46.67	68.33	7.67	5.42	4.17
L,S.D	NS	NS	35.86	1.36	NS	7082	0.66	2.68	2.68	7.99	4.54	2.79	NS	2.63

Key: A = Attributes, C = Cultivars, L.S.D. = Least significant different, VL = Vine length, WAP = Week after planting, NL = Number of leaves, NB = Number of branches, DF = Days to 1st flower initiation, D 50 % F = Number of days to 50 % flowering, MF = Number of male flowers, FF = Number of female flowers , MFN = Mean fruit number

Conclusions, Summary and Recommendations

A field experiment conducted at University of Calabar teaching and research farm in 2005 cropping season to characterized two exotic and two local cultivars of *Cucumis sativus* in a randomized complete block designed (RCBD) with three replications. Most agronomic traits showed some variations among the cultivars tested. The indigenous cultivars produced high number of branches , leaves , pollen (male flowers) and flowers earlier that exotic cultivars which produced low number leaves, branches, pollen but high yield and early fruiting. Genetic similarity existed between exotic cultivars in number of leaves at two weeks after planting. The exotic cultivars fruited earlier than the indigenous cultivars. The exotic cultivars (Markmore 76) with high performance of (8.50 t /ha) for mean fruit number than Ex – Eket (3.67 t / ha) and other varieties is recommended for cultivation in the humid agro – ecological Zone.

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CGBPB 018

SCREENING OF RICE GENOTYPE FOR BLAST RESISTANCE

Usman A.[1], G. G. Mowobi [1*], M. Y. Yeye[1], M. Yusuf[1] and O. Alabi[1]

[1]Institute for Agricultural Research Samaru, Ahmadu Bello University Zaria
Corresponding Author: mowobig@gmail.com +2348068672841, +2348056155336)

ABSTRACT

*Rice blast rice blast (Magnaporthe oryzae) is one the most important destructive disease of rice that can lead to 80% yield lost under severe condition. In view of this, the present study was conducted to identify sources of resistance to blast in rice. Four rice genotype was screened to determine their status for rice blast disease at Institute for Agricultural Research Samaru, Ahmadu Bello University Zaria. The genotypes were evaluated in completely randomized design with 3 replications in the screen house of the Department of Crop Protection, Institute for Agricultural Research Samaru in 2019. Data were collected on plant height, number of plant infected with blast, Seedling vigour, tillering ability, blast disease score and leaf blast estimated. Analysis of variance computed showed highly significant difference ($P \leq 0.01$) between the genotypes for seedling vigour (0.03**) and disease index (17.24**) while significant ($P \leq 0.05$) variation between the genotypes was observed for number of leaf (3.79*). In contrast, there was no significant ($P > 0.05$) variation noted for plant height, and tillering ability. The highest PCV and GCV values were computed for leaf blast the highest broad sense heritability was also computed for leaf blast. IRAT 109 with blast disease computed value of 0.6 depicted a high resistance to blast, JAMILA was moderately susceptible (Blast score 4.0) while FARO 52 with blast value of 7.3 and FARO 66 with blast value of 6.1 was susceptible. This significant difference observed among the genotype implies that there may be sufficient variation among the genotype screened which suggest that progress can be made following selection.*

Keywords: *Genotype, Resistance, Disease, Blast, Screening*

Introduction

Rice (*Oryza sitiva*) with genome size of 430mb (2n = 24) is the most widely consumed staple food for a large part of the world's human population (Perera and Dahanayake, 2016; Amanullah *et al.*, 2016). It may have originated in southern India and now cultivated all over the world (Amanullah *et al.*, 2016). In Africa, a total of 14.2 million hectares of land area was cultivated in 2018 with 33.2 million tons harvested with productivity of 2.3 ton/ha; Nigeria had 6.8 million metric tons paddy production on a land area of 3.3 million hectares and a productivity of 2.03 tons/ha (FAOSTAT, 2018). Rice is an important staple food crop for more than half of the world population and it provides 27% of the calories in low and middle income countries (Patil and Sharanagouda, 2017).

Despite rice being the main food for more than half of the world's population, yield loss poss a major threat to food security. Even though the world rice production increases from 257 million tons in 1966 to 782 million tons in 2018 (FAOSTAT, 2018), the increase has not kept up with the demand for rice because of the corresponding increase in human population. It is stated that rice production must increase by 40% in 2030 to meet the ever increasing demand (Khush *et al.,* 2001). Hence population increasing at an alarming rate, making the food security the major challenge in the future.

Disease are among the most important limiting factors that affect rice production. More than 70 disease carried by fungi, bacterial, viruses or nematodes have been reported on rice and in severe cases, these

losses could be up to 70-80% in some rice ecosystems (Deepak and Prasanta, 2017). Among the biotic stress, blast disease is the most important. Since there have been several rice blast outbreak, effort have been made to develop new resistant varieties of rice. Because the disease is polygenic, it is highly influenced by environment. The continuous studies on blast is important in order to overcome this disease problem there by sustaining rice production in the future. The objectives of this studies is to identify sources of resistance to blast in rice.

Material and Method

The research was conducted at the screen house of the Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria, Kaduna State, located in Samaru on latitude $11^0$11'N, longitude 7^0 38'E and 686 m above sea level in the Northern Guinea Savannah Ecological Zone of Nigeria. The average annual rain fall of the area is about 1,058 mm which is distributed within 160 days (Olanuga, 1979).

Genetic Materials: The genetic materials comprises of 4 rice genotypes, IRAT 109, FARO 52, and FARO 66 where obtained from African Rice (IITA, Ibadan, Nigeria) while JAMILA was obtained from the Zaria. IRAT 109 is resistant to blast with maturity day of 90 – 100 days, FARO 52 is highly susceptible to blast with maturity of 100 – 110 days, FARO 66 is also blast susceptible and matures at 100 – 110 and JAMILA is also a blast susceptible genotype.

Fungal Isolation: Plant samples infected with blast were collected from a rice field in Dogarawa, Bomo Village, and Samaru Kaduna State, Nigeria. Diseased leaves and nodes of rice panicles were placed on wet filter papers in a Petri-dish for sporulation.
Media: Potato Dextrose Agar with streptomycin (PDAs) growth media was used. 200 g of sliced peeled potatoes was weighed in 1 liter of water and boiled for 30 minutes. It was then filtered out, 20g of agar agar and 20g of Dextrose were then weighed into the solution and mixed well. Media was then autoclaved at 121°C for 15 minutes and left to cool to about 40^0C. It was then dispensed into sterile Petri dishes with a diameter of 9 cm.
Leaf Preparation: Infected leaf samples were cut into small portion and sodium hypochlorite was added to it for 3 min and rinsed 3 times with distilled water.
Culturing: Leaf sample were placed on the media in a petri-dish in the micro flow chamber, before been taken to an incubator for observation of blast and viewed under microscope (7-14 days).

Inoculation: Inoculum was harvested from the cultured plate and blended (mixed) in a 200 ml of water and solution was sieved using muslim cloth. River sand was sieved and sterilized using oven. It was then used to create injury (rubbing) on the surface of the leaf to aid proper penetration of the inoculum. The inoculum was sprayed on the surface of the plant leaf and the residue was also used to inoculate the soil in the screen house. The strength of the inoculum was determine using hemocytometer.

Spore Storage: The cultured pathogen was sub-cultured into McCartney bottle using sterile picking pin. It was then kept for further use in other not to lose the pathogen.

Screening Of Genetic Material: The 4 genetic materials were screened for blast disease during the dry season of 2019 (February – March). The genotypes were raised in completely randomized design with 3 replications in the screen house of the Department of Crop Protection IAR in a pot of 14 cm diameter wide and 12 cm deep. The material were planted in 2 rows of 5 pots each. FARO 52 Plants were used as spreaders and inoculated with conidia harvested from mycelia of a *Magnaporthe oryzae* isolate. The test material (IRAT 109) was surrounded by 6 stand of susceptible rice cultivar as spreader rows. At the fourth-leaf stage (3-4 weeks after sowing), the seedlings were sprayed with spores of *Magnaporthe oryzae* and about 30 - 40 ml of the spore suspension of the blast pathogen, soil inoculation was done

alongside the leaf inoculation. Water was sprayed 3 - 4 times a day to maintain high humidity. Inoculated seedlings were monitored for the development of blast lesions. The disease reaction of each genotype was recorded after 30 days of inoculation, following standard 0 - 9 scale (SES IRRI, 2013) Table 1. Data were collected on plant height (cm), seedling vigor (scale of 1-9, tillering ability (1-9), number of leaves affected and leaf blast (1-9) as described by Standard Evaluation System of International Rice Research Institute (SES IRRI, 2013).

Statistical Analysis: The data collected was subjected to analysis of variance (ANOVA) using General Linear Model procedure of Statistical Analysis System (SAS, 2002) and where there is significant difference between treatment means, Fisher's protected Least significant difference (LSD) test was used for comparison.

RCBD linear model: $y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$

Where,

y_{ijk} = Responds of the experimental i^{th} treatment unit with the j^{th} replicate and k^{th} block.

μ = The overall mean

α_i = Effect of treatment

β_j = The effect of block j

e_{ijk} = Random error

i = Number treatment unit; j = Number of replication; k = Number of block.

Table 1. Description of the Standard Evaluation System Scale for rice blast disease Scoring

Grade	Disease Severity	Host response
0		Highly Resistant
1	Small brown specks of pin point size	Resistant
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin Lesions are mostly found on the lower leaves	Moderately resistant
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately resistant
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions of 3mm or longer infecting 4 10% of the leaf area	Moderately Susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly Susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible

Source: (SES IRRI, 2013)

Result

Analysis of variance: The result of the analysis of variance is presented in Table 2. The result revealed highly significant (P < 0.01) variation for seedling vigour, number of infected leaves and leaf blast and no significant (P< 0.05) difference was depicted by plant height and tillering ability.

Mean performance of genotypes: Plant height has a mean of 25.38 and ranged from 23.88 - 29.25, seedling vigour has a mean of 0.90 and ranged from 0.8 – 2.0, tillering ability has a mean of 10.30 and ranged from 6.10 - 1 2.30, number of leaf has a mean of 2.26 and ranged from 2.10 – 3.60 while leaf blast has a mean of 4.50 and ranged from 0.60 - 7.30.

The range and mean performance of the 4 genotypes for the 5 characters studied are presented in Table 4. The result showed presence of significance difference for the traits studied at 5% probability level that was further confirm by mean comparison test using the respective LSD values. The mean performance indicated the different response to the blast as there was variation.

Estimated variance Component: The result of the estimated variance component (Table 3), genotypic coefficient of variation (GCV), phenotypic coefficients variation (PCV) and broad sense heritability (Hb) were the 5 traits presented in Table 4. The PCV value computed for the 5 traits ranged from 12.83 for seedling vigour to 65.24 for leaf blast while GCV ranged from 5.93 tillering ability to 64.88 leaf blast. The value of phenotypic coefficient of variation were generally slightly higher than the corresponding genotypic coefficient of variation for all traits studied. High GCV was observed for leaf blast and number of leaf while moderate GCV was observed for seedling vigour. High PCV was observed for leaf blast and number of leaf while tillering ability, seedling vigour and plant height showed moderate PCV.

Heritability in broad sense: Broad sense heritability (Hb), which is an estimate of the total contribution of genetic variance to total phenotypic variance ranged from 4.52 tillering ability to 98.88 leaf blast. The heritability was high for leaf blast, number of leaf and seedling vigour which might be due to environmental influence on the expression of the traits. Low heritability in broad sense was observed for plant height and tillering ability (Table 3).

Table 2: Anova Table of Rice Genotype for Blast Fungus

Source	Degree of Freedom	Plant Height	Seedling Vigour	Tillering Ability	Number of Leaves Affected	Leaf Blast
Rep	1	1.98	0.01**	9.68	0.02	0.02
Genotype	3	18.52	0.03**	16.52	3.79*	17.24**
Error	3	13.44	0.01	15.77	0.22	0.19

**: highly significance difference at (P 0.01) probability level, * significance difference at (P 0.05) probability level,

Table 3: Variance component of Rice Genotype for Blast Fungus

	Variance Component					
Traits	σ_e^2	σ_g^2	σ_p^2	GCV %	PCV %	Hb
Plant Height	13.44	2.54	11.80	6.28	13.54	21.54
Seedling Vigour	0.01	0.01	0.01	10.14	12.83	62.50
Tillering Ability	15.77	0.37	8.26	5.93	27.90	4.52
Number of Leaves Affected	0.22	1.79	1.90	59.41	61.21	94.20
Leaf Blast	0.19	8.52	8.62	64.88	65.24	98.88

Table 4: Mean Performance of Rice Genotype for Blast Fungus

Traits	Genotype	Mean	Range	CV%
Plant Height	FARO 52	23.93	23.88-29.25	16.96
	FARO 66	23.88		
	IRAT 109	24.47		
	JAMILA	29.25		
	Mean	25.38		
	LSD	13.7		
Seedling Vigour	FARO 52	0.80	0.8-1	0.01
	FARO 66	0.80		
	IRAT 109	1.00		
	JAMILA	1.00		
	Mean	0.90		
	LSD	0.00		
Tillering Ability	FARO 52	6.10	6.10 - 12.30	38.56
	FARO 66	12.30		
	IRAT 109	12.00		
	JAMILA	10.80		
	Mean	10.30		
	LSD	12.64		
Number of Leaves Affected	FARO 52	3.60	2.10-3.60	20.85
	FARO 66	2.90		
	IRAT 109	2.40		
	JAMILA	2.10		
	Mean	2.26		
	LSD	1.49		
Leaf Blast	FARO 52	7.30	0.60-7.30	9.77
	FARO 66	6.10		
	IRAT 109	0.60		
	JAMILA	4.00		
	Mean	4.50		
	LSD	1.40		

Discussion

The national average productivity of rice is still very low as compared to other rice producing countries in the world. This is mainly due to insufficient improved rice varieties, disease and other environmental factors affecting rice productivity. As a result of this, the present study accounted to screen 4 rice genotypes for resistance to rice blast and pattern of genetic variance present in the rice genotype. The presence of highly significant among the genotype for all characters except for tillering ability and plant height that were non-significant implies the presence of considerable variation among the genotype. Observations recorded after 30-40 days after sawing based on leaf blast severity following SES 2013 scale showed that IRAT 109 is highly resistant and this was visible both in growth and vigour. The resistant ability of these genotypes may be genetics as it suppresses development of the organism causing these diseases. Although FARO 66 is susceptible, it was less susceptible when compare to FARO 52 which is the most susceptible among the four genotype and the fungus was very visible at 40 days after sawing on the leaves, while JAMILA was moderately susceptible. The different response of the rice genotypes used in this study is important in the selection of resistant varieties. These findings inspire carrying out further genetic studies to improve the genotypes through hybridization and selection programs. This result agrees with Spyridon *et al.* (2009), who reported that varietal differences significantly contributed to the resistance or susceptibility of the rice to leaf blast and also in line with the work of Gbadeyan et al. (2018) who worked on screening of blast and genotype by environment interaction of rice.

In general high heritability was observed for leaf blast, number of leaves and seedling vigour. It suggest that selection base on these characters would be effective for future crossing programme. This result agrees with the work of Tuhina-Khatun *et al.* 2015 and Kamara *et al.* 2017 who also reported high broad sense heritability in rice. As explained above, the GCV values were relatively lesser than PCV for all traits, however, the magnitude of the difference between the PCV and GCV was relatively low for tillering ability, plant height and vegetative vigour. This implies that the marked influence of environmental factor for the phenotypic expression of genotype was low; therefore there is higher chance of improving this traits through selection based on the phenotypic value of the traits. On the other hand, the difference in magnitude between the PCV and the GCV values were relatively high for number of leaf and leaf blast (Table 4) (Ikramullah et al., 2011)..

High heritability estimate was observed for seedling vigour, number of leaf and leaf blast. The high estimated heritability value for this traits indicated that the variation observed was mainly under genetic control and was less influenced by the environment hence, the possibility of progress from selection. This may be attributed to the uniform environment in the screen house (Muhder *et al.,* 2020).

Conclusion

The study highlighted presence of significant genetic variations for agronomically important traits (such as seedling vigour, leaf blast and number of leaf) among the 4 rice genotypes. The promising genotypes of IRAT 109 exhibited significantly level of resistance than JAMILA, FARO 52 and FARO 66. Hence, IRAT 109 can be considered as candidates for blast resistant variety for possible progress.

Significance Statement

This study discovered the presence of significant genetic variation among the tested genotypes for the 5 traits considered. The study also highlighted the level of resistance and susceptibility to blast of rice among the tested genotype

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CGBPB 019

CHARACTERIZATION OF COWPEA (*Vigna unguiculata* [L.] Walp.) USING MORPHOLOGICAL TRAITS

[1]Abdullahi W.M., [2]Echekwu., C.A., [2]Oyekunle., M., [2]Mohammed., M. S., [1]Togola., A., Oyatomi O., [3]Lawan Z. M., [2] Ahmed, H.O., [3]Mohammed, S.G and [1]Boukar, O.

International Institute of Tropical Agriculture, Kano Station.
Institute for Agricultural Research Samaru, Ahmadu Bello University Zaria.
Department of Agronomy, Bayero University, Kano.
Corresponding Author:aswaj@yahoo.com

ABSTRACT

Knowledge of genetic diversity among cowpea accessions is important for the preservation of local varieties and will be a basis for the development of improved varieties. Screenhouse experiment was conducted at the International Institute of Tropical Agriculture (IITA) Kano Station in 2020 to estimate the phenotypic variation among cowpea accessions. Highly significant (P≤0.01) difference was detected for all tested traits among the accessions. Principal Component Analysis (PC) showed that 92% of the total variability among tested accessions which were due to seed colour, seed shape and seed size contributed mainly to PC1. Leaf colour, leaf area, growth habit and growth pattern contributed to PC2. Cluster analysis of the phenotypic traits resulted in five distinct groups. Among the accessions, TVu-10033, TVu-16510, TVu-16545 and TVU-12470 were associated with desirable seed characteristics. TVu-3717 and TVu-16467 were identified to possess good vegetative traits. The phenotypic traits therefore provide a useful measure of genetic distances among the cowpea accessions and thus, will enable the identification of potential parental materials for future breeding programme.

Keywords: *Accessions, growth habit, seed size, variability and Principal Component Analysis.*

Introduction

Cowpea, *Vigna unguiculata* (L.), is an important grain legume grown in the tropics where it constitutes a valuable source of protein in the diets of millions of people as well as soil fertility enhancement following N fixation (Boukar *et al.*, 18). All the evidences indicate that cowpea originated in Africa but the exact place of domestication is uncertain. Ethiopia, Central Africa, South and West Africa all have been considered as probable centers of domestication as reviewed by Ng and Marechal (1985). According to Simmonds (1976) West Africa and India both are modern centers of diversity for this crop. The centre of maximum diversity of cultivated cowpeas and land races is found in West Africa in a region comprising the Sudan savannah zone of Nigeria. Collection, characterization and evaluation of available cowpea germplasm, quantification of the magnitude of diversity and classification into groups facilitate identification of genetic variability that enables breeders to select traits of interest for an improvement programme. Information on the nature and degree of genetic diversity would assist plant breeders in choosing the best genotypes as parents for

hybridization (Souza and Sorrells 1991). Therefore, the objectives of the study were to (1) assess the extent of genetic diversity among cowpea

genotypes using phenotypic traits (2) study the magnitude of association between vegetative growth and seed traits and (3) select superior lines with desirable agronomic traits.

Due to the often large number of accessions in gene banks, many of them are typically not utilized for crop improvement. It is reasonable to believe that the cowpea germplasm lines have remained in West and Central Africa sub-region over a long period after having been domesticated there. The plants have become adapted to the agro-ecologies prevalent in the sub-region rather than countries especially since a common farming system of intercropping is mostly practised in the different countries.

Materials and methods

Plant material and study site

Cowpea accessions were obtained from gene bank of International Institute of Tropical Agriculture, (IITA) Ibadan. The genotypes names are given in Table 1. A screenhouse experiment was conducted at the Kano Station. Augmented design 9 x 9 with 2 replications was done in the screenhouse to phenotype 33 cowpea accessions with 1 check during the 2020 off-season (April-July 2020). Plastic pots 20.4cm length and depth of 23cm were filled with sterilized sieved sand and top soil (sandy loam). Three seeds each of the accessions and checks were sown in pots filled with 6kg soil. The seeds were sown at uniform depth in holes made with the help of the thumb and kept in a greenhouse.

Data Collected

The phenotypic traits evaluated were measured using the International Board for Plant Genetic Resources descriptors for cowpea (IBPGR, 1983). To avoid border effects, five randomly selected plants were taken from each plot in the center row for the measurement of the phenotypic traits. The selected plants were tagged at an early growth stage. Pod length and number of seeds per

pod were recorded using 10 pods from randomly selected plants. A data was subjected to SAS (Statistical Analysis System, SAS Institute, N.C) Version 9.4.

Results

Genetic variation

Analysis of variance revealed highly significant differences for all phenotypic traits among the 33 cowpea genotypes studied indicating a high level of genetic variation. This high variation revealed a large scope for breeding and provided the necessary genetic information for the selection of useful traits for use in the cowpea improvement programme. The genetic and phenotypic variances further indicated inherent genetic variability among different cowpea genotypes.

All accessions studied had determinate growth pattern with diverse growth habit from Acute erect, erect, semi erect prostate and climbing. Seed from India were white and brown with rhomboid shape and intermediate seed texture with dark and intermediate green leaf colour. The erect accessions have been reported to have potential for good returns in high intercrop adaptability and high reproductive efficiency (Cobbinah *et al.*, 2011). For seeds from Togo 88% are white seeded and kidney shaped (Table. 1) while 13% had brown seed colour. For accessions from USA 60% had white seed while others were brown with kidney and rhomboid shapes. For seed texture 48% had intermediate, 39% has smooth texture while the rough seed were 13%. For leaf colour 55% had dark green leaf colour, 33% had intermediate green leaf colour and 12% with pale green leaf colour. For number of branches the lowest was 4 while the highest was 17 and this goes with Abe *et al.,* (2015) who reported 10 as the mean number of branches in cowpea. Over 67% of seed from Cameroun

were white and the remaining were Brown and Variegated.

The value for number of branches ranged from 4.00 to 17 with an overall mean 7.89

(Table 2). The highest and lowest numbers of branches were obtained from accessions TVu-7605 and TVu-4 and TVu-16545 respectively. The highest leaf area were recorded in TVu 3718 and TVu-7598 (101 and 100), whereas the lowest was in TVu-16393. The leaf area recorded in this study was lower than the values reported by Idahosa et al. (2010) in Nigeria.

The largest seed area was found TVu-12470 and TVu-9321 with 60.85 and 59.37 while the lowest was in TVu 3717. Hundred seed weight ranged from 3-23g (Table. 2) and this is harmony with the work of Abe *et al.* (2015) who reported 13g as the mean hundred seed weight in South Africa. These values were within the range of what Egbe et al. (2010) reported in Nigeria, but higher than those reported by Idahosa et al. (2010). Shoot and dry weight was highest in TVu-3818 (30.00) while the lowest was TVu-8191 (0.04). Root dry weight ranged from 0.04-5.54

Correlation

The simple correlations between each pair of phenotypic traits clearly depicted the close association between some of the traits. Selection of associated traits can be used to improve important traits of interest. Strong correlation was observed between pod length and number of seeds per pod. Number of branches had a moderate positive correlation with leaf area, seed area, hundrend seed weight, shoot dry weight and root dry weight. Leaf area showed a moderate negative correlation with hundrend seed weight and a moderate positive correlation with shoot and root dry weight. Furthermore, seed area had a strong positive correlation with hundrend seed weight, shoot and root dry weight and a weak negative correlation with number of branches. Shoot dry weight was moderately and positively correlated with seed area and root dry weight.

Principal component Analysis

In general, the degree of genetic diversity tends to have a positive correlation with the number of countries from which the accessions were collected. Which suggested that cowpea came to the USA through slaves who may have brought them along from West Africa. We also observed that some USA lines are in close proximity with accessions from India. Principal components (PCs) indicated about 92.22 % of the total genetic variation. The first PC accounted for the largest Eigen-value and accounted for the greatest amount of variance in the original data 71% and the second 22% accounted for the greatest amount of variation in the residual variation, which was unaccounted for by the first principal axis

The variables with high scores on PC1 were quantitative traits: Seed colour, seed texture, growth habit, growth pattern and leaf colour. The variables with highest scores on PC2 were: numbers of branches, leaf area, seed area, hundred seed weight, shoot dry weight and root dry weight. Hence, the variables with high coefficient in the first and second PCs were considered the most relevant as they explained over half of the total variation.

Hierarchical Clustering

Cluster analysis for phenotypic traits showed a clear demarcation between the cowpea genotypes (Figure. 2) were all the genotypes were distinctly separated from each other and the genotypes with similar phenotypic traits were grouped together. Clustering of genotypes based on their similarity/dissimilarity is valuable for cowpea breeders in that the most important genotypes in the population may be selected from different clusters for improvement of cowpea (Figure. 2)

Conclusions

For improving trait of interest characterizing and evaluation of cowpea germplasm is important. This study has revealed that there is sufficient genetic variability among the accessions studied which can be exploited for use in the cowpea improvement programme. Accessions TVu-3717 and TVu-16467 were identified as possessing vegetative traits and these genotypes could be used as parents when breeding for fodder production. Similarly, accessions TVu-10033, TVu-16510, TVu-16545 and TVU-12470 were associated with desirable seed characteristics and are suitable parental lines for improvement of grain production. These lines are recommended for further evaluation across environments in Nigeria.

Table 1: List of cowpea genotypes evaluated with origin and growth habit

Sn	Origin	Accession	Seed colour	Seed shape	Seed texture	Growth habit	Growth pattern	Leaf colour
1	Cameroun	TVu-8228	White	Rhomboid	Intermediate	Semi-prostrate	Determinate	Intermediate green
2	Cameroun	TVu-10032	White	Rhomboid	Intermediate	Erect	Determinate	Pale Green
3	Cameroun	TVu-10033	White	Rhomboid	Intermediate	Prostrate	Determinate	Pale Green
4	Cameroun	TVu-10037	White	Ovoid	Smooth	Erect	Determinate	Dark green
5	Cameroun	Tvu-16393	Brown	Rhomboid	Smooth	Acute erect	Determinate	Dark green
6	Cameroun	Tvu-16399	Varigated	Rhomboid	Smooth	Erect	Determinate	Intermediate green
7	India	TVu-16467	White	Rhomboid	Intermediate	Erect	Determinate	Dark green
8	India	TVu-16510	Brown	Rhomboid	Intermediate	Acute erect	Determinate	Intermediate green
9	Nigeria	TVu-4	White	Kidney	Rough	Semi-erect	Determinate	Dark green
10	Nigeria	TVu-3710	White	Kidney	Rough	Climbing	Determinate	Dark green
11	Nigeria	TVu-3717	Varigated	Kidney	Smooth	Climbing	Determinate	Pale Green
12	Nigeria	TVu-3718	White	Kidney	Rough	Climbing	Determinate	Dark green
13	Nigeria	TVu-3818	White	Kidney	Rough	Erect	Determinate	Dark green
14	Nigeria	TVu-4275	Varigated	Kidney	Smooth	Climbing	Determinate	Dark green
15	Nigeria	TVu-7456	White	Ovoid	Intermediate	Semi-erect	Determinate	Dark green
16	Nigeria	TVu-12415	Varigated	Ovoid	Smooth	Prostrate	Determinate	Intermediate green
17	Nigeria	TVu-12470	Brown	Ovoid	Smooth	Semi-erect	Determinate	Pale Green
18	Nigeria	TVu-16545	Brown	Kidney	Smooth	Climbing	Determinate	Intermediate green
19	Nigeria	IT07K-297-13	White	Rhomboid	Intermediate	Erect	Determinate	Dark green
20	Nigeria	IT08K-150-12	White	Kidney	Intermediate	Erect	Determinate	Dark green
21	Togo	TVu-7509	White	Rhomboid	Intermediate	Erect	Determinate	Dark green
22	Togo	TVu-7560	White	Kidney	Intermediate	Prostrate	Determinate	Intermediate green
23	Togo	TVu-7562	White	Kidney	Intermediate	Erect	Determinate	Dark green
24	Togo	TVu-7566	White	Kidney	Intermediate	Acute erect	Determinate	Dark green
25	Togo	TVu-8131	Brown	Kidney	Smooth	Climbing	Determinate	Intermediate green

26	Togo	TVu-8182	White	Kidney	Smooth	Prostrate	Determinate	Dark green
27	Togo	TVu-8191	White	Kidney	Intermediate	Erect	Determinate	Dark green
28	Togo	TVu-9321	White	Rhomboid	Intermediate	Acute erect	Determinate	Intermediate green
29	USA	TVu-7598	White	Kidney	Intermediate	Semi-erect	Determinate	Intermediate green
30	USA	TVu-7605	White	Kidney	Intermediate	Climbing	Determinate	Intermediate green
31	USA	TVu-8016	Brown	Rhomboid	Smooth	Acute erect	Determinate	Dark green
32	USA	TVu-9201	Brown	Rhomboid	Smooth	Erect	Determinate	Intermediate green
33	USA	TVu-11960	Brown	Kidney	Smooth	Climbing	Determinate	Dark green

Table. 2 showing mean performance of growth traits in 33 cowpea genotypes

Sn	Accession	Number of branches	Leaf area	Seed area	Hundred seed weight	Shoot dry weight	Root dry weight
1	TVu-4	4.00	54.00	38.45	15.00	9.00	2.61
2	TVu-3710	9.00	74.20	40.89	14.00	12.00	0.59
3	TVu-3717	11.00	29.28	22.69	3.00	11.00	0.53
4	TVu-3718	6.00	101.26	37.53	12.00	23.00	1.80
5	TVu-3818	7.00	60.00	57.00	17.00	30.00	5.54
6	TVu-4275	10.00	70.00	22.56	5.00	19.00	0.89
7	TVu-7456	6.00	61.20	49.31	17.00	20.00	0.56
8	TVu-7509	5.00	72.00	47.51	13.00	7.00	1.11
9	TVu-7560	6.00	72.00	51.13	20.00	19.00	4.04
10	TVu-7562	5.00	48.60	42.77	14.00	31.00	0.07
11	TVu-7566	9.00	35.75	50.75	16.00	10.00	0.60
12	TVu-7598	9.00	100.00	43.99	10.00	23.00	0.99
13	TVu-7605	17.00	86.45	40.27	14.00	17.00	1.05
14	TVu-8016	6.00	44.00	37.63	16.00	19.00	0.66
15	TVu-8131	9.00	39.00	25.52	9.00	11.00	1.93
16	TVu-8182	6.00	37.80	30.22	9.00	5.00	0.30
17	TVu-8191	8.00	102.00	49.45	15.00	4.00	0.04
18	TVu-8228	13.00	47.00	46.33	14.00	9.00	0.72
19	TVu-9201	11.00	27.45	28.60	11.00	8.00	0.51
20	TVu-9321	5.00	52.20	59.37	20.00	16.00	0.80
21	TVu-10032	10.00	44.00	50.58	23.00	14.00	0.63
22	TVu-10033	8.00	55.20	50.60	14.00	8.00	0.80
23	TVu-10037	9.00	70.35	30.78	12.00	19.00	0.43
24	TVu-11960	10.00	74.10	17.40	4.00	4.00	0.93
25	TVu-12415	10.00	68.00	45.85	18.00	15.00	0.59
26	TVu-12470	5.00	69.30	60.85	22.00	3.00	0.81
27	Tvu-16393	9.00	22.00	31.28	9.00	14.00	0.40
28	Tvu-16399	5.00	54.00	29.05	9.00	14.00	0.44
29	TVu-16467	6.00	93.60	45.70	7.00	26.00	0.38
30	TVu-16510	9.00	37.26	52.26	21.00	19.00	1.47
31	TVu-16545	4.00	40.88	38.37	12.00	13.00	4.20
32	IT07K-297-13	10.00	59.85	61.71	21.00	16.00	2.14
33	IT08K-150-12	5.00	64.05	51.40	19.00	22.00	4.34
Mean	7.939	57.715	40.609	13.788	14.848	1.300	7.939
Se±	0.478	3.766	2.179	0.928	1.211	0.223	0.478

Table. 3 Principal component analysis of quantitative characters in cowpea accessions showing latent vector loading

	Latent vectors				
Traits	1	2	3	4	5
Number of branches	0.01987	-0.01175	0.02485	-0.99264	-0.05044
Leaf area	0.94906	-0.30258	0.07368	0.02233	0.04257
Seed area	0.28334	0.86984	0.13620	0.01866	-0.37971
Hundrend Seed weight	0.07090	0.36719	0.08092	-0.05539	0.91929
Shoot dry weight	0.11635	0.12618	-0.98299	-0.03053	0.02099
Root dry weight	0.00735	0.03079	-0.05089	0.09906	0.07702

Table. 4 showing Pearson correlation for seed and vegetative traits

	Nbrnc	Lfa	SeedA	S100W	Shtwt	Rtwt
Nbrc	1.00000					
Lfa	-0.01362	1.00000				
SeedA	-0.22055	0.18614	1.00000			
S100w	-0.16625	-0.00772	0.85502**	1.00000		
Shtwt	-0.13907	0.20974	0.19216	0.10979	1.00000	
Rtwt	-0.28459	0.01857	0.26772	0.27510	0.31534	1.00000

Key: Nbrnc=Number of branches per plant, Lfa= Leaf are, SeedA= Seed area, S100W= Hundrend seed weight, Shtwt= Shoot dry weight, Rtwt= Root dry weight

Figure. 1 biplot of the Principal component Analysis based on country of origin

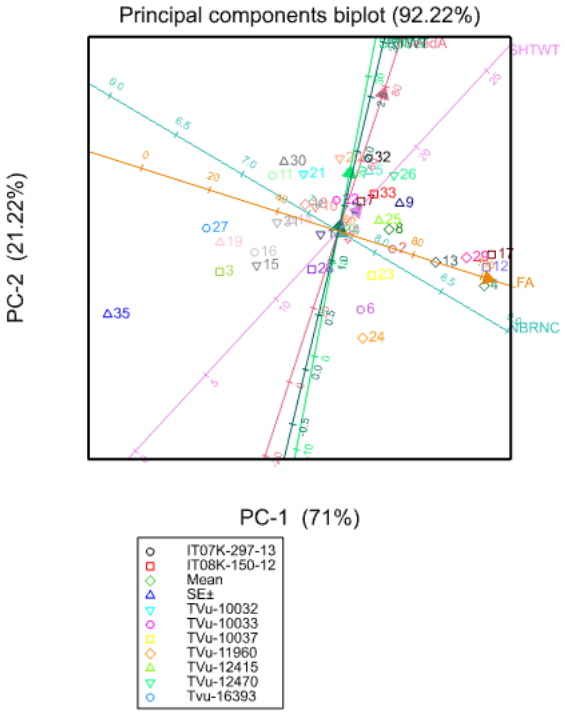
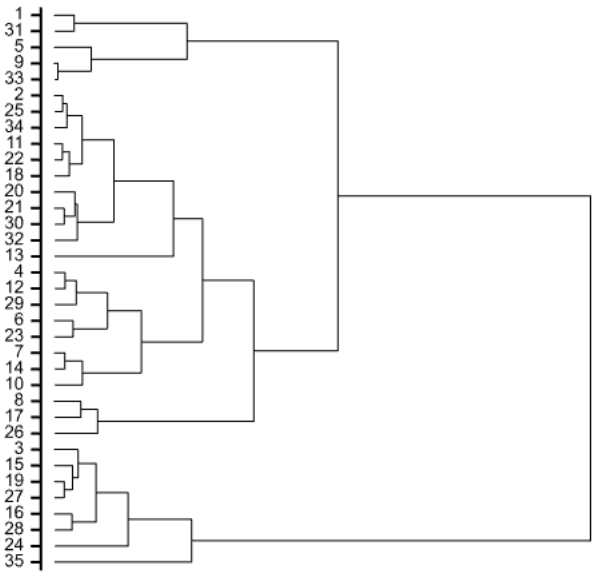


Figure. 2 showing hierachial clustering of the studied traits



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EFFECT OF VARIETIES AND PLANTING DATES ON THE GROWTH AND YIELD OF GREEN BEANS (*PHASEOLUS VULGARIS L.*)

Jibrin, M. S[1]., Abubakar, A[2]., Abubakar, N[2]., Ahmad, Y[2]., Chimdi, G. O.,[3] and Garba, Y[4].

[1]Department of Plant Science, Ahmadu Bello University, Zaria
[2]Department of Horticulture and Agricultural Extension Bauchi State College of Agriculture
[3]Department of Agricultural Technology, Federal Polytechnic, Bauchi, Nigeria.
[4]Department of Crop production, Ibrahim Badamasi Babangida University, Lapai, Niger State
Corresponding author: mohasjibrin@gmail.com +2348063529669

ABSTRACT

The study of the effect of varieties and different planting dates on the growth and yield of green beans was conducted in Bauchi State College of Agriculture greenhouse field during the 2017 rainy season. The factorial treatment consisted of three varieties (Yar-salena, Yar-gora and Gyadan beans) and three different planting dates (17th June, 1st July and 15th July) respectively. The treatment combinations were laid out in a Randomized Complete Block Design (RCBD) with three replications. The data collected on growth and yield parameters were subjected to statistical analysis to test the level of significance among the treatments. Significant differences ($P \leq 0.05$) were observed among the treatments. Effect of variety indicated that Yar-selena recorded the tallest plant (124.9cm) at an advanced stage of growth, while Yar-gora gave the highest number of leaves (22.00) at 3 WAP. On leaf area, Yar-gora and Yar-selena recorded the largest leaf area as compared to Gyadan beans, while the highest value for number of seeds per plant and seed yield per plot was recorded by Yar-gora and Yar-selena respectively. Planting dates showed no much effect on most of the parameters measured. Yar-gora and Yar-selena varieties germinated and flowered earlier as compared to Gyadan beans. Significant positive and negative correlations were observed among the growth and yield parameters measured. Yar-gora and Yar-selena varieties can be adopted as suitable varieties in the study area using early planting. Further study should be conducted to ascertain these findings and make strong recommendations.

Key word: *Green bean, variety, planting date, growth and yield parameters*

Introduction

Green beans (*Phaseolus vulgaris L.*) is one of the most important leguminous vegetable crops in the world. It originated in central and south America. In 2010, China produced about 13 million tons, the second highest producer that same year. Africa is the second most important region, producing about 2.5 million metric tons, with Uganda, Kenya, Rwanda, Burundi, Tanzania and Congo playing major roles (Abdel-Mawgoud *et al.*, 2005). In Nigeria, there is no exact information as to when green beans was first introduced. The crop is cultivated in different major growing areas of the country and in states like Benue, Plateau and Kaduna.
Green beans contain excellent levels of vitamin A, powerful antioxidants that helps to protect against high cholesterol, heart disease and cancer. They also contain good amounts of vitamins and minerals. (www.organicfacts.net and www.towergaden.com).
Despite the health and nutritional benefits of green beans and its high potential in improving

the economic status and the standard of living of smallholder farmers and vendors. Its production is constrained by biotic and abiotic factors; absence of genotype screening for different agro-ecological zones, high postharvest losses as a result of absence of suitable storage and processing facilities and lack of cultural practices, such as appropriate sowing date, plant spacing and nutritional management among others.

Green beans are found to have a lot of health and economic benefits but, information on its cultural practices is scanty and therefore, there is need for such information to encourage its cultivation among our local farmers in Bauchi. In view of the afore-mentioned this study was carried out to determine the suitable variety and sowing date for Bauchi agro-ecology and to study the relationship between growth and yield parameters.

Materials and Methods

The experimental was conducted during the rainy season of 2017 and 2018 at the Bauchi State College of Agriculture Research Farm, situated about 5km south of Bauchi near Abubakar Tafawa Balewa University (A.T.B.U) Yelwa campus. The experimental area is located at longitude $10^{0}28^{1}$ N and latitude $90^{0}51^{1}$ E, 609.5m above the sea level in the northern guinea savannah ecological zone of Nigeria. The total annual rainfall of the area is about 1150-1300 mm per annum (Nimet, 2013). The temperature of the area ranges between 18^{0}C to 35^{0}C, the soil type of the experimental site is sandy loam. A Randomized Complete Block Design (RCBD) with three replication and single row plot of 3m long were used. Three varieties of green beans; Yar gora, Yar selena and Gyadan beans were used for the research.

Data collection and Analysis

Data on growth, yield and yield related agronomic traits: seed germination percentage (%), number of days to 50 % flowering, plant height (cm), leaf area (cm^2), number of branches per plant, pod weight per plant (g), pod weight per plot, number of seed per plant, seed yield per plot (kg) and 100 grain weight (g), were recorded from 5 randomly selected plants. The data collected were subjected to analysis of variance using SAS version 9.0 to test for significance among the treatments. Duncan multiple range test was used to separate the means. Correlation analysis was also conducted to test the relationship between growth and yield parameters.

Results and Discussions

Table 1 presents the effect of variety and planting dates on the days to 50 % germination of green beans. Yar-gora variety had the lowest number of days (4.00 days) to 50 % flowering; followed by Yar-salena (5.00 days) and the longest day to 50 % flowering was recorded by Gyadan beans variety. The effect of planting date showed that planting date of 17th June, 2017 and 1st July 2017 gave the longest days to 50 % germination of 6.00 days each. However 15th July, 2017 had 5.00 days to 50 % germination. The result on days to 50 % flowering, as affected by variety showed that Gyadans beans had the longest days to 50 % flowering (50.00 days) and the shortest days to 50 % flowering (34.00 days) was recorded by Yar-gora. Effect of planting date showed that early planting on 17th June, 2017 had the longest days to flowering of 47 days. While planting of 1st July, 2017 and 15th July, 2017 recorded 36 days and 35 days to 50 % flowering respectively.

TABLE 1: Effect of variety and planting dates on days to 50 percentage (%) germination and Flowering of green beans during 2017 rainy season in Bauchi, Nigeria

Treatments		
Variety	DT 50% germination	DT 50% Flowering
Yar Gora	4.000[c]	34.00[b]
Yar Selena	5.00[b]	35.00[b]
Gyadan Beans	7.00[a]	50.00[a]
Planting date		
17 June 2017	6.00[a]	47.00[a]
1st July, 2017	6.00[a]	36.00[b]
15th July, 2017	5.00[b]	35.00[b]
Variety*Planting date	**	**
CV	7.74	3.26

DT = Days to, CV = coefficient of variation.

Table 2 present the effect of variety and planting date on plant height of green beans at different growth stages. At 3 weeks after planting (WAP) the plant height ranged from 12.9cm to 20.91cm, where Yar-gora variety recorded the tallest plant height followed by Yar-selena and the shortest plant height was recorded by Gyadan beans. At 5WAP Yar-selena recorded the tallest plant height (60.59 cm) which is statistically the same as Yar-gora with 39.48 cm followed by Gyadan beans with 33.37cm plant height. Similarly at 7WAP Yar-selena significantly recorded the tallest plant (124.92 cm) and the shortest plant height (40.07 cm) by Yar-gora which is statistically at par with Gyadan beans with the height of 66.21 cm. The differences recorded on plant height as affected by the variety can be attributed to difference in genotype. Where Yar-gora and Yar-selena consistently recorded taller plants as compared to Gyadan beans across the different growth stages. More so, Yar-gora and Yar-selena are morphologically taller than Gyadan beans that can be attributed to the differences in genotype it also portrays the possibility of their adaptability to the study area. The result here agrees with Yoldas and Esiyok (2017) who reported significant difference among the varieties in their study titled "Effects of Sowing Dates and Cultural Treatments on Growth, Quality and Yield of Processing Beans" .On the contrary Gatechew *et al.*, (2014) reported no significant difference on plant height. However the effect planting date on plant height showed no significant difference except at 3 WAP were 1st July, 2017 had the tallest plant height of 19.29 cm. the shortest plant height was recorded by 17th June, 2017. The difference observed at 3 WAP could be attributed long period for vegetative growth in early planting of 1st July, 2017 as compared to 15th July, 2017. These decreases in plant height could be attributed to a shorter vegetative period (Yoldas and Esiyok 2017). (Gatechew *et al.*, 2014 and Vieira, 1990) reported significant difference on plant height as affected by sowing date.

Table 2: Effect of variety and planting dates on the plant height (cm) of green beans during 2017 rainy season in Bauchi, Nigeria

Treatments	Weeks after planting		
Variety	3	5	7
Yar Gora	20.91[a]	39.48[ab]	40.07[b]
Yar Selena	16.21[b]	60.59[a]	124.92[a]
Gyadan Beans	12.97[c]	33.37[b]	66.21[b]
Planting date			
17 June 2017	16.11[b]	47.35[a]	68.93[a]
1st July, 2017	19.29[a]	48.78[a]	86.78[a]
15th July, 2017	14.69[b]	37.31[a]	75.49[a]
Variety*Planting date	NS	NS	NS
CV	16.74	54.31	70.49

CV = Coefficient of variation

Table 4 shows the effect of variety and planting dates on leaf area of green beans at different growth stages. At 3WAP Yar-gora variety recorded the largest leaf area (30.88cm^2) and the lowest is recorded by Gyadan beans variety with leaf area of 4.52 cm^2. At 5WAP Yar-gora variety also recorded the largest leaf area of 73.86 cm^2 and then followed by Yar-selena variety which recorded the leaf area of 63.44 cm^2 and then the smallest is recorded by Gyadan beans variety with a leaf area of 5.59 cm^2. At 7WAP Yar-selena variety gave the largest leaf area which is statistically at par with Yar-gora variety while the smallest leaf area is recorded by Gyadan beans variety 6.98 cm^2. The significantly larger leaf area of Yar-gora and Yar-selena as compared to Gyadan beans could be attributed to the climbing nature of the former two varieties which gave them ability to trap solar radiation there by causing their leaves to expand.

At 3WAP the planting dates, 17th June, 2017, 1st July, 2017 and 15th July, 2017 had no significant effect on leaf area. At 5WAP statistically significant differences ($P \leq 0.05$) was observed among the planting dates where planting at 17th June, 2017 recorded the highest number of leaf area and the lowest is planting at 1st July, 2017 which recorded the leaf area of 42.22 cm^2. At 7WAP effect of planting dates shows that planting at the three different dates shows no significant effect on leaf area. The leaf area were not significantly affected by the planting date. This can be related to short growth period of crop. The large leaf area recorded at early plating date (17th July, 2017) at 5WAP could be attributed to the fact that at early planting date the crop have access to growth and development (leaf area, taller plants and others). Contrary to the result of this study Gatechew *et al*., (2014) and Singer *et al*., (1996) reported that higher leaf area was recorded for late sowing than early sowing of green beans.

Table 4: effect of variety and planting date on leaf area (cm^2) of green beans during 2017 rainy season in Bauchi Nigeria leaf area

Treatment	Weeks after planting		
Variety	3	5	7
Yar Gora	30.88[a]	73.86[a]	74.46[a]
Yar Selena	28.83[a]	63.44[b]	95.71[a]
Gyadan Beans	4.52[b]	5.59[c]	6.98[b]
Planting			
17th June, 2017	18.91[a]	53.39[a]	74.37[a]
1st July, 2017	24.23[a]	42.22[b]	44.06[a]
15th July, 2017	21.09[a]	47.27[ab]	58.73[a]
Variety*planting date	NS	NS	NS
CV	27.11	18.20	73.47

CV = Coefficient of variation

Table 5 present the effect of variety and planting dates on number of branches of green beans at 3WAP, 5WAP and 7WAP growth stages. At 3WAP the result shows that there is no significantly different on the number of branches. At 5WAP also the number of branches were all statistically the same. However, at 7WAP the number of branches ranged from 22.00 to 36.00 where Yar-selena variety recorded the highest number of branches and Gyadan beans recorded the least number of branches. The differences on the number of branches expressed at the later stage of the crop circle can be attributed to the variance in the varieties. At 3, 5 and 7WAP planting dates had no significant effect the number of branches. This is attributed to short duration of the crop. Seyum (2014) reported that the apparent discrepancy in the number of primary branches between early and late sowing dates could be attributed to the difference in moisture content of the soil; early sowing leading to more moisture availability than late sowing and thus affecting vegetative growth including primary branch development. On the other hand, the difference between the two varieties in terms of primary branch production is probably due to their genetic makeup. Yusufali *et al.* (2006) reported more number of primary branches with an early sowing than late sowing of field beans in Karnataka (India).

Table 5: Effect of variety and planting date on Number of branches of green beans during 2017 rainy season in Bauchi Nigeria leaf area

Treatment	Weeks after planting		
Variety	3	5	7
Yar Gora	5.00[a]	18.00[a]	30.00[ab]
Yar Selena	5.00[a]	19.00[a]	36.00[a]
Gyadan Beans	5.00[a]	13.00[a]	22.00[b]
LSD			
Planting date			
17th June, 2017	4.00[a]	16.00[a]	32.00[a]
1st July, 2017	5.00[a]	18.00[a]	27.00[a]
15th July, 2017	5.00[a]	15.00[a]	29.00[a]
LSD			
VAR*PLD	NS	NS	NS
CV	19.22	38.16	35.44

CV = Coefficient of variation

Number of Pods

The effect of variety and planting on yield and yield components of green beans is presented in Table 6. Effect of variety on number of pods per plants shows that Yar-selena significantly ($p<0.05$) produced the highest number of pods (26.00) that is statistically at per with Yar-gora (25.00) and the lowest number of pods (7.00) was recorded by Gyadan beans. Yar-selena and Yar-gora production of more number of pods as compared to Gyadan beans can be attributed to their growth pattern (tall plants) and early flowering as compared to Gyadan beans. This corroborates with the findings of Dauda et al., (2015) who reported early flowering varieties had more number of pods. Similar result was reported by Anjani et al., (2009).

Utilization growth factors (water, sunlight and soil nutrient) that translate into yield component (number of pods). At the different planting dates, plating on 17th June, 2017 had the highest number of pods (25.00) that is statistically the same as 1st July planting. The lowest pod number was recorded by planting date of 15th July, 2017.

Pod Weight (g)

Table 6 also present the effect of variety on pods weight and it shows that all the varieties were all statistically at per with one another. In contrast to the findings of Dauda *et al.*, (2015) who reported significant difference on effect of varieties on pod weight. The planting dates, also had no significant effect on pod weight.

Number of seeds per pods

Effect of variety on number of seed per plant shows that Yar-selena significantly ($p<0.05$) produced the highest number of seed per plot (71.00) that is statistically at par with Yar-gora variety (53.00) and the lowest number of seed per plot (17.00) was recorded by Gyadan beans variety. Yar-selena produces the more number of seed per plant hence the variety produce the highest number of pod compare to Yar-gora and Gyadan beans. The differences recorded on seed yield per plant can be attributed to differences on number of pod where Yar-selena and Yar-gora consistently recorded the highest number of seed yield. Gatechew et al., (2014) however reported on the contrary.

At the different planting dates, the different planting dates had no any effect on the varieties. Similar result was reported by Gatechew *et al*., (2014).

Seed yield per pods (g)

Effect of variety on seed yield per plot shows that Yar selena variety significantly ($p<0.05$) produced the highest number of seed yield per plot (329.89 g) that is statistically at par with Yar-gora (248.33 g) and the lowest number of seed yield per plot (68.56) was recorded by Gyadan beans. The differences recorded on seed yield per plot can be attributed to differences on number of pod. Where Yar-selena and Yar-gora consistently recorded the highest number of seed yield per plots as compare to gyadan beans.

At the different planting dates, no significant differences among the three planting dates.

100 Grain weight (g)

Effect of variety in 100 grain weight shows that Gyadan beans variety significantly present the highest weight (21.48 g) followed by Yar-selena with the weight of (18.38 g) that is statistically at par with Yar-gora (16.83 g). The weightiest 100 grain weight recorded by Gyadan beans can be related to the big seed size of this variety as compared to the other two varieties.

Also at the different planting dates, no significant difference was observed.

Interaction between varieties and planting dates on days to 50 % flowering is presented in figure 1. The result indicated that Yar-gora and Yar-salena varieties sowed on 15th July, 2017 recorded the shortest days to 50 % germination; while the longest day to 50 % germination was recorded when variety Gyadan beans was planted on 15th July, 2017.

Figure 2 presents the interaction effect between variety and planting date. Yar-gora and Yar- selena variety planted on 15th July 2017 recorded the shortest day to 50 % flowering. The longest day to 50 % flowering was recorded by Gyaden beans variety planted on 15th July, 2017. This can be attributed to their early days to 50 % germination as Yar-gora and Yar-selena varieties germinates earlier than Gyaden beans respectively. Similar trend was observed for the varieties when planted on 17th June, 2017

Table 6: Effect of variety and planting date on yield and yield components of green beans during 2017 rainy season in Bauchi, Nigeria

Treatments					
Variety	NP	PW (g)	NSPP	SYPP (g)	100 GW (g)
Yar Gora	25.00[a]	6.04[a]	53.00[a]	248.33[a]	16.83[b]
Yar Selena	26.00[a]	6.04[a]	71.00[a]	329.89[a]	18.38[b]
Gyadan Beans	7.00[b]	4.21[a]	17.00[b]	68.56[b]	21.48[a]
Planting date					
17th June, 2017	25.00[a]	5.76[a]	47.00[a]	211.56[a]	18.85[a]
1st July, 2017	21.00[a]	4.27[a]	58.00[a]	274[a]	18.27[a]
15th July, 2017	13.41[b]	6.26[a]	35.00[a]	161.22[a]	19.57[a]
Variety*planting date	NS	NS	NS	NS	NS
CV	35.33	109.90	51.45	56.23	12.66

DTFG = Days to 50 % germination, DTFF = Days to 50 % flowering, PHT = plant height, NL = number of leaf, NB = Number of branches, NPPP = Number of pod per plant, PWPP = Pod weight per plant, NSPP = number of seed per plant, SYPP = Seed yield per plant, GW = Grain weight, NS = Non-significant, * = Significant, ** = Highly significant and CV = C

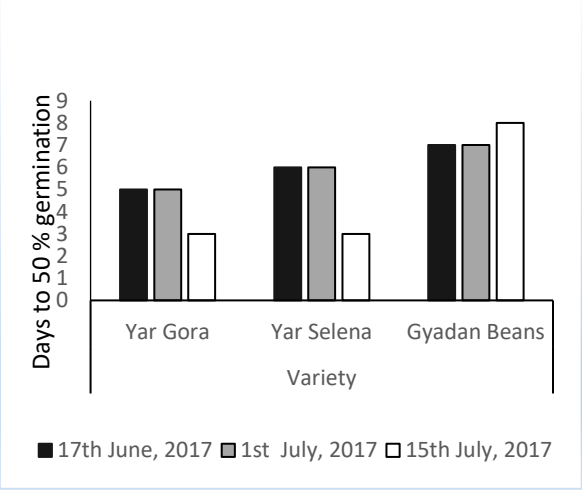

Figure 1: I
date on day
during 2017 rainy season in Bauchi, Nigeria

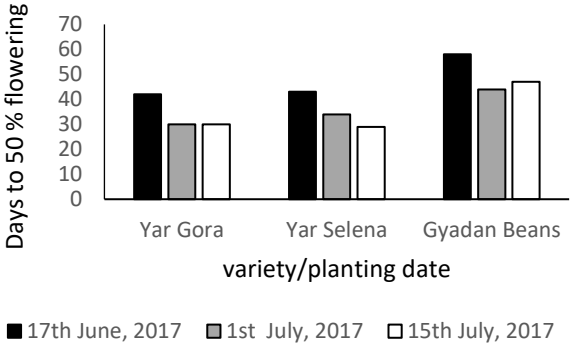

Figure 2: Interaction effect of variety and planting date on days to 50 % flowering of green bean during 2017 rainy season in Bauchi, Nigeria

Correlation coefficient of variation.

The relationship between growth and yield parameters measured is presented in table 9. Days to 50 % germination showed a positive and highly significant correlation with days to 50 % flowering (r = 0.7871**) and 100 grain weight (r = 0.3970**) respectively. The correlation between Days to 50 % germination and (leaf area and number of branches) was negative and highly significant (r = -0.6594** and r = -0.4134**). Days to 50 % flowering correlated positively and highly significant (r = 0.4506**) with 100 GW. The plant was positively and highly significantly correlated with (number of leaf (r = 0.7134**), number of branches (r = 0.4913**) number of seed per plant (r = 0.5785**) and seed yield per plant (r = 0.5531**)). Positive and significant correlation was observed between leaf area and most of the measured parameters, except for 100 grain weight that exhibited negative and highly significant correlation with leaf area (r = -0.5491**). Number of pod per plant had positive and highly significant correlation with number of seed per plant (r = 0.6763**) and seed yield per plot (r = 0.6445**) respectively. Number of seed per plant recorded positive and highly significantly correlation with seed yield per plot (r = 0.9865**).

Correlation coefficient analysis helps to determine the nature and degree of relationship between any two or more measurable characters. An understanding of the direction and extent of association of the component characters with economic yield is an essential prerequisite for formulating best selection strategy (Acquaah 2007).

Summaries and Conclusions

Statistically significant difference ($P<0.05$) were observed among the studied characters. The varieties Yar-salena and Yar-gora performed better as compared to Gyadan beans on most of the measured traits. Yar-gora variety had lowest days to 50% germination (4.00) and the longest days to germination (7.00) by Gyadan beans. Highest pod number (26.00) was recorded by Yar-gora and the lowest (8.00) by Gyadan- beans similar trends was observed on the parameters measured It can be concluded from the results obtained that planting Yar-gora and Yar-selane varieties, varieties early in Bauchi can boast the production and income of local farmers at early planting. Further studies should be carried out to ascertain the result obtain across seasons and locations.

Table 9: Correlation between growth and yield parameters of green beans grown during 2017 rainy season in Bauchi, Nigeria.

	DTFG	DTFF	PHT	NL	LA	NB	NPPP	PWPP	NSPP	SYPP	100 GW
DTFG	1.0000	0.7871**	-0.0818^{NS}	0.2357^{NS}	-0.6594**	-0.4134**	-0.3277^{NS}	0.3296^{NS}	-0.2643^{NS}	-0.2715^{NS}	0.3970**
DTFF		1.0000	-0.1316^{NS}	0.1996^{NS}	-0.6024**	-0.3103^{NS}	-0.3377^{NS}	0.1920^{NS}	-0.3621ns	-0.3671*	0.4506**
PHT			1.0000	0.7134**	0.1129^{NS}	0.4913**	0.1914^{NS}	-0.0925^{NS}	0.5785**	0.5531**	0.0753^{NS}
NL				1.0000	-0.2648^{NS}	0.2822^{NS}	-0.0856^{NS}	0.1280^{NS}	0.2349^{NS}	0.2320^{NS}	0.3337^{NS}
LA					1.0000	0.3706*	0.76891**	-0.2602^{NS}	0.4656**	0.4237**	-0.5491**
NB						1.0000	0.3477^{NS}	-0.1633^{NS}	0.6362**	0.6105**	-0.1714^{NS}
NPPP							1.0000	-0.2106^{NS}	0.6763**	0.6445**	-0.4717**
PWPP								1.0000	-0.2714^{NS}	-0.2628^{NS}	-0.0256^{NS}
NSPP									1.0000	0.9865**	-0.3813**
SYPP										1.0000	-0.4323**
100 GW											1.0000

DTFG = Days to 50 % germination, DTFF = Days to 50 % flowering, PHT = plant height, NL = number of leaf, NB = Number of branches, NPPP = Number of pod per plant, PWPP = Pod weight per plant, NSPP = number of seed per plant, SYPP = Seed yield per plant, GW = Grain weight.

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BREEDING STRATEGIES TO ENHANCE DROUGHT TOLERANCE IN SORGHUM (*Sorghum bicolor* L. Moench): A REVIEW

Jibrin, M.S[1]., Yeye, M[1]., Oyekunle, M[1]., Mohammed, S. M[1]., and Angarawai, I. I.[2]

[1]Institute for Agriculture Research (IAR), Ahmadu Bello University
[2]International Crop Research Institute for Semi Arid Tropics (ICRISAT).
Corresponding Author: mohasjibrin@gmail.com +2348063539669

ABSTRACT

Plants are constrained by an array of environmental factors having negative consequence on their performance. These hostile factors greatly affect the earthly distribution of plants, as well as their growth and productivity. Drought a complex phenomenon presents itself as one of the major constrained to agricultural production globally, due to its varying intensity, frequency and its further exacerbated by climate change. Sorghum is the fifth most important cereal crop and occupies the second position among the staple food grains in semi-arid tropics. It requires relatively less water than other important cereals such as maize, rice and wheat. However, the yield potential of the crop is significantly limited due to drought. The drought resistance of plants can be divided into four basic types; drought avoidance, drought tolerance, drought escape, and drought recovery. Plant adaptation to drought stress is manifested by several modifications at morphological, anatomical and physiological levels. Several mechanisms including osmotic adjustments, stay green, leaf rolling, waxiness on the stem, root morphology and its architecture, transpiration efficiency, secretion of soluble solutes are known to play important role in bringing drought response. Improving the drought adaptation of C4 crops such as Sorghum is key in mitigating occurrence and severity of drought episodes due to higher evapotranspiration and rising temperatures. However, selection for drought tolerance is difficult due to genotype by environment interactions. Advances in genomics and other aspects of breeding offers an opportunity to produced sorghum cultivars adaptable to the changing climate. This paper outlines the main effects of drought on sorghum growth, development, and yield. In this review, mechanisms and breeding approaches related to drought in sorghum are discussed.

Keywords: *Drought tolerance, breeding, genetics, mechanisms, Sorghum bicolor Stay green*

Introduction

Sorghum is one of the most important crops worldwide after wheat, rice, maize and barley, providing food, fodder and bioenergy feedstock (FAO 2006). It is one of the most important dry land food crops grown in marginal lands and dietary food for more than half a billion poor and most food insecure people living in the sub-tropical and semi-arid regions of Africa and Asia (FAO, 2017). Sorghum is produced in intensive and commercialized in developed world with average yields of 3-5 t /ha largely used for feed, while, in the developing countries, it is grown in low-input, extensive production systems, with productivity of being 1t/ha mostly for food (Kumar, 2016; Reddy, 2017). It grows in low-rainfall, arid to semi-arid environments considered marginal for other cereal crops such as maize and wheat. It has exceptional tolerance to drought, high temperature stresses and low soil fertility, making it the crop of choice by millions of farmers in marginal agro-ecologies. The majority of sorghum production was in Africa (41%), followed by the Americas (38%) and then Asia (18%).

Cereal crops such as rice, wheat, maize, barley, sorghum and pearl millet are the major source of food for millions of population globally. More than 50% of the caloric intake is derived from cereal grains consumption. Among the cereals, sorghum is widely grown in the arid and semiarid tropics covering an area of 44 million ha with production estimates of 62.5 million tonnes and productivity of 1.6 tons/ha globally. Among the sorghum producing countries, the United States, India, Mexico, Nigeria, Sudan, Ethiopia and Australia are the major contributors representing 77% of the world production and 70% of the sorghum area (Rakshit *et al.* 2014). African and Asian countries together contribute around 83% of the sorghum area and 57% of the production. There has been a drastic decline in the area under grain sorghum due to various biotic (grain mold, shoot fly and stem borer) and abiotic stresses (drought, cold and osmotic). In addition, low support prices and less profitability of sorghum as compared to other commercial food and oilseed crops in the Asian countries lead to major shift in the sorghum area. Cultivation of genetically modified corn and cotton hybrids with high productivity has gradually replaced the sorghum area in the USA (Smith 2000). African countries have experienced steady increase in the sorghum area since last decade. Recently, Asian countries have witnessed drastic reduction in the consumption of sorghum in due to the changing food habits, urbanization and improvement in the economic conditions of the people (House *et al.* 2000). In spite of the decline in the area and consumption, most of the sorghum growing countries have witnessed steady increase in sorghum yields (1.1–1.4 tons/ha) (Murty *et al.* 2007). This yield gain is mainly attributed to genetic improvement programs and crop management practices (nutrient and water management). Mainly, projects undertaken to exploit heterosis through hybrid development has led to better yield gain in the developed countries. While, cultivation of historical varieties and landraces have led to slow yield gain in African countries and Indian states (Cothren *et al.* 2000). Sorghum bicolor, the primary species in cultivation has the ability to produce yields under adverse conditions. It is a buffer crop for the marginal farmers providing grain and fodder under minimal inputs (Badigannavar *et al.* 2018).

Water stress occurs when plants are unable to meet evapotranspiration demand. It is induced by unavailability of water due to erratic rainfalls or inadequate irrigation but can be exacerbated by other factors such as soil salinity and physical properties and high air or soil temperature (Rauf *et al*, 2015). Soil salinity makes water unavailable to plants by modifying soil water potential and inducing osmotic stress (Munns 2002). Soil texture and structure determine various properties such as porosity and surface roughness, which in turn affect water infiltration, soil holding capacity and retention. Water stress symptoms appear more rapidly on plants grown on sandy soil as compared to clay-textured soil (Sullivan 2002). High temperatures also affect water availability by increasing transpiration and may cause temporary wilting of the plant due to higher rate of water losses than water absorption by roots (Istanbulluoglu *et al.* 2009).

A drought is actually a meteorological event which implies the absence of rainfall for a period of time, long enough to cause moisture-depletion in soil and water deficit with a decrease of water potential in plant tissues (Kramer (1980). But from an agricultural point of view its, working definition would be the inadequacy of water availability, including precipitation and soil-moisture storage capacity, in quantity and distribution during the life cycle of a crop plant, which restricts the expression of the full genetic potential of the plant (Sinha (1986). It affects plant growth, survival and productivity in the world (Boyer (1982); Bohnet and Jensen (1996). It is a major production constraint in agriculture worldwide. It is estimated that cultivation on the earth is only possible on 16% of the potentially arable area due to limited availability of water (Alexandratos and Bruinsma 2012). Drought is occurring on all

continents with varying intensity and frequency. Its effect is more pronounced in the semiarid tropics (SAT), where rainfall is generally low and erratic in distribution.

The horn of Africa is strongly affected by drought almost every 12 years but drought intensified during the years 2009-2011 (Rauf *et al.* 2015). Around 17% of the global cultivated area was affected by drought during the period 1980-2006 (Dai *et al.* 2013). Drought principally affects crops cultivated under rain fed conditions, which represent 80% of the total cultivated area worldwide. The part of the cultivated area permanently affected by drought at the world level is estimated to be around 28% in sorghum, (Li *et al.* 2009).

Drought can have major consequences on growth, development and yield of crops by affecting several physiological, morphological and biochemical processes (Simpson, 1981). It is the major cause of poor crop performance and low yields, and sometimes it causes total crop failure. In the tropics, the probability of drought is highest at the start and the end of the growing season.

Drought can occur at both seedling, pre-flowering and post-flowering stages of development, and has the most adverse effect on yield (Tuinstra *et al.*, 1997). Drought stress at the seedling stage of development will severely affect plant establishment (Baalbaki *et al.*, 1999). If it occurs at flowering, or in the grain filling stages, it may cause reduced yields, or complete crop failure (Blum, 1996). Researchers have classified drought as either pre- or post-flowering stress. The reactions of genotypes to these stresses are variable and controlled by different genetic mechanisms. Pre-anthesis moisture stress has effects on yield components such as stand count, tillering capacity, number of heads and number of seeds per head, while post-anthesis moisture stress affects transpiration efficiency, CO_2 fixation and carbohydrate translocation. The latter factors, in turn, results in low yields and premature plant senescence (Thomas and Howarth, 2000; Xin et al., 2008).

Generating crop that can adopt to the changing climate, drought in particular, there is need to study the agronomical, physiological, biochemical and molecular basis of drought tolerance by integrating crop modelling and genomic prediction tools (Tardieu *et al.* 2018). In addition, the combination of phenotyping and selection of traits under moisture stress conditions is important as it involve complex interaction within the plant and with environment. In this way, the real assessment of the drought stress and its effect on crop plants can be studied by generating genetic variability for morphological, physiological and yield contributing traits, formulating efficient screening techniques at laboratory and field level and precise phenotyping methods to screen genotypes/mutants in a controlled condition. In this context, the present review discuss mechanisms pertaining to drought stress, plant response to various phases of moisture stress and genetic and molecular breeding methods to combat these problems.

Sorghum have developed several mechanism in response to drought, (Verma *et al.*, 2013) reported that There are four different mechanisms which help in survival of plants under moisture deficit conditions. Which are:

Drought escape: Drought Escape refers to natural or artificial adjustment of the growth period, life cycle, or planting time of plants to prevent the growing season from encountering local seasonal or climatic drought (Mitra, 2001). Farmers usually choose crop varieties with short life cycles, which complete their life cycle by avoiding the seasonal drought stress in agricultural production. The simplest way of survival under drought conditons is to escape drought. Generally, drought occurs either in the mid or late crop season. Drought escape is most common in case of plants grown in desert regions.

Drought avoidance: Drought avoidance refers to ability of the plant to maintain a favourable internal water balance under moisture stress. In other words, plants which avoid drought retain high water contents in their tissues. Drought avoidance is as the ability of plants to conserve water at the

whole plant level through decreasing water loss from the shoots or by more efficiently extracting water from the soil (Ludlow and Muchow, 1990). Drought avoidance can permit a longer growth period in the crop through reduced water use or increased water uptake.

The root system plays a critical role in response to water deficit stress. Some plants have the robust ability to increase root growth at the early stage of drought stress to absorb the water in deep soil (Hu and Xiong, 2014). Drought avoidance is principally characterized by the maintenance of high plant water potentials in the presence of a water shortage (Mitra, 2001)

Drought Tolerance: Drought tolerance refers to the ability of plants to sustain a certain level of physiological activities under severe drought stress conditions through the regulation of thousands of genes and series of metabolic pathways to reduce or repair the resulting stress damage (Mitra, 2001). In other words, Drought tolerance is the ability of plants to withstand water deficit while maintaining appropriate physiological activities to stabilize and protect cellular and metabolic integrity at tissue and cellular level (Tuinstra *et al.*, 1997; Xiong *et al.*, 2006). Plants accumulate a variety of organic and inorganic substances (such as sugars, polyols, amino acids, alkaloids, and inorganic ions) to increase their concentration in the cytochylema, reduce the osmotic potential, and improve cell water retention in response to water stress. This phenomenon is defined as osmotic adjustment (OA) (Rhodes and Samaras, 1994) a significant strategy for plant drought tolerance.

Drought recovery: Drought recovery refers to the plant capability to resume growth and gain yield (for crops) after exposure to severe drought stress which causes a complete loss of turgor pressure and leaf dehydration. Levit (1980) pointed out that the determination of drought resistance is much more difficult than that of other stress resistances.

According to Kidanemaryam, (2019) in various plants, physiological traits that are linked with drought tolerance when a plant is endangered to drought stress include greater cell growth, photosynthesis and biomass accumulation during pre-flowering stress, high pollen viability, seed set and seed numbers at flowering and improved stay green, photosynthesis and seed size during post flowering drought (Kidanemaryam, 2019). Other traits are (i) leaf rolling and wax content which will help in reducing leaf temperature, (ii) yield traits such as seed filling duration and seed filling rate which will increase seed size, and (iii) root traits such as increased root growth and water absorption which increases water uptake (Mutava, 2009). In order to develop climate resilient plants with improved water use efficiency, there is need to study the agronomical, physiological, biochemical and molecular basis of drought tolerance by integrating crop modelling and genomic prediction tools (Tardieu et al. 2018). In addition, the combination of phenotyping and selection of traits under moisture stress conditions is important as it involve complex interaction within the plant and with environment. In this way, the real assessment of the drought stress and its effect on crop plants can be studied by generating genetic variability for morphological, physiological and yield contributing traits, formulating efficient screening techniques at laboratory and field level and precise phenotyping methods to screen genotypes/mutants in a controlled condition. In this context, the present review discuss mechanisms pertaining to drought stress, plant response to various phases of moisture stress and genetic and molecular breeding methods to combat these problems.

Physiological response to drought tolerance

Kidanemaryam (2019) in his review titled "Mechanisms of Drought Tolerance in Sorghum (Sorghum bicolor (L.) Moench) Basis and Breeding Methods" enumerated the following as physiological drought tolerance mechanism in crop:

Physiological mechanisms of drought tolerance

Leaf rolling and stomatal conductance

Root system architecture

Osmotic adjustment, dehydration tolerance and transpiration efficiency

Solute accumulation and storage sugar solutes
stay-green / non-senescence
Chlorophyll fluorescence and reflection indices
Anatomical modifications to reduce water loss (sunken stomata/glaucousness/ epicuticular wax/leaf pubescence)
Canopy temperature
Genetics of drought tolerance
Drought resistance is a complex trait, expression of which depends on action and interaction of different morphological (earliness, reduced leaf area, leaf rolling, wax content, efficient rooting system, awn, stability in yield and reduced tillering), physiological (reduced transpiration, high water-use efficiency, stomatal closure and osmotic adjustment) and biochemical (accumulation of proline, polyamine, tetrahalide, etc., increased nitrate reductase activity and increased storage of carbohydrate) characters. Very little is known about the genetic mechanisms that condition these characters (Mitra, 2001). A number of traits related to drought resistance have been identified and mapped; however, the stay-green trait is recognized as the most crucial drought resistance trait in sorghum.
Breeding methods for drought tolerance
The main goal of plant breeders and biologist is to understand the mechanism of drought tolerance so as to assist them in developing drought resistant crops. the biological basis for drought tolerance is still largely unknown and few drought tolerance determinants have been identified. The slow pace in revealing drought tolerance mechanisms has hampered both traditional breeding efforts and use of modern genetics approaches in the improvement of drought tolerance of crop plants (Xiong *et al.*, 2006).
In view of the limited space, we would highlight some of the Breeding methods for drought tolerance as pointed out by (Kidanemaryam 2019) follows: (i) Molecular breeding approaches through identification of QTL and marker-assisted selection offer an opportunity for significant improvements in the drought tolerance of crops. (ii) Biotechnological Methods to Improving Drought Tolerance; there are different biotechnological approaches for drought improvement. Mainly Genomics, Proteomics, Metabolomics, Genetic engineering and others. (iii) Drought Evaluation Methods; In order to realize the balance between the different drought tolerance traits and their values to plants, it is critical that drought evaluation studies include measurement of both plant growth condition (soil water status) and plant responses including tissue water status and its regulators such as leaf area and stomatal conductance. With the transgenic and mutant approaches to characterize gene function under stress, the most important requirement is reliable and repeatable drought evaluation methods. Specific physiological and biochemical conditions had to be met to test these plants in growth chambers, greenhouses or in field conditions. (iv) Role of Marker assisted selection; In utmost breeding programmes, the genetic enhancement for drought resistance is accomplished through selection for yield because of low heritability of yield under stress and the spatial as well as temporal variation in the field environment, conventional breeding approaches are slow. Whereas molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic (RAPD) and isozyme will facilitate to develop drought resistant genotypes more effectively as their expressions are independent of environmental effects (Mitra, 2001).

Conclusions and Recommendations

Drought a complex phenomenon has presents itself as one of the major constrained to agricultural production globally, due to its varying intensity, frequency and its further exacerbate by the climate change. Sorghum requires relatively less water than other important cereals such as maize, rice and wheat. However, yield potential of the crop is significantly limited due to drought. The drought resistance of plants can be divided into four basic types; drought avoidance, drought tolerance,

drought escape, and drought recovery. Plant adaptation to drought stress is manifested by several modifications at morphological, anatomical, physiological levels. Several mechanisms including osmotic adjustments, stay green, leaf rolling, waxyness on stem, root morphology and its architecture, transpiration efficiency, secretion of soluble solutes are known to play important role in bringing drought response. Improving the drought adaptation of C4 crops such as Sorghum is key in mitigating occurrence and severity of drought episodes due to higher evapotranspiration and rising temperatures. However, selection for drought tolerance is difficulty due to genotype by environment interactions. Adoption of modern breeding/molecular techniques offer opportunities in developing crop plant that can adapt to changing climate as occasioned by drought and other indicators of climate change.

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FIELD EVALUATION OF SOME MAIZE VARIETIES IN A GUINEA SAVANNAH AGRO-ECOLOGY

[1]Manggoel, W.; [2]Dasbak, M.A.; [2]Jibung, G.G.; [3]Badi, S.H. [3]Da'ar, J.W. and [3]Mashat, I.M.

[1]Department of Agricultural Technology, Plateau State College of Agriculture, Garkawa
[2]Department of Crop Production Technology, Plateau State College of Agriculture, Garkawa
[3]Department of Horticultural Technology, Plateau State College of Agriculture, Garkawa
Corresponding Author: maarufbs@fukashere.edu.ng. +2348065673636

ABSTRACT

Crop varieties differ in performances and it is on this basis that varieties with economically important agronomic traits should undergo extensive evaluation in order to recommend them for commercial production. Twenty six maize (Zea mays L.) varieties obtained from National Seed Council of Nigeria were evaluated for cob and seed yields at the Teaching and Research Farm of Plateau State College of Agriculture, Garkawa, Nigeria in 2018 and 2019 cropping seasons. The maize varieties were laid out in a randomized complete block design (RCBD) with three replications. The result showed that the maize varieties differed significantly ($p<0.05$) in mean husk weight plant^{-1}, cob weight plant^{-1}, seed weight plant^{-1}, number of seeds plant^{-1} and grain yield ha^{-1}. Frequency of better performance than grand mean for traits quantified identified eight varieties having high frequencies (3-5; 5=100%) for traits with significant treatment means. The varieties within this category included: SAMAZ 15, SC651, OBA98, SDM-2, SAMAZ 45, SAMAZ 48, DUPONT P4226 and OBA SUPER 3. These varieties also had high rank scores (90 - 130) and were within the 1st and 8th positions of ranking among the 26 maize varieties. On the bases of the superior cob and grain yield ranking, these varieties were recommended for commercial maize production in the study area.

Keywords: *Characterization, grand mean, maize, rank scores, varieties*

Introduction

Maize (Zea mays L.) is a major staple food crop in sub-Saharan Africa. Its high energy content has made it very important in human and animal diets (Akinwale *et al.*, 2013). The crop is considered a model system for the study of genetics, evolution, and domestication (Lu *et al.*, 2009). In the global context, the genetic improvements in maize, combined with suitable agronomic practices, have allowed increase in grain yield by an average of 111 kg ha^{-1} yr^{-1} between 1965 and 2012 (USDA, 2015).

Maize provides a major source of calories in Nigeria as well as other parts of the world (Ado *et al.*, 2013). The crop is used as source of food for human beings and feed for animals, for production of biofuel as well as manufacturing of industrial products like starch, syrup, alcohol, acetic acid and lactic acid (Fentaw et al., 2015). According to West Africa Agricultural Productivity Programme (WAAPP, 2014) maize is one of the most important staple food crops in Nigeria.

Crop varieties with outstanding performance should undergo extensive multi-location testing and promotion for adoption for commercial production. Consequently, much work have been done in the characterization of

maize germplasm and this has led to continued improvement of the adaptive characteristics in relation to yield (Olaiya *et al.*, 2019; Asare-Bediako, 2019), pest and disease resistance (Buso *et al.*, 2019; Asare-Bediako, 2019; Craven and Fourie, 2011), striga resistance (Akinwale *et al.*, 2013) and other adaptive features. Improved varieties have been developed which are suitable for cultivation in specific ecological zones. Field trials of these varieties have been conducted across several locations. For instance, two test locations, Mokwa and Abuja, both in the southern guinea savannah zones of Nigeria, have been routinely used for the evaluation of maize genotypes in the IITA Maize programmes (Akinwale *et al.*, 2013).

The emergence of several seed companies in the West Africa sub-region have necessitated intensified efforts towards hybrid development and extensive testing. This is because the improved varieties vary in performances across locations. Consequently, the evaluation of the performances of cultivars in different ecological zones for adaptability is imperative and should be carried out on a continuous basis (Manggoel and Panwal, 2009). Akinwale *et al.* (2013) also posits that hybrids with outstanding performance should undergo extensive multi-location testing and promotion for adoption for commercial production. This study was aimed at the characterization of 26 maize varieties at Garkawa in the southern guinea savannah agro-ecology and to recommend outstanding varieties for commercial production of the crop in the study area.

Materials and Method

Field experiments were carried out at the Teaching and Research Farm of Plateau State College of Agriculture, Garkawa, (Lat. 10.11'N and Long. 8.21'E) in the Guinea Savanna ecological zone of Nigeria in 2018 and 2019 cropping seasons. The climate is characterized by two distinct wet and dry seasons. The wet season starts late April and ends in October while the dry season starts November and ends mid April. The mean annual rainfall is about 1,450mm and a relative humidity of 60%. The mean monthly maximum and minimum temperature are 22^0C and 15^0C, respectively; and an altitude of 1,195 m.a.s.l (Da'ar *et al*, 2014).

The experimental materials (treatments) were made up of 26 maize varieties; namely: SDM 2, DUPONT P4226, OBA SUPER 3, SAMAZ 14, OBA SUPER 6, SAMAZ 48, SAMAZ 19, SDM 1, SAMAZ 37, SAMAZ 24, DUPONT P4063W, SC651, DUPONT 30Y87, SAMAZ 40, DUPONTP3 966W, SC719, SC649, SAMAZ 17, OBA SUPER 11, SAMAZ 39, OBA 98, SAMAZ 33, SAMAZ

18, SAMAZ 15, SDM 6 and SAMAZ 45 obtained from the National Seed Council (NSC) of Nigeria.

The experimental design used was randomized complete block design (RCBD) with three replications. The land was ploughed, harrowed and ridged to give a fine tilth before the seeds were sown. The spacing was 75cm x 25cm between rows and between plants. The seeds were sown and each plot had 20 maize plants, implying 53,333 plants per hectare based on standard recommendations (Iken and Anusa, 2004). Weeding was done manually at 3 and 6 weeks after sowing (WAS). Fertilizer application was done in two split doses at the rate of 150 kg ha^{-1} NPK (15:15:15) and 100kg ha^{-1} NPK (20:10:10). Other agronomic practices were carried out according to standard practices for maize production. Harvesting was carried out when the crops reached physiological maturity. This was when the cobs and shoots were dried.

The number of cobs produced on five sampled plants were counted and recorded to obtain the mean number of cobs/plant. The husk of each cob of the sampled plants was weighed and seed rows per cob counted. The numbers of seeds on each cob of the sampled plants were counted. The shelled seeds on each cob were weighed and the mean recorded as grain yield in tones ha^{-1}. The data collection was subjected to analysis of variance using *Genstat discovery*

edition Software. Means that were found to be statistically significant ($p<0.05$) were separated using the least significant difference (LSD) as described by Obi (2002).

The frequencies of better performance than grand variety means were recorded for significantly different treatment means. This was done by comparing each variety mean with the grand mean. Varietal performances were ranked and scored: 1st = 26 points, 2nd = 25 points…26th = 1 point. The total rank score was plotted by variety according to the method adopted by Manggoel and Panwal (2009).

Results and Discussion

The mean, range, mean squares and coefficient of variations for the reproductive traits assessed and averaged over two cropping seasons (2018 and 2019) for the 26 maize varieties are presented in Table 1. The analysis of variance showed that the means for the varieties differed significantly ($p<0.05$) for husk weight plant^{-1} (HW/P), cob weight plant^{-1}(CW/P), seed weight plant^{-1} (SW/P), number of seed plant^{-1} (NS/P) and grain yield (GY). The significant differences in the mean and wide range for the traits considered implied there were discernable evidences of inherent genetic variability among the varieties, hence a wider scope for improvement of the crop (Manggoel *et al.,* 2012). Goshime *et al.* (2020) reported significant variation among genotypes for yield and other traits when the authors recently evaluated the performance of some selected new maize hybrids under sole and inter crop production systems.

Results obtained for the two cropping seasons (2018 and 2019) were statistically at per and variety x year interaction were not significant ($p<0.05$); hence the data were averaged over the two cropping seasons (Table 2). The variety SAMAZ 15 recorded the highest mean value for HW/P (112.3g) which was above the grand mean (62.3g); and was statistically similar to the mean husk weight of SDM-2 (106.8g), SC651 (98.3g), OBA98 (91.1g), SAMAZ 48 (86.1g), OBA SUPER3 (83.2g), DUPONT P4226 (79.8g) and SAMAZ 45 (75.8g). The least mean husk weight was recorded for the variety DUPONT P3966W (32.9g), which was below the grand mean. Mean CW/P followed similar trend, with the variety SAMAZ 15 being distinct having mean value of CW/P of 669.0g. The mean value for CW/P was still low for the variety DUPONT P3966W (185.0g), implying that maize varieties with higher husk weight plant^{-1} had corresponding higher cob weight plant^{-1}. The significant statistical differences in mean husk weight and cob weight obtained in this study are evidence of variations in the yield potentials of the maize genotypes. Damiyal *et al.* (2017) reported significant treatment effect ($p \leq 0.05$) for husk weight plant^{-1} in an earlier report when the authors evaluated some hybrid maize varieties.

Though differences were recorded among the maize varieties in number of seed rows cob^{-1} (SR/C), the differences were not significant ($p<0.05$). The non-significant differences in mean number of seed rows cob^{-1} underscore the importance of this trait as Abdel-Moneam *et al.* (2015) listed number of seed rows per cob as one of the most important yield components of grain yield in maize.

The result of the analysis showed that the mean values for seed weight plant^{-1} (SW/P), number of seed cob^{-1} (NS/C) and grain yield ha^{-1} (GY/ha) were statistically significant ($p<0.05$) and ranged from 157.5g-479.5g, 341.7-744.0 and 1.58 - 4.29t/ha, in that order. The maize variety SAMAZ 15 was outstanding for SW/P (479.5g), NS/C (744.0) and GY/ha (4.29t), and was above the grand variety mean (SW/P=263.91g; NS/C=462.77; GY/ha= 2.65t) for the three traits. The mean values for SW/P, NS/C and GY/ha for this variety (SAMAZ 15) were however statistically similar to that of SC651 (SW/P=418.0g; GY/ha =4.16t; NS/C=704.5). Other maize varieties

with SW/P, NS/C and GY/ha above the grand mean included SDM-2, DUPONT P4226, OBA SUPER3, SAMAZ48, OBA98, SAMAZ15, and SAMAZ 45.

The number of seeds plant^{-1} obtained in this study falls within that obtained when improved varieties were grown under optimum organic manure (cattle) recommended application of 5t/ha, which gave the highest number of seeds plant^{-1} of 625 (Damiyal *et al.*, 2017). The mean grain yield obtained in this study (1.58-4.29t/ha) is similar to the grain yield (1.84-3.48t/ha) reported by Sorsa and Kassa (2015). A recent study (Goshime *et al.*, 2020) however, reported higher values (8.10-10.10t/ha) for grain yield of maize for some new selected maize hybrids under sole and inter crop systems in Ethiopia

Frequency of better performance than grand means for parameters quantified (Table 3) identified eight varieties having high frequencies (3-5) for the five traits considered. Varieties within this category included: SAMAZ 15, SC651, OBA98, SDM-2, SAMAZ 45, SAMAZ 48, DUPONT P4226 and OBA SUPER3. These varieties also had high rank scores of between 90 and 130 (Fig. 1) and were within the 1st and the 8th position of ranking (Table 4). These varieties were regarded to have performed better (adapted) at the Garkawa agro-ecology. Three other varieties (SAMAZ 33, SAMAZ 14 and DUPONT P4063W) had moderate frequencies (1-2) of better varietal performance than grand mean as well as moderate rank scores (77-87). Frequencies of better performance than grand mean was used by Manggoel and Panwa (2009) to recommend seven elite varieties of cowpea within the Makurdi agro-ecology.

Conclusion

On the bases of the superior cob and seed yields, eight (8) varieties had mean cob and grain yield above grand mean. These varieties included: SAMAZ 15, SC651, OBA98, SDM-2, SAMAZ 45, SAMAZ 48, DUPONT P4226 and OBA SUPER3. The varieties also had high rank scores (90 - 130) and were within the 1st and the 8th positions of ranking among the 26 maize varieties. On the bases of the superior cob and grain yield ranking, these varieties were recommended for commercial maize production in the study area.

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Table 1: Mean, range, mean squares, Fisher's probability and coefficient of variations for 7 reproductive traits in maize averaged over two cropping seasons

Characters	**Mean**	**Range**	**MS**	**F_{pr}**	**CV (%)**
Husk weight/plant(g)	62.3	32.9 - 122.3	41.34**	0.044	18.3
Cob weight/plant(g)	322.0	185.0 - 669.0	123.67**	0.002	10.0
Number of cobs/plant	1.23	1.0 – 1.6	0.89^{ns}	0.825	4.4
Seed row/cob	13.04	12.0 -15.4	1.27^{ns}	0.674	1.8
Seed weight/plant (g)	263.91	157.5 - 479.5	167.40**	<.001	18.4
Number of seed/plant	462.8	341.7 – 744.0	89.35**	<.001	15.4
Grain Yield t/ha	2.65	1.58 – 4.29	236.49**	0.029	4.9

Fpr = Fisher's probability; MS = Mean square (Genotype); CV = Coefficient of variation (%), ** = Significant at 1% probability; ns = not significant

Table 2: Mean cob and seed/grain yields of 26 Maize varieties averaged over two growing seasons (2018 and 2019)

S/N	Varieties	HW/P (g)	CW/P (g)	NC/P	SR/C	SW/P (g)	NS/P	GY/ha (t)
1	SDM 2	106.8	429.4	1.1	12.7	342.6	526.8	3.43
2	DUPONT P4226	79.8	416.7	1.3	12.2	342.6	498.8	3.41
3	OBA SUPER 3	83.2	363.0	1.1	12.7	294.6	412.4	2.91
4	SAMAZ 14	59.1	294.9	1.4	13.2	247.2	467.2	2.46
5	OBA SUPER 6	57.4	244.9	1.2	13.2	194.5	402.4	1.94
6	SAMAZ 48	86.1	388.8	1.1	12.8	345.7	600.4	3.45
7	SAMAZ 19	43.7	271.5	1.3	12.8	220.0	376.5	2.20
8	SDM 1	60.6	274.2	1.3	13.2	223.4	429.3	2.21
9	SAMAZ 37	45.6	299.7	1.4	12.9	253.0	446.9	2.53
10	SAMAZ 24	45.6	282.7	1.0	12.2	243.5	379.1	2.44
11	DUPONT P4063W	51.1	306.0	1.2	13.2	261.6	467.4	2.62
12	SC651	98.3	488.3	1.3	15.4	418.0	704.5	4.16
13	DUPONT 30Y87	38.5	260.8	1.4	15.2	227.8	438.9	2.27
14	SAMAZ 40	59.9	279.0	1.2	12.2	226.3	412.1	2.26
15	DUPONTP3 966W	32.9	185.0	1.0	13.2	157.5	385.8	1.58
16	SC719	45.5	254.1	1.1	14.2	215.9	403.1	2.14
17	SC649	62.2	269.1	1.4	13.3	229.1	376.1	2.28
18	SAMAZ 17	57.9	225.1	1.5	13.2	179.7	353.4	1.79
19	OBA SUPER 11	39.8	256.5	1.0	12.4	215.5	442.3	2.15
20	SAMAZ 39	39.9	212.8	1.2	12.0	178.1	341.7	1.71
21	OBA 98	91.1	418.1	1.1	13.2	353.6	590.9	3.52
22	SAMAZ 33	60.2	381.0	1.4	12.5	235.8	467.7	3.32
23	SAMAZ 18	50.8	273.0	1.1	12.7	227.1	363.3	2.25
24	SAMAZ 15	112.3	669.0	1.6	13.8	479.5	744.0	4.29
25	SDM 6	35.5	216.2	1.2	12.2	185.1	409.8	1.82
26	SAMAZ 45	75.8	412.9	1.1	13.2	364.6	591.9	3.64
	MEAN	**62.3**	**322.0**	**1.23**	**13.04**	**263.91**	**462.77**	**2.65**
	LSD (p<0.05)	**37.23**	**107.6**	**NS**	**NS**	**63.25**	**159.62**	**1.01**

HW/P (g) = Hush weight/plant, CW/P (g) = Cob weight/plant, NC/P = Number of cobs/plant, SR/C = Seed row/cob, SW/P (g) = Seed weight/plant (g), NS/P = Numbers of seed/plant, GY t/ha = Seed weight/ha, NS = Not significant

Table 3: Frequency of better performance than grand variety mean** for parameters quantified*

5	4	3	2	1	0
SAMAZ 15	-	OBA SUPER 3	SAMAZ 33	SAMAZ 14	SAMAZ 37
SC651				DUPONT P4063W	SAMAZ 40
OBA 98					SDM 1
SDM 2					SAMAZ 24
SAMAZ 45					SC649
SAMAZ 48					DUPONT 30Y87
DUPONT P4226					SAMAZ 18
					OBA SUPER 11
					SAMAZ 19
					SC719
					OBA SUPER 6
					SAMAZ 17
					SDM 6
					SAMAZ 39

***Parameters quantified**	****Grand variety means**
Hush weight/plant (g)	62.30
Cob weight/plant (g)	322.00
Seed weight/plant (g)	263.91
Seed weight (t/ha)	2.65
Numbers of seed/plant	462.77

Table 4: Rank score and position for better performance than grand variety mean

S/N	VARIETY	RANK SCORE	POSITION
1	SDM 2	112	4th
2	DUPONT P4226	103	7th
3	OBA SUPER 3	90	8th
4	SAMAZ 14	77	11th
5	OBA SUPER 6	35	22nd
6	SAMAZ 48	110	6th
7	SAMAZ 19	37	20th
8	SDM 1	60	14th
9	SAMAZ 37	74	12th
10	SAMAZ 24	59	15th
11	DUPONT P4063W	81	9th
12	SC651	124	2nd
13	DUPONT 30Y87	50	17th
14	SAMAZ 40	61	13th
15	DUPONTP3 966W	10	26th
16	SC719	36	21st
17	SC649	57	16th
18	SAMAZ 17	25	25th
19	OBA SUPER 11	39	19th
20	SAMAZ 39	12	25th
21	OBA 98	114	3rd
22	SAMAZ 33	87	10th
23	SAMAZ 18	47	18th
24	SAMAZ 15	130	1st
25	SDM 6	22	24th
26	SAMAZ 45	112	4th

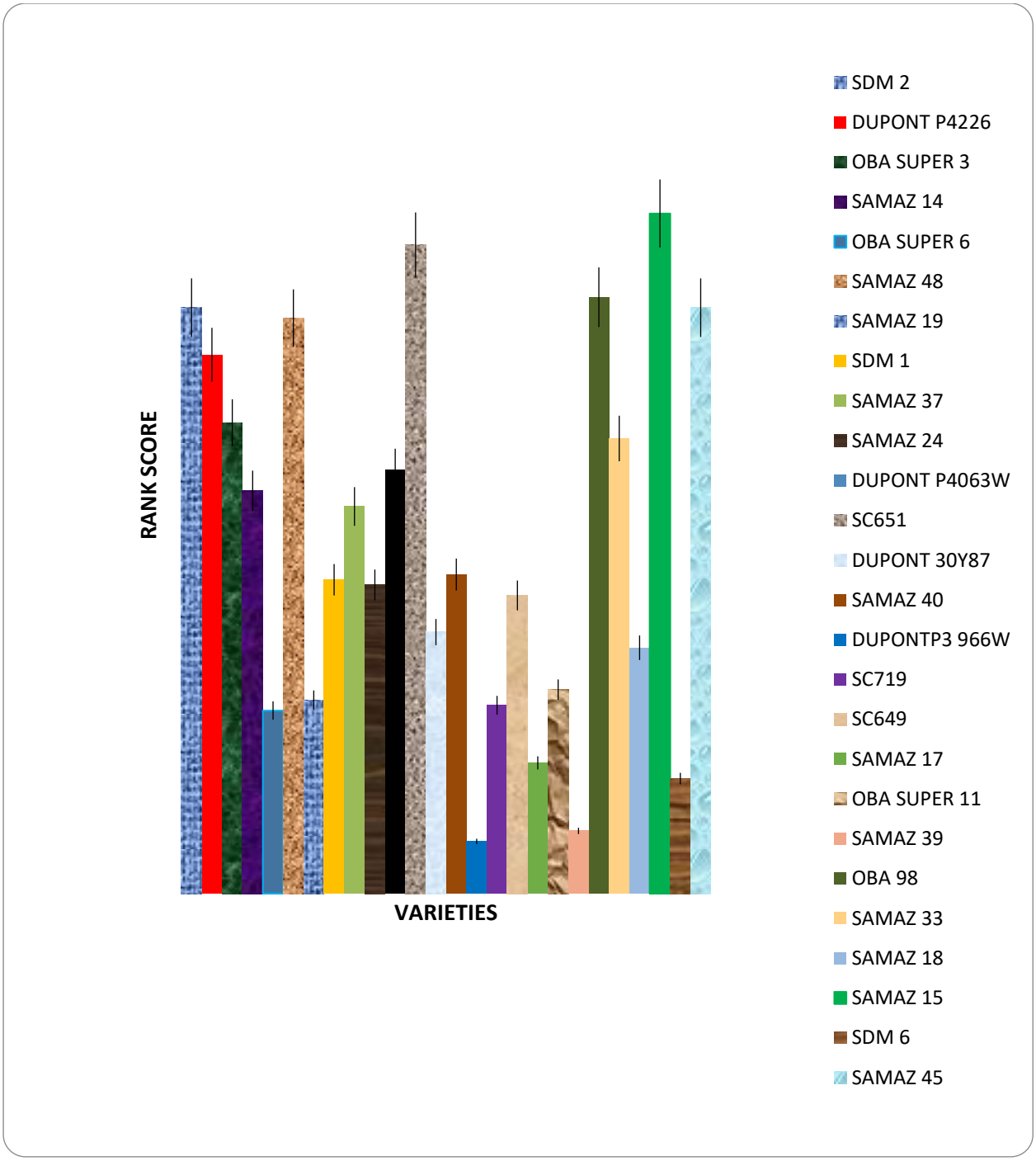

Fig. 1: Rank score summed over cob and seed yields for 26 maize varieties

CGBPB 023

ESTIMATION OF GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE IN SOME MORPHOLOGICAL AND YIELD CHARACTERS OF SWEETPOTATO (*Ipomoea batatas* (L.) LAM) INDUCED WITH CHEMICAL MUTAGENS

Orji[1*], I. E., Eka[2], M. J., Okocha[2], P. I., and Harriman[2], J. C.

[1]Dangote Fertilizer Limited, KM 15 Okunraye Bus stop, Lagos Free Trade Zone, Ibeju-Lekki, Lagos state
*Corresponding Author: orjilla@gmail.com; +2348039548747

ABSTRACT

An investigation was carried out to estimate the extent of genetic variability, heritability and genetic advance induced by sodium azide and colchicine mutagens in some of the characters in Sweetpotato (Ipomoea batatas (L.) Lam). Vine cuttings of four (4) varieties of sweetpotato commonly grown in South-eastern Nigeria were soaked in sodium azide (SA) and colchicine (COL) mutagens at various concentrations: 0%, 0.03%, 0.05% and 0.07%, for 2 hours and were used to establish the M_1V_1 generation. M_2V_2 generation plants were raised using vine cuttings selected at random for each treatment from the best performed M_1V_1 generation plants. Genetic variability, heritability and genetic advance was estimated for some morphological and yield characters. The genotypic variance (VG) was higher than the error variance (VE) for total number of leaves at 12 WAP, total number of branches at 12 WAP, stem girth at 12 WAP, main vine length at 12 WAP, leaf area at 12 WAP and total number of storage roots/m^2, while the error variance was higher in total weight of roots (kg)/m^2 and total weight of storage roots (t)/ha. Leaf area at 12 WAP recorded the highest genetic advance estimate of 119.06%, followed by total number of leaves at 12 WAP and total number of branches at 12 WAP, both of which recorded 65.73% and 51.90% respectively.

Keywords: *Heritability, Genetic advance, mutagens, sweetpotato, variability*

Introduction

Genetic variability is the basis of all selections in a breeding program. The wider the genetic variability, the better and easier the selection would be. Since one of the main objectives of any breeding program is to develop high-yielding and better quality lines for release as varieties to farmers (Ehdaie and Waines, 1989), mutation induction serves as a useful means of inducing a higher quantity of such variability within the population to boost the achievement of this target.

As the estimate of phenotypic variability cannot distinguish between the effect of genetic and environmental effects, so the study of genetic variability is useful in partitioning out the real genetic differences (Sanjay *et al.*, 2012). The observed variability is a combined estimate of genetic and environmental causes, of which only the former one is heritable (Keke, 2015). Therefore it is necessary to partition the total variation into heritable and non-heritable constituents with the help of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability and genetic advance (Maniee *et al.*, 2009).

Detection of significant genetic variability thus indicates the presence of genetic

variance in the population alone while broad-sense heritability estimate presents information on relative magnitude of genetic and environmental variation in germplasm pool (Peddi and Dhaduk, 2014). Estimates of heritability alone do not provide a suggestion about the expected gain in the next generation, but have to be measured in conjunction with estimates of genetic advance (Shukla *et al.*, 2006).

High heritability in some yield traits in groundnut treated with sodium azide was observed by Mensah and Obadoni (2007). In okra variety GO-2, Peddi and Dhaduk (2014) in their work on induction of genetic variability in okra by a chemical and physical mutagen reported high heritability and genetic advance for fruit length, fruit weight and fruit yield/plant.

Genetic variability studies on sweetpotato have been reported by many researchers (Afuape *et al.*, 2014, Dai *et al.*, 1988 and Jones, 1977) but not much have been specifically reported on mutagenesis studies in sweetpotato. Hence the objective of this study was to estimate the extent of genetic variability, heritability and genetic advance induced by sodium azide and colchicine mutagens in some characters in Sweetpotato (*Ipomoea batatas* (L.) Lam).

Materials and Methods

Vine cuttings of four (4) commonly cultivated sweetpotato varieties used in this study (Butter milk, TIS87/0087, UMUSPO/3 and UMUSPO/1) were obtained from the National Root Crops Research Institute (NRCRI) Umudike, Abia state.

The parent plants (Butter milk, TIS87/0087, UMUSPO/3 and UMUSPO/1) were mutated using sodium azide (SA) and colchicine (COL) mutagens at concentrations of 0 %, 0.03 %, 0.05 % and 0.07 %. These cuttings were soaked in these concentrations for 2 hours at room temperature and periodically agitated. Finally, they were rinsed with running tap water for 1 hour to wash out the chemical residues before taking them to the field. The vine cuttings with 0% treatments were used as control. Vine cuttings selected at random for each treatment from the best performed M_1V_1 generation plants were used to establish the M_2V_2 generation including the control. M_1V_1 and M_2V_2 were the first and second mutative vegetation respectively.

Total number of leaves per plant was determined by counting the total number of leaves at 12 WAP and expressed per plant by dividing the total number of leaves by number of plants sampled. Total number of branches per plant was determined by counting the total number of branches at 12 WAP and expressed per plant by dividing the total number of branches by number of plants sampled. An electronic vernier calliper was used to measure stem girth at 12 WAP and expressed per plant in mm by dividing the total stem girth by number of plants sampled. Main vine length per plant was determined at 12 WAP with the use of a calibrated measuring tape and expressed in cm per plant by dividing the total vine length by number of plants sampled. For linear measurement of leaf area (cm^2) in sweetpotato, the leaf factor for sweetpotato with non-lobed leaves is 0.45; while that with multilobed leaves is 1.24 (Ramanujam and Indira, 1978). The leaf area was therefore estimated using the following mathematical relationship as described by Ogoke *et al.* (2003):

Leaf area = Length × Width × Leaf factor

The storage roots were harvested at 16 WAP (Ezulike *et al.*, 2001). Yield data were obtained by counting, measuring and weighing the roots of harvested plants. The following data on yield were obtained: total number of storage roots/m^2, total weight of storage roots (kg)/m^2, and total weight of storage root per hectare (t/ha).

Estimation of genetic component and heritability was analyzed using the software: Analysis of Genetic Designs (AGD-R), of Francisco *et al.* (2015). The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were computed according to Singh and Chaudhury (1985) as:

GCV (%) = $\frac{\sqrt{\partial^2 g}}{\bar{x}} \times 100$; PCV (%) = $\frac{\sqrt{\partial^2 p}}{\bar{x}} \times 100$

Where: ∂^2_g = genotypic variance, ∂^2_p = phenotypic variance, and $\bar{x}$ = sample mean.

Expected genetic advance (GA) and genetic advance as a percentage of mean (GAM) were computed according to Engida *et al.* (2007) as:

GA = $\frac{K \times \sqrt{\partial^2 p} \times \partial^2 g}{\partial^2 p}$; GAM = $\frac{GA}{\bar{x}} \times 100$

Where: k = standardized selection differential at 5% selection intensity (k = 2.063), ∂^2_p = phenotypic variance, ∂^2_g = genotypic variance and $\bar{x}$ = sample mean.

Results and Discussion

Table (1) showed the estimates of the phenotypic (VP), genotypic (VG), error variances (VE), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2B) and genetic advance expressed as a percentage of mean (GAM) in some morphological and yield traits of sweetpotato treated with chemical mutagens.

Wide variations were observed in some of the morphological traits studied while the yield traits showed narrower range of variation indicating that the mutagens induced more variations in the morphology than in the yield traits. This is an indication that some of the morphological traits might be controlled by major genes.

Phenotypic variance (VP) ranged from 0.08 for total weight of roots (kg)/m^2 to 4198.68 for number of leaves at 12 WAP. The phenotypic variance was partitioned into heritable (genotypic variance) and non-heritable (error variance) components. The genotypic variance (VG) was higher than the error variance (VE) for total number of leaves at 12 WAP, total number of branches at 12 WAP, stem girth at 12 WAP, main vine length at 12 WAP, leaf area at 12 WAP, and total number of storage roots/m^2 while the error variance was higher in total weight of roots (kg)/m^2 and total weight of storage roots (t)/ha.

The higher genotypic variance observed for total number of leaves at 12 WAP, total number of branches at 12 WAP, stem girth at 12 WAP, main vine length at 12 WAP, leaf area at 12 WAP, and total number of storage roots/m^2 compared to a lower environmental influence (error variance) suggests that these characters may be under genetic control rather than environment. In similar mutagenesis studies, Jehangir and Chandrasekharan (1978); Gonge and Kale (1996); and Senthil Kumar *et al.* (1998) working on various crops reported an increase in genotypic variability for all traits studied.

Phenotypic coefficient of variability (PCV) ranged from 25.71 % to 65.73%. Total weight of roots (kg)/m^2 had the lowest PCV while leaf area at 12 WAP had the highest. The genotypic coefficient of variability ranged from 14.20% for total weight of storage roots t/ha to 61.59% for leaf area at 12 WAP. The PCV recorded higher estimates than GCV for all the traits studied indicating the influence of environment on the expression of these traits. Contrary to the report of Sanjay *et al.* (2012), it was observed in this study that the difference between PCV and GCV for each of the respective traits was not more than 15% indicating that the variability due to the genetic constitution was more than the variability exerted by environmental factors hence, selection of performance based on phenotype is likely to be reliable.

GCV alone is not enough to determine the amount of heritable variability as heritability and Genetic Advance with GCV are required to assess the heritable portion of the total variation (Peddi and Dhaduk, 2014). High heritability estimate for a trait depicts that the control of the trait is more under genetic control than the environment, and that breeding progress can be made by phenotypic observation and mass selection (Afuape *et al.*, 2014).

For heritability in broad sense (H^2B), leaf area at 12 WAP recorded the highest estimate (88%) followed by stem girth at 12 WAP (79%). Total number of leaves at 12 WAP (75%), total number of branches at 12 WAP (68%), main vine length at 12 WAP (63%)

and total number of storage roots/m^2 gave heritability estimates that were more than 50%. Total weight of roots (kg)/m^2 and total weight of roots (t)/ha recorded heritability values of 38% each which was the least.

Jones *et al.* (1986) suggested that in sweetpotato, a heritability estimate above 60% are quite adequate for good selection advance and estimates as low as 40% could be considered favourable provided that the selection techniques have enough precision. Based on the result of this study for heritability in broad sense (H^2B), leaf area at 12 WAP recorded the highest estimate (88%) followed by stem girth at 12 WAP (79%). Total number of leaves at 12 WAP (75%), total number of branches at 12 WAP (68%), main vine length at 12 WAP (63%) and total number of storage roots/m^2 gave heritability estimates that were more than 50%. This is an indication that the effect of mutagens could have caused an additive gene effect which played an important role in the expression of these traits. Thus selection for these characters for each variety at specific mutagenic treatment will be effective for improvement (Peddi and Dhaduk, 2014).

Sheeba *et al.* (2003) reported that broad-sense heritability estimate would be reliable only when accompanied with high Genetic Advance. Johnson *et al.* (1955) reported that the heritability estimates along with genetic advance is more useful than the resultant effect for selecting best genotypes (mutants), as it suggests the presence of additive gene effects.

Genetic advance expressed as a percentage of mean (GAM) ranged from 16.04% for total weight of storage roots (t)/ha to 119.06% for leaf area at 12 WAP. Leaf area at 12 WAP recorded the highest genetic advance estimate of 119.06%, followed by total number of leaves at 12 WAP and total number of branches at 12 WAP both of which recorded genetic advance estimates of 65.73% and 51.90% respectively. Since these traits also recorded high heritability values, they can be said to have additive and epistatic gene effects, which are fixable and can provide the desirable gain (Sheeba *et al.*, 2003).

Stem girth at 12 WAP, main vine length at 12 WAP and total number of storage roots/m^2 recorded moderate genetic advance estimates of 49.90%, 44.32% and 37.43% respectively. High heritability and moderate Genetic Advance indicates non-additive gene effects including dominance and epistasis. This indicates the role of environment in the expression of the traits (Peddi and Dhaduk, 2014).

Table 1: Estimate of the phenotypic (VP), Genotypic (VG), Error variances (VE), Phenotypic coefficient of variation (PCV), Genotypic coefficient of variation (GCV), Heritability (H^2b) and Genetic Advance expressed as a percentage of mean (GAM) in some morphological and yield attributes of sweetpotato treated with chemical mutagens

Attributes	VP	VG	VE	PCV (%)	GCV (%)	H^2B (%)	GAM (%)
Number of leaves @ 12 WAP	4198.68	3164.17	1034.51	42.28	36.70	75.00	65.73
Number of branches@12 WAP	13.35	9.09	4.26	36.94	30.48	68.00	51.90
Stem girth @ 12 WAP	3.79	2.99	0.80	30.66	27.23	79.00	49.90
Main vine length @ 12 WAP	3815.67	2391.67	1424.00	34.28	27.14	63.00	44.32
Leaf Area @ 12 WAP	2585.25	2269.74	315.51	65.73	61.59	88.00	119.06
Total no of storage roots/m^2	2.47	1.42	1.05	31.56	23.93	57.00	37.43
Total weight of storage roots/m^2	0.08	0.03	0.05	25.71	15.75	38.00	19.89

Total weight of storage roots/ha	0.10	0.03	0.07	25.92	14.20	38.00	16.04

Conclusion

The purpose of this study was to estimate the extent of genetic variability, heritability and genetic advance induced by sodium azide and colchicine mutagens in some of the characters in four varieties of sweetpotato (Butter milk, TIS87/0087, UMUSPO/3 and UMUSPO/1). The estimates showed that improvement by direct selection is possible in number of leaves at 12 WAP, number of branches at 12 WAP and leaf area at 12 WAP.

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CGBPB 024

PERFORMANCE EVALUATION OF RELEASED SORGHUM (*SORGHUM BICOLOR* L. MOENCH) VARIETIES FOR YIELD AND RELATED TRAITS

Adekanye, P.S., Abdulmalik, R.O, Sami, R. A., Usman, M., Ahmed, H.O., Hassan, L.B.

Department of Plant science, Institute for Agricultural Research, Samaru, Ahmadu Bello University, Zaria.
Corresponding Author: preciousadekanye@gmail.com

ABSTRACT

Performance of released sorghum varieties is crucial for assessing progress made in breeding for improved sorghum varieties. The present study was carried out to evaluate the performance and determine the association of released sorghum varieties for grain yield and other agronomic traits. A total of 10 varieties comprising eight released varieties and two checks were evaluated at Samaru during 2017/2018 dry season. Data collected on grain yield and other agronomic traits were subjected to analysis of variance using appropriate statistical software. Mean squares were significant for all measured traits except plant height and stem girth. The grain yield of the varieties ranged from 667 kg ha^{-1} for SAMSORG 9 to 2000 kg ha^{-1} for SAMSORG 45 with an average of 1179 kg ha^{-1}. The top yielding variety, SAMSORG 45 out-yielded the best check, CF35:5 by 40 %. Grain yield was significantly and positively associated with plant height, (r = 0.47), number of leaves (r = 0.65), panicle weight (r = 0.82) and 100 seed weight (r = 0.68). The performance of the released varieties can be re-evaluated in multiple environments to assess progress made in breeding for improved sorghum varieties in terms of grain yield and other agronomic traits.

Keywords: *Genetic variance; genotype; phenotype; grain yield, Sudan Savanna.*

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world after rice, wheat, maize and barley (FAO, 2017). In Africa, Nigeria is the largest producer of sorghum, and the third largest in the world, after U.S.A and India (FAO, 2015). It is an important affordable staple in Nigeria that is highly versatile because of its adaptability to varying ecological growing conditions and contribution to food security (Olaniyan, 2015). It has also commanded industrial demands for drinks, alcoholic and non-alcoholic, feed and confectioneries including baby foods. It is also the cheapest source of energy and micro-nutrients after pearl millet for the vast majority of the population in Africa and India (Parthasarathy *et al.,* 2010). The increased uses of sorghum as food in the sub-tropical Africa could alleviate the problem of chronic undernourishment (Dasai *et al.,* 1992). The optimum temperature for sorghum seed germination is 25–35°C, but temperatures as low as 21°C will not dramatically affect growth and yield (Plessis, 2008). Sorghum is well adapted to an annual rainfall ranging from 400 mm to 700 mm and it can also be cultivated in areas of up to 1000 mm of rainfall per year (Plessis, 2008). Although there is no known official figure on national production demand and supply of sorghum in Nigeria, the local demand is more than the current supply owing to increasing demand from local industries in Nigeria.

Sorghum in general possesses a wide range of genetic variability (Sharma *et al.,* 2006). Adequate variability provides options from which selections are made for improvement and possible hybridization. The Yield is a quantitative trait, which depends upon many

other independent contributing traits. Information on the association between yield and its components helps in evaluating the contribution of different components towards yield, this in turn will allow simultaneous selection for many characters associated with yield (Swamy *et al.,* 2018). The most important objective of the Institute for Agricultural Research (IAR), Ahmadu Bello University is to develop crop varieties that are high yielding, with good quality grains for food, feed and industrial utilization. (Abad *et al.,* 2005). Hence they have released a number of open pollinated sorghum varieties for improved grain yield and other agronomic traits. To assess progress made in breeding, the released sorghum varieties need to be evaluated for yield and other related traits. Hence, this study aimed at evaluating the performance and association of the released varieties for yield and other agronomic traits.

Materials and Methods

The experiment was conducted at the Institute for Agricultural Research (IAR) farm located at Samaru during the 2017/2018 dry season. Samaru is (11^0 11^1 and 07^0 38E) located in the Northern Guinea Savannah ecological zone of Nigeria on longitude 7^0 38' 65" E, at latitude 11^0 14'44" and an annual rainfall of 1100mm, and an altitude of 686m above sea level (Yerima, 2014). The plant materials used in the study comprised 10 IAR sorghum released varieties along with one check. The variety, maturity and yield range are presented in Table 1 as follows:

Table 1: Ten IAR Sorghum Released Varieties

S/N	Variety	Maturity	Yield Potential
1.	SAMSORG 3	Early maturity	1500 – 2500kg/ha
2.	SAMSORG 9	Early maturity	1800 – 3500kg/ha
3	SAMSORG 38	Early maturity	1800 – 3000kg/ha
4.	SAMSORG 39	Early maturity	1800 – 2500kg/ha
5.	SAMSORG 40	Early maturity	2000 – 2500kg/ha
6.	SAMSORG 41	Early maturity	2200 – 2700kg/ha
7.	SAMSORG 45	Early maturity	2500 – 3000kg/ha
8.	SAMSORG 46	Early maturity	2500 – 3000kg/ha
9.	CF35:5	Early maturity	2500 – 3000kg/ha
10.	NR71151	Early maturity	1500 – 2000kg/ha

The evaluation trial was laid out in a Randomized Complete Block Design (RCBD) consisting of two replications. Each plot consisted of a single row of 5cm long with an inter and intra row spacing of 0.75 m and 0.30 m respectively. Three seeds were planted per hill and later thinned to two per stand at 2 weeks after sowing (WAS). A compound fertilizer, NPK 15:15:15 was applied at the rate of 60 kg N ha^{-1}, 60 kg P ha^{-1}, and 60 kg K ha^{-1}, at planting and an additional 60 kg N ha^{-1} of urea was applied four weeks later. In each trial, the field was kept weed-free through the application of a mixture of Gramoxone and Primextra at 5 l ha^{-1}. Subsequent manual weeding was carried out at two and six weeks after sowing (WAS) to keep the trials weed-free.

Observations were made on each plot for days to 50% heading as the number of days from planting to when 50% of the plants have fully emerged panicles; days to 50% flowering as the number of days from planting to when 50% of the plants have shed pollen; plant height as as the distance from the base of the plant to the tip of the panicle; stem girth as the distance around the stem at maturity; number of leaves as the total number of leaves of 10 random plants/plot; panicle length measured in centimeters (cm) from the base of the panicle to the tip; panicle weight measured in kilograms (kg) as the total panicles harvested per plot; 100 grain weight: Weight of 100 grains threshed from panicle in kg ; Grain weight: Total weight of grains from harvested panicles in

grams (g).;. Grain yield: Grain yield (obtained from grain weight and converted to kg ha^{-1}). Grain yield (kg/ha) = Grain yield (kg/plot) x 10,000 m^2 / Plot area (m^2)

Data collected were subjected to Analysis of variance (ANOVA) using the generalized linear model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc, 2008). Means were compared using least square difference (LSD). The form of the ANOVA and expected mean squares (EMS) for one location is shown in Table 2 .The linear model for the RCBD for one environment is $Y_{ij} = \mu + R_i + G_j + \varepsilon_{ij}$

Y_{ij} = An observation value for the j^{th} progeny in the i^{th} replication; μ = the overall Mean; R_i = the effect of the i^{th} replication; G_j = the effect of the j^{th} genotype; ε_{ij} = residual effects

Table 2: Form of Analysis of Variance for Randomize Completely Block Design

Source of variation	df	MS	EMS
Replication	$r-1$		
Genotype	$g-1$	MS_g	$\sigma_e^2 + r\sigma_g^2$
Error	$(r-1)(g-1)$	MS_e	$\sigma^2{}_e$

$$\sigma^2 g = \frac{MS_g - MS_e}{r}, \qquad \sigma^2{}_e = \frac{MS_e}{r},$$

$$\sigma^2 = \frac{M_{sg}}{r}$$

Where:

$\sigma^2 g$ = genotypic variance; $\sigma^2{}_e$ = error variance; r = number of replicate; MS_g= Mean square due to genotype; g = number of genotypes; MS_e = error mean square

Correlation coefficients that measure the degree of association between one trait and another were estimated using the Pearson correlation coefficient formula given by Singh and Chaudhary (1985).

$$r_{xy} = \frac{Cov(xy)}{\sqrt{\sigma^2(y)_+ \sigma^2(x)}}$$

Results and Discussions

The means square for genotype were highly significant (P≤ 0.01) for days to 50% heading, days to 50% flowering, number of leaves, panicle weight, grain weight, 100 seed weight and grain yield while, it was significant (P≤ 0.05) only for panicle length (Table 3). In contrast, no significant difference was recorded for plant height and stem girth. Analysis of variance showed significant differences amongst the genotypes for all the traits tested except for the stem girth and plant height suggesting existence of adequate genetic variability among the selected materials and they could be exploited for sorghum improvement. Similar result was reported by Tesfamichael *et al.* (2015), who also reported significant mean squares for grain yield and other agronomic. The mean performance of the released varieties for grain yield ranged from 667 kg ha^{-1} for SAMSORG 9 to 2000 kg ha^{-1} for SAMSORG 45 with an average of 1179 kg ha^{-1}. The top yielding variety, SAMSORG 45 out-yielded the best check, CF35:5 by 40 % (Table 4). Days to 50% flowering varied from 76 days for NR 71151 to 92 days for SAMSORG 39, while plant height ranged from 140 cm for SAMSORG 3 to 214 cm for SAMSORG 45. Grain weight ranged from 0.25 kg for SAMSORG 9 to 0.75 kg for SAMSORG 45

and 100 seed weight varied from 3.35 for SAMSORG 41 to 4.1 for CF35:5.

Grain yield was significantly and positively correlated with plant height (r = 0.47), number of leaves (r = 0.65), panicle weight (r = 0.82), 100 seed weight (r = 0.68) and grain weight (r = 1.00). In contrast, there was no significant correlation between grain yield and days to 50% heading (r = 0.25), days to 50% flowering (r = 0.21), stem girth (r = 0.08), and panicle length (r=0.40). This suggests that improvement in these traits will bring about an associated increase in grain yield (Table 5). Similar results was observed by Mahajan *et al.* (2011) and Warkad *et al.* (2010), who reported significant and positive correlation between grain yield and other agronomic traits in sorghum.

Conclusions

From the studies, it was inferred that the released varieties performed better than the checks and grain yield had significant and positive association with, plant height, number of leaves, panicle weight, 100- seed weight and grain weight. Hence, in the further improvement programme due importance may be given for these traits to improve genetic yield potential.

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Table 3: Analysis of variance for grain yield and other agronomic traits of ten sorghum varieties evaluated in Samaru, 2018.

Source of variation	df	Grain yield	Days to 50% heading	Days to 50% flowering	Plant height	Stem girth	Number of leaves	Panicle weight	Panicle length	Grain weight	100 seed weight
Replication	1	426320.00	0.80	5.00	1755.93	2.52	4.05	0.17	22.05	0.05	0.04
Genotype	9	361371.85**	66.33**	64.53**	1170.95	0.43	3.78**	24.38**	0.04*	0.05**	0.13**
Error	9	12729.87	5.91	7.88	419.47	09.6	0.59	0.0012	7.72	0.0017	0.013

*, ** Significant at 0.05 and 0.01, respectively.

Table 4: Mean Performance for grain yield and other agronomic traits of ten I.A.R sorghum varieties evaluated at Samaru in 2018

Variety	Grain yield (kg ha^{-1})	Days to 50% heading	Days to 50% flowering	Plant height (cm)	Stem girth (cm)	Number of leaves	Panicle weight (kg)	Panicle length (cm)	Grain weight (g)	100 seed weight (kg)
SAMSORG 3	933	76	79	140	7.30	11	0.70	23.50	0.35	3.45
SAMSORG 9	667	80	83	151	7.40	13	0.65	21.00	0.25	3.45
SAMSORG 38	1107	84	87	182	8.20	13	0.58	27.50	0.42	3.70
SAMSORG 39	800	89	92	176	7.60	10	0.60	19.00	0.30	3.45
SAMSORG 40	1300	74	77	206	8.20	12	0.85	27.00	0.47	3.50
SAMSORG 41	920	75	78	180	8.10	12	0.75	24.00	0.34	3.35
SAMSORG 45	2000	85	87	214	8.20	14	1.10	26.00	0.75	3.95
SAMSORG 46	1800	85	87	209	8.90	14	0.80	27.50	0.67	3.90
CF35:5	1200	75	78	181	8.90	13	0.80	19.00	0.45	4.10
NR 71151	1067	73	76	168	7.80	14	0.80	20.00	0.40	3.50
Mean	1179	80	82	181	5.58	12.60	0.76	23.45	0.44	3.64
C v	9.56	3.0	3.4	11.3	12.3	6.2	4.7	11.5	9.6	3.2
LSD	255.2	5.5	6.4	46.3	2.2	1.7	0.1	6.1	0.1	0.3

Table 5: Pearson correlation coefficients for grain yield and other agronomic traits of ten IAR released varieties evaluated at Samaru in 2018

	Heading date	Flowering date	Plant height	Stem Girth	Number of leaves	Panicle length	Panicle weight	Grain weight	100seed weight	Grain yield
Heading date	1									
Flowering date	0.99**	1								
Plant height	0.29	0.24	1							
Stem girth	0.01	-0.02	0.42	1						
Nos. of leaves	-0.12	-0.13	0.19	0.074	1					
Panicle length	0.12	0.05	0.42	0.29	0.30	1				
Panicle weight	-0.07	-0.07	0.25	-0.05	0.57**	0.08	1			
Grain weight	0.25	0.21	0.47*	0.08*	0.65**	0.40	0.82**	1		
100 seed weight	0.19	0.17	0.37	0.31	0.45*	0.03	0.48*	0.68**	1	
Grain yield	0.25	0.21	0.47*	0.08	0.65**	0.40	0.82**	1.00**	0.68**	1

*, ** Significant at 0.05 and 0.01, respectively. Nos. = number

CGBPB 025

ANATOMICAL CHARACTERIZATION OF CASTOR PLANT TOWARDS ASSESSING GENETIC DIVERSITY

*** Odeje S. C., Adamu, A. K[1]., Alonge S. O[1]., Adelanwa, M. A[1]. and Aminu M. A[1].**

*Department of Biological Sciences, Nigeria Police Academy, Wudil, Kano
Corresponding Author: chrissaliuodeje@gmail.com; +2347066815535, +2348053576274

ABSTRACT

Castor oil plants present in several anatomical forms are yet to be characterized in Nigeria. Thus information on the diverse nature of this plant is scarce. The aim of this research was to characterize the accessions using anatomical markers towards assessing their genetic diversity. The research materials comprised of 80 accessions obtained from the seed bank of the Institute for Agricultural Research, Ahmadu Bello University (A. B.U), Zaria, and National Cereals Research Institute (NCRI), Bida. The research was carried out in the Post-Graduate Laboratory of the Department of Biological Sciences, A.B.U., Zaria. Anatomical studies were done following the procedures of Abubakar (2010) and Lux et al. (2005). Data were collected from systematically sampled plants. Anatomical traits indicated low variation. The stomata type was predominantly tetracytic (88.8 %) while their epidermal cell walls were Sinous, Undulating and Straight, with the sinous type being the majority (46.25 %). In the stem, collenchyma tissues had mainly the angular type (88%), while the sclerenchyma tissues were predominantly fibrous. The cross-section of the leaf lamina revealed round (65 %) and flat (30 %) midribs. Coefficient of variation was generally low. Principal components analysis revealed that six principal components accounted for about 55.69 % of total variance with the first principal component only taking about 12.63 %. It can therefore be concluded that, anatomical traits were not significantly diverse in castor but promising traits can be selected for future studies.

Keywords: *Castor, Anatomy, Assessment, Diversity and Characterization*

Introduction

Castor belongs to the family Euphorbiaceae (Kyoung-in *et al.*, 2011). It has a monotypic genus (Ricinus) (Chakrabarti and Ahmad, 2008). It is distantly related to cassava, croton, true rubber tree (*Vasconcelos et al.*, 2012). It contains predominantly ricinoleic acid, an 18-carbon, monounsaturated fatty acid (Lijun, *et al.*, 2010). HIV protease inhibitor (Zhang *et al.*, 2001)

This plant has been reported to have a number of important uses. These include its use as an anti-fungal agent (Yalamanchili *et al.*, 2016; The oil has also been employed in Ayurvedic medicine to enhance memory (Dua *et al.,* 2009). It is also a great additive and powerful laxative that serves as remedy for ailments like Multiple Sclerosis, Parkinson's disease and Cerebral Palsy. It relieves pain from Rheumatism and Gastro-intestinal problems (Salihu *et al.*, 2014).

Global castor seed production is around one million tonnes per year (FAO, 2008). India is the world's leading producer with about 830, 000 tonnes annually (FAO, 2008). Interestingly, Nigeria produces just about 2000 tonnes of this non-edible but highly important crop annually (Daily Trust, 2014).

The greatest problem of castor oil plant is the low level of genetic variability which has been identified as a prerequisite for any genetic improvement programme. In Nigeria different forms of castor plants are seen but the lack of proper record of these diverse forms makes proper identification and classification difficult. Knowledge about the genetic wealth of available germplasms helps

in the development of new cultivars. Germplasm collection constitutes the world's most readily available source of plant materials (Allard *et al.*, 1991). Anatomical features are known to support morphological evidence and have been used to classify other plants (Ali *et al.*, 2009; Abdel-Raouf, 2012). In view of this, carrying out taxonomic characterization of plant species provides a wide range of data on the status of that species.

Materials and Methods

The field study was carried out at the Institute for Agricultural Research (IAR) farm, Department of Biological Sciences, Ahmadu Bello University, Zaria. Nigeria on Latitude 11° 12' North, Longitude 7° 33' East and on Altitude 610 metres above sea level (Osuhor *et al.*, 2004). The anatomical study was carried out in the Post-Graduate Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria. Nigeria. Zaria'

Collection of Plant Materials

Forty seed accessions of castor oil plant were each collected from the Institutes for Agricultural Research (IAR), Ahmadu Bello University (ABU), Zaria and National Cereals Research Institute (NCRI), Badeggi, Niger State. The research materials consisted of 27 accessions from the Guinea savanna agro-ecological region of Nigeria, including the Federal Capital Territory (FCT); 20 from the Sudan savanna and 4 from the Sahel savanna. Others include 15 accessions from the Rain-forest regions and 14 exotic genotypes. All accessions bearing 'Z' as the initial letter were obtained from Zaria, while those bearing 'B' were obtained from Badeggi (Table 1). They were put in paper bags and labeled accordingly until required for use.

Table 1 *Ricinus communis* accessions collected from different agro-ecological regions in Nigeria

	Agro-ecological Regions					
	Ricinus communis accessions code					
SN	GS	SUSSAS	SERF	SWRF	EX	
1	04Buam	04Zbkb	05Zngur	32Ziko	38Bikoy	52Bpt
2	06Blaf	05Zshk	19Zmgm	33Zmke	39Bleoy	53Bpt
3	10Bk/ala	06Zdub	23Zmtm	34Zigo	40Bgbm	57Bpi
4	10Bnkp	07Zsg	24Zmd	35Zoba	41Balj	61Bpb
5	16Bdkik	08Zdtm	-	36Zabi	47Bed	63Bpi
6	16Bdk	09Zjb	-	37Zakz	48Biwo64Bpi	
7	18Bdek	10Bkala	-	38Zumod	-	67Bpa
8	19Bdk	11Zkt	-	39Zisia	-	74Bpaf
9	22Bof	12Zbas	-	42Zowr	-	75Bag
10	23Bofu	13ZKat	-	-	-	86Bmo
11	24Blkj	14Zexs	-	-	-	89Bpsaf
12	26Bils	17Zang	-	-	-	94Bpru
13	27Bkw	18Zzng	-	-	-	01Zbr
14	28Basa	20Zshka	-	-	-	03Zbra
15	29Bile	21Zjib	-	-	-	-
16	32Bbd	26Zjig	-	-	-	-
17	36Bncng	27Zkdw	-	-	-	-
18	37Bbwd	28Zdtm	-	-	-	-
19	46Bom	29Zrm	-	-	-	-
20	50Bilw	30Ztwn	-	-	-	-
21	103Basa	-	-	-	-	-
22	02Zkab-	-	-	-	-	
23	15Zkba-	-	-	-	-	
24	16Zabj	-	-	-	-	-
25	22Zkab-	-	-	-	-	
26	41Ziga	-	-	-	-	-
27	24Zrmp	-	-	-	-	-

Key

SUS-Sudan savanna
GS-Guinea savanna
SAS-Sahel savanna
SERF-South-eastern rainforest
SWRF-South-western rainforest
EX-Exotic materials

Stem/Leaf Anatomical Studies

Stems and leaves of matured castor plants were harvested from the field at 10 weeks after planting (WAP). They were washed thoroughly in tap water, fixed in Formalin Acetic Acid (FAA) for 24 hours and transferred into 70 % ethanol (Abubakar, 2010). The parts to be processed were dehydrated by immersing in graded alcohol with the concentrations of 30 %, 50 %, 70 %, 95 % and absolute alcohol (100 %) in which the plant materials stayed for 2 hours in each grade of the alcohol (Saheed and Illoh, 2010). They were then cleared using the following procedure:

The plant materials were first put in a solution containing Alcohol (3 parts): Chloroform (1part) for 2 hours. After this,

the materials were transferred to a solution containing 50 % Alcohol (1 part) and 50 % Chloroform (1part) for 2 hours. This was followed by 25 % alcohol (1 part) and 75 % Chloroform (3parts) for 2 hours. The samples were then transferred to a solution of 0 % alcohol (0 parts) and pure chloroform (pure) for 2 hours after which it was left in pure chloroform overnight. The tissues were impregnated with paraffin wax for 24 hours in an embedding oven maintained at 70 °C.
The tissues were then embedded on the wooden block using Embedding Oven (Gallenhamp) Hotbox set at 60 ^{0}C with labels and orientation. They were trimmed and the block was mounted on the Rotary microtome for sectioning. Thin sections were cut from both the stem and leaf using a microtone machine version C740521 and set at 6 microns (µ). The sections were then mounted on the slide using Meyer's albumin and kept on the dryer (SUNVIC) for 24 hours. The slide dryer was maintained at 70 ^{0}C. They were later cleared with xylene for 10 minutes according to the technique of Lux *et al.* (2005). The procedure involved gumming the specimens to the slides by using adhesive then immersing in different changes of alcohol, i.e., 30 % -50 % - 70 % - 95 % for 2 minutes each. After this, it was transferred into two changes of absolute alcohol for 4 minutes each. The tissues were then transferred to xylene for 10 minutes. This was followed by staining with Safranin O, then counter-stained with fast-green and covered with cover slip on which was added a mountant (DPX). The specimens were viewed under light (Olympus and Celestron) microscopes using the low power objective lens, then high power objective lens.

Stomatal Studies

Epidermal peels were obtained using a pair of sharp forceps and razor blade on the lower surface of the leaves. (Abubakar, 2010) The peels were placed on a plain slide and stained with safranine and methylene blue before microscopic examination and data collection.

Data Collection

The following data were collected: -
Epidermal cell and Stomata numbers were manually counted using a tally counter; Stomata length (µm) and Width (µm) were measured using a calibrated eyepiece micrometer against the stage micrometer following the procedures of Gill and Nyamuame (1990) and Abubakar (2010) while, Stomata Index was calculated using the formular; SI = S/(S+E) × 100 (Tripathi and Mondal, 2012)
Where
S= No of stomata per unit area; E= No of epidermal cells per unit area
Stomata, cell wall patterns and, conducting tissues were classified into types.

Data Analysis

Mean and Standard deviation as well as coefficient of variation of the different anatomical parameters of the accessions were calculated.
Cluster Analysis was used to group the accessions into groups using Ward's hierarchical algorithm based on Squared Euclidean distances.
Data was processed using Statistical Analysis Software (SAS) version 10.

Results

Stomata Studies

The results showed that three types of stomata were observed. These included; tetracytic, anomocytic and anisocytic stomata. with the tetracytic type constituting about 88.8 % of the total accessions (Figure 1). The epidermal cell wall patterns were majorly Sinous, Undulating and Straight, with the sinous type being the majority (46.25 %). However, a few combination of the main types was also observed (Figure 2). In the stem, collenchyma tissues had mainly the angular type (88%) and a few plate collenchyma (Plate 2) while the sclerenchyma tissues were predominantly fibrous. The cross-section of the leaf lamina revealed round (65 %) and flat (30 %) midribs.

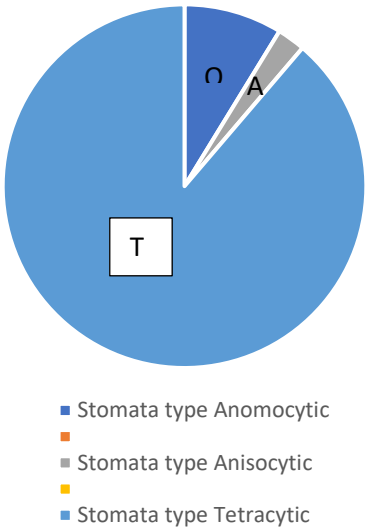


Fig 1: Percentage stomata types of 80 castor accessions
Key
T – Tetracytic (88.75%); O – Anomocytic (8.75%) ' A – Anisocytic (2.5%)

Plate collenchyma

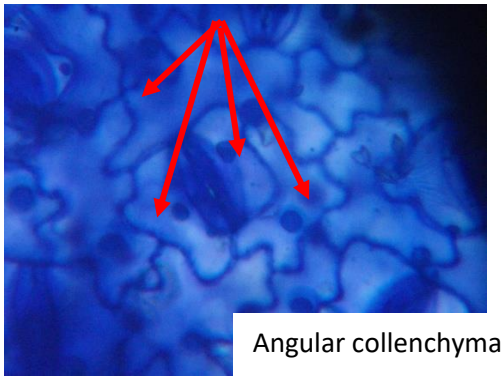


Plate 1: Tetracytic

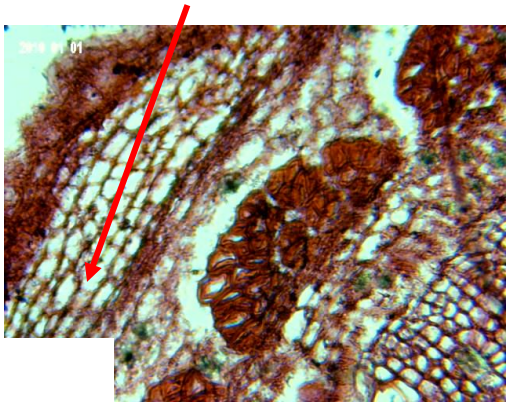


Plate 2: Plate Collenchyma

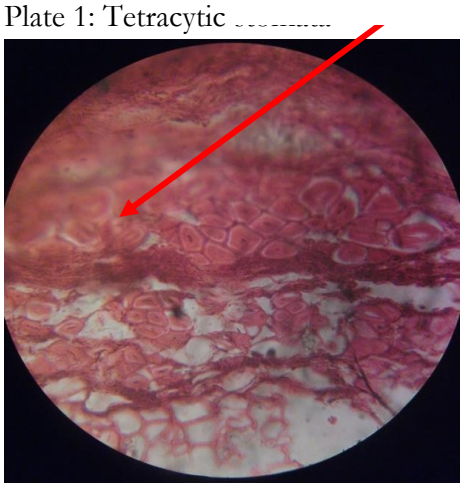


Plate 3: Angular collenchyma

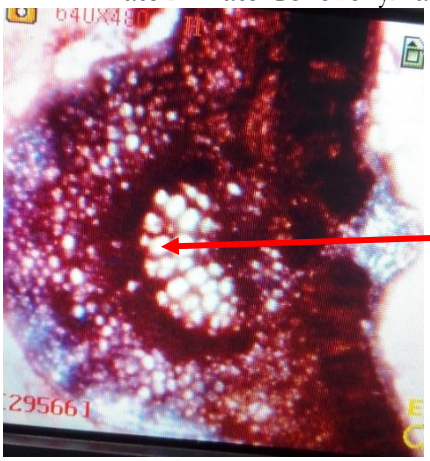
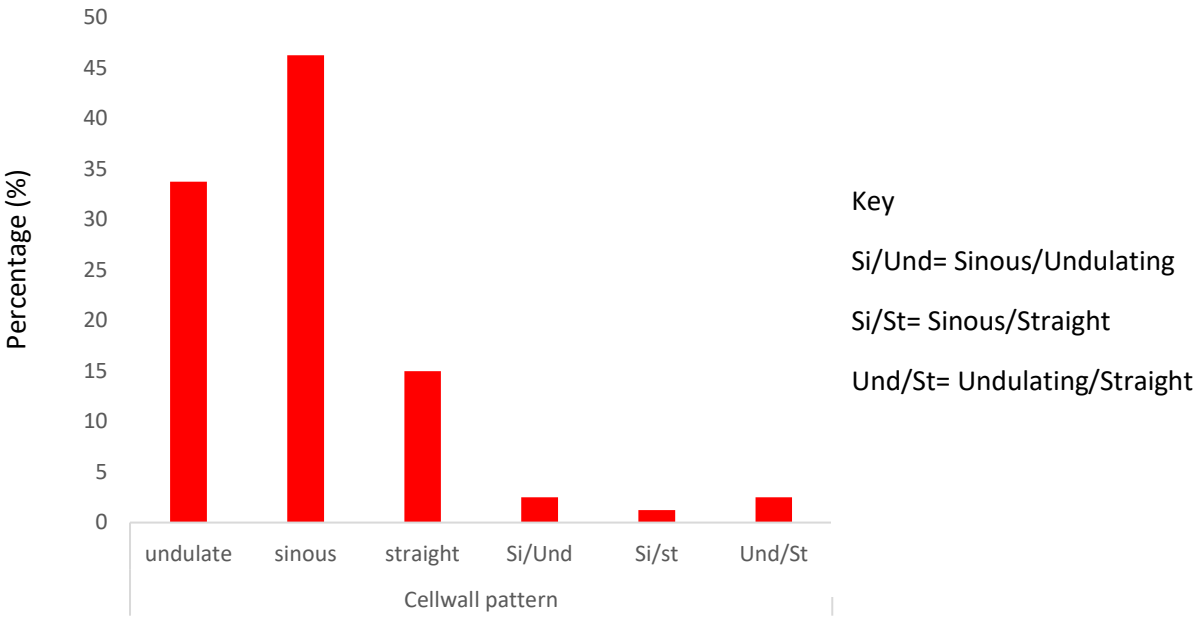


Plate 4: Centric vascular bundle



Cell wall pattern

Fig 2: Percentage cellwall pattern of 80 castor accessions

Cluster Analysis

The analysis indicated that, the 80 castor accessions were grouped into four distinct clusters with cluster 3 having the largest number of accessions (37). Twenty (20) of these accessions were obtained from Bida. However, Cluster 1 is bifolious with accessions from Katsina State (11Zkt) and Abia State (36Zabi) (Figure 3).

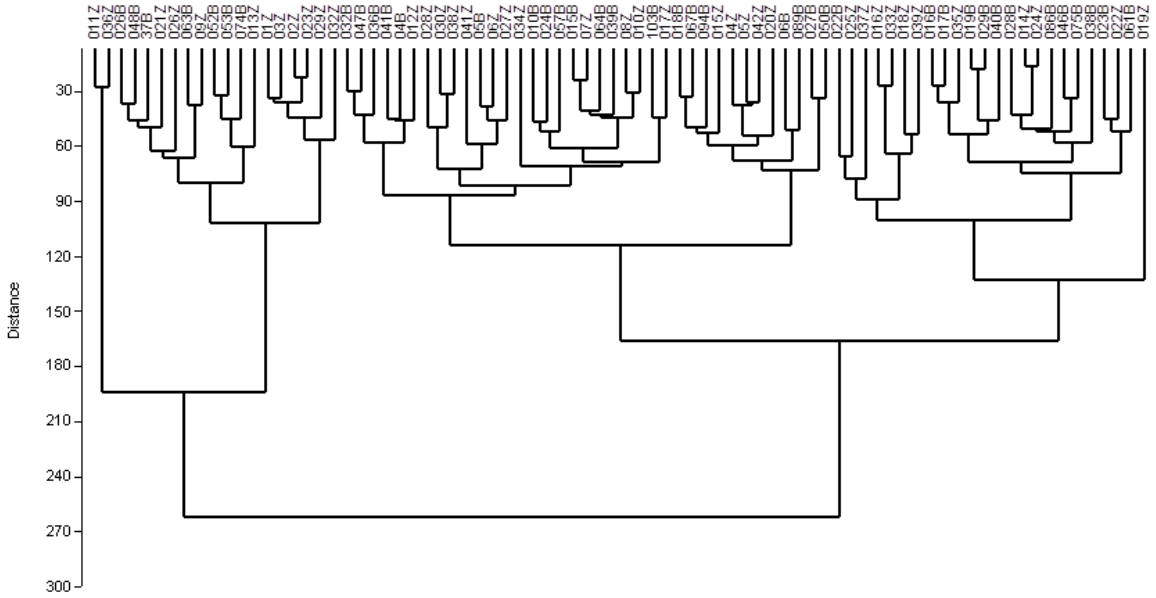


Figure 3: UGMA based on anatomical traits of 80 accessions using Euclidean similarity distance

The results further showed that the anatomical characteristics studied were significantly different (Table1). The results further revealed that the epidermal cell number, stomatal number length and width, as well as collenchyma thickness were observed to have maximum values of 182, 28.00 8.00, 47.90, 22.00 and 8.00.

The factor scores, eigen values and the total variance for each trait of the 80 accessions of castor oil plant. The five principal components accounted for about 56.02 % of total variance. The first principal component only accounted for about 16.69 %. The relative discriminating power of the principal axis was high (3.84) for axis 1 and low (1.52) for axis 5.

Table 2: Statistics of Anatomical traits record in 80 castor bean accessions

Trait	max	mini	mean	std	mean sq	cov	error
ECN	132.00	50.00	87.29	3.56	432.78**	1.04	0.82
STL	28.00	11.00	18.33	3.26	21.38**	4.38	0.65
STW	8.00	2.00	4.20	1.52	4.86* 17.46	0.54	
STI	47.90	12.30	28.21	6.52	81.51**	2.62	0.55
STN	22.00	6.00	13.53	3.02	17.56**	5.61	0.58
CoT	8.00	3.00	5.01	2.12	2.73* 17.80	0.79	

Discussion

The stomata length ranged from 11 to 28 µm between accessions, while the guard cell length ranged from 20 to 38 µm. This is an indication of distinct variation between accessions, however, these sizes are termed medium since stomata sizes less than 15 µm are regarded as small and those above 38 µm are termed large (Abdulrahaman and Oladele, 2003). The result of this research is in agreement with that of Taha *et al.* (2012) who reported a stomata size range of 20 to 35 µm in *Callistemon viminalis* and 20-30.83 µm in *Eucalyptus camaldulensis* but differs greatly with that of Abdulrahaman and Oladele (2003) who reported a range of 48.20 to140 µm between *Corchorus olitorius* and *Celosia argentea.*

Stomatal density was also observed to be low compared to that recorded by Abdulrahaman and Oladele (2003). This result shows that, in majority (86 %) of the accessions there were very few or no stomata on the upper epidermis of the leaves indicating low diversity. However, the mean stomata index of 28.21 % indicates a moderate occurrence as pointed out by Abdulrahaman and Oladele (2003) who stated that, a stomata index of 14 % is low while 73 % stomata index observed in *Amaranthus cruentus* is high. The narrow base of the stomata index observed indicates low diversity in the stomata distribution of these castor accessions used in this study. A similar result was reported by Tripathi and Mondal (2012).

Stomatal complexes are believed to be a significant character in the classification of plant species (Taha *et al.*, 2012). The work of Noraini and Cutler (2009) also confirmed the utilization of stomata and epidermis that are associated with it in the taxonomic significance of eight species of *Parashorea.* The stomatal complexes observed in this research showed that, despite other types of stomata such as anisocytic and anomocytic stomata observed in some instances, there was basically tetracytic type of stomata among the castor oil accessions studied. Considering that 90 % of the accessions possessed tetracytic stomata type, it can be said that, there was low diversity among these accessions for this trait. Gole *et al.* (2013), however, reported the predominant presence of paracytic stomata in *Hevea* species, a genus in the Euphorbiacea family, same as in castor, suggesting that, this family may not be the same in the type of stomatal complexes. Anisocytic and anomocytic stomata were observed in only few accessions. Bondada *et al.* (2006) also confirmed the presence of anomocytic stomata in Chinese brake fern, just as the importance of the diversity of this structure has been reported by Noraini and Cutler (2009) on eight species of *Parashorea* species.

The distribution of stomata in the present research revealed hypostomatic stomata in all the accessions. The lack of stomata on the adaxial surface could have resulted from the degeneration of guard cells with thickened walls, disintegrated nuclei and vacuolated cytoplasm (Gupta *et al.*,1968; Abubakar, 2010) or, it may also be as a result of adaptation to overcome certain environmental stress. Similarly, Radman (1985) reported a relationship between environmental conditions and stomata distribution in oat. According to the author amphistomatic stomata leaves would be selected for habitats in which water supply and demand fluctuate widely in seasonal or diurnal scale. However, the lack of stomata on the adaxial surface compared with the abaxial surface could have resulted from the degeneration of guard cells with thickened walls, disintegrated nuclei and vacuolated cytoplasm (Gupta *et al.*,1968; Abubakar, 2010). This indicates low diversity, especially as it cuts across all the accessions despite collecting them from different geographic locations.

The cellwall pattern ranges from sinous to undulating though it was rarely straight. These variations were seen intra- and inter-allelically, suggesting significant variation. Bondada *et al.* (2006) also observed such sinous anticlinal walls, while Abubakar (2010) also reported straight as well as undulating cell wall patterns alongside the sinous cell wall. The author's result on *Moringa oleifera* is in consonance with the results obtained from the present work. However, the author reported more undulating patterns compared with the sinous observed in the present work. Mbagwu *et al.* (2007) reported pentagonal, rectangular to hexagonal shapes of epidermal wall in some species of *Solanum*. This kind of discrepancy may be due to the different species of plant in response to different environmental conditions, and this may serve as a means of delimiting the accessions taxonomically. Furthermore, such different wall patterns may however be due to prevailing environmental conditions which could have led to increase in cellular turgidity and hence more waviness. A similar situation had been reported by Abubakar (2010) and Taha *et al.* (2012). However, Belhadj *et al.* (2007) opined that ecotypes can be different based on epidermal characteristics due to adaptation to the environment on which they grow.

Thus, the type of mesophyll was homogenous because, only dorsiventral type of mesophyll was observed in all the accessions studied, indicating low diversity in this trait. Dorsiventral types of mesophyll have also been reported in other plant species (Kantachot *et al.*, 2007; Armstrong *et al.*, 2012 and Taha *et al.*, 2012). However, it has also been reported that, isobilateral mesophyll types exist in the leaves of plant species such as *Eucalyptus* and *Eugenia* (Watson and Dallwitz, 1992). These were however not observed in the present work. This may serve as a delimiting factor for this accession. However, it is an indication of low diversity.

The shape of the vascular bundles which were usually in clusters showed that, some were rings, others were crescent or even a semi-circular arc, while others were in the form of an open arc. However, majority of the accessions showed crescent type of vascular bundles in their midribs. Crescent-shaped vascular bundles have also been reported in *Kalanchoe tormentosa* (Abdel-Raouf, 2012) in which the author also reported that, all the taxa studied showed this type of shape except *K. longiflora* which had ring form of vascular tissues.

The anatomical data used for clustering of the 80 accessions grouped the accessions into four distinct clusters which, like the morphological data, did not reflect any region-specific pattern. However, cluster 1 was observed to be bifolious, a characteristic of high variability. Ndir *et al* (2013) reported a similar finding in *Jathropha curcas*. The two accessions (11Zkt and 36Zabi) grouped together were from two different regions that is, Katsina in the North-west (11Zkt) and Abia (36Zabi) in the South-east, respectively. The two accessions may be the same as the seed characters, stomata tye and vascular bundle types suggest. Their stomata type was tetracytic and their vascular bundles were crescent.

Conclusion

In conclusion, the anatomical features of castor oil plant suggest low genetic diversity as structural variations at the anatomical level were observed to be few. Over 80 % of the accessions had tetracytic stomata, while 60 % of them had crescent vascular bundles. Also, collenchyma tissues were basically of the angular type with over 70 % of the accessions having this type of collenchyma. The cluster analysis revealed that, the anatomical dendrograms had few clusters, with large number of accessions clustered together. Cluster 3 had 37 accessions clustered together while, cluster 4 had 24 accessions.

Recommendation

Molecular analysis is recommended to ascertain the true identity of the accessions studied so as to enhance proper classification of this important non edible but very important crop.

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CGBPB 026

SPEED BREEDING: A POWERFUL TOOL FOR TRUNCATING BREEDING CYCLE THROUGH RAPID GENERATION ADVANCEMENT

***Yahaya, A.I.,[1] Mohammed, M.S.,[1] Alhassan, U.,[1] Mohammed, U[1]. and Bugaje, S.M.[1]**

Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria-Nigeria.
*Corresponding Author: abdulyahyu@gmail.com

ABSTRACT

With the world population growing at an unprecedented rate, more food will be needed to feed the populace. However, this could only be achieved if present breeding approaches are complemented with new emerging breeding techniques that could at least double or triple the present crop output. Development of new varieties is time consuming as it depends on the number of generation periods of a crop. Speed Breeding (S.B.), a new and exciting approach to breeding promises to develop new crop varieties faster, offering hope for global food security. It greatly shortens the generation time thereby accelerating the breeding time. The technique involves growing plants under continuous light (20–22 hours). This allows plants to photosynthesize for longer, resulting in faster growth. With this technique, four to six generations of wheat plants can be grown per year instead of two generations under normal growth conditions. The result is researchers develop new crop varieties quicker. Well established and standardized breeding protocols for other major crop species like barley, canola and perennial fruit crops like apple have been developed. The objective of this review write-up is to overlook speed breeding activities as carried out in different crops and its importance

Keywords: *Speed breeding, Crop improvement, wheat, Growth chambers, Greenhouse*

Introduction

According to Mundia (2020), Speed Breeding is a new and exciting approach to breeding originally inspired by the United States National Aeronautics and Space Administration (NASA) that promises to develop new crop varieties faster, offering hope for global food security. Speed breeding involves treatment of plants in a better quality of light, higher intensity of light and longer day length. Development of new varieties in any crop is time-consuming as it is dependent on the generation period of a crop. Speed breeding or accelerated plant breeding is an emerging strategy among plant breeders to develop new cultivars in a short span of time. This is because scientists are in a virtual race against time to produce better crops that will enable us to face future challenges. Among these challenges, the length of time taken to grow the plants from seed to seed stands foremost. The faster we shorten the cycle, the faster the results and produce crops faster. Under normal conventional breeding approach, it takes up to twelve years to transfer new sources of genetic resistance into adapted germplasm.

Pioneer efforts towards truncating generation periods of crops started with the wheat breeding activities of Norman Borlaug in Mexico (1945) referred to as "Shuttle Breeding". This involved growing wheat in two successive plantings per year-one during summer on the low soil fertility area under rainfed conditions and another during winter

at almost 2000km apart in the irrigated area. The locations therefore had contrasting growing conditions and soil fertility. Through this effort, two generations of wheat were grown in a year instead of one thereby cutting the breeding time by half.

Another breakthrough aimed at shortening breeding process was attained with the coming of the Doubled Haploid (D.H.) technique in the 80's. D.H. is a genotype formed when haploid cells undergo chromosome doubling. Artificial production of doubled haploids is important in plant breeding. According to SYNGENTA (2021), all corn-breeding companies nowadays use haploids to shorten the time required to produce parent lines by several years. Reduced time and increased efficiencies for scientists to develop new hybrids have the potential to bring about higher-yielding and better-adapted seed options for growers at a faster pace.

However, in Speed Breeding, the plants are grown in controlled growth chambers or greenhouses using optimal light intensity and quality, particular day length and temperature, these accelerate various physiological processes in plants especially photosynthesis and flowering, thus shortening the generation time. Speed breeding can be used to achieve up to 4–6 generations per year instead of 2–3 generations under normal glasshouse conditions using the technique,

It can therefore be used to transfer new sources of genetic resistance into adapted germplasm. According to Gosh *et al.* (2018), speed breeding approaches and protocols are well established and standardized for major crop species like wheat, barley and canola. This strategy is now being applied, and standardization protocols are in progress for other crops including perennial fruit crops like apple. Speed breeding could serve as a basic platform for integrating high-throughput phenotyping and genotyping techniques, marker-assisted/genomic selections and gene editing for improvement of the traits in crop species.

Application of speed breeding in different crops

The outlook of speed breeding activities carried out in different crops and its importance in present situation of crop improvement are as follows:

Wheat (*Triticum aestivum*)

NASA experiments to grow wheat in space were the inspiration for University of Queensland (Australia) scientists to develop the world's first 'speed breeding' procedures here on planet Earth. NASA experiments involved using continuous light on wheat which triggered early reproduction in the plants. With speed breeding, wheat generation from seed to seed can now be achieved in just 8 weeks. This means that it is now possible to grow as many as 6 generations of wheat every year – a threefold increase on the shuttle-breeding techniques currently used by breeders and researchers.

Through Speed Breeding, researchers are working to develop new crop varieties faster. This is due to the fact that the technique involves growing plants under continuous light (20–22hours). This allows plants to photosynthesize onger, resulting in faster growth. With this technique, four to six generations of wheat plants can be grown per year instead of two generations under normal growth conditions. The result is researchers develop new crop varieties quicker.

Canola

Speed breeding can be used to achieve up to four (4) generations for canola (*Brassica napus*) which is a form of rapeseed. This is a significant increase compared with widely used commercial breeding techniques which can result in 2-3 generations under normal glasshouse conditions.

Barley

Speed breeding can be used to achieve up to 6 generations per year for barley (*Hordeum vulgare*).

Chickpea (*Cicer arietinum*)

Compared to a glasshouse with a natural photoperiod, where only 2-3 generations of chickpea can be achieved per year, speed breeding enables 4-6 generations of this crop to be grown in a year.

Other crops

Speed breeding is likely to reduce generation time for other crop species, such as

sunflower (*Helianthus annus*), pepper (*Capsicum annuum*), and radish (*Raphanus sativus*), which have been shown to respond well to extended photoperiod. Speed breeding methods have already been successfully applied to accelerate breeding objectives for amaranth (*Amaranthus* spp.) and peanut (*Arachis hypogaea*). For species that require short days to trigger the reproductive phase, such as rice (*Oryza sativa*) and maize (*Zea mays)*, the speed breeding technique could be used to promote rapid vegetative growth prior to reducing the photoperiod. Recent advances in genomic tools and resources and the decreasing cost of sequencing have enabled plant researchers to shift their focus from model to crop plants. Despite such advances, the slow generation times of many crop plants continue to impose a high entry barrier. We envisage that combining these tools and resources with speed breeding will provide a strong incentive for more plant scientists to perform research on crop plants directly, thus further accelerating crop improvement research. In a breeding context, rapid generation advance to homozygosity following crossing will facilitate genetic gain for key traits and allow more rapid production of improved cultivars by breeding programs.

According to Mundia (2020): 'The amount of power needed for the lights and temperature control makes this too expensive for farmers to use. Breeders, however, often have to put their plants through many generation cycles, and in this context Speed Breeding can save both time and money. Therefore, Improved varieties can be made available quicker.'

Generally, crops that make significant contributions to Africa's food security have a lengthy generation time and complex biology. Therefore, Speed Breeding presents researchers and plant breeders with unique opportunities to fast track genetic improvements for important traits.

Establishing a Speed Breeding platform in Africa, can simultaneously increase access to modern and innovative methods of crop improvement while increasing the efficiency and cost-effectiveness of breeding for under-researched crops'.

Africa sits at the frontline of a changing climate system and is very vulnerable to climate change. Agriculture in sub-Saharan Africa needs a boost to feed the 600 million people currently experiencing food insecurity, and the extra 1 billion people expected to live in the next 30 years on the continent. In this light, developing better yielding, more nutritious and climate-resilient crop varieties faster is a major priority for Africa's researchers.

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CGBPB 027

GENE AND ENVIRONMENT INTERACTION WITH ESTIMATION OF VARIANCE COMPONENT FOR SEED YIELD IN SESAME (*Sesame indicum* L.)

[1]Ogah, G.O., [2]Onovo, J.C., [2]Allanana J.A. and [3]Babanlungu Z.A

[1]Department of Biotechnology, Federal University of Technology, Owerri, Imo State
Corresponding Author: omame14real@yahoo.com +2347030746477

ABSTRACT

The research was conducted at three different locations in Nasarawa State, Nigeria. The state lies within the guinea savannah region of Nigeria. The state has tropical climate with moderate rainfall (annual mean rainfall of 1100-1300mm). The soil is characterized by sandy clay loam, silt clay and clay loam. The locations selected include the Southern, Western and the Northern parts of the state. The areas include Lafia (Agricultural Field, Nasarawa State College of Agriculture Lafia), and Keffi (Plant Science and Biotechnology Garden, Nasarawa State University, Keffi), and Akwanga (Agricultural Farm, College of Education, Akwanga). The experiment was carried out during the 2012 cropping season (July to December). The varieties of sesame used include E8, Ex-Sudan, Boroko local, and NCRIBEN 01M. The varieties were obtained from National Cereals Research Institute Baddegi (NCRI) Niger State. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data were collected on the number of seeds/plant, above ground plant weight (g), 1000 seed weight (g), harvest index (%): value of the weight of seed divided by the above ground weight multiply by 100 gives the harvest index, seed production efficiency (%): weight of seed divided by weight of capsule, number of pods per axil. Data collected were subjected to Statistical Package for the Social Sciences (SPSS 16.0.1) and Additive Mean Multiplication Interaction (AMMI) Model was used to test for significance. The result obtained from the combined analysis of variance (ANOVA) for seed yield show that the effects due to environment, genotype and (GXE) were highly significant at (P <0.05). Keffi with the highest environmental index of 201.9 is recommended environment for higher seed yield whereas E8 with the highest yield performances in all the locations is also recommended as the best variety.

Keywords: *Gene, Environment, Interaction, Variance, Seed yield, Sesame*

Introduction

Sesame is an annual plant growing to 50 to 100 cm (1.6 to 3.3ft) tall, with opposite leaves 4 to 14 cm (1.6 to 5.5ft) long with an entire margin; they are broad lanceolate to 5 cm (2in) broad, at the base of the plant, narrowing to just 1cm(0.4in) broad on the flowering stem (Baydar *et al.,*2012). The flowers are yellow tubular, 3 to 5 cm (1.2 to 2.0in) long with a four-lobed mouth. The flower may vary in colours with some being white, blue or purple (Bedigian, 2011). Sesame fruit is a capsule, normally pubescent, rectangular in section and typically grooved with a short triangular beak. The length of the fruit capsule varies between 0.5 to 2.0 cm and the number of loculi from 4 to 12. The fruit naturally splits open (dehisces) to release the seeds by splitting along the septa from the top to bottom or by means of two apical pores, depending on the varietal cultivar, the degree of dehiscence is of importance in breeding for mechanised harvesting as is the insertion height of the first capsule (Bedigian, 2011). Sesame seeds are small, the size form and colours vary with the thousands of varieties now known (Beheshti & Fard, 2010). Typically, the sesame seeds are about 3 to 4 mm long by 2 mm wide and 1mm

thick. The seeds are ovate, slightly flattened and somewhat thinner at the eye of the seed (hilum) than at the opposite end. The weight of the seed is between 20 and 40 mg. The seed coat may be smooth or ribbed (Bhat *et al.,* 2012). Sesame seed come in many colours depending on the cultivar harvested. The most traded variety is off white colour. Other common colours are buff, tan, gold, brown, reddish, gray and black (Bisht *et al.,* 2011).Sometimes sold with its seed coat removed (decorticated). This is the variety often present on top buns in developed economies (Black-samulsson *et al.,* 2012). The aim of the research work was to investigate gene and environment interaction and estimation of variance component for seed yield in sesame.

Materials and Methods

The field experiment was carried out at three different locations within Nasarawa State, Nigeria. Nasarawa State lies within the guinea savannah region of Nigeria. The state has tropical climate with moderate rainfall (annual mean rainfall of 1100-1300mm). The soil is characterized by sandy clay loam, silt clay and clay loam. The locations were selected to include the Southern, Western and the Northern parts of the state. The areas include Lafia (Agricultural Field, Nasarawa State College of Agriculture Lafia), and Keffi (Plant Science and Biotechnology Garden, Nasarawa State University, Keffi), and Akwanga (Agricultural Farm, College of Education, Akwanga). The experiment was carried out during the 2012 cropping season (July to December). The varieties of sesame used include E8, Ex-Sudan, Boroko local, and NCRIBEN 01M. The varieties were obtained from National Cereals Research Institute Baddegi (NCRI) Niger State. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data were collected on the number of leaves, branches, flower, number of pods per axil, pod shape, pod beak, pod length and seeds per pods, plant height (meter rule was used to measure the plant height), lea shape, leaf length, leaf colours, flower colours, days to 50% flowering and days to maturity. Data collected were subjected to Statistical Package for the Social Sciences (SPSS 16.0.1) Analysis of Variance (ANOVA) and Additive Mean Multiplication Interaction (AMMI) Model was used to test for significance.

Results and Discussion

The result from the combined analysis of variance (ANOVA) for seed yield show that the effects due to environment, genotype and (GXE) were highly significant at ($P < 0.05$), this result is in conformity with the findings of (Bisht *et al.*, 2011)) in linseed. The mean seed yield of Sesame genotypes average over environments is presented on table 1. The mean seed yield at the individual environments ranges from 266.44kg/ha in Akwanga to 518.95kg/ha in Keffi. Keffi had the largest environmental index of 201.9 and therefore the most suitable environment for higher seed yield. On the other hand Akwanga recorded the least environment index of -178.1 and hence the poorest environment.

Table 1: Environmental mean seed yield (kg/ha), IPCA scores and index of sesame tested at three locations.

Environment	Environmental Index	Environmental mean (kg/ha)	IPCA1	IPCA2
Keffi	201.9	18.95	5.557	1.262
Akwanga	-178.1	266.44	3.850	0.484
Lafia	-108.1	410.82	-1.697	-1.231

Table 2: Genotype mean seed yield (kg/ha), IPCA1 and IPCA2 scores of sesame tested at three locations.

Genotype	yield/ha	IPCA1	IPCA2
E8	528.78kg/ha	4.851	2.921
Ex-sudan	351.38kg/ha	-1.127	0.782
Boroko local	251.56kg/ha	2.860	-0.323
NCRIBEN 01M	458.23kg/ha	-1.092	2.840

Additive Main Multiplication Interaction (AMMI)

The result from Additive Main Multiplication Interaction (AMMI) analysis showed that the first principal component axis (IPCA1) of the interaction capture 51.46% of the interaction sum of squares with 23 degree of freedom. Similarly, the second principal component axis (IPCA2) explained a further 27.42% of the interactions. The mean squares of both IPCA1 and IPCA2 were significant at P= 0.05 and cumulatively contributed 78.88% of the total Genotype and Environment Interactions (GXE). The result indicates that the AMMI model fits the data well; variability in both main effect and interaction (IPCA1) of the environment and genotypes for mean seed yield is shown in fig 2. Environment Keffi (kef) was the most favourable for all genotypes where maximum mean seed yield was recorded. Environment Lafia (Laf) also showed suitability of performance of all genotypes. Akwanga (Akw) was the least favourable environment for the performance of all genotypes and the lowest mean seed yield was also recorded in the location

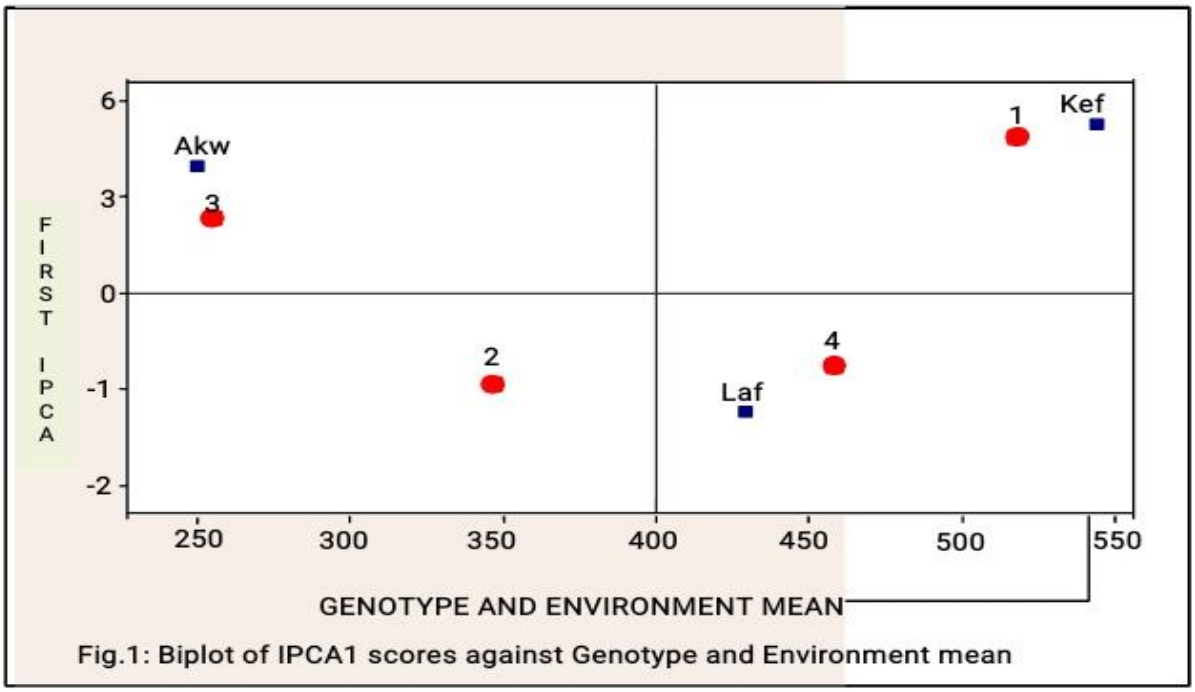

Fig.1: Biplot of IPCA1 scores against Genotype and Environment mean

Fig.1: Biplot of IPCA1 scores against genotype and environment mean (kg/ha) Kef=Keffi; Laf=Lafia; Akw=Akwanga, Gen (1) = E8, Gen (2) = Ex-Sudan, Gen (3) = Boroko Local, Gen (4) = NCRIBEN 01M

As indicated in (Fig.1), Environment Keffi (kef) shows suitability for performance for all the genotypes E8 (1), Ex-Sudan (2), Boroko local (3) and NCRIBEN 01M (4) in seed yield. Similarly, environment Lafia (Laf) showed suitability for performance of genotypes E8 (1), Ex Sudan (2), NCRIBEN 01M (4) While environment Akwanga (Akw) showed suitability for performance of genotypes E8 (1), Boroko Local (3). According to interaction principal component analysis (IPCA1) scores, genotypes that are concentrated around the origin were considered stable. As shown on Fig.1, genotype E8 was stable with high yield and genotypes Ex Sudan and NCRIBEN 01M are stable with average yield. Genotype Boroko local was stable with low yield, however, these genotypes shown high performance to environment. From the biplot (Fig.1) of the main effects and the first principal scores of interaction (IPCA1) of both genotypes and environments, the differences among genotypes in terms of direction and magnitude along the x-axis (environment mean yield) and y-axis (interaction principal component axis) IPCA1 scores are important. In the biplot genotypes or environment that appears almost on a perpendicular line of a graph had similar mean yields and those that fall almost on a horizontal line had similar interaction (Crossa *et.al,* 2012). Thus, the relative variability due to genotypic difference Genotypes or environments on the right side of the midpoint of the perpendicular line have higher yields than those on the left side. As a result, genotypes including Gen1 (E8), Gen 4 (NCRIBEN 01M), were generally high yielding (528.72 and 458.23kg/ha), in contrast, Genotype Gen2 (Ex Sudan) was generally average with 351.38 kg/ha while the genotype gen 3 (Boroko local) was having a low yield of 251.56kg/ha in comparison with the other three. Environments Keffi, Env1 and Lafia, Env2 were always on the right hand side of the midpoint of the main effect axis seemed to be favourable environments, while environment Akwanga, Env 3 on the left hand side was less favourable environment. Genotype or environment with large negative or positive IPCA scores have

high interactions while those with IPCA scores near zero (close to horizontal line) have little interaction across environments are considered the most stable than those further away from the line. In the biplot, genotype Gen1 and Gen4 were vertical distant apart; however, they did not fall close to the horizontal line. This implies that these genotypes lack stability but had high yield potential in favourable environments.

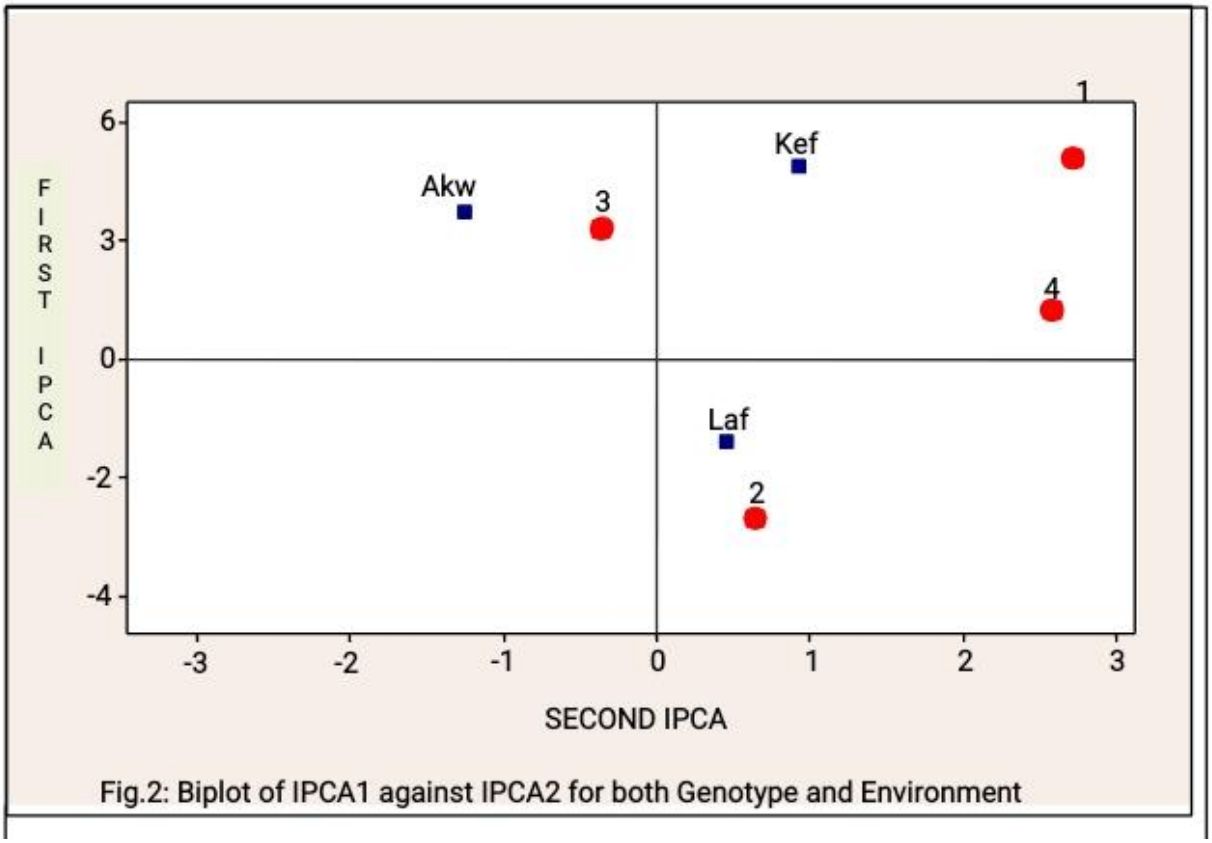

Fig 2: Biplot of IPCA1 scores against the IPCA2 scores for genotype and environment since, IPCA2 scores were also important in explaining genotype x environment interaction, the ballot of the first two IPCAs were also used to demonstrate the relative magnitude of the GEI for specific genotypes and environments (Figure 2). The IPCA scores of genotypes in the AMMI analysis or adaptation

Over environments (Gauch and Zonel, 2011), the greater the IPCA scores, the more specifically adapted is a genotype to certain environment (Sanni *et al.,* 2012). The more the IPCA scores approximate to zero, the more stable or adapted the genotype is over all the environments sampled. The biplot of the first two IPCA does not show the best adapted genotype and 1 or genotype to most environments. However, genotype E8 (gen1), Ex-Sudan (gen2), and Boroko local (gen3) NCRIBEN 01M (gen4) were adapted to high yielding environment of Keffi (Env.1) while genotype E8, NCRIBEN 01M were well adapted to the high yielding environment of Lafia (Env.2). The environments fell into three sections: the best genotypes with respect to Env.1 were gen1, gen2, and gen4, Env2 gen1 and gen4, Env.3 gen1. (Fig2.) Considering the environment tested in this study, no single location had both IPCA1 and IPCA2 close to zero line. This indicates that all the environments had potential for large GEI.

Conclusion

In conclusion, the environment Keffi was the most favorable for all genotypes where maximum mean seed yield was recorded. Environment Lafia also showed suitability of performance of all genotypes. Akwanga was the least favorable environment for the performance of all genotypes and the lowest mean seed yield was also recorded in the environment. On the other hand, genotype E8 performed better than the other three genotypes in the entire environments. Genotype NCRIBEN 01M perfomed very well in keffi and fairly in Akwanga and Lafia, genotype Ex sudan performed well in Akwanga and fairly in Lafia, genotype Boroko local performed fairly in Keffi and Lafia

Recommendation

Keffi with the highest environmental index of 201.9 is recommended environment for higher seed yield whereas E8 with the highest yield performances in all the locations is also recommended as the best variety.

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ASSESSING VARIABILITY FOR ROOT SYSTEM ARCHITECTURAL TRAITS IN FIELD-GROWN COWPEA WITH AND WITHOUT ADDED PHOSPHATE FERTILIZER

Abdulrazaq, Y. T[1]., *Mohammed, S. B[1]., Umar, M. L[1]. and Ishiyaku, M. F[1].

Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria Corresponding Author:bsmohammed@abu.edu.ng +2348069299881

ABSTRACT

A field experiment was conducted at one of the Institute for Agricultural Research low phosphorus field, Samaru Zaria in 2018 to assess the variability for root system architectural traits in field-grown cowpea. Twenty cowpea genotypes were grown at an intra and inter-row spacing of 0.20 m and 0.70 m respectively, with the application of 60 kgha^{-1} P_2O_5. The treatments were laid out in a randomized complete block design and replicated two times with two factors (low and high phosphorus). Data were collected on shoot dry weight, stem diameter, adventitious root number, basal root number, nodule number, nodule size, adventitious root growth angle, basal root growth angle, and taproot diameter at 5, 10, and 15 cm. The results showed that root architecture traits in cowpea varied between genotypes and in response to contrasting soil P conditions. Application of 60 kgha^{-1} of SSP resulted in enhanced dry fodder production and expression of root characteristics. A significant positive association was found between stem diameter and root architectural traits. The presence of correlation between stem diameter and root traits could be used to select for desired root phenotype, since stem diameter has the advantage of being easily determined non-destructively during the growth period unlike dry weight or P uptake efficiency. These findings will be useful for cowpea breeding programmes targeting the selection of genotypes with a well-developed root system for the development of drought-tolerant and P efficient varieties.

Keywords: *Cowpea, Root System Architecture, Phosphate fertilizer, Phosphorus use efficiency*

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a grain legume of global importance especially in sub-Saharan Africa, Brazil, India, and southern parts of the United States (Boukar *et al.,* 2018). Africa accounts for more than 65% of global production with Nigeria and Niger producing over 50% of the World cowpea grains (FAOSTAT, 2016). Cowpea grains are rich in protein and supplement major cereal-based meals in forming a nutritious diet in many areas (Snapp *et al.,* 2018). All parts of the crop including grains, fresh pods, leaves, and stalks serve as either food for humans or feed for livestock (Dixon *et al.,* 2003). Cowpea is cultivated in most parts of West Africa in a mixed intercrop with major cereals (Mohammed et al., 2021). Owing to its ability to fix atmospheric nitrogen (N) in association with *Bradyrhizobium* species, the crop replenishes soil fertility by increasing N availability (Olufajo & Singh, 2002).

Cowpeas are known to be relatively tolerant to drought and heat when compared with other cereals, but its productivity is constrained by several factors. Its yield in smallholder farmers' fields is low (< 600 kg/ha) as against the genetic potential of 2, 000 – 2, 500 kg/ha (Boukar *et al.,* 2018) as a result of several stresses including insect pests, diseases, and parasitic weeds, drought, heat, poor soil fertility especially low nitrogen (N) and soil phosphorus (P) (Mohammed *et al.,* 2021). In addition, poor agronomic and cultural practices like low plant population because of intercropping and low to no use

of phosphate fertilizers are among the reasons for the low productivity of the crop (Mohammed *et al.,* 2021; Olufajo & Singh, 2002).

Soils in most growing areas of West Africa are low in available P, an important nutrient required for normal growth, several biological processes, N fixation, maturity and development of cowpea and other legumes (Hussain, 2017; Sanginga *et al.,* 2000). Some reports have indicated that over 75% of African arable lands are P deficient (Cordell *et al.,* 2009). The easy and quick fix for soil P deficiency would be the use of synthetic P based fertilizers like single super phosphate (SSP), triple superphosphate (TSP), or diammonium phosphate (DAP). However, there are several limitations to the use of this alternative; first, the World rock phosphate reserves, the main raw material; rock phosphate (RP) for producing phosphate fertilizer is limited and fast depleting (Cordell *et al.,* 2009). In most African countries, the cost of producing P fertilizer is too high and coupled with the fact that there are few fertilizer producing plants in the continent, and fertilizers are manufactured and transported from abroad, thereby making it cost-prohibitive for smallholder farmers to apply sufficient quantity and causing untimely availability (Mohammed *et al.,* 2020). Similarly, many farmers are of the wrong view that legumes including cowpea do not require fertilizer application and thereby do not mostly supply cowpea plants with the recommended fertilizer package (Mohammed *et al.,* 2020). There is also the problem of P fixation in many soils due to their acidic nature, thereby leading to low uptake of P by plants and leaving a larger amount of P in the soil in unavailable forms (Mehra *et al.,* 2015). Due to the challenges associated with P fertilization for cowpea production, the most sustainable strategy to address these problems is breeding programmes targeting genetic improvement for P acquisition and use of efficiencies in cowpea. There is a body of literature that showed sufficient genetic variability and potential to select cowpea lines for adaptation to low P soil and response to added P fertilization (Saidou *et al.,* 2012; Sanginga *et al.,* 2000).

Most of these earlier investigations have exploited the above-ground plant parameters like shoot biomass production, yield components, harvest index to predict crop adaptation and response to P under limited and optimum P conditions (Sanginga *et al.,* 2000). Roots are important for anchorage, uptake of water and mineral elements including N, P, and K from the soil for plant use (Paez-Garcia *et al.,* 2015). Roots are also involved in interactions between the plant and the microorganisms within the rhizosphere such as creating a barrier that inhibits pathogen and pests penetration into the plant (Richardson *et al.,* 2011). The root system arises from the coordinated control of an endogenous genetic system and the action of environmental stimuli such as the pH, temperature, water level, microbial and chemical constituents. The nutrient level, health, and type of substrate strongly influence the rooting characteristics, that is; the root growth, structure, and architecture of the root system, as such, a healthy root system plays a vital role in crop productivity (Wissuwa *et al.,* 1998).

There have been limited attempts to explore the utility of cowpea root system architecture (RSA) in the genetic improvement programmes, partly due to its underground nature and limited phenotyping approaches that will permit screening a large number of plants that are usually conducted in most breeding programmes. However, there are success stories on how RSA has been successfully deployed to improve crop plants for tolerance to drought, low soil N in crops such as soybean, common bean, maize (Lynch, 2013; Zhan *et al.,* 2015). Targeting and deploying RSA traits in crop plants including in cowpea selection schemes, presents a novel strategy to develop varieties that will be of great utility under sub-optimal soil fertility and changing climate conditions, and enhanced capacity for exploration of soil environments for better water and nutrient absorption (Paez-Garcia *et al.*, 2015). Some of the earliest work on cowpea RSA have indicated four root classes, namely; tap,

adventitious, basal, and lateral roots as main root classes for field-grown cowpeas (Burridge *et al.,* 2016; Kahn & Schroeder, 1999; Kahn & Stoffella, 1987) while other reports have argued that cowpea root system consisted of adventitious, lateral and taproots (Lynch, 2013), suggesting that basal roots do not exist in cowpea. A framework for an RSA is hereby given as taproot (the first root to emerge from the seed), adventitious roots (first-order lateral roots (shoot-borne roots) emerging from the hypocotyl), lateral roots (which are branches of other roots) and basal roots (root which develops from the hypocotyl, that is, the organ which is between the base of the shoot and base of the taproot (Zobel & Waisel, 2010).

There is limited information on the extent of genetic variation for cowpea root classes under different soil P conditions and the need to validate the root classes expressed by cowpea under field conditions. Therefore, the present work focused on the root system architecture of cowpeas grown under field conditions with and without P fertilization and was conducted to achieve the following objectives: (i) to determine the root classes of field-grown cowpeas under varying P fertility of the soil, (ii) to quantify genetic variation for cowpea root traits under low soil P, and (iii) to investigate the relationship between cowpea root traits and stem diameter.

Materials and Methods

Plant Materials

Twenty elite cowpea genotypes, sourced from the cowpea breeding unit of the Institute for Agricultural Research, Ahmadu Bello University Zaria (IAR/ABU) were used in this study. Table 1 below shows the names of the lines.

Table 1: the list of elite cowpea genotypes used for the study

1	IT82E-18	11	SAMPEA 12
2	IT84S-2049	12	SAMPEA 14
3	IT84S-2246-4	13	SAMPEA 15
4	IT86D-1010	14	SAMPEA 16
5	IT90K-822-7	15	SAMPEA 17
6	IT97K-556-6	16	Sanzi
7	IT99K-573-1-1	17	SuVita2
8	SAMPEA 1	18	TN-256-87
9	SAMPEA 10	19	Vita-7
10	SAMPEA 11	20	Yacine

Description of the experiment site

The experiment was carried out at one of the low P fields of IAR/ABU Samaru in 2018. Samaru is located on 11°10'31.7"N and 7°36'43.9"E *(Garmin GPS$_{map}$ 78s)* in the northern guinea savannah of Nigeria and has a unimodal rainfall with annual precipitation of 1,000 – 1, 200 mm (NAERLS *et al.,* 2017).

Experimental design and field management

The experimental area was 15 m x 6 m (90 m^2). The land was sprayed with glyphosate (*Round-up*) at the rate of 4 l/ha, then ploughed and harrowed twice. A randomized complete block design with two replications and two factors (low and high soil phosphorus and twenty cowpea genotypes) was used. Table 1 shows the list of the genotypes used. P was supplied to the high P treatment at 60 kg/ha^{-1} on the seventh day after sowing (DAS). The low P treatments did not receive an external application of SSP fertilizer. Before sowing, seeds were treated with a broad-spectrum commercial fungicide *(AllStar),* at a rate of 4 kg of seeds to a sachet of 10 g based on the manufacturer's recommendation. Sowing was done on the 5th July 2018 at an intra and inter-row spacing of 0.20 m and 0.75 m. The plot size was one row of 2 m. Plants were

protected against insect pests, and weeds using recommended insecticides and hoe weeding, respectively.

Root sampling and phenotyping

At eight weeks after sowing, two plants were uprooted from each plot using shovels, a procedure known as *Shovelomics* (Burridge *et al.,* 2016; Trachsel *et al.,* 2011) for measurement of shoot dry weight and root architecture traits on the root crowns. To excavate a root, a shovel was placed at a radius of around 20 - 25 cm from the base of the plant and to a depth of 15 - 25 cm into the soil, and the plant is then gently uprooted. Secateur was used to detach the shoot from the root crown. The excavated roots were washed, labelled, tagged and inserted into a container of water before architectural traits were measured.

Data collection and analysis

The following data: shoot dry weight, stem diameter, adventitious root number, basal root number, nodule size, nodule number score, adventitious root growth angle, basal root growth angle, taproot diameter at 5, 10 and 15 cm were measured with the aid of a compass-like root scoreboard. The stem and taproot diameters were recorded using a digital Vernier calliper. Shoot samples were air-dried in the screenhouse under ambient temperature until the stable dry weight was attained and the weight was recorded with a digital scale. Data collected were analyzed using the general linear model in SAS for significant differences. Means were generated and used for comparison between high and low P treatments. Pearson correlation coefficients were estimated using the R *corrplot* package.

Results

Expression of various root classes of field-grown cowpeas under varying soil P conditions

The results revealed that four classes of root types are produced by cowpea genotypes grown under field conditions in response to soil P nutrients. The root classes are adventitious, basal, taproots and lateral roots found on them. The root types were found on cowpea plants under both low and high P treatments, though were generally more and larger in high P soil condition. The number of these root types expressed by cowpea plants varied between genotypes and in response to P treatments imposed on the soil. For instance, the basal roots ranged from 4 – 17 and 6 -15 under high and low P conditions, respectively. The width of the tap roots were assessed by taking the diameter (mm) at different intervals of 0 – 5, 5 – 10 and 10 – 15 cm of the tap root length (Table 2). In this study, lateral roots which are secondary roots that emerged from adventitious, basal and tap root roots were observed across all the genotypes evaluated, but were not measured.

Table 2: Cowpea root classes expressed in field soils with contrasting phosphorus concentration at Samaru in 2018

	High P					Low P				
Genotypes	ADRN	BRN	TAP5	TAP10	TAP15	ADRN	BRN	TAP5	TAP10	TAP15
IT82E-18	11	9	5.2	3.2	1.0	13	14	4.2	2.3	1.0
IT84S-2049	11	10	4.1	3.3	0.6	10	7	5.3	3.6	0.8
IT84S-2246-4	7	14	6.2	4.4	2.2	7	10	3.3	2.1	0.7
IT86D-1010	8	10	6.1	3.8	2.2	7	9	2.7	1.0	0.1
IT90K-822-7	9	15	4.4	3.3	1.1	11	8	3.5	0.6	0.0
IT97K-556-6	7	7	5.6	3.6	1.2	6	7	1.9	1.0	0.7
IT99K-573-1-1	8	4	6.8	5.2	2.6	7	12	3.3	1.5	0.8
SAMPEA-1	13	17	5.5	2.8	1.5	11	13	1.7	1.2	0.2
SAMPEA-10	9	8	4.1	2.9	1.9	5	7	3.0	1.7	0.4
SAMPEA-11	11	13	4.7	3.6	1.6	9	15	3.3	2.0	1.0
SAMPEA-12	9	13	5.0	3.0	1.4	10	9	2.8	1.2	0.7
SAMPEA-14	8	10	5.7	2.9	1.5	6	11	3.0	2.1	1.0
SAMPEA-15	7	7	6.0	13.0	2.0	6	9	3.1	0.9	0.5
SAMPEA-16	11	12	6.4	2.7	1.7	10	13	3.0	1.5	0.5
SAMPEA-17	7	8	6.6	3.8	1.6	9	6	3.3	2.3	0.8
Sanzi	8	7	3.6	2.2	2.0	8	10	1.7	1.0	0.4
SuVita2	7	11	7.5	2.9	1.6	12	7	3.7	1.8	0.7
TN-256-87	11	16	4.1	2.5	1.3	7	6	2.0	0.8	0.2
Vita-7	6	10	5.6	4.0	1.9	10	9	4.4	2.9	0.9
Yacine	13	8	5.1	2.8	5.0	8	9	3.4	1.6	0.4
Mean	9	10	5.4	3.8	1.8	8	9	3.1	1.6	0.6
Min	6	4	3.6	2.15	0.6	5	6	1.7	0.6	0.01
Max	13	17	7.5	13	5	13	15	5.3	3.6	1.0

ADRN = adventitious root number, BRN = basal root number, TAP5,10, & 15 = Taproot diameter at 5, 10, & 15 cm. *Values are means of the genotype for the traits measured.

Effect of P-deficiency (low P) relative to P fertilized (high P) conditions on the performance of cowpea genotypes

The P availability of the field used in this study was low at 2 - 6 mg Pkg^{-1} (Bray I- P). The low P availability was the main growth-limiting factor of the genotypes evaluated in P-deficient treatment, as shown by a 60.5% reduction in dry fodder weight, 36% in stem diameter, 20% nodule number, and 68% in taproot diameter at 10 - 15 cm of taproot length (Table 3). These reductions were caused by the low inherent P content of the soil leading to decrease in P uptake by the genotypes. The response of the genotypes was contrasting in the two P treatments, with performance under high P being generally superior over the low P. Though, some genotypes such as IT82E-18, IT84S-2246-4 and SAMPEA 15 produced high fodder dry weight compared to others under the low P treatment (Table 4).

Table 3: Relative reduction in performance of cowpea genotypes under contrasting soil P conditions evaluated as Samaru in 2018

	High P	Low P	Percent reduction (%)
DRYFODWT	35.5	14.0	60
SD	10.0	6.4	36
ADRN	8.9	8.3	6
BRN	10.3	9.4	9
NNS	2.3	1.9	20
NSS	1.8	1.1	38
TAP5	5.4	3.1	43
TAP10	3.8	1.6	57
TAP15	1.8	0.6	68

DRYFODWT = dry fodder weight (g), SD = stem diameter (mm), ADRN = adventitious root number, BRN = basal root number, NNS = nodule number score, NSS = nodule size score, TAP5,10, & 15 = Taproot diameter at 5, 10, & 15 cm. HP plots received the equivalent of 60 kg P_2O_5 ha^{-1}, while LP plants were grown on low P soil (2 - 6 mg Pkg^{-1} soil Bray I -P).

Table 4: Differential response of cowpea genotypes to varied P concentration in a low P field at Samaru in 2018

	Dry fodder weight (g)			**Stem diameter (mm)**			**Adventitious root number**			**Basal root number**		
Genotypes	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean	HP	LP	Mean
IT82E-18	30.5	14.2	22.4	8.4	7.4	7.9	11	13	12	9	14	11
IT84S-2049	16.3	11.6	14.0	6.3	7.7	7.0	11	10	11	10	7	9
IT84S-2246-4	49.2	14.0	31.6	13.4	8.7	11.0	7	7	7	14	10	12
IT86D-1010	39.1	8.3	23.7	10.4	6.2	8.3	8	7	8	10	9	9
IT90K-822-7	42.3	4.3	23.3	10.4	6.1	8.2	9	11	10	15	8	12
IT97K-556-6	37.2	7.8	22.5	15.0	5.5	10.2	7	6	6	7	7	7
IT99K-573-1-1	38.6	8.4	23.5	12.0	7.1	9.5	8	7	7	4	12	8
SAMPEA-1	44.3	5.4	24.9	12.6	6.2	9.4	13	11	12	17	13	15
SAMPEA-10	25.0	4.4	14.7	8.7	5.0	6.8	9	5	7	8	7	8
SAMPEA-11	43.5	12.2	27.8	11.7	5.5	8.6	11	9	10	13	15	14
SAMPEA-12	32.2	8.0	20.1	8.7	6.1	7.4	9	10	9	13	9	11
SAMPEA-14	30.0	7.7	18.8	7.6	6.9	7.2	8	6	7	10	11	10
SAMPEA-15	37.2	16.7	27.0	5.4	5.5	5.4	7	6	6	7	9	8
SAMPEA-16	27.0	9.5	18.2	11.4	6.0	8.7	11	10	10	12	13	13
SAMPEA-17	33.9	7.2	20.6	9.9	5.4	7.6	7	9	8	8	6	7
Sanzi	30.0	12.1	21.0	6.5	4.5	5.5	8	8	8	7	10	9
SuVita2	45.4	10.0	27.7	11.5	8.3	9.9	7	12	9	11	7	9
TN-256-87	37.2	6.1	21.6	8.9	4.9	6.9	11	7	9	16	6	11
Vita-7	39.4	5.6	22.5	11.3	7.9	9.6	6	10	8	10	9	10
Yacine	31.1	9.7	20.4	10.4	7.1	8.7	13	8	10	8	9	9

* HP plots received the equivalent of 60 kg P_2O_5 ha^{-1}, while LP plants were grown on low P soil (2 - 6 mg Pkg^{-1} soil Bray I -P).

Relationship between root traits of cowpea genotypes grown under two soil P conditions and shoot parameters

The correlation coefficients between the root architecture traits measured revealed a highly significant correlation between stem diameter and dry fodder weight at high P (r = 0.6), whereas the adventitious root number was moderately correlated with stem diameter at low P (r = 0.4) (Figure 1). Similarly, the following pairs of traits had significant positive correlations; stem diameter and Tap5 in LP (r = 0.7), stem diameter and Tap10 in LP (r = 0.6), adventitious root growth angle and stem diameter in LP (r = 0.4),) while a significant negative correlation was observed between Tap5, Tap10, stem diameter (all in LP) and adventitious root growth angle at HP (Figure 1).

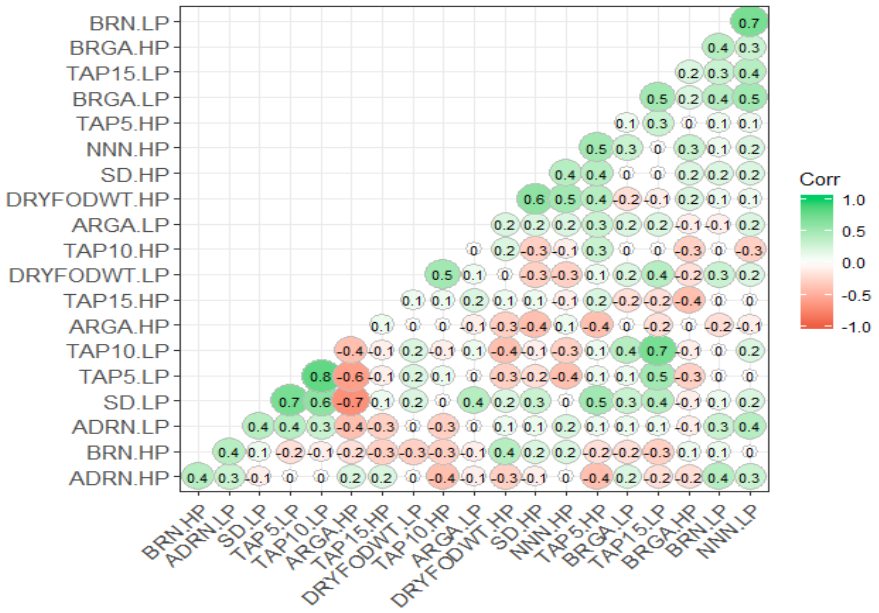

Figure 1: Association of various root architecture traits of cowpea and shoot parameters measured from contrasting soil P conditions. * HP plots received the equivalent of 60 kg P_2O_5 ha^{-1}, while LP plants were grown on low P soil (2 - 6 mg P kg^{-1} soil Bray I -P). Positive correlation coefficients in blue and negative correlation coefficients in red, specify a positive and negative association, respectively, between given traits. The higher the intensity of the green colour, the stronger is the strength of the association, while the higher the intensity of the orange-red colour, the weaker is the relationship.

Discussion

Root architecture traits of 20 genotypes of cowpea assessed under two contrasting soil P conditions in the field revealed the presence of four root types, namely; adventitious, basal, taproot and lateral roots. The laterals were not measured in this present study but were observed on adventitious, basal and taproot systems. This report corroborated the first known reports on cowpea root architecture, where four classes were identified for cowpeas grown under field conditions in the USA (Kahn & Schroeder, 1999; Kahn & Stoffella, 1987, 1991). This report presents clearer evidence over the explanation given in a review by Lynch

(2013) that the cowpea root system does not express the basal root class. The conclusion by Lynch (2013) may be because basal roots are not quite distinct in some cowpea genotypes, a phenomenon observed in this study. Appropriate knowledge of the morphological root characteristics of the crop will permit efficient characterization for selection and breeding of varieties with target root types suitable for specific edaphic conditions such as areas prone to drought, and those with low available plant P. P is reported to be more available in the upper subsoil (0 - 20 cm), thus, genotypes with topsoil foraging ability are suitable in exploring soil environment for efficient uptake of P (Ho et al., 2005; Liao et al., 2001; Miguel et al., 2015).

Findings from this study also revealed, there are significant differences between genotypes evaluated for dry fodder weight under two soil P nutrients, with the performance of high P treatments superior over the performance of plants in the low P plots. P is the most crucial micronutrient after N that plays a vital role in supporting normal growth, and several biochemical processes especially for legumes including cowpea (Krasilnikoff *et al.* 2003; Sanginga *et al.* 2000). The high dry fodder yield for high P treatments over the low P is attributed to the application of 60 kg P_2O_5 ha^{-1} (SSP). The differential response to P fertilizer will enable the selection of genotypes with good P uptake ability since not all genotypes have high P uptake from soils even when P is adequately applied. P uptake from fertilized soils has been reported to be low in most tropical environments, with estimates ranging from 10 - 30% P uptake in the first year, while the uptake in the succeeding years decreases, thereby making uptake of P by plants below optimum (Reynolds *et al.* 2012). Furthermore, a significant positive correlation was found between stem diameter and some root traits, such association could be used as an indirect selection tool and will enable non-invasive characterization of root architecture traits of the crop when validated.

Conclusion and Recommendation

From the results of this experiment, it is concluded that four root classes are expressed by cowpea under field conditions. The root architecture traits in cowpea varied between genotypes and in response to contrasting soil P conditions. Application of 60 kg ha^{-1} of SSP resulted in enhanced production of dry fodder and expression of root characteristics. The presence of correlation between stem diameter and root traits could be used to select for desired root classes, since stem diameter has the advantage of being easily determined non-destructively during the growth period unlike dry weight or P uptake efficiency. It is recommended that these genotypes should be further evaluated in more locations and under different levels of soil P content to determine the consistency of root traits expression, and performance of the genotypes in P deficient soils. Stem diameter can be used as an indirect indicator of measuring root architecture traits in cowpea, if it is validated in a further study.

Acknowledgements

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DECIPHERING EFFECTS OF SEED SIZE AND COTYLEDON CLIPPING ON LOW PHOSPHORUS TOLERANCE OF COWPEA

Sani, I., *Mohammed, S. B., Umar, M. L. and Ishiyaku, M. F.

Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria
Corresponding Author: bsmohammed@abu.edu.ng; +2348069299881

ABSTRACT

Cowpea is a vital grain legume especially in sub-Saharan Africa. The productivity of the crop is constrained by several biotic and abiotic factors. Drought and poor soil phosphorus (P) are major abiotic factors limiting the productivity of the crop in the tropics. Earlier works have shown the presence of genetic diversity for the tolerance of cowpea genotypes to low soil P and its response to applied phosphate fertilizers. The aim of the current study was to determine the effect of seed borne P on the performance of cowpea in low P growth medium through cotyledon clipping. To achieve the objectives of the study, nine genotypes of cowpea of varying seed size were screened in a low soil P medium by clipping their cotyledons after emergence in a screenhouse at the Department of Plant Science, Ahmadu Bello University, Zaria. Cotyledon clipping was carried out at 6, 7, 8, 9 and 10 days after sowing. Data were collected on days to flowering, plant dry (shoot and root) weight, pod dry weight, and seed dry weight. The results showed that, no significant differences for all the variables measured among the genotypes differing in seed size, indicating that cotyledon clipping of the germinating seeds has no substantial effect on the performance of the crop in a low P soil. These findings are important and will be useful for cowpea breeding programmes and agronomists targeting the development of P efficient varieties and deciphering mechanisms of low P tolerance of cowpea.

Keywords: *cowpea, cotyledon clipping, seed size, phosphorus*

Introduction

Cowpea *(Vigna unguiculata (L.)* Walp*)* is considered a staple food in many parts of the World including Brazil, India and many parts of Africa. The crop is a multi-functional legume native to Africa. It provides food for humans, feeds for livestock and is a dependable source of revenue for farmers and its value chain actors (Boukar et al., 2018). More than 65% of World cowpea grains are produced in Africa, with Nigeria and the Republic of Niger being the top World producers, producing around 3 million tons and 1.1 million tons of grain cowpea, respectively (FAOSTAT, 2020). Its seeds contain over 25% protein, thereby making it a good substitute for animal protein sources like fish and meat for people that cannot afford meat and fish (Ajeigbe et al., 2008).

Cowpea has a wide range of growing habits (Ehlers & Hall, 1997). It is a short-day plant, and many accessions are photoperiod sensitive. Grain yield of cowpea is limited by biotic and abiotic factors. Drought and poor soil fertility are major abiotic factors responsible for low yield of the crop in tropics and semi-arid tropics (Sanginga et al., 2000). Tropical soils are inherently low in some vital nutrients, most especially, nitrogen (N) and phosphorus (P) (Bationo et al., 2002). The crop is a heavier feeder of P than N, as it can fix atmospheric N in symbiotic association with *Bradyrhizobia* spp. There are numerous reports in the literature of genetic variability in cowpea for tolerance to low soil P and response to the application of phosphate fertilizers (Mohammed et al., 2021; Sanginga et al., 2000). Different

mechanisms have been attributed to cowpea's tolerance to low soil P. One possible mechanism for tolerance to low soil P, is seed-borne P as reported in other legumes. Seed-borne P is stored in cotyledons, as cotyledons are known for storing nutrients for seedling uptake before roots are fully formed.

The removal of cotyledons to determine the effects of seed-borne P on crop performance has been previously used to study tolerance to low P in common beans (Hernandez *et al.*, 2007) and peanut (Wissuwa and Ae, 2001). Ambika *et al.* (2014) reported seed size as an important component that has an effective role directly on cultivar adaptation and seedling vigour. Furthermore, Ojo *et al.* (2007) reported that a genetic evaluation of cowpeas P utilization showed that larger seeds experienced higher P uptake from the soil. The seed weight has been shown to be the main factor that determines the rate of water uptake and has been strongly correlated with a relative growth rate in cowpea (Mukhtar and Alhassan 2006). The current work aimed to investigate the effect of seed size and cotyledon clipping, if any, on the performance of cowpea grown on soil with low phosphorus concentration and determine if there is any relationship between the seed size of cowpea genotypes and clipping of cotyledons.

Materials and Method

Experimental site

The experiment was carried out at the phosphorus screenhouse of the cowpea breeding unit at the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. Before planting, the screenhouse was decontaminated with a broad-spectrum insecticide, to ensure a pest-free environment.

Plant materials and experimental design:

A total of nine (9) genotypes, namely, TVu-297, IAR-18-2-001 (IFE-1), TVu-887, 58-77, B301, TVu-9929-5, SAMPEA14, SAMPEA 10 and IT97K-556-6 were used. The genotypes were grouped based on seed size into small, medium, and large sizes, with each category consisting of three genotypes. The experiment was laid out in a randomized complete block design with two replications.

Planting and description of treatments

Prior to planting, river sand was sieved, and acid-washed using a ratio of 1:10 of water and HCl (10 L of water to 100 ml of conc. hydrochloric acid) to 10 kg of the river sand. The acid-washed sand was later dried at room temperature. A 5 kg of acid-washed river sand was weighed into each pot of 22.5 x 19.5 cm diameter by depth dimension, lined with polyethene material, that was gently perforated with a needle to ensure appropriate drainage.

Four seeds were sown per pot at the depth of 2 cm and later thinned to 2 per pot at 5 days after sowing (DAS). Seeds were sown into 54 pots per replication, giving a total of 108 pots for the two replications. Cotyledon clipping was carried out at 6, 7, 8, 9 and 10 DAS, while the cotyledons of the control pots were not clipped. A low dose of NPK (20:10:10) fertilizer was applied to individual pots, by mixing 1 g of the fertilizer into 100 ml of water at 7 DAS, this was to avoid confounding effects of major nutrient elements. Pots were watered daily, kept weed-free and plants were protected against insect pests and disease by spraying insecticides and fungicides. At maturity, pods were harvested, kept into properly labelled envelopes and manually threshed.

Data collection and Analysis

Data was collected on seed physical characteristics such as the length, thickness, width and weight of 100 seeds before planting. Data were collected on days to first flowering, plant dry (shoot and root) weight (g), pod dry weight (g), and seed weight (g) were measured with a sensitive weighing balance. The analysis of variance was carried out using the generalized linear model procedure- PROC GLM in SAS (SAS 9.4). Means of the genotypes were generated and compared using the least significant difference test (LSD).

Results

Mean squares of analysis of variance for cowpea genotypes evaluated in low phosphorus soil

The results of the analysis of variance revealed significant differences in the performance of the genotypes for days to first flowering, plant dry weight (shoot and root dry matter) and seed dry weight. The clipping of cotyledons of emerging seeds did not affect the performance of the plants for all the traits measured except for days to first flowering (Table 1). In addition, all the seed dimension traits measured; seed length, thickness, and weight of 100 seeds taken prior to planting were significantly different between genotypes except seed width that was not statistically different between the genotypes used (Table 2).

Table 1: Mean squares of cowpea genotypes evaluated for performance in low soil phosphorus

Source of variation	Days to first flowering	Plant dry weight (g)	Pod dry weight (g)	Seed dry weight (g)
Rep	7.3	15.2	0.6	0.0
Genotypes	106.5**	195.7**	0.3	3.2*
Clipping	21.6*	16.4	0.4	0.8
Error	7.5	16.9	0.4	1.5
Mean	50	8.9	0.9	1.9
CV	5.5	46.0	66.9	62.4

* Indicates significant difference at ($p < 0.05$), ** indicates significant difference at ($p < 0.01$), whereas, values without significant difference are without an asterisk.

Table 2: Mean squares of analysis of variance for seed dimension traits of nine cowpea genotypes

Source of variation	Seed length (mm)	Seed thickness (mm)	Seed width (mm)	Weight of 100 seeds (g)
Rep	0.4	0.4	11.0	0.0
Genotypes	66.2**	28.1**	21.4	1424.9**
Error	0.5	0.5	10.8	0.0
Mean	8.9	6.8	5.5	20.1

** Indicates significant difference at ($p < 0.01$), whereas, values without significant difference are without an asterisk.

Performance of cowpea genotypes with varying seed size evaluated in low soil phosphorus

In comparing the means for days to first flowering (DFF) of the genotypes grown in low P soil, genotypes 58-77, B301, TVu-9929-5, SAMPEA-10 and IT97K-556-6 had DFF below the average of all the genotypes (50 days). Genotypes with the shortest DFF appeared to be mostly small seeded. The mean plant dry matter ranged from 4.3 to 14.8 g for B301 and TVu-297, respectively. The genotypes with smallest plant dry matter and largest dry matter fall within the category of small and large-seeded genotypes. The same pattern was observed for all other genotypes, small seeds mostly had smaller dry weight while the highest dry weight was observed on the large-seeded genotypes. The same pattern was observed for plant dry weight as well as pod and seed dry weight with slight deviations in some genotypes (Table 3). The seed dimension traits were different between the genotypes. The seed length ranged from 5.5 to 13.0 mm for SAMPEA-14 and IT97K-556–6. Similarly, the seed thickness varied from 5.1 to 9.5 mm, and the seed width ranged from 4.0 to 8.0 mm (Table 4), indicating that the genotypes used in the study had varying seed physical characteristics.

Table 3: Means of the nine cowpea genotypes evaluated for effects of cotyledon clipping on the growing plants on a low P soil

Genotypes	Days to first flowering	Plant dry weight (g)	Pod dry weight (g)	Seed dry weight (g)	Seed size score
TVu-297	53	14.8	1.8	1.4	Large
IAR-18-2001	54	12.9	0.7	0.3	Large
TVu-887	53	13.1	0.8	0.1	Large
58-77	46	5.6	0.9	2.0	Small
B301	48	4.3	2.2	1.0	Small
TVu-9929-5	48	4.5	1.9	1.3	Small
SAMPEA-14	50	10.9	2.4	0.9	Medium
SAMPEA-10	48	7.3	1.5	0.7	Medium
IT97K-556-6	48	7.0	2.5	1.1	Medium
Mean	50	8.9	1.6	1.0	
LSD	2.2	3.3	0.9	1.7	

Least significant difference (LSD)

Table 4: Means of the seed dimension attributes of nine cowpea genotypes evaluated for effects of clipping cotyledons on plants growing on a low P soil

Genotypes	Seed length (mm)	Seed thickness (mm)	Seed width (mm)	100 seed weight (g)
TVu-297	12.0	8.8	6.2	35.7
IAR-18-2001(IFE-1)	10.2	8.1	6.0	27.0
TVu-887	9.5	6.9	4.9	17.8
58-77	9.4	6.5	5.1	19.5
B301	8.7	6.7	8.0	16.1
TVu-9929-5	6.2	4.9	4.0	8.2
SAMPEA-14	5.5	5.1	3.8	17.1
SAMPEA-10	6.6	5.1	4.2	9.3
IT97K-556-6	13.0	9.5	7.1	20.1
LSD	0.7	0.6	2.9	NA

Least significant difference (LSD)

Effects of seed size and cotyledon clipping on cowpeas grown on low soil phosphorus

Performance of the genotypes in terms of plant dry weight (shoot and root dry weights) was influenced by seed size on low P soil, as large-seeded genotypes produced more total dry matter over small and medium seed genotypes (Fig. 1A). However, the seed size of the genotypes did not significantly affect the number of days it takes from sowing to the emergence of the first flower of the plants (Fig. 1A). The clipping cotyledons of the genotypes had no significant effect on all the parameters measured, the performance of the genotypes across all treatment (clipping days of the cotyledons) was not significantly different when compared to the unclipped control treatment with intact cotyledons (Fig. 1B).

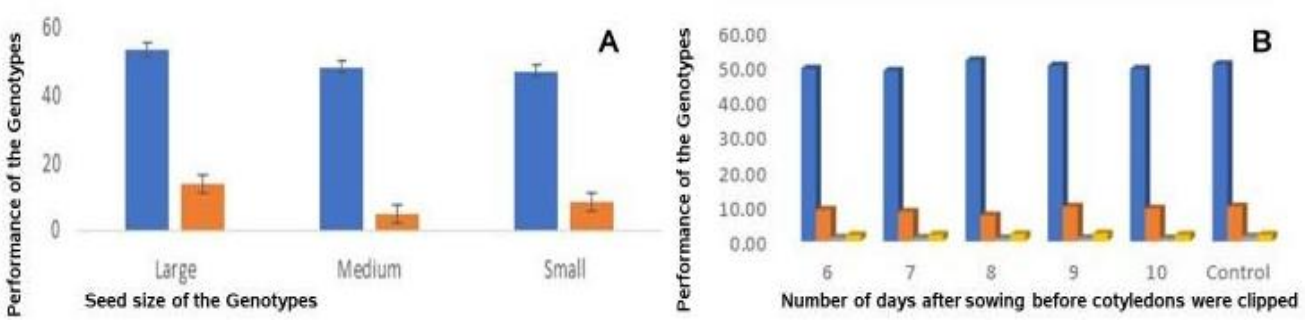

Figure 1: (A) Effects of seed size on the days to flowering (Blue bars) and plant dry weight (shoot and root dry mass in grams) Orange bars), (B) Effects of cotyledon clipping on the performance of cowpea genotypes, where in X-axis 6, 7, 8, 9 and 10, signifies cotyledons were clipped at 6, 7, 8, 9, & 10 days after sowing, Blue bars = days to first flowering, Orange bars = shoot dry weight (g), Grey bars = pod dry weight (g) and Yellow bars = seed dry weight (g).

Discussion

The results of this current study revealed significant differences between cowpea genotypes for days to first flowering, plant dry weight (shoot and root dry weights) and seed dry weight grown on low P soil. This finding is consistent with other reports indicating the presence of significant diversity for growth and yield parameters for cowpea lines grown on low P soils (Sanginga *et al.,* 2000; Ojo *et al.,* 2006). Seed size of the genotypes appeared to be a predictor of the plant biomass production since biomass of the genotypes with large seeds were well over genotypes with medium and small seeds, this is very likely as the seed is the nutrient reservoir for the developing seedling and more nutrients are stored in seeds with larger endosperm compared to small endosperm seeds, a similar observation was made by Alexender (2014), that large-seeded cowpea lines produced more biomass than small-seeded types.

Breeding programmes, especially in Nigeria, have often time selected for large seeds due to consumer preference for large-seeded varieties in most areas of northern Nigeria, where there is significant cultivation of the crop. Larger seeded genotypes may be benefiting from overall improved performance. Breeders do select for desired traits in conjunction with large seed phenotypes in most breeding programmes, therefore, selecting for low P tolerance might have directly or indirectly led to selection for increased seed size. Furthermore, the clipping of cotyledons at different intervals (clipping of cotyledons on 6, 7, 8, 9 and 10 days after seedling emergence) compared with the control treatment with intact cotyledons produced no significant effect on the performance of the genotypes grown on low P soil. This result corroborated the report of Alexander (2014), who investigated if seed size had any influence on the performance of cowpea lines with cotyledons clipped at different periods and grown on different concentrations of P media.

Conclusions And Recommendation

The study was designed and conducted to determine the effect of seed-borne P on the performance of cowpea in low P soil through cotyledon clipping. Nine genotypes of varying seed size were grown in low soil P medium with their cotyledons clipped after emergence on the 6, 7, 8, 9 and 10 days after emergence. The study showed that seed size had a significant effect on the plant biomass production in cowpea, while the clipping of cotyledons of cowpea genotypes did not result in any significant effects on the performance of cowpea grown on soil low in available P. The current work did not address the response of cowpea on different growth media varying in P concentrations. A future study to investigate the effect if any of cotyledon clipping and seed size of cowpea on different concentrations of soil P is recommended.

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SCREENING OF SOYBEAN GENOTYPES (*Glycine max* (L.) Merrill) FOR CUCMBER MOSAIC VIRUS DISEASE RESISTANCE IN MINNA, NIGERIA

[1*]Adamu, A. S., [1]Gana, A. S., [1]Salaudeen, M. T., [2]Daudu, O. A. Y., [3]Ehirim, B. O. and [3]Shaahu, A.

[1]Department of Crop Production, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria.
Corresponding Author: aahakym@gmail.com +2348036315890[2]

ABSTRACT

Development of resistant crop is a key component of pest control generally. The study therefore was conducted to determine the effect of cucumber mosaic virus disease on some soybean genotypes under screenhouse conditions. The experiment was laid out in Completely Randomized Design with three replications. Seeds of healthy soybean genotypes were planted as control (uninoculated). Data were subjected to analysis of variance (ANOVA). All the infected genotypes elicited disease incidence except NCRISOY ACC.62 (0 %) which was found to be resistant, likewise all the healthy genotypes. In addition, ACC.62 exhibited the highest number of leaves (30) and 100-seed weight (11.3 g). Phenotypic variance (81.39) was higher than the genotypic variance for all the characters, indicating high environmental influence on the parameters. Highest Phenotypic coefficient of variation (26) and genotypic coefficient of variation (17) were recorded in infected 100-seed weight per plant. The highest heritability was found for infected 100-seed weight (42). Genotypes ACC.62 and ACC.66 which gave a better result under CMV disease stress is recommended for crop improvement.

Keywords: *Cucumber mosaic virus, Disease incidence and severity, Genotypes, variation.*

Introduction

Soybean (*Glycine max* [L.] Merrill.) is an important global legume crop that grows in the tropical and subtropical climate. It belongs to the botanical family Fabaceae in the subfamily Papilionideae. It has 40 chromosomes (2n=2x=40) and is a self-fertile with less than 1 % out-crossing (Shurtleff and Aoagi, 2007). Although limited by lack of methionine, soybean is the world's leading source of oil (21%), protein (40%) and in minerals (iron and calcium) and vitamin (Krober, 2009). Soybean, from the nutritional point of view, is classified as first class protein because of its complete essential amino acid profile which compare to those of animal protein (Umar *et al.*, 2009). Soybean, in itself, is adept of fixing nitrogen through symbiotic relationship with nitrogen-fixing soil bacteria *Bradyrhizobium japonicum* (McNeil, 2010). In Nigeria, the recent drive to increase soybean production is mainly attributed to the increasing demand for edible oil proteins. Many varieties have since been produced by introduction, breeding and selection, with the most exceptional varieties released for large scale production across different ecological zones of the country (Arogundade *et al.*, 2010). According to FAO (2013) about 6 million tonnes of soybean were produced from 6 million hectares of land in Nigeria. Yield per unit area is usually less than 1 tonne per hectare in tropical Africa despite the large land area utilised. However, according to Thuzar (2010), factors militating against the level of soybean production and productivity are ascribed to biotic and abiotic factors.

In Nigeria, a number of diseases have been reported to affect soybean crop, which includes bacteria blight, *cercospora leaf spot,* red leaf blotch and soybean mosaic virus, been the most frequently isolated virus of soybean that occurs everywhere soybean is grown (Arogundade *et al*., 2010). Cucumber mosaic virus (*Cucumis sativus* L.) belongs to the genus *Cucumovirus* and family Bromoviridae. The virus has the widest host range of all the viruses and attacks a greater variety of weeds, ornamentals, vegetables and other plants (Crescenzy, 1993). The virus is economically important, in that, it reduces seed yield and quality considerably and exist in all ecological zones of the country, particularly the rainforest zone, derived savannah, northern and southern Guinea savanna zones of the country (Alegbejo, 2015). In addition, the virus could be a serious disease of soybean particularly when the crop is grown near cucurbits (cucumber) and solanaceous crops (pepper and tomato). Cucumber mosaic virus is seed-borne and also transmitted by over 75 species of aphids in a non-persistent manner (O'keefe *et al*., 2007). Symptoms of infection include yellow mosaic, leaf chlorosis, blistering and leaf curling (Adamu, 2015). However, information on disease incidence and severity of CMV will allow for management interpolations intended at containing the disease through breeding of resistant varieties as well as increasing soybean productivity in Sub-Saharan Africa.

Materials and Methods

Experimental Site

The study was conducted at the screenhouse of the Department of Crop Production, Federal University of Technology, Minna, Niger State, Southern Guinea-Savanna agro-ecological Zone of Nigeria. Temperature varies between 35 to 37^{0}C with relative humidity of between 40 to 80 %.

Source of Planting Materials

Thirty two soybean accessions used for the research are as follows; NCRISOY's ACC.2, ACC.3, ACC.5, ACC.7, ACC.9, ACC.10, ACC.16, ACC.17, ACC.18, ACC.20, ACC.21, ACC.22, ACC.24, ACC.25, ACC.28, ACC.29, ACC.60, ACC.61, ACC.62, ACC.63, ACC.64, ACC.65, ACC.66, ACC.67, ACC.68, ACC.69, ACC.72, ACC.73, ACC.76, ACC.77, ACC.78, ACC.79. They were collected from the soybean breeding unit of the National Cereals Research Institute, Badeggi, Niger State.

Source of inoculum, Propagation and Inoculation procedure

The CMV isolate used for the experiment was obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna, Niger State. CMV inoculum was multiplied on cowpea plants by mechanical inoculation at 10 days post sowing to ensure full coverage of the treatments. This was achieved by gently dusting Carborundum (600-mesh) on the axial leaf surface of the cowpea cultivar Ife-Brown with cotton wool. CMV-infected leaves were ground in inoculation buffer, at pH 7.2 (0.1 sodium phosphate dibasic, 0.1 M potassium phosphate monobasic, 0.01 M ethylene diamine tetra acetic acid and 0.001 M L-cystine per litre of distilled water) at 1:1 weight/volume (1 g of leaf in 1 Ml of buffer) using a pre-cooled mortar and pestle. Five microliters of β-mecarpto-ethanol was added to the extract prior to application. The extract was applied on the leaf surface previously dusted with carborundum powder by gently rubbing the leaves using cheesecloth. In order to remove excess inoculum, leaves were lightly rinsed with distilled water afterwards. Symptomatic leaves enough to cover for the experiment were harvested twelve days after inoculation. The leaves were further preserved in an airtight vials containing anhydrous calcium chloride ($CaCl_2$) at the base, with a thin layer of non-absorbent cotton wool lying in-between the leaf sample and the anhydrous salt. The samples were kept at room temperature prior to inoculation in the field (screenhouse).

Treatment, Experimental Design, Sowing and inoculation

Thirty two soybean genotypes were inoculated (infected) with CMV which served as the treatment. The experiment was laid out

in a Completely Randomized Design (CRD) with three replications. Five soybean seeds were sown in black poly bags (30 cm diameter and 30 cm height) containing 3 kg heat sterilized soils. After germination, seedlings were thinned to three plants per pot. CMV disease free (healthy) soybean seeds were planted as control (Adamu *et al.*, 2018). Throughout the period of evaluation, plants were watered daily. Sap inoculation and inoculation procedure are as detailed in source of inoculum, propagation and inoculation procedure above.

Observation and Data Collection

Both the infected and healthy soybean genotypes were observed for disease incidence and severity. Disease incidence was taken as percentage of seedlings showing typical symptoms of CMV infection

at one, two and three weeks post inoculation (WPI). Below is the formula used to achieve percentage disease incidence;

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Disease severity was observed at one, two and three weeks post inoculation (WPI) using the scoring scale of 1-5 (Arif and Hassan, 2002). Number of leaves per plant, number of pods at harvest, pod length and 100 seed weight were the morphological characters observed.

Table 1: Visual assessment of CMV disease according to Arif and Hassan (2002)

Symptom score	Percentage rating	Reaction
1	0 %	No symptoms (apparently healthy plants)
2	10-30 %	Slightly mosaic leaves
3	31-50 %	Mosaic
4	51-70 %	Severe mosaic, leaf distortion and stunting
5	> 70	Severe mosaic, stunting and death of plants

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using statistical analysis system (SAS, 2012) version 9.0. Significant difference was determined at $p \leq 0.05$ and means were separated using Duncan's Multiple Range Test (DMRT) at $p=0.05$. Estimate of variance component were generated. Variability parameters (Genotypic and Phenotypic variances) were estimated according to Panse (1957).

$$\delta^2 g = \frac{MSg - MSe}{r}$$

$$\delta^2 ph = \frac{MSg - MSe}{r}$$

Where:

$\sigma^2 g$ = genotypic variance, $\sigma^2 ph$ = phenotypic variance, $\underline{MSg}$ = mean square of genotypes, $\underline{MSe}$ = mean square of error, r = number of replications

The genotypic and phenotypic coefficients of variation were estimated using the formula below and further categorized as low (0-10 %), moderate (10-20 %) and high (>20 %) according to Sivasubramanian and Menon (1973):

$$\text{GCV (\%)} = \frac{\sqrt{\sigma 2g}}{X} \times 100$$

$$\text{PCV (\%)} = \frac{\sqrt{\sigma 2ph}}{X} \times 100$$

Where: GCV = genotypic coefficient of variation, $\delta^2 g$ = genotypic variance, PCV = phenotypic coefficient of variation, $\delta^2 ph$ = phenotypic variance, x = population mean.

Broad sense heritability (h^2) was calculated as the ratio of the genotypic variance to the phenotypic variance using the formula below according to Allard (1960) and were further categorized as low (<50%), moderate (50-70%) and high (>70%) as suggested by Robinson (1966). $h^2 = \frac{\sigma 2g}{\sigma 2ph} \times 100$

Where: h^2 = broad sense heritability (%), $\sigma^2 g$ = genotypic variance, $\sigma^2 ph$ = phenotypic variance.

Genetic advance (GA) was calculated with method suggested by Singh and Chaudhury (1985): $GA = K.h^2$,

Where: K-costant= 2.06 at 5% selection intensity, σ^2ph=phenotypic variance, h^2 = heritability in broad sense.

Results

Disease Incidence and Severity

Symptoms of CMV disease were first observed at seven days post inoculation (7 WPI). Visible symptoms of mosaic, blistering, chlorosis, leaf curling and mild mottling were observed on some of the infected (CMV-inoculated) genotypes. At 1 WPI, disease symptom varied between 0 and 77.8 % with 75 % of the infected genotypes showing varying symptoms. At 2 WPI, percentage of plant genotypes exhibiting CMV disease symptoms increased to 84 % with incidence variation of between 0 to 88.9 %. At 3 WPI, symptom score varied between 0 to 100 %, and also percentage of genotypes exhibiting disease symptoms increased to 96.9 % where only genotype ACC.62 remained symptomless all through the evaluation. In contrast to the CMV-infected genotypes, the healthy (uninoculated) genotypes were apparently symptomless (Symptom score=1). Genotypes NCRISOY ACC.9, ACC.67, and ACC.73 elicited 100 % disease incidence.

Disease severity varied significantly (p<0.05) among the genotypes evaluated (Table 4.2). At 1 WPI, severity score varied between 1.0 and 3.0. But at 2 and 3 WPI, severity score varied between 1.0 to 3.67 and 1.0 to 4.33, respectively. Genotype's ACC.63 and ACC.66 recorded the lowest disease severity during the course of the evaluation. However, in ACC.62, there was no disease incidence observed, hence, the severity score of 1 (apparently healthy plants) from the severity rating scale.

Growth and Yield Parameters

Effect of CMV on number of leaves per plant significantly (p<0.05) varied with soybean genotypes (Table 3). Number of leaves per plant varied between 13.33 (ACC.60) to 30.00 (ACC.66) in the infected genotypes. On the other hand, number of leaves per plant varied between 8.00 (ACC.20) and 32.67 (ACC.5) in the healthy genotypes. The effect of CMV on number of pods per plants varied amongst genotypes (Table 3). Infected genotypes produced number of pods per plant ranging from 20 (ACC.72) and 44 (ACC.16 and ACC.62) whereas number of pods per plant ranged between 21 (ACC.20) and 47 (ACC.66 and ACC.67) for the healthy genotypes.

Cucumber mosaic virus significantly (p<0.05) affected pod length per plant (Table 4). Infected genotypes produced shorter pods with values ranging from 2.00 cm (ACC.60 and ACC.73) to 4.33 cm (ACC.25 and ACC.62) contrary to the healthy genotypes which had a range of 1.33 cm (ACC.20) to 4.67 cm (ACC.62). Relatedly, CMV disease significantly (p<0.05) affected one hundred seed weight per plant (Table 4). Some of the seeds of the healthy genotypes appeared bigger and heavier than that of the infected genotypes. While one hundred seed weight of the infected genotypes ranged from 4.64 g (ACC.60) to 11.33 g (ACC.66) that of the healthy, ranged from 4.15 g (ACC.20) to 12.54 g (ACC.62).

Genetic Variability for Growth and Yield Components

Table 5 presents the combined means, estimates of genotypic and phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability and genetic advance of both the CMV-infected and healthy environments. The highest phenotypic and genotypic variances in all the traits observed were recorded in number of pods per plant (81.40 and 22.61), respectively. Similarly, high phenotypic and genotypic variances were observed in number of leaves per plant (29.67, 34.88 and 12.12), respectively.

Generally, PCV was high in all the traits observed and estimates ranged between 22 % of the healthy number of leaves and 100-seed weight to 26 % of infected 100-seed weight

per plant. Contrary to the PCV, the GCV estimates ranged between 7.9 % of healthy pod length and 17 % of infected 100-seed weight. GCV of infected (11) and healthy (13) number of leaves, infected number of pods (14) and infected 100-seed weight (17) were moderate, while healthy number of pods (8), infected (9.5) and healthy (7.9) pod length and healthy 100-seed weight (10) were low. Furthermore, healthy number of pods per plant recorded the lowest GCV value of 8 % while the highest GCV value of 18 % was observed in infected 100-seed weight per plant. Generally, heritability in the broad-sense estimate varied from 11 % of the healthy pod length per plant and 42 % of the infected hundred seed weight per plant. High heritability was observed for healthy number of leaves (0.35) and infected 100-seed weight (4.2). Genetic advance was highest for infected number of pods (5.2). Healthy number of leaves showed high heritability (0.35) coupled with high genetic advance (4.26).

Discussion

All the genotypes were susceptible to CMV disease except genotype ACC.62, which had no visible symptom all through the period of evaluation. The genotype could be said to exhibit immunity to the virus. This agrees with the findings of McCreight *et al.* (2008) who stated that Immunity is the highest level of resistance and is partly exhibited as absence of visible symptoms in inoculated plants. Genotype ACC.17, ACC.25, ACC.63 and ACC.66 were apparently symptomless for about 20 DPI, this suggests that the genotype possess high level of tolerance to CMV disease. This observation is in agreement with Gergerich and Dolja (2006) who stated that susceptibility and resistance to virus infection is determined by plant genotype. In addition, disease severity also varied amongst the genotypes observed. The severity of infection increased over time in some genotypes, which is in agreement with Aliyu *et al.* (2012) when some cowpeas were inoculated with *Blackeye cowpea mosaic virus* (BlCMV). However, the symptoms of infection observed on the CMV-infected genotypes are in agreement with those reported by Salaudeen *et al.* (2015) when some okra genotypes were infected with CMV. The obvious variations in the morphological and yield data of the infected and healthy genotypes are clear attestation of pathogenicity of CMV on the evaluated genotypes. This corroborates the findings of Adamu *et al.* (2015) when some soybean cultivars were infected with CMV.

The estimates of phenotypic and genotypic variance were the highest for infected number of pods and healthy number of leaves, respectively. As expected, phenotypic coefficient variation were generally higher than the genotypic coefficient of variation, which indicates low genotypic contribution and greater contribution of the environmental influence in the expression of these traits, hence, the need for careful selection. This corroborates the findings of Karnwal and Singh (2009). The result of heritability in broad sense further showed low heritability for all the traits except for healthy number of leaves and infected 100-seed weight. This agrees with the findings of Sajjad (2012) who stated that it could be due to the increase in environmental variance or decrease in genetic variance. Panse (1957) stated that high heritability coupled with high genetic advance indicates the additive gene effect while high heritability coupled with low genetic advance indicates the non-additive gene effects for control of the particular character as observed in infected 100-seed weight.

Conclusion

As revealed in the study, there exist adequate variability amongst the genotypes evaluated in terms of susceptibility, tolerance and resistance to cucumber mosaic virus disease. The result further revealed reduction in productivity of soybean genotypes due to CMV disease. Information on disease incidence, disease severity, morphological and yield components coupled with components of variance, broad sense heritability and genetic advance could be

utilized as a guide for the improvement of soybean.

Recommendation

Genotypes NCRISOY ACC.62 and ACC.66 were identified to possessing resistance and a level of tolerance to CMV disease, respectively. Also, both genotypes were found to have performed better with regards to some favourable traits under CMV disease stress, hence, recommended as parents when breeding for resistance and higher productivity. In addition, the genotypes are recommended for further evaluation across environments.

Table 2: Disease incidence and severity in soybean plants infected with *Cucumber mosaic virus* under screenhouse conditions in Minna, Nigeria, 2018

		Disease incidence (%)			Disease severity (scale 1-5)		
S/N	Accession no.	1WPI	2WPI	3WPI	1WPI	2WPI	3WPI
1	ACC.2	0.00e	33.33c-f	55.55b-d	1.00e	2.33dc	3.00c-e
2	ACC.3	33.33b-e	44.44b-e	55.55b-d	2.33a-c	2.67b-d	3.00c-e
3	ACC.5	33.33b-e	33.33c-f	33.33d-f	2.00b-d	2.00de	2.33ef
4	ACC.7	33.33b-e	33.33c-f	55.55b-d	2.33a-c	3.00a-c	3.00c-e
5	ACC.9	33.33b-e	88.88a	99.99a	3.0a	3.33ab	3.67a-c
6	ACC.10	11.11de	33.33c-f	33.33d-f	1.33de	2.00de	2.00fg
7	ACC.16	22.22c-e	33.33c-f	44.44c-e	1.67c-e	3.00a-c	3.00c-e
8	ACC.17	0.00e	0.00f	44.42c-e	1.00e	1.00f	2.33ef
9	ACC.18	33.33b-e	44.44b-e	44.44c-e	2.33a-c	3.00a-c	3.33b-d
10	ACC.20	22.22c-e	55.55a-d	66.66a-d	2.67ab	3.00a-c	3.00c-e
11	ACC.21	0.00e	11.11ef	44.44c-e	1.00e	1.33ef	2.00fg
12	ACC.22	44.44a-d	55.55a-d	77.77a-c	2.00b-d	2.67b-d	3.00c-e
13	ACC.24	0.00e	44.44b-e	55.55b-d	1.00e	2.33cd	2.33ef
14	ACC.25	0.00e	0.00f	33.33d-f	1.00e	1.00f	2.33ef
15	ACC.28	11.11de	33.33c-f	44.44c-e	1.33de	2.00de	2.00fg
16	ACC.29	33.33b-e	33.33c-f	44.44c-e	2.00b-d	2.00de	2.33ef
17	ACC.60	44.44a-d	55.55a-d	77.77a-c	2.33a-c	3.00a-c	3.67a-c
18	ACC.61	55.55a-c	66.66a-c	66.66a-d	2.33a-c	3.67a	4.00ab
19	ACC.62	0.00e	0.00f	0.00f	1.00e	1.00f	1.00h
20	ACC.63	0.00e	0.00f	11.11ef	1.00e	1.00f	1.33gh
21	ACC.64	33.33b-e	33.33c-f	33.33d-f	2.00b-d	2.00de	2.67d-f
22	ACC.65	44.44a-d	44.44b-e	55.55b-d	2.33a-c	2.67b-d	3.00c-e
23	ACC.66	0.00e	0.00f	11.11ef	1.00e	1.00f	1.33gh
24	ACC.67	55.55a-c	88.88a	99.99a	2.67ab	3.00a-c	3.33b-d
25	ACC.68	22.22c-e	44.44b-e	66.66a-d	1.67c-e	2.67b-d	2.67d-f
26	ACC.69	66.66ab	66.66a-c	77.77a-c	2.67ab	3.33ab	4.33a
27	ACC.72	66.66ab	77.77ab	77.77a-c	2.67ab	2.67b-d	4.00ab
28	ACC.73	44.44a-d	66.66a-c	99.99a	2.67ab	3.33ab	3.33b-d
29	ACC.76	22.22c-e	22.22d-f	55.55b-d	1.33de	1.33ef	2.67d-f
30	ACC.77	22.22c-e	44.44b-e	44.44c-e	1.33de	3.00a-c	3.33b-d
31	ACC.78	77.77a	77.77ab	77.77a-c	2.33a-c	2.67b-d	3.00c-e
32	ACC.79	77.77a	88.88a	88.88ab	2.33a-c	3.00a-c	3.67a-c
	±SE	11.28	10.94	10.76	0.28	0.24	0.27

Means with dissimilar letters within the column differ significantly ($p \leq 0.05$) by the Duncan Multiple Range Test (DMRT)

KEY: Accession no.=Accession Number, 1=ACC.2, 2=ACC.3, 3=ACC.5, 4=ACC.7, 5=ACC.9, 6=ACC.10, 7=ACC.16, 8=ACC.17, 9=ACC.18, 10=ACC.20, 11=ACC.21, 12=ACC.22, 13=ACC.24, 14=ACC.25, 15=ACC.28, 16=ACC.29, 17=ACC.60, 18=ACC.61, 19=ACC.62, 20=ACC.63, 21=ACC.64, 22=ACC.65, 23=ACC.66, 24=ACC.67, 25=ACC.68, 26=ACC.69, 27=ACC.72, 28=ACC.73, 29=ACC.76, 30=ACC.77, 31=ACC.78, 32=ACC.79.

Table 3: Number of leaves per plant and number of pods at harvest in soybean plants infected (inoculated) and healthy (uninoculated) with Cucumber mosaic virus in Minna, 2018.

S/N	Accession no.	Leaves per plant (no.)		Number of pods (no.)	
		Infected	Healthy	Infected	Healthy
1	ACC.2	23.00a-d	28.67ab	33a-d	33ab
2	ACC.3	24.67a-d	30.33ab	34a-d	43ab
3	ACC.5	26.00a-c	32.67a	38a-d	46a
4	ACC.7	23.00a-d	26.67ab	32a-d	38ab
5	ACC.9	24.00a-d	25.67ab	33a-d	40ab
6	ACC.10	15.67cd	24.33ab	21cd	37ab
7	ACC.16	24'00a-d	25.67ab	44a	45a
8	ACC.17	28.00ab	31.00a	36a-d	42ab
9	ACC.18	24.67a-d	28.33ab	35a-d	39ab
10	ACC.20	21.67a-d	8.33c	42a	21b
11	ACC.21	21.33a-d	27.00ab	33a-d	38ab
12	ACC.22	25.00a-d	30.00ab	39a-c	40ab
13	ACC.24	14.00d	26.33ab	22b-d	38ab
14	ACC.25	29.00a	27.33ab	42a	39ab
15	ACC.28	23.33a-d	27.67ab	40ab	45a
16	ACC.29	21.00a-d	27.00ab	37a-d	40ab
17	ACC.60	13.33d	28.00ab	22b-d	46a
18	ACC.61	21.67a-d	30.00ab	34a-d	46a
19	ACC.62	28.00ab	29.33ab	44a	45a
20	ACC.63	24.00a-d	19.00b	43a	29ab
21	ACC.64	26.67a-c	27.67ab	40ab	41ab
22	ACC.65	23.00a-d	28.33ab	37a-d	38ab
23	ACC.66	30.00a	27.67ab	43a	47a
24	ACC.67	23.67a-d	29.33ab	40ab	47a
25	ACC.68	24.67a-d	19.00b	36a-d	27ab
26	ACC.69	22.00a-d	31.67a	36a-d	45a
27	ACC.72	16.33b-c	36.00a	20d	36ab
28	ACC.73	15.00cd	28.00ab	22b-d	37ab
29	ACC.76	27.67ab	31.67a	40ab	44ab
30	ACC.77	25.00a-d	31.67a	43a	44ab
31	ACC.78	19.00a-d	27.67ab	30a-d	37ab
32	ACC.79	24.33a-d	29.33ab	36a-d	36ab
	CV%	25.60	21.21	26.11	29.41
	±SE	3.39	3.37	5.31	6.74

Means with dissimilar letters within the column differ significantly ($p \leq 0.05$) by the Duncan Multiple Range Test (DMRT)

Table 4: Pod length and Number of 100-seed weight in soybean plants infected (inoculated) and healthy (uninoculated) with Cucumber mosaic virus in Minna, Nigeria, 2018.

S/N	Accession no.	Pod length (cm)		100 seed weight (g)	
		Infected	Healthy	Infected	Healthy
1	ACC.2	3.00a-c	3.57ab	9.60a-e	10.43ab
2	ACC.3	3.33a-c	3.73ab	10.18a-c	11.77ab
3	ACC.5	4.00ab	3.37ab	10.23a-c	10.74ab
4	ACC.7	3.33a-c	3.67ab	10.59a-c	11.87ab
5	ACC.9	2.67a-c	3.10ab	9.01a-e	10.44ab
6	ACC.10	2.67a-c	3.90ab	6.60c-f	11.59ab
7	ACC.16	3.33a-c	3.60ab	9.58a-e	9.31ab
8	ACC.17	3.33a-c	3.53ab	8.97a-e	9.45ab
9	ACC.18	3.00a-c	3.60ab	9.08a-e	12.00ab
10	ACC.20	3.33a-c	1.33c	7.74b-f	4.15c
11	ACC.21	3.00a-c	3.37ab	9.70a-d	10.88ab
12	ACC.22	4.00ab	3.17ab	8.64a-f	9.26ab
13	ACC.24	2.33bc	3.23ab	6.00d-f	11.42ab
14	ACC.25	4.33a	4.33a	10.65a-c	11.81ab
15	ACC.28	2.33bc	3.27ab	8.04b-f	9.71ab
16	ACC.29	3.33a-c	3.73ab	9.17a-e	11.31ab
17	ACC.60	2.00c	3.67ab	4.64f	8.50a-c
18	ACC.61	3.33a-c	3.37ab	7.32b-f	8.48a-c
19	ACC.62	4.33a	4.67a	12.20a	12.54a
20	ACC.63	4.00ab	2.40bc	10.38a-c	8.02a-c
21	ACC.64	3.33a-c	3.37ab	7.65b-f	10.20ab
22	ACC.65	3.33a-c	3.80ab	7.24b-f	11.32ab
23	ACC.66	3.00a-c	3.33ab	11.33ab	12.40a
24	ACC.67	3.00a-c	3.43ab	8.03b-f	9.71ab
25	ACC.68	3.00a-c	2.67bc	8.02b-f	7.05bc
26	ACC.69	3.00a-c	3.10ab	8.02b-f	9.78ab
27	ACC.72	2.33bc	3.37ab	4.66f	11.99ab
28	ACC.73	2.00c	3.37ab	5.51ef	10.78ab
29	ACC.76	3.00a-c	3.77ab	10.89ab	10.68ab
30	ACC.77	3.33a-c	3.60ab	8.13a-f	9.10ab
31	ACC.78	3.33a-c	4.10ab	10.30a-c	11.96ab
32	ACC.79	2.67a-c	3.50ab	9.99a-d	11.06ab
	CV%	28.38	26.58	23.79	23.77
	±SE	0.51	0.53	1.19	1.41

Means with dissimilar letters within the column differ significantly ($p \leq 0.05$) by the Duncan Multiple Range Test (DMRT)

Table 5: Estimates of genetic variability for growth and yield components of thirty two soybean genotypes infected (inoculated) with CMV and healthy (uninoculated) soybean genotypes in Minna, Nigeria, 2018

TRAITS	Mean		GV		PV		GCV (%)		PCV (%)		h^2B %		GA	
	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
NOL	22.90	27.54	6.7	12.12	29.66	34.88	11	13	24	22	27	35	3.03	4.26
NOP	35.21	39.67	22.61	9.35	78.93	81.40	14	8	25	23	29	12	5.3	2.23
POL	3.14	3.43	0.09	0.07	0.61	0.63	9.5	7.9	25	23	15	11	0.24	0.18
100SW	8.69	10.3	2.09	1.12	4.94	5.12	17	10	26	22	42	22	1.9	1.02

NOL (No.)-Number of leaves per plant; NOP (No.)-Number of pods per plant; POL (cm)-Pod length per plant; 100SW (g)- 100 seed weight per plant; GV-Genotypc variance; PV-Phenotypic variance; GCV (%)-Genotypic coefficient of variation; PCV (%)-Phenotypic coefficient of variation; h^2B-Heritability in broad-sense; GA-Genetic advance.

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MUTAGENIC EFFECTS OF VARIOUS DOSES OF FAST NEUTRON IRRADIATION ON GERMINATION, SEEDLING GROWTH AND SEEDLING SURVIVAL OF OKRA (*Abelmoschus esculentus* (L.) MOENCH)

Umar, U.U[1]., Aliyu, S[1]., *Nafiu, M. I.,[2] Bayero, U.,[3] Bello, T. A.,[4] Abdullahi, R. I.[5] and Mohammed, A. S.[4]

[1]*Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria*
[2]*Department of Biological Sciences, Federal University Gusau, Zamfara, Nigeria*
[3] *Department of Biology, Ahmadu Bello University, Zaria, Nigeria*
[4] *School of Basic and Remedial Studies (SBRS), Ahmadu Bello University, Zaria, Nigeria*
[5] *Department of Botany, Ahmadu Bello University, Zaria, Nigeria*

*Corresponding Author: nafiumuhammadib@gmail.com +234803 4640652

ABSTRACT

Mutagenic effects of various doses of fast neutron irradiation on germination parameters of okra (Abelmoschus esculentus [L.]) using an Americium-Beryllium source with a flux of 1.5 × 104 n.cm²/s for six different irradiation exposure periods (IEPs): 120hours, 96hours, 72hours, 48hours, 24hours and 0hour (control) were studied. The treated seeds and the control were presoaked in distilled water for 18hours to initiate biochemical reaction. The experiment was set out in Randomized Complete Block Design (RCBD) with 30 poly-pots per block. Data were collected on germination percentage, germination speed, seedling length, vigor index and seedling survival. The analysis of variance revealed that the treatment mean square for all the parameters was only significant for seedling length (P≤0.05). The optimum dosage that created useful variability was 72 hours in all the germination, seedling growth and seedling survival of okra which was found to be better than the control. This showed that at 72 hours fast neutron concentration was the best treatment for most of the trait evaluated. Highest means values observed at 72 hours in all the germination, seedling growth and seedling survival could be used for improvement of these agronomic traits in okra.

Keywords: *Mutagens; Okra; Trait; Selection; Yield.*

Introduction

Okra or Okro (*Abelmoschus esculentus* (L.) Moench) is known in many countries as "Ladies finger, Bhindi, Bamia, Ochro or Gumbo, is a flowering plant in Malvaceae family. Malvaceae contains 243 genera and 4225 species. Okra family contains well-known members which are cotton and cacao and hibiscus. The largest genera in terms of a number of species include *Hibiscus* (300 species), *Sterculia* (250 species), *Dombeya* (250 species), *Pavonia* (200 species) and *Sida* (200 species) (Benjawan, 2007). It is a flowering perennial plant in the mallow family, often cultivated annually in temperate climates, and it grows to around 2m tall (Duvauchelle, 2012). The leaves are broad, palmately lobed with 5 to 7 lobes and 10-20cm long. The flowers are 4 to 8 cm in diameter, containing five white or yellow petals, with a red or purple spot at the base of each. Okra pods are variable in colors (white, red, green and purple). The fruit is capsule-like and the length can be up to 18 cm long with pentagonal cross-section, containing numerous seeds. The crop is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. The

genus Abelmoschus belongs to family Malvaceae and is represented by 12 species in which the most important vegetable crop is okra (Alzahrani, 2021). Okra is the sixth important popular vegetable crop that is widely grown under varying climatic conditions (Duvauchelle, 2012).

In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus, increases the frequency of mutations above the natural background level (Farooq *et al.,* 2010). Induced mutations have played a pivotal role in enhancing world food security, as new food crop varieties with various induced mutations have brought about a significant increase in crop production at locations people could directly access (Kharkwal and Shu, 2010). Induced mutations are significant as novel mutations are being isolated for enhanced nutrition quality of crop plants, for example micronutrients, protein, amino acids, fatty acids and vitamins (Navnath and Mukund, 2014). Mutations can be induced in various ways, such as exposure of plant propagules, including seeds, tissues and organs, to physical or chemical mutagens (FAO, 2010). Physical mutagens are mostly electromagnetic radiations such as gamma rays, X-rays, UV light and particle radiation, including fast and thermal neutrons as well as beta and alpha particles.

The knowledge and understanding of the reaction of okra to different doses of fast neutron irradiation as well as the effective irradiation dose for induction of variability among okra is either scanty or currently unavailable. Mutation, whether induced or natural, has played an important role in increasing variability leading to production of varieties of crop.

The aim of this research is to study the mutagenic effects of various doses of fast neutron irradiation on germination, seedling growth and seedling survival of okra. The objectives are to determine the effect of different doses of fast neutron irradiation on germination,

seedling growth and seedling survival of okra and to compare the germination, seedling growth and seedling survival of fast neutron irradiated okra and control.

Materials and Methods

Study Area

The study was carried out during the raining season 2018 at the Botanical Garden of the Department of Biological Sciences, Kaduna State University (longitude 10° 20'N and latitude 7° 45'E) in the northern Guinea savannah ecological zone of Nigeria.

Experimental Material

One improved variety of okra (Jokoso) was obtained from Institute of Agricultural Research (I.A.R), Ahmadu Bello University, Zaria.

Treatment of seeds with the mutagen

Fresh untreated okra seeds were divided into five sets of 200 hundred seeds and were irradiated at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria with Fast Neutron Irradiation (FNI) using an Americium-Beryllium source with a flux of 1.5 × 104 n.cm2/s for five different irradiation exposure periods (IEPs): 120hrs, 96hrs. 72hrs,48hrs, 24hrs.The equipment was a Miniature Neutron Source Reactor (MNSR) designed by the China Institute of Atomic Energy (CIAE) and licensed to operate at a maximum power of 31 KW (SAR, 2005). A control seeds was kept separately without irradiation with FNI.

Experimental Design and Procedure

The treated seeds and the control were presoaked in the distilled water for 18 hours to initiate biochemical reaction. The experiment was set out in Randomized Complete Block Design (RCBD) with 30 poly-pots per block. The experiment was replicated three times, with a total of 90 pots. Ten seeds were planted per pot. Three weeks after planting, each pot was thinned to two plants per pot. A total of 5 pots for each treatment and control was

used.

Data Collection

Data was collected on the following parameters:

1. Seed germination: seed germination of each replication was recorded on the 18th day after sowing and the average will be recorded as expressed below:

Seed germination (%) = Number of seeds geminated x100
Number of seeds planted

2. Seedling Length (SL) (cm): this will be measured from five randomly selected plants using ruler from the ground level to the tips of the highest leaves at two weeks after sowing and the average will be recorded.

3. Seedling Survival: this will be observed after four weeks of seedling emergence and per cent survival will be calculated as follows:

Seedling survival (%) = Number of seedlings survived x100
Number of seeds germinated

4. The germination speed (GS) and the vigor index (VI): calculated as described by Maguire (1962) and Abdul-Baki and Anderson (1973) respectively:

$$\mathbf{GS} = \sum_{\mathbf{I}}^{\mathbf{i}} \frac{\mathbf{ni}}{\mathbf{Di}} \qquad \mathrm{VI} = \frac{\mathrm{SL\ (cm)} \times \mathrm{GS\ (\%)}}{100}$$

Where ni is the number of seeds germinated on ith day and Di is the number of days from the start of the experiment. SL is seedling length

Data Analysis

The data were subjected to analysis of variance (ANOVA) appropriate to Randomized
Complete Block Design (RCBD) technique using the SAS computer software programme,
version 9.2 (SAS, 2008). When significant difference existed between treatment means, comparison of the means was done using Duncan Multiple Range Test (DMRT) at 5% level of significance (Rangaswamy, 2010).

Results and Discussion

Analysis of Variance for Germination Parameters in Induced Mutants of Okra

Analyses of variance for germination parameters in induced mutant okra are presented in
Table 1.0 The mean square for dose was only significant difference (P≤0.05) for seedling length while for germination percentage, germination speed and vigour index were non-significant.

Mutagenic Effects of various Doses of Fast Neutron Irradiation on Germination Parameters of Okra

Effects of the various doses of the sodium azide on germination parameters are presented in
Table 2.0 the germination percentage ranged from 84.47% to 96.70%. The highest germination percentage was observed in 72 hour (96.70%) which was found to be higher than the control, while it was lowest in 24 hours (84.47%). The germination speed varied
from 1.41 to 1.61. The highest germination speed was observed in 72 hour and 120 hours (1.61 each), while it was lowest in 24 hours (1.41). The seedling length ranged from 10.73cm to 12.50cm. The highest seedling length was observed in 72 hour (12.50cm), while it was lowest in control (10.73cm). The vigour index ranged from 0.16 to 0.20. The highest vigour index was observed in 72 hours (0.20) while the lowest was observed in 24
hours, 48 hours and the control (0.16). The seedling survival found to be constant (100%)
at all the treatments.

The result obtained in this experiment showed that the different doses of Fast Neutron Irradiation (FNI) significantly affected only seedling length and did not affect germination
percentage, germination speed, vigour index and seedling survival. The significant reduction in germination percentage and germination speed at 24 hours and 96 hours exposure to fast neutrons may be due to the induction of oxidative damage in some cells by production of the free radical oxygen that

lead to higher frequency of chromosomal aberrations and DNA damage due to the accumulative genotoxicity and chromosomal aberrations (Uhl *et al.,* 2003). Similar result was reported on Niger (*Guizzotia abyssinica*) treated with gamma rays (Poornananda and Hosakatee, 2009). The significant increase observed in seedling length and vigor index from control to 72 hours and 120 hours could have been as a result of irradiation doses having biopositive effects on the gene resulting to a new altered germplasms. Fast Neutrons irradiation had bio-positive effects at specific dose probably by increasing number of amino acid produced in a protein (Haliem *et al.,* 2013). Fast neutron may cause special interference with DNA leading to induction of structural changes in DNA, such as chromosomal rearrangement, strand breaks, base deletions, pyrimidine dimers, cross-links and base modifications, and other effects (Gill and Tuteja, 2010). Similar finding were reported in *Capsicum annum*, *Capsicum frutescens* treated with fast neutrons that an increase in irradiation exposure period lead to increase in morphological traits (Falusi *et al.,* 2012). The steady values (no increased) observed in vigor index from control to 48 hours and in seedling survival throughout the treatment with increase in the dosage of fast neutron may be that the gene that confer this characters where not affect by fast neutrons at those treatments thus showing no effect morphologically or the mutations on these loci are recessive or the dosage was not enough to induce mutations in this traits. The not significant difference in percentage germination, germination speed, vigor index and seedling survival could be because the dosage of fast neutron was not enough to induce missense mutation. Highest means values observed at 72 hours in all the morphological traits could be used for improvement of these agronomic traits in okra.

Table 1: Mean Square for Germination Parameters in Induced Mutant of Okra

Source of Variation	Df	GP	GS	SL	VI	SS
Treatment	5	85.4430	0.0237	1.0622*	0.0009	0.0000
Replication	2	15.3100	0.0042	6.9238	0.0020	0.0000
Error	1	53.4200	0.0147	0.7112	0.0005	0.0000

* Significant at 0.05 probability level of significance.
Key - GP: germination percentage, GS: germination speed, SL: seedling length, VI: vigour index, SS: seedling survival.

Table 2: Mutagenic Effects of various Doses of Fast Neutron Irradiation on Germination Parameters of Okra

Treatments	GP (%)	GS	SL (cm)	VI	SS (%)
Control	88.87[a]	1.48[a]	10.73[b]	0.16[a]	100.00[a]
24 hours	84.47[a]	1.41[a]	11.17[ab]	0.16[a]	100.00[a]
48 hours	85.57[a]	1.43[a]	11.40[ab]	0.16[a]	100.00[a]
72 hours	96.70[a]	1.61[a]	12.50[a]	0.20[a]	100.00[a]
96 hours	92.20[a]	1.54[a]	11.60[ab]	0.18[a]	100.00[a]
120 hours	96.67[a]	1.61[a]	11.73[ab]	0.19[a]	100.00[a]

Means with same letter(s) in a column are not significantly different according to DMRT at 5% level of probability
Key - GP: germination percentage; GS: germination speed: SL: seedling length; VI: vigor index; SS: seedling survival.

Conclusions

The treatment means square for all the parameters was only significant for seedling length in this study. The optimum dosage that created useful variability was 72 hours in all the germination, seedling growth and seedling survival for fast neutron which was found to be better than the control. This showed that at 72 hours fast neutron concentration was the best treatment for most of the traits evaluated.

Recommendations

1. Further studies should be carried on more generations such as M2, M4, on okra.
2. Further trials for disease resistance, water tolerance, drought tolerance and so on could be carried out in subsequent generations to determine mutants that can adapt
and produce maximally under such conditions.
3. It is recommended that further experiment should be carried out at higher exposure periods that may produce promising traits to be exploited further for future breeding programme of okra in the study area.

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GENETIC VARIABILITY STUDIES IN SOME GINGER MUTANT LINES GROWN IN HUMID TROPICAL AGROECOLOGY OF NIGERIA

Abua, M. N.*, Obok, E.E, Ittah , M .A, Agah, L.J, Edugbo , R.E and Iwo, G. A

[1] Department of Crop Science, University of Calabar. PMB 1115 Calabar, Cross River State. Nigeria.
Corresponding Author:marynjei@yahoo.com; njeiabua@unical.edu.ng +2348069026641

ABSTRACT

An experiment was conducted, in the Teaching and Research Farm, Department of Crop Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, Calabar, Nigeria in 2017 on some ginger lines to evaluate the genetic variability, heritability and genetic advance in order to obtain necessary information useful in ginger improvement programmes .The experiment was laid out in a Randomized Complete Block Design in three replications. Heritability values for rhizome length (76.94 %), yield (68.91 %) and rhizome fingers (61.53 %) were high. Genetic advance as percent of the mean was high in rhizome yield (67.9%), rhizome length (41.86%) and number of rhizome fingers per plant (45.39 %) indicating less environmental effect and thus proffers the possibility of carrying out selection for these traits. The high values of heritability together with genetic advance as percent of the mean observed in these traits shows that phenotypic selection for these traits will be effective .On the other hand , number of leaves (39.23%), sprouting percentage (31 %), leaf length (20.2 %), leaf area (13.94%), establishment count (5.78 %) and number of tillers per plant (5.0 %) recorded low heritability values Genetic advance was also low in leaf width (27.47 %), plant height (25.30 %), number of tillers per plant (3.0 %), establishment count (2.46 %), leaf area (7.22 %), leaf length (6.2 %), sprouting percentage (18.49 %) and number of leaves per plant (17.4 %).The low heritability values as well as low values of genetic advance as percent of the mean showed that environmental factors play prominent role in the expression of these characters and indicates slow progress through selection for these traits .

Keywords: *Variability, Selection, Heritability, Improvement and Genetic advance.*

Introduction

Ginger, (*Zingiber officinale*) is mainly vegetatively propagated since it is prone to sexual reproduction constraints (Jatoi and Watanabe, 2013) and this had attributed to the narrowness of its genetic variation (Babu *et al.,* 2013). The sexual reproduction constraint thus limit ginger improvement mainly to clonal selection (Nisar and Ghofoor, 2011). Due to the fact that conventional breeding procedures like hybridization cannot be adopted for its improvement, the other alternative to improving the crop is to create variability through mutation. Inducing mutation has been considered an established method for increasing genetic variability in many crops and according to FAO (2006), 2,540 varieties in different crops have been commercially released through mutation breeding and this include vegetatively propagated crops like garlic, cassava, turmeric and potato, The aim of ginger improvement is to develop high yielding cultivars with vast adaptations, high quality, high content of Oleoresin, diseases and lodge resistance (Peter *et al.*, 2007) .

Generally, the achievement from any crop improvement programme depends to a large extend on the behaviour and degree of the genetic variability in the available breeding material. Therefore, success on plant breeding activities entirely depends on the existence of genetic variability with respect to desired traits and selection skills of the plant breeder (Adhikari *et al.,* 2018). Since inheritance of most economic characters such as yield are complicated in nature and are really affected by different environmental conditions, the study of heritability and genetic advance is most important in estimating the potential for improvement by selection. The improvement of yield could be carried out through selection, using existing genetic variability and positive/negative effect of traits on the dependent trait (yield). The behaviour and degree of genetic variability brings about the genetic improvement of desired traits in crops (Ibrahim, 2010). The progress of a breeding programme is conditioned by the nature of the genotypic and phenotypic variations in the various characters. The genotypic and phenotypic coefficient of variation are helpful in exploring the nature of variability in the breeding population whereas, the estimate of heritability provides index of transmissibility of characters.

The objective of this study therefore, is to evaluate the components of genetic variability, heritability and genetic advance in some ginger lines in order to provide necessary information useful in ginger improvement programmes.

Materials and Methods

This research was carried out in the Teaching and Research farm of the Department of Crop Science, University of Calabar , Calabar , Nigeria. during the 2017 cropping season (March to December). Calabar is located in the South Eastern zone of Nigeria with latitude (4.9757° N, and longitude 8.3417° E) and about 39m above sea level and has a biomodal annual rainfall distribution that ranges from 2915 to 3,500mm with a mean annual temperature range of 27 ^{0}C to 35 ^{0}C and relative humidity between 75-85% (NI 2017)

Seventeen ginger genotypes consisting of fifteen (15) mutant lines (UG1-11-07, UG1-13-02, UG1-2-35, UG1-5-04, UG1,-5-18, UG1-5-22, UG1-5-31, UG1-5-35, UG1-5-38, UG1-5-48, UG1-5-49, UG1-5-52, UG1-7-24, UG2-11-03, UG2-9-01) and two local landraces (UG1 and UG2) were used for this experiment. These genotypes were evaluated in the field in a randomized complete block design in three replications. Genetic component analysis was calculated for all the traits. Genotypic variance, environmental variance and phenotypic variance were estimated according to the methods of Singh and Chaudhary (1995) as follows:

$$\text{Genotypic variance } (\sigma_g^2) = \frac{MS_g - MS_e}{r}$$

$$\text{Environmental variance } (\sigma_e^2) = MS_e$$

$$\text{Phenotypic variance } (\sigma_p^2) = \sigma_g^2 + \sigma_e^2$$

Where

MS_g = Genotypic variance

MS_e = Error mean square

Genotypic and phenotypic coefficient of variability (GCV % and PCV %) were estimated according to Singh and Chaudhary (1995) as follows

$$GCV\ (\%) = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times \frac{100}{1}$$

$$PCV(\%) = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times \frac{100}{1}$$

Where GCV = Genotypic coefficient of variability

PCV = Phenotypic coefficient of variability

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

$\bar{x}$ = Mean

Broad sense heritability and genetic advance were estimated according to Johnson *et al.*, (1955) as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times \frac{100}{1}$$

$$GA = \frac{\sigma_g^2}{\sqrt{\sigma_p^2}}$$

$$GAM = \frac{GA}{\bar{x}} \times \frac{100}{1}$$

Where H^2 = Broad-sense heritability

GA = Genetic advance

GAM = Genetic advance as percent of mean

K = 2.06 at 5 % selection differential

$\sqrt{\sigma_p^2}$ = Square root of phenotypic variance

Results

The results of the genetic components analysis are presented in Table 1. Genotypic Coefficient of Variability (GCV) ranged from 4.95 % in establishment count to 40.64 % in yield. Phenotypic coefficient of variability (PCV) ranged from 14.37 % in leaf length to 48.96 % in yield. Phenotypic Coefficient of Variability (PCV) was higher than genotypic coefficient of variability (GCV) .Heritability values ranged from 5.0 % in number of tillers to 76.94 % in rhizome length.

Genetic advance as percent of mean was high in rhizome yield (67.9%), rhizome length (41.86%) and number of rhizome fingers per plant (45.39 %) .On other the hand , the values were low in leaf width (27.47 %), plant height (25.30 %), number of tillers per plant (3.0 %), establishment count (2.46 %), leaf area (7.22 %), leaf length (6.2 %), sprouting percentage (18.49 %) and number of leaves (17.4 %).

TABLE 1
Genetic components analysis for growth and yield traits of ginger lines

Trait	Mean	σ_g^2	σ_e^2	σ_p^2	GCV (%)	PCV (%)	H^2 (%)	GA	GAM
Sprouting percentage(%)	69.06	123.3	272.60	395.90	16.0	28.8	31	12.77	18.49
Establishment count(%)	76.16	14.22	231.50	245.72	4.95	20.58	5.78	1.87	2.46
Plant height (cm)	55.65	89.76	82.46	172.22	17.0	23.58	52.1	14.08	25.30
Number of leaves	16.29	4.59	6.579	11.17	13.15	20.51	39.23	2.83	17.4
Leaf length (cm)	20.00	1.755	6.934	8.689	6.6	14.73	20.2	1.23	6.2
Leaf width(cm)	2.221	0.1521	0.1086	0.2607	17.55	22.98	58.34	0.61	27.47
Leaf area (cm^2	32.95	9.585	59.14	68.73	9.395	25.16	13.94	2.38	7.22
Number of tillers	13.33	0.76	13.57	14.33	6.70	28.39	5.0	0.41	3.07
Number of rhizome fingers	14.98	17.74	7.087	28.83	28.0	35.84	61.53	6.80	45.39
Rhizome length (cm)	16.53	14.66	4.394	19.054	23.0	26.4	76.94	6.92	41.86
Yield(t/ha)	16.05	60.2	19.20	61.76	40.64	48.96	68.91	11.2	67.9

σ_g^2=Genotypic variance; σ_e^2 =Environmental variance σ_p^2=Phenotypic variance; GCV=Genotypic coefficient of variation; PCV=Phenotypic coefficient of variation; H^2=Heritability; GA=Genetic advance; GAM=Genetic advance as percent of mean

Discussion

The phenotypic coefficients of variability (PCV) values were higher than genotypic coefficients of variability (GCV) values in all the traits, suggesting that these traits were highly influenced by the environment. The effectiveness of a breeding program depends on the degree and behaviour of the genotypic and phenotypic variations in the various traits. The study of heritability and genetic advance is very essential in estimating the potential for improvement by selection. The usefulness of heritability shows how reliable the genotype will be identified by its phenotypic expression (Chandrababu and Sharma, 1999). Deshnukhet *et al.,* (1986), reported that PCV and GCV values greater than 20% are regarded as high whereas values less than 10% are considered to be low while values between 10% and 20% are medium. Based on this, the result showed that rhizome yield , number of rhizome fingers per plant and rhizome length had high GCV and PCV values while plant height, leaf width, sprouting percentage and number of leaves recorded medium GCV and PCV values. This result implies that selection may be successful on these traits. The high magnitude of GCV further revealed that greater extent of variability is present in these characters thereby suggesting good scope for improvement through selection of this crop. Studies of genetic variability create very useful information on the effectiveness of genetic improvement of crops for yield and quality attributes. A prerequisite for response to selection is genetic variability and thus, an understanding of the extent and nature of phenotypic and genotypic variability is one of the fundamental needs for the breeders for further improvement of crops (Adam, 2008). The degree of genotypic and phenotypic variability that exist in a crop is important in developing superior varieties and in starting a breeding programme. High genotypic variance allows easy selection for improvement and widens the probability for heritability of traits from parents to the offspring (Ayalneh *et al.*, 2012). Very high significant genotypic variance in diverse attributes of ginger was reported by Ravishanker *et al.*, (2013). Jatoi and Watanabe, (2013) observed a high to moderate variance for plant height, rhizome weight, rhizome thickness, sheat length, tillers per plant and leaf length in ginger landraces .

Heritability helps the breeder in selection on the basis of phenotypic performance . Singh (2001), reported that heritability is very high when the values are greater than 80%, moderately high when the values are between 60-79%, medium when the values are between 40-59% and low when the values are less than 40%. According to this groupings, heritability values for rhizome length (76.94%), yield (68.91%), and number of rhizome fingers per plant (61.53%) were moderately high; those of leaf width (58.34%) and plant height (52.1%) were medium while those of number of leaves (39.23%), sprouting percentage (31%), leaf length (20.2%), leaf area (13.94%), establishment count (5.78%) and number of tillers per plant (5.0%) were all low. The moderately high heritability values recorded in rhizome length, yield, and number of rhizome fingers per plant indicated that these characters are least influenced by the environment suggesting that the largest part of the observed variation of these traits was genetic in nature and the effect of the environment was smaller, therefore there is a possibility of obtaining a satisfactory selection. This conform with the findings of Omoigui *et al.*,(2006) that high heritability estimates allows easy transmissibility of characters from parents to offspring. Hence, selection for improvement of these characters will be successful. Conversely, the low heritability values observed in number of leaves per plant, sprouting percentage, leaf length, leaf area, establishment count and number of tillers per plant showed that these traits were highly affected by the environment and genetic improvement through selection will be slow. Heritability estimates gives information about the proportion of the physical expression of traits which is attributed to genetic differences and this plays a greater role in selection (Ndukauba *et al.*,2015). When

heritability estimates are high, the traits are expected to remain stable in different environmental conditions and could easily be improved through selection (Siddique *et al.*, 2006). There is a direct relationship between heritability and response to selection which is referred to as genetic progress. The expectation of a response to selection is called genetic advance (G.A.). High genetic advance coupled with high heritability estimate offers the best effective condition for selection (Larik *et al.*, 2000). Johnson *et al.* (1955) and Shulka *et al.* (2006) also stated that heritability and genetic advance should be jointly considered for reliable selection. In other words, heritability values in addition to estimates of genetic advance were better than heritability alone in predicting effective selection. Therefore, genetic advance is an important indicator in selection. Genetic advance is of considerable importance because it indicates the magnitude of the expected genetic gain from one cycle of selection (Hamdi *et al.,* 2003). Hussein (2006) stated that , Prediction of the response of an individual to selection are more reliable when GCV, estimate of heritability in the broad sense and genetic advance are combined, instead of relying on estimates of heritability alone. Hence, high values of heritability and genetic advance as a percent of the mean along with high genotypic coefficient of variation in traits like rhizome length, number of rhizome fingers per plant and rhizome yield indicates that improvement of these traits through effective selection will be easy. Medium heritability values accompanied with low genetic advance as percent of mean recorded for leaf width (58.34%, 27.47%), and plant height (52.1%, 25.30%) indicates that these characters were affected more by the environment. Low heritability with low genetic advance observed in number of tillers (5.0%, 3.0%), establishment count (5.78%, 2.46%), leaf area (13.94%, 7.22%), leaf length (20.2%, 6.2%), sprouting percentage (31%, 18.49%) and number of leaves per plant (39.23%, 17.4%) indicate slow progress through selection for these traits. In other words, selection may not be useful for the improvement of these traits because of the narrow range of phenotypic variation among the genotypes in respect to these characters, therefore the improvement of these characters could be achieved through heterosis or progeny testing (Abua ,*et al.,*2018)

Summary and Conclusion

The genetic components analysis showed that the variance components, heritability as well as the genetic advance values for number of rhizome fingers per plant, rhizome length and rhizome yield were moderately high suggesting that selection for improvement of these traits will be successful. The relative contribution of the components of genetic variance and environmental variance helps in choosing the best selection methods. The information obtained from this research work will be important in the development of excellent selection procedures for the improvement of ginger genotypes and also serve as a source of useful information for the cultivation of ginger. Further testing of these genotypes is recommended.

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CGBPB 033

GENOTYPE × ENVIRONMENT INTERACTION OF WHITE MAIZE HYBRIDS IN SOUTHERN GUINEA SAVANNA ECOLOGY OF NIGERIA

Aboderin O.S. [1], Bankole F.A.[2]

[1]Department of Agronomy, University of Ilorin, Ilorin, Nigeria
Corresponding Author: olawaleaboderin@yahoo.com, +2348032161832

ABSTRACT

A proper understanding of the effects of G x E interactions on variety evaluation is vital for cultivar recommendations. This study was conducted to determine the grain yield performance, stability and adaptability of white maize hybrids in the Southern Guinea savanna of Nigeria. Seven maize hybrids were evaluated at six locations in the southern Guinea savanna (SGS) ecology of Nigeria in 2015 and 2016. The mean square from analysis of variance showed that significant differences ($\rho \leq 0.01$) existed across environment and genotypes for grain yield and other measured traits. Genotype by environment effect was also significant ($\rho \leq 0.05$) for grain yield and days to anthesis only. The yield of the hybrid ranged from 3.25 (EEWH) to 5.98 t/ha (EEWH-59). The analysis of GGE biplot showed that EEWH- 59 and EEWH-48 were not only the highest yielders but also were the most stable across the environments except in Ballah where EEWH-43 performed better than the rest of the genotypes. Mokwa based on discriminating and representative effect of the GGE biplots appears to be the ideal test environment for selecting adapted genotypes while Omu-Aran and Ballah were highly discriminating but non-representative and are therefore useful for selecting specifically adapted genotypes. EEWH-59 and EEWH-48 were the closest to the ideal genotype and may be considered as superior hybrids for further testing in farmers' fields for the purpose of registration and release for the southern Guinea Savanna.

Keywords: *Cattle, breeds, genetic, resources, variatio- Genotype x environment interaction, GGE biplot, Zea mays L., grain yield, Southern Guinea Savanna, Stability.*

Introduction

Maize (*Zea mays* L.) is the third most important crop after wheat and rice in terms of production in the world (IITA, 2009). It is not only an important source of human nutrition but also a basic element of animal feed and raw material for manufacture of many industrial products which include corn starch, maltodextrins, corn oil, corn syrup etc. Maize is a versatile crop grown over a range of agro-climatic zones with an unmatched sustainability in diverse environments (Downsell, 1996). It is grown from 58^0 N to 40^0 S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Downsell *et al.*, 1996) and with a growing cycle ranging from 3 to 13 months (CIMMYT, 2000).

Grain yield is the most important trait because it is the one that gives an economic benefit to the consumers. Hence, good varieties should give high yield and should be stable across different environments in which they are grown (Finlay and Wilkinson, 1963). However, the quantitative nature of grain yield predisposes it to the effect of genotype x environment interaction (GEI) which is the differential response of cultivars across two or more environments. The effect of GEI often cause complications in varietal selection activities and identification of genotypes for wide adaptation in most

breeding programmes (Magari and Kang, 1993, Badru-Apraku *et al.,* 2011). A large portion of the observed GEI in grain yield responses of maize genotypes in multilocational trials may be due to varying environmental conditions prevailing at the different agroecological zones where maize

is grown, which often hampers the identification of high yielding genotypes.
Numerous studies have shown that a proper understanding of the environmental and genetic factors causing the interactions as well as an assessment of their importance in the relevant GEI system could have a large impact on plant breeding (Magari and Kang, 1993; Basford and Cooper, 1998, Badru-Apraku *et al.,* 2011). GEI occurs universally when genotypes are evaluated in several different environments (Becker and Léon, 1988; Magari, 1989; Kang, 1990, Badru-Apraku *et al.,* 2003). The study of the GEI allows the classification of genotypes by their behaviour into two different situations, either stable or adapted to a particular environment in terms of their yield or some other interesting agronomic features. Generally, the term stability refers to a genotype's ability to perform consistently, whether at high or low yield levels, across a wide range of environments. On the other hand, adaptability refers to the ability of the genotype to be high-yielding with respect to a given environment or given conditions to which it is adapted (Cooper *et al.,* 1996).
Some authors, however, have applied the yield stability concept with respect to consistency in time of genotype performance, using the adaptation concept in relation to consistency in space (Lin and Binns, 1988; Evans, 1993). Only genotype × location (GL) interaction, rather than all kinds of GE interaction, is useful for depicting adaptation patterns, as only this interaction can be exploited by selecting for specific adaptation or by growing specifically adapted genotypes (Piepho *et al.*, 1998).
Several statistical procedures have been deployed in revealing GxE patterns, the GGE biplot developed by Yan, Hunt, Sheng, and Szlavnics (2000) respectively is the most efficient. It is an efficient tool for identification of the best performing genotype in a given environment and the most suitable environment for each genotype (Yan et al., 2000).

Crop breeders have therefore, focused on developing genotypes with superior grain yield, quality and other desirable characteristics which can be exhibited over a wide range of different environmental conditions. Therefore, this study was designed to investigate the influence of genotype by environment interaction on the expression of grain yield and related traits on white maize hybrids in Southern Guinea savanna ecology of Nigeria.

Materials and Methods

Seven Extra Early white maize hybrids with tolerance/resistance to multiple stress factors (drought, striga, low N etc) which were developed by maize scientists at the International Institute of Tropical Agriculture (IITA), Ibadan were used for this study. The lists of the genetic materials are presented in Table 1. The experiment was set up on each site in a Randomized Complete Block Design with an alpha lattice arrangement, 5 m long with inter and intra-row spacing of 0.75 and 0.5 m, respectively. Two (2) seeds were planted per hill and each set of trial was replicated three (3) times. The genetic materials were evaluated at six (6) locations in the southern guinea savanna (SGS) ecologies of Nigeria between 2015 and 2016. The locations were Ballah, Lapai, Mokwa, Omu-Aran, Kishi and Ilorin. The details of the testing site are presented in Table 2. Data was recorded for grain yield (GY) (shelled grain weight per plot adjusted to 15% grain moisture and converted to tons per hectare), anthesis date (number of days after planting when 50% of the plants shed pollen), silking date (number of days after planting when 50% of the plants extrude silks), anthesis-silking interval (ASI) (the difference between silking date and anthesis date), Plant height (cm), Ear height (cm), and Plant and ear aspects (rated on a scale of 1 to 5).

Data Analysis

Data collected were subjected to analyses of variance using SAS 9.0 edition statistical software. Combined analyses of variance (ANOVA) across locations were performed to determine differences in genotypic performances across locations and also to determine whether there were significant differences among the locations. Genotypes (G) and environments (E) were considered as fixed effects while replications and genotypes x environment (GE) were considered random factors. Subsequently, the hybrids with high grain yield means were further subjected to genotype main effect plus genotype by environment interaction (GGE) biplot analysis to separate the GEI into principal component axes. This analysis was carried out using GGE biplot, a window application that fully automates biplot analysis (Yan, 2001). The GGE biplots were constructed using the first two principal components (PC1 and PC2). Information on yield performance and stability of the hybrids were analyzed by the biplot based on the position of their label relative to abscissa (the horizontal axis) and ATC coordination axis (vertical axis) respectively of the vector view of the biplot. The genotypes were ranked along the average-tester axis (ATA abscissa), with an arrow pointing to a greater value based on their mean performance across all environments. The double-arrowed line separates entries with below-average means from those with above-average means. The stability of the genotype is measured by their projection onto the double-arrow line average-tester coordinate (ATC) axis.

Table 1: - List of extra-early and early maturing hybrids used in the study.

S/N	Extra-Early White Hybrids
1.	EEWH-43
2.	EEWH-44
3.	EEWH-47
4.	EEWH-48
5.	EEWH-50
6.	EEWH-52
7.	EEWH-59

Table 2: Description of the test locations used in the study.

Location	State	Latitude	Longitude	Altitude (m)	Mean annual Rainfall (mm)
Ilorin	Kwara	$8^0$30'N	$4^0$32'60E	289	1318
Ballah	Kwara	$13^0$22'0N	$5^0$34'60E	249	1150
Mokwa	Niger	$9^0$16'60N	$5^0$26'0E	87	1250
Kishi	Oyo	$9^0$46'0N	$3^0$51'0E	372	2000
Omu- Aran	Kwara	$8^0$08'18.85"N	$5^0$06'9.4"E	536	1132
Lapai	Niger	$9^0$3'0N	$6^0$9'0E	117	1300

Results and Discussion

Combined Analysis of variance (ANOVA) for grain yield and other agronomic traits in 7 extra-early maturing white maize hybrids evaluated in six environments in the SGS of Nigeria

The results of the combined analysis of variance (ANOVA) across 6 environments for grain yield and related traits in the 7 extra early maturing white maize hybrids revealed significant effects of the environments ($\varrho<0.01$) and genotypes ($\varrho<0.01$) for grain yield and other agronomic traits investigated (Table 3). This is an indication of differential response of the genotypes to the environments and underscores the need to identify high yielding and stable genotypes across the environments. This result is consistent with the findings of Badu-Apraku *et al.,* (2003) and Mohammadi *et al.,* (2009) who reported that the largest proportion of total variation in multi- environment trials were attributed to locations. The genotypes also differed significantly for days to anthesis, plant and ear heights while variety GEI was significant only for grain yield and ear height indicating the greater importance of GEI during selection for a high yielding maize cultivar. The observed significant GEI for grain yield also indicates the influence of the different climatic and soil factors at each location on expression of grain yield. Significant differences among the genotypes for a particular trait is an indication that there is sufficient variability among the genotypes for selection or improvement (Badru-Apraku *et al.,* 2013). The test environments used for the study contributed significant proportion of variation in grain yield compared to the genotypes themselves and there was alteration in genotype ranking from one testing site to another.

Grain yield ranged from 3.25 t/ha (EEWH-47) to 5.98 t/ha (EEWH-59) with a mean of 4.28 t/ha. Majority of the hybrids were similar for ASI while hybrid EEWH-47 had the longest interval of four (4) days (Table 4). The difference in height among the hybrids was 14 cm with hybrid EEWH-59 being the tallest while hybrid EEWH-50 was the shortest. Hybrid EEWH-43 had the highest ear placement while hybrid EEWH-47 had the lowest and the difference was 9 cm. The hybrids were however similar for ears per plant (EPP) across environments.

Polygon view of seven extra-early maturing white maize hybrids across six environments

The polygon view of the GGE biplot showed in respect of grain yield of seven top ranked extra-early maturing maize hybrids is presented in Figure 1. The principal component (PC) axis 1 explained 67.9% of the total variation while PC2 explained 22.2% and thus, PC1 and PC2 accounted for 90.1% of the total variation. According to Yan *et al.,* (2005), the vertex cultivar in each sector in the polygon view represents the highest yielding cultivar in the environments that fall within that particular sector. Thus, hybrids EEWH-59 and EEWH-48 were the highest yielding in the environment. EEWH- 43 was the best (vertex cultivar) for Ballah.

Performance and stability of seven extra-early maturing white maize hybrids evaluated in six environments

The mean versus stability view of the GGE biplot displayed the performance as well as the stability of the cultivars at the six environments in the SGS of Nigeria (Fig. 2). The genotypes were ranked along the average-environment axis (AEA abscissa), with an arrow pointing to a greater value based on their mean performance across all environments. The double-arrowed line separates entries with below-average means from those with above-average means. The average yield of the cultivars is approximated by the projections of their markers on the average-tester axis, (Yan and Tinker, 2006). Based on this, EEWH-48 and EEWH-59 with short vector pointing toward a greater value based on the mean performance along the ATC were the most stable and high yielding cultivars while EEWH-50 with a long vector pointing towards greater value based on the mean

performance along the ATC was the least stable and also low yielding cultivar.

Discriminating power versus representativeness view of the testing sites for seven extra-early maturing white maize hybrids evaluated in six environments

Discriminating power versus representativeness view of GGE biplot analysis for the 6 test locations used in the evaluation of the 7 extra early maturing maize hybrids in the SGS of Nigeria in the year 2015 and 2016 is presented in Figure 3. In the biplot, the length of the environment vector is a measure of its discriminating power and the angle between any vector and average tester axis approximates the correlation coefficient between the vector and the ideal test location, which is an indication of the representativeness of the testing site, (Yan and Tinker, 2006). On this basis, Mokwa happens to be the closest to the ATC and the most representative of all the test environments and therefore the best test environment for selecting generally adapted genotypes while Omu aran and Ballah have Discriminating but non-representative effect and are therefore useful for selecting specifically adapted genotypes.

Table 3: Mean squares from combined ANOVA for grain yield and other agronomic traits in 15 extra-early maturing white maize hybrids evaluated in six environments in the Southern Guinea Savannah of Nigeria (2015 and 2016)

Source	Df	Grain Yield	Days to 50% anthesis	Days to 50% silking	Anthesis-silking interval	Plant Height	Ear height	Ear per plant
Environment (E)	5	17595767.9**	174.88**	201.34**	171.51**	12981.19**	6930.29**	0.16**
Rep (E)	12	3034488.4	12.32**	6.97	2.88	148.16	20.92	0.01
Genotype (G)	6	22250583.6**	15.30**	14.37*	1.15	554.99	186.53*	0.02
G x E	30	2253473.8*	4.45*	4.61	1.85	211.72	88.83	0.02
Pooled Error	72	2232190.8	2.78	3.51	174.10	180.43	69.48	0.02
CV (%)		34.89	3.21	3.44	64.24	9.94	15.30	15.85

Table 4:- Mean Performance for grain yield and other agronomic characters of 7 extra-early maturing white maize hybrids across six environments in the SGS of Nigeria (2015 and 2016).

HYBRIDS	Grain yield (t/ha)	Days to anthesis (days)	Days to silking (days)	ASI (days)	Plant height (cm)	Ear height (cm)	Ears per plant (no)
EEWH-43	4.97	50	53	3	140	55	1
EEWH-59	5.98	53	55	2	141	51	1
EEWH-50	3.46	53	56	3	127	49	1
EEWH-44	3.58	52	53	2	136	53	1
EEWH-47	3.25	52	56	4	131	46	1
EEWH-48	5.33	52	54	2	132	52	1
EEWH-52	3.42	52	54	3	140	53	1
Mean	4.28	52	54	2	135	51	1
LSD	0.99	1.11	1.24	1.03	8.93	5.20	0.10
CV(%)	34.89	3.21	3.44	64.24	9.94	15.30	15.85

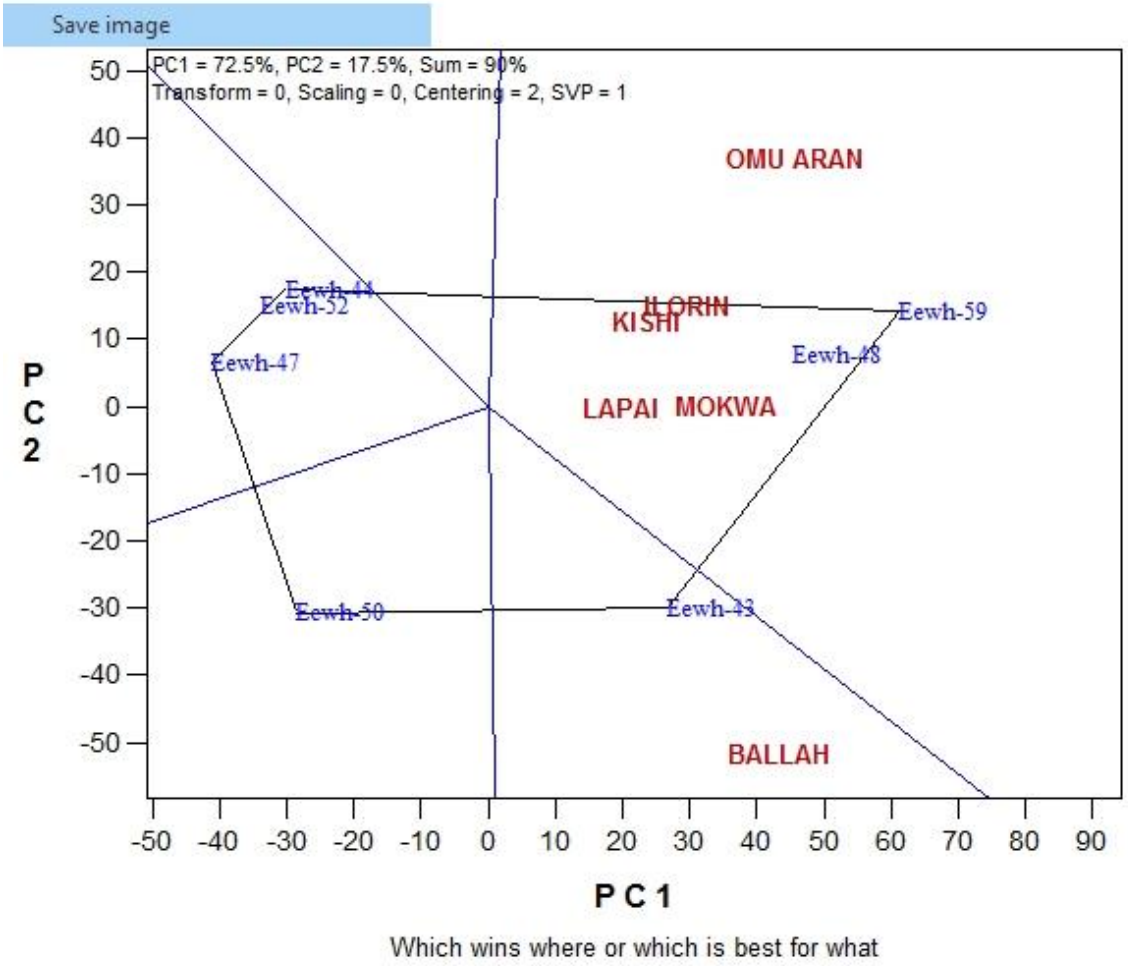

Figure 1:- Polygon view of genotype main effect plus genotype x environment biplot of seven extra early maturing white maize hybrids evaluated at 6 environments in Southern Guinea savanna of Nigeria

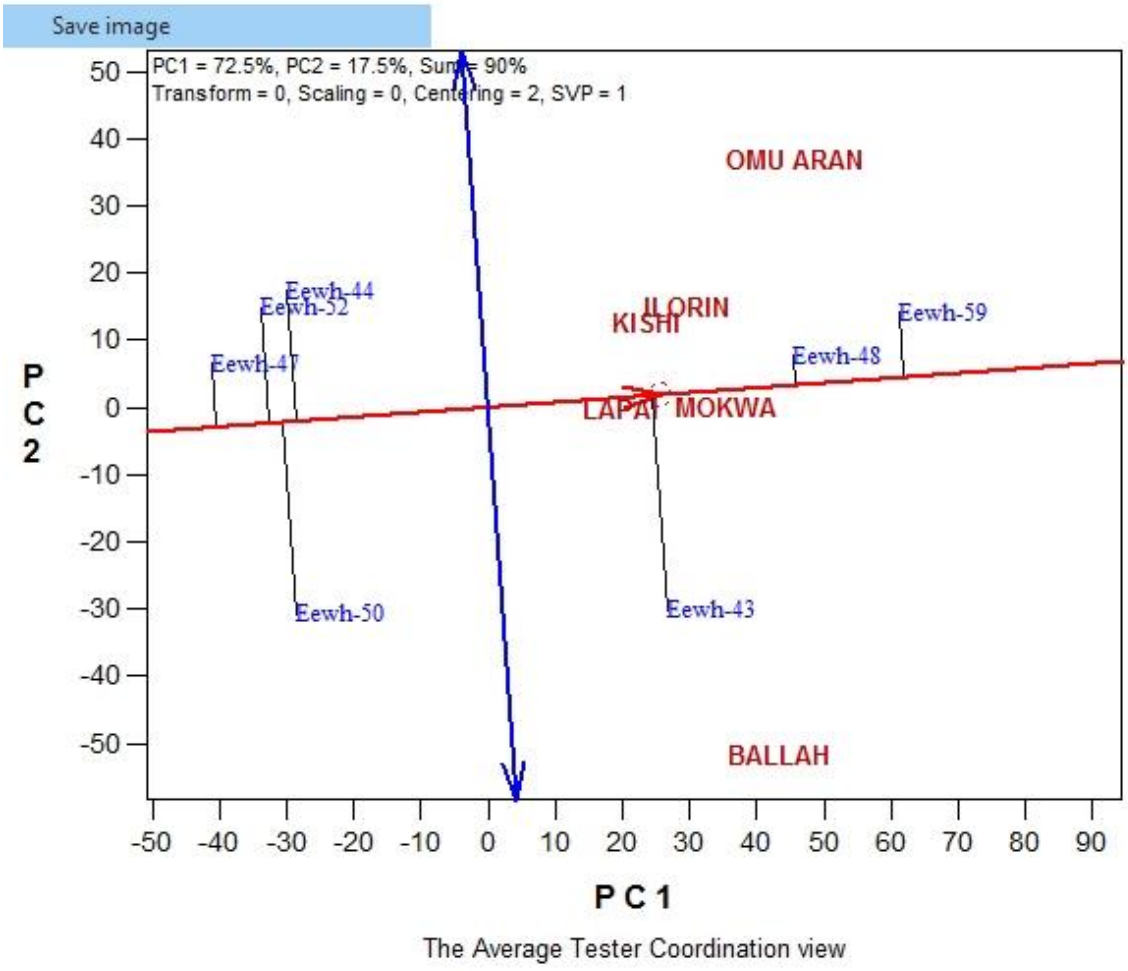

Figure 2: Mean versus stability view of GGE biplot of seven extra-early maturing white maize hybrids evaluated at 6 environments in Southern Guinea Savanna of Nigeria

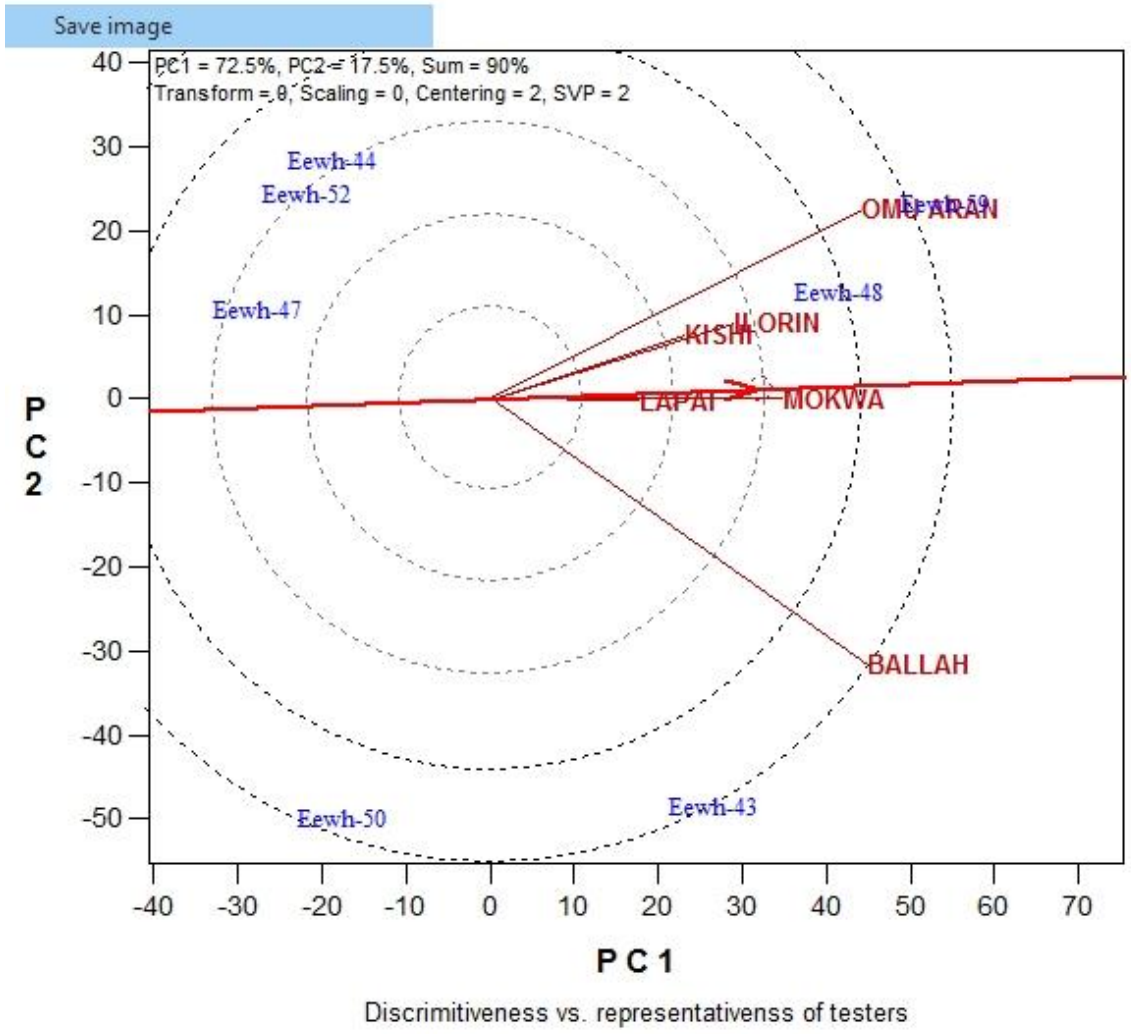

Figure 3: vector view of genotype main effect plus genotype by environment interaction (GGE) biplots showing ideal environments for the seven extra-early maturing white maize hybrids based on their discriminating power and representativeness.

Conclusion and Recommendations

Identification of superior genotypes based on their performance is usually complicated by the phenomenon of genotype by environment interaction (GEI). Large occurrence of GEI causes the relative rankings of genotypes to change from location to location. Thus, it is important to have a proper understanding of the effects of GEI on variety evaluation to aid decisions on cultivar recommendations

Mokwa based on discriminating and representative effect on the GGE biplots appears to be the ideal test environment for selecting generally adapted genotypes while Omu aran and Ballah have Discriminating but non-representative effect and are therefore useful for selecting specifically adapted genotypes. EEWH-59 and EEWH-48 were the closest to the ideal genotype and may be considered as superior varieties for further testing in farmers' fields for the purpose of release.

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GENETIC VARIABILITY AMONG SETS OF EARLY AND EXTRA-EARLY MAIZE (*ZEA MAYS* L.) INBRED LINES UNDER DROUGHT

S. Zakariya*, M. Oyekunle*, D. A. Aba, S. M. Bugaje

Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
* Corresponding Author: zacqkz@gmail.com; +2348037151311

ABSTRACT

Information on genetic variability among diverse germplasm is required to facilitate the identification of lines that would produce crosses possessing high levels of heterosis. This study was conducted to determine the genetic variability for grain yield and agronomic traits of different maturity groups of maize inbreds for tolerance to drought and identify promising drought-tolerant maize inbred lines that can be used to develop drought tolerant maize hybrids for drought-prone regions. Twenty one inbred lines of different maturity groups and endosperm-modification were screened under induced drought and optimum growing conditions at Samaru in 2016. Results of the analysis showed significant ($p < 0.05$) difference among genotypes for grain yield, days to anthesis, days to silking, and plant and ear heights under induced drought Grain yield of the inbreds ranges from 0.61 t ha^{-1} for TZEI 13, ENT 3 and TZEEI 29 to 1.23 t ha^{-1} for TZEQI 33 under induced drought and 0.97 t ha^{-1} for TZEQI 24 to 2.07 t ha^{-1} for TZEE-W-Pop STR C_5 under optimum growing conditions. The highest drought tolerance index (4.38) was recorded for TZEQI 24 having a yield reduction of 10.8%, while TZEI 29 had the lowest tolerance index of -5.90 a with yield reduction of 40.8%. Genes for tolerance to drought in the identified inbreds could be introgressed into maize germplasm for development of drought tolerant maize hybrids for drought prone areas of Nigeria.

Keywords: *Base Index, Drought tolerance, Inbreds, Induced drought, Yield reduction*

Introduction

Maize (Zea mays L.) is ranked third to wheat and rice in the world's production of cereal crops (Cooper *et al.,* 2014). It is widely grown throughout the world in a wide range of agro ecological environments. Being a C4 species, maize utilizes moisture and sunlight efficiently to produce high yield and total dry matter (Bell, 2017). The demand for global maize production is increasing as a source of food, forage, oil, and biofuel, for the ever-increasing world human population. However, the annual maize yield loss due to drought was estimated to be about 15% of potential yield on a global basis (Edmeades, 2008 and Adewale *et al.,* 2018). Major maize producing areas will become warmer, drier, and subject to an array of new maize diseases and pests under climate change that may lead to alarming impacts on maize production under the hotter climate scenarios, although the degree of the impact varies across sites and rainfall pattern change (Edmeades, 2013). This scenario calls for incorporating drought and heat tolerance traits into maize germplasm to offset predicted yield losses and sustain maize productivity in vulnerable sites (Tesfaye *et al.,* 2018).

Even in those lowlands with adequate precipitation for maize production, periodic drought may occur at the most sensitive stages of the crop such as flowering and grain filling. While drought will impact the growth and ultimate performance of a crop at any stage, it is of most detriment at flowering and

grain-filling resulting in yield penalties of between 40 and 90% (Menkir and Akintunde, 2001; Badu-Apraku *et al*., 2011; Badu-Apraku and Oyekunle, 2012). Therefore, improved tolerance to drought is an important breeding objective to stabilize maize production. Information on the performance of inbreds is crucial for hybrid development programs. It is of great importance for maize breeders to identify potential inbred lines that would produce hybrids exhibiting high levels of heterosis without making all possible crosses among inbreds available in a breeding program. Inbred line information is desirable to reduce costs and time associated with hybrid production and evaluation.

Selection of the suitable genotypes under water-stress conditions is one of the most important goals in breeding programs (Richards *et al.,* 2002). Fernandez & Kuo (1993) stated that the identification of genotypes based on drought tolerance could be difficult when the interaction between genotypes and the environment happened. Fernandez & Kuo (1993) classified plants according to their reactions in drought-stress and non-stress environments in 4 groups: A) genotypes with the best and highest performance under both conditions, B) with the best performance only in optimal conditions, C) best performance in drought-stress condition, D) weak performance in both conditions. Various drought indices were determined and used for the identification of best drought-tolerant genotypes. Several reports demonstrate the important role of Drought Tolerance Index (DTI) and Geometric Mean Productivity (GMP) as the most suitable indices to identify resistant genotypes in drought-stress conditions (Darkwa *et al.,* 2016; Gholinezhad *et al.* 2014; Golabadi *et al.,* 2006; Mehrabi *et al.,* 2011; Moghaddam & Hadizadeh, 2002; Naghavi *et al.,* 2013; Ramirez-Vallejo & Kelly, 1998; Rosielle & Hamblin, 1981).

This study determined the genetic variability among inbred lines of different maturity groups and endosperm modification and uses Drought Tolerance Index to classify the lines for drought tolerance.

Experimental Site

The research was carried out at the Institute for Agricultural Research (IAR), Samaru Research farm at Zaria. The Institute for Agricultural Research (IAR) Research farm, Zaria is located on $11^0$11'N, $07^0$38'E with altitude of 686 m above sea level, in the northern Guinea savanna agro-ecological zone of Nigeria, and the soil type is loamy with mean annual rainfall of about 1045 mm.

Genetic Materials and experimental procedures

Twenty one early and extra-early maize inbred lines with different kernel modification sourced from IITA were used for this experiment. The 21 inbred lines were evaluated under optimum growing conditions at Samaru during the 2015 rainy season and managed drought stressed in the same location during 2015/2016 dry season. A randomized complete block design with three replications was used for the evaluation. Row length was 4 m long with 22 plants per row. Row and hill spacing were 0.75 m and 0.4 m respectively. Three seed were planted per hill and seedlings were thinned to two per stand about two weeks after emergence, giving a population density of 66,666 plants per hectare. A compound fertilizer (NPK 15:15:15) was applied at the rate of 60 kg N ha^{-1}, 60 kg P ha^{-1} and 60 kg K ha^{-1} two weeks after planting. An additional 60 kg N ha^{-1} urea was top-dressed two weeks later in the drought experiment and 4 weeks

later in the well-watered experiment. Irrigation was supplied twice every weak using furrow irrigation system. The managed drought stress was achieved by supplying irrigation water twice a week up to 35 days after planting. Thereafter, the irrigation water was withdrawn in the drought experiment, so that the maize plants relied on stored water in the soil for growth and development. On the other hand, the experiment under optimum growing conditions, continue to receive irrigation until physiological maturity. Except for the water treatment, all management practices were the same for both the optimum and drought experiments. The experiments were kept weed-free by the application of gramaxone, a contact herbicide just after planting. Subsequently, manual weeding was done at two and four weeks after planting to keep the trials field weed-free.

Data Collection

Days to anthesis and silking were recorded for each plot as the number of days from planting to when 50% of the plants in a row had shed pollen and had emerged silks, respectively. Anthesis-silking interval (ASI) was computed as the interval in days between days to silking and anthesis. Plant and ear heights were calculated as the average of measurements on 10 competitive plants (excluding plants at the edges) per plot and were measured after anthesis as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant and ear aspects were rated on a scale of 1 to 5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Stay green characteristic was taken as leaf death score (LDS) and was recorded on a scale of 1 to 10, where 1 = 10% dead leaf area; 2 = 20% dead leaf area; 3 = 30%dead leaf area; 4 = 40% dead leaf area; 5 = 50% dead leaf area; 6 = 60%dead leaf area; 7 = 70% dead leaf area; 8 = 80% dead leaf area; 9 = 90% dead leaf area; 10 = 100% dead leaf area, for the drought stressed plots at 63 and 70 days after planting (DAP) respectively. The number of ears per plant (EPP) was computed as the total number of ears at harvest divided by the number of plants at harvest. Under drought environments, all ears harvested from each plot were shelled to determine percentage grain moisture and grain weight. Grain yield, adjusted to 150 g kg^{-1} moisture, was computed from the shelled grain weight. On the other hand, under well-watered environments, harvested ears from each plot were weighed and representative samples of ears were shelled to determine percent grain moisture. Grain yield adjusted to 150 g kg^{-1} moisture, was computed from ear weight and grain moisture assuming a shelling percentage of 80% (800 g grain kg^{-1} ear weight).

Statistical Analysis

The data collected were subject to analysis of variance (ANOVA) using individual plot means. Analysis was computed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS) software, version 9.2 (SAS, Institute 2004). To characterize the inbred lines for tolerance to drought, a base index that combined increased grain yield under drought stress with good plant and ear aspects, delayed leaf senescence, short ASI, increased number of ears per plant and grain yield under well-watered conditions was developed according to Badu-Apraku et al. (2011) and Oyekunle et al. (2013). Since each parameter was standardized with a mean of zero and standard deviation of 1 to avoid the effects of different scales, a positive value indicated tolerance of lines to drought while a negative value indicated susceptibility of lines to drought. The base index value was calculated using the following equation:

Base index = $\left[(2 \times Yield_{DS}) + Yield_{WW} + EPP - ASI - Plant\ aspect - ear\ aspect - LDS\ II\right]$

Results

Performance of Inbred Lines under Drought and Optimum Growing Conditions

Results of the analysis of variance of inbreds showed significant difference ($p < 0.05$) among the genotypes for grain yield, days to anthesis, days to silking, and plant and ear heights under induced drought stress (Table 1). In contrast, under optimum growing conditions, there was no significant difference among the inbreds for all measured traits except grain yield and plant height (Table 1).

Grain yield of the inbreds ranges from 0.61 t ha^{-1} for TZEI 13, ENT 3 and TZEEI 29 to 1.23t ha^{-1} for TZEQI 33 under induced drought and 0.97 t ha^{-1} for TZEQI 24 to 2.07 t ha^{-1} for TZEE-W-Pop STR C_5 under optimum growing conditions (Table 2). TZEQI 24 had the lowest yield under optimum growing conditions but had the highest drought tolerance index (4.38) because of its lowest yield reduction percentage (10.83) when compared with its performance under induced drought stress and optimum growing conditions. TZEI 29 had the lowest drought tolerance index (-5.90). Under drought, the grain yield of TZEQI 24 represents 89.17% of what it would have produced in the same environment under optimal growing. The greatest reduction in yield was observed in TZEI 13 (62.07%). Twelve of the twenty one inbreds in the present study had positive index value and thus regarded as tolerant to drought while the remaining nine had negative index value and thus regarded as susceptible to drought stress.

Table 1; Mean squares for grain yield and other agronomic traits of maize parental inbred lines evaluated under drought during 2016 dry season and optimum growing conditions in Samaru in 2015 rainy season.

Source	DF	Yield	Days Male	Days Female	ASI	Plant Height	Ear Height	Root Lodge	Stalk Lodge	Husk Cover	EPP	Ear Aspect	Plant Aspect	Cob Length	Cob Diametr	Row/ Cob	Kernels/ Row	100 GW	LDS 1	LDS 2
Drought stress																				
Rep	1	0.084	0.095	0.214	3.429	360.2	6.095	0.095	0.024	0	0.008	0.214	0.024	36.03*	0.214	1.929	88.6	100.59*	0.381	0.857
Entry	20	0.068*	11.53**	25.15**	1.995	1037.46**	401.28**	1.095	0.507	0.557	0.007	0.199	0.218	7.785	0.549	11.28	86.36	22.73	0.367	1.064
Error	20	0.052	1.745	6.764	2.029	259	80.5	0.795	0.674	0.5	0.005	0.164	0.161	6.556	0.372	7.079	64.5	20.15	0.181	0.807
Optimum Growing Conditions																				
Rep	1	0.248	2.881	10.5*	2.38*	214.9	77.36	0.095	0.149	0.095	0.006	0.292	0.006	36.03*	0.214	1.929	88.6	100.60*		
Entry	20	0.17*	3.745	3.431	0.445	868.22*	180.6	0.183	0.145	0.07	0.013	0.356	0.392	7.785	0.549	11.28	86.36	22.73		
Error	20	0.086	2.431	1.75	0.331	343.8	116.3	0.283	0.149	0.095	0.011	0.317	0.206	6.556	0.372	7.079	64.5	20.15		

Table 2; Grain yield of parental inbred lines maize used in Diallel crosses evaluated under induced drought stress in 2016 dry season and optimum growing conditions in 2015 at Samaru.

Name	Pedigree	Drought Response	Grain Yield (t/ha)		Yield Reduction (%)	Base Index
			DS	WW		
TZEQI 24	TZE-W Pop x 1368 STR S7 Inb 2 x Pool 15 SR QPM BC2S6 31-42-1-1-1-6	T	0.87	0.97	10.83	4.38
TZEI 60	TZE-W Pop STR Co S6 Inbred 90-1-3	T	0.98	1.28	46.87	3.72
TZEQI 33	TZE-W Pop x 1368 STR S7 Inb 2 x Pool 15 SR QPM BC2S6 15-42-1-1-1-5	T	1.23	1.93	36.32	3.37
TZEI 86		T	0.94	1.42	32.49	3.04
TZEI 108	WEC STR S7 Inbred 7	T	1.19	1.74	31.28	2.43
TZEI 65	TZE-W Pop STR Co S6 Inbred 141-1-2	T	0.89	1.10	41.33	2.20
TZEEI 21	TZEE-W Pop x LD S6 (Set B) Inb.44	T	1.18	1.72	42.68	1.81
TZEE-W Pop STR C5	TZEE-W-POP STR Extra	T	1.15	2.07	46.00	0.97
TZE-Y Pop DT STR C4		T	1.15	1.64	30.29	0.84
TZEI 63	TZE-W Pop STR Co S6 Inbred 136-2-3	T	1.12	1.69	34.79	0.81

TZEI 59	TZE-W Pop STR Co S6 Inbred 80	T	0.96	1.40	52.59	0.81
TZEI 24	TZE-Y Pop STR Co S6 Inbred 142-2-2	T	1.03	1.69	39.29	0.46
TZEEI 6	TZEE-W SR BC5 x 1368 STR S7Inb. 100	S	0.91	1.59	43.59	-0.90
TZEI 25	TZE-Y Pop STR Co S6 Inbred 171-1-2	S	0.83	1.43	42.22	-1.44
TZEI 13	TZE Comp5-Y C6 S6 Inbred 12	S	0.61	1.62	62.07	-1.63
TZEI 124	TZE-Y Pop STR Co S6 Inbred 3-1-3	S	0.84	1.50	44.43	-2.16
TZEQI 4	TZE-W Pop x 1368 STR S7 Inb 1 x Pool 15 SR QPM BC2S6 1-1-1-1-4-11	S	0.89	1.63	45.81	-2.59
ENT 3	M37W/ZM607#bF37sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-2-1-6 x [KILIMA ST94A]-30/MSV-03-2-10-B-1-B-B-xP84c1 F27-4-1-6-B-5-B]3-1-2-B/CML442)-1-1	S	0.61	1.11	43.15	-4.28
TZEI 87	TZE-W Pop x 1368 STR S7 Inb.	S	0.69	1.36	49.14	-4.30
TZEQI 25	TZE-W Pop x 1368 STR S7 Inb 2 x Pool 15 SR	S	0.83	1.52	45.62	-4.68

	QPM BC2S6 1-42-2-4-1-12					
	TZEE-W SR BC5 x 1368					
TZEEI 29	STR S7 Inb. 27	S	0.61	1.07	40.84	-5.90
MEAN			**0.93**	**1.50**		
S.E			**0.04**	**0.20**		

T- tolerant, S- susceptible

Discussion

Breeding for drought tolerant cultivars/hybrids is an essential step towards sustaining and increasing crop productivity, especially as the negative impacts of climate change on agricultural production escalate. Screening for, and characterization of, plant germplasm with better stress tolerance traits are prerequisites for the success of such breeding programs. This study evaluated a set of maize inbred lines for drought and tolerance under field conditions and identified inbred lines with superior drought and tolerance traits. The results suggest great genetic variation among maize germplasm for drought tolerance. The significant variation among inbreds for grain yield under drought and optimum water conditions indicated that adequate genetic variation existed among the different maize inbreds. The presence of these genetic variability implied significant progress could be made from selection for improvements in grain yield for the development of productive maize hybrids for drought prone and optimal growing environments. This result corroborates the findings of Rosielle and Hamblin (1981), Badu-Apraku *et al.* (2011) and Badu-Apraku and Oyekunle (2012). Inbred lines identified for their superior drought tolerance will serve as essential genetic material for breeding drought tolerant hybrids. The increased anthesis-silking interval, reduced number of ears per plant, grain yield, deterioration in plant and ear aspect under drought is consistent with the results of several earlier workers (Bolanos and Edmeades, 1993; Edmeades *et al.,* 1993). The high grain yield reduction observed among inbreds and revealed that the level of drought stress imposed during the flowering and grain-filling stages were severe enough to elucidate the differences among the inbreds. The low yield reduction observed in inbreds TZEQI 24, TZEI 108, TZEI 65 and TZE-Y-Pop DT STR C_4 under drought suggested that these inbreds possess drought-tolerant genes, which could be introgressed into populations and for the development of drought-tolerant hybrids and synthetic varieties. Inbreds with positive base index values are drought tolerant whereas those with negative base index values indicated susceptibility (Badu-Apraku *et al.*, 2011). The severe grain yield reduction observed under induced drought stress in the present study falls within the range reported by earlier workers (Badu-Apraku *et al.*, 2005; Campos *et al.*, 2006; Derera *et al.*, 2008; Badu-Apraku *et al.*, 2011).

Conclusion

Identification and characterization of inbred lines with superior drought tolerant traits are prerequisites for the success of a breeding program. The higher grain yield reduction that was observed among the inbreds under induced drought stress showed that the level of drought stress imposed were enough to elucidate the differences among the inbreds. Inbred lines TZEQI 24, TZEI 108, TZEI 65 and TZE-Y-Pop DT STR C_4 had the least yield reduction under drought. These inbred lines should be useful to maize breeders interested in breeding drought tolerant hybrids and synthetic cultivars for the drought prone regions of Nigeria.

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CORRELATION AND PATH COEFFICIENT ANALYSIS IN MAIZE (*Zea mays* L.) UNDER NON-STRESS AND WATER STRESS CONDITIONS

U.U. Umar *[1], S.G. Ado[2], A. Usman[2], M.D. Abdulrahman[1] and A.M. Iyad[2]

[1]Department of Biological Sciences, Kaduna State University, Kaduna State
*Corresponding author: biologistforlife09@yahoo.com, +2348065658743
[2]Department of Plant Science, Ahmadu Bello University, Zaria

ABSTRACT

The research was conducted at the Institute for Agricultural Research farms located at Samaru (11°11'N; 07°38'E) and Kadawa (11°39'N; 08°02'E) under non-stress and water stress conditions. The objectives were to determine traits affecting grain yield in maize under non-stress, intermediate stress and severe water stress conditions and to establish the nature of relation between grain yield and other traits by partitioning the correlation coefficients into direct and indirect effects using path analysis. The results showed grain yield correlated positively with plant height, ear height and ears per plant under the three conditions at phenotypic and genotypic levels except plant height under severe stress which was negative at phenotypic level. Days to 50% tasseling, days to 50% silking and anthesis-silking interval (ASI) correlated negatively with grain yield under the three conditions at both levels except 50% tasseling and days to 50% silking under non-stress which were positive but non-significant. In the path coefficient analysis, ear height (1.532, 1.224, 0.486), days to 50% tasseling (0.812, 0.782, 1.054) and ears per plant (1.194, 0.152, 0.441) should be considered as the main yield components because these traits showed positive direct effects towards increasing grain yield under the three conditions. These traits may be used as an effective selection criterion to improve yield potential of maize genotypes under non-stress and water stress conditions while ASI, days to 50% silking and plant height should be selected against as these traits showed negative direct effects towards increasing grain yield under the three conditions.

Keywords: *Correlation, non-stress, intermediate, severe, path, maize*

Introduction

Maize (*Zea mays* L.) has high potential for production and productivity in the Savanna ecology of sub-Saharan Africa due to high solar radiation and low night temperatures (Undie *et al.*, 2012). Despite the potential of maize in the Savanna ecology and the level of adaptation it displays, low yields are obtained due to biotic and abiotic stress. Biotic factors limiting maize production include insect pests, diseases, and parasitic weeds. The most important abiotic stresses limiting maize production are drought and low soil fertility, and these two are among the most important stresses threatening maize production, food security and economic growth in sub-Saharan Africa (Kamara *et al.*, 2004). Drought remains the most important constraint and losses in maize yield of about 15% each year has been attributed to it in Sub-Saharan Africa (Badu-Apraku *et al.*, 2005). Therefore, development of high yielding drought tolerant maize is very important.

Yield is a complex quantitative trait, considerably affected by environment. Therefore, selection of genotypes based on yield is not effective. Selection has to be made for the components of yield. A positive correlation between two desirable traits

makes the job of the breeder easy for improving both traits simultaneously. Even lack of correlation is useful for the joint improvement of the two traits. On the other hand, a negative association between two desirable traits impedes or makes it impossible to achieve a significant improvement in both traits (Ezeaku and Mohammed, 2006). Correlation studies only do not clearly reveal such sort of information and inadequate knowledge of interrelationships of heritable traits may lead to negative results (Bhatt, 1972). On the other hand, path coefficient analysis measures the direct and indirect effect for one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effect (Dewey and Lu, 1959). Path analysis focused direct and indirect effect of component traits on yield. The present research work was therefore carried out to detect correlation and path coefficient for increased yield in maize under non-stress and water stress conditions. The information thus obtained will be used to define the suitable selection criteria for yield improvement under non-stress and water stress conditions.

Materials and Methods

This study was carried in two locations: Samaru, Zaria (11°11'N, 07°38'E, 686 m above sea level) in the northern Guinea Savanna ecological zone of Nigeria and Kadawa, Kano (11°39'N, 08°02'E, 496 m above sea level), in the Sudan Savanna ecological zone of Nigeria under non-stress, intermediate and severe water stress conditions. Experimental materials for the present study consisted of 7 female inbred lines which were crossed with 6 drought tolerant male inbred lines to produce 42 hybrids during 2012 rainy season using North Carolina mating design II (Comstock and Robinson, 1948). Thirteen parents, resulting 42 F_1's and a commercial check were sown in a 7 x 8 simple lattice design replicated two times under each condition during the 2012/2013 dry season for evaluation. Each replication had one row of 3m length for each genotype while plant-to-plant and row to row distance was 0.25m and 0.75m, respectively. All other agronomic practices were kept uniform in both experiments except the irrigations.

Non-stress plot continued to receive irrigation water once every week until the end of physiological maturity. In intermediate stress plot water stress was imposed by withdrawing irrigation water as from 6 weeks after planting until the end of the growing season, to ensure drought stress at grain filling stage. The crop was allowed to mature only on stored soil water. In severe stress plot water stress was imposed by withdrawing irrigation water as from 5 weeks after planting to ensure drought stress at flowering stage. Because the average anthesis-silking interval was between 3-5 days at Samaru, an additional irrigation was applied at about 14 days after the end of male flowering to ensure that the small amounts of grains formed were filled adequately (Banziger *et al.*, 2000). No further irrigation water was applied at Kadawa because the average anthesis-silking interval was less than 3 days. The three conditions were separated from each other by 2.5 m alley to prevent spill-over at the water stress sites during the period of imposed water stress and at the beginning and end of each replication; non experimental lines were raised to minimize the edge border effects.

Observations and measurements were recorded from each plot for the following characters: days to 50% tasseling, days to 50% silking, anthesis-silking interval (ASI), plant height (cm), ear height (cm), ears per plant and grain yield (kg/ha). The estimates of correlation coefficient among various characters were worked out according to Singh and Chaudhary (1985). Path coefficients analysis was performed to assess direct and indirect effects of the measured traits on grain yield following the procedure given by Dewey and Lu (1959).

Results and Discussion

Correlation Analysis

In present study, plant height and ear height under the three conditions was positively and significantly correlated at both levels with

grain yield except plant height at phenotypic level which was negative and significant and ear height at genotypic level which was positive and non-significant under severe stress. Bello *et al.* (2010) and Wannows *et al.* (2010) also reported positive and significant association of grain yield with plant height and ear height while Prakash *et al.* (2006) reported negative correlation between grain yield and plant height. Ears per plant showed positive and significant genotypic association with grain yield under intermediate stress at both levels and under non-stress at genotypic level while under severe stress at both level and non-stress at phenotypic level were positive but non-significant. Aminu *et al.* (2013) and Aminu and Izge (2012) also reported positive association between grain yield and number of cops per plant. The study revealed that grain yield under non-stress condition correlated positively and significantly with days to 50% tasseling and silking at phenotypic level, while at genotypic level were positive but non-significant. Similar results were reported by Bello *et al.* (2010) between grain yield and days to 50% tasseling at phenotypic level while Selvaraj and Nagarajan (2011) reported positive and non-significant genotypic correlation of days to 50% tasseling and silking with grain yield. This shows that selection for any of these traits may result in corresponding increase in grain yield.

Grain yield was negatively and significantly associated with days to 50% tasseling and days to 50% silking under severe stress at both levels and under intermediate stress at genotypic level while at phenotypic level were negative but non-significant. Aminu and Izge (2012) also reported negative correlations of days to 50% tasseling and silking with grain yield under drought conditions. Grain yield was negatively and significantly associated with ASI under severe stress at both levels and under non-stress at genotypic level, while under intermediate stress at both levels and under non-stress at phenotypic level were negative but not-significant. Similar results were reported in maize by Bello *et al.* (2010) and Kumar *et al.* (2011). This contradicts the reports of Aminu *et al.* (2013) and Aminu and Izge (2012) who reported positive association between ASI and grain yield. This indicates that an increase in any of these traits may results in a corresponding decrease in grain yield and this suggests that grain yield can be improved by selecting for early tasseling and silk emergence and shorter ASI under drought conditions.

Table 1 Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients of maize traits under non-stress, intermediate and severe stress conditions across locations

Traits	DYTS	DYSK	ASI	PLHT	EHT	EPP	GY
Non-stress condition							
Days to 50% tasseling	1	0.986**	0.384*	-0.456*	-0.275	-0.272	0.155
Days to 50% silking	-0.350*	1	0.779**	0.749**	-0.348*	-0.425*	0.155
Anthesis-silking interval	-0.471*	-0.309*	1	-0.036	-0.393*	0.991**	-0.377*
Plant height	-0.457*	-0.344*	-0.064	1	0.993**	-0.289	0.748**
Ear height	-0.394*	-0.224	-0.026	-0.505*	1	-0.268	0.316*
Ears per plant	-0.425*	-0.330*	-0.054	-0.409*	-0.429*	1	0.823**
Grain yield	0.997**	0.998**	-0.065	0.974**	0.989**	0.176	1
Intermediate stress condition							
Days to 50% tasseling	1	0.989**	-0.045	-0.206	-0.833**	0.990**	-0.644**
Days to 50% silking	-0.235	1	0.169	-0.243	-0.992**	0.024	-0.779**
Anthesis-silking interval	-0.307*	-0.131	1	-0.352*	0.024	-0.009	-0.259
Plant height	-0.281	-0.271	-0.048	1	-0.855**	0.991**	-0.643*
Ear height	-0.173	-0.205	-0.021	-0.604**	1	-0.992**	0.376*
Ears per plant	-0.261	-0.223	-0.017	-0.472*	-0.398*	1	0.341*
Grain yield	-0.207	-0.196	-0.028	0.974**	0.992**	0.367*	1
Severe stress condition							
Days to 50% tasseling	1	0.994**	-0.321*	-0.797**	-0.537*	0.002	-0.383*
Days to 50% silking	-0.436*	1	-0.168	0.997**	0.776**	0.993**	-0.991**
Anthesis-silking interval	-0.460*	-0.131	1	-0.430*	-0.334*	0.022	-0.701**
Plant height	-0.458*	-0.349*	-0.335*	1	0.832**	0.719**	0.691**
Ear height	-0.488*	-0.415*	-0.321*	-0.579*	1	-0.073	0.289
Ears per plant	-0.464*	-0.394*	-0.281	-0.455*	-0.571*	1	0.159
Grain yield	-0.497*	-0.431*	-0.353*	0.669**	0.886**	0.159	1

*, **Significant at 0.05P and 0.01 probability levels, respectively. DYTS-days to 50% tasseling, DYSK-days to 50% silking, ASI-anthesis-silking interval, PLHT-plant height, EHT-ear height, EPP- ears per plant, GY-grain yield

Path Coefficient Analysis

The estimates of correlation coefficients revealed only the relationships between yield and the associated characters but did not show the direct and indirect effects of different traits on yield *per se*. This is because the attributes that are in association do not exist by themselves, but are linked to other components. Path coefficient analysis for grain yield confirm the direct contributions of days to 50% tasseling, ear height and ears per plant under the three conditions, ASI under non-stress and days to 50% silking and plant height under severe stress. Thus, these effects could be explored more confidently as selection criteria for yield improvement in the studied materials and a slight increase in one of the above traits may directly contribute to grain yield. Positive direct effect was also observed for plant height on grain yield by Ashraf *et al.* (2002), while Qadir and Saleem (1991) reported positive direct of ears per plant on grain yield.

In the present study, days to 50% tasseling recorded negative and significant correlations with grain yield but positive direct effect on grain yield under non-stress and intermediate stress conditions. Days to 50% silking also recorded negative and significant correlations with grain yield but positive direct effect on grain yield under severe stress condition. These results were supported by Bekavac *et al.* (2007) who reported that correlation identifies mutual associations between the various parameters irrespective of causation, while path coefficient analysis, on the other hand, specifies the causes and measure their relative importance and therefore, should be applied in addition to correlation analysis as more powerful tool according to Bhatt (1972) and Dewy and Lu (1959). They also added in some instances, path coefficient analysis gives somewhat different picture of net effects than do correlation analysis. That was the case here the positive direct effects of days to 50% tasseling and silking on grain yield, although the significant and negative correlations between them. The negative direct effects of days to 50% tasseling and ear height under severe stress, days to 50% silking and plant height under non-stress and intermediate stress and ASI under intermediate and severe stress on grain yield shows that these traits have no true relation with grain yield and hence not effective for the improvement of grain yield. These results are consistent with findings of Selvaraj and Nagarajan (2011).

Positive indirect effects showed that plant height under non-stress and intermediate stress, ASI under the three conditions, days to 50% silking and tasseling under non-stress and ear height under severe stress condition exerted positive indirect effects on grain yield via almost all the traits. Indirect effects on grain yield indicated that such traits effected grain yield via other component characters. Negative indirect effects of some characters on grain yield showed that there is no true relation of such characters on grain yield as negative effects were showed by ears per plant under the three conditions, ear height under intermediate stress, days to 50% tasseling and silking under intermediate stress and severe stress and plant height under severe stress. Negative indirect effects showed that these characters are not effective on grain yield; hence selection of such traits for improvement of yield could be less reliable.

Conclusion

The results of the current study showed that plant height, ear height and ears per plant should be considered as selection criteria for maize yield improvement under non-stress and water stress conditions. The current study confer that ears per plant, ear height and days to 50% tasseling showed maximum direct positive effect on grain yield should be considered suitable for future maize breeding under non-stress and water stress conditions. The direct effects of days to 50% tasseling was positive but the correlations were negative under three conditions; in such situation direct selection for this trait should be practiced to reduce the undesirable indirect effect. This study, therefore suggests that ears per plant, ear height and days to

50% tasseling are the main characters to be considered for improvement of grain yield under non-stress and water stress conditions.

Table 2 Direct (diagonal in bold) and indirect path coefficients of different maize traits on grain yield under non-stress, intermediate stress and severe stress conditions across locations

Traits	DYTS	DYSK	ASI	PLHT	EHT	EPP	Total effect
Non-stress condition							
Days to 50% tasseling	0.812	-0.361	0.056	0.394	-0.421	-0.325	0.155
Days to 50% silking	0.801	-0.366	0.114	0.647	-0.533	-0.507	0.155
Anthesis-silking interval	0.312	-0.285	0.146	0.031	0.602	-1.183	-0.377
Plant height	0.167	0.274	-0.005	-0.864	1.521	-0.345	0.748
Ear height	-0.223	0.127	0.058	-0.858	1.532	-0.320	0.316
Ears per plant	-0.221	0.156	-0.145	0.250	-0.410	-1.194	0.823
Intermediate stress condition							
Days to 50% tasseling	0.782	-0.474	0.023	0.195	-1.020	-0.151	-0.644
Days to 50% silking	0.774	-0.480	-0.086	0.230	-1.214	-0.004	-0.779
Anthesis-silking interval	-0.035	-0.081	0.507	0.334	0.029	0.001	-0.259
Plant height	0.099	0.117	0.179	-0.948	1.047	0.151	0.643
Ear height	-0.652	0.476	-0.012	-0.811	1.224	-0.151	0.376
Ears per plant	0.775	-0.012	-0.005	0.940	-1.214	-0.152	0.341
Severe stress condition							
Days to 50% tasseling	1.054	-1.055	0.187	-0.833	0.261	-0.001	-0.383
Days to 50% silking	-1.048	1.062	-0.098	-1.042	0.377	-0.438	-0.991
Anthesis-silking interval	0.338	-0.178	-0.584	-0.449	0.162	-0.010	-0.701
Plant height	-0.846	-1.059	0.251	1.045	-0.404	-0.317	-0.696
Ear height	-0.566	-0.824	0.195	0.869	0.486	-0.032	0.289

Ears per plant	-0.002	-1.054	0.013	0.752	0.035	0.441	0.159

DYTS-days to 50% tasseling, DYSK-days to 50% silking, ASI-anthesis-silking interval, PLHT-plant height, EHT-ear height, EPP- ears per plant, GY-grain yield

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CGBPB 036

COMBINING ABILITIES FOR MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS RELATED TO DROUGHT TOLERANCE IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) GENOTYPES

[1*]Abdurrasheed N., [2]Usman A.,[2] Mohammed M.S., [2]Isah H.,and [2]Mowobi, G.G.

[1]Department of Forestry and Wildlife Management, Federal University of Dutsin-Ma, Katsina State
Corresponding Author: nabdurrasheed@fudutsinma.edu.ng, rasheednafisat@gmail.com. +2348167147343

ABSTRACT

Seven parents made of four drought susceptible and three drought tolerant randomly selected and crossed in a half diallel mating design to generate 21 F_1 progenies. The 7 parents, 21 F_1 progenies and two checks were evaluated under two water moisture regimes (water stress and well water). The objective of this study was to assess the nature of gene action controlling drought tolerant traits in groundnut. The experiment was laid in randomized complete block design with split plot and randomized twice. Data was collected for number of pods per plant and pod yield; physiological traits such as SPAD chlorophyll meter reading at 60 days after sowing and harvest index. The mean squares due to GCA and SCA were significant (P≤0.05) significant for most of the traits under non stress and water stress condition indicating importance of additive and non-additive types of gene action in controlling these traits. Significant of GCA and non-significance of SCA for pod yield under water stress condition could imply the that the performance of the crosses can be adequately predicted from the performance of the parents, i.e high yielding parent will result to high yielding crosses. The ratio of GCA to SCA also portrays the importance and the predominance of non-additive genetic effects because the SCA was higher than the GCA variance and all of the ratios were less than unity. The importance of additive effect under water-stress suggests the need to achieve acceptable progeny performance under drought stress condition.

Keywords: *Additive, gene action, GCA, SCA, non-additive.*

Introduction

Drought is widely known as the major limiting factor to groundnut production in sub Saharan Africa. In semiarid zones, especially West Africa, drought can occur at any stage of crop cycle and often leads to devastating effects in groundnut growth, development, metabolism, pod yield and seed quality Hamidou *et al.* (2013).

The use of drought resistance and variation is an important strategy to combat drought problem, this variation should be able to provide high pod yield under dry condition. Direct selection for yield under water stress condition may be effective; the limitations of this approach are high resource investment and poor repeatability of the results due to the large genotype x environment (G x E) interaction that results in slow breeding progress (Wright *et al.,* 1996). Therefore, rapid progress may be achieved by considering physiological traits such as SPAD chlorophyll meter reading (SCMR). SCMR have been used as surrogate traits for Water Use Efficiency (WUE).

Effective selection of genotype for particular traits under improvement depends on sufficient additive genetic variation of the traits that are expressed as heritability. Information on the inheritance of drought tolerance for morphological and

physiological traits in groundnut is available to help breeders choose appropriate breeding method, but information that would assist selecting appropriate genotypes for their breeding program is limited. The objective of this study was to examine the *per* se performance and the combining ability estimates of groundnut genotypes for morphological and physiological traits related to drought tolerance.

MATERIAL AND METHODS

Seven genotypes consisting of four droughts susceptible (ICGV-IS-07813, RS006F_4B_1-50, ICGV-IS-07841 and ICG 12989) and three drought tolerant (SAMNUT 23, ICG 5195 and ICG 3312) groundnut genotypes were used as parents. The parents were crossed in a half diallel mating design as described by Dabholkar (1992). The F_1 seeds were multiplied to obtain enough F_2 seeds for evaluation as suggested by Hallauer *et al.* (2010) for self-pollinating crops. Evaluation of the 7 parents, 21 F_1 progenies and two checks was performed in screen house of Department of Plant Science (11°11'N, 07°38' E and 686 m above sea level) in the Northern Guinea savannah ecological zone of Nigeria ecological zone of Nigeria. The research was carried out during the 2019 dry and raining seasons. The genotypes were laid out in a randomized complete block design in a split plot arrangement with water regimes in main plot and genotypes in subplot with two replications. All recommended cultural practices and operation (planting, irrigation and weeding) were conducted. Morphological traits such as 50% flowering, plant height, number of pods per plant and pod yield were measured; physiological traits such as SPAD chlorophyll meter reading at 60 days after sowing (DAS) and harvest index were also measured.

Data from each water condition was subjected to ANOVA separately to detect the significance of genotypic difference (Gomez and Gomez, 1984). Combining ability analysis was carried out according to Hallaeur and Miranda (1988). Mean squares were portioned into difference due to crosses and parents. The general combining ability (GCA) of the parents and Specific Combining Ability (SCA) among the crosses were estimated using a general linear model (GLM) procedure of SAS (SAS Institute, 2004).

The linear model for diallel mating design for Model II, Method II

The model for the analysis of variance is:

$$X_{ijk} = \mu + r_k + g_i + g_j + s_{ij} + p_{ijk}$$

Where, μ = mean, r_k = replication effect, g_i and g_j are GCA effects.

s_{ij} is the SCA effect and p_{ijk} is the experimental error for the X_{ijk} observation.

Results

Combining Ability Mean Squares and Variances for Morphological and Physiological traits

The mean squares due to general combining ability (GCA) under non-stress condition were highly (P≤0.01) significant for number of pods per plant and SCMR at 60 DAS while pod yield and harvest index were significant (P≤0.05). Under water stress condition, GCA showed significant (P≤0.05) differences for all traits recorded except pod yield, which showed a non-significant (P>0.05) difference (Table 1).

Under non-stress condition, magnitude of SCA variances were higher than GCA variances for all traits. GCA variances were positive for number of pods per plant and SCMR at 60 DAS while GCA variances were negative for pod yield and harvest index. SCA variances were generally higher than corresponding GCA. Positive SCA variances were recorded for all traits. The ratios of GCA to the SCA variance for all the traits were less than unity, positive GCA/SCA ratios for all the traits. Under water-stress condition, the magnitudes of SCA variances were greater than GCA variances for all traits. GCA variances were positive for all the traits. SCA variances were generally higher than corresponding GCA. Positive SCA variances were recorded for all traits. The ratios of GCA to the SCA variance for all the traits were less than unity; positive GCA/SCA ratios were recorded for all the traits (Table 2).

Table 1: Mean squares for GCA and SCA estimates for morphological and physiological traits of groundnut genotypes evaluated under non-stress and water-stress condition at Samaru in 2019

Source of variation	DF	NPP		Pod yield		SCMR 60DAS		HI	
		NS	WS	NS	WS	NS	WS	NS	WS
GCA	6	118.76**	31.46*	18.31*	7.60*	25.06**	8.81*	58.81*	85.04*
SCA	21	53.23*	32.66*	27.48**	2.74	15.20*	7.14	131.03**	44.82*
Error	29	20.51	12.62	5.18	4.74	4.03	5.04	25.90	30.62

GCA= General Combining Ability, SCA= Specific Combining Ability, NS =non-stress, WS= water stress, NPP= Number of pods per plant, SCMR = SPAD chlorophyll meter reading, DAS= Days after sowing, HI = Harvest Index

Table 2: General combining ability and specific combining ability variances for morphological and physiological traits of groundnut genotypes evaluated under non-stress and water-stress condition at Samaru 2019

Traits	σ_e^2		σ_{GCA}^2		σ_{SCA}^2		GCA:SCA	
	NS	WS	NS	WS	NS	WS	NS	WS
Number of pods/plants	10.26	6.31	7.28	-0.13	32.72	20.04	0.82	0.66
Pod yield	2.59	2.37	-1.02	0.10	22.30	2.00	0.57	0.69
SCMR at 60DAS	2.02	2.52	1.1	0.19	11.17	2.1	0.77	0.71
Harvest Index	12.95	15.31	-8.02	4.47	105.13	14.2	0.47	0.79

NS =non-stress, WS= water stress σ_e^2 = Error variance, σ_{GCA}^2 = General Combining Ability variance, σ_{SCA}^2 = Specific Combining Ability variance, =GCA:SCA ratio, SCMR = SPAD chlorophyll meter reading, DAS= Days after sowing.

Combining Ability Effects

General combining ability effects

The estimates of general combining ability effects under non-stress and water-stress conditions are presented in (Table 3). Under water-stress condition, number of pods per plant recorded a positive significant (P≤0.05) GCA effect for ICG 12989 (0.80) and ICG 3312 (0.94). Significant (P≤0.05) positive GCA effects were recorded for pod yield for ICG 5195 (0.06). Non-significant difference was recorded for SCMR 60DAS. However, ICG 12989 recorded the lowest negative GCA effect (-0.09) for SCMR 60DAS. Significant (P≤0.05) positive GCA effects were recorded for Harvest index for SAMNUT 23 (3.52), ICG 5195 (1.01) and ICG 3312 (0.38).

Specific Combining Ability effects

The estimates of specific combining ability effects under non-stress and water-stress condition are presented in (Table 4). Under water-stress condition, number of pods per plant recorded positive highly (P≤0.01) significant SCA effects were recorded for ICGV-IS-07813 x ICG 5195 (7.49). Pod yield recorded a significant (P≤0.05) positive SCA effects for ICG 12989 x 5195 (1.32) while negative significant (P≤0.05) SCA effects were recorded for SAMNUT 23 x ICG 5195(-0.71) and ICGV-IS-07841 x ICG 3312 (-1.27). SCMR at 60DAS, a negative highly (P≤0.01) significant SCA effects were recorded for ICG 5195 x ICG 3312(-5.33). Negative significant (P≤0.05) SCA effects were recorded for ICGV-IS-07841 x SAMNUT 23 (-6.04) for SCMR at 80DAS. For harvest index, a positive significant (P≤0.05) SCA effects was recorded for ICGV-IS-07841 x SAMNUT 23 (8.04).

Table 3: General combining ability effect estimates for some morphological and physiological traits of groundnut genotypes evaluated under non-stress and water-stress condition in Samaru 2019.

Genotypes	NPP		PYD		SCMR 60DAS		HI (%)	
	NS	WS	NS	WS	NS	WS	NS	WS
ICGV-IS-07813(P_1)	0.99	0.87	0.14	-0.21	-1.42*	-1.19	1.8	-1.23
RS006F4B1-50(P_2)	0.20	-2.13	-0.38	-0.90*	0.37	0.44	-1.76**	-2.72
ICGV-IS-07841(P_3)	-3.58*	-1.92	0.49	-0.85	1.51*	0.67	1.22	2.52
ICG 12989(P_4)	0.20	0.80*	-0.92	0.52	-0.24	-0.09	-0.81	1.55
SAMNUT 23(P_5)	-3.08*	0.65	-1.48*	0.65	-1.60*	0.7	-1.86*	3.52*
ICG 5195(P_6)	4.42*	0.80	1.78*	0.06**	-0.09	0.26*	2.79	1.01*
ICG 3312(P_7)	0.85	0.94*	0.36	0.72	1.47*	-2.16	-1.37*	0.38*
SE ±	1.40	1.09	0.71	0.42	0.63	0.70	1.59	1.38

NS =non-stress, WS= water stress,DFF= Days to 50% flowering, NPP= Number of pods per plant, PYD= Pod yield, S.E= Standard Error, SCMR = SPAD chlorophyll meter reading, DAS=Days after sowing, HI = Harvest Index.

Table 4: Specific combining ability effect estimates for some morphological and physiological traits of groundnut genotypes evaluated under non-stress and water-stress condition in Samaru 2019

Genotypes	Crosses	NPP		Pod yield		SCMR 60DAS		HI	
		NS	WS	NS	WS	NS	WS	NS	WS
ICGV-IS-07813 x RS006F4B1-50	P_1P_2	-3.2	-6.58*	-3.62	-1.99	-1.07	-1.85	-8.4	-5.92
ICGV-IS-07813 x ICGV-IS-07841	P_1P_3	0.08	-2.80	3.61	-0.69	-0.84	0.83	20.33**	3.88
ICGV-IS-07813 x ICG 12989	P_1P_4	6.3	-1.51	5.17**	0.18*	4.55**	-0.70	6.16	0.37
ICGV-IS-07813 x SAMNUT 23	P_1P_5	-2.92	1.63	-3.07	0.81	-2.26	1.97	-7.54	-2.14
ICGV-IS-07813 x ICG 5195	P_1P_6	-3.92	7.49**	-0.13	1.65**	-1.95	0.53	-0.22	5.49
ICGV-IS-07813 x ICG 3312	P_1P_7	7.64	7.93	0.93	1.72	-4.36	-5.71	1.07	1.84
RS006F4B1-50 x ICGV-IS-07841	P_2P_3	-4.13	1.20	-5.27**	-0.35	-3.52*	0.72	-7.75	-1.68
RS006F4B1-50 x ICG 12989	P_2P_4	-0.42	1.49	0.44	0.33	1.49	0.5	-2.01	0.84
RS006F4B1-50 x SAMNUT 23	P_2P_5	1.87	3.13	3.85*	0.55	1.71	-1.04	7.22	-1.98
RS006F4B1-50 x ICG 5195	P_2P_6	5.87	-3.01	-0.31	-0.16	-0.71	-0.75	4.97	-2.4
RS006F4B1-50 x ICG 3312	P_2P_7	-4.14	5.93	-1.09	0.43	-3.08	1.07	-0.59	-5.24
ICGV-IS-07841 x ICG 12989	P_3P_4	-7.13	-3.72	-4.23*	-1.62	2.23	1.05	-10.39	-5.01
ICGV-IS-07841 x SAMNUT 23	P_3P_5	1.65	3.42	1.28	1.05	-3.24	-2.90	-0.13	8.04*

ICGV-IS-07841 x ICG 5195	P_3P_6	12.65	2.28**	7.07**	0.49*	1.56	1.98*	9.12*	2.0*
ICGV-IS-07841 x ICG 3312	P_3P_7	-1.43	-7.36	-1.17	1.27*	2.18	0.50	4.87	-1.47
ICG 12989 x SAMNUT 23	P_4P_5	1.37	1.3	0.54	0.98	-3.81**	-0.16	7.58	4.77
ICG 12989 x ICG 5195	P_4P_6	-0.63	3.56	0.28	1.32*	0.95	-0.73	-1.04	3.39
ICG 12989 x 1CG 3312	P_4P_7	-6.64	-0.64	-2.33	1.60	-1.86	0.1	-1.46	5.53
SAMNUT 23 x ICG 5195	P_5P_6	-4.35	-2.3	-0.86	-0.71*	3.01	2.86	-1.99	3.41
SAMNUT 23 x ICG 3312	P_5P_7	-7.93	-6.29	-5.34	-0.47	-2.67	1.86	-5.16	3.03
ICG 5195 x ICG 3312	P_6P_7	7.07	-1.14	2.86	1.24	-0.31	-5.33**	4.89	2.78
SE ±		9.82	3.73	4.99	1.42	2.15	2.4	5.41	4.73

DFF= Days to 50% flowering, PLTHT = plant height, NPP= Number of pods per plant, SEED WGT = Seed Weight, SCMR = SPAD chlorophyll meter reading, HI = Harvest Index, S. E= Standard Error.

Discussion

In any breeding strategy, germplasm diversity is of paramount importance when creating a breeding population (Kiwuka *et al.*, 2012). Investigation of the influence of different genetic systems can facilitate a better understanding of the nature of gene interactions that could influence drought tolerance (Guo *et al.*, 2017). This study was performed to investigate the mechanism of inheritance of drought tolerance in the crosses of some groundnut genotypes as a prerequisite in planning an efficient breeding program for drought tolerance in groundnut. Significant GCA mean squares indicated the importance of additive gene effect governing the inheritance pod yield, seed weight, SCMR at 60DAS and harvest index. Selection of superior genotypes in segregating generations should be possible for these traits. These results are in agreement with those of Jogloy *et al.*, (2005) who found significant GCA mean squares for the inheritance of pod yield, seed yield and 100 - seed weight. Green *et al.* (1983) and Swe and Branch (1986) found that GCA and SCA mean squares were significant for yield and yield component traits.

The GCA/SCA ratio was > 50% for all the traits recorded under both conditions, these results agree with the findings of Chiona (2009) who reported that the ratio of GCA/SCA for storage root yield was 0.68. Baker (1978) indicated that high ratios of GCA/SCA mean that the additive gene action makes a greater contribution to the expression of specific traits than non-additive gene action. The GCA/SCA ratio was > 50% for most of the traits recorded. These results agree with the findings of Chiona (2009) who reported that the ratio of GCA/SCA for yield was 0.68. Baker (1978) indicated that high ratios of GCA/SCA mean that the additive gene action makes a greater contribution to the expression of specific traits than non-additive gene action. This study revealed that the additive gene action had important

Combining Ability Effects

Combining ability effects are effective genetic information used in planning the next phase of breeding programs (Amongi *et al.*, 2015) .Consideration of the GCA effects of the parents involved in the superior specific cross-combinations identified for all the target traits indicated that the combining ability status of the crosses was dependent on the GCA effect status of the parents involved (Khodadadi *et al.*, 2017). From this study, the positive significant GCA effects recorded for ICG 5195 (P_6) and ICG 3312 (P_7) with respect to pod yield, SCMR at 60 DAS and harvest index under water stress are desirable indicators of drought tolerance therefore these genotypes are desirable effects for producing more drought-tolerant progenies. This is in agreement with Franco *et al.* (2001) findings where they reported that crosses involving parents with higher estimates of general combining ability for traits where high values are desirable should be potentially superior for the selection of lines in advanced generations. Given the high SCA effects recorded in F_2 individuals of ICGV-IS-07813 x ICG 5195 (P_1P_6) for number of pods/plant and pod yield and ICGV-IS-07841 x ICG 5195 (P_3P_6) for number of pods/plants, pod yield, SCMR at 60 DAS and harvest index would indicate that the means of these F_2 individuals were higher than predicted for the mentioned indicators of drought stress. These would affects imply that genotypes ICGV-IS-07813, ICG 5195 and ICGV-IS-07841 could be considered as good combiners for use in future drought breeding programs in groundnut. This finding agrees with the findings of Amongi *et* al. (2015) in common beans.

Conclusion

In both water conditions, the presence of additive and non-additive effects controlling the traits evaluated indicates that the seven parents used in the study contributed differently in the crossings they participated in. The parents ICG 5195 (P_6) and ICG 3312 (P_7) are recommended for breeding programs aimed at drought tolerance, due to its general combining ability, considering pod yield. The progenies ICGV-IS-07813 x ICG 5195 (P_1P_6)

and ICGV-IS-07841 x ICG 5195 (P_3P_6) under drought stress, results in higher pod yield considering the specific combining ability.

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ESTIMATION OF GENETIC PARAMETERS FOR SOME AGRO MORPHOLOGICAL TRAITS AMONG NIGERIA EGUSI MELON GENOTYPES.

Gado, A.A., Muhammad, M.L., Daudu, O.A.Y., Dangana, M.C., Abubakar, A. Kolo, J.T.

Department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria.
*Corresponding Author: gado.aishatu@futminna.edu.ng +234 8036218125

ABSTRACT

In order to assess some genetic parameters among Nigerian Melon genotypes; the Melon genotypes were evaluated for their morphological and yield attributes at the Department of Biological sciences experimental garden, Federal University of Technology, Minna during 2015/2016 and 2016/2017 growing seasons, using a complete Randomized Block Design (CRBD) with three replicates. The agromorphological parameters were investigated using standard procedures. The results on the agromorphological parameters showed significant difference ($p \leq 0.05$) for most of the parameters studied. The study revealed that all of the agromorphological parameters were influenced by genetic factors such parameters are suitable for selection. Higher estimate for genotypic variances than environmental variances were observed for all the parameters which indicate good characters for selection and improvement of the crop. The highest genetic advance as percentage of mean (2924.2%) was obtained for weight of fruits ; whereas, number of seeds per fruit had the lowest (14.40%). High values of broad sense heritability estimates were observed for plant height at week 4 up to maturity, number of flowers per plant, number of flower buds per plant, days to germination and number of fruits per plant. Therefore, combination of high heritability estimates with genetic advance in the selection program is vital for selection of the crop in the future. Emphasis should be made on those agro-morphological parameters that shows greater genetic importance for selection and improvement of the crop in Nigeria.

Keywords: *Genotypic variance, Phenotypic variance Heritability, Egusimelon,*

Introduction

Citrullus colocynthis (L.) is a variety of melon seeds, which is popularly called 'egusi' in West Africa. It belongs to a large family called Curcubitaceae, which consist of 119 genera and about 925 species. It is one of the most important vegetable crops in the tropical, subtropical and Mediterranean zones of the world (Schippers, 2000). It is a native of Africa, which has perhaps been introduced to Asia, Iran and Ukraine (Schippers, 2000). Its common names include egusi in Yoruba, agushi in Hausa, epingi or paragi in Nupe and eashi in Gwari. Dialect names for this crop include egusi-itoo. It produces climbing vines up to 4 meters long, which are covered with stiff hairs. The heart-shaped or roughly palmate leaves are up to 12 centimeters long and 14cm wide. It bears small yellow male and female flowers with petals less than a centimeter in length. The fruit is egg-shaped or an elongated ovate shape, up to about 19 centimeters long and 8cm wide, and cream in colour with green streaks. The plant is a creeping annual plant and an intercropping plant used in traditional farming practices; it grows well on light rich soil in the hot climatic regions of Africa. It has been known to tolerate low rainfall. In the Southeastern part of Nigeria, the crop is best cultivated after the first rainfall of the year (Akpambang *et al.*, 2008). Thirteen

weeks after planting the first fruits are harvested. The different species of Cucurbitaceae have served humans for over 10,000 years as important food and as source of many useful products (Ajuru and Okoli, 2013). In Nigeria, they are used for different purposes in different parts of the country. It is important to improve the productivity of the crop to satisfy the demands of dietary needs and raw materials for industrial processing to edible oil and livestock feedstuff through breeding programs. The success of increasing the productivity of any crop through breeding largely depends on the presence of variability among the breeding materials (Adeyemo and Ojo, 1991). Broad genetic variability is the basis for successful plant breeding and the successful development of adaptations to environmental conditions. Generally breeding programs depends on knowledge of the nature and magnitude of variations in the available materials, magnitude of association of characters with yield, extent to which these characters are heritable as well as extent of environmental influence on them (Aruah *et al.,* 2012; Ndukauba *et al.,*2015). Various morphological and physiological characters contribute to yield. Each of these component characters has its own genetic systems. Further, these yield components are influenced by environmental fluctuations. Therefore, it is necessary to separate the total variations into heritable and non-heritable components with the help of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability and genetic gain (Maniee *et. al.*, 2009). Furthermore, knowledge of the association between yield and its components can improve the efficiency of selection in plant breeding (Izge *et al.*, 2001). This study was undertaken to estimate the genetic variability, heritability, character association among the different egusi-melon genotypes.

Materials and Methods

The morphological parameters were investigated using standard procedures after the techniques of Akinyele and Osekita (2006); Hegazi and Hamideldin (2010); Idehen *et al.* (2014). Specifically, the days to germination (DG) were determine as the interval between sowing of seeds and day a germinating seedling emerges above soil level. The number of leaves per plant (NL) at maturity was determined by counting the number of leaves attached to the plants. The length of vine of the plants at two weeks interval up to maturity was measured in centimetres (cm) using a metre rule. Sexual maturity (SM) was determined as the interval between emergence of seedling and appearance of flowers. For each of the morphological parameters mentioned above, mean value per plant was determined. The leaf colour and seed colour were determined using a Royal horticultural colour chart; leaf shape and seed shape were determined using a chart. Leaf texture was determined using fingertips (IPGRI, 2003).

The yields from the different accessions of Melon were determined using the following indices: number of fruits per plant (NF), number of seeds per pod (NSP), and weight of fruit (WF). For NSP and WF, ten fruits each were selected at random for all the accession and the values were recorded for further statistical analysis.

NF were determined by counting the total number of fruits a plant produced at the completion of the life cycle. NSP were determined by opening the fruit and counting the number of viable seeds which were determined by their relatively large size and firmness. WF were determined by measuring the pods on a weighing balance, mean values of yield parameters per fruit or plant were determined for the Melon plants.

Genetic Parameters Estimates

Broad Sense Heritability (h^2) was estimated according to Falconer (1989) using:

$$h^2 = \frac{\sigma 2g}{\sigma 2ph}$$

(equation1)

Where σ^2g is the genotypic variance; σ^2ph is the phenotypic variance. Phenotypic and

Phenotypic variances were obtained from the analysis of variance table using equations 2 and 3 as follows:

$$\sigma^2 g = \frac{MS1 - MS2}{rXs} \quad (2)$$

$$\sigma^2 ph = \frac{MS1}{rXs} \quad (3)$$

(Where r: replication, s: season, MS1: Mean square for cultivar, MS2: Mean square for cultivar X season).

The mean values were used for genetic analyses to determine Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), using equation 4 and 5 as follows:

$$GCV\ (\%) = \frac{\sqrt{\text{Genotypic Variance}}}{\text{Grand Mean}} \text{ X } 100 \quad (4)$$

$$PCV\ (\%) = \frac{\sqrt{\text{Phenotypic Variance}}}{\text{Grand Mean}} \text{ X } 100 \quad (5)$$

Genetic advance (GA) was calculated with the method suggested by Singh and Chaundry (1985) using equation 6 as follows:

$$GA = k.\ \sigma ph.\ h^2 \quad (6)$$

Where K: constant = 2.06 at 5% selection intensity, σph: square root of phenotypic variance, h^2: Heritability

GA as percentage of mean (GAM) = (GA/Grand Mean) X 100 (7)

Results and Discussion

Genotypic variance, phenotypic variance, Environmental variance, broad sense Heritability. Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV) and Genetic advance for eleven characters are presented in (Table 1) The result revealed considerable genotypic variances among the various accessions for the characters under consideration. The result revealed consistency in the environmental and genotypic variance. In all the eleven characters studied, the genotypic variance was quite higher than the environment variance.

Genotypic variance (GV) was higher than environmental variance (EV) for all the eleven (Table 1). However, the influence of the environmental factors on the expression of other characters as indicated by the magnitude of the EV was not evident. This indicates that the phenotypic variance (PV) was not caused by environmental influences of those characters. Consequently, such character possesses promising genetic variability; so, selection for them is very efficient and successes very high.

The higher GV (7916.65) was for plant height at maturity, this was followed by number of seed per fruit (6834.17), then number of flower buds per plant. The least GV (0.28) was recorded for weight of fruit per plant. Phenotypic variance (PV) was also highest in plant height at week 10 (10022.14) followed by number of seeds per fruit (8778.28), then number of flowers per plant (2973.55), and followed by plant height at maturity (7964.65); the lowest PV (0.40) was found in weight of fruit per plant (Table 1).

Genotypic coefficient of variation (GCV) was higher for number of fruits per plant (131.71%), then followed by number of branches per plant at maturity (86.77%), this was followed by number of flowers per plant (73.87%); the least GCV (17.54%) was found in fruit diameter. Genotypic coefficient of variation (GCV) ascertains the degree of genetic variability present in various quantitative traits. High GCV indicates the presence of exploitable genetic variability for these traits which may facilitate selection (Yandav, 2009). Polygenic variation may be phenotypic, genotypic or environmental and relative values of these three coefficients for a trait will give an idea about the magnitude of its variability (Nausherwan *et al*, 2008).

Genotypic coefficient of variation, which is the real indicator of the extent of genetic variability in a population, was high for all the characters, except for fruit diameter and days to flowering. For all the tested character, higher PCV than GCV values were obtained.

The highest PCV (138.02%) was for number of fruits per plant, followed by number of branches per plant (97.41%), then followed by number of flowers per

plant (75.75%); the least PCV (20.50%) was found in fruit diameter (Table 1). High PCV is an indication of the presence of substantial horizon for selection of the trait under consideration which dependent on the amount of variability present. Thus, a greater potential is expected in the selection for number of fruits per plant, number of flowers per plant and number of branches per plant among the genotypes under study while there is a narrow scope for selection of fruit diameter and days to flowering on account of low amount of variability among genotypes studied. (Khan *et al*, 2009) reported that high PCV is an indication of the existence of greater scope for selection of the trait under consideration which is dependent on the amount of variability present.

The highest broad sense heritability (h^2) of (100%) was recorded for plant height at week 10 with an expected genetic advance over percentage of mean (GAM) of 46.10%. this was followed by plant height at maturity 99% with an expected GAM 17.58%, followed by plant height at week 6 (97%) with expected respective GAM of 103.37%. Number of leaves per plant at maturity produced the lowest heritability values (67%) and a corresponding lowest GAM values (54.09) (Table 1).

Heritability suggests the extent of genetic control for the expression of a particular trait and the reliability of phenotype in predicting its breeding value (Chopra, 2000). High heritability indicates less environmental influence in the observed variation (Mohanty, 2003; Eid, 2009). Heritability in the broad sense (h^2_{bs}) indicates only whether there is sufficient genetic variation present in a population or not, which implies whether a population will respond to selection pressure or not (Milatovic *et al*, 2010).

Table 1: Estimation of Some Components of Genetic Parameters for some Agromorphological Characters among the Melon Accessions

Characters	Grand mean	Genotypic variance (σ2g)	Phenotypic variance (σ2p)	Environmental variance (e2)	Broad sense heritability (h2)/%	Genotypic coefficient of variation (GCV)/%	Phenotypic coefficient of variation (PCV)/%	Genetic advance (GA)	GA as a % of mean
Plant height at 4weeks (cm)	74.45	702.62	738.27	35.65	95	35.60	36.50	71.55	96.10
Plant height at 6 weeks after (cm)	81.86	1167.44	1200.55	33.10	97	41.74	42.33	84.79	103.57
Plant height at 8 weeks (cm)	137.98	1589.53	1695.73	106.20	94	28.89	29.84	57.63	41.76
Plant height at 10 weeks	211.23	995.94	10022.14	26.20	100	47.33	47.39	97.38	46.10
Plant height at Maturity	322.40	7916.65	7964.65	48.34	99	27.60	27.68	56.68	17.58
Days to germination	5.24	6.02	6.55	0.53	92	46.86	48.88	92.53	1765.83
Number of branches per plant at maturity	5.71	24.55	30.94	6.39	79	86.77	97.41	159.23	2784.58
Number of leaves per plant at maturity	100.49	1051.00	1570.99	519.99	67	32.26	39.44	54.36	54.09
Days to flowering	32.78	43.08	56.96	13.88	76	20.02	23.02	35.86	109.39
Number of flower bud per plant	82.52	3127.10	3292.86	165.76	95	67.77	69.54	136.04	1211.19
Number of flowers per plant	74.93	3063.65	3221.49	157.83	95	73.87	75.75	148.41	197.51

Number of fruits per plant	10.98	208.95	229.46	20.51	91	131.71	138.02	258.92	2358.1
Weight of fruit (g)	1.77	0.28	0.40	0.12	70	30.02	35.86	51.76	2924.2
Number of seeds per Fruit	322.91	6834.17	8778.28	1944.11	78	25.60	29.02	46.53	14.40
Fruit diameter	40.60	50.14	69.44	19.30	72	17.44	20.50	30.53	164.85

Conclusion

In conclusion, broad genetic variability was observed among the melon accessions that could be useful for future breeding purposes. The results of this study indicate that there is considerable genetic variation present in most of the traits to warrant selection for better genotypes. These traits can therefore be given special attention in selections aimed at melon improvement. In other to access the selection effect on trait more effectively, heritability accompanied with genetic advance is more useful than heritability alone.

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CGBPB 038

EVALUATION OF THE POTENTIALS OF SOME CASTOR SEED ACCESSIONS TO DORMANCY BREAKING METHODS

Usman Abubakar Sadiq[1], Sami Rukaiya Aliyu[1], Abubakar Hussaini[1], Usman Alhassan[1], Usman Muhammad[1]

Department of Plant Science, Ahmadu Bello University, Zaria

ABSTRACT

Castor bean (Ricinus communist L.) is an important non-edible oilseed crop that has potential for biodiesel production. It is well adapted to Nigeria climatic conditions but the major limitation to its large-scale production throughout the world has been the problem of poor germination due to seed dormancy. However, treatments like scarification and stratification are crucial in overcoming external and internal dormancy. It is in this view that this work was carried out to determine the germinations and vigor of the castor bean genotypes after treatment with different methods of breaking seed dormancy. Therefore, seeds of five castor accessions (IARCAS204, IARCAS201, IARCAS005, IARCAS007, IARCAS028) were treated with five different methods of breaking dormancy as T1 T2 T3 T4 and T5. Laboratory germination using sand as substratum was conducted at seed testing laboratory of the IAR Samaru in 2020. The experiment was laid in a completely randomized design (CRD) replicated three times. Data was collected on germination and vigor parameters then subjected to statistical analysis (ANOVA) and differences between treatments means was compared using LSD-test at probability level of 0.05. The mean squares from analysis of variance for accessions showed highly significant ($p<0.01$) for germination percentage and some vigour traits. Also, the treatments were highly significant ($p<0.01$) for all the parameters and accessions and treatment interaction was highly significant ($p<0.01$) for germination percentage and vigor. It is concluded that IARCAS005 performed better and the best method of treatment was the removal of seed coat to break dormancy before planting so as to achieve maximum stand count which could results to maximum yield

Keywords: *accessions, castor, dormancy, treatments*

Introduction

Castor plant, (*Ricinus communis* L.) is a species of flowering plant to the family Euphorbiaceae, which has numerous number of plants which are mostly native to the tropics (Salihu, Gana, Apuyor, & Research, 2014). The name 'Ricinus'was derived from the Latin word for tick. The plant is named so probably because its seed has markings and a dump at the end which looks like a certain tick (Salihu et al., 2014). Castor bean is an important non-edible oilseed crop that has potential for biodiesel production. The oil produced from this crop is considered to be of importance to the global chemical industry because it is the only commercial source of a hydroxylated fatty acid.

Dormancy is a mechanism of higher plants to adapt to adverse conditions by halting growth and development, it can take place in different organs like seeds and buds (Soppe & Bentsink, 2016). Dormancy is influenced by both genetic and environmental factors. It helps them to survive periods that are adverse for growth like drought, cold or heat. The development of dormancy during evolution has led to plants been able to inhabit environments that would have been

lethal for their continuous growth without a dormant phase. During the dormant state, metabolic activity reduces to the lowest minimum and development comes to a stop. Dormancy is often associated with low moisture levels and protective structures like a seed coat in seeds or protective scales in buds and bulbs. Induction usually happens towards the termination of a growth season and release at the beginning of the next one (Soppe & Bentsink, 2016).

Freshly harvested seeds of castor usually have some degree of dormancy manifested in slow, erratic and low germination probably due to seed coat impermeability to water (Msaakpa, Obasi, Kortse, & Science, 2013). Although it is an reliable mechanism to guarantee the survival and perpetuation of the species, seed dormancy limits the process of seed germination (Msaakpa et al., 2013). and has been the major limitation to its large-scale production throughout the world. Although the seed germination biology of many plant species has been investigated, there is lack of adequate information concerning that of castor. Little research has been done on the aspects of the type, causes and control of dormancy in castor seeds. Previous studies on pre-sowing treatments to break dormancy in castor seeds have also not received desired attention. It is in this view that this work was carried out to determine the germinations and vigor of the castor bean genotypes after treatment with different methods of breaking seed dormancy.

Materials and Methods

Seeds of five castor accessions (IARCAS204, IARCAS201, IARCAS005, IARCAS007, IARCAS028) were obtained from the castor breeding unit of Institute of Agricultural Research Samaru - Zaria. One hundred seeds were used for each treatment as T1 which is the control, T2 were soaked in water for (48 hours), T3, was soaked in boiled water up to 100^{o}C for two minutes T4 seed coat were removed and T5 caruncle was removed. The treatments were subjected to germination and vigour test in the seed testing laboratory in Complete Randomised Design (CRD).

Germination Percentage: Four replicates of twenty-five seeds for each treatment were planted in germination tray using sand as substratum. The trays were watered every other day and emergence was recorded from the first count day and final germination was evaluated on the 14th day after planting.

First Count: The percentage of germinated seedlings in the first count day (day 7) after planting for castor bean seed according to (ISTA, 2015) was recorded and expressed as percentage

Speed of germination (GI): Was measured according to (Czabator, 1962). Germination count was taken every day until germination was completed. An index of the speed of germination was then calculated by adding the quotients of the daily counts divided by the number of days of germination. The higher the index the more vigorous is the seed sample.

Data collected was subjected to statistical analysis (ANOVA) and differences between treatment means was compared using LSD-test at probability level of 0.05.

Results and Discussion

The mean squares from analysis of variance for accessions showed highly significant ($p<0.01$) for germination percentage, speed of germination. However, no significance ($p>0.05$) for accessions for first count was observed table 1. The treatments also showed highly significant ($p<0.01$) for all the parameters. The accessions and treatment interaction was also highly significant ($p<0.01$) for germination percentage, first count, and speed of germination table 1.

Table 1 Analysis of variance for germination and vigour traits of castor evaluated at Samaru in 2020

Source of variation	d.f	Germination %	First Count	Speed of Germination
Accessions (A)	4	407.35**	54.08	13.85**
Treatment (T)	4	2027.34**	1248.67**	117.37**
A×T	15	276.16**	225.93**	16.46**
Error	61	76.43**	66.93	4.91**

d.f–degree of freedom, **-highly significant

In terms of germination percentage, IARCAS 005 when seed coat was removed had the highest value and IARCAS 204 when caruncle was removed had lowest though, not statistically different with former when seed coat was removed. This indicates that response of dormancy treatments is genotype specific. Hot water treatment had the lowest germination percentage in all the accessions followed by cold water except in IARCAS 201 (Table 2). (Finch-Savage, Cadman, Toorop, Lynn, & Hilhorst, 2007) reported that dormancy can be overcome by high or low temperatures, depending on the specie. However, in this finding castor bean seed does not exhibit such scenario because all the treatments outperformed the cold and hot water treatments

Table 2 Means of accessions and treatments for germination percentage

Treatments	**IARCAS005**	**IARCAS007**	**IARCAS028**	**IARCAS201**	**IARCAS204**
Control	35.00c	19.00f	20.00f	35.00c	32.00d
Cold Water	**10.00j**	**6.67k**	**17.00h**	**4.00l**	**17.25g**
Hot Water (100℃)	9.00k	5.00l	14.00h	13.33i	14.00i
Seed Coat Removed	**46.00a**	**38.00b**	**37.00b**	**9.33j**	**0.00m**
Caruncle Removed	18.67g	23.00e	31.00d	26.00e	44.00a
CD	**8.57**				
Range	4–46				

CD-Critical difference

As in the germination percentage, IARCAS 005 when seed coat was removed had the highest first count values indicating that it is more vigorous than all the other accessions though, statistically similar to IARCAS 007 (Table 3). Similar trend was observed as in the germination percentage among the treatments with hot water been lower followed by cold water treatment except in IARCAS 204. Seed coat removed had the highest vigour in three out of the five accessions used in this study. However, IARCAS 204 had the lowest values when seed coat was removed as in the germination percentage indicating that response of dormancy treatments is genotype specific.

Table 3 Means of accessions and treatments for first day count

Treatments	**IARCAS005**	**IARCAS007**	**IARCAS028**	**IARCAS201**	**IARCAS204**
Control	30.00bc	14.00f	15.00f	29.00cd	23.00e
Cold Water	**9.33h**	**6.67k**	**13.00g**	**8.00hij**	**6.50k**
Hot Water (100℃)	8.00hij	6.00l	8.00hij	5.33l	7.00j
Seed Coat Removed	**40.00a**	**32.00a**	**31.00b**	**8.00hij**	**0.00m**
Caruncle Removed	12.00g	19.00e	26.00d	30.00bc	29.00cd
CD	**8.02**				
Range	5.33–40				

CD-Critical difference

The accession IARCAS005 with seed coat removed was significantly higher than all the accessions with regard to speed of germination (Table 4). This confirmed the results of germination percentage and first count. Also, as in first count hot water treatment had lower values followed by cold water treatment except in IARCAS 204. Seed coat removed had the highest vigour in three out of the five accessions used in this study (Table 4). This re affirms that response of dormancy treatments is genotype specific. It is also in agreement with finding of (Msaakpa et al., 2013) who reported that when castor bean seed accessions were treated with different chemicals to break the dormancy, the accession LAF – 4 treated with either coconut milk or KNO3 or aluminum tetrafluoride produced higher final germination count and germination speed index.

Table 4. Means of accessions and treatments for speed germination

Treatments	IARCAS005	IARCAS007	IARCAS028	IARCAS201	IARCAS204
Control	9.10b	3.79h	4.36f	7.19d	6.01e
Cold Water	**2.11j**	**1.84k**	**3.96g**	**1.07m**	**2.57h**
Hot Water (100℃)	1.61l	1.21l	2.52i	1.69k	2.47i
Seed Coat Removed	**11.93a**	**9.72b**	**8.46c**	**2.31j**	**0.00m**
Caruncle Removed	3.87g	4.92f	7.08d	5.79e	7.90c
CD	**2.18**				
Range	1.07-11.93				

CD-Critical difference

Conclusions

This finding revealed that response of dormancy treatments is genotype specific. Among the accessions studied, IARCAS2005 performed best in the parameters measured and removal of seed coat can be the best method to overcome dormancy in castor bean seed.

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CGBPB 039

VARIABILITY, HERITABILITY AND GENETIC ADVANCE OF YIELD AND RELATED CHARACTERS IN OKRA

David, Grace S., Iwo, G. A., Ittah, Macauley A. and Funyowei, Felicia D.

Department of Crop Science, University of Calabar, Calabar, Nigeria.
Corresponding Author: graceukoha@gmail.com +2348066292593

ABSTRACT

Okra (Abelmoschus esculentus (L.) Moench) is an important vegetable crop grown in various countries of the world. Exploiting the genetic variability is important for the continuous improvement of this crop. A field experiment was carried out in Calabar in 2016 to assess the variability, heritability and genetic advance of yield and related characters in selected genotypes of okra. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Data collected on both morphological and reproductive traits were subjected to analysis of variance which was used to partition the gross (phenotypic) variability into the components due to genetic (hereditary) and non-genetic (environmental) factors and to estimate the magnitude of these. The results obtained from this study showed considerable variability among the genotypes for plant height at all sampling periods except 4 weeks after planting (WAP) and number of days to 50% flowering. Phenotypic coefficient of variation ranged from 4.78 – 24.05 and genetic advance (GA as % of mean) ranged from 8.35 – 36.67 with the lowest value recorded in number of days to 50 % flowering and the highest in plant height at 6 WAP. The highest heritability of 85% and 77% were recorded in number of days to 50% flowering and plant height at 8 WAP respectively, this was closely followed by plant height at 6 WAP with heritability value of 74 %. This shows that selection is possible for these traits and could be exploited for further breeding studies.

Keywords: *Okra, variability, heritability, trait, selection.*

Introduction

Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop that is grown in the tropical and sub-tropical regions of the world (Schippers, 2000). India ranked the highest in okra production with 6,126,000 tonnes, Nigeria comes second with 1,978,286 tonnes yearly production and Sudan third. Total okra production worldwide is 9,872,826 tonnes (FAOSTAT, 2020). Okra is of economic importance because the fruits, buds, flowers are often eaten. It is also medicinal and the leaves can serve as feed to animals (Siesmonsa and Kouame, 2004). Genotypes show variability in various characters such as yield, number of days to first flowering, number of pods per plant and height (Jagan *et al.*, 2013). Heritability and genetic advance are suitable measures for accessing the genetic portion of total variability and this aids selection for various characters. Adeoluwa and Kehinde (2011) in their study on 35 accessions of okra observed a wide variability for all characters except leaf and petal colour. Phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) thus

revealing the effect of environmental factors. High PCV and GCV were observed for pod yield per plant and peduncle length respectively. Likewise, Nwangburuka *et al.,* (2012) reported high GCV broad sense heritability and genetic advance for plant height, fresh pod length and width, number of branches and pod weight per plant.

Okra is an important vegetable crop in Nigeria and West Africa and to meet the increasing demand for this crop, it is important to evaluate various genotypes also, assessing variability for continuous improvement of this crop. The objective of this study was to assess the genetic variability in some okra genotypes and identify traits that will provide basis for selection.

Materials and Methods

The experiment was carried conducted at Agricultural Development Project (ADP), Calabar, Cross River State from April – August 2016. Calabar has a bimodal annual rainfall of about 2000–3500mm and temperature ranging from 27 – 35 °C. The experiment was laid out in a randomized complete block design (RCBD) with three replications. A total of 17 genotypes were used. Seeds were sown at a spacing of 40 x 30 cm giving a plant population of 8,333 plants per hectare. All plots received ring application of NPK 15:15:15 3 weeks after planting (WAP). Weeding was also done manually at 3 and 6 WAP. Pods were harvested every 2 – 3 days at maturity. Data was collected on the following: numbers of days to 50% emergence, plant height, number of leaves, leaf area, leaf area index, number of days to 50 % flowering, number of pods, fresh weight of pods. Data collected was analyzed using genstat 10.3 which was used to partition variability into components due to genetic and non - genetic factors. Variance components (PCV, GCV and error variances) were estimated according to Uguru (1995) using the formula:

$$Vg = \frac{Msg - Mse}{r}$$

$$Vp = \frac{Msg}{r}$$

$$Ve = \frac{Mse}{r}$$

where Msg, Mse and r = mean squares of genotype, error and replication respectively. PCV and GCV and H^2bs were computed according to Allard (1960) as:

$$PCV = \frac{(Vp)^{0.5}}{x} \times 100$$

$$GCV = \frac{(Vg)^{0.5}}{x} \times 100$$

$$H^2bs = \frac{Vg}{Vp} \times 100$$

$$GA = k \times sp \times H^2bs$$

where Vp, Vg and x = phenotypic variance, genotypic variance and grand mean for the trait under consideration.

H^2bs, GA, k and sp= Heritability in broad sense, genetic advance, constant 2.06 at 5 % selection pressure and phenotypic standard deviation respectively.

Results and Discussions

Results showed considerable variation among traits. Mean square genotype was generally higher than mean square error for the traits studied (Table 1). Phenotypic variances were generally higher than the genotypic variances for the characters studied (Table 2). This is due to the environmental influence, as phenotypic variance is a component of both genetic and environmental factors. The highest phenotypic and genotypic variance in the characters considered was observed and recorded for plant height at 12 WAP. PCV generally ranged from 4.78 -24.05 with the highest PCV for plant height at 6 WAP and the lowest in number of days to 50 % flowering respectively (Table 3). Similarly, the genotypic coefficient of variability GCV ranged from 4.41 - 20.66 with the highest GCV in plant height at 6 WAP.

Generally, broadsense heritability varied from 66 – 85 % with the lowest value in number of days to 50 % flowering and the highest for plant height at 6 WAP. Also, genetic advance (% of mean) ranged from 8.35 for number of days to 50 % flowering to 36. 64 for plant height at 6 WAP respectively. Genetic variability is useful in selection and overall crop improvement. The significant differences observed in the performance of genotypes for the traits under consideration is an indication of variability which implies that this crop can be improved by selection, hybridization and other breeding methods.

Table 1: Mean squares (genotype and error), variance ratios and grand mean of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	Mean squares			
	Genotype	Error	Variance ratio	Mean
Number of days to 50 % flowering	25.00	3.76	6.65**	60.33
Plant height at 6 WAP	70.72	18.52	3.82*	20.19
Plant height at 8 WAP	81.38	18.89	4.31**	25.49
Plant height 10 WAP	111.48	37.64	2.96*	33.77
Plant height 12 WAP	158.03	49.47	3.19*	39.35

* , ** Significant at 5 % and 1% level respectively

Table 2: Phenotypic (Vp), genotypic (Vg) and error (Ve) variances of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	Vp	Vg	Ve
Number of days to 50 % flowering	8.33	7.08	1.25
Plant height at 6 WAP	23.57	17.40	6.17
Plant height at 8 WAP	27.13	20.83	6.30
Plant height 10 WAP	37.16	24.61	12.55
Plant height 12 WAP	52.68	36.19	16.49

Table 3: Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) broad sense heritability (H^2bs) and genetic advance (GA) of okra genotypes for yield and related traits evaluated in Calabar 2016

Attributes	PCV	GCV	H^2b (%)	GA	GA (% Mean)
Number of days to 50 % flowering	4.78	4.41	85	5.04	8.35
Plant height at 6 WAP	24.05	20.66	74	7.4	36.67
Plant height at 8 WAP	20.43	17.91	77	8.26	32.41
Plant height 10 WAP	18.05	14.69	66	8.29	24.54
Plant height 12 WAP	18.44	15.29	69	10.32	26.22

This result agrees with Nwangburuka *et al.,* (2011) who reported high variability among some okra genotypes. From this study, the highest PCV and GCV was observed in plant height at 6 WAP and the lowest in number of days to 50 % flowering which is an indication of high variability for these traits. The highest estimate of genetic advance was observed in plant height at 12 WAP while the lowest was observed in number of days to 50 % flowering. Furthermore, there was high heritability estimates for number of days to association between characters at genetic level.

This is similar to the findings of Ibrahim and Hussein (2006) on roselle (*Hisbiscus sabdariffa).*
From this study, variability shows that there are considerable potentials in improvement of this crop. Any trait with high heritability and genetic advance shows the presence of additive genes and the transmissibility of this trait to subsequent generations.

Conclusion

Okra is an important vegetable crop with great potentials for improvement. The variability observed among genotypes from this study is a clear evidence which is also in consonant with reports from other researchers. For further studies, more emphasis should be given to number of days to 50 % flowering and plant height at 6 WAP.

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50% flowering and plant height at all sampling periods, this suggests that the genotypic factor had greater effect on the phenotypic performance of these traits. Hence, selection based on the phenotypic performances of these characters will be reliable and effective. Similarly, the highest estimate of genetic advance was observed in plant height at 12 WAP while the lowest value was observed in number of days to 50 % flowering. The high GCV observed in the characters suggest very strong inherent

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CGBPB 040

DIVERSITY IN POLLEN MORPHOLOGY AND VIABILITY IN *CORCHORUS OLITORIUS* L. (BUSH OKRA)

Mikail, L.I.M[1,2] and Mustapha, O.T[2]

Science Department, Kogi State Polytechnic, Lokoja[1]
Plant Biology Department, University of Ilorin[2]
Corresponding Author: latifahmikail47@gmail.com, +2348059924333

ABSTRACT

Eleven varieties of Corchorus olitorius L. seeds were collected from NIHORT and IAR, Ibadan, Nigeria and grown till maturity. The anthers were harvested from the field into experimental bottle and dusted on to clean slide to expel the pollen grains. A drop of aceto-orcein stain was added to and viewed under the Olympus microscope. The pollen morphology and percentage pollen viability showed variations and similarities. Pollen grains were spherical in shape and exhibited three different types of pollen (monocolpate, dicolpate and tricolpate). pollen wall layer was differentiated into exine and intine.NG/To/02/12/179 exhibited multiple pollen. Highest pollen index was recorded in both NG/SA/DEC/07/0403 and NG/OA/JUN/09/001 while NHGB/09/147 had the lowest.

Keywords: *Corchorus olitorius L., pollen, viability, variation, morphology and index*

Introduction

West African sorrel or bush okra is known as a native to both tropical and subtropical region of the World. It has many local names in Nigeria, 'Ewedu' in Yoruba, 'ahihiara' in Igbo and 'malafiya' or 'rama' in Hausa, somewhat similar to 'malukhiyah' called by Arabs in North-Eastern Africa. It exists both as wild and cultivated leafy vegetables Generally, jute is a highly perishable vegetable and it has also been observed that the wilder *Corchorus* species in Africa are the good sources of genetic variability. (Makinde *et al.* 2009).

Corchorus is a flowering plant in the genus *Corchorus* L. belonging to the family Malvaceae, formerly Tiliaceae, and of recent Sparrmanniaceae. *Corchorus* has been classified in a number of families including Capparaceae, Cistaceae, Papaveraceae, and Tiliaceae (Whitlock *et al.* 2003). It consists of about 40-100 species of which 30 are found in Africa (Makinde *et al.* 2009). Alongside cotton it is one of the major fiber crops in the World especially in Indian subcontinent (Basu et al. 2009). It grows all year round provided the required conditions (rainfall, light intensity, soil humidity and soil structure) are met. *Corchorus olitorius* is most frequently cultivated as vegetable in Nigeria and Africa is said to be the center of origin (Roy *et al.* 2006). However, the presence of wilder *Corchorus* species in Africa leads to larger genetic diversity with *Corchorus olitorius.* It is found presently in all tropical Africa countries and is the leading leafy vegetable in countries like Cote d'Ivorè, Benin, Nigeria, Cameroon, Sudan, Kenya and Zimbabwe (Makinde *et al.* 2009). According to Maity et al. 2012, the wild varieties are considered important genetic resources for both biotic and abiotic stress tolerance and fine fiber traits.

The two most common types of *Corchorus olitorius* in Nigeria are 'Amugbadu', which grow tall with large finely serrated leaves that are oblong in shape and 'Oniyaya', which is widely branched with broad, deeply and

irregularly serrated (*Corchorus incisifolus*) leaves and highly mucilaginous. In Cameroon and other West Africa countries, there are numerous local types varying among others in height, stem colour, leaf and fruit shape. 'Oniyaya' in Cameroon is more deeply lobed than those found in Nigeria though there are overlaps. A large, broad-leaved Accession from Cameroon with shorter swallow tails that is commonly cultivated near Yaoundé is called Greant de Bertoua. Its leaf tips are rounder than those of 'Amugbadu', the most common one in Nigeria, which resembles the Cameroonian Ewondo (Pal et al. 2006). There are several other minor local morphotypes, for example 'Eleti ehoro' with small ovate leaves like the ear of a hare, oblong and with fine serration and 'Eti eku' with a leaf shape like the ear of a rat. Another Accession is 'Yaga' (Makinde *et al.* 2009).

Ayurveda's use of the leaves for ascites, pain, piles, and tumors have been reported elsewhere, the leaves are used for cystitis, dysuria, fever, and gonorrhea. The cold infusion is said to restore the appetite and strength (James, 1983). Fresh leaves of *Corchorus olitorius* are rich source of vitamin A and vitamin C, β-carotene, iron, calcium, etc. The leaves are used in the treatment of chronic cystitis, gonorrhea, and dysuria, for toothache (Hillocks, 1998). In Nigeria the seeds are used as a purgative and febrifuge, the leaves infusion to cure malaria and consume during pregnancy. Also, in the South Eastern part of the country, it is used as a remedy for irregular menstrual flow in women. Jute also possesses broad antibacterial properties (Pal *et al.* 2006).

The genus *Corchorus* is highly diversified in terms of health benefits (strong bones and teeth, good vision, protection against chronic diseases, wound healing, high immunity, etc.) FAO 1972; Duke and Wain, 1981; James, 1983; Hillocks, 1998 and Pal *et al.* 2006) and economically as fabrics, handcrafts, floor coverings, pharmaceuticals, cosmetics, paints industries, home textiles, geotextiles, building materials and floor coverings, potting bags, technical textiles, etc. (Basu *et a*l. 2009 and Chattopadhyay *et al.* 2004). It can also be intercropped with food crops and vegetables such as yam, groundnut, watermelon, okra, tomato and many more (NIHORTPROTA Nigeria, 2002). According to Ragho 2015, Pollen morphology are is a vital tool in detection of adulteration in crude drugs/herbal medicine thereby safe guarding food and health security in the nation.

The aim of this work therefore, is to critically assess the *Corchorus* species of different accessions under study with a view to identifying the best accession suitable for this locality by evaluating variations in the pollen compositions. In view of this, the study is of significance due to the fact that variation within species is essential for the success of breeding programs for crop improvement and sustainability.

Materials and Methods

Eleven varieties of *Corchorus olitorius* L. obtained from NIHORT and IAR Ibadan, Nigeria (NG/OA/JUN/09/001, NG/SA/DEC/07/0403, NHGB/09/147, NG/SA/DEC/09/0403, NG/AA/06/12/177, NHGB/09/145, NHCo-2, NG/OA/02/11/008, NG/To/02/12/179, NG/AA/SEP/09/173 and NG/To/02/12/180). The seeds were grown till maturity and the anthers were harvested in to experimental bottles for the pollen analysis in all the varieties under study. Five pollen grains were examined for each of the accession studied and the average recorded. The pollen grains with the aid of forceps and needle were separated from the anthers and were dusted on a clean slide and a drop of aceto-orcein stain was added. This was covered with a clean cover slip and then viewed using X40 lens of Olympus microscope. The pollen shape was revealed and the measurement was taken with WF10X Olympus eye piece graticule and photographs obtained with photomicrograph.

Percentage pollen viability (PPV):

At maturity, the pollen grains from the anthers of each accession were dusted on a clean glass slides and a drop of aceto-orcein stain was added and carefully covered with clean cover slip. The fully stained pollen

grains were considered viable while the unstained were considered non-viable. The pollen viability was determined using X40 objective of Olympus light microscope and PPV calculated as follows:

% Pollen Viability (PPV) = Number of viable pollens (PV) X 100 (Koshy *et al.*, 2013)

Total number of pollens observed (TP) 1

The photomicrographs were taken using Samsung lens ES95 on the Olympus light microscope.

Results and Discussion

Pollen morphology revealed similarities and slight variations in the varieties under study. The pollen grains observed in all the accessions were similar that is, there were no obvious differences among the pollen grains in terms of shape, surface and structure. The pollen grains observed were all spherical in shape and exhibited three different types of pollen namely: monocolpate (NG/OA/JUN/09/001, NG/SA/DEC/07/0403, NHGB/09/147 and NG/To/02/12/179), dicolpate (NHCo-2, NG/AA/06/12/177, NHGB/09/145, NG/To/02/12/179 and NG/To/02/12/180) and tricolpate (NG/SA/DEC/09/0403 and NG/AA/SEP/09/173). The spherical shape of pollen grains studied are in line with the work of Nighat et al. (2009) on Abutilon and Hibiscus pollens of Malvaceae family. Spheroidal shape of pollen grains and some pollen type(tricolpate) agrees with Debasmita et al. (2015) work on Nyctaginaceae family. The exine and intine layer of the pollen wall were well differentiated as indicated in plate 1-10 palynological observations. Variation and similarities were also observed in the pollen size.

Pollen viability revealed the level at which each of the varieties studied is successful independently. NG/SA/DEC/09/0403 had the highest percentage viability while NHCo-2 had the least however; all the accessions are highly viable as indicated in Table 2. The variations of pollen grain viability were also recorded in the researched varieties.

Table 1: Pollen morphology in *Corchorus olitorius* accessions studied.

Accessions	Pollen length(µm)	Pollen width(µm)	Pollen index
NG/OA/JUN/09/001	12	9	1.33
NG/SA/DEC/07/0403	**12**	**9**	**1.33**
NHGB/09/147	10	12	0.83
NG/SA/DEC/09/0403	**14**	**12**	**1.17**
NG/AA/06/12/177	10	9	1.11
NHGB/09/145	**10**	**9**	**1.11**
NHCo-2	11	13	0.85
NG/OA/02/11/008	**13**	**11**	**1.18**
NG/To/02/12/179	12	11	1.09
NG/AA/SEP/09/173	**12**	**11**	**1.09**
NG/To/02/12/180	12	14	0.86

Table 2: Percentage pollen viability for the accessions of *Corchorus olitorius*

Accessions	Total number of pollens counted	No. of Viable Pollens (PV)	Number of non-viable pollens	Percentage Pollen Viability (PPV)
NG/OA/JUN/09/001	92	82	10	89.13
NG/SA/DEC/07/0403	**75**	**71**	**4**	**94.67**
NHGB/09/147	94	89	5	94.68
NG/SA/DEC/09/0403	**94**	**90**	**4**	**95.74**
NG/AA/06/12/177	76	67	9	88.16
NHGB/09/145	**41**	**35**	**6**	**85.37**
NHCo-2	59	49	10	83.05
NG/OA/02/11/008	**47**	**43**	**4**	**91.49**
NG/To/02/12/179	90	85	5	94.44
NG/AA/SEP/09/173	**90**	**81**	**9**	**90.00**
NG/To/02/12/180	99	94	5	94.95

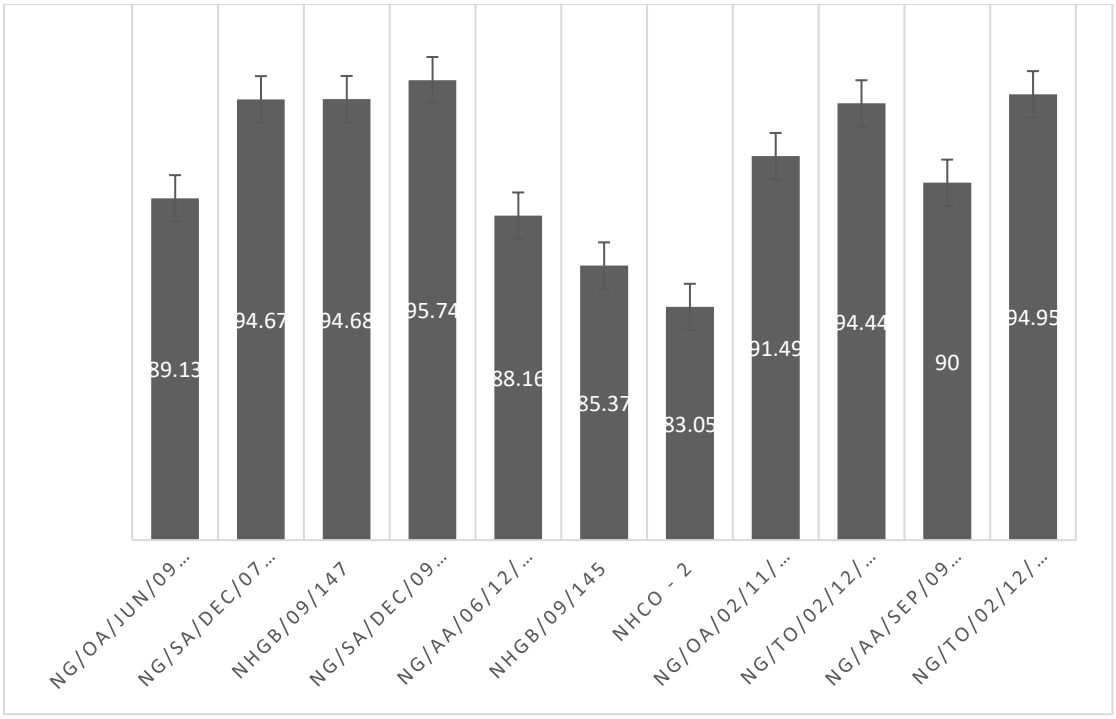
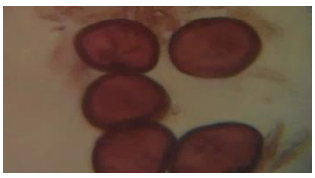


Figure 1: Histogram showing percentage pollen viability for *Corchorus olitorius* accessions studied.



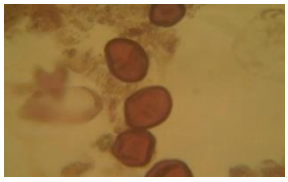


Plate1: Dicolpate Pollen of NHCo-2 Plate2: Monocolpate Pollen of NG/SA/DEC/07/0403

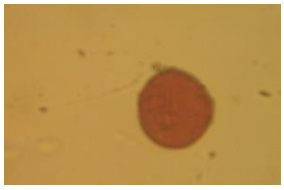


Plate3: Monocolpate pollen of NG/OA/JUN/09/00 Plate4: Dicolpate pollen of NG/AA/06/12/177

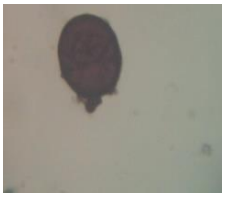


Plate5: Tricolpate pollen of NG/SA/DEC/09/0403 Plate6: Dicolpate pollen of NG/To/02/12/179

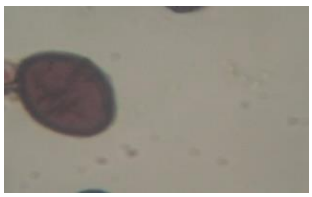


Plate 7: Dicolpate pollen of NG/To/02/12/180

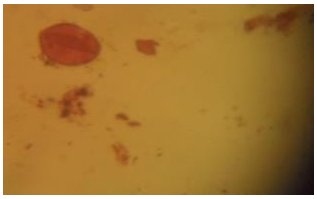


Plate 8: Monocolpate pollen of NHGB/09/147

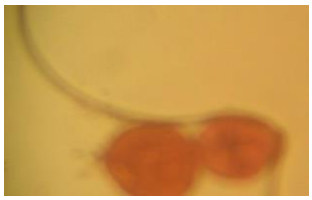


Plate9: Tricolpate pollen of NG/AA/SEP/09/173

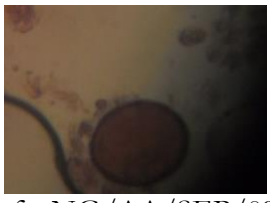


Plate10: Monocolpate pollen of NG/To/02/12/179

Result shows that percentage pollen viability is not directly correlated with the seed and pod production. Generally, the pollen viability was fairly high for all the accessions studied. The pollen grain shape, size and surface were similar in all the cultivars examined this might be as a result of genetics or other factors which could be environmental.

Certain accessions experienced similarities in their pollen length and width while others differ. It ranges from 10-14µm in length and 9-14µmin width. This research also demonstrated that a variety can possess more than one type of pollen as shown by NG/To/02/12/179.

Conclusions and Recommendations

The result from pollen shows that plants belonging to the same family of *C. olitorius* displayed similarities in shape, size and type of surface structures in pollen grains therefore, pollen morphological characters are significant in classification hence, can be used in taxonomy of *C. olitorius* species from other species of *Corchorus* as they have similar types of pollen and could as well encourages cross breeding most especially in varieties with multiple pollen types (superior varieties) so as to develop new hybrids.

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CGBPB 041

CHARACTERIZATION OF SOYBEAN [*GLYCINE MAX* (L.) Merril] GENOTYPES USING SEEDS QUALITATIVE MORPHOLOGICAL TRAITS

*** ISAH, A.[1], ALIYU, R. E.[1] AND ADAMU, A. K.[1]**

[1]Departyment of Botany, Ahmadu Bello University, Zaria. Kaduna State, Nigeria.
*Email of corresponding author: abuisaf2016@gmail.com, Phone Number: 08130597618 and Postal Address: Department of Botany Ahmadu Bello University, Zaria. Kaduna State, Nigeria.

ABSTRACT

This research was conducted at the physiology laboratory of the department of botany Ahmadu Bello University, Zaria to identify and characterize soybean genotypes through seed qualitative morphological traits. A total of twenty three genotypes were used for the study. The seed qualitative morphological characteristics like the seed coat colour, hilum colour, seed coat luster and seed shapes were evaluated using standard protocols for soybean. Results obtained revealed wide variations among the soybean genotypes for the qualitative morphological traits. Seed qualitative morphological characteristics are thus summarized: seed coat colour was not reliable for the differentiation of these genotypes because all the genotypes were yellow. Hilum colour grouped the genotypes into: imperfect black 3 (13.04%), black 4 (17.39%), dark brown 1 (4.35%), brown 5 (21.74%), light brown 9 (39.13%) and imperfect yellow 1 (4.35%). Seed coat luster grouped the genotypes into shiny 11 (47.83%) and intermediate 12 (52.17%) while the seed shapes categorized the genotypes into spherical flattened 14 (60.87%), elongated 1 (4.35%) and elongated flattened 8 (34.78%). Cluster analysis grouped the genotypes into two at 25 rescaled distances based on the seed qualitative morphological traits. Useful information on the variability in seed qualitative traits among the studied soybean germplasm will serve to characterize the diversity of soybean landraces.

Keywords: *Soybean, Genotype, Qualitative Traits, Hilum, Seed Coat and Shape*

Introduction

Soybean [*Glycine max* (L.) Merril.; 2n = 40] is economically a very important leguminous seed crop for feed and food products which is rich in seed protein (about 40%) and oil (about 20%) (Singh, 2017) belonging to the pea family fabaceae that grows in the tropical, subtropical, and temperate regions of the world. Soybean is hailed as the most protective bean because it has the highest protein content amongst plant products and is the only vegetable food that contains all eight essential amino acids. Soybean has a wide range of latitudinal adaptation in both North and South hemispheric geographical locations; this reflects its complex domestication, origin and subsequent breeding history (Carter *et al.*, 2004).

The knowledge of existing plant genetic diversity is quite essential for effective management of crop genetic resources. The variability in the genome of a species can be grouped in to visible and nonvisible characteristics (Atnafua and Endashaw 2004). In plant diversity study, qualitative traits are regarded as the most important characters in describing a particular genotype, this is because the traits are mostly controlled genetically and as such, they are less dependent to the environmental response. According to Xiabo *et al.* (2011), the appearance of qualities of soybean which include seed shape, seed colour, hilum colour, glossy seed coats, rate of mottling seed length, width and thickness are important parameters in analyzing genetic diversity in soybean.

Govindarao (2010) also characterised 24 varieties of soybean using the seed qualitative

morphometric traits and was able to group the 24 genotypes into three groups baesd on the seed coat lustre viz: shiny, intermediate and dull; he also classified same 24 varieties based on their seed shapes into four groups as spherical, spherical flattened, elongated and elongated flattened while for the seed coat colour, he grouped all the genotypes into two either as yellow or brown coloured.

Xiabo *et al.,* (2011) classified 235 varieties of chinese soybean mini collection on the basis of seed appearance qualities and reported that, most of the seed coats (56.65%) were yellowish in colour while for the hilum colour 73.91% and 21.74% were brown and yellow respectively. Bonj *et al.*, (2007) reported the classification of ten *Phaseolus vulgaris* cultivars into three groups based on their seed shapes as long, elliptical and egg.

The selection and subsequent recommendation for release of most of germplasm have been based mainly on subjective traditional analysis of the yield data from a number of locations, with little or no emphasis on the aspect of their characterization (Ojo 2012). Therefore, the aim of this study is to characterize the seeds of soybean genotypes based on the seed qualitative traits.

Materials and Methods

The seed qualitative study was conducted at the Physiology laboratory in the Department of Botany, Ahmadu Bello University Zaria. Twenty three soybean genotypes (Seventeen land races and six improved varieties) were utilized for the study. The land races were obtained from local farmers in Kaduna, Kastina and Zamfara states while the improved varieties were collected from the seed unit of plant science department, Ahmadu Bello University Zaria (Table 1).

Thirty seeds each of the twenty three genotypes were randomly divided into three replicates in petri-dishes with each replicate having ten seeds. The petri-dishes were labeled appropriately. The seed attributes like seed coat color, hilum color, Seed coat luster, and shape were determined and recorded. The Seed coats and hilum colors were observed under natural day light and the colors were authenticated by matching with a standard color chart pattern (Govindarao 2010). The seed shapes based on height/length ratio and height/thickness ratio were measured using Vernier caliper and the ratios were compared based on the USDA-ARS Soybean descriptors 2010 to obtain the correct shapes.

Results

The soybean seed attributes which includes: seed coat color, hilum color, seed coat luster and shapes are presented in Table 2. There was no difference in the seed coat color as all the twenty three genotypes were found to be yellow. The hilum colours varied significantly as seven different colors were observed. These are: imperfect black 3 (13.04%), black 4 (17.39%), dark brown 1 (4.35%), brown 5 (21.74%), light brown 9 (39.13%) and imperfect yellow 1 (4.35%). Two types of seed coat luster were obtained 11 genotypes (47.83%) were shiny while the remaining 12 genotypes (52.17%) were found to be intermediate. Three different types of seed shapes were observed. These are: spherical flattened, elongated flattened and elongated. 14 (60.87%) genotypes were observed to be spherical flattened, 8 (34.78%) genotypes were observed to be elongated flattened and while 1 (4.35%) genotype was observed to be elongated in shape. It was also observed that, five out of the 7 improved varieties were elongated flattened with the remaining two having elongated flattened shapes.

Table 1: Collection locality and species of the soybean genotypes used for the study

S/n	Genotype	Category	Source	State
1	Karara	Land race	Pambegua	Kaduna
2	Bakin hanci	Land race	Funtua	Kastina
3	Idan kwdo	Land race	Giwa	Kaduna
4	Idan fara	Land race	Giwa	Kaduna
5	Daneka	Land race	Giwa	Kaduna
6	Zafa	Land race	Dandume	Kastina
7	Kwankwaso	Land race	Funtua	Kastina
8	Idan kwado	Land race	Dandume	Kastina
9	Danbulagi	Land race	Funtua	kastina
10	Kwankwaso	Land race	Dandume	Kastina
11	Gamagantlinka	Land race	Maru	Zamfara
12	Idan kwado	Land race	Funtua	Kastina
13	silba	Land race	Maru	Zamfara
14	silba	Land race	Kaura	Zamfara
15	Kwankwaso	Land race	Kaura	Zamfara
16	Janhanchi	Land race	Kaura	Zamfara
17	TGX-1835	Improved	IAR	Kaduna
18	TG-923-2E	Improved	IAR	Kaduna
19	TGX-1989-19F	Improved	IAR	Kaduna
20	TGX-306-036c	Improved	IAR	Kaduna
21	TGX- 1951-3E	Improved	IAR	Kaduna
22	TGX-1987-10E	Improved	IAR	Kaduna
23	TGX-1485-10	Improved	IAR	Kaduna

Table 2: Frequency distribution for qualitative morphological traits in soybean genotypes

S/n	Traits	Colour	Frequency	Percentage
1	Seed coat colour	Yellow	23	100
2	Hilum colour	Imperfect black	03	13.04
		Black	04	17.39
		Dark brown	01	4.35
		Brown	05	21.74
		Light brown	09	39.13
		Imperfect yellow	01	4.35
3	Seed coat luster	Shiny	11	47.83
		Intermediate	12	52.17
		Dull	00	0.00
4	Seed shapes	-		

SPFLND	-	14	60.87
ELNG	-	01	4.35
ELNFLND	-	08	34.78

KEY: SPFLND- Spherical Flattened, ELNG- Elongated, ELNFLND- Elongated Flattened

Clustering of Soybean Genotypes Based on the Seed Qualitative Traits

Hierarchical cluster analysis using the seed qualitative traits grouped the twenty three genotypes into two clusters I and II at 25 rescaled distances. (Figure 1)

Cluster I consisted of twenty genotypes: 4, 8, 3, 2, 17, 7, 12, 18, 16, 5, 15, 19, 23, 11, 20, 1, 10, 13, 22 and 14; this cluster could further be grouped into two sub clusters I and II at 5 rescaled distances. Sub cluster I comprised of eleven genotypes: 4, 8, 3, 2, 17, 7, 12, 18, 16, 5 and 15 which are apart from one another at 1 rescaled distance. Sub cluster II consisted of nine genotypes: 19, 23, 11, 20, 1, 10, 13, 22 and 14 which could further be subdivided into two sub groups A and B at 2 rescaled distances. Sub group A consisted of six genotypes: 19, 23, 11, 20, 1 and 10 that are apart from one another at 1 rescaled distance while sub group B consisted of three genotypes: 13, 22 and 14 which are also apart from one another at 1 rescaled distance.

On the other hand, cluster II consisted of three genotypes: 6, 9 and 21. This cluster could further be divided into two sub clusters I and II at 13 rescaled distances. Sub cluster I consisted of two genotypes: 6 and 9 that 1 rescaled distance apart from each other. The sub cluster II consisted only of one genotype 21. (Figure 1)

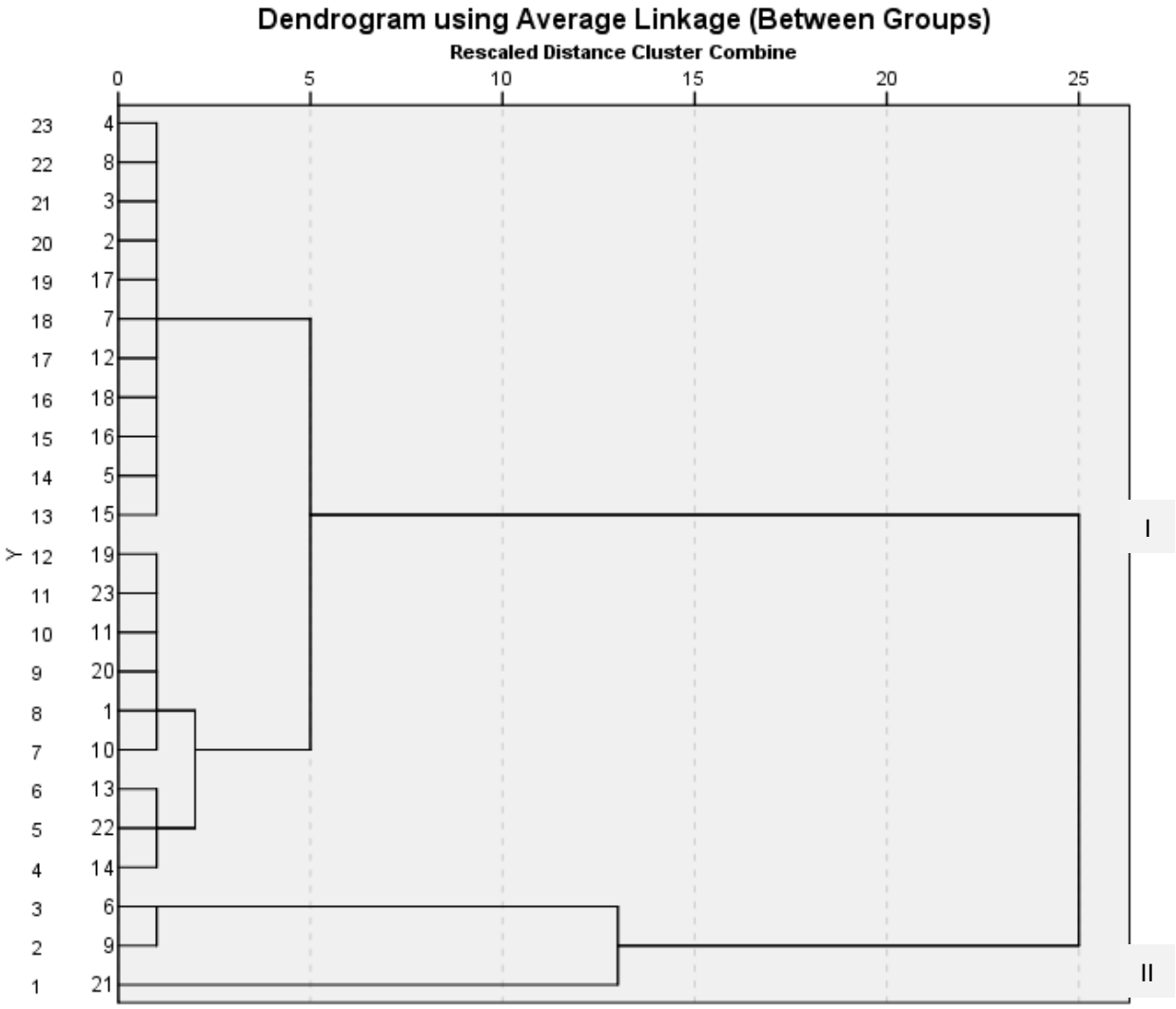

Figure 1 Hierarchical Cluster Analysis for Twenty Three Soybean Genotypes Analyzed for Seed Qualitative traits.

KEYS: 1-Karara, 2- Bakin hanci, 3- Idan kwdo, 4- Idan fara, 5- Daneka, 6- Zafa, 7- Kwankwaso, 8- Idan kwado , 9- Danbulagi, 10- Kwankwaso, 11- Gamagantlinka, 12- Idan kwado, 13- silba , 14- silba 15- Kwankwaso, 16- janhanchi, 17- TGX-1835, 18- TG-923-2E, 19- TGX-1989-19F, 20- TGX-306-036, 21- TGX-1951-3E, 22- TGX-1987-10E, 23- TGX-1485-10.

Discussion

The seed coat colour will not be reliable for identification of the genotypes because all the genotypes were observed to be yellowish. However, the variation in the hilum colour observed will be a reliable mean of identifying cultivars. This finding is supported by Xiabo *et al.,*(2011) who classified 235 varieties of chines soybean mini collection on the basis of seed appearance qualities and found out that, majority of the seed coats (56.65%) were yellowish in colour while for the hilum colour 73.91% and 21.74% were brown and yellow respectively.

The variation observed in the seed coat luster as well as the seed shapes could be due to the genetic factors and the environmental influence as well. This result corroborated the finding of Govindarao *et al*., (2010) who classified 24 varieties of soybean into three groups viz: shiny, intermediate and dull based on the seed coat luster; he also characterized same 24 varieties based on their seed shapes into four groups as spherical, spherical flattened, elongated and elongated flattened. It is also supported by Bonj *et al*., (2007) who classified ten *Phaseolus vulgaris* cultivars into three groups based on their seed shapes as long, elliptical and egg.

Cluster analysis plays an important role in revealing complex relationship existing in populations of diverse origin in a simplified manner. It is also essential for the indication of genotypes possessing useful traits from different clusters for breeding purposes. The hierarchical cluster analysis for the seed qualitative traits put the twenty three soybean genotypes into two major clusters. The fact that the twenty three genotypes occupied two clusters revealed that, genetic diversity existed among the soybean genotypes. Three genotypes 6, 9 and 21 clustered separately, from the remaining genotypes; this is an indication that these three genotypes possessed similar genes for qualitative attribute as supported by Alege *et al.,* 2017. However, genotype 21 clustered singly, 9 and 6 clustered in pair as well as 14, 22 and 13 making them the most divergent genotypes. Dughdugh *et al*., (2017) mentioned that, such divergent genotypes are strongly recommended to be explored by breeders and the genetic engineers. These divergent genotypes therefore may be good parental stock when breeding for improved qualitative traits. Also, the genotypes did not cluster based on their locations (sources) and seed coat colour; this is an indication that, there is no justification to select these genotypes based on their geographical origin and seed coat colour, this finding is in agreement with the work of Sadia *et al*., (2012) who reported that, cultivar in a cluster may not necessarily comprise of all the cultivars from the same origin.

Conclusions and Recommendations

In conclusion, wide variation in the evaluated seed qualitative traits was obtained; this indicated the existence of wide diversity in the studied genotype (both the land races and the improved varieties). The seed qualitative traits of the soybean genotypes at 25 rescaled distances also showed distinct variations. This study therefore recommends that, Soybean genotypes with highest genetic dissimilarities and traits of interest could be selected and utilized in breeding programs as these variations may be help in exploring new germplasm to be used as parental stock in subsequent breeding programmes.

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PHENOTYPING OF SORGHUM LINES FOR RESISTANCE TO AFRICAN STEM BORER (*SESAMIA CALAMISTIS*)

Badiru, Evilla[1], Yeye M.Y.[2], Ishiyaku, M.F. [2] and Usman, I.S. [2]

[1]National Biotechnology Development Agency, P.M. B 5118.Wuse. Abuja
Corresponding Author: evybiyi@yahoo.com, +2348033003730.

ABSTRACT

Stem borer (Sesamia calamistis) is a serious insect pest of sorghum (Sorghum bicolor) resulting in grain yield losses ranging between 15-80%. However, genotypes showing complete resistance to these borers have not been identified in Nigeria. Utilization of resistant varieties in combination with other methods of control would offer a sustainable strategy for S. calamistis management in sorghum production. The objective of this study was to validate the acclaimed resistance in the materials received from Kenya and India in Nigerian environment and to screen and ascertain the status of some Nigerian Sorghum to Sesamia calamistis. Eighty-eight sorghum lines were artificially infested with the eggs of the stem borers at two different environment (Field and Screen House) using alpha-lattice design, consisting of 11 plots in eight blocks, replicated twice. Data were collected on leaf feeding, number of dead-hearts, cumulative stem tunnel length, number of exit holes, and selected agronomic traits. There were significant ($p<0.01$) differences among the test genotypes for all the traits measured. Based on the selection index, 23% of genotypes were categorized as resistant, 33% each as moderately resistant/moderately susceptible and 11% as susceptible.13 genotypes showed resistance across the environments (field and screen house): ICSB464, ICSL71086, SSV20041-2YELLOW, ICSL71018, ICSR94032, ICSV700, ICSL71193, ICSR94030, ICSL71253, ICSL71268, ICSL71023, ICSL71061 and ICSL71137 were resistant with selection index ranging from 0.0 to 0.50. These sorghum lines with various resistance to S. calamistis could be used as source of resistance and as parents in sorghum improvement programme in breeding for resistance to stem borer.

Keywords: *Genotypes, selection Index, Sesamia calamistis, Sorghum bicolor,*

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] commonly referred to as Guinea Corn in West Africa is the fourth most important cereal in the world after rice, wheat and maize (Dogget, 2013). Nearly 80% of the cultivated area lies in Asia and Africa and widely cultivated for food, forage, ethanol, and sugar production (Liu *et al.*, 2009). Nigeria currently produced about 6.57 million metric tons of sorghum (Statista2021). Sorghum production especially in tropical Africa is constrained by a number of anthropod pests, the stem borers belonging to lepidoptera being the most important. Stem borers cause grain yield losses ranging from 15-80% depending on crop variety, phenological stage of attack and agro ecological environment (Karaya *et al.*, 2009, Muturi *et al.*, 2012). The damage caused are leaf feeding, dead heart formation, exit holes and stem tunneling damage (Kishore *et al.*, 2007; and Muturi *et al.*, 2012). The spotted stem borer (*Chilo partellus* (Swinhoe)) Pyralidae, African stem borer

(*Busseola fusca* Fuller), and African pink borer (*Sesamia calamistis*) are among the most damaging insect pests that greatly reduce sorghum grain yield in African environments (Sharma *et al*., 2005; Mwimali *et al*., 2015). Among the several stalk borer species, pink stem borer (*Sesamia calamistis* (Hampson)) is the most important pest of sorghum in the Nigerian savannah (Ajayi 1998, Anaso and Thilza, 2006). Host plant resistance forms an important part of integrated pest management as it provides inherent control without environmental issues and is compatible with other pest management approaches (Singh *et al.,* 2012). Effective breeding methods for resistance to borer damage could, therefore, be designed by plant breeders using both improved and identified sources of stem borer resistance.

Management of *S. calamistis* in sorghum has mainly focused on cultural control, burning of crop residues, intercropping and predominantly the use of pesticides (Amsalu *et al*., 2008). However, use of these strategies would invariably increase the cost of cultivation of sorghum, which is not a feasible option for the resource poor farmers of the semi-arid tropics. Furthermore, the use of chemical pesticides could be harmful to both the environment and human health. Hence, the exploitation of host plant resistance is the only viable option both in terms of economic and environmental sustainability for controlling stem borer in sorghum (Tadele *et al.,* 2011). Considering multiple stem borer damage traits are useful since resistance to stem borers is quantitatively inherited thus selecting for resistance based on a single parameter would not be effective.

High yielding varieties in Nigeria are susceptible to stem borers. Thus, there is the need to increase the levels of tolerance in elite genotypes without sacrificing grain and Stover yield. Existing sorghum germplasm in Nigeria has not been evaluated for resistance to stem borers. Therefore, it is important to profile sorghum genotypes with reference to stem borer resistance. In Nigeria, little research attention has been accorded to stem borers like *S. calamistis* in cereals and there is paucity of information on the status (resistance/susceptibility) of the sorghum germplasm. Screening of all the elite germplasm becomes necessary as the first phase of resistance breeding programme. Such information will be useful in developing an appropriate strategy to produce stem borer-resistant open pollinated varieties and hybrids for cultivation by the farmers in the semi-arid tropics. Therefore, the objective of this study was to assess the levels of resistance of elite sorghum germplasm obtained from ICRISAT Kenya and India and Institute for Agricultural Research (IAR) released sorghum varieties.

Materials and Methods

The research was conducted at the IAR screen house and research farm at Samaru, Zaria. Nigeria. The farm is located on 11°11'N, 7°38'E, 640 m asl, 1200 mm annual rainfall) in the Northern Guinea Savannah. Eighty-eight sorghum lines comprising forty recombinant inbred lines (RILs) from ICRISAT Nairobi, Kenya, eight varieties from ICRISAT Patancheru, India, twenty-eight elite germplasm lines from Nigeria and twelve IAR released varieties were planted in IAR research farm under rain-fed condition. These lines were arranged in an alpha-lattice design, consisting of 11 plots in eight blocks, replicated twice. Each plot consisted of one row; 5 m long. The inter and intra row spacing was 75 cm and 30 cm, respectively. Three seeds were planted and later thinned to two plants per stand after two weeks of sowing. At 16 days after sowing, the crops were sprayed with cypermethrin (synthetic pyrethroid) to minimize shoot fly infestation, since this insect interferes with screening for resistance to stem borers (Makueti *et al.,* 2012).

Twenty-one days after sowing, plants were artificially infested with 50g of stem borer eggs

placed between the stem and the last unfolded leaf sheath using a forcep. Eggs of *S. calamistis* used in this study were obtained from the International Institute for Tropical Agriculture (IITA) Ibadan. To avert drowning of eggs in the water held in leaf whorls, sorghum seedling whorls were tapped gently before infestation. Infestations were carried out early in the morning to encourage egg survival. All other recommended cultural practices were observed. At the screen house, the same source of eggs of *S. calamistis* used on the field were used. One sorghum line/variety was sown in one pot repeated twice. Three seeds were planted and later thinned to two plants per pot after 2 weeks of sowing. Infestation was carried out as described for field above.

On the field, 4 plants within each row were tagged for infestation while in the screen house two plants per pot were sampled and data were taken systematically from the marked plants. The observations were recorded on per plant basis at two and four weeks after the artificial infestation. Percentages of plants with leaf damage were computed by expressing the number of plants showing pinholes damage as a percentage of the total number of plants sampled (Muturi *et al.,* 2012). Dead heart incidence was computed by counting the number of plants showing dead heart damage and expressed as a percentage of the total number of plants sampled (Kumar *et al.,* 2005). At harvest, number of stem borer exit holes on the stem were counted on each sampled plant. The main stem of plants infested was split open from the base to the apex, and the cumulative tunnel length measured with a ruler in centimeters. Seedling vigour was scored at 2 weeks after sowing on a scale of 1 – 5, where 1 = low vigour (plants showing minimum growth, less leaf expansion and poor adaptation; 3=Moderate vigor; 5=high vigor tall plants with expanded leaves and robustness) (Kishore *et al.,* 2007, Muturi *et al.,* 2012). At physiological maturity, plant height was measured in centimeter from the base of the plant to the tip of the panicle using a calibrated pole. Days to panicle emergence was recorded as the number of days from the date of sowing to the date when 50% of panicle emerged in a plot. Days to 50% flowering was recorded as the number of days from the date of sowing to the date of anthesis of 50% of plants in a plot. After harvest, sorghum panicles were sun-dried and hand threshed. Grain yield and hundred seed weight were recorded in grams for each of the sampled plants using an electric weighing balance.

Data were first analyzed on individual environment basis and combined across the 2 environments. The screen house and the field were equated to 2 environments. Data on percentages were angular transformed while those of counts were log transformed before the analysis of variance (Kishore *et al.,* 2007). The mean values of all the traits for each replicate were used to compute the analysis of variance using SAS GLM version 9.2. Treatment means were compared using a protected Fishers' least significant difference (LSD) test at P = 0.05. Selection index was calculated based on leaf damage (2^{nd} and 4^{th} week), dead heart (2^{nd} and 4^{th} week), stem tunneling and exit holes by adding the ratios between the values for each genotype and the overall mean for each parameter, and divide by 6 (number of damage parameters considered) (Tadele *et al.,* 2011). Genotypes were grouped into four categories namely, resistant, moderately resistant, moderately susceptible and susceptible (Tadele *et al.,* 2011, Muturi *et al.,* 2012). The genotypes with selection index values less than 0-0.5 were regarded as resistant, 0.6-1.0 moderately resistance, 1.1-1.5 as moderately susceptible and those with a selection index greater than 2.1 as susceptible (Bergvinson *et al.,* 2004; Tadele *et al.,* 2011 and Muturi *et al.*,2012).

Results and Discussion

For the combined analysis, environment mean squares were significant (P = < 0.05-0.01) differences for all traits except for dead heart at 4 weeks, exit holes and yield (Table1). There

were highly significant (P = < 0.01) differences among genotypes for all traits measured except for leaf damage at 4 weeks, dead heart at 2weeks and tunnel length. The

interactions between environment and genotype were significant for leaf damage at 2week, dead heart at 2 and 4 weeks but highly significant (p<0.01) for seedling vigor, days to 50% flowering, plant height, panicle length,100 grain weight and exit hole (Table1).

Mean Performances

Out of the 88 genotypes screened, only 13 top resistant and nine worst susceptible genotypes are presented in Table 2. Across the two environments, the mean performance for leaf damage at 2 weeks ranged from 1.9% for KAT487 to 52.2% for SAMSORG17 with a mean of 34.2%. Leaf damage at 4 weeks range from 0.0% for ICSL71086, ICSR94032 to 48.2% for SAMSORG 3 with an average of 16.2%. Leaf damage incidence was greater at 2 weeks compared to 4weeks. Genotypes observed to show low leaf damage at 4 weeks were ICSL71086, ICSR94032, ICSL71268, SSV20041-1 White and ICSB464 (in increasing order of leaf damage incidence). Dead heart at 2 weeks ranged from 0.0 % to 64.8% with an average of 8.8%. SAMSORG 17 recorded the highest number of dead heart (64.8%) at 2 weeks. At 4 weeks, it ranged from 0.0% to 100% with a mean of 26.5%. Genotypes ICSB 464, ICSV700, ICSL71193, ICSR94030, ICSL71253, ICSL71023 and ICSL71018 suffered the least dead heart damage at 4 weeks. ICSR94032, ICSL71086 and SSV20041-2 Yellow recorded fewer exit holes of 0.5, 1.3 and1.5 respectively. Stem tunnelling length range from 10.8cm for ICSL71137 to 97.5cm for SSV 20041-1 White, with a mean of 49.4cm. Seedling vigour scores ranged from 2.0 to 4.0 with an average of 3.3. Only three lines indicated less vigour (ICSR94032, ICSL71193 and ICSL71023) (Table2). Days to panicle emergence (HD) were longest for SAMSORG 17 (92 days) while ICSL71061 took 68 days for the panicle to emerge. The mean heading date observed was 76 days. Days to 50%

flowering ranged from 70 to 94 days for ICSL71061 and SAMSORG 17 respectively with a mean of 78 days. Plant height ranged from 93.8 cm for ICSL 71137 to 223.4 cm for SAMSORG8 with a mean of 143 cm. Panicle length ranged from 14.2 cm for ICSV700 to 25.1cm for SAMSORG8 and closely followed by SAMSORG 44(24.9cm) with an average of 19.5cm. Hundred grain weight ranged from 0.2 g for ICSL 71219 to 4.1 g for Yar Washa with a mean was 2.1g. High grain yield (> 59 kg/ha) were observed on ICSL71137, SAMSORG17, SAMSORG72.3, SAMSORG44 and SSV20041-1 White while lower grain yield (<20kg/ha) was recorded on ICSL71018, KAT487, and ICSL71086.

Table 1. Combined means squares for the reaction of sorghum to stemborer (*S. calamistis*) and agronomic traits.

Source of variation	DF	SV	AN	HD	PLT HT, Cm	PAL, Cm	100-GW (g)	Yield,	LD2. % WK	LD4, % WK	DH2, % WK	DH4, % WK	E H	TL Cm
Loc	1	1.16**	819.9**	946.23**	25170.6**	66.04*	4.33**	2785.0	7.79**	0.75**	2.27**	1.56	0.05	24951.7**
Rep (Loc)	2	0.13	5.11	1.933	358.6	3.20	0.38*	1772.0	0.02	0.04	1.80**	3.74**	0.04	605.4
Block (Loc x Rep)	28	0.23	3.93	3.353	318.9	5.65	0.07	652.0	0.05	0.03	0.09	0.14	0.08	1427.1
Entry	87	1.58**	112.7**	115.07**	2926.9**	45.05**	0.99**	1537**	0.09**	0.04	0.12	0.34**	0.15**	1077.8
Loc x Entry	87	0.64**	27.6**	30.197**	1003.7**	23.84**	0.24**	1067.0	0.08*	0.04	0.13*	0.22*	0.10**	942.8
Error	14	0.17	3.8	2.6414	225.4	8.16	0.08	804.1	0.05	0.03	0.09	0.16	0.06	873.2

*and ** significance at 0.05 and 0.01 levels of probabilities, respectively.
SV=Seedling Vigor, AN=Days to 50%Anthesis, HD=Heading Date, PLT HT=Plant Height (cm), PAL=Panicle Length (cm),100GW=100Grain Weight(g), Yield(kg/ha) %LD2WKS= Percentage leaf damage at 2 weeks, %LD4WKS=Percentage leaf damage at 4 weeks,%DH2WKS= Percentage Dead Heart at 2 weeks ,%DH4WKS= Percentage Dead Heart at 4 weeks, Exit Holes and TL=Tunnel Length in centimeter

Table 2. Mean Performance of Top13 resistant and nine Worst Susceptible sorghum genotypes to *Sesamia calamistis* and some agronomic traits evaluated under infestation across two environments.

Line	LD2W, % 2WK	LD4W, % 4WK	DH2W, % 2WK	DH4W, % 4WK	EH	TL, cm	SI	CAT	SV	HD	AN	PLT HT, cm	PAL, cm	GW, kg	Yield, kg/ha
ICSB464	28.4	5.0	0.0	0.0	2.7	29.0	0.2	R	3.0	81.1	81.8	125.4	19.8	1.8	54
ICSL71086	17.2	0.0	0.0	25.4	1.3	20.2	0.2	R	3.0	71.9	74.8	100.7	17.5	2.3	17.7
SSV20041-2 YELLOW	5.1	8.6	0.0	9.6	1.5	51.0	0.3	R	3.0	87.0	88.4	196.5	17.3	3.0	40.5
ICSL71018	34.6	8.6	0.0	0.4	5.8	21.7	0.3	R	4.0	75.6	77.8	119.5	17.0	2.4	8.7
ICSR94032	26.7	0.0	0.0	8.7	0.5	45.5	0.4	R	2.0	89.0	90.1	141.9	21.3	1.6	42
ICSV700	44.1	13.0	0.0	0.0	5.2	18.2	0.4	R	3.0	82.9	85.2	155.9	14.2	1.8	34.6
ICSL71193	44.7	11.6	0.0	0.0	5.8	42.8	0.5	R	2.0	72.0	74.6	132.0	19.6	2.1	29.7
ICSR94030	40.8	17.1	0.0	0.0	5.2	33.2	0.5	R	3.0	78.6	79.1	113.2	24.8	1.3	31.4
ICSL71253	22.3	13.9	0.0	0.0	10.0	39.7	0.5	R	4.0	73.4	75.4	129.9	20.5	2.2	33.3
ICSL71268	24.8	0.2	0.0	11.0	6.9	37.9	0.5	R	4.0	69.2	70.9	125.0	15.8	1.8	43.3
ICSL71023	25.8	9.6	0.0	0.0	5.9	41.9	0.5	R	2.0	73.2	76.4	115.7	17.1	2.2	41.4
ICSL71061	40.6	6.1	0.0	8.9	6.8	50.4	0.5	R	3.0	67.9	69.9	135.6	17.0	1.7	22.5
ICSL71137	29.7	17.1	0.0	34.8	4.1	10.8	0.5	R	3.0	76.5	78.1	93.8	15.8	1.8	59.2
SAMSORG44	37.3	9.9	32.3	49.2	6.6	53.4	1.6	S	4.0	75.9	77.5	141.7	24.9	2.6	82.1
SAMSORG8 (KSV14)	34.0	28.5	28.0	59.6	3.6	26.5	1.6	S	4.0	84.0	85.7	223.4	25.1	3.2	72.3
SSV20041-1 WHITE	21.6	2.2	33.0	53.4	8.2	97.5	1.6	S	3.0	86.1	87.2	204.8	22.9	3.3	90.6
SAMSORG3 (KSV4)	38.8	48.2	28.0	47.1	4.4	45.5	1.8	S	4.0	75.3	77.4	154.7	20.6	1.9	56.1
ICSL71005	43.3	13.9	30.6	69.7	7.3	83.5	1.9	S	4.0	72.4	74.8	145.6	20.6	1.9	31.2
MAIMADARA-2	47.9	42.4	33.7	55.0	3.5	27.2	1.9	S	3.0	74.8	76.0	147.3	21.6	1.8	52.0
SAMSORG42	22.1	19.2	42.4	69.9	7.7	72.4	2.0	S	4.0	88.4	90.1	163.1	22.3	3.1	58.9
KAT487	1.9	25.1	53.8	86.2	2.8	44.6	2.1	S	3.0	86.6	88.8	154.5	24.6	2.5	16.6
SAMSORG17	52.2	22.2	64.8	100.0	3.1	57.9	2.7	S	4.0	92.2	94.1	142.7	18.3	3.0	60.2
Mean	34.2	16.2	8.8	26.5	5.6	49.4	1.0		3.3	76.1	78.0	143.0	19.5	2.1	43.2

CV	51.5	84.9	229.7	95.1	50.6	59.7	12.5	2.1	2.5	10.4	14.6	13.3	65.7
LSD	34.3	27	39	49.3	5.6	57.9	0.8	3.2	3.8	29.4	5.6	0.5	55.6

%LD2WKS= Percentage leaf damage at 2 weeks, %LD4WKS=Percentage leaf damage at 4 weeks, %DH2WKS= Percentage dead heart at 2 weeks, DH4WKS = Percentage dead heart at 4 weeks, EH=Exit Holes, TL=Tunnel Length in centimeters, SI=Selection Index, CAT= Category, SV=Seedling Vigor, HD=Heading Date, AN=Days to 50%Anthesis, PLT HT=Plant Height (cm), PAL=Panicle Length (cm),100GW=100Grain Weight(g),Yield(kg/ha),

This study identified sorghum genotypes with resistance to *S. calamistis* based on leaf damage, dead heart formation, stem tunneling and exit holes following artificial infestation of seedling whorls with stem borer eggs. Multiple stem borer damage was considered because resistance to stem borers is a multi-mechanism quantitative trait, and thus, selecting for resistance based on a single parameter would not be effective (Singh *et al.,* 2011). 88 genotypes were screened but only 22 genotypes based on top resistance and worst susceptibility was reported in this manuscript. The analysis of variance revealed significant variation among the genotypes for all characters examined. Out of the 22 genotypes reported, 13(59%) of the genotypes with selection index from 0.0 to 0.50 showed resistance across the environment: ICSB464, ICSL71086, SSV20041-2YELLOW, ICSL71018, ICSR94032, ICSV700, ICSL71193, ICSR94030, ICSL71253, ICSL71268, ICSL71023, ICSL71061, and ICSL 71137(in a descending order Table 2). Sorghum resistance to *C. partellus* based on reduced dead heart damage was reported by Sharma *et al.,* (2006) for ICSV700 and ICSB464. Out of the identified resistance lines to *S. calamistis* in this study, 11 are common to lines earlier identified by (Chinwada *et al.,* 2001; Sharma *et al.,* 2007, Muturi *et al*., 2012) to possess resistance to both *C. partellus* and *Busseola fusca.* They include: ICSB464, ICSL71086, ICSL71018, ICSR94032, ICSV700, ICSL71193, ICSR94030, ICSL71268, ICSL71023, ICSL 71137, ICSL71061and ICSL71253 .This implies that these genotypes possess multiple resistance across the three species of stem borers, i.e. *B.fusca, C.partellus and S.calamitis* and thus can be used as resistant check in screening trials and as potential donors in the breeding for multiple resistance to the multiple agents of stem borer infection.

Genotypes with selection index of 0.6 to 0.10 were designated as moderately resistant, (data not shown). Out of the 29 moderately resistant genotypes, 6 were elite germplasm: DALWANDA, KAURA MAIGUNDUMA, YAR GUMEL, MACIA, MORI JIGAWA, AMARYA DA ANGO, 2 regional germplasm: 89MW1005, IS36555, and 2 IAR released varieties SAMSORG 6 and SAMSORG14.

Moderately susceptible genotypes with selection index 1.1 to 1.5 were: SAMSORG41, KAURA KADUNA-1,ICSL71001, ICSL71055, IS30768,SAMSORG40, RIB*98-SB-F3-78, ICSL71187, ICSV745, SAMSORG5, ICSL71080, FARA 2 BAUCHI, IS17562, ICSL71016,ICSL71213, MAIMADARA-1,ICSL71246, ICSL71215, KL-2, , SAMSORG38, ICSL71007, DANJIBE, RIBDAHU, KL-1,ZAUNA INUWA, TWIN SEEDED, SAMSORG39, SAMSORG17, and YAR WASHA (data not shown). Some genotypes previously reported as resistant from India (Sharma *et al.,* 2007) and Kenya (Muturi *et al.,* 2012) were found to be moderately susceptible to this pest at the test sites in Nigeria. They include: ICSL71001, ICSL71055, IS30768, ICSL71187, ICSV745, ICSL71080, IS17562, ICSL71016, ICSL71213, ICSL71246, ICSL71215 and ICSL71007, this could be attributed to the insect species and genotype by environment interactions that influenced expression of resistance to damage by *S. calamistis.*

Genotypes with selection index 1.6 to 2.0 were designated as susceptible: SAMSORG44, SAMSORG8, SSV20041-1 WHITE, SAMSORG3, ICSL71005, MAIMADARA-2, SAMSORG 42, KAT487 and SAMSORG17. (Table 2). Genotype ICSL71005 previously reported resistant to *C. partellus* in India (Sharma *et al.,* 2007) and to *B. fusca* in Zimbabwe (Chinwada *et al.,* 2001), was found susceptible to *S.calamitis* in Nigeria. This could be attributed to the insect species and genotype by environment interactions.

Conclusion

This study demonstrated that there are genotypic differences in resistance/susceptibility to damage by *S. calamistis*. Resistance to *S. calamistis* is polygenic, thus, the use of numerous traits facilitates identification of superior

genotypes. The sorghum materials could be grouped into resistant, moderately resistant, moderately susceptible and susceptible. This study identified sorghum genotypes with resistance to *S. calamistis*. The 13 Sorghum genotypes resistant to *S. calamistis* identified can serve as donor parents in the breeding for stem borer resistance. Their use as potential donor parents is further buttressed by the fact that 12 out of the 13 resistant parents possess multiple resistance to the causative agents of stem borer: - *Chilo partellus*, *Busseola fusca* and *Sesamia calamistis* in sorghum. In addition, eight elite germplasm and two IAR varieties were identified to be moderately resistant. Cultivation of genotypes with resistance to stem borers would greatly improve food security and income of the resource poor farmers in areas prone to African pink borer.

Recommendations

The genotypes identified to have multiple resistance can be used in breeding for multiple resistance to the multiple agents of infection. Genotypes that showed combined resistance to the three borers and with good agronomic performance may be deployed to areas where these borers exist. However, breeding for resistance to these borers should continue besides deployment of these stem borer resistant varieties. The most susceptible genotype, SAMSORG 38, SAMSORG 40 and SAMSORG 44 could be utilized as a susceptible check in screening for resistance to *S. calamistis* and improved further to incorporate resistance. The 13 Sorghum genotypes resistant to *S. calamistis* identified can serve as potential donor parents in the breeding for stem borer resistance, also they can be used as resistant check in screening trials. The eight elite germplasm and two IAR varieties identified to be moderately resistant can be capitalized on as takeoff genotypes to build upon their resistance.

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HETEROTIC GROUPINGS OF MAIZE (*Zea mays* L.) INBRED LINES UNDER ARTIFICIAL *STRIGA* INFESTATIONS

S. O. Afolabi[1], A. Menkir[2], M. Oyekunle[1], U. S. Abdullahi , Melaku Gedil[2], Meseka Silvestro[2], Mengesha Wende[2]

[1]Institutes for Agricultural Research Ahmadu Bello University Zaria
[2]International Institute for Tropical Agricultural (IITA), PMB 5320, Ibadan 200001, Nigeria

ABSTRACT

Classification of available Striga resistant inbreds into distinct heterotic groups is crucial for the development of superior hybrids. This study seeks to access the performance of testcrosses, determine the mode of gene action controlling the inheritance of Striga resistant traits and classify the lines into heterotic groups. Ninety testcrosses derived from three inbred testers and 30 Striga resistant lines using line x tester mating scheme were evaluated under artificial Striga infestation along with two checks at Mokwa, Abuja, and Samaru in 2017 and 2018. Significant mean squares observed for most traits indicated the presence of genetic variability. There was predominance of additive gene action in controlling the inheritance of most traits. SCA of grain yield classified 16 inbreds into three groups, while GCA of multiple traits classified the 30 inbreds into three groups. Lines from opposing groups can be intermated to develop complementary populations to facilitate the development of outstanding Striga resistant hybrids and synthetics.

Keywords: *Maize, heterotic group, Striga*

Introduction

Maize (*Zea mays* L.) is an important cereal crop of the world. It has great worldwide significance as human food, animal feed and as a source of hundreds of industrial products (Kumar, 2012) and plays an important role in the diet of millions of African people (Fandohan *et al.*, 2005). It is a preferred staple food for over 900 million poor consumers, 120-140 million poor farm families and about one third of malnourished children (CIMMYT and IITA, 2010). However, several constraints including drought, parasitic and perennial weeds and low soil fertility have been reported as stress factors limiting maize yields in the savannas. *Striga hermonthica* (Del.) Benth is the most devastating biotic stress factor that limits maize production in the savannas. It severely affects an estimated 40 million hectares of land devoted to cereal production in West Africa causing yield losses ranging from 41 to 91% in susceptible maize genotypes (Kim, *et al.,* 1998; Lagoke *et al.,* 1991). The development and deployment of maize varieties with polygenic resistance to *S. hermonthica* has thus been considered a critical component of an integrated control strategy to minimize yield losses in farmers' fields as most of the maize cultivars currently grown by farmers may suffer close to 100% yield loss under severe Striga infestation (Kling et al., 2000; Menkir et al., 2007). Classification of the *Striga* resistant inbreds developed by maize breeders over the years into distinct heterotic groups is essential to the development of superior hybrids, synthetics, and breeding populations for *Striga* resistance which is currently lacking. The objectives of this study therefore were, to determine the combining ability of 30 tropical maize

inbreds, classify them into heterotic groups under Striga-infested environments.

Materials and Method

Thirty (30) *Striga* resistant maize inbred lines and three testers were crossed to generate 90 testcrosses in a line x tester mating scheme. The testcrosses were generated under irrigation during the 2015/16 dry season at the nursery field of IITA, Ibadan, Oyo State. The 90 testcrosses along with two checks (standard tolerant and susceptible checks) were evaluated under both artificial *Striga*-infested and *Striga*-free conditions at Mokwa, Abuja and Zaria during the growing seasons of 2017 and 2018. *Striga* seeds were collected, prepared and applied as described by Berner *et al.* (1996); Kim and Winslow (1991). The experiment was laid in 4 × 23 randomized incomplete block design with two replications. Each plot consisted of 4m row plots, one infested and one non-infested arranged adjacent to each other with a 1.5m alleyway. Row and hill spacing of 0.75 m and 0.25 m, respectively were used to give a maize population density of 53,333 plants ha^{-1}. Two to three maize seeds were placed in the same hole with the *Striga* seeds and later thinned to one maize plant per hill at two weeks after planting (WAP). Fertilizer application was delayed until about 30 days after planting, and 30 kg/ha each of N, P, and K were applied as 15-15-15 NPK, on the *Striga*-infested trials across the three locations. Additional 10 - 20 kg N ha^{-1} fertilizer was applied at 6 WAP. This delay in application and reduced rate of fertilizer was necessary to enhance good germination of *Striga* seeds and attachment of *Striga* plants to the roots of their host plants in *Striga*-infested plots (Kim, 1991). Weeds other than *Striga* were controlled by hand weeding in both infested and non-infested fields. Each location-year combination in the current study was regarded a test environment to conduct analyses of variance for all traits measured using PROC MIXED procedure in SAS (SAS, 2013). In these analyses, environments, replication (environments), block (replication × environments), were considered as random effects, whereas testcrosses were regarded as fixed effects. Further line x tester analysis was conducted to partition the testcross mean square into lines, testers, line x tester, environment x line, environment x tester and environment x line x tester effects using a SAS program following the procedure of Singh and Chaudhary (1977). The combining ability effects and grain yields of the testcrosses were used to classify the lines into different heterotic groups. To belong into a heterotic group, the line must have significant ($P < 0.05$) positive SCA effects with one of the testers and significant ($P < 0.05$) negative SCA effects with the other testers, along with a mean yield equal to or greater than 1 S.E above the grand mean of all testcrosses involving the positive SCA tester. Lines that have zero or non–significant SCA effects was not classified into either heterotic group (Agbaje *et al.*, 2007).

Results and Discussions

Results of the combined ANOVA for the two years combined across the three locations (Abuja, Mokwa and Samaru) under artificial *Striga* infestation as well as *Striga*-free environments showed that the mean squares for the testcrosses were highly significant ($P < 0.01$) for all traits measured under both research conditions (Data not shown). This indicated the presence of adequate genetic variability among the genotypes to allow for good progress from selection for improvement of grain yield and other *Striga*-resistant adaptive traits. Highly significant differences due to environment were also observed for all the traits measured under both artificial *Striga* infestation and *Striga*-free environments. This indicated differences in the climatic conditions across environment and that each environment was unique and highly variable, emphasizing the need for testing the hybrids in more than one environment over several years. Similarly, genotype × environment interaction (GEI) mean squares were highly significant ($P < 0.01$) for all traits under *Striga*-infestation except for days to anthesis and anthesis-silking interval. This indicated that the crosses behaved somewhat differently across environments for those traits and that the

Striga emergence and damage was different across the different environments. This results are also consistent with the findings of Badu-Apraku et al. (2011b), BaduApraku et al. 2011a; Badu-Apraku and Oyekunle 2012) The mean squares due to GCA of lines and GCA of testers for the testcrosses evaluated under artificial *Striga* infestation were significant ($P < 0.01$) for most of the traits measured, except for *Striga* rating at 10WAP, while the mean squares due to SCA of the testcrosses were also significant for most of the traits, including grain yield. However, the mean square due to environment was significant for all the traits, while the mean square due Environment x Tester interaction was only significant for grain yield, ears aspect, plant height, ears per plant, *Striga* rating at 10WAP, *Striga* count at 8WAP and *Striga* count at 10WAP (Table 1). These results indicated that both additive and non-additive gene actions were important in the inheritance of most of the traits of interest. The preponderance of GCA over SCA sum of squares for days to silk, days to anthesis, plant height, *Striga* rating at 8 and 10 WAP, *Striga* count at 8 and 10 WAP, ear aspect, number of ears per plant and grain yield under both research conditions implied that additive gene action was more important than non-additive gene action for these traits. The results support the findings of Yallou *et al.* (2009) and Badu-Apraku *et al.* (2011) who reported that additive gene action was more important than non-additive gene action for most traits evaluated under artificial *Striga* infestation.

Table 1: Analysis of variance for combining ability effects of different traits of the 90 testcrosses evaluated under artificial *Striga* infestation in Abuja, Mokwa and Samaru in 2017 and 2018.

Source of Variation	Df	Grain yield/ha (Kg ha^{-1})	Striga Rating (10WAP)	Striga Count (10WAP)
Evn	5	108048690.40**	53.80**	313120.02**
Rep(evn)	6	7514207.60**	7.26	1657.78**
Blk(evn*rep)	264	1329164.60**	1.13	978.71**
Line	29	14421619.80**	10.51	2415.23**
Tester	2	144002172.10**	271.83	85258.06**
Line x tester	145	1790442.50**	2.14	1299.23**
Env x line	10	21480020.70	45.67	15450.29**
Env x tester	58	1971877.10**	1.03	751.93
Evn x line x tester	290	1071728.20	0.93	778.98
Error	539	1121033.00	1.13	747.26

The three testers were only able to classify sixteen (16) of the thirty inbred lines into three heterotic groups based on the SCA of grain yield (data not shown). Four (4) inbred lines (L1, L2, L16 and L30) exhibiting negatives significant SCA effects with the resistant tester (T1) were assigned to group A, while seven (7) inbred lines (L8, L9, L13, L15, L17, L27 and L28) that exhibit significant negative SCA effects with the tolerant tester (T2) and positive significant effects with either of T1 and T3 were assigned to group B. Five (5) inbred lines (L4, L19, L20, L21 and L23) were assigned to group C, since they show significant negative SCA effects with the susceptible tester (T3) and positive significant SCA effects with either/both of the other two testers (T1 and T2). The remaining fourteen (14) inbred lines (L3, L5, L6, L7, L10, L11, L12, L14, L18, L22, L24, L25, L26 and L29) could not be grouped based on SCA of grain yield because they did not exhibit significant negative SCA effects with any of the three testers. Similar

groupings of the lines were observed under *Striga*-free condition. Several workers (Menkir et al. 2004; Melani and Carena 2005; Fan et al. 2009) have used SCA effects of grain yield for classifying maize inbreds into heterotic groups.

The results of the dendrogram constructed based on the general combining ability of multiple traits showed that the lines were clustered into three groups based on their genetic origin. Majority of the lines clustered in the first group are of the ACRSYN-W-S2-173-B*4/TZLComp1C4S1-37-5-BBB) origins; two of the lines L25 and L26 are of same origin (TZLComp.1C4S1-37) while the resistant and tolerant testers T1 and T2 both of which are from two backgrounds were also clustered in this group. Three of the lines in the second group are of IWD-SYN-STR-C3 origin, while the other two L11 and L13 are of 1393/ZDiploBC4 origin. The third group consist of 16 inbred lines most of which are of ZDiplo.BC4 origin. The susceptible tester (T3) and a susceptible line (1393) were both clustered within the same sub-group in this group. The clustering method tended to put most of the inbred lines with significant positive GCA for grain yield into Group 1 and the ones with significant negative GCA into Group 3, while the all the lines in Group 2 do no exhibit any significant GCA for grain yield. A similar trend was observed for the clustering of genotypes evaluated under *Striga*-free condition, but the lines were clustered into five groups. This corroborates the finding of Badu-Apraku *et. al.* (2013).

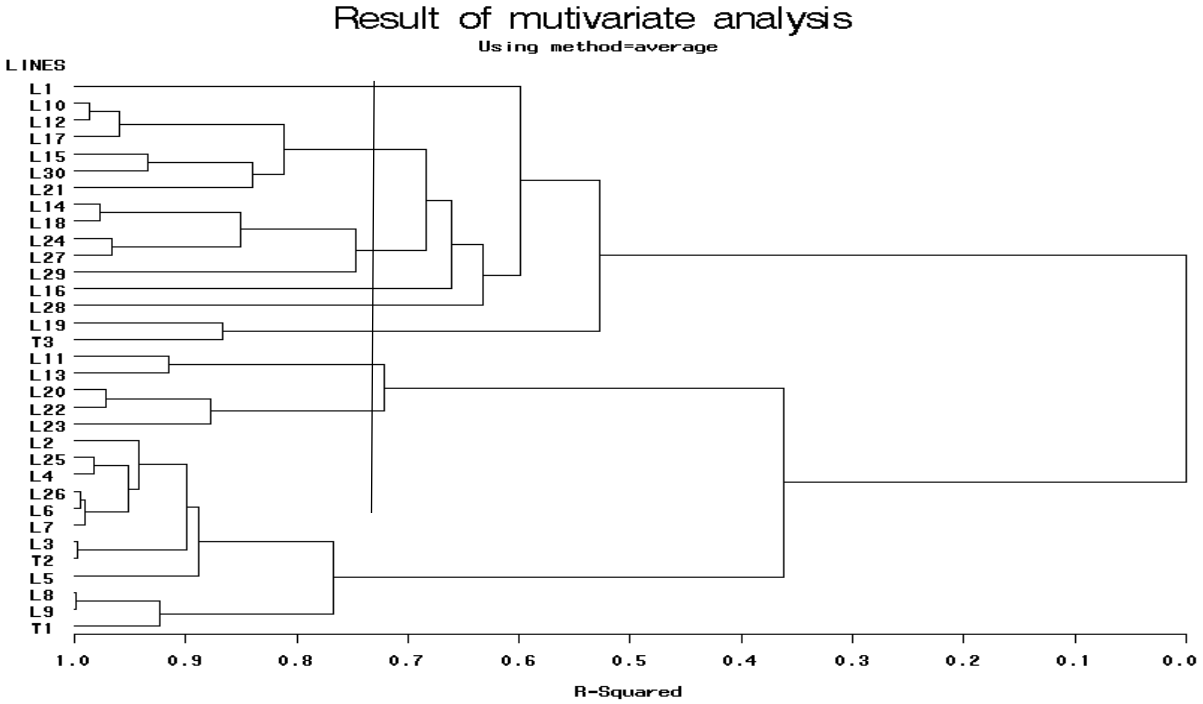

Fig 1: Clustering based on the general combining ability of multiple traits of the 30 Striga resistance inbred lines and 3 inbred testers evaluated under artificial Striga infestations and Striga-free conditions in Abuja, Mokwa and Samaru in 2017 and 2018

Conclusion and Recommendation

In conclusion, there was considerable genetic variability among the testcrosses under both research conditions with identification of L9 x T1, L2 x T2, L8 x T1, L4 x T1, L9 x T2, L20 x T1, L5 x T2 and L23 x T1 hybrids as the most outstanding hybrids. Additive and non-additive gene actions were significant with predominance of additive gene action in controlling the inheritance of the traits studied. Classification based on GCA effects of multiple traits gave a better, more predictable and usable heterotic grouping of the lines. Therefore, the genetic diversity detected and the high level of resistance fixed in the following maize inbred lines; L9, L8,

L6, L2, L4, L23, L24 and L19 can be exploited readily in breeding programs as parents of hybrids and synthetics and also as sources of favourable alleles to improve resistance to *Striga* in locally adapted germplasms.
Furthermore, L9 x T1, L2 x T2, L8 x T1, L4 x T1, L9 x T2, L20 x T1, L5 x T2 and L23 x T1 identified as the most ideal hybrids across test environments can be further tested extensively in multi-locations to confirm the consistency of their performance and promoted for adoption and commercialization for use in order to improve food security in the sub-region. And inbred lines from the opposing tester groups identified in this study under each research environment could be intermated separately to develop complementary populations to facilitate the development of outstanding hybrids and synthetic varieties.

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CORRELATION BETWEEN GRAIN YIELD AND PHYSIOLOGICAL TRAITS IN THE EVALUATION OF DROUGHT TOLERANCE OF MAIZE (*ZEA MAYS* L.) INBRED LINES

S. Zakariya*, M. Oyekunle*, A. M. Abubakar, I. E. Ahmed

Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
* Corresponding Author: zacqkz@gmail.com; +2348037151311

ABSTRACT

Climate change sets new challenges to major crop adaptation at stressful environments. Improvement for maize drought tolerance is a major objective for breeders and plant physiologists. For such a purpose, the measurement of physiological traits related to maize response to drought might prove to be useful indices. The objective of this study is to establish whether the physiological traits can be used as reliable physiological traits to evaluate the performance of maize genotypes under both drought and normally watered conditions. Fifty five single-cross hybrids generated from a diallel cross of eleven early and extra-early maize inbred lines plus one check were evaluated across Samaru and Kadawa. There was significant ($p<0.05$) correlation between grain yield and all other measured traits except root lodge, stuck lodge and husk cover under both drought and optimum watered condition and row per cob under optimum condition. This shows that there was a very good association between the physiological characteristics studied and grain yield under both drought and optimum watered condition. It also shows that the physiological characteristics can facilitate the selection of stress-adaptive genotypes under both research condition conditions and may permit modern maize to be grown at a wider range of environments

Keywords: *Correlation, Drought tolerance, Inbreds, Leaf senescence, Induced drought, Single-cross.*

Introduction

Maize (Zea mays L.) is one of the most important cereals in the world, but global climate change adversely effects maize production in various ways (Erdal *et al.,* 2015). Among abiotic stresses, drought has significant influence on the various stage of maize life cycle such as seedling, vegetative and reproductive growth. Drought is the single most important production constraint affecting maize production and productivity in the sub region. Edmeades *et al.* (1995) reported an estimated 15% annual maize yield loss from drought in the West African savannas and indicated that localized losses may be much higher in the marginal areas where the annual rainfall is below 500 mm and soils are sandy or shallow.

Maize grain yield is determined by many factors such as plant height (PH), leaf senescence (LS), plant aspect (PA), ear aspect (EA), anthesis-silking interval (ASI), and ears per plant (EPP) (Fuad-Hassan *et al.,* 2008). When water

shortage happens before flowering, it does have extreme harmful influence on anthesis, thus silk emergence from husks is delayed, and causes an increased ASI (Bassetti and Westgate, 1993b; Edmeades *et al.,* 2000). In many studies, the sensitivity of

silk growth toward water shortage has been defined as the growth rate of silk emergence (Herrero and Johnson, 1981; Westgate and Boyer, 1985; Bassetti and Westgate, 1993b). Increased ASI ultimately leads to low grain yield (Edmeades *et al.,* 1992). For this reason, ASI plays important roles as drought tolerance index (TI). Drought tolerance has been adapted for diminishing ASI in maize (Edmeades *et al.,* 1993; Bolaños and Edmeades, 1996; Bruce *et al.,* 2002). A short ASI is correlated with quantitative trait loci (QTLs) of increased grain yield (Ribaut *et al.,* 1997). Information on the interrelationship among grain yield and yield related traits is desirable for designing appropriate breeding strategies most especially under stress conditions. The heritability of grain yield under stress conditions is usually low (Badu-Apraku *et al.,* 2012). Maize breeders in IITA utilize a selection index that integrates increased grain yield under drought and well-watered environments with a short anthesis-silking interval, increased ears per plant, good stay green characteristic, and good scores for plant aspect and ear aspect under drought for improvement of maize germplasm for tolerance to drought (Menkir and Akintunde, 2001; Badu-Apraku *et al.,* 2004, Oyekunle *et al.,* 2015). Badu-Apraku *et al.* (2011) reported that the most reliable traits for selection for improved grain yield under drought in the early maturing germplasm were ear aspect, ears per plant, anthesis-silking interval, and plant aspect. The objectives of the present study were to (i) evaluate for drought tolerance and identify the drought tolerant genotypes using yield related traits under drought and (ii) asses relationship between grain yield and yield-related traits under drought and well-watered conditions.

Experimental Site

The research was carried out at the Institute for Agricultural Research (IAR), Samaru

Research farm at Zaria. The Institute for Agricultural Research (IAR) Research farm, Zaria is located on $11^0$11'N, $07^0$38'E with altitude of 686 m above sea level, in the northern Guinea savanna agro-ecological zone of Nigeria, and the soil type is loamy with annual rainfall of about 1045 mm.

Genetic Materials and experimental procedures

Twenty one early and extra-early maize inbred lines with different kernel modification sourced from IITA were used for this experiment. The 21 inbred lines were evaluated under optimum growing conditions at Samaru during the 2015 rainy season and managed drought stressed in the same location during 2015/2016 dry season. A randomized complete block design with three replications was used for the evaluation. Row length was 4 m long with 22 plants per row. Row and hill spacing were 0.75 m and 0.4 m respectively. Three seed were planted per hill and seedlings were thinned to two per stand about two weeks after emergence, giving a population density of 66,666 plants per hectare. NPK 15:15:15 was applied at the rate of 60 kg N ha^{-1}, 60 kg P ha^{-1} and 60 kg K ha^{-1} two weeks after planting. An additional 60 kg N ha^{-1} urea was top-dressed two weeks later in the drought experiment and 4 weeks later in the well-watered experiment. Irrigation was supplied twice every weak using furrow irrigation system. The managed drought stress was achieved by supplying irrigation water twice a week up to 35 days after planting. Thereafter, the irrigation water was withdrawn in the drought experiment, so that the maize plants relied on stored water in the soil for growth and

development. On the other hand, the experiment under optimum growing conditions, continue to receive irrigation until physiological maturity. Except for the water treatment, all management practices were the same for both the optimum and drought experiments. The experiments were kept

weed-free by the application of gramaxone, a contact herbicide just after planting. Subsequently, manual weeding was done at two and four weeks after planting to keep the trials field weed-free.

Data Collection

Days to anthesis and silking were recorded for each plot as the number of days from planting to when 50% of the plants in a row had shed pollen and had emerged silks, respectively. Anthesis-silking interval (ASI) was computed as the interval in days between days to silking and anthesis. Plant and ear heights were calculated as the average of measurements on 10 competitive plants (excluding plants at the edges) per plot and were measured after anthesis as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant and ear aspects were rated on a scale of 1 to 5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Stay green characteristic was taken as leaf death score (LDS) and was recorded on a scale of 1 to 10, where 1 = 10% dead leaf area; 2 = 20% dead leaf area; 3 = 30%dead leaf area; 4 = 40% dead leaf area; 5 = 50% dead leaf area; 6 = 60%dead leaf area; 7 = 70% dead leaf area; 8 = 80% dead leaf area; 9 = 90% dead leaf area; 10 = 100% dead leaf area, for the drought stressed plots at 63 and 70 days after planting (DAP) respectively. The number of ears per plant (EPP) was computed as the total number of ears at harvest divided by the number of plants at harvest. Grain yield, adjusted to 150 g kg^{-1} moisture, was computed from the shelled grain weight. On the other hand, under well-watered environments, harvested ears from each plot were weighed and representative samples of ears were shelled to determine percent grain moisture. Grain yield adjusted to 150 g kg^{-1} moisture, was computed from ear weight and

grain moisture assuming a shelling percentage of 80% (800 g grain kg^{-1} ear weight).

Statistical Analysis

The data collected were subject to analysis of variance (ANOVA) using individual plot means. Analysis was computed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS) software, version 9.2 (SAS, Institute 2004).

Results

Traits for drought tolerance

Table 1 represents the results of the mean performance of the genotypes for Anthesis Silking interval, Plant and ear aspects, number of ears per plant and Leaf Senescence I and II under drought stress and well-watered conditions.

Anther-silking interval (ASI)

Under drought condition, the mean values for ASI are significantly increased as compared to the well-watered condition. ASI of TZEI108 x TZEI65 and TZEI87 x TZEEI21 is 4.929/1.036 and 5.643/1.5 days under drought and well-watered conditions, respectively. ASI for TZEEI29 x TZEI87, TZEQI4 x TZEEI21, TZEEI29 x TZEEI21, TZEI86 x TZEEI6 and TZEI59 x TZEEI21 are recorded to be more than 6 days under DS conditions. ASI is a major secondary trait for the drought tolerance selection in maize (Bolaños and Edmeades 1996; Ribaut *et al.,* 1997; Bänzinger *et al.,* 2000; Ziyomo and Bernardo, 2012). Previous study reported silk senescence occurred following a few days after first silk appearance, and the flower function loss happened 4 to 10 days after the first silks emergence according to genotypes (Bassetti

and Westgate 1993). Rowland (1993) reported a few days of retardation of anthesis in days among genotypes under drought stress. According to Rowland (1993), drought stress at silking stage interrupted silk

emergences from the cob husk, caused silks dryness, and inhibited pollen tube growth. Drought stress at vegetative, flowering, and reproductive stage led to reduction in grain yield (Bawa *et al.,* 2015).

Ears per plant (EPP)

Regarding EPP, the worst ten performing hybrids recorded less than 0.5 number of ears per plant, on the other hand, the best ten performing hybrids shows more than 0.5 increases in the number of ears per plant under drought stress condition. This result relates EPP to grain yield under drought stress. This result corroborates the findings of Kim *et al* (2016). Grain yield is ultimate goal for drought tolerance. EPP is an important factor conferring drought tolerance.

Leaf senescence (LS)

We observed leaf senescence under the Drought Stress conditions. Leaf senescence was elevated in all genotypes but genotypes with better grain yield have better score for leaf senescence as compared to those with poor grain yield under drought stress condition. This result is coinciding with the report of Quarrie and Jone (1977). Inhibition of leaf development resulted in the reduction of cell expansion and cell division under drought stress. In addition, drought stress caused reduction in the leaf area, radiation use efficiency, and harvest index by overproducing ROS and accelerated leaf senescence (Nogués and Baker, 2000; Earl and Davis, 2003). Leaf senescence was inversely correlated to grain yield. Grain filling

needs nitrogen uptake, however higher leaf senescence limits nitrogen supply (Masclaux-Daubresse *et al.,* 2010)

Correlation among grain yield and other traits under drought and well-watered conditions

Significant positive phenotypic correlations were observed between grain yield and plant height (r_p = 0.09), ear height (r_p = 0.01), EPP (r_p = 0.64), cob length (r_p = 0.82), cob diameter (r_p = 0.78), number of rows per cob (r_p = 0.57), number of kernels per row (r_p = 0.88), 1000 kernel weight (r_p = 0.27) while significant negative phenotypic correlations were observed between grain yield and days to silking (r_p = -0.25), ASI (r_p = -0.52), ear aspect (r_p = - 0.83), plant aspect (r_p = -0.66), leaf senescence I (r_p = -0.38), and leaf senescence II (r_p = -0.28) under drought (Table 2). Apart from root lodge and husk cover, EPP was significantly correlated with all other measured traits under drought. Under well-watered conditions, positive and significant correlations were detected between grain yield and plant height (r_p = 0.04), ear height (r_p = 0.04), EPP (r_p = 0.45), cob length (r_p = 0.39), cob diameter (r_p = 0.34) kernel per row (r_p = 0.53), and 1000 kernel weight (r_p = 0.14) while significant negative correlations were observed between grain yield and days to anthesis (rp = -0.03), days to silking (rp = -0.09), ASI (rp = -0.16), ear aspect (rp = -0.45) and plant aspect (rp = -0.19) (Table 2)

Table 1; Mean grain yield and other agronomic traits of top 10 and worst 10 single-cross hybrids evaluated under induced drought stress at Samaru and Kadawa in /20152016 dry season.

GENOTY PES	**Yield**		**ASI**		**Plant Aspect**		**Ear Aspect**		**EPP**		**LS 1**	**LS 2**
	DS	**W W**	**DS**	**W W**	**DS**	**W W**	**DS**	**W W**	**DS**	**W W**		
TZEE-W-Pop STR C5 x TZEI87	2.5 24	4.6 79	4.0 36	1.5 71	2.8 57	1.8 57	2.8 57	1.7 5	0.8 43	1.1 6	1.3 57	3.4 64
TZEI108 x TZEI65	2.4 94	4.5 14	4.9 29	1.0 36	2.6 43	2.1 79	2.2 86	1.7 68	0.8 53	1.0 18	0.7 86	2.4 29
TZEEI29 x TZEQI24	2.4 58	4.4 38	3.4 84	1.2 5	2.8 87	2.1 43	2.6 41	2.2 68	0.6 97	1.1 11	1.2 98	2.9 13
SAMAZ 42	2.3 43	4.4 19	4.4 52	1.2 1	2.9 82	2.0 24	2.4 46	1.5 12	0.7 95	1.1 16	1.2 66	3.1 71
TZEI87 x TZEI65	2.3 33	4.3 53	3.9 52	4.2 3	3.0 97	2.0 02	2.8 12	1.8 39	0.7 81	1.0 34	1.4 92	2.8 61
TZEI86 x TZEI65	2.0 93	4.2 61	3.1 31	2.5 08	2.8 57	2.0 42	2.6 81	2.2 94	0.6 53	1.0 33	0.9 6	2.6 87
TZEI108 x TZEEI29	1.9 79	4.2 55	4.5 99	2.4 92	2.8 27	2.0 46	2.4 86	1.5 65	0.7 48	1.0 36	1.1 79	2.6 94
TZEI108 x TZEI87	1.9 63	4.2 18	4.3 21	2.1 87	3.1 03	2.3 65	2.8 19	0.8 99	0.9 68	0.9 85	1.4 33	3.4 44
TZEQI24 x TZEEI21	1.8 46	4.1 55	4.6 87	2.2 3	3.2 24	1.8 08	2.9 19	1.4 76	0.6 7	1.0 86	0.8 21	3.0 04
TZEQI24 x TZEEI6	1.8 26	4.1 54	4.6 11	2.0 63	3.1 31	2.0 83	3.0 04	1.8 49	0.7 11	1.0 68	1.2 7	3.0 95
TZEI59 x TZEQI24	0.6 2	2.7 53	4.8 41	1.8 17	3.4 27	2.5 22	3.6 07	2.8 89	0.3 34	1.0 09	0.9 01	3.8 97
TZEE-W-Pop STR C5 x TZEQI24	0.5 96	2.7 32	5.6 9	1.3 37	4.2 74	2.4 15	4.2 78	2.3 41	0.3 81	1	2.1 51	4.2 86
TZEEI29 x TZEI87	0.5 53	2.6 7	6.0 79	3.7 86	3.8 47	2.4 29	4.1 69	2.5 18	0.4 8	0.8 6	2.3 77	4.3 49

TZEI87	x	0.5	2.5	5.6	1.5	3.9	2.4	4.3	2.4	0.4	0.8	2.3	4.6
TZEEI21		24	89	43		46	11	04	29	76	94	21	43
TZEI87	x	0.5	2.5	5.8	2.2	4.3	2.3	4.3	2.5	0.4	0.9	2.7	5.2
TZEQI4		24	39	57	14	04	75	04	71	61	98	86	14
TZEI86	x	0.5	2.5	4.9	2.3	3.9	2.2	4.2	2.2	0.3	0.9	1.8	4.3
TZEEI29		16	09	64	06	11	28	14	88	64	4	57	21
TZEQI4	x	0.4	2.3	6.6	2.6	4.2	2.4	4.3	2.3	0.2	0.8	2.1	4.6
TZEEI21		67	34	67	83	48	4	67	45	78	36	31	63
TZEEI29	x	0.3	2.3	7.0	3.2	4.1	1.9	4.3	2.9	0.3	0.9	2.0	5.1
TZEEI21		62	08	36	18	96	76	39	44	56	36	71	43
TZEI86	x	0.3	2.1	6.7	3.4	4.2	2.4	4.6	2.4	0.4	0.7	1.9	4.3
TZEEI6		5	87	94	44	44	5	39	27	45	7	29	06
TZEI59	x	0.1	2.1	6.4	2.6	4.3	2.3	4.4	3.3	0.1	0.9	2.2	4.9
TZEEI21		83	61	25	47	95	95	8	12	99	27	98	56

Table 2; Correlation among grain yield and other measured traits of 55 single-cross hybrids evaluated under induced drought stress (above diagonal) and under optimum growing conditions (below diagonal) in 2016 dry season.

	Yield	**Days to anthesis**	**Days to silking**	**ASI**	**plant Height**	**Ear Height**	**Root Lodge**	**Stock Lodge**	**Husk cover**	**EPP**	**Ear Aspect**	**Plant Aspect**	**Cob Length**	**Cob Diameter**	**Row / Cob**	**Kernels/ Row**	**1000 KW**	**LS I**	**LS II**
Yield		0.11*	-0.25**	-0.52**	*0.09	*0.01	0.04	-0.01	-0.06	0.64**	-0.83**	-0.66**	0.82**	0.78**	0.57**	0.88**	0.27**	-0.38**	-0.52**
Days to anthesis	-0.03*		0.75**	-0.13	-0.05	0.08	-0.09	-0.02	0.08	0.09	-0.13	-0.12	0.13*	0.11	0.04	0.15*	0.04	-0.17**	-0.20**
Days to silking	-0.09*	0.92**		0.56**	-0.20**	0.02	0.02	0.08	0.07	-0.16*	0.19**	0.31**	-0.22**	-0.26**	-0.22**	-0.23**	-0.12	0.14*	0.21**
ASI	-0.16*	0.29**	0.64**		-0.24**	-0.07	0.14*	0.14*	0.003	-0.35**	0.45**	0.63**	-0.50**	-0.53**	-0.37**	-0.53**	-0.24**	0.43**	0.57**
Plant Height	*0.04	0.40**	0.44**	0.27**		0.56**	-0.13*	-0.22**	-0.16*	0.10	-0.07	-0.27**	0.14*	0.12	0.09	0.14*	0.20**	-0.20**	-0.25**
Ear Height	*0.04	0.46**	0.47**	0.25**	0.83**		0.06	-0.05	-0.07	0.05	-0.02	-0.03	0.07	-0.01	0.06	0.06	0.08	-0.05	-0.02

Root Lodge	0.05	0.28**	0.22**	-0.02	0.08	0.09		0.15*	-0.04	0.02	0.002	0.18**	0.03	-0.10	-0.003	-0.002	-0.06	0.15*	0.14*
Stalk Lodge	-0.02	0.02	7E-04	-0.03	-0.06	0.04	0.03		0.16*	0.04	0.07	0.26**	-0.07	-0.11	0.03	-0.07	-0.09	0.13*	0.18**
Husk Cover	-0.04	0.05	0.06	0.04	-0.01	-0.08	0.13*	0.11		-0.05	0.01	0.05	-0.04	-0.12	0.02	-0.07	-0.06	-0.02	0.04
EPP	0.45**	-0.08*	-0.1*	-0.08*	0.02*	-0.07*	4E-04	-0.16*	-4E-04		-0.61**	-0.43**	0.65**	0.53**	0.52**	0.65**	0.23**	-0.27**	-0.34**
Ear Aspect	-0.45**	0.15*	0.18**	0.13	0.11	0.08	0.15*	-0.03	0.12	-0.13		0.62**	-0.79**	-0.70**	-0.56**	-0.80**	-0.29**	0.38**	0.49**
Plant Aspect	-0.19**	0.05	0.02	-0.04	0.04	0.12	0.35**	0.29**	0.13	-0.20**	0.31**		-0.63**	-0.62**	-0.45**	-0.64**	-0.21**	0.52**	0.71**
Cob Length	0.39**	0.05	0.04	-3E-04	0.05	0.05	0.13	0.13	-0.01	0.24**	-0.14*	0.16*		0.73**	0.65**	0.83**	0.28**	-0.41**	-0.48**
Cob Diameter	0.34**	0.05	0.02	-0.06	0.11	0.07	0.04	0.01	-0.06	0.13*	-0.14*	-0.07	0.08		0.56**	0.76**	0.35**	-0.39**	-0.54**
Row/Cob	0.02	0.04	0.02	-0.02	0.03	0.14*	0.02	0.07	-0.11	-0.05	-0.05	0.04	-0.07	0.34**		0.58**	0.28**	-0.32**	-0.42**
Kernels/Row	0.53**	-0.09	-0.13	-0.14*	-0.05	-0.11	0.03	0.03	0.02	0.25**	-0.28**	-0.04	0.61**	0.08	-0.18**		0.29**	-0.37**	-0.54**

1000 KW	0.14*	0.20**	0.15*	-0.02	0.08	0.04	0.22**	0.08	0.08	0.11	0.04	0.07	0.05	0.48**	-0.10	0.01	-0.14*	-0.23**

*, **, significant at 0.05 and 0.01 levels of probability

ASI: anthesis silking interval; EPP: ears per plant; LS 1: leaf senescence I; LS 2: leaf senescence II; 1000 KW: 1000- kernels weight

Discussion

The presence of significant positive phenotypic correlations between grain yield and plant height, ear height, EPP, cob length, cob diameter, kernel per row and 1000 kernel weight indicated that improvement in these traits would contribute to significant progress in grain yield under drought and well-watered conditions. Similarly, the existence of negative correlations between grain yield and days to silking, ASI, ear aspect, plant aspect, and leaf senescence under drought and well-watered conditions indicated that these traits might have direct or indirect effects on grain yield under the two research conditions. These results justified the inclusion of most of these traits in the base index for selection of genotypes for tolerance to drought. This result is in agreement with the findings of earlier workers (Badu-Apraku et al., 2011; Badu-Apraku and Oyekunle, 2012; Oyekunle et al., 2015).

Since the results of the present study revealed that plant and ear aspects, ear height, and number of kernel per row were the most reliable traits under both drought and well-watered conditions, improvement of grain yield under well-watered conditions would indirectly result in improved grain yield in drought environments.

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GENETIC VARIABILITY STUDIES FOR GROWTH AND YIELD ASSOCIATED TRAITS OF SOME FINGER MILLETS (*ELEUCINE CORACANA* [L.] GAERTN) LANDRACES IN NIGERIA

***SALISU, A.,[1] ADAMU, A.K.,[2] ADELANWA, M.A.[2] USMAN, A.[3] AND NURA, S.[4]**

1. Rabiu Musa Kwankwaso College of Advanced and Remedial Studies, Tudun Wada Kano.
2. Department of Botany, Ahmadu Bello University, Zaria.
3. Department of Plant Science, Ahmadu Bello University, Zaria.
4. Department of Biology, Ahmadu Bello University, Zaria.

*Corresponding Author: salisuabdulqadirbc@gmail.com or salisunura40@yahoo.com

ABSTRACT

A research was conducted to determine the extent of variability for growth and yield related traits in five local landraces of finger millets (Eleusine coracana) in Nigeria with the aim of selection for yield associated traits. Seeds of five (5) finger millet genotypes: PLT 1, PLT 2, PLT 3, SMK and KN01 were obtained from Plateau, Kaduna and Kano states, Nigeria. The seeds were sown in a plot in a Randomized Completely Block Design (RCBD) with three replications to raise the first generation which after harvest was advanced to second generation. The data obtained was subjected to Analysis of Variance with Duncan's New Multiple Range Test used to separate means that were significant. The genetic parameters estimates were determined at second generation. The results obtained revealed highly significant difference (P≤0.01) were found in the number of seeds per spikelet and 1000 seed weight. Significant difference (P≤0.05) was found for height at maturity, High variability values were found in the yield associated traits of among the 5 finger millets genotypes. The high variability values recorded among the genotypes suggest that selection will be effective in breeding programs of finger millets. Thus, genotype PLT 1 is recommended for selection and other improvement programs in the crop due to high number of seeds produced..

Keywords: *Genotypes, Heritability, Millet.*

Introduction

Finger Millet (*Eleusine coracana*) is one of the members of millets group (Ramashia *et al.*, 2021) known popularly as "Tamba" in Nigeria (Umar and Kwon-Ndung, 2014). It is a cereal grain that belongs to the family Poaceae (Sood *et al.*, 2016) and is a gluten-free grain (Gebre, 2019). It is ranked 4th among other millets in the world in importance after sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica*). It is cultivated in some parts of African countries such as Ethiopia, Zimbabwe, Nigeria and South Asian country India (Opole, 2019). It is an allopolyploid with basic somatic chromosome number of 2n= 36 from 2 diploid genome *Eleusine indica* and *Eleusine floccifolia*. Finger Millet has several medicinal and nutraceutical values. Regular consumption of finger millet can prevent the risk of cardiovascular diseases, type II diabetes, gastrointestinal cancers and range of other disorders (Mckeown, 2002). It is also an internal remedy for leprosy and liver diseases (Van Wyk and Gericke, 2000). It has potential health benefits in all age groups and people with chronic diseases (Ramashia *et al.*, 2019). The grains contain zinc (Zn), amino acids, and vitamin B complex. The grain contains the highest amount of carbohydrates, protein, fat, and crude (Saleh *et al.*, 2013). It also contains antioxidant properties such as polyphenols that protect the body against degenerative diseases (Udeh et al., 2017). Finger millet is

utilized to produce weaning foods, traditional beer, and extruded products (Shobana et al., 2013).

However, despite the importance of the crop, its cultivation in Nigeria is beyond subsistence farming due to poor yields (Lawrence *et al.*, 2014) and neglect by the scientific communities and the authorities concern and it is often termed an Orphan crop (Kumari and Pande, 2010). Farmers who grow the crop solely depend on local landraces which are low yielding (Fetene *et al.*, 2011). There is paucity of information on finger millet genetic variability in Nigeria compared to other cereal crops such as wheat, maize, barley, rice and sorghum (Gopal *et al.*, 2009). Study of genetic diversity and population structure between genotypes has long been a major goal for crop development (Egbadzor *et al.* 2014). The identification of genetic variability in finger millet will provide a basis for genetic improvement of the crop for quantitative and qualitative traits. This study therefore aimed at studying the genetic variability in finger millet for improved growth and yield associated traits.

Materials and Methods

Study Site

The field experiments were conducted in the Botanical Garden of the Department of Botany, Ahmadu Bello University Zaria, Nigeria (Lat 11° 11′ N, Long 07° 38′ E, and Altitude 686m above sea level), for 2 years (during 2018 and 2019 rainy seasons).

Sources of Seeds

The seeds of five finger millets genotypes were obtained from PLT 1, PLT 2, PLT 3, SMK and KN01 were obtained from Plateau, Kaduna and Kano states, Nigeria.

Experimental Design

The Five finger millet genotypes were sown to raise the first generation in one row plot of 1 metre per entry/genotype, with spacing of 25cm between the rows and 15cm between the plants. The genotypes were laid in a randomized completely block design (RCBD) with three replications. The seedlings were thinned three weeks after sowing and weeded thrice at third, sixth and ninth weeks after sowing, a basal dose of 50kg N/ha, 25 kg P/ha and 25 kg K/ha NPK 20: 10: 10 was applied. The first generation was harvested and advanced to second generation. Data were taken on Height at maturity, leaves number and size, finger length, number of fingers. number of seeds/spikelets and 1000 seeds weight. The data obtained were analyzed using Analysis of Variance (ANOVA) with Duncan's New Multiple Range Test (DNMRT) used to separate the means. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated based on the formula described by Syukur *et al.* (2012) as follows:

$$\sigma_g^2 = [\underline{MSG - MSE}]$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 / r^{r}]$$

$$PCV\ (\%) = \sqrt{\frac{(\sigma_p^2)}{x}} \text{ x } 100$$

$$GCV\ (\%) = \sqrt{\frac{(\sigma_g^2)}{x}} \text{ x } 100$$

Where:

σ_g^2 = genotypic variance,

σ_p^2 = phenotypic variance,

MSG = mean square of genotypes,

MSE = error mean square,

r = number of replications,

x = grand mean of a character.

The Broad sense Heritability (H^2) and Genetic Advance (GA) were estimated according to the formula described by Singh and Choudhury (1985) as follows:

$$H^2\ (\%) = \frac{\sigma_g^2}{\sigma_p^2} \text{ x } 100$$

Results

The mean performance of 5 finger millet genotypes is presented in Tables 1. The result showed that, PLT 2 is the tallest (70.00 cm) and produced 11 leaves and 6 fingers. PLT 1 has the largest leaves (172.10 cm^2) and

number of seeds/spikelets. PLT3 has the longest finger (7.26 cm) while KNO 1 has the highest value in terms of seeds weight.

The results for the Analysis of variance of the 22 different finger millet genotypes in the second generation are presented in Table 2. The results revealed significant difference ($P \leq 0.01$) for number of seeds per spikelet and 1000 seed weight. Significant difference ($P \leq 0.05$) was found for height at maturity.

Table 1: Mean Performance of the 5 finger millet genotypes in Second Generation

Genotypes	Height (cm)	No. of Leaves	Leaf Area (cm^2)	Finger Length (cm)	No. of Fingers	No. of Seeds / Spikelet	1000 Seeds Weight (g)
PLT I	60.33^{c}	10.66^{a}	172.10^{a}	5.64^{b}	5.33^{a}	7.33^{a}	2.43^{a}
PLT 2	70.00^{a}	11.00^{a}	170.26^{a}	6.83^{a}	6.00^{a}	6.99^{b}	2.47^{a}
PLT 3	69.66^{a}	11.00^{a}	168.80^{a}	7.26^{a}	6.00^{a}	6.99^{b}	2.80^{a}
SMK	66.33^{b}	10.33^{a}	170.26^{a}	6.26^{a}	5.33^{a}	6.99^{b}	2.80^{a}
KN O1	68.00^{a}	10.33^{a}	170.03^{a}	6.93^{a}	6.00^{a}	7.00^{b}	2.83^{a}
Mean							
S.E (±)							

N.B: Means with the same superscript(s) down a column are not significantly different at P=0.05

Table 2: Degree of Freedom and Mean Squares for morphological traits of the 22 finger millet genotypes in 2019

Source of variation	Df	Height at Maturity (cm)	No. of Leaves	Leaf Area (cm^2)	Finger Length (cm)	No. of Fingers	No. of Seeds/ Spikelet	1000 Seeds Weight (g)
Replication	2	150.14	0.02	75.85	0.84	0.28	0.07	0.01
Genotype	4	25.63*	0.19	14.68	0.18	0.20	0.31**	0.19**

Error	8	6.07	0.18	6.68	0.07	0.07	0.05	0.01

Key: *= Significantly Different (P≤0.05) **= Highly significantly Different (P≤0.01)

The result for the genetic parameters estimates in 5 finger millets genotypes grown in 2019 is presented in Table 3. The result showed slight difference between PCV and GCV values for all the studied traits. High PCV values of 21.82% in 1000 seeds weight and 20.44% in number of seeds per spikelet were found. Moderate PCV values were obtained for height at maturity (14.68%), finger length (13.32%) and number of fingers (11.45%). Low PCV values were obtained for the remaining traits. Similar result was found in the GCV values except for number of fingers in which low GCV value is found. High heritability (>60%) values were found for all the studied traits except in leaf area where moderate heritability value (59.91%) is obtained. The Genetic Advance values for all the selected traits of 22 finger millets were high (>60%). Similar results were obtained for the GAM values.

Table 3: Estimates of genetic parameters in 5 finger millet genotypes

Characters	PCV %	GCV %	H_b %
Height at maturity	14.68	12.96	77.91
No. of leaves	8.56	7.31	73.07
Leaf area	4.38	3.39	59.91
Finger length	13.32	11.95	81.44
No. of fingers	11.45	9.80	73.33
No. of seed/spikelet	20.44	20.00	95.04
1000 seed weight	21.82	21.17	94.18
No of days to maturity	9.18	8.34	82.62

N.B: PCV and GCV: 0 – 10 % = Low, 10 – 20% = Moderate and >20% = High H_b: 0 – 30 % = Low, 30 – 60 % = Moderate and >60 % = High. GA: 0 – 10 % = Low, 10 – 20 % = Moderate and >20 % = High.

Discussion

The presence of majority of the phenotypic classes for each trait in the genotypes reported by the present study implied that diversified germplasm is available for improvement as reported by Lule *et al.* (2012). The highly significant variations observed in the morphological traits of the 5 finger millet genotypes indicated the presence of adequate variability that exists in the gerrmplasm; which subsequently reflects the heterogeneity of the collected finger millet genotypes. This finding is in agreement with the findings of Upadhyaya *et al.* (2007) who reported similar finding among finger millets genotypes in India. Similar report was found in the work of Bhattacharjee *et al.* (2007) and Khairwa *et al.* (2007) among pearl millets genotypes.

Evaluation of the phenotypic characters for the different genotypes showed that the phenotype could have genetic diversity for all the studied traits except number of leaves and leaf area implying that genetic variability exists in the finger millet genotypes studied. This finding agrees with that of Shahryani *et al.* (2011) and Garavandi and Kabrizi (2010) who individually reported genetic diversity for plant height, 1000 seed weight, spikelet in bread wheat genotypes and similar crops. More so, previous findings by Upadhyaya *et al.* (2007) in pearl millet germplasm, Similar result was found in the work of Abubakar *et al.* (2019) who reported wide range of variability among selected traits of pearl millets (*Pennisetum glaucum*) landraces obtained from Northern Nigeria.

The slight difference between the PCV and GCV values indicates little influence of the environment in the expression of the traits. This confirms the presence of high genetic variability that exists in the finger millet genotypes for morphological parameters. Singh *et al.* (2018) also obtained similar results. Similarly, the presence of high heritability values reported by this study

agrees with the findings of Shinde *et al.* (2014) who reported high heritability for ear-head width, number of ear heads/plant and plant height in finger millet genotypes. Heritability estimate is reliable in pin pointing characters enforcing selection, since heritability is influenced by environment. The high heritability obtained for all the studied traits indicate the importance and reliability of these characters for selection. High broad sense heritability have been attributed to the effects of additive and non – additive gene actions (Singh *et al.,* 2018). Selection based on these traits could assist in successful isolation of desirable genotypes (Shanmugapackiam and Raguchander, 2018), especially where the additive component of GCV is high.

Conclusion

There is genetic variability among the phenotypic traits of the finger millet genotypes in terms of height at maturity, finger lengths and 1000 seeds weight. The genetic parameters among the 5 finger millets genotypes showed that PCV values were slightly higher than the GCV values in all the trials which signifies less influence of the environment to the phenotypic expression. Genotype is PLT 1 recommended for breeding programs in Nigeria due to high number of seeds produced.

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CGBPB 046

BIO-PRESERVATIVE EFFICACY OF *ALOE VERA* GEL ON POSTHARVEST QUALITY OF TOMATO FRUITS (*LYCOPERSICON LYCOPERSICUM* MILL)

Tinuoye O.O.,[1] Negbenebor H.E.,[1] Muhammed S.G.[2] and *Nura S.[2]

Department of Biological Science, Baze University, Abuja, Nigeria.
Department of Biology, Ahmadu Bello University, Zaria.
*Corresponding Author: salisunura40@yahoo.com

ABSTRACT

A study was carried out to determine the bio-preservative efficacy of Aloe vera gel coating on the postharvest quality of tomato fruits (Lycopersicon lycopersicum). Two varieties of tomato: ROMA and a local variety were collected from Ahmadu Bello University farm in Zaria, washed thoroughly with distilled water and treated with Aloe vera gel coatings at three (3) different concentrations: 10%, 50%, 100% and 0% as control. The treated tomato fruits were left in storage at room temperature for a period of sixteen days arranged in a completely randomized design with three replications during which physical properties relating to tomato qualities were recorded appropriately. Data obtained were analysed using Analysis of Variance with Duncan's New Multiple Range Test used to separate means that were significant at 5% level. The result obtained revealed significant difference (P≤0.05) in the effects of Aloe vera concentrations on the tomato fruit weight and decay rate. The effect is concentration dependent, increased with increased in concentration. Thus, 100% Aloe vera coating preserved tomato fruit for as long as 16 days and can be use as bio-preservative.

Keywords: *Coating, Roma, Shelf-life, UTC.*

Introduction

Tomato (*Lycopersicon lycopersicum*) is one of the most important vegetable crops in Nigeria (Ogwu *et al.*, 2019). In Nigeria, about one million hectares of total land area is used for its cultivation and this crop makes up about 18% of the average daily consumption of vegetables in Nigerian homes (Babalola *et al.,* 2010). Nigeria ranks second to Egypt in Africa and 13th globally in tomato production (Ebimieowei *et al.,* 2013). Tomato is an important condiment in daily diets, consumed both fresh and in paste form and a very cheap source of vitamins A, C, E and minerals which protect the body against diseases. It contains lycopene, a flavonoid antioxidant together with carotenoids which protect the body cells and other structures in the human body (Ogo-Oluwa and Liamngee, 2016). Despite the high nutritional quality of tomato and its various usage, tomato fruits are of a highly perishable nature, with a short shelf life of between 12 hours to 72 hours (Ejale and Abdullah, 2004). Tomato postharvest life as a climacteric fruit is relatively short since many processes cause loss of quality and storability, including high respiration rates, transpiration, postharvest diseases and acceleration in ripening process and senescence (Zapata *et al.* 2008). Tomato quality changes continuously after harvesting. Fruit quality aspects include firmness, flavour, colour and nutritional value, as well as shelf life, processing attributes and resistance to pathogens (Ju *et al.*, 2000). Tomatoes deteriorate rapidly after harvest and in some cases during or after transport and marketing.

Due to the high perishable nature of tomato fruits, a significant quantity rot before they get to the various areas where they are not cultivated and where the demand is high (Irokanulo *et al.,* 2015). This results in heavy

quantitative and nutritional losses to farmers and consumers as well as the rural and urban dwellers living far from areas of production who will have to pay more for few healthy fruits that gets to them (Ejale and Abdullah, 2004). Its preservation and storage is therefore important to the economy of individual homes, farmers and the country considering the vital role it plays in the health of people (Irokanulo *et al.,* 2015). Therefore the fruit requires appropriate technologies that can improve its shelf-life. Bio-preservation is seen as the most appropriate technology that aims at extending storage/shelf life of fruits and vegetables by utilizing plant-based products applied in food engineering for a long time. Bio-preservation is a noble food preservation method defined for extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds (Ananou *et al*., 2007).

Aloe vera extract is one of the promising bio-preservative which has a great potential to become a common use for most fresh fruits and vegetables. *Aloe vera* is applied to fruits as an edible coating which has been widely used for most fruits and vegetables. Edible coatings have various favourable effects on fruits such as imparting a glossy appearance and better colour, retarding weight loss, or prolonging storage/shelf life by preventing microbial spoilage (Dang *et al*., 2008). *Aloe vera* extract appears to contain various antibiotic and antifungal compounds that can potentially delay or inhibit microorganisms that are responsible for food borne illness in food spoilage. *Aloe vera* extract based edible coatings have been shown to prevent loss of moisture and firmness, control respiratory rate and maturation development, delay oxidative browning, and reduce microorganism proliferation on sweet cherries (Lin and Zhao, 2007). This study therefore aimed at assessing the efficacy of *Aloe vera* gel as a bio-preservative agent that improves the shelf-life of tomatoes.

Materials and Methods

Sample Collection

Two varieties of tomato fruits: Roma and a local variety used for the research were collected from a farm at Kadawa, Kano state. The plant materials where authenticated at the Herbarium of the department of Botany while *Aloe vera* leaves were collected from the Botanical Garden, Department of Botany, Ahmadu Bello University, Zaria. The plant materials were authenticated at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria.

Treatments and Experimental Design

The preparation of *Aloe vera* extract followed the method described by Liamngee *et al.* (2018). Matured fresh leaves of *Aloe vera* having a length of approximately 55 to 85 cm were selected and washed thoroughly with distilled water. The tapering point of the leaf top and the short sharp spines located along the leaf margins were removed by a sharp knife and then the knife was introduced into the mucilage layer below the green rind avoiding the vascular bundles. The top and bottom parts were removed and the *Aloe vera* extract was macerated in clean and sterilized glass bottles. Serial dilutions of the *Aloe vera* gel were prepared to give 10, 50 and 100% respectively. To obtain gel concentration of 10%, 10 milliliters of *Aloe vera* gel was measured in a measuring cylinder and 90 milliliters of sterile distilled water was added. To obtain 50% gel concentration, 50 milliliters of the gel was measured in a measuring cylinder and 50 milliliters of sterile distilled water was added. To obtain 100% gel concentration, the gel in its undiluted state was used.

The different varieties of tomato fruits were washed with distilled water and left to air dried at room temperature. The tomato fruits were immersed completely into each respective *Aloe vera* concentration of 10, 50 and 100% respectively for three minutes. The treated tomato fruits were removed from the gel and kept on plastic crates stored at room temperature. The experiment was laid out in a completely randomized design (CRD) with three replications where physicochemical properties were analysed at 3 days intervals

during the storage period. Data were collected from percentage weight loss (g) using digital weighing balance and rate of decay. The number of decayed fruits was counted on each day of storage and calculated using the formula:

Decay rate = Number of fruits decaying x100

Total number of fruits

The number of days the tomato fruits still remain marketable and had eating quality during the storage period was recorded and it was decided based on appearance of the fruit. The pH value was measured by means of digital pH meter and the values were recorded.

Data Analysis

The data obtained were analysed using Analysis of Variance with Duncan's New Multiple Range Test used to separate significant means at 5% level.

Results

The result obtained for the effects of *Aloe vera* coatings on fruit weight of Roma variety is presented in Table 1. The result showed significant difference ($P \leq 0.05$) in the effects of various concentrations of *Aloe vera* on fruits weight. The result indicated significant decreased in weight of tomato fruit with decrease in concentration from 7.28% among 100% coatings to as high as 48.94% weight loss among the controls.

Table1: Effect of *Aloe vera* coating on fruit weight of ROMA variety

Conc	Fruit Weight (g)						
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	% Weight Loss
0%	58.23[a]	53.90[c]	46.86[c]	41.16[c]	35.13[c]	29.73[c]	48.94%
10%	56.10[a]	52.16[c]	47.13[c]	43.86[c]	38.46[c]	33.76[c]	39.82%
50%	58.90[a]	55.53[b]	52.00[b]	49.16[b]	46.80[b]	42.40[b]	28.01%
100%	67.76[a]	65.50[a]	63.23[a]	62.10[a]	60.70[a]	62.83[a]	7.28%
Mean	60.24	56.77	52.31	49.07	45.27	42.18	
CV	6.32	12.15	18.92	18.46	11.25	12.62	
P-values	0.02	0.02	0.10	0.07	0.00	0.00	

N.B: Means with the same superscript(s) down a column are not significantly different at P≤0.05

Similarly, the result for the effect of *Aloe vera* gel coating on percentage weight loss of Local variety is shown in Table 2. The result indicated significant difference (P≤0.05) in the effect of various concentrations of *Aloe vera* in protecting weight loss of tomato. The result showed marked reduction in percentage weight loss of local variety of tomato from 17.00% treated with 100% coating to as high as 41.62% in the controls in 16 days.

Table 2: Effect of *Aloe vera* gel coatings on fruit weight of Local variety

Conc	Fruit Weight (g)						
	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16	% Weight Loss
0%	68.63[b]	62.63[c]	56.86[b]	51.03[b]	46.90[c]	40.06[c]	41.62%
10%	64.66[c]	60.53[c]	55.30[b]	51.53[b]	47.20[c]	42.03[c]	34.99%
50%	71.46[a]	68.46[a]	64.70[a]	61.50[a]	58.16[b]	54.90[b]	23.17%
100%	70.80[a]	68.80[a]	65.83[a]	63.93[a]	61.70[a]	58.76[a]	17.00%
Mean	68.88	65.11	60.67	56.99	53.49	48.93	
CV	8.42	11.24	5.63	7.83	7.50	8.19	
P-values	0.28	0.42	0.30	0.38	0.32	0.35	

N.B: Mean(s) with the same superscript down a column are not significantly different at P≤0.05

The result for the effect of *Aloe vera* coating in preserving tomato (Variety Roma) from decay is presented in Table 3. The result indicated total protection from decay symptomatology for a period of 16 days due to 100% coating. However, all the controls showed decay symptoms at 16 days of storage.

More so, the result for the effect of *Aloe vera* coating (Table 4) in preventing decay of the local tomato variety showed that 33.33% of the fruits treated with 100% concentration decayed at 16 days of storage while 100% of the controls decayed at the same period.

Table 3: Effect of *Aloe vera* coating on decay rate of Roma variety

	Decay percentage (%)					
Conc	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
0%	0.00[a]	0.00[a]	0.00[a]	66.66[a]	100.00[a]	100.00[a]
10%	0.00[a]	0.00[a]	0.00[a]	33.33[b]	66.66[b]	66.66[b]
50%	0.00[a]	0.00[a]	0.00[a]	33.33[b]	33.33[c]	66.66[b]
100%	0.00[a]	0.00[a]	0.00[a]	0.00[c]	0.00[c]	0.00[c]
Mean	0.00	0.00	0.00	33.33	49.99	58.33
CV	0.00	0.00	0.00	0.00	4.08	4.08
P-values	0.00	0.00	0.00	<0.0001	<0.0001	<0.0001

N.B: Means with the same superscript(s) down a column are not significantly different at P≤0.05

Table 4: Effect of *Aloe vera* coating on decay rate of Local variety of tomato

	Decay percentage (%)					
Conc	Day 1	Day 4	Day 7	Day 10	Day 13	Day 16
0%	0.00[a]	33.33[a]	66.66[a]	66.66[a]	100.00[a]	100.00[a]
10%	0.00[a]	0.00[b]	33.33[b]	33.33[b]	33.33[b]	66.66[b]
50%	0.00[a]	0.00[b]	0.00[c]	33.33[b]	33.33[b]	66.66[b]
100%	0.00a	0.00[b]	0.00[c]	0.00[c]	33.33[b]	33.33[c]
Mean	0.00	8.33	24.99	33.33	49.99	66.66
CV	0.00	0	1.34	4.04	4.08	4.08
P-values	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

N.B: Means with the same superscript(s) down a column are not significantly different at P≤0.05

Discussion

Application of an edible coating is a technique that can be used to increase fruit storability (Chrysargyris *et al.*, 2016). The bio-preservative efficacy of *Aloe vera* gel coatings was reported by the present study to improve the shelf-life of tomatoes for as long as 16 days under storage at room temperature. This finding agrees with that of Liamngee *et al.* (2018) who reported similar findings in tomatoes obtained from Makurdi. Similarly previous studies (Jaiswal *et al.*, 2017; Thushara and Mini, 2018; Tzortzakis *et al.*, 2019) have reported the effectiveness of *Aloe vera* coatings in improving the shelf-life of tomato fruits. Similarly, Alberid *et al.* (2015) reported the high efficacy of *Aloe vera* gel in the maintenance of grapes quality.

The present report by this study that *Aloe vera* gel has the ability to lower the decay

percentage of tomato fruits agrees with the finding of Ibrahim *et al.* (2019) who reported antimicrobial activity and bio-preservative efficacy of *Aloe vera* coatings. This preservative ability of *Aloe vera* was attributed to the presence of approximately 110 potentially active constituents from six different classes: Chromone and its glycoside derivatives; anthraquinone and its glycoside derivatives; flavonoids; phenylpropanoids and coumarins; phenylpyrone and phenol derivatives; and phytosterols and others. Similar result was reported by Padmaja and John (2014) in Jujube fruits coated with *Aloe vera* which results in lowered decay percentage due to the ability of *Aloe vera* to prevent the growth of fungi responsible for spoilage of fruits and reduction of shelf life. The finding of this study is also similar to that of Martinez-Romeo, (2006) who reported that *Aloe vera* gel coating is effective as a physical barrier and thus reduced the weight loss and lowered the respiration rate during post-harvest storage of table grapes and cherries. This can probably be attributed to hygroscopic properties which enable formation of a barrier to water diffusions between the fruit and the environment, thus allowing its external transference as noted by Morillion *et al.,* (2002).

Conclusion

Aloe vera is a potent preservative that has the ability to preserve effectively the post harvest qualities of tomato fruits by protecting heavy weight loss and fruit deterioration. The effect of *Aloe vera* extract is concentration dependent, increases with increase in concentration. Thus, 100% concentration of *Aloe vera* is the desirable rate for application to tomato.

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A REVIEW INTO CYTOGENETICS OF THE GENUS *DIGITARIA* (POACEAE)

***Armiya'u M.A.,[1] Abdul S.D.,[2] Umar T.,[1] Yalli A.A.,[1] Salau I.A.[3] & Basheer A.[4]**

Department of Biology, Shehu Shagari College of Education, Sokoto.
Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi.
Department of Biological Science, Federal University, Gusau.
School of Basic and Remedial Studies, Ahmadu Bello University, Funtua.
*Corresponding Author: armeeya@gmail.com salisunura40@yahoo.com

ABSTRACT

The genus Digitaria is one of the genera that formed the family Poaceae (=Gramineae). The genus comprises of a number of important species such as Digitaria exilis and Digitaria iburua that were neglected by research. Cytogenetic studies of the genus will aid in a broad critical analysis of chromosomes evolution in the clade. This study therefore reviewed the cytogenetics of the genus Digitaria with the aim of providing information that could be utilized in the genetic improvement of this genus. Cytogenetics play a significant role in providing information about chromosomes that are the carriers of genetic materials responsible for controlling plants ontogeny and morphogenesis. It is the central pivot on which understanding of the mechanisms of change in the structure of plants is built upon. It provides a clear understanding of Chromosome evolution, structure and behavior that aid in the analysis of genomes and genetic transmission systems of breeding materials. Thus, cytogenetic studies of the genus digitaria provide a clear insight into its functional genomics that assist in its genetic improvement.

Keywords: *Acha, Iburua, Karyotype, Mapping*

Introduction

The genus Digitaria comprises of the most oldest African cereals cultivated for thousands of years in West Africa across the dry savannas from Senegal to Cameroun since about 5000 B.C (Chukwu and Abdulqadir, 2008). There are over 300 *Digitaria* species grown as fodder; out of which only three or four are grown as cereals (CIRAD, 2004). The genus comprises of plant species belonging to Poaceae (=gramineae) family, sub-family of Panicoideae, tribe of Paniceae and the genus *Digitaria*. Members are annual and perennial grasses with a wide geographic distribution in the tropics and subtropics (Clayton and Renvoze, 1986). A number of wild species are valuable forage grasses throughout the tropics; many others were harvested in the past for food in times of famine or food scarcity in Africa (Haq and Ogbe, 1995; Adoukonou-Sagbadja *et al.*, 2006). *Digitaria exilis* for instance was a staple food crop on the Fouta Djallon in Guinea and the Bauchi plateau in Nigeria (Purseglove, 1972). The cultivation of *D. exilis* alone scatters from Cape Verde in theWest to the Lake Chad in the East, from the edge of the Sahara in the North to the beginning of the rain forest in the South. *D. iburua* is currently much more limited in cultivation being found only in Northern Nigeria, Togo and Benin that, almost certainly, represent a relic of formerly wider cultivation (Haq and Ogbe, 1995).
However, molecular study (Hilu *et al.*, 1997) advocated the possibility of multiple domestications of Digitaria species associated to different centers of diversification. In their molecular phylogenetic approach based on RAPD markers, Hilu *et al.* (1997) confirmed the high genetic relatedness of *D. longiflora* and *D. ternata* to *D. exilis* and *D. iburua* respectively. Large genetic divergence of *D. fuscescens* to both cultivated species was found. These

findings have provided up to date the clearest insight on the fonio origin and evolution. However, as suggested by the authors, other approaches such as cytological investigations and artificial crosses between taxa or exploration of other related species (such as *D. barbinodis* Henr.) are also needed.

Cytogenetics being the study of character transmission in relation to structure and function of chromosomes has played a significant role in the development of plants genetics and breeding. Cytogenetic manipulation of the chromosomes in crop plants continues to be one of the most important methods available to plant breeders for introducing new variation into varieties of crops (Gale and Miller 1987). Interspecific hybridization in plants is an important evolutionary phenomenon involved in the dynamics of speciation that receives increasing interest in the context of possible gene escapes from transgenic crop varieties. Crops are able to cross-pollinate with a number of related species and exchange chromosome segments through homologous recombination into a recipient species leading to evolution of new hybrids. Observation of interspecific hybrids between different taxa in nature is rare but recurrent (Ellstrand *et al.*, 1996; Arnold, 1997). The advancement in cytological techniques has uncovered the phenomena involved in angiosperms evolution (Rieseberg and Wendel, 2004). The reality of ancient introgression is difficult to establish but phylogenetic studies are increasingly providing indirect evidence. Interspecific gene transfers cancel the accumulated nucleotide divergences between species. Then the phylogenetic trees obtained for a gene that was kept isolated within each species and for a gene exchanged after their evolutionary differentiation can be inconsistent. These phylogenetic incongruities support a reticulate evolution pattern (Vriesendorp and Bakker, 2005; Marhold and Lihova, 2006).

Molecular cytogenetic analysis of the chromosomes of crops is a vital part of understanding their evolution, genetics, genetic recombination and karyotypic stability (Heslop-Harrison and Schwarzacher, 1993). Plant chromosomes have traditionally been a fruitful material for almost every kind of cytogenetic research. However, with the development of cytomolecular techniques, mainly FISH (Fluorescent In Situ Hybridization) and its numerous variants, plant cytogenetics research has greatly advanced, revealing unexpected details of chromosome behavior and evolution (Guerra, 2005). Plant cytogenetics has continued to flourish and make essential contributions to genomics projects by delineating marker order, defining contig gaps and revealing genome rearrangements (Debbie and Hank, 2010). This study therefore aimed at reviewing the significance of cytogenetic studies in crop improvement.

Cytogenic Studies in Crop Plants

Cytogenetics study through karyomorphological studies and chromosomes mapping techniques aided by molecular approaches have proven vital in the uproper understanding of functional genomics in economic plants thereby pave easiest ways for their improvement.

Karyomorphological Studies in Plants

The concept of Karyotype denotes a distinct and constant individuality of species somatic chromosomes and that closely related species have more similar chromosomes than those of more distantly related ones (Sharma, 1991). Chromosomal differences also reflect differences in the source of genetic variation, while morphological, physiological and biochemical differences reflect differences in the product of gene action, modified by environmental factors (Stebbins, 1971). According to Stace (2000) the evolution of karyotype in many genera takes place through a series of alterations in chromosome morphology. According to him, karyotype is the phenotypic appearance of the somatic chromosomes, in contrast to their genotypes and suggested that evolution in the form and shape of chromosomes has resulted in the progressive asymmetry of the karyotype.

However, karyotypes are grouped into symmetrical and asymmetrical karyotypes. A

symmetrical karyotype is one in which the chromosomes are all of approximately the same size, and have median or sub-median centromeres (Stace, 2000). Where as asymmetrical karyotype consist of chromosomes of unequal sizes with submedian and subterminal centromere. Asymmetry karyotype can occur either through the shift of centeromere position from median to sub terminal or terminal, or through the accumulation of differences in relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous (Johnson, 2003). Moreover, a number of asymmetrical karyotypes consist of distinctly large and small classes of chromosomes. Such karyotypes are referred to as bimodal karyotypes.

The importance of karyotype analysis in distinguishing plant species is well known. The role of alteration of chromosome morphology in speciation and in determining interrelationships between species, varieties and even strains has been reviewed earlier by Sharma and Sharma (1959). It has been reported that species often show similarity in gross morphology but they differ from each other in small details in chromosome morphology like the centromere, secondary constriction, number and size of satellites and variation in total chromatin length (Yin *et al.*, 2010). Darlington (1973) stated that the basic chromosome number will be increased or decreased as a consequence of unequal translocations and depends largely on whether the regions about the centromeres are active or inert.

Frame (2001) reported that karyotypic diversity often provides indications of on-going speciation events. He stressed that, the relative homogeneity of *Schoenocaulon*'s chromosome morphology reflects its relative evolutionary status. Liu *et al.* (2000), in his karyomorphological studies on *Biebersteinia stefan* (Geraniaceae) has found out that this species has a unique karyomorphology which is congruent with embryological, anatomical, chemical and molecular data. Janadharan and Thopil (2003) showed the abundance of nealy submedian chromosomes which is an advanced karyomorphological feature.

Chromosomes Mapping

According to Debbie *et al.* (2012) the field of cytogenetic mapping in plants was initiated by the pioneer work of McClintock in 1929. She was the first identified the ten pachytene bivalents of maize (*Zea mays* L., 2n=20) and to narrow the position of a linkage group, involving genes for colored aleurone (C), shrunken endosperm (Sh) and waxy endosperm (Wx1), to the long arm of chromosome 9. A carmine-based stain was used to visualize endogenous cytological features such as chromatic (darkly staining) regions, achromatic (lightly or nonstaining) regions, chromomeres (darkly staining granules), nucleolus organizing regions, and centromeres on these classical cytological maps.

Another class of fairly recent genetic tools are the molecular linkage maps. These are not physical chromosome tools but are very useful in chromosome and genome identification and monitoring alien introgressions. RAPD markers were basically random base sequences that were extensively used to tag genes controlling agronomic traits (Williams *et al.*, 1990). The next class of markers were RFLPs. Extensive maps consisting of 1000 RFLP markers were generated in wheat (Gale *et al.*, 1995). These markers were characterized as too expensive and cumbersome for application to marker assisted selection but played a significant role in uncovering relationships between diverse grass genomes (Gale and Devos, 1998). For instance, overall colinearity between wheat, barley, maize and rice genomes was established (Feuillet and Keller, 1999) which permitted the sharing of markers across the maps. Because the rice genome was fully sequenced (Goff *et al.*, 2002), this permitted the extensive use of rice sequences to perform fine mapping in wheat genetic studies (Liu *et al.*, 2006).

RFLP technology, particularly the use of cDNA probes has been and still is a valuable method of monitoring alien chromatin introgression into the wheat genome, mainly

because of sequence conservation and systemic relationship among the Triticeae. In more recent fine mapping experiments it is being discovered that the collinearity between chromosomes of species such as wheat and rice is not as close as initially assumed (La Rota and Sorrels, 2004). Other molecular maps were developed with PCRbased markers including RAPDs (William *et al.*, 1990) AFLPs (Vos *et al.*, 1995) and microsatellites (SSRs) (Roder *et al.*, 1998). High density microsatellite maps coupled with high throughput capillary electrophoresis are the essential components of a marker assisted breeding program. The high density consensus map of Somers *et al.*, 2004 is adequate for QTL detection. High density maps are essential for map based cloning by increasing the probability of discovering markers flanking the gene in question and by reducing the number of BAC clones containing the gene.

Another class of methodologies that facilitate the identification of specific genomes, individual chromosomes or chromosomal segments is the use of fluorescent signals. The first procedure to use fluorescent labels to distinguish plant chromosomes was the process of genomic in situ hybridization (GISH) (Shwarzacher *et al.*, 1989). This technique was then used to identify parental genomes and genome organization, plus alien genome/chromosome introgression (Jiang and Gill, 1994). The identification of the three genomes of hexaploid wheat has been achieved, but proved to be difficult to repeat consistently (Mukai *et al.*, 1993; Sanchez Moran *et al.*, 1999). A modification of the multicolour GISH technique (Han *et al.*, 2004) permitted the unequivocal identification of all three wheat genomes plus the presence of alien chromosomes and translocations. For instance, using a combination of FISH, Afa and PSc119.2. Kubalakova *et al.* (2005) were able to identify all chromosomes of durum wheat. Additional tools that complement the use of FISH technology for cytogenetic analysis of individual chromosomes are 5S and 25S genes plus BAC clones that can be differentially stained to identify individual chromosomes (Zhang *et al.*, 2004). The fiber FISH technique, using appropriate probes which is applied to stretched somatic chromatin can provide better resolution of gene

Cytogenetics of Genus Digitaria

The cytogenetics of grasses has always been the most advanced area in plant cytogenetics and when combined with FISH and GISH (Genomic *In Situ* Hybridization) it furnishes a flexible model to study the behavior of individual genomes, individual chromosomes, or chromosomal fragments in natural and artificial hybrids. Since most plants, notoriously the cultivated ones, originated from one or two hybridization events followed by polyploidization, this technique has become the most common way to analyze allopolyploids and again grasses. In several aspects, the cytogenetics of hybrids has also received special attention in relation to plant breeding. Since hybrid derivatives may contain a variable number of alien chromosomes or chromosome arms, the use of GISH opens the doors to a clear genome distinction where before there was alot of speculations (Guerra, 2005).

Following hybridization, gene flow may result in the introgression of chromosome segments from one species to another by direct and recurrent back crosses. Alternatively, interspecific hybrids might give birth to new fertile species either via spontaneous and instantaneous chromosome doubling (allopolyploidy; as in wheat and oilseed rape) or via the fixation of viable recombinant chromosome sets (Rieseberg and Carney, 1998). These stable and fertile allopolyploid and homoploid forms can also constitute bridges and gene reservoirs for subsequent gene flows back to their diploid progenitors. Introgression can then act on ecosystems by species invasion or replacement (Ellstrand, 2003) and may thus have an impact in biological diversity. In the crop-wild context, with or without genetically transformed varieties, evolution towards nasty weeds is also a major rising concern (Burke *et al.*, 2002; Lu and Snow, 2005).

Weeds incorporating transgenes carrying insect or disease resistance, or resistance to environmental stresses, are likely to display increased overall fitness and competitiveness, and their spread may have serious agronomic and environmental implications (Warwick *et al*, 2003; Watrud *et al*., 2004; Reichman *et al*, 2006). Natural selection of a favourable allele in an interspecific context has long been neglected but while the spread of an introgressed allele is possible through neutral evolution, it can thrive much more successfully through natural selection even with low selective advantage (Arnold *et al*., 1999; Rieseberg and Burke, 2001).

Most Poaceae species show broad chromosome size and number diversity (Schapova, 2012), with basic numbers varying from n=2 to n=18 and somatic numbers, from 2n=4 to 2 n = 263–265 (De Wet 1987). This cytological diversity is believed to be related to aneuploidy, euploidy and hybridization processes during evolution of Poaceae species (Hilu 2004; Schapova 2012). However, for most tropical and subtropical species, current data available are as yet scarce (Honfi *et al*., 1991; Sede *et al*., 2010). Although n=9 and n=10 are the predominant basic chromosome numbers in the subfamily Panicoideae, other basic numbers can also be found in this subfamily (Hilu 2004).

The genus *Digitaria* is cytologically variable with a basic chromosome number n=9 (Purseglove, 1972) that is typical for most genera of the Paniceae tribe. The genus is characterized with very small chromosomes and polyploidy is known to have played important role in its evolution. Karyological analysis of various *Digitaria* species revealed a wide range of chromosome numbers/ploidy levels ranging from diploid (2n=18) to dodecaploid (2n=108) (Zeven and de Wet, 1982; Wipff and Hatch, 1994; Bennett *et al*., 2000; Caponio and Rua, 2003). The basic chromosome numbers in the genus are n=9, 15 and 17 with the most frequent chromosome number being 2n=18, 36, 54 and 72, but variants with 24, 27, 30, 34, 35, 40, 45, 60, 68,70 and 76 have also been reported, as well as intra-specific variability in chromosome number (Goldblatt and Johnson, 2000).The vast majority of the species are polyploids with tetraploid and hexaploid levels being the most commonly found. Some species like *D. cognate* subsp. Pubiflora Wipff have more than one ploidy level (Wipff and Hatch, 1994); a presence of B-chromosomes is also reported in *Digitaria eriantha* Steud (Pozzobon *et al*., 2006). In cultivated fonio, *D. exilis* is contradictory reported to be diploid, tetraploid or hexaploid. Both tetraploid (Zeven and de Wet, 1982) and hexaploid (Wanous, 1990) levels have been proposed for *D. iburua*. The disparity in the reports and mainly the lack of unequivocal karyotypic information on these crops argue for the need of wide cytological reinvestigations for the effective use of fonio landraces in breeding. Except the chromosome number, little is known about other cytogenetic parameters of *Digitaria*. However, Vodouhè and Achigan Dako (2006) reported the somatic chromosome number of *D. exilis* to be 2n=54 .

Conclusion

It was concluded that, unravelling the karyomorphology of members of the genus Digitaria provides a clear insight into its functional genomics that will assist in its genetic improvement.

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GENETICS OF BRIX CONTENT IN SOME SORGHUM *(SORGHUM BICOLOR (L.) Moench)* LINES IN NIGERIA

[1]Saidu G., [1]D.A. Aba, [2]A. Usman., [2]M.S. Muhammad, [3]Hamisu H.S.

Corresponding author e-mail:briefbalagado@gmail.com

ABSTRACT

Sweet sorghum (Sorghum bicolor (L.) Moench is a type of cultivated sorghums and has been recognized widely as potential alternative source of bio-fuel because of its high fermentable sugar content in the stalk. A substantial variation of brix content and related traits is rarely known to exist in sweet sorghum. The objectives of the study were to estimate the nature and magnitude of gene effects for Brix content and its component traits in sweet sorghum through generation mean analysis. The experimental materials consist of six basic generations, namely P1, P2, F1, F2, BC1P1, BC1P2 of three crosses involving six parents were evaluated in zaria during rainy 2019. The Generation mean analysis indicated the presence of epistasis in expression of Brix content and its components. The mean performance of the F1 in all the crosses indicated dominant gene effect for all the characters. The interaction was of duplicate epistasis, therefore, in addition to the main genetic effects, ([d], [h] components, the interaction components were also taken into consideration to develop the breeding strategy. Prevalence of significant additive effects in the crosses IS23525xKSV15(C1), NRS005x SAMSORG45(C2), indicated effectiveness of direct selection for sugar/brix content improvement. All the interaction components are highly variable; therefore, specific breeding strategy, for direct selection and development of pure lines are appropriate for Brix content improvement.

Keywords: *Epistasis, Gene actions, Brix Content.*

Introduction

Sweet sorghum is similar to cultivated grain sorghum except for sugar rich stalks and is recognized widely as a potential source of biofuel. Besides having rapid growth, high sugar accumulation, and high biomass production potential, sweet sorghum has wider adaptability and offers comparable grain yields Reddy *et al.* (2008). It can be grown with limited water under minimal inputs and can be harvested within a span of four months. The economic superiority is contributed by characters such as stalk yield, stalk sugar content (Brix %), stalk juice extractability, content of non-reducing and reducing sugars and grain yield. Bala Ravi *et al.* (1996). The sugar content in the juice extracted from sweet sorghum stalks varies from 16-23%. Sweet sorghum is best suited for ethanol production because of its higher fermentable sugar content in the stalk compared to sugarcane Reddy *et al.* (2008). The feasibility of converting stalk sugars to ethanol. syrup. jaggery on or near farms, and the adaptability of sorghum to a wide range of environments prompted researchers to evaluate the potential of sweet sorghum as an alternative crop for ethanol production Daniel *et al.* (1991). The bagasse after extraction of juice from sweet sorghum can be used for animal feed, vermi-composting and co-generation of power (Reddy *et al.*, 2005; Srinivasa Rao *et al.*, (2009). Further, the bagasse has\ a higher biological value than the bagasse from sugarcane when used as forage for animals, as it is rich in micronutrients and minerals Seetharama *et al.* (2002).

The bagasse has similar levels of cellulose and sugarcane bagasse and therefore has a good

prospect as a raw material for pulp product. The sweet sorghums have not been a major focus of commercial breeding programmes; hybrids have been developed between grain and sweet sorghums, usually for fodder or dual purpose use (grain and fodder). Thus, increasing stalk sugar yields is becoming an important objective in sweet sorghum breeding Murray *et al.* (2009). Genetic enhancement of the crop for increased sugar yield is very critical to make sweet sorghum more profitable to the farmers and the industry, while sustaining grain yield, juice volume, plant height, plant girth and other important components. The choice of an efficient breeding programme depends to a large extent on knowledge of the type of gene action involved in the expression of the character. The knowledge on nature of gene action for sugar yield and its component traits like Brix content at dough stage, and fresh juice volume in the breeding material can provide useful information for selecting proper breeding procedure for future genetic enhancement. Inheritance of stalk diameter, Brix content and fresh biomass in stem diameter was subject to both additive gene effect and non additive gene effect, but mainly controlled by non additive genes Zhou *et al.* (2005). However, the literature regarding inheritance of these traits and their genetic interactions in sweet sorghum is scanty. Keeping this in view, an attempt has been made to understand the gene action controlling Brix content and its component traits through generation mean analysis using different genotypes of sweet sorghum with varied Brix% and juice content.

Material and Methods

Eighteen genotypes of sweet sorghum and non sweet sorghum (Table 1). Were selected based on their Brix% (5-16%) to provide the basic material in the study. Six generations viz., P1, P2, F1, F2, BC1P1 and BC1P2 of four inter-varietal crosses namely, IS23525xKSV15(C1), NRS005x SAMSORG45(C2), and SAMSORG44 x NRS003(C3). Were developed and raised in randomized complete block design (RCBD) with three replications at Institute of Agricultural Research (IAR) Research station Samaru .Zaria during the rainy 2018. The parents and F1's were planted in two rows of 2 m length; BC1P1 and BC1P2 were planted in six rows of 2 m length; F2's were planted in twelve rows of 2 m length each accommodating 20 plants in a row with spacing of 45 ×15 cm. All the recommended agronomic practices\ were adopted to raise a healthy crop. 20 plants from each of the parents and F1's, 60 plants from BC1P1 and BC1P2 generations and 120 plants from F2 population were randomly selected avoiding border rows for recording data on\ days to 50% flowering, plant height (cm), number of leaves, number of internodes, stalk length (mm), stalk weight (g), Fresh juice weight (g),Fresh juice volume (ml), Brix content at dough, Brix content at milk stage, Brix content at maturity, Fresh bagasse, Dry biomass, leave length, Brix content was measured using Refractometer.

Table 1: Description of sweet sorghum and non sweet sorghum with their source

Genotypes/varieties	High sweet	Source	
IS23525	SWEET	IAR	-
SWSV DAN SADAU	SWEET	IAR	-
NRSSSOO5	SWEET	IAR	-
NRSSSOO3	SWEET	IAR	-
CSR 01	NONSWEET	IAR	-
CSR 02	NONSWEET	IAR	
KSV 04	NON SWEET	IAR	
KSV 12	NON SWEET	IAR	
SKSV 13	NONSWEET	IAR	
KSV 15	NON SWEET	IAR	
SAMSORG 40	NONSWEET	IAR	
SAMSORG 44	NONSWEET	IAR	
SAMSORG 41	NONSWEET	IAR	
SAMSORG 45	NONSWEET	IAR	
SAMSORG 46	NONSWEET	IAR	
SAMSORG 38	NONSWEET	IAR	
SAMSORG 39	NONSWEET	IAR	
SAMSORG 03	NONSWEET	IAR	

Statistical Analysis

The analysis of variance (ANOVA) was carried out through Statistics10. To confirm the data adequacy, Mather's (1949) scaling test (A, B, C, and D) was performed for confirmation of additive-dominance model reported by Singh and Chaudhary (2012).

A ¼ P1 þ F1−2BC1 ¼ 1=2ð½i−½j þ ½lÞ:

C ¼ P1 þ P2 þ 2F1−4F2 ¼ 2½i þ ½l

B ¼ P2 þ F1−2BC2 ¼ 1=2ð½i þ ½i þ ½lÞ:

D ¼ 2F2−BC1−BC2

Estimates of various gene effects, allelic interaction, and their test of significance were computed by a sixparameter model of Hayman (1958) and Jinks and Jones (1958) by the following equations: m ¼ Mean ¼ F 2

d ¼ Additive effect ¼ BC1−BC2

h ¼ Dominance effect ¼ 2BC1 þ 2BC2 þ F1−4F2−ð1=2ÞP1−ð1=2ÞP2

I ¼ Additive Additive genetic interaction ¼ 2BC1 þ 2BC2−4F2

J ¼ Additive Dominance genetic interaction ¼ 2 BC 1−P1−2BC2 þ P2

l ¼ Dominance Dominance genetic interaction

¼ P1 þ P2 þ 2F1 þ 4F2−4BC1−4BC2

Result

Mean analysis of different generations

The mean performance of P1, P2, F1, F2, BC1P1 and BC1P2 families of three crosses are shown in Table 2. The mean performance of the two parental lines for each cross were different from each other for all the fourteen traits viz., number of leaves, number of internodes, days to 50% flowering, plant height, stem diameter, stalk weight, fresh juice volume, dry biomass, Brix content at dough stage, Brix content at milk stage, Brix content at maturity, leave length. Also the mean performances of F1 and F2 for the above said traits were different from those of both the parents for each cross and they tended towards their respective female parents (P2) which are of high Brix% except in the case of C3 where F2 means were closer to the lower parent. The F1 means were greater than the respective mid-parent mean values for all the given characters. The BC1P1 and BC1P2 family means were tended towards their respective parents and overlapped with each other which indicate gene interactions. Joint scaling test revealed that (Table 3) both additive and dominance gene effects were highly significant for all the traits in all the crosses except in C3 for stem diameter, in C1 for plant height, fresh juice weight, bagasse and Brix yield where only additive gene effects were non-significant. Similarly, dominant gene effects were non significant for days to 50% flowering in C1, C2 and C3, for stem diameter and Brix% in C1, for plant height, stalk length, fresh juice volume, and juice weight in C3. In general the estimates of dominance gene effects were positive and higher compared to additive gene effects which are negative. Significance of chi-square values were reported for all the traits in three crosses. Six parameter model (Table 4) of Jinks and Jones (1958) revealed significance of main genetic effects, [d] and [h] in general. However, additive [d] gene effects were not significant for stalk weight, cane weight, juice weight, Brix %, bagasse and sucrose in C1 and for stem girth in C3. In the same manner dominance [h] gene effect was not significant for Brix% in C3. The magnitude of dominance gene effects [h] was substantially higher than that of additive gene effects [d] in all the crosses for fourteen traits. The magnitudes of dominance× dominance [l] gene effects were higher than additive ×additive [i] and additive × dominance [j] gene effects irrespective of direction of their effects for all the characters in three crosses. Further, the net sign of dominance × dominance [l] gene effects were positive in general for traits studied and in particular for Brix% and sugar content in all the crosses.

Discussion

The mean values of F1 and F2 families tended towards that of sweet sorghum parent (P2) except in case of C2 where F2 means were closer to P1 which may be ascribed to large error variance (Table 2).

The F1 means were greater than the respective mid-parent mean values for all the given traits, indicating dominance for important traits like plant height, stalk diameter, fresh juice volume, Brix% content conforming the report of Semenova (1988). The BC1P1 and BC1P2 family means were tended towards their respective parents and overlapped with each other which indicate gene interactions. Chi-square significance of joint scaling test indicated the inadequacy of additive-dominance model which in turn indicated the presence of non-allelic interactions in the present study involving three crosses suggesting possible involvement of digenic interactions for some trait. Significance of additive and dominance gene effects was observed in all the crosses revealing the importance of both of these in such a way that the negative sign associated with additive effects [d] in all the three crosses for each trait indicates the combination of genes from both the parents did not add up to the improvement of the characters suggesting dominance effect (Table 3).

Importance of both additive and non-additive gene effects for Brix traits of sorghum was revealed in previous reports by Ramalingam and Rangasamy (1987); Saxena *et al.* (1999). Further, dominance component [h] of generation mean observed was positive and greater in magnitude than additive gene effect [d] for all the characters in the three crosses which strengthen the fact that dominance component played a major role in the inheritance of all these characters. The sign for dominance effect is a function of the F1 mean value in relation to the mid parental value and indicates which parent is contributing to the dominance effect (Cukadar-Olmedo and Miller, 1997). The predominant role of non-additive gene action for plant height, stm diameter, Brix content at maturity, stalk yield, and fresh juice volume in sweet sorghum was reported by Sankarapandian *et al.* (1994). Similarly Gupta and Baliwal (1976) reported nonadditive gene action for total soluble solids (Brix %). The negative sign found associated with the dominance effect for days to 50% flowering in C1 and C3 indicate the dominance effect for decreasing alleles as it reduced the number of days to 50% flowering in hybrid combinations in which it was close to their lower parent. In contrary, Dangi *et al.* (1978) reported predominant role of additive gene action for days to 50% flowering as well as for plant height and stem diameter. Among the digenic\ interactions, additive×additive (i) interaction was positive for most of the traits in all the three crosses, except for days to 50% flowering in C1 and C2, plant height in C3 and for Brix% in C1 and C3 respectively (Table 4). It was found that magnitude of i (additive×additive) was significant and higher than both j (additive ×dominance) and l (dominance×dominance) components revealing the presence of associated pair of genes for all the traits in three crosses. Opposite signs of dominance [h] and dominance× dominance (l) gene effects revealed duplicate epistasis for all the traits except for days to 50% flowering in C3 revealing consistency of gene action over crosses. The negative sign associated with additive (d) and positive sign associated with additive×additive (i) component in the three crosses for majority of traits indicate positive additive gene action consisted of positive additive digenic interaction whereas, the balance of the additive gene effects of the genes controlling these traits was negative. Kearsay and Jinks (1968) suggested that the two parental lines have equal opportunity to contribute to the expression of additive by additive effects when averaged cross all possible F2 genotypes. Accordingly the combination of genes from both the parents would have contributed to expression of sugar yield and its component traits in the crosses under study. Non-allelic interactions are known to either reduce or enhance the extent of heterosis depending upon their direct Ion and magnitude of action. Confounding epistatic effects in the study suggested that inheritance of these traits is complex and polygenic Warnock *et al.* (1998). Higher

magnitude of dominance gene effects and dominance gene interactions could not be exploited for heterosis breeding due to presence of duplicate epistasis in the present crosses as it minimizes the manifestation of heterosis Kearsey and Pooni (1996). Hence, selection for high sugar yielding genotypes would be effective if dominance and epistatic effects were first reduced by few generations in subsequent segregating generation or population improvement methods may possibly serve the purpose of developing high sugar yielding genotypes of sweet sorghum.

Conclusions

Generation mean analysis of three crosses in the present study explicated the presence of epistasis for the characters involved. The presence of epistasis has important implications for any plant breeding program. Although the results of this experiment may be applicable to the germplasm used herein, the identification of dominance and epistatic effects suggest that additional research is necessary to further advance the breeding of Brix content in sweet sorghum.

Table 1: Generation Means of the Families for Morphological, Physiological, Brix Content and Its Components in Sweet Sorghum During Wet Season 2019 in Zaria (Means ± Standard Errors)

CROSS	Gen	DFF	PLHT	NOLV	NOIN	STDM	FBM	FJV	FJW	DBM	BXD	BXM
Cross1	P1	83.67±1.76	176.73±4.63	8.93±0.13	8.00±0.20	5.87±0.17	179.89±60.10	90.00±10.00	93.70±9.90	32.90±8.04	5.97±2.13	11.92±
	P2	77.00±1.00	187.13±6.33	7.87±0.52	6.87±0.52	5.94±0.19	130.37±25.28	103.00±1.53	110.67±3.28	33.72±3.10	6.72±2.85	7.15±2
	F1	83.67±0.67	168.73±9.91	7.93±0.41	6.93±0.41	5.85±0.20	237.92±±69.89	153.33±31.80	161.17±30.72	27.90±4.64	6.88±1.52	7.98±0
	F2	79.00±1.73	209.93±13.12	7.60±0.12	6.60±0.23	4.93±0.49	169.61±30.33	133.33±46.76	140.07±49.80	26.30±2.96	2.24±1.79	8.90±0
	B1	78.33±5.49	171.40±10.18	7.93±1.07	6.93±1.07	5.20±0.96	170.37±40.28	136.67±13.33	149.47±9.41	41.08±0.50	5.94±2.52	13.41±
	B2	81.00±2.52	173.00±8.19	9.20±0.53	8.20±0.53	5.54±0.16	145.69±32.39	121.67±40.86	127.83±42.83	35.23±11.45	9.19±0.19	9.57±2
Cross2	P1	81.67±1.38	152.07±3.92	7.47±0.61	6.47±0.61	5.75±0.20	160.43±37.79	95.00±1.83	103.53±1.76	31.71±2.09	6.81±1.62	9.71±0
	P2	80.00±0.97	186.40±3.70	7.53±0.17	6.53±0.17	6.23±0.11	135.36±19.10	120.00±11.11	126.53±9.81	32.46±2.52	5.11±0.80	7.38±1
	F1	82.00±2.76	169.13±9.48	8.07±0.59	7.07±0.59	5.32±0.53	134.43±12.93	91.00±0.63	97.77±2.32	40.28±1.96	9.10±1.46	9.74±0
	F2	86.67±0.76	190.33±5.98	7.53±0.08	6.47±0.11	5.29±0.25	199.84±37.66	183.33±27.89	193.23±26.59	36.15±2.03	6.81±0.79	4.68±1
	B1	79.00±1.10	191.20±1.81	8.13±0.23	7.27±0.28	4.99±0.17	180.61±23.49	211.67±21.16	221.77±20.89	36.05±2.14	8.13±1.83	7.25±1
	B2	83.33±1.38	181.27±5.58	7.67±0.17	6.67±0.17	4.91±0.13	190.38±6.44	148.33±1.05	154.17±2.71	40.25±2.28	8.04±0.39	10.39±
Cross3	P1	82.33±0.50	187.60±6.91	8.87±0.12	7.93±0.13	6.41±0.23	197.70±17.94	171.67±18.05	146.37±15.48	25.59±1.21	8.01±0.67	10.45±
	P2	76.00±0.50	199.40±1.76	8.60±0.51	7.60±0.51	6.06±0.27	189.496.05	181.67±10.24	194.97±11.96	41.89±1.95	7.9±90.40	7.01±0
	F1	82.33±1.83	192.00±2.17	9.13±0.18	8.13±0.18	6.13±0.19	196.06±24.58	246.67±12.02	255.10±13.65	53.12±2.11	9.40±0.98	9.48±0
	F2	81.00±0.50	161.33±2.99	7.93±0.15	6.93±0.15	5.31±0.16	185.98±12.31	200.00±10.00	216.67±7.68	45.51±1.60	7.30±0.63	7.86±1
	B1	81.67±1.42	185.13±6.03	7.87±0.20	6.87±0.20	5.19±0.12	152.11±7.96	173.33±20.48	189.17±19.25	28.33±1.84	7.71±0.67	8.25±0
	B2	80.00±0.58	197.73±2.19	8.93±0.32	7.93±0.32	5.89±0.06	286.75±11.42	211.67±23.47	221.70±24.65	36.47±4.32	6.15±0.64	6.71±0
	Mean	81.04	182.25	8.18	7.19	5.60	180.17	154.02	161.33	35.66	7.08	8.77
	CV	5.24	10.47	11.02	12.76	12.27	33.27	33.02	31.37	26.99	41.28	31.96
	LSD	4.03	18.13	0.86	0.87	0.65	56.94	48.32	48.08	19.56	2.78	2.66

DFF: days tofifty percent flowering, PLHT: plant height, NOLV: number leaves, NOIN: number internode, BXM: brix at maturity BXD:brix at dough BXM:brix at milk stage STDM: stalk diameter, LVLT:leave length

Table 2. Joint Scaling Test for Assessing the Adequacy of Additive-Dominance Model for Brix Contents and its Related Traits in Sweet Sorghum in three crosses evaluated in Zaria in the Wet Season 2019

	Cross1				Cross2				Cross3			
Traits	A	B	C	D	A	B	C	D	A	B	C	D
DFF	-10.67	1.33	-12.00	**-1.33	*-5.67	4.67*	21*	11.00*	**-1.33	1.67*	1.00	0.33
PLHT	*-2.67	*-9.87	138.4**	75.47**	61.20*	7.00*	84.6**	8.20*	-9.33	4.07**	-125.7	-60.2
NOLV	*-1.00	2.60	**-2.27	**-1.93	0.73	**-0.27	**-1.00	**-0.73	**-2.27	0.13	**-4.00	**-0.93
NOIN	*-1.07	2.60	**-2.33	**-1.93	1.00	**-0.27	**-1.27	**-1.00	**-2.33	0.13	**-4.07	**-0.93
STDM	*-1.31	**-0.71	*-3.77	**-0.87	**-1.08	**-1.73	**-1.45	0.68	**-2.17	**-0.41	**-3.5	**-0.46
FBM	-77.07	-76.9	-107.7	23.16*	66.36**	110.98	234.72**	28.69**	-89.54	187.95**	-35.4	-66.9
FJV	30**	**-13	33.67**	8.33*	237.33**	85.67**	336.33**	6.67*	-71.67	-5	-46.67	15.00*
FJW	44.07*	*-16.17	33.57**	2.83	242.23**	84.03**	347.33**	10.53*	-23.13	-6.67	15.13*	22.47**
DBM	21.36*	8.84*	-17.22	-23.71	0.10	7.75*	**-0.13	*-3.99	-22.06	-22.08	8.33*	26.23**
BXD	*-0.97	4.77	-17.48	*-10.64	0.36	1.86	**-2.89	**-2.56	**-1.98	-5.08	*-5.6	0.73
BXM	6.91*	4.00	0.56	*-5.18	*-4.94	3.66*	-17.86	*-8.29	**-3.43	*-3.07	*-4.98	0.76
BXMT	3.19*	4.63	*-4.46	*-6.14	*-5.75	2.93*	-15.3	*-6.24	**-2.05	**-2.05	*-5.85	**-0.87
LVLT	*-1.49	6.55*	1.39	**-1.84	5.43*	**-1.14	13.34*	4.52*	0.21	*-4.01	16.98*	10.39*
STKLT	0.03	**-1.49	3.08	2.27	1.67	0.37	0.34	**-0.85	5.05	1.60*	**-1.51	**-4.08

DFF: days tofifty percent flowering, PLHT: plant height, NOLV: number leaves, NOIN: number internode, BXM: brix at maturity BXD:brix at dough BXM:brix at milk stage STDM: stalk diameter, LVLT:leave length.

Table 3. Estimates of Additive, Dominance and Digenic Epistatic Gene Effects of Brix Content and Related Traits in Three Crosses of Sweet Sorghum Evaluated in Zaria in Wet Season 2019

	Estimates	DFF	PLHT	NOLV	NOIN	STDM	FBM	FJV	FJW	DBM	BXD	BXM	BXMT	LVLT
	M	79.00**	209.93**	7.60*	6.60**	5.29**	169.61**	133.33**	140.07**	26.30*	2.24*	8.90**	7.04**	42.69*
Cross1	d (a)	-2.67**	-1.60**	-1.27**	-1.27**	0.08	24.67*	15.00	21.63	5.85*	-3.25*	3.84*	0.98	-3.30**
	h (d)	6.00*	-164.13	3.40*	3.37*	-2.03**	36.47*	40.17*	53.32*	42.01	21.83**	8.79*	11.17*	1.75
	I (aa)	2.67	-150.93	3.87*	3.87*	-1.36**	-46.32	-16.66	-5.67**	47.42	21.29**	10.35*	12.27*	3.68*
	j (ad)	-6.00*	3.60	-1.80**	-1.83**	0.32	-0.09**	21.50	30.12	6.26	-2.87**	1.46	-0.72**	-4.02**
	l (dd)	6.67*	163.46**	-5.47*	-5.40*	4.17*	200.29**	-0.34**	-22.23*	-77.62	-25.09*	-21.26*	-20.09*	-8.74**
	χ2	183.24**	42.38**	169.03**	103.37**	95.61**	167.28**	77.97**	222.21**	292.11**	64.50**	246.42**	340.81**	105.52**
	TE	C	-	D	D	D	C	D	D	-	D	D	D	-
	M	86.67**	190.33**	7.53**	4.93*	0.00								
	d (a)	-4.33**	9.93	0.47	-0.34**	-1.07**	199.84**	183.33**	193.23**	36.15	6.81*	4.68*	6.50*	44.17**
Cross2	h (d)	-20.83	-16.50*	2.03*	1.69	37.73**	-9.78*	63.33*	67.60**	-4.20	0.10	-3.14**	-3.41**	1.01
	I (aa)	-22.00	-16.40*	1.47	1.75	29.60**	-70.85	-29.83	-38.33*	16.18	8.26*	17.77**	15.13*	-8.23**
	j (ad)	-5.17**	27.10	0.50	-0.30**	-1.07**	-57.38	-13.33*	-21.06*	7.99	5.12*	16.58**	12.47*	-9.04**
	l (dd)	23.00**	-51.80*	-1.93**	0.27	-42.93	-22.31	75.83	79.10*	-3.83	-0.75**	-4.30**	-4.34**	3.29*
	χ2	203.25**	278.78**	141.85**	99.68**	132.55**	-119.96	-309.67	-305.20	-15.84	-7.34**	-15.29*	-9.65**	4.74*
	TE	-	C	D	-	-	337.92**	416.95**	222.21**	297.37**	2279.58**	66.12**	344.11**	60.33**
	M	81.00**	161.33**	7.93**	6.93*	5.31**	-	-	-	-	D	D	D	D
	d (a)	1.67*	-12.60**	-1.07**	-1.07**	-0.70**								
	h (d)	2.50*	118.90*	2.27*	2.23*	0.81	185.98**	200.00**	216.67**	45.51	7.30*	7.86**	7.96**	45.37*
Cross3	I (aa)	-0.67**	120.40*	1.87	1.87	0.92	-134.64	-38.33*	-32.53*	-8.14	1.56	1.54	0.33	2.84
	j (ad)	-1.50*	-6.70**	-1.20**	-1.23**	-0.88**	136.27*	40.00	39.50*	-33.09	-0.06**	-0.77**	1.53	-22.98*
	l (dd)	0.33	-115.13	0.27	0.33	1.66	133.80*	-30.00*	-44.93	-52.47	-1.46**	-1.52**	1.75	-20.78*
	χ2	92.11**	55.55**	50.43**	84.41**	205.71**	-138.75	-33.33*	-8.23	0.01	1.55	-0.18**	0.00	2.11
	TE	-	-	-	-	-	-232.21	106.67**	74.73**	96.60	8.53*	8.02**	2.36	24.58**
							249.67**	167.53**	148.43**	123.45**	85.18**	103.03**	115.55**	66.12**
							-	-	C		D	D	-	D

m= mean effect; [d] = additive; [h] = dominance; [i]= additive x additive; [j]=additive x dominance; [l]= dominance x dominance.

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STUDIES OF GENETIC VARIABILITY IN TOMATO (*Lycopersicon lycopersicum*) GENOTYPES UNDER MOISTURE STRESS AT BAGAUDA, KANO STATE

[1]Hamisu, H.S., [1]Afolayan, S.O., [2]Mohammed, M. S., [1]Idris, B.A., [1]Muhammad, S.M., Gwammaja[1] M. Y. [1]Yaduma, J.J., [2]Usman, A., [2]Saidu, G., Idris, A.U., [1]Hudu, A.H, [1]Hudu. M., [3]Dawaki, K.D., [4]Musa V.H. and [1]Giginyu, Y.D.

[1]National Horticultural Research Institute, P.M.B 3390, Bagauda Station, Kano State.
[2]Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, P.M.B 1044, Samaru, Zaria.
[3]Department of Crop Science, Kano University of Science and Technology, Wudil.
[4]Department of Crop Production, Faculty of Agriculture, Kogi State University, Anyigba, P.M.B. 1008, Anyigba, Kogi State, Nigeria
Corresponding Author e-mail: saleh00073hamisu@gmail.com

ABSTRACT

Drought stress due to climatic change causes significant fruit yield loss in tomato (Lycopersicon lycopersicum). Knowledge on the nature and extent of magnitude of genetic variability for various traits under moisture conditions; are pre-requisite for any crop improvement that will assist for selection tolerant genotype for reduced fruit yield losses due to drought stress in drought prone ecologies of Nigeria. A screen house experiment was conducted in completely randomized design with three repetitions under water stress and non-stress conditions during the 2012 dry season. Thirteen traits were measured on five plants for plant height, number of branches, number of leaves, leaf length, leaf width, leaf area, number of flowers, number of marketable and number of non-marketable fruits, total number of fruits per plant, average fruit weight, biomass and fruit yield. The estimates of PCV were higher than GCV for all studied traits. High GCV and PCV values were recorded for all traits suggesting high genetic variability for these traits. Broad-sense heritability ranged from 4.92% to 62.25%. The estimates of GA showed a wide range from 4.57 to 1089.51. High estimates of Broad-sense heritability coupled with high GAM were observed for the number of branches per plant, suggests additive gene effects for the hereditary pattern. From the results of the current study, simple selection of drought tolerance based on phenotypic performance can be achieved under moisture stress condition.

Keywords: *Drought, moisture, stress, tomato, GCV and PCV*

Introduction

The tomato is one of the most cultivated and consumed as a second most important vegetable throughout the world after potato (FOASTAT, 2005). Tomato is very rich in minerals, vitamins, essential amino acids and high level of lycopene anti-oxidants. In Nigeria, the major production areas of tomato lie between latitudes 7. 5°11' and 13.0° N, longitude in the Sahel-Sudan Savannah ecologies. Despite the wide cultivation and importance of tomato for improving health and income generation, drought stress is one of the major causes of fruit yield reduction in the Sahel and Sudan agroecologies as well as erratic rainfall and higher temperature due to elevating climate change. The present varieties

in use across the country are very sensitive to drought. Information of genetic variability tolerance to drought in available tomato germplasm is a pre-requisite for genetic improvement of tomato under moisture stress. This breeding effort will ensure increased production and productivity of tomato possessing economic fruit yield under drought environment. Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) are useful criteria for the assessment of variability among the crop spp. Heritability as the proportion of genetic variance to the total variance will assist in determining the influence of location in the expression of the trait and the extent to which improvement is feasible after selection (Robinson, *et al.,* 1949). However, heritability alone is not enough to make an efficient selection in segregating generation. High heritability accompanied by Genetic Advance (GA) is appropriate parameter for selection of a trait and serves as an indication of additive gene action for such trait. Showemimo *et al.* (2007) reported high GCV and PCV for number of fruits per plant, plant height and fruit weight at 14 days watering interval, while high broad sense heritability was recorded for these traits at the same watering interval among the tomato genotypes. (Hamisu *et al.*, 2016) observed high values of GCV and PCV for number of fruits per plant whereas high heritability values were recorded for number of fruits per plant and fruit yield per plant of tomato genotypes. High values of GCV and PCV were observed for number of fruits per plant, average fruit weight, marketable fruits and yield per plant; high Broad sense heritability coupled with GA were recorded for plant height, average fruit weight, number of fruits per plant and fruit yield (Ligade *et al.,* 2017). The study was conducted to estimate genetic variability of tomato genotypes under moisture stress so that drought tolerant genotype(s) would be identified and use as starting materials in the tomato drought tolerance breeding program.

Materials and Methods

Sixty-five tomato genotypes were evaluated using a completely randomized design with three repetitions in the screen house at the National Horticultural Research Institute, Bagauda research farm (11°33´N latitude and 8°23´E longitude of the equator) during the 2012 dry season. Bagauda is in the Sudan savanna whose climate is characterized by mean of annual rainfall of 830mm with mean daily temperature ranged from 18.4°C to 45°C. Seedlings were raised in nursery and transplanted into pots about 30 days after sowing (4-5 leaf stage). Non-stress plants were irrigated with 900mm of water a week, while stressed plants were irrigated with 300mm of water a week. The treatments were imposed two weeks after transplanting. Data were measured on three plants for plant height, number of branches, number of leaves, leaf length, leaf width, leaf area, number of flowers, number of marketable fruits, number of non-marketable fruits, total number of fruits per plant, average fruit weight, Biomass and fruit yield. Data obtained were analyzed using Statistix 10. The estimates of the Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV) and classified according to Shivasubramanian and Menon (1973) as: low = 0-10%, moderate = 10-20% and high = 20% and above. Broad sense heritability was calculated according Singh and Chaudhary (1985) and categorized according to Robinson *et al,* (1949) as follows: low = 0-30%, moderate = 30-60% and high = 60% and above, while Genetic Advance (GA) and Genetic Advance as a percent of Mean (GAM) were computed according to formula of Johnson *et al,* (1955).

Results and Discussion

The results of analysis of variance (Table 1) revealed highly significant (P = 0.01) differences for plant height, number of branches, number of leaves, number of fruits

per plant and number of marketable fruits and significant differences (P = 0.05) for leaf length, leaf width and leaf area, indicating presence of sufficient variability existing for the traits among genotypes selected for the study which can be exploited through selection and breeding under moisture stress. Similar results were reported by Dutta *et al.* 2018, Nwosu *et al.* 2018 and Dasta and Shimelis, 2021. Akhoundnejad 2020 recorded significant differences among three genotypes for fruit length, diameter and average fruit weight under drought stress and Oliveira *et al.* 2021 observed significant differences for number of fruits per plant, plant height, number of leaves and average fruit weight. However, number of flowers per plant, number of non-marketable fruits, average fruit weight and fruit yield per plant recorded non-significant differences revealing that selection cannot be practiced. Genotypic variance was higher than the environmental variance for plant height, number of branches, and number of leaves (Table 2), indicating more contribution of the genetic variance to the total variation and therefore these traits could be considered and be exploited for selection in early generations. Higher environmental variance than genotypic variance for leaf length, leaf width, leaf area, number of flowers, number of marketable fruits, number of non-marketable fruits, biomass, total number of fruits per plant, average fruit weight, and fruit yield were observed, revealing strong influence of the environment on the expression of these traits and therefore these traits could be exploited for selection in later segregation generations. Results corroborated with findings of Anuradha *et al.* 2020 and Dasta and Shimelis, 2021. Genotypic coefficient of variation and phenotypic coefficient of variation are important in studying of the nature and magnitude of variability of different traits, because it measures the range of variability which is prerequisite for any crop improvement. The phenotypic coefficient of variation values were higher than the genotypic coefficient of variation, thereby suggesting the strong influence of the environmental factors on the expression of the traits (Table 2). The results revealed that GCV value was high for plant height, number of fruits per plant, number of leaves, leaf area, number of flowers per plant, biomass, number of fruits per plant, number of marketable fruits, number of non-marketable fruits, average fruit weight and fruit yield per plant; indicating that genetic variance contributing more to total variation, hence selection could be carried out in earlier generations. Moderate GCV values were observed for leaf length and leaf width. Similar results were observed by Showemimo *et al.* 2007, Dutta *et al.* 2018, Nwosu *et al.* 2018, Anuradha *et al.* 2020 and Dasta and Shimelis, 2021. Highest estimates of broad sense heritability (Table 2) were observed for number of branches per plant (62.25%); whereas moderate heritability was recorded for plant height (50.09%), number of leaves (56.76%), leaf length (37.43%), leaf width (36.36%), leaf area (37.56%), number of fruits per plant (59.82%) and number of marketable fruits (58.20%) suggesting both additive and non-additive gene actions are important in influencing the expression of these traits under moisture stress. Low heritability was recorded for number of flowers per plant (24.44%), biomass (19.47%), number of non-marketable fruits (4.92%),, average fruit weight (27.71%), and fruit yield per plant (18.95%). Similar results for high heritability were reported for number of branches per plant and moderate heritability for plant height and number of leaves (Nwosu *et al.* 2018 and Anuradha *et al.* 2020). Low broad-sense heritability might be due to confounded effects of other stresses such as temperature and mineral imbalance; therefore, selection should be delayed to later segregating generations. However, high heritability alone is not adequate to show response to selection. Hence, high heritability coupled with high genetic advance as percent of mean are important parameters in response to selection of a trait. High broad-sense

heritability (62.25%) and genetic advance as percent of mean (69.45%) were recorded for number of branches per plant (Table 2), indicating additive gene effects for the inheritance of the traits. Anuradha *et al.* 2020 also recorded similar result of high broad-sense heritability and genetic advance as percent of mean for number of branches per plant. The trait can be considered and selected for further improvement through simple breeding methods like pure line, single seed descent and mass selection for higher fruit yield under moisture stress conditions.

Table 1: Mean squares for thirteen agronomic traits under moisture stress during 2012 dry season at Bagauda, Kano

Source of variation	Df	PHT (cm)	NBPP	NL	LL (cm)	LW (cm)	LA (cm^2)	NFLPP	BM (Kg)	NFRPP	NMFR	NNMFR	AFW (g)	FRYPP (Kg)
Genotype	64	176.88**	7.92**	14.06**	3.82*	1.43*	1.97*	3.6	997.4	234.5**	227.3**	0.61	21.18	2330
Error	128	88.28	2.99	6.08	2.39	0.91	1.23	2.72	803.2	94.2	95.02	0.58	15.31	1888.4

df = Degree of freedom, PHT = Plant height, NBPP = Number of branches per plant, LL = Leaf length, LW = leaf width, LA = Leaf area, NFLPP = Number of flowers per plant, BM = Biomass, NFRPP = Number of fruits per plant, NMFR = Number of marketable fruits, NNMFR = Number of non-marketable fruits, AFW = Average fruits weight and FRYPP = Fruit yield per plant.

Table 2: Genetic parameters for thirteen agronomic traits under moisture stress during 2012 dry season at Bagauda, Kano

Parameter	PHT	NBPP	NL	LL	LW	LA	NFLPP	BM	NFRPP	NMFR	NNMFR	AFW	FRYPP
Mean	25.83	3.00	7.75	4.70	2.91	2.38	1.74	27.87	8.82	8.49	0.33	5.55	50.82
σ_e^2	29.43	1.00	2.03	0.80	0.30	0.41	0.91	267.73	31.41	31.67	0.20	5.10	629.47
σ_g^2	29.53	1.64	2.66	0.48	0.17	0.25	0.29	64.73	46.76	44.09	0.01	1.96	147.20
σ_{ph}^2	58.96	2.64	4.69	1.27	0.48	0.66	1.20	332.47	78.17	75.77	0.20	7.06	776.67
GCV (%)	21.02	42.73	21.04	14.69	14.31	20.87	31.13	28.87	77.53	78.21	30.30	25.20	23.87
PCV (%)	29.70	54.16	27.93	24.01	23.73	34.05	62.96	65.42	100.24	102.53	136.64	47.88	54.84
h_b^2 (%)	50.09	62.25	56.76	37.43	36.36	37.56	24.44	19.47	59.82	58.20	4.92	27.71	18.95
GA	792.32	208.35	253.11	87.02	51.72	62.71	55.16	731.34	1089.51	1043.52	4.57	151.70	1088.07
GAM (%)	30.65	69.45	32.66	18.51	17.77	26.34	31.70	26.24	123.52	122.91	13.84	27.33	21.41

df = Degree of freedom, PHT = Plant height, NBPP = Number of branches per plant, LL = Leaf length, LW = leaf width, LA = Leaf area, NFLPP = Number of flowers per plant, BM = Biomass, NFRPP = Number of fruits per plant, NMFR = Number of marketable fruits, NNMFR = Number of non-marketable fruits, AFW = Average fruits weight and FRYPP = Fruit yield per plant.

Conclusion

The results revealed that, there was adequate and significant variability for majority of the traits among sixty-five tomato genotypes, which could be used through selection and improvement of the traits under moisture stress. Moderate heritability was recorded for most of the desired traits revealing both additive and non-additive gene actions are important in influencing the expression of these traits under moisture stress. High broad-sense heritability and genetic advance as percent of the mean were recorded for number of branches per plant, indicating additive gene effects for the inheritance of the traits. The trait can be considered and selected for further improvement through simple breeding methods like pure line, single seed descent and mass selection for higher fruit yield under moisture stress conditions.

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INVESTIGATION ON COMMUNITY-BASED INTERVENTION THROUGH COCKREL EXCHANGE PROGRAMME FOR SUSTAINABLE IMPROVED RURAL CHICKEN PRODUCTION IN NASARAWA STATE, NIGERIA

Mundi[1], H. L., Yakubu[2], A. and Mundi[3], I.

[1]Department of Animal Science, Faculty of Agriculture, Federal University Lafia.
[2]Animal Science Department, Faculty of Agriculture Shabu-Lafia Campus, Nasarawa State University Keffi.
[3]Department of Animal Breeding and Physiology, College of Animal Science, University of Agriculture Makurdi.
Author for correspondence: mundihariratladi@hotmail.com, contact: 08062125888

ABSTRACT

Local chickens play an important role for smallholder and contribute significantly to food security of households in rural and semi-urban communities. However, their productivity in Nigeria is low. The present study aimed at improving the performance of local chicken in Nasarawa State through cockerels exchange programed. A total of one hundred and eighty (180) 25-week old cockerels were obtained from a reputable farm in the State and distributed to some household. Fourty eight (48) households from Danka Sarki Extension Village (University Extension Model Village) and ten (10) households from College of Agriculture Lafia staff quarters were randomly selected. Each participating farmer (household) was given cockerels depending on the number given in return for his/her own cock(s). Pre and post trial information was obtained on flock size, number of chicks, growers, cocks and hens including number of clutch/bird, eggs/clutch, eggs hatched/clutch, mortality, body weight, body length, wing length, shank length, thigh length, thigh circumference, neck length, neck circumference and breast circumference. Average flock size (21.40±1.45 versus 17.42±1.12), growers (5.33±0.30 versus 3.98±0.26) and hens (4.10±0.39 versus 3.04±0.26) were higher in post-intervention birds. There were no significant (P>0.05) differences in the number of clutches/bird, number of eggs/clutch, number of eggs hatched per clutch and number of growers mortality. However, chicks, cocks and hens mortality was significantly (P<0.05) lower in post-intervention compared to pre intervention birds. Body (1.66±0.05 versus 1.46±0.04) and breast circumference (26.71±1.80 versus 22.89±1.18) of post interventions cocks were significantly (P<0.05) higher. The distribution of cockerels (improved/exotic birds) to the rural livestock farmers in the study area appeared to contribute to improved village chicken production.

Keywords: *body linear measurement, clutch size, flock structure, growth performance, mortality*

Introduction

Insufficient animal protein intake in developing countries according to FAO (1991) is still far below the recommended level of 67g per head per day of which 58% must be of animal origin. Nigerians consume only 5.5g of animal protein per person per day as against 38.86g per person per day recommended by FAO (1991). Protein intake, particularly of animal origin below recommended level has adverse consequences on health, productivity and development of the human being especially children, aged and pregnant women who are most susceptible to health challenges due to low protein intake. Diversification into improved production of local chicken via cockerel exchange could be a viable option in ameliorating shortage of protein intake among the populace in developing countries especially Nigeria (Muthukumar and Dev Roy, 2005). Village chickens are generally birds of indigenous breeds living

in almost symbiotic relationship with human communities (Yakubu, 2010) which are usually on free range. Out of a total of 72,400,856 chickens in Nigeria, 86.17% are on free-range (RIM, 1992). Development of village chicken production can be a useful way of helping to meet the nutritional, income, employment and gender needs of the rural population (Olwande *et al.*, 2010). The traditional system of chicken production is advantageous due to free feed resources in the surrounding environment and kitchen leftovers, ability to incubate and brood naturally (Pedersen *et al.*, 2002; Muchadeyi *et al.,* 2005). However, poor reproductive performance, poor growth rates, diseases and high mortality are some of the major constraints in smallholder village chicken production (Salum *et al.*, 2002; Conroy *et al.*, 2005; Yakubu, 2010). Ajayi *et al.* (2012) suggested the suitability of exotic chickens for crossbreeding programmes, which when mated with indigenous stocks, will ultimately improve the growth and carcass traits potentials of Nigerian indigenous chickens. Village chicken production in the Nasarawa State is faced with low productivity, poor feeding, insufficient capital and incidence of pest and diseases (Ajayi *et al.,* 2007a and b). In order to address the problem of low productivity of village chickens in Nasarawa State; crossbreeding, an elementary technique such as introduction can be used. The study is therefore aimed at determining the improvement of the performance of local chicken, their offspring and their adaptability to natural environment.

Materials and Methods

Location and Site of the Study

The experiment was carried out at two communities: Danka Sarki and College of Agriculture staff quarters in Lafia Local Government Area of Nasarawa State. Nasarawa State falls within the Southern Guinea Savannah zone of Nigeria. The state lies between latitude 7°c and 9°c North and Longitude 7°cand 10°cEast. It has a climate typical of the tropical zone because of its and a temperature ranging from 20°c in October to 36°c in March while rainfall varies from 13.73 cm in some places to 14cm in others (NiMET, 2019).

Management of cockerel on-station

A total of one hundred and eighty 25-week old cockerels were obtained from a reputable farm in Nasarawa State. The birds were reared on deep litter at the Livestock Unit of the Teaching and Research Farm, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus. They were fed growers' ration containing 16% CP and 2750kcal ME/kg from the 25th to 27th week of age. Fresh clean water was also supplied *ad libitum*. Routine management practices were strictly adhered to.

Selection of participating communities/households

48 households from Danka Sarki Extension Village (University's Extension Model Village) (48 households) and 10 households from College of Agriculture, Lafia (COAL) Staff quarters were randomly selected.

Training of selected village chicken farmers

Prior to the commencement of the on farm trial involving the distribution of cockerels to farmers, they were trained for one day to afford them the opportunity to understand the whole project concept. It also equipped farmers with techniques to follow after the project phase for the sake of sustainability of the project. Training covered various aspects relating to rural poultry management, supplementation and disease control.

Management of cockerel's on-farm

Each participating farmer (household) was given cockerels raised in the breeding centre depending on the number given in return for his/her own cock(s). The cockerels were managed under the traditional free range system where they mated randomly with village hens.

Data collection

For the on-farm trial (free range system), data were obtained on flock size, number of chicks, growers, cocks and hens including number of clutch/bird, eggs/clutch, eggs hatched per clutch, mortality, body weights of chicks and growers and production traits such as body size, Pre-trial information on village chicken production in the selected villages was obtained using structured questionnaires and interview schedules.

Statistical analysis

Data on flock composition were estimated using the mean procedure of SPSS (2010). The independent-samples T Test was used to compare data on pre-trial and post-trial (after 20 months of intervention) village chicken production in the selected areas.

Results

The flock composition (mean ± SE) of chickens in the study area is shown in Table 1. The average flock size (17.42±1.12 versus 21.40±1.45), growers (3.98±0.26 versus 5.33±0.30) and hens (3.04±0.26 versus 4.10±0.39) were significantly ($P<0.05$) higher in post-intervention than pre-intervention birds. However, there was no significant ($P>0.05$) difference in the average number of chicks (8.48±0.71 versus 9.94±0.81) and cocks (2.02±0.16 versus 2.44±0.23) before and after the introduction of exotic cockerels.

The performance characteristics of the birds are shown in Table 2. There were no significant ($P>0.05$) differences in the number of clutches/bird, number of eggs/clutch, number of eggs hatched per clutch and number of growers' mortality. However, chick, cock and hen's mortality was significantly lower in post-intervention birds.

The body weight (g±SE) and linear body measurements (cm±SE) of birds are shown in Table 3. The body weight and breast circumference of cocks were significantly ($P<0.05$) higher in post-intervention birds compared to their pre-intervention counterparts.

The body weight (g±SE) and linear body measurements (cm±SE) of hens are shown in Table 4. There were no significant ($P>0.05$) differences in all the body parts measured.

Table 1. Flock composition (mean ± SE) of chickens in Nasarawa State

Flock structure	Pre-intervention	Post-intervention
Flock Size	17.42±1.12[b]	21.40±1.45[a]
Chicks	8.48±0.71[a]	9.94±0.81[a]
Growers	3.98±0.26[b]	5.33±0.30[a]
Cocks	2.02±0.16[a]	2.44±0.23[a]
Hens	3.04±0.26[b]	4.10±0.39[a]

[ab] Means in the same row for each parameter with different superscripts are significantly different (P<0.05)

Table 2. Performance indices (mean±SE) of chickens in Nasarawa State

Parameters	Pre-intervention	Post-intervention
No of clutches/bird/year	3.15±0.08[a]	3.27±0.10[a]
No of eggs/clutch	10.50±0.20[a]	10.94±0.22[a]
No of eggs hatched/clutch	8.54±0.21[a]	8.60±0.21[a]
No of Chick mortality/clutch	2.65±0.19[a]	2.19±0.14[b]
No of Grower's mortality/clutch	1.17±0.14[a]	0.92±0.12[a]
No of Cock's mortality/clutch	0.30±0.0[a]	0.15±0.05[b]
No of Hen's mortality/clutch	1.15±0.17[a]	0.52±0.10[b]

[ab]Means in the same row for each parameter with different superscripts are significantly different (P<0.05).

Table 3. Morphometric traits of cocks in Nasarawa State

Traits	Pre-intervention	Post-intervention
Body weight	1.46±0.04[b]	1.66±0.05[a]
Body length	26.55±0.78[a]	28.55±0.86[a]
Wing length	15.93±0.36[a]	16.76±0.36[a]
Shank length	6.95±0.18[a]	7.38±0.19[a]
Thigh length	15.32±0.47[a]	16.48±0.49[a]
Thigh circumference	7.35±0.21[a]	8.02±0.31[a]
Neck length	7.75±0.14[a]	7.89±0.15[a]
Neck circumference	14.81±0.15[a]	15.19±0.15[a]
Breast circumference	22.89±1.18[b]	26.71±1.18[a]

[ab] Means in the same row for each parameter with different superscripts are significantly different (P<0.05).

Table 4. Morphometric traits of hens in Nasarawa State

Traits	Pre-intervention	Post-intervention
Body weight	1.43±0.04	1.53±0.05
Body length	24.77±0.62	26.47±0.80
Wing length	15.03±0.30	15.93±0.40
Shank length	6.55±0.16	6.92±0.18
Thigh length	14.19±0.38	15.18±0.47
Thigh circumference	6.65±0.22	7.26±0.29
Neck length	7.30±0.08	7.53±0.14
Neck circumference	14.47±0.13	14.81±0.15
Breast circumference	21.81±0.77	24.22±1.11

Means in the same row for each parameter are not significantly different (P>0.05).

Discussion

Livestock keepers are a rich source of information about breeds and production systems and also important diseases which affect their animals (Abdelqader *et al*., 2007; Yakubu *et al*., 2012). Backyard producers have yet to make any major commitment to improve their chicken productivity (Sambo *et al*., 2015). The performance values obtained in this study are higher than those to those reported by Yakubu (2010). The average flock size is also higher than the value (13.4±1.10) reported for chickens in Ghana (Blackie, 2015). Since the mortality rate was lower in chicks, cocks and hens during post-intervention, it implies that more birds will reach the adult stage. This will lead to the availability of more birds for procreation with probable increase in number of offspring and birds that will be taken for sale and consumption. The superior body weight and breast circumference in post-intervention birds is an indication that the birds may attract better price when taken to the market.

The better performance of birds crossed with cockerels could be attributed to heterosis, which tend to confer great advantage on the progeny as a result of the combination of the good productive ability of the exotic cockerel and the adaptive capability of the Nigerian indigenous chickens. According to Rajkumar *et al.* (2011), heterosis is almost exclusively the aggregate of all single locus dominance effects, and because these are usually positive or beneficial, heterosis can be expected to be usually in the favorable direction

Conclusion

The distribution of cockerels (improved exotic birds) to the rural livestock farmers in the study areas appeared to contribute to improved village chicken production with reduced number of chick, cock and hen's mortality including better body weight and breast circumference in cocks in post

intervention birds. Cockerel exchange programed may be a way out of poverty at the village level. Therefore, it is recommended that Nasarawa State government and other policy makers should endeavor to encourage farmers to adopt the cockerel exchange programmed holistically because of its potential towards better chicken performance. However, efforts should be made to ensure that the germplasm of the Nigeria indigenous chickens is conserved for future use.

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CGBPB 051

IDENTIFICATION OF INBRED AND HYBRID TESTERS IN MAIZE (*Zea mays* L.)

M.A. Abubakar, *M. Oyekunle, and I.E. Ahmed

Department of Plant Science, Institute for Agricultural Research, A.B.U, Zaria
*Corresponding Author: moyekunle@abu.edu.ng; +2348059884805,

ABSTRACT

Maize (Zea mays L.) is an important staple food crop in Nigeria. Information on the combining ability and identification of testers is crucial for hybrid development. This study was conducted to determine mode of gene action for grain yield and identify testers of newly developed maize inbred lines. Nine inbred lines were mated in diallel mating design. The 36 crosses and four checks were evaluated in two environments at Zaria. The results of analysis of variance showed significant difference (P<0.05) among genotypes for grain yield and most traits across environments. Specific coming ability sum of square were larger than general combining ability for most measured traits, indicating importance of non-additive gene action in controlling the traits. Inbreds SMLP-45, SMLP-50 and SMLP-61 and hybrids SMLP-50 × SMLP-58 were identified as best inbred and hybrid testers. Testers identified should be used for grouping of other inbreds in tropical maize hybrid breeding programs.

Keywords: *Combining ability; Diallel cross; Heterotic groups; Zea mays L.*

Introduction

Maize (*Zea mays* L.) is a major staple food crop in sub-Saharan Africa. Its high energy and of recent protein content has made it very important and alternative in human and animal diets. Its production is hindered with quality and productive seeds. However, maize production can be improved with the adoption of maize hybrids. To promote rapid development and the deployment of hybrids, it is important to determine the usefulness of available inbred lines in hybrid combinations through combining ability studies (Badu-Apraku *et al.* 2013). Information on the combining ability of newly developed inbred lines is necessary to guide breeding strategies. Classifying inbred lines into different heterotic groups will help to reduce the development and evaluation of less productive crosses, while exploiting maximum heterosis by crossing opposing inbred lines (Terron *et al.*, 1997).

Maize scientists at Institute of Agricultural Research (IAR), Samaru has developed several locally adapted maize inbred lines. Information on mode of gene action, and appropriate testers is completely lacking, therefore, this study was design to (i) determine mode of gene action for grain yield and (ii) identify inbred and hybrid testers of newly developed maize inbred lines.

Materials and Methods

The experiment was carried out at Zaria, located in the Northern Guinea Savanna of Nigeria (Latitude and longitude). Nine inbred lines obtained form IAR were mated in diallel mating design to generate 36 crosses. The 36 crosses along with four checks (Name the checks here) were evaluated at two environments (where and where?). In each environment, the experiment was planted in 5 x 8 lattice design replicated two times. Each

entry was planted in a row plot of 5 m long and 0.75 m apart with a distance of 0.50 m between plants within a row. The trials were hand planted with three seeds per hill, which

later were thinned to two seed per hill to get a total plant population of 53,333 per hectare.
Compound fertilizer (NPK 15-15-15) was applied two weeks after planting (WAP) at the rate of 60 kg N, P_2O_5 and K_2O per ha-^{1} and additional 60 kg N ha-^{1} were applied as top dressing six weeks after planting (WAP). The trial was kept weed-free by application of atrazine and gramoxone as pre- and post-emergence herbicides at 5 l/ha^{-1} follows by hand weeding.
Data were collected on grain yield (kg/ha), days to anthesis, days to silking, anthesis-silking interval, plant height (cm), ear height (cm), number of ears per plant (EPP), plant aspect, and ear aspect. Data collected were analyzed using SAS. The proportionate contribution of each agronomic trait was computed as percentage of the sum of squares for the crosses, modified Baker (1978) by Hung and Holland (2012). The SCA effects for grain yield were used for classifying maize inbreds into heterotic groups. (Vasal *et al.* 1992). The criteria proposed by (Pswarayi and Vivek 2008; Badu-Aparaku *et al.*, 2013) was adopted to identify inbred and hybrid testers.

Result and Discusion

In the combined ANOVA of the diallel crosses, the mean squares for environments and hybrids revealed significant for all measured traits across test environment except mean squares crosses for EPP, (Table 1). The significant variation observed among hybrids for grain yield and most measured traits across environments indicated that adequate genetic variation existed among the hybrids. The presence of genetic variation among the hybrids implied significant progress could be made from selection for improvements in grain yield and yield related traits for the development of productive maize hybrids. The presence of significant environmental variation for all measured traits was a demonstration of the uniqueness and variability of the environments emphasized

the need for testing the genotypes in several environments. Partitioning of the variations due to genotype into components revealed that GCA mean squares were significant for all measured traits except ear height and EPP (Table 1). Similarly, SCA mean squares were significant ($p < 0.05$) for all measured traits except for plant aspect, ear aspect and EPP indicating the importance of additive and non-additive gene action for the measured traits.
Significant ($p < 0.05$) positive GCA effects for grain yield were observed for inbred SMLP-61 (Table 2). In contrast, SMLP-86 had significant ($p < 0.05$) negative GCA effects for grain yield. The observed significant positive GCA effects for grain yield for inbred lines SMLP-61 across environments suggested that inbred lines would contribute favourable alleles for improved grain yield to their progenies.
You presented GCA x Env and SCA x Env. in the Table No results presentations and discussions was made
Baker's ratio was used to determine the relative importance of GCA and SCA effects. The predictability of mode of gene action based on GCA Baker, (1978) is higher when the ratio is almost equal to one. The SCA sums of square were larger than those of GCA sums of square for all measured traits except days to anthesis, days to silking and plant aspect indicating the predominance of non-additive gene actions in controlling these traits.
Vasal *et al.* (1992) showed that in the classification of inbred lines into heterotic groups, those with positive SCA effects are in opposite heterotic groups and those with negative SCA effects belong to the same heterotic groups. Based on the direction of SCA effects of grain yield and the absolute value of the difference between SCA effects of

a parent cross with SMLP-45 and SMLP-50 (Table 3), inbred lines are classified into three heterotic groups, group I comprises of SMLP-

10, SMLP-45 and SMLP-86 group II consist of SMLP-36, SMLP-50, SMLP-58 and SMLP-81 and group III are SMLP-4 and SMLP-61

The inbred in each heterotic group may be recombined to form heterotic populations which could be improved through recurrent selection.

Table 1. Mean squares of analysis of variance for grain yield and other agronomic traits of maize hybrids evaluated across two environments at Zaria in 2019.

Source of Variation	DF	Grain yield, (kg/ha^{-1})	Days to anthesis	Days to silking	Plant height, cm	Ear height, cm	Plant aspect	Ear aspect	EPP
Environment	1	49743564.41**	656.10**	705.60**	3020.64**	6039.31**	50.63**	6.60**	0.17*
Replication (Env.)	2	525073.80	0.43	0.93	292.31	156.67*	0.70*	0.45	0.13*
Block (Rep .× Env.)	16	1937435.24**	8.73	8.68	471.59**	177.68**	0.29	0.33	0.06*
Genotype	39	1853264.70**	29.73**	27.53**	344.16**	110.92**	0.79**	0.36*	0.04
GCA	8	2053265.63*	81.72**	73.58**	475.25*	100.97	1.92**	0.54*	0.06
SCA	27	1442892.28*	15.69**	14.24**	449.29**	162.24**	0.47	0.30	0.03
Genotype × Env.	39	657914.21*	5.27	5.52	187.90*	104.19**	0.60**	0.17	0.03
GCA × ENV	8	708941.86*	3.54	2.91	236.27	159.34*	1.34**	0.28	0.04
SCA × ENV	27	790622.20*	6.41	6.07	183.11	107.59	0.47	0.18	0.02
Error	62	723550	5.27	5.47	113.9	42.16	0.28	0.21	0.03

**: highly significant difference at (P≤0.01) probability level, * significant difference at (P≤0.05) probability level, DF= Degree of freedom ENV= Environment, Rep= Replication, GCA= General Combining Ability, SCA= Specific Combining Ability

Table 2. GCA Effects of inbred parents for grain yield and other agronomic traits evaluated across two environments at Zaria in 2019.

Lines	Grain yield, (kg/ha^{-1})	Days to anthesis	Days to silking	ASI	Plant height, cm	Ear heigt, cm	Plant aspect	Ear aspect	EPP
SMLP-4	-51.03	0.55	0.44	-0.028	0.25	-0.74	0.27	0.18	-0.02
SMLP-10	-57.75	-3.50**	-3.26**	0.294*	-6.05*	-0.61	0.13	0.15	-0.01
SMLP-36	-346.51	-0.63	-0.45	-0.028	-0.35	-1.48	0.41**	0.05	-0.06
SMLP-45	223.99	-1.13	-1.17	0.044	-5.87*	-2.63	-0.30	-0.02	0.10**
SMLP-50	201.68	-0.06	-0.34	-0.206	4.67	3.11	-0.18	0.10	-0.05
SMLP-58	63.30	-0.45	-0.56	-0.099	4.99*	0.39	-0.05	-0.04	0.02
SMLP-61	410.74*	0.55	1.08	0.187	4.61*	3.08	-0.39*	-0.18*	0.02
SMLP-81	-11.67	2.12**	1.76**	-0.206	0.77	-0.44	0.00	-0.24**	0.01
SMLP-86	-432.75*	2.57**	2.49**	0.044	-3.02	-0.70	0.10	0.00	-0.01
LSD(0.05)	345.95	0.78	0.70	0.34	6.12	5.19	0.48	0.22	0.08
Baker's ratio	0.30	0.61	0.60	0.18	0.24	0.16	0.55	0.35	0.38

**: highly significant difference at (P≤0.01) probability level, * significant difference at (P≤0.05) probability level, GCA= General Combining Ability, SCA= Specific Combining Ability, LSD=Least Significant Difference

Table 3. SCA of grain yield absolute value differences between SMLP-45 and SMLP-50

Lines	SMLP-4	SMLP-10	SMLP-36	SMLP-45	SMLP-50	SMLP-58	SMLP-61	SMLP-81	SMLP-86	Groups
SMLP-4		-482	557	382	-405	-380	-757*	810*	276	C
SMLP-10			-62	-757*	-637	827*	187	549	376	A
SMLP-36				795*	64	81	-15	-1062*	-357	B
SMLP-45					857*	-593	357	-260	-780*	A
SMLP-50						-640	260	33	468	B
SMLP-58							713	186	-195	B
SMLP-61								-606	-138	C
SMLP-81									350	B
SMLP-86										A
SMLP-45	382	-757	795		857	-593	357	-260	-780	
SMLP-50	-405	-637	64	857		-640	260	33	468	
Absolute value differences	789	120	731	857	857	47	97	293	1248	
LSD(0.05)	644	644	644	644	644	644	644	644	644	

The choice of potential lines as testers for classifying other lines into heterotic groups was based on the (a) display of high positive GCA effects of grain yield, (b) classification into heterotic groups, and (c) per se grain yield, (Pswarayi and Vivek 2008). The identification of a single-cross tester among the hybrid was based on (a) display of reasonably good GCA effects of the inbred lines constituting the single cross; (b) grouping of the inbred lines constituting the single crosses to the same heterotic group; (c) a reasonably good yielding ability of the potential single-cross tester to qualify its use as a seed parent in successful three-way and double-cross hybrids for high seed production, (Badu-Aparaku *et al*., 2013). Base on this criteria therefore, inbreds SMLP-45, SMLP-50 and SMLP-61 and hybrids SMLP-50 × SMLP-58 were identified as best inbred and hybrid testers for different heterotic implied that the inbred could be used as parent to classify the other lines into heterotic groups and to develop high-yielding hybrids

In conclusion, additive and non-additive gene actions are important with non-additive gene action plays a predominant role in the inheritance of grain yield and most traits in the set of the inbreds used. Inbred lines were classified into three heterotic groups. Inbred parents SMLP-45, SMLP-50 and SMLP-61 and hybrid SMLP-50 x SMLP-58 were identified as inbred and hybrid testers.

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CGBPB 052

GENETIC ANALYSIS OF DROUGHT-RELATED TRAITS IN MAIZE (*Zea mays* L.) INBREDS AT SEEDLING STAGE

I.E. Ahmed, *M. Oyekunle, and M.A. Abubakar

Department of Plant Science, Institute for Agricultural Research Samaru, Ahmadu Bello University, Zaria.
Corresponding Author: Email: moyekunle@abu.edu.ng; +2348059884805

ABSTRACT

Information on the mode of gene action for drought tolerance at seedling stage is essential for development of drought tolerant maize genotypes that is currently limited. The study was conducted to determine the mode of gene action in the inbred lines. Nine inbred lines were crossed in diallel mating scheme to generate 36 hybrids. The hybrids plus four checks were evaluated at the screen house at Samaru in 2019 using randomize complete block design with two replications. Data were collected on what? and at what stage of development? The results revealed a significant ($p < 0.05$) different among hybrids for fresh shoot weight, fresh root weight, number of death leaves per plant, number of leaves per plant, seedling height and leaf area. There was preponderance of GCA over SCA which implied that additive gene action controlled the inheritance of the traits in the inbreds. Hybrids, SMLY-186 x TZEI-17 and TZEI-29 x TZEI-11were identified as the most drought tolerant at seedling stage.

Keywords: *Additive gene action, Diallel mating scheme, Drought, Maize hybrids*

Introduction

Maize (Zea mays L.) belongs to the grass family Poaceae, commonly known as corn. It is the third most important food crop worldwide (Frova *et al,* 1999). It is used in many ways than any other cereal. Therefore it is considered as a multi-purpose crop and has been put to a wider range of uses such as human food, animal and poultry feed and for hundreds of industrial purposes. It is an important cereal crop worldwide and is ranked third after wheat and rice for its nutritional quality and uses (Cassamon, 1999: Ali et al., 2014ab). Abiotic stresses like moisture stress, high and low temperature stress, salinity; nutrient stresses etc. frequently limit growth and productivity of major crop species such as maize, rice, sorghum etc. Biotic stress factors are caused by pathogens, insect pests, weeds or intraspecific competition resources (Hill *et al.*, 1998). Drought stress is very unpredictable and it occurs at any developmental stage of the growth cycle of a plant.

Previous breeding projects have been mainly focused on drought tolerance at flowering and grain filling stages; consequently giving less attention to seedling drought stress tolerance (Qayyum et al., 2012). Drought stress occurring at seedling stage can reduce crop stand (Khayatnehad et al., 2010). Methodology of screening maize genotype for drought tolerance at seedling stage has been reported (Akinwale et al, 2016). It is therefore necessary to evaluate some maize inbred lines and hybrids for rooting and seedling traits under drought condition using the established screening methodology. The objectives of this study were to; (i) determine the mode of gene action in the inbred lines and (ii) determine the relationship among drought related-traits at seedling stage.

Materials and Methods

Nine inbred lines were crossed in diallel mating scheme to generate 36 hybrids. The 36 hybrids

plus 4 checks were layout in a randomized complete block design with two replications at the screen house Department of Plant Science Faculty of Agriculture ABU Samaru, Zaria. The polytene bags were filled with 5 kg of soil and 5 seeds of each experimental material were planted per bag. Water was applied to each bag at the rate of 0.6 liter for the period of three weeks. drought condition while water was supplied to the treatment under optimum condition throughout the period of experiment 6 (WAP). Emergence of seedling were assessed from 4 days after planting (DAP) to 9 DAP. All the agronomic practices were carried out as when due. At 42 days when the experiment terminated, the plants were carefully uprooted from each polytene bag and the roots were washed under a running tap water to remove the sand. The roots were detached from the shoots at the cotyledonary node, and data were recorded on the following seedling traits; number of leave, seedling height, (cm) leaf area (cm^2), number of plant per bag, Number of leaves per plant, number of dead leaves, primary root length (cm), fresh shoot weight (g), fresh root weight (g), dry shoot weight (g), dry root weight (g), weights of fresh biomass and dry biomass.

The data collected was subjected to analysis of variance using generalized linear model procedure of SAS package (SAS, 2009). Computation procedures were based on linear model for RCBD design._GCA effects of the parents and SCA of the crosses were estimated in the diallel crosses following Griffing's method 4 model 1 (Griffing, 1956). Identification of drought tolerant hybrids was done using rank summation index (RSI) were computed following Mulumba and Mock (1978), and Oyekunle and Badu-apraku (2017) and Phenotypic and genotypic correlations were estimated using the standard procedure suggested by Kashiani and Saleh (2010) from the corresponding variance and covariance components using the equations.

Results and Discussion

The results of analysis of variance revealed a significant ($p< 0.05$) different among hybrids for fresh shoot weight, dry shoot weight, fresh biomass, dry biomass, number of leaves per plant, number of death leaves per plant, and number of leaves at 4 and 5 (WAP), leave area at 4 (WAP) and number of death leave at 4 and 5 (WAP) (Table 1). The presence of genetic variability in the study materials indicates that improvement can be made through selection. The presence of significant mean squares for GCA and SCA for some of measured traits under both research conditions indicates the importance of additive and non-additive gene action for controlled traits. However, the preponderance of GCA over SCA for the measured traits under drought condition indicates that additive gene effect play major roles in controlling the traits under both research conditions.

The rank summation index (RSI) of best 10 single-cross hybrids and worst performing single-cross hybrids range from 63.0 to 267.0 for SMLY-86 x TZEI-11 and TZEI-23 x TZEI-129 hybrids under drought condition and from 271.0 to 363.0 for SMLY-86 x SMLY-186 and SMLY-84 x SMLY-86 to 363.0 for SMLY-84 x SMLY-86 (data not shown). Under optimum condition, the rank summation index (RSI) range from 66.0 to 159.0 for SMLY-41 x TZEI-17 and SMLY-186 X TZEI-17 hybrids and ranges from 220.0 to 332.0 for SMLY-84 x SMLY-186 and TZEI-129 x TZEI-17 to 332.0 for TZEI-129 x TZEI-17 for worst hybrids under optimum condition (data not shown). The hybrids were ranks based on their RSI, thus, the lower the RSI of any individual or hybrid the greater is its drought tolerant. This finding is in agreement with those of Umoh *et al.* (2020) who reported similar result on the variation, ranking and component analysis of ten maize population using the method of Mulumba and Mock (1978), and Oyekunle and Badu-apraku (2017) reported similar result on agronomic performance of drought-tolerant maize hybrids in diverse environments of lowland tropics.

The result of correlation analysis among the traits measured revealed that number of leave per plant is significantly and negatively correlated with dry shoot weight (-0.29**), dry root weight (-0.41**), and dry biomass (-0.39**), plant aspect (-0.28*), dry root weight (-0.28*), fresh biomass (-0.25*) and dry biomass (-0.27*) under drought stress and negatively correlated with fresh root weight (-0.31*) under optimum growing condition. Fresh shoot weight had significant positive correlation with all the measured traits under both conditions except primary root length and number of leave per plant under drought condition and plant aspect and number of dead leaf under optimum condition. However, fresh root weight had significant positive correlation with dry shoot weight (0.25*), dry root weight (0.56**), fresh biomass (0.65**) and dry biomass (0.39**) under optimum condition and significant positive correlation with fresh shoot weight (0.42**) under drought condition. Likewise, under optimum condition dry shoot weight had positive correlation with fresh biomass (0.35**) and dry biomass (0.95**). The phenotypic relationships that exist between root and shoot traits suggested that seedling roots and shoots traits could be used as indirect selection criterion for drought tolerant genotype at seedling stage.

In conclusion, additive gene action was found to control most of the measured traits of inbreds at seedling stage and seedling aspect and other secondary traits could be used in the base index to identify drought tolerant at seedling stage.

Table 1. Mean square analysis of variance for measured traits hybrids under drought stress evaluated at Samaru in 2019.

Source of variation	DF	Number of leave		Seedling height		Leave area		Root	Plant	Fresh	Fresh	Dry	Dry	Fresh	Dry biomass	% number
		4WAP	5WAP	4WAP	5WAP	4WAP	5WAP	Length, cm	aspect	shoot weight	root weight	Shoot weight	root weigh	biomass		Of death leave Per plant
Replication	1	0.8	0.1	162.3	1155.4	241.8	4156.1*	0.1	6.5	1853	361.3	35.6	85.3	3850.6	231.1	88.6
Genotype	39	0.5*	1.2*	82.9	97.7	1156.5*	1476.9	68.2	2.3	250.5	21.1	8.5	2.6	315.3	13.9	66.7
GCA	8	0.3*	1.2*	100.9	177.8*	1613.3	2608.9**	62.4	1.6	100.2	26.8*	1.9	1.8	133.5	4.8	74.3
SCA	27	0.4*	0.7*	71.6	105.7	829.4	1617.2*	85.3	1.5	132.1	23.6*	3.8	1.6	196.7	7.3	67.2
Error	39	0.4	0.3	55.2	70.2	579.8	1395.2	63.3	1.3	251.4	31.6	7.6	2.8	350.4	15.4	66.7

**: highly significance difference at (P 0.01) probability level, * significance difference at (P 0.05) probability level, DF = degree of freedom PASP = plant aspect, PRL = primary root length, TNLP = total number of leaf per plant, FSW and FRW, and DSW and DRW = fresh and dry shoot and root weight, FBM and DBM = fresh and dry biomass, FRSR and DRSR = fresh and dry shoot to root ratio, GCA = general combining ability, SCA = specific combining ability

Table 2. Correlation among the measured traits of single-cross hybrids evaluated under induced drought stress (below diagonal) and under optimum growing conditions (above diagonal) in Samaru in 2019.

	Number of leave per plant	Root length, cm	Fresh shoot weight	Fresh root weight	Dry shoot weight	Dry root weight	Fresh biomass	Dry biomass	Plant aspect	Number of death leave per plant
TNLP		0.11	-0.09	-0.31**	0.01	-0.23	-0.21	-0.07	-0.23	-0.19
PRL	-0.05		0.19	0.09	0.07	0.12	0.19	0.09	0.12	-0.02
FSW	0.19	0.17		0.32**	0.31**	0.53**	0.93**	0.43**	0.01	-0.24*
FRW	-0.05	0.19	0.42**		0.25*	0.56**	0.65**	0.39**	-0.22	-0.15
DSW	0.21	0.17	0.78**	0.48**		0.46**	0.35**	0.95**	0.12	-0.19
DRW	0.22	0.27*	0.46**	0.64**	0.46**		0.65**	0.71**	0.13	-0.27*
FBM	0.15	0.19	0.97**	0.64**	0.81**	0.57**		0.49**	-0.08	-0.25*
DBM	0.24*	0.24*	0.76**	0.64**	0.91**	0.79**	0.83**		0.14	-0.27*
MST_CNT	-0.11	-0.15	0.23*	-0.09	-0.29*	-0.41**	0.17	-0.39**	-0.21	-0.01
PASP	-0.28*	0.02	-0.29*	-0.08	-0.21	-0.28*	-0.27*	-0.27*		
TNDL	-0.19	-0.02	-0.29	-0.15	-0.19	-0.27*	-0.25*	-0.27*	0.52**	

**: highly significance difference at (P 0.01) probability level and *: significance difference at (P 0.05) probability level
PASP = plant aspect, PRL = primary root length, TNLP = total number of leaf per plant, FSW and FRW, and DSW and DRW = fresh and dry shoot and root weight, FBM and DBM = fresh and dry biomass, FRSR and DRSR = fresh and dry shoot to root ratio.

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CGBPB 053

ESTIMATION OF GENETIC PARAMETERS FOR SOME AGRO MORPHOLOGICAL TRAITS AMONG NIGERIA EGUSI MELON GENOTYPES.

Gado, A.A., Muhammad, M.L., Daudu, O.A.Y., Dangana, M.C., Abubakar, A.

Kolo, J.T.

Department of Plant Biology, Federal University of Technology, Minna, Niger State, Nigeria.
*Corresponding author. E-mail: gado.aishatu@futminna.edu.ng +234 8036218125

Abstract

In order to assess some genetic parameters among Nigerian Melon genotypes; the Melon genotypes were evaluated for their morphological and yield attributes at the Department of Biological sciences experimental garden, Federal University of Technology, Minna during 2015/2016 and 2016/2017 growing seasons, using a complete Randomized Block Design (CRBD) with three replicates. The agromorphological parameters were investigated using standard procedures. The results on the agromorphological parameters showed significant difference ($p \leq 0.05$) for most of the parameters studied. The study revealed that all of the agromorphological parameters were influenced by genetic factors such parameters are suitable for selection. Higher estimate for genotypic variances than environmental variances were observed for all the parameters which indicate good characters for selection and improvement of the crop. The highest genetic advance as percentage of mean (2924.2%) was obtained for weight of fruits ; whereas, number of seeds per fruit had the lowest (14.40%). High values of broad sense heritability estimates were observed for plant height at week 4 up to maturity, number of flowers per plant, number of flower buds per plant, days to germination and number of fruits per plant. Therefore, combination of high heritability estimates with genetic advance in the selection program is vital for selection of the crop in the future. Emphasis should be made on those agro-morphological parameters that shows greater genetic importance for selection and improvement of the crop in Nigeria.

Keywords: *Genotypic variance, Phenotypic variance Heritability, Egusimelon*

Introduction

Citrullus colocynthis (L.) is a variety of melon seeds, which is popularly called 'egusi' in West Africa. It belongs to a large family called Curcubitaceae, which consist of 119 genera and about 925 species. It is one of the most important vegetable crops in the tropical, subtropical and Mediterranean zones of the world (Schippers, 2000). It is a native of Africa, which has perhaps been introduced to Asia, Iran and Ukraine (Schippers, 2000). Its common names include egusi in Yoruba, agushi in Hausa, epingi or paragi in Nupe and eashi in Gwari. Dialect names for this crop include egusi-itoo. It produces climbing vines up to 4 meters long, which are covered with stiff hairs. The heart-shaped or roughly palmate leaves are up to 12 centimeters long and 14cm wide. It bears small yellow male and female flowers with petals less than a centimeter in length. The fruit is egg-shaped or an elongated ovate shape, up to about 19 centimeters long and 8cm wide, and cream in colour with green streaks. The plant is a creeping annual plant and an intercropping plant used in traditional farming practices; it grows well on light rich soil in the hot climatic regions of Africa. It has been known to tolerate low rainfall. In the Southeastern part of Nigeria, the crop is best cultivated after the first rainfall of the year (Akpambang *et al.*, 2008). Thirteen weeks after planting the first fruits are harvested. The different species of Cucurbitaceae have served humans for over 10,000 years as important food and as source of many useful products (Ajuru and Okoli, 2013). In Nigeria, they are used for different purposes in different parts of the country. It is

important to improve the productivity of the crop to satisfy the demands of dietary needs and raw materials for industrial processing to edible oil and livestock feedstuff through breeding programs. The success of increasing the productivity of any crop through breeding largely depends on the presence of variability among the breeding materials (Adeyemo and Ojo, 1991). Broad genetic variability is the basis for successful plant breeding and the successful development of adaptations to environmental conditions. Generally breeding programs depends on knowledge of the nature and magnitude of variations in the available materials, magnitude of association of characters with yield, extent to which these characters are heritable as well as extent of environmental influence on them (Aruah *et al.,* 2012; Ndukauba *et al.,*2015). Various morphological and physiological characters contribute to yield. Each of these component characters has its own genetic systems. Further, these yield components are influenced by environmental fluctuations. Therefore, it is necessary to separate the total variations into heritable and non-heritable components with the help of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability and genetic gain (Maniee *et. al*., 2009). Furthermore, knowledge of the association between yield and its components can improve the efficiency of selection in plant breeding (Izge *et al*., 2001). This study was undertaken to estimate the genetic variability, heritability, character association among the different egusi-melon genotypes.

MATERIALS AND METHODS

The morphological parameters were investigated using standard procedures after the techniques of Akinyele and Osekita (2006); Hegazi and Hamideldin (2010); Idehen *et al.* (2014). Specifically, the days to germination (DG) were determine as the interval between sowing of seeds and day a germinating seedling emerges above soil level. The number of leaves per plant (NL) at maturity was determined by counting the number of leaves attached to the plants. The length of vine of the plants at two weeks interval up to maturity was measured in centimetres (cm) using a metre rule. Sexual maturity (SM) was determined as the interval between emergence of seedling and appearance of flowers. For each of the morphological parameters mentioned above, mean value per plant was determined. The leaf colour and seed colour were determined using a Royal horticultural colour chart; leaf shape and seed shape were determined using a chart. Leaf texture was determined using fingertips (IPGRI, 2003).

The yields from the different accessions of Melon were determined using the following indices: number of fruits per plant (NF), number of seeds per pod (NSP), and weight of fruit (WF). For NSP and WF, ten fruits each were selected at random for all the accession and the values were recorded for further statistical analysis.

NF were determined by counting the total number of fruits a plant produced at the completion of the life cycle. NSP were determined by opening the fruit and counting the number of viable seeds which were determined by their relatively large size and firmness. WF were determined by measuring the pods on a weighing balance, mean values of yield parameters per fruit or plant were determined for the Melon plants.

Genetic Parameters Estimates

Broad Sense Heritability (h^2) was estimated according to Falconer (1989) using:

$$h^2 = \frac{\sigma 2g}{\sigma 2ph} \qquad \text{(equation1)}$$

Where σ^2g is the genotypic variance; σ^2ph is the phenotypic variance. Phenotypic and Phenotypic variances were obtained from the analysis of variance table using equations 2 and 3 as follows:

$$\sigma^2 g = \frac{MS1 - MS2}{rXs} \quad 2$$

$$\sigma^2 ph = \frac{MS1}{rXs} \quad 3$$

(Where r: replication, s: season, MS1: Mean square for cultivar, MS2: Mean square for cultivar X season).

The mean values were used for genetic analyses to determine Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV), using equation 4 and 5 as follows:

$$GCV\ (\%) = \frac{\sqrt{\text{Genotypic Variance}}}{\text{Grand Mean}} X\ 100 \quad 4$$

$$PCV\ (\%) = \frac{\sqrt{\text{Phenotypic Variance}}}{\text{Grand Mean}} X\ 100 \quad 5$$

Genetic advance (GA) was calculated with the method suggested by Singh and Chaundry (1985) using equation 6 as follows:

$$GA = k.\ \sigma ph.\ h^2 \quad 6$$

Where K: constant = 2.06 at 5% selection intensity, σph: square root of phenotypic variance, h^2: Heritability

GA as percentage of mean (GAM) = (GA/Grand Mean) X 100 7

RESULTS AND DISCUSSION

Genotypic variance, phenotypic variance, Environmental variance, broad sense Heritability. Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV) and Genetic advance for eleven characters are presented in (Table 1) The result revealed considerable genotypic variances among the various accessions for the characters under consideration. The result revealed consistency in the environmental and genotypic variance. In all the eleven characters studied, the genotypic variance was quite higher than the environment variance.

Genotypic variance (GV) was higher than environmental variance (EV) for all the eleven (Table 1). However, the influence of the environmental factors on the expression of other characters as indicated by the magnitude of the EV was not evident. This indicates that the phenotypic variance (PV) was not caused by environmental influences of those characters. Consequently, such character possesses promising genetic variability; so, selection for them is very efficient and successes very high.

The higher GV (7916.65) was for plant height at maturity, this was followed by number of seed per fruit (6834.17), then number of flower buds per plant. The least GV (0.28) was recorded for weight of fruit per plant. Phenotypic variance (PV) was also highest in plant height at week 10 (10022.14) followed by number of seeds per fruit (8778.28), then number of flowers per plant (2973.55), and followed by plant height at maturity (7964.65); the lowest PV (0.40) was found in weight of fruit per plant (Table 1).

Genotypic coefficient of variation (GCV) was higher for number of fruits per plant (131.71%), then followed by number of branches per plant at maturity (86.77%), this was followed by number of flowers per plant (73.87%); the least GCV (17.54%) was found in fruit diameter. Genotypic coefficient of variation (GCV) ascertains the degree of genetic variability present in various quantitative traits. High GCV indicates the presence of exploitable genetic variability for these traits which may facilitate selection (Yandav, 2009). Polygenic variation may be phenotypic, genotypic or environmental and relative values of these three coefficients for a trait will give an idea about the magnitude of its variability (Nausherwan *et al*, 2008).

Genotypic coefficient of variation, which is the real indicator of the extent of genetic variability in a population, was high for all the characters, except

for fruit diameter and days to flowering. For all the tested character, higher PCV than GCV values were obtained.

The highest PCV (138.02%) was for number of fruits per plant, followed by number of branches per plant (97.41%), then followed by number of flowers per plant (75.75%); the least PCV (20.50%) was found in fruit diameter (Table 1). High PCV is an indication of the presence of substantial horizon for selection of the trait under consideration which dependent on the amount of variability present. Thus, a greater potential is expected in the selection for number of fruits per plant, number of flowers per plant and number of branches per plant among the genotypes under study while there is a narrow scope for selection of fruit diameter and days to flowering on account of low amount of variability among genotypes studied. (Khan *et al*, 2009) reported that high PCV is an indication of the existence of greater scope for selection of the trait under consideration which is dependent on the amount of variability present.

The highest broad sense heritability (h^2) of (100%) was recorded for plant height at week 10 with an expected genetic advance over percentage of mean (GAM) of 46.10%. this was followed by plant height at maturity 99% with an expected GAM 17.58%, followed by plant height at week 6 (97%) with expected respective GAM of 103.37%. Number of leaves per plant at maturity produced the lowest heritability values (67%) and a corresponding lowest GAM values (54.09) (Table 1).

Heritability suggests the extent of genetic control for the expression of a particular trait and the reliability of phenotype in predicting its breeding value (Chopra, 2000). High heritability indicates less environmental influence in the observed variation (Mohanty, 2003; Eid, 2009). Heritability in the broad sense (h^2_{bs}) indicates only whether there is sufficient genetic variation present in a population or not, which implies whether a population will respond to selection pressure or not (Milatovic *et al*, 2010).

CONCLUSION

In conclusion, broad genetic variability was observed among the melon accessions that could be useful for future breeding purposes. The results of this study indicate that there is considerable genetic variation present in most of the traits to warrant selection for better genotypes. These traits can therefore be given special attention in selections aimed at melon improvement. In other to access the selection effect on trait more effectively, heritability accompanied with genetic advance is more useful than heritability alone.

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Table 1: Estimation of Some Components of Genetic Parameters for some Agromorphological Characters among the Melon Accessions

Characters	Grand mean	Genotypic variance (σ2g)	Phenotypic variance (σ2p)	Environmental variance (e2)	Broad sense heritability (h2)/%	Genotypic coefficient of variation (GCV)/%	Phenotypic coefficient of variation (PCV)/%	Genetic advance (GA)	GA as a % of mean
Plant height at 4weeks (cm)	74.45	702.62	738.27	35.65	95	35.60	36.50	71.55	96.10
Plant height at 6 weeks after (cm)	81.86	1167.44	1200.55	33.10	97	41.74	42.33	84.79	103.57
Plant height at 8 weeks (cm)	137.98	1589.53	1695.73	106.20	94	28.89	29.84	57.63	41.76
Plant height at 10 weeks	211.23	995.94	10022.14	26.20	100	47.33	47.39	97.38	46.10
Plant height at Maturity	322.40	7916.65	7964.65	48.34	99	27.60	27.68	56.68	17.58
Days to germination	5.24	6.02	6.55	0.53	92	46.86	48.88	92.53	1765.83
Number of branches per plant at maturity	5.71	24.55	30.94	6.39	79	86.77	97.41	159.23	2784.58
Number of leaves per plant at maturity	100.49	1051.00	1570.99	519.99	67	32.26	39.44	54.36	54.09
Days to flowering	32.78	43.08	56.96	13.88	76	20.02	23.02	35.86	109.39
Number of flower bud per plant	82.52	3127.10	3292.86	165.76	95	67.77	69.54	136.04	1211.19
Number of flowers per plant	74.93	3063.65	3221.49	157.83	95	73.87	75.75	148.41	197.51
Number of fruits per plant	10.98	208.95	229.46	20.51	91	131.71	138.02	258.92	2358.1
Weight of fruit (g)	1.77	0.28	0.40	0.12	70	30.02	35.86	51.76	2924.2
Number of seeds per Fruit	322.91	6834.17	8778.28	1944.11	78	25.60	29.02	46.53	14.40
Fruit diameter	40.60	50.14	69.44	19.30	72	17.44	20.50	30.53	164.85

CGBPB 054

L-ASCORBYL-2-MONOPHOSPHATE FORTIFIED PRACTICAL FISH DIETS MODULATES *COLLA1, IGF-1*, AND *IGF-II* GENE mRNA EXPRESSIONS IN *MACROPTERUS SALMOIDES* JUVENILES

[1,2] Abdullateef Yusuf, [2]Huang Xuxoing [1]Abrak Dambo, [3]Adamu Auwal, [4]Umar Rilwanu [5]Mahmoud Suleiman Jada and [6]Imodagbe O. Moshud
[1]Department of Biology, Ahmadu Bello University, Zaria, Nigeria
[2]Shanghai Engineering Research Center of Aquaculture, Shanghai, 201306, China
[3]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria
[4]NAERLS, Ahmadu Bello University, Zaria, Nigeria
[5]Department of Biochemistry, Modibo Adamawa University of Science and Technology, Yola, Nigeria
[6]Samaru College of Agriculture, Division of Agricultural colleges Zaria (DAC), Ahmadu Bello University Zaria, Nigeria
For Correspondence: yusufabdullateef@yahoo.co.uk , +2348038869278

Abstract

A 60 days feed trial was conducted to assess the effect l-ascorbyl-2-monophosphate fortified practical fish diets on modulation of colla1, igf-1, and igf-ii gene mRNA expressions in Macropterus salmoides juveniles. Eight isonitrogenous, isocaloric experimental diets supplemented with different l-ascorbyl-2-monophosphate level (0 (basal), 25, 50, 75, 100, 125, 150, and 175 mg/kg) were formulated. Each diet was randomly assigned to triplicate group of 30 juvenile fish with an average initial weight of 10.87 ±0.17g, fish were fed thrice daily. After feed trial, liver, and muscle were collected for total RNA extraction. The study revealed that l-ascorbyl-2-monophosphate significantly elevated colla1, igf-i and igf-ii in group fed AMP100, AMP125, AMP150 and AMP175. Conclusively, l-ascorbyl-2-monophosphate fortified practical fish diets significantly up-regulates colla1, igf-i and igf-ii, largemouth bass.

Keywords: L-ascorbyl-2-monophosphate, Largemouth bass, *igf-i, igf-ii*, *colla1*

Introduction

The main objectives of finfish aquaculture industry are to enhance fast growth and to produce high-grade fish. Micronutrients have been used to improve growth and quality of fish. L-ascorbyl-2-monophosphate form of vitamin c could be one of such micronutrients as growth promoters.

Vitamin C (VC), which is also known as ascorbic acid, is a multifunctional micronutrient in aquatic animals (Dawood and Koshio, 2016). Several studies have used different exogenous derivatives of ascorbic acid in fish diets such as L-ascorbic-2-glucose, ascorbate-2-sulfate, L-ascorbyl-2-monophosphate and L-ascorbyl-2-polyphosphate by Wang *et al.* (2003a, 2003b), Ai *et al.* (2006), Lin and Shiau (2005), and Xiao *et al.* (2010) respectively. Amongst all, the phosphate derivatives of VC have been reported to be more effective ascorbic acid form (NRC, 2011).

Limited information relating to the effect of ascorbic acid on growth and collagen related gene mRNA expressions in fish are available. This made it imperative to investigate the modulatory potentials of l-ascorbyl-2-monophosphate fortified practical fish diets on *igf-1, igf-ii,* and *colla1* gene mRNA expression in *Macropterus salmoides* juveniles.

MATERIALS AND METHODS

Experimental feed preparation

Eight (8) isonitrogenous (47.47 % crude protein) and isolipidic (11.77 % crude lipid) experimental diets were supplemented with VC (Ascorbic acid monophosphate (AMP) 35 % VC concentration) at 0, 25, 50, 75, 100, 125, 150, and 175 mg ascorbic acid equivalent kg^{-1} representing AMP0, AMP25, AMP50, AMP75, AMP100, AMP125, AMP150 and AMP175 respectively. Experimental diets had equal amount of fishmeal, wheat gluten meal, sprayed-dried blood meal, shrimp meal, fermented soybean meal, corn gluten meal, Brewer's yeast meal, squid paste, concentrated soybean phospholipid, Cr2O3, vitamin mix (without VC), mineral mix, Ca(H2PO4)2, soybean oil and α-cassava starch containing 420.00, 30.00, 40.00, 50.00, 90.00, 110.00, 20.00, 20.00, 25.00, 5.00, 10.00, 10.00, 10.00, 40.00 and 80.00 g/kg respectively, zeolite power was used as filler as the VC supplement changed across experimental diets. All feed ingredients ground dough primed with required water and pellets were made 1 mm in size. All experimental diets were stored at -20 ℃ to prevent VC degradation.

Experimental fish

Prior to experimental feeding trial, a total of nine hundred and sixty (960) fish were allowed to adapt into experimental environment for two weeks under routine examination. At the beginning of feeding experiments, fish with an average weight (10.87 ± 0.17 g, body weight) were fasted for 24 h and randomly allotted to 32 cages (1.5 × 1.0 ×1.2 m with 30 fish each) suspended in an indoor flow-through concrete tank (5.0 ×3.0 ×1.2 m) system corresponding to quadruplicate cages per each dietary treatment. They were continuously supplied with oxygen to maintain the dissolved oxygen level. Fish were spatially and sparingly fed by hand to apparent satiation trice daily (8.00, 12.00 and 16.00h) for 60 days. During the experimental period, water quality parameters; temperature, dissolve oxygen, pH, ammonia and nitrate were monitored using kits and electronic meter, and the natural light cycle was adopted.

Total RNA isolation and reverse transcription analysis

After feed trial, three fish were sampled from each tank, liver and dorsal muscle tissues were excised and total RNA was extracted from liver and muscle samples according to RNA Iso-plus protocol aided by the manufacturer's guide, and then dissolved in DEPC treated water. Isolated RNA quantity was determined using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, USA), with absorbance at 260 and 280 nm and RNA ratios (A260:A280) from 1.8 to 2 was used for further experiments. Total RNA extracted was converted to cDNA using PrimeScript TM RT reagent Kit (TaKaRa) and stored at -20 ºC ready for gene expression experiment. Effect of l-ascorbyl-2-monophosphate on the expressions of insulin-like growth factor I and II (IGF-I and IGF-II) and Type 1 collagen (colla1) gene were evaluated via RT-qPCR. Specific primers of each gene were designed based on published *M. salmoides* cDNA using Primer 5.0 software (http://www.premier biosoft.com/primerdesign/index.html) (Table 1) were produced based on the obtained gene sequences by Shanghai Sangon Biological Engineering Technology & Services CO., Ltd. (Shanghai, China). Real-time PCR was conducted on a Mini Option Real-time PCR machine (Bio-Rad). The 20-µl reaction contained 1-µl cDNA sample, 10 µl SYBR green I Master Mix (TaKaRa), 0.5µl of each primer and 8 µlH2O. PCR amplification was performed in triplicate wells, following the protocol: 3 min at 95C, 45 cycles consisting of 10s at 95 ºC, 15s at 63 ºC and 25 s at 72 ºC.

Data analyses

All data were presented as mean values ± standard error. The statistical analysis was carried out using analysis of variance after exploring the normality and homogeneity of data assumption, all data from each group were subjected to turkey-Kramer test to determine if dietary VC significantly ($P<0.05$) affected response variables

using the SPSS 20 (SPSS Inc., Michigan Avenue, Chicago, IL, USA).

RESULTS AND DISCUSSION

Colla1 gene mRNA expression in liver were significantly higher in AMP125, AMP150 and AMP175 (Fig 1A), and lowest in AMP0. *Colla1* gene expression level was ascorbic acid dependent, fish fed AMP175 had highest *colla1* mRNA level, however, was not significantly different (P>0.05) from fish AMP100, AMP125 and AMP150.

Growth related gene mRNA levels showed a significant elevation of mRNA level as ascorbic acid level in diet increased (Fig.1B-E). The AMP0 had lowered growth-related gene expression levels while AMP100, AMP125, AMP150 and AMP175 significantly elevated Insulin-like growth factor I and II (IGF-I and II). AMP0 and AMP25 were not significantly different from AMP50 group, similar trend was observed in liver.

In this study, type 1 collagen synthesis was impaired, phenotypically observed in AMP0 (1.33mg/kg diet), showing characterized slow growth, survival rate, broken back syndrome and hemorrhagic fin and lip exacerbation as reported in our previously published article Yusuf *et al.* (2021). In our study, AMP100, AMP125, AMP150 and AMP175 actuated an increased *colla1* regulation compared to AMP0 group.

Insulin-like growth factors are mitogenic hormones released in multiple organs which regulate somatic growth in vertebrates. Also, fish IGF system are influenced by nutritional conditions (Kawanago *et al.*, 2014). They are hormone primarily involved in the regulation of growth, cell differentiation and fetal development, and osteoblast maturation in vertebrate (Carinci *et al.*, 2005; Plasna *et al.*, 2000). The insulin-like growth factors (IGF) axis is an important neuroendocrine parameter regulating growth in fish (Cheng *et al.*, 2017). Sea bass larva fed low VC content displayed lower level of IGF-I inducing an impairment of osteoblast maturation (Hughes *et al.*, 2006). Ascorbic acid sustains pre-osteoblast proliferation and commitment through production of *colla1*, reacting with α2 – and β1- integrin, by kick-starting the mitogen-activated protein kinase pathway and phosphorylation (Carinci *et al.*, 2005), it also attenuates expression of osteoclast differentiation genes, such as receptor activator of nuclear factor kappa-B, receptor activator of nuclear factor kappa-B ligand, tartrate-resistant acid phosphatase, and cathepsin K by promoting steoblast formation and blocking osteoclastogenesis (Choi *et al.*, 2019). Skeletal muscle plasticity and individual fiber growth result from a balance between protein synthesis and degradation, increased protein degradation in myoblasts due to ascorbic acid increased (Mitosumoto *et al.*, 1994; Shima *et al.*, 2011; Duran *et al.*, 2019). Ascorbic acid supplementation is able to accelerate and advance the beginning of myogenesis in fish myoblasts, and Igf1 which is among the most studied and best characterized muscle growth-promoting factors that triggers several downstream cascades which culminate the activation of mechanistic target of rapamycin (Mtor) and other processes that integrates signals from nutrients, energy status and growth factors, controlling protein synthesis among its other functions (Duran *et al.*, 2019). Pacu fish showed high expression of mtor in the myoblasts treated exclusively with ascorbic acid which is an indication of increased protein synthesis (Duran *et al.*, 2019). Pufferfish (*Takifugu obscurus*) fed with dietary VC up-regulated growth hormone (GH) and *igf-1* as dietary VC level increased (Cheng *et al.*, 2017), which implies that dietary VC not only improves feed utilization, but also influences regulation of growth and collagen related gene as observed in AMP125 and AMP150 group.

CONCLUSION

L-ascorbyl-2-monophosphate fortified practical fish diets significantly up-regulates *colla1, igf-i* and *igf-ii,* largemouth bass.

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Table 1: Targeted gene primer sequences used for real-time quantitative PCR (qPCR)

Target Gene	**Forward/reverse sequence (5'–3')**	GenBank Accession no./ Source
IGF-I	CTTCAAGAGTGCGATGTGC-**F** GCCATAGCCTGTTGGTTTACTG-**R**	Chen et al. (2012)
IGF-II	CGTTGTGGAAATAGCCTCGG- **F** TATCCAAACAGATGTGCGCG-**R**	GQ328049.1
COLLA 1	AGGCATCCCAGAACATCACA-F CAATGTCGATGATGGGCAGG-R	EF413588
beta-actin	ATCGCCGCACTGGTTGTTGAC-**F** CCTGTTGGCTTTGGGGTTC- **R**	Chen et al. (2012)

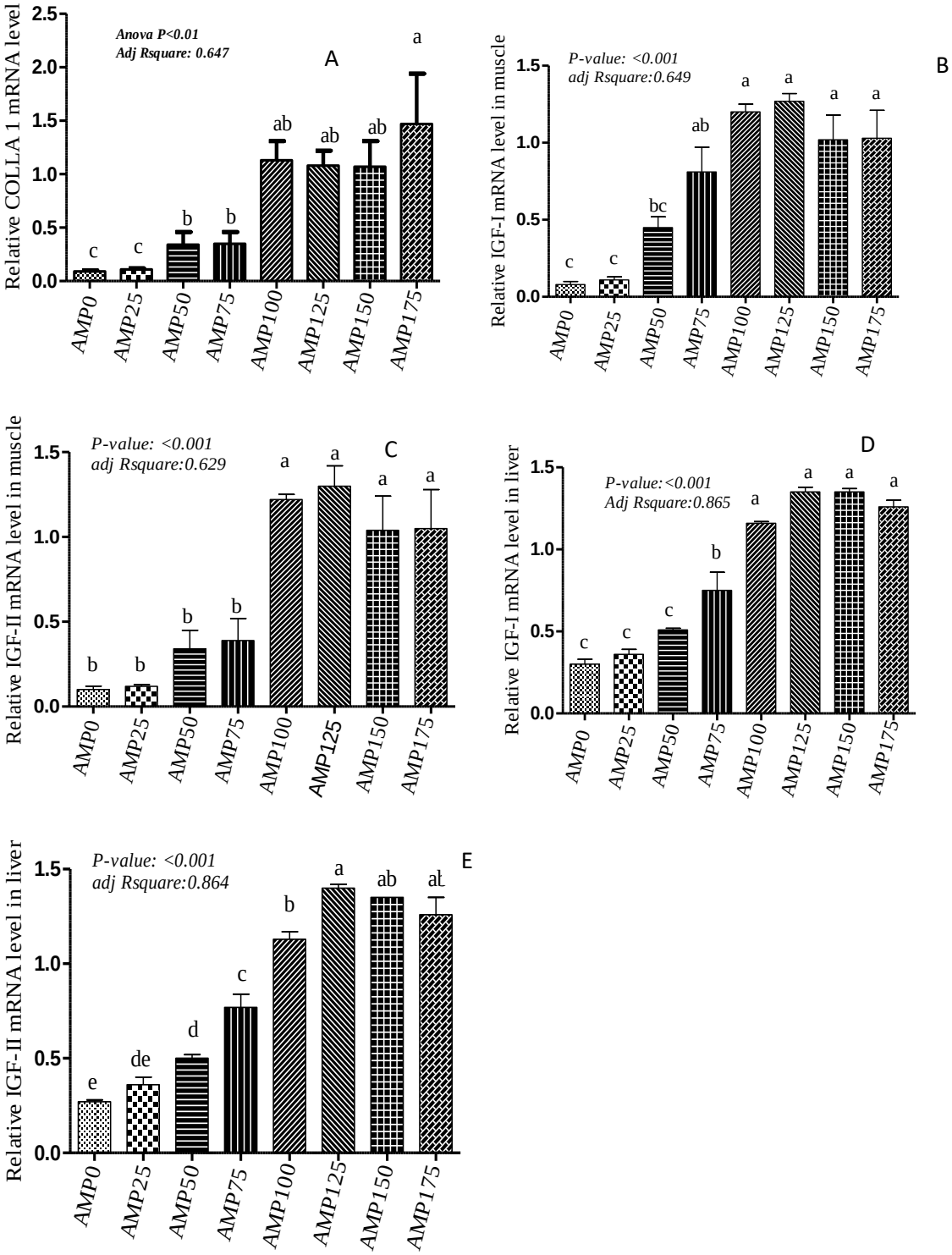

Fig. 1: Relative gene mRNA expression level (A) *colla1* gene in liver (B) *igf-i* gene in muscle (C) *igf-ii* gene in muscle (D) *igf-i* gene in liver (E) *igf-ii* gene in liver of largemouth bass fed l-ascorbyl-2-monophosphate fortified practical fish diets

CGBPB 055

COMBINING ABILITY ANALYSIS FOR STRIGA RESISTANCE AMONG PEARL MILLET (*Pennisetum glaucum* L R. Br) INBREDS IN A LINE × TESTER CROSS

G.S. Balam[-1*] and A.U Izge[2]
[*]Department of Crop production, Faculty of Agriculture and Agricultural Technology Abubakar Tafawa Balewa University, P. M. B. 2044 Bauchi State, Nigeria.
[2]Department of Crop Science, Faculty of Agriculture, Federal University Dutse, P. M. B. 7156, Ibrahim Aliyu By-Pass, Dutse Jigawa State, Nigeria

Corresponding Author E-mail: byelharastanley@gmail.com +2348026053617

Abstract

Pearl millet production has been constrained by Striga hermonthica in Sudan Savannah of Nigeria leading up to 10 - 95 % grain loss. Breeding for resistant hybrid would be a promising alternative for a reduction in cost of production and increased yield. The objectives of this study were to determine gene action and effects among parents and hybrid under striga. It was also to evaluate the response of pearl millet to Striga hermonthica and identify high yielding genotypes and hybrid under striga infestation. Twenty nine F_1 hybrids and two checks were evaluated under Striga infestation in Bauchi and Maiduguri 2018 rainy season in a randomized complete block design. Data were collected on number of plant at emergence, days to 50 % flowering, days to 100 % flowering, plant height, panicle length, number of plants at harvest, number of leaves/plant, number of panicle/plot, striga count at 90 days after sowing, 1000 grain weight and grain yield. The general combining ability and specific combining ability variances were significant for most traits. The results revealed that both additive and non-additive genetic variances were important in determining the performance of the traits. However, non-additive genetic variance were preponderant than additive genetic variance in controlling the traits. The result indicated SOSAT C-88, LCIC 9702, Ex-Baga and PEO 5984 have been identified as good general combiners for some desirable traits especially for grain yield and resistance to striga. In another development, SOSAT × Ex-Monguno, LCIC 9702× Ex-Gubio and PEO 5984 × Ex-Baga has been identified as good specific combiners for grain yield. The hybrids; SOSAT ×Ex-Baga and PEO 5984 ×Ex-Baga are the best specific combiners for striga tolerance. High genetic variability exists among the population.

Key words: *Millet, Striga, Randomized Complete Block Design, General combine Ability, Specific Combine Ability.*

Introduction

Pearl millet known as *Gero* in northern Nigeria is the sixth most important cereal cultivated as rain fed crop on about 26 million ha in arid and semi-arid areas of Africa and the Indian sub-continent (Atif *et al.* 2012). It is grown predominantly in Africa and Asia in over 40 countries as a staple grain and as source of feed and fuel and construction material. It has wide adaptability to local environments and grown in West Africa from the oases of the Sahara desert under irrigation to northern Sahel under as little as 250 mm of rainfall per annum. Pearl millet can grow on hot and dry soils unfavorable to sorghum. Its

improvement program especially in Nigeria has been geared towards higher yield for human food which may likely play a major role in easing food shortage as population skyrockets, Izge, (2006) reported that the purpose for expanding pearl millet production in these regions to meet growing demand for food will depend on success of research, cultivation arid hybrid development programs. *Izge et al.* (2005) reported higher potentials for making progress in the selection of desirable traits in pearl millet towards higher grain yield, because there are tremendous levels of genotypic variability existing among landraces.

Parasitic weed (*Striga hermonthica Del. Benth.*) has been a major biotic constraint among many others to pearl millet production, particularly in northern Nigeria. The exceptional degree to which the weed damages pearl millet is one of several characteristics which make *Striga hermonthica* the most serious of all parasitic weed (Parker and Riches, 1993). Estimated grain losses have been put between 10 -95% depending on varietal reaction, ecology and cultural practices, *Wilson et al* (2004). All the known methods of Striga control have shortcomings; conventional herbicides are prohibitive in cost and ineffective since damage is done before *Striga* emerges from the soil. Other disadvantages in the use of herbicides are pollution of environment, disturbance of ecological balance, toxicity in cost of production.

Measures that minimize impact on crop loses, deplete *Striga* seed bank in the soil, reduce further *Striga* seed production and diminish spread of *Striga* to farms are essential in their control. Host plat resistance or tolerance when effectively deployed offers, many benefits with an insignificant increase in cost of production. The objectives of the study was therefore, to determine the combining ability variances and estimate the general combining ability effects of parents and the specific combining ability effect of hybrids under striga infestation in order to identify the mode of gene action determining resistance to striga and genotypes with good yield potential.

MATERIALS AND METHODS

Nursery trial through line × tester mating design was conducted in 2017 dry season at the Lushi Irrigation Station, Bauchi. The area is located on latitude 10^0 18 N; 9^0 50 E at an altitude of 628 m above sea level. In 2018 raining season, the 20 F_1^S, and the parents were evaluated in two locations viz: Bauchi and Maiduguri.

The pearl millet cultivars; PEO 5984, Super SOSAT, SOSAT C-88, Ex-Borno and LCIC 9702 were obtained from the mandate Research Institute, the Lake Chad Research Institute in Maiduguri, Nigeria. In addition, wild millet *Monoddi* having Striga resistance genes was obtained from International Crop Research Institute of the Semi-Arid Tropics (ICRISAT) in Niamey. The three local ones were named Ex-Monguno, Ex-Baga, and Ex-Gubio based on the locations they were obtained from. The wild millet obtained from ICRISAT Niamey was PS202. The wild millets were used as males as well as testers. The cultivars were used as well as females as well as lines. The pearl millet commercial cultivars obtained from LCRI Maiduguri and ICRISAT and the wild types are described in Table 1.

Nursery experiment was conducted during dry season of 2017 to form initial F_1 population through line × tester mating. The procedure was as described in the work of Izge, (2017). The 20 F_1 including nine parental lines were grown for evaluation at Bauchi and Maiduguri, Nigeria during cropping season of 2018. The treatment were laid in randomized complete block design made up of three replications. Data collection was carried out on number of plant at emergence, days to 50% flowering, days to 100% flowering, plant height, panicle length, number of plant at harvest. Number of leaves /plant, number of panicle/ plot, striga count at 90 days after sawing, 1000 grain weight and on grain

yield. Data collection were analyzed using Statistical Analysis of System (SAS) as described by Singh and Chaudhary (1985).

RESULTS AND DISCUSSION

The mean squares from the analysis of variance across locations are presented in Table 2: The results indicated that there were significant or highly significant differences across locations in number of panicle/plot (14941.0**), striga count at 90 DAS 86779.41and 1000 grain weight (338.1**), however all other remaining traits did not show any significant differences across location. The result also indicated that significant or highly significant differences existed in number of plant at emergence (154.07**), number of panicle/plot (900.55*), number of plant at harvest was significant different (38.9*) foe line × tester interaction across locations. There was however, significant difference in number of leaves per plant (2.43*) and striga count at 90 DAS (10520.82*) in location × line × tester interaction. All other traits did not show any significant difference for location × line × tester interaction.

Falconer (1989) reported similar result and found tremendous level of genetic variability among maize and millets. It is important to note that the amount of genetic improvement that can be obtained by selection among number of hybrids has been reported to be dependent on the amount of variability existing in a population. The results indicated also the influence of the environment on the performance of genotypes, DFF, DHF, PLF, PLH and STC 90 days after sawing as their Rep × Location was × line × tester interaction. Drabo 2016.

Analysis of variance for combining ability, estimates of genetic variance and proportional contribution to total variance at Bauchi and Maiduguri combined are presented in Table 3. The result indicated that there was significant or highly significant difference in GCA effect for lines in number of plant at emergence (154.07**), days to 50% flowering (266.89**), days to 100% flowering (277.18**) and number of plant at harvest (151.76*). The GCA variance for tester however indicated no significant difference in all traits. The lines × tester SCA was highly significant (33.97**) for days to first flowering. All other traits were not statistically significant.

The genetic component of variance on the other hand shows that covariance half sib for lines was highest in grain yield (7074.21) followed by plant height (78.30) and number of panicle/plot (41.49). The covariance half sib for testers shows that plant height had highest value of 22.17 followed by number of panicle/plot with value of 5.42. The result also indicated that the covariance full sib was higher in grain yield, striga count at 90 DAS, number of panicle/ plot and days to 50% flowering, with values of 10930.62, 105.64 and 31.09 respectively.

The combining ability indicated that SCA variance were higher than GCA variance in all the traits, expect in plant height, number of plant at harvest and grain yield indicating preponderance of SCA or non-additive effects over GCA effects over GCA effects in control of most of the traits.

Significant mean square observed among parents and hybrids for different agronomic traits imply that both parent and hybrids derived from them would likely respond to selection. The observed significant therefore could be attributable to the kind of genetic differences in the parents. The variance among hybrids could be a gene action. Falconer (1989) and Izge *et-al* (2005) have reported similar result and found astounding level of genetic variability among crop plants. The amount of genetic improvement that can be obtained by selection among hybrids would be dependent on the amount of variability.

The result of proportional contribution to the total variance shows that the contribution of lines were greater than for tester or line × tester in all the traits. In the same vein, the contribution of line × tester to total variance were greater than testers in all the traits. The ratio of δ^2 GCA /

δ^2SCA was less than unity for all the traits, there by indicating the preponderance of non-additive gene effects in the expression of these traits. These result are in accordance with the findings of Dangariya et-al., (2009), Bachkar et-al., (2014) and Chittora and Patel (2016).

The significant mean square observed in GCA of parent and SCA of hybrids for some traits shows the importance of additive and non-additive genetic effects in their inheritance, a similar findings was reported by Azhaguvel et-al., (1996). This further confirm the presence of genetic variability in the materials evaluated and in means therefore that the materials could be used for improvement in grain yield and other desirable agronomic traits. The results for GCA effects are resented in Table 4. Note that negative effects for days to 50% flowering, days to 100% and striga count at 90 DAS are desirable. These traits are important in breeding programmer. Significant GCA effects for these character were also recorded by Khon and Dubey (2015) and Nandariya et-al., (2016). The result indicated that Ex-Baga is the best general combiner among tester with desirable performance in days to 50%, 100% flowering, plant height, number of panicle/plot. Number of plant at harvest and 1000 grain weight. The second best general combiner is Ex-Monguno with desirable performance in five traits. The result also indicated that the best general combiners among the lines are SOSAT C-88 and LCIC 9702 with desirable combining ability effects in eight traits each. Both SOSAT C-88 and LCIC 9702 are excellent general combiners for grain yield among the testers with combining ability values of 64.9 and 48.98 respectively. Worst general combiner among all the lines and the tester is Ex-Borno while the best general combiner for grain yield among the tester is PS 203 with a value of 39.63. Negative GCA effect for days to 50% flowering or 100% flowering implies that the parents when crossed to another parent with negative GCA effect would produce hybrids that would mature earlier. This has been reputed by Martnex et-al., (1993).

The specific combining ability effects of the hybrids are presented in Table 5. SCA provide information on the role of non-additive gene action (intra and inter-allelic interaction) in the expression of gene action. The result showed that the hybrids Super SOSAT × Ex-Gubio, Ex-Borno × Ex-Gubio and SOSAT C-88 × Ex-Monguno are the best specific combiners as all of them were able to combine very well in five different traits. The best specific combiner for grain yield were; SOSAT C-88 × Ex-Monguno, LCIC 9702 × Ex-Gubio and PEO 5984 × Ex-Baga. The best specific combiner for striga tolerance on the other hand were; SOSAT C-88 × Ex-Baga and PEO 5984 × Ex Baga. The result shows that the best specific combiners in striga tolerance were not among the best specific combiners for grain yield, SPSAT C-88, LCIC 9702 and PEO 5984 were among the best general combiners that produced among the best hybrids in striga tolerance and grain yield. Similar result have been found to confirm that the best general combiners are likely to produce best hybrids when they are crossed together.

Specific combining ability effects are used to identify the best cross-combination in hybrids production as reported by Izge *et-al.*, 2007. This study identifies a number of desirable hybrids for some of the traits that can be utilized for improvement of pearl millet grain yield.

CONCLUSION AND RECOMMENDATION

The present study reviled that there is genetic variability in the material used and it can be exploited for genetic improvement for striga resistance. SCA was greater than GCA line and GCA tester, therefore non additive gene action plays a predominant role in the inheritance of the traits studied for striga infestation. The proportional contribution of lines was greater than that of tester and line × tester. Ex- Baga was the best combiner among the testers and SOSAT C-88 and LCIC 9702 for lines with desirable

performance in most of the traits. The best specific combining ability of hybrids are Super SOSAT × Ex-Gubio, Ex-Borno × Ex-Gubio and SOSAT C-88 × Ex-Monguno and on the other hand best SCA combiner for striga are SOSAT C-88 × Ex-Baga and PEO 5984 × Ex-Baga. Significant difference was observed in all the traits among lines, testers and hybrids in terms of performance and hence, can be recommended to farmers for cultivation. Therefore it can be employed breeding programmer targeted at improving certain traits of interest.

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Table 1. Description of cultivars used as parents (lines and testers) in the study

Parental lines	Source	Description
Lines		
*Ex-Borno	LCRI	Medium maturing/medium sized seeds and adapted to the Sahel region of Nigeria.
* SOSAT-C88	LCRI	Long/compacted panicle, early maturing and large seeded.
* Super SOSAT	LCRI	Long panicle and meduim maturing.
* LCIC 9702	LCRI	Long compacted panicle, early maturing and large seeded.
*PEO 5984	LCRI	Medium maturing, medium sized and compacted panicle.
Testers		
** PS 202	ICRISAT	Short and small seeded panicle, hairy, profuse tillers and striga resistant.
* Ex-Gubio	LCRI	Medium height and small yellow-seeded panicle, profuse tillers, late maturing and tolerant to striga.
* Ex-Baga	LCRI	Medium and small brown-seeded panicle, profuse tillers, late maturing and tolerant to striga.
*Ex-Monguno	LCRI	Medium and small purple-seeded panicle, hairy, profuse tillers and tolerant to striga.

Source: *LCRI, Maiduguri, Nigeria**- ICRISAT, Niamey, Niger Republic

Table 2: Mean square (ms) from analysis of variance of pearl millet genotype across Bauchi and Maiduguri 2018 rainy season in a line x tester analysis

Source of Variation	df	NPE	DFF	DHF	PLH	PNL	NPH	NLP	NPP	STC 90	GRY	TGW
Location	1	880.21	1591.41	907.5	13568.13	85.01	1435.21	267.01	14941.01**	86779.41**	207954.3	338.1**
Rep Vs Location	4	13.43	94.82**	83.12**	1956.36*	8.88	10.92	0.43	491.67	20140.98**	541373.9	17.33
Hybrid	19	60.39	83.55	73.14	762.32	23.57	58.97	1.39	525.5	10819.86*	59871.69	37.05
Line	4	154.07**	266.89	277.18	1430.64	27.43	151.76	1.08	900.55*	4489.35	129960.1	49.67
Tester	3	31.88	37.43	9.43	956.6	29.81	15.54	1.32	516.5	1972.82	25629.16	36.25
Line x tester	12	36.3	33.97	21.05	490.98	20.72	38.9*	1.51	402.73	15141.78	45069.52	33.24
Loc x Hybrid	19	18.96	16.99	14.78	535.08	16.78	21.82	2.36	506.11	7896.67	33321.09	35.52*
Loc x Line	4	36.73	11.01	9.48	510.2	10.36	29.48	2.97	533.82	5560.72	39830.18	20.45
Loc x Tester	3	24.23	11.25	8.9	304.73	29.1	35.83	1.28	302.94	514.7	39522.27	52.01*
Loc x Line x Tester	12	11.72	20.41	18.02	600.96	15.84	15.77	2.43*	547.67	10520.82*	29601.1	36.42*
Error	76	27.95	21	15.59	795.04	17.64	20.04	1.23	343.54	5604.1	32671.18	37.15

2018 Rainy Season
KEYS

NHE= Number of plant at emergence 30 days after sowing
DFF= Days to 50% flowering
DHF= Days to 100% flowering
PLH= Plant height
PNL= Panicle length
NPH= Number of plant at harvest
NLP=Number of leaves per plant
NPP= Number of panicle per plot
STC 90= Striga count at 90 Days
GRY= Grain yield
TGW= 1000 grain weight

**= Significant at 1% level of probability.
* = Significant at 5% level of probability

Table 3: Analysis of variance for combining ability, estimates of genetic variance and proportional contribution total variance among eleven traits of pearl millet at Bauchi and Maiduguri combined during 2018 rainy season.

Source of variation	df	NPE	DFF	DHF	PLH	PNL	NPH	NLP	NPP	STC90	GRY	YGW
Rep	2	18.78	181.76**	167.89**	6065.81**	33.63	14.07	3.005	754.30	51546.10**	600312.53**	1.07
Lines (GCA)	4	154.07**	266.89**	277.18**	1430.64	27.43	151.76*	1.07	900.55	4489.35	129960.09	49.07
Testers (GCA)	3	31.88	37.43	9.43	956.60	29.81	15.54	1.32	516.50	1972.83	25629.16	36.25
L x T(SCA)	12	36.30	33.97**	21.05	490.98	20.72	38.90	1.51	402.73	15141.78	45069.52	33.24
Error	60	20.37	13.67	11.58	969.70	16.95	17.04	1.29	275.97	5700.41	32238.35	26.72
Genetic components of variance												
Cov H.S Lines		9.81	19.41	21.34	78.30	0.56	9.41	-0.04	41.49	-887.70	7074.21	1.32
Cov H.STester		-0.21	0.17	-0.55	22.17	0.43	-1.11	-0.01	5.42	-627.09	-925.73	0.14
Cov F.S		16.95	31.09	28.27	-13.08	2.92	16.40	0.01	105.64	642.17	10930.62	4.12
σ^2GCA		2.28	4.64	4.95	23.16	0.22	1.10	-0.01	10.96	-34.48	1484.85	0.34
σ^2SCA		21.23	27.05	12.63	-638.28	5.02	-29.15	0.30	169.00	12588.49	1.71	8.69
Ratio GCA/ SCA		0.11	0.17	0.37	0.07	0.04	0.04	-0.03	0.06	-0.03	868.33	0.04
Proportional contribution to total variance												
Lines		53.71	67.25	79.78	39.51	24.50	54.18	16.27	36.08	8.74	45.70	27.88
Tester		8.33	7.07	2.04	19.81	19.97	4.16	14.98	15.52	2.88	6.76	15.45
Lines x Testers		37.96	25.68	18.18	40.68	55.52	41.66	68.75	48.40	88.39	47.54	56.67

KEYS

NHE= Number of plant at emergence 30 days after sowing
DFF= Days to 50% flowering
DHF= Days to 100% flowering
PLH= Plant height
PNL= Panicle length
NPH= Number of plant at harvest
NLP=Number of leaves per plant
NPP= Number of panicle per plot
STC 90= Striga count at 90 Days
GRY= Grain yield
TGW= 1000 grain weight

**= Significant at 1% level of probability.
* = Significant at 5% level of probability

Table 4: General combining ability effects for eleven traits in pearl millet evaluated at Bauchi and Maiduguri during 2018 rainy season

Genotypes Traits	NPE	DFF	DHF	PLH	PNL	NPH	NPP	NLP	STC 90	GRY	TGW
Tester											
PS 202	-o.8	-1.04	-0.48	-3.37	0.51	-0.49	-0.341	-0.09	9.38	39.63	-0.60
Ex-Baga	-1.08	-0.88	-0.48	4.53	-1.49	0.11	5.96	-0.06	4.18	-2.41	1.64
x-Gubio	0.23	1.13	0.55	5.03	0.41	-0.59	-2.08	-0.16	-6.49	-30.74	-0.42
Ex-Monguno	1.33	0.79	0.42	-6.20	0.58	0.98	-0.48	0.31	-7.06	-6.48	-0.62
SE+	1.39	1.14	1.05	9.61	1.27	1.27	5.13	0.35	23.30	55.41	1.60
Lines											
PEO 5984	1.72	-5.01	-5.18	-1.14	-1.73	2.57	2.14	-0.19	0.92	9.40	-0.86
Super SOSAT	0.09	2.61	2.82	-3.77	0.14	0.07	-3.82	6.23	-5.67	-0.98	-0.39
SOSAT C-88	0.84	-0.02	0.48	11.78	0.81	0.78	-0.11	0.23	18.29	64.90	2.54
Ex-Borno	-4.37	3.40	3.10	-0.27	-0.15	-4.18	-7.15	-0.19	-18.78	-122.31	-0.53
LCIC 9702	1.72	-0.98	-1.23	1.40	0.93	0.78	8.93	-0.07	5.25	48.98	-0.76
SE+	1.84	1.51	1.39	12.71	1.68	1.69	6.78	0.46	30.59	73.30	2.11

KEYS

NHE= Number of plant at emergence 30 days after sowing
DFF= Days to 50% flowering
DHF= Days to 100% flowering
PLH= Plant height
PNL= Panicle length
NPH= Number of plant at harvest
NLP=Number of leaves per plant
NPP= Number of panicle per plot
STC 90= Striga count at 90 Days
GRY= Grain yield
TGE= 1000 grain weight

Table 5: Specific combining ability effects of F1 hybrids for eleven traits of pearl millet across locations, (Bauchi and Maiduguri) 2018 rainy season.

Entries	Traits Hybids	NPE	DFF	DHF	PLH	PNL	NPH	NLP	NPP	STC 90	GRY	TGW
1	PEO 5984 × PS 202	0.52	1.58	0.98	16.24	2.53	-0.13	-0.24	3.83	12.58	-0.33	-0.83
2	Super SOSAT × PS 202	-0.03	3.13	1.32	-7.13	-0.34	-0.62	-0.16	2.45	-51.83	-18.13	0.42
3	SOSAT C88 × PS 202	3.89	-0.42	1.32	2.49	0.16	2.66	0.18	2.41	49.88	70.33	-1.56
4	Ex-Borno × PS 202	-3.23	-0.83	-0.64	3.87	0.62	-0.88	0.43	-1.05	31.46	-34.29	0.82
5	LCIC 9702 × PS 202	-1.15	-3.46	-2.98	-15.47	-0.97	-1.01	-0.20	-7.63	-42.08	-17.58	1.14
6	PEO 5984 × Ex-Baga	0.62	0.25	0.15	0.51	-1.47	3.60	0.23	-2.88	10.95	77.86	-1.24
7	Super SOSAT × Ex-Baga	1.41	-2.54	-1.52	5.97	0.33	1.27	-0.69	-7.92	101.37	-54.26	-2.37
8	SOSAT C88 × Ex-Baga	0.49	-0.75	-1.35	-2.08	0.66	-0.94	0.31	-6.13	-59.09	-18.96	5.91
9	Ex-Borno × Ex-Baga	-0.63	1.83	1.86	-4.87	0.12	-2.28	0.06	-4.25	-37.34	60.40	-0.73
10	LCIC 9702 × Ex-Baga	-1.88	1.21	0.86	0.47	0.37	-1.44	0.10	21.17	-15.88	-65.05	-1.57
11	PEO 5984 × Ex- Gubio	-1.02	1.42	0.78	-6.49	0.97	-2.70	0.66	-0.34	-21.88	-11.63	1.49
12	Super SOSAT × Ex- Gubio	0.78	-1.54	-1.05	1.80	0.93	-0.03	0.41	2.78	-31.30	38.58	1.44
13	SOSAT C88 × Ex- Gubio	-4.31	2.08	1.78	-5.58	0.43	-3.58	-0.43	-0.59	-19.76	-168.30	-2.67
14	Ex-Borno × Ex- Gubio	2.07	-2.00	-1.68	-0.37	-1.78	3.22	-0.18	3.78	15.49	32.58	0.24
15	LCIC 9702 × Ex- Gubio	2.48	0.04	0.16	10.63	-0.53	3.09	-0.47	-5.63	57.45	108.78	-0.49
16	PEO 5984 × Ex-Monguno	-0.12	-3.25	-1.92	-10.26	-2.03	-0.77	-0.64	-0.61	-1.65	-65.90	0.58
17	Super SOSAT × Ex-Monguno	-2.16	0.96	1.25	-0.63	-0.91	-0.06	0.44	2.68	-18.23	33.81	0.52
18	SOSAT C88 × Ex-Monguno	-0.08	-0.92	-1.75	5.16	-1.24	1.86	-0.06	4.31	28.98	116.93	-1.68
19	Ex-Borno × Ex-Monguno	1.80	1.00	0.46	1.37	1.05	0.15	-0.31	1.52	-9.61	-58.69	-0.33
20	LCIC 9702 × Ex-Monguno	0.55	2.21	1.96	4.37	3.13	-0.64	0.57	-7.90	0.52	-26.16	0.92
	SE±	3.69	3.02	2.78	25.43	3.36	3.37	0.93	13.56	61.65	146.60	4.22

KEYS

NHE= Number of plant at emergence 30 days after sowing
DFF= Days to 50% flowering
DHF= Days to 100% flowering
PLH= Plant height
PNL= Panicle length
NPH= Number of plant at harvest
NLP=Number of leaves per plant
NPP= Number of panicle per plot
STC 90= Striga count at 90 Days
GRY= Grain yield
TGW= 1000 grain weight

CGBPB 056

VARIATION IN THE PROXIMATE COMPOSITION OF SOME *JATROPHA CURCAS* L. (JATROPHA) GENOTYPES FROM SOKOTO AND KEBBI STATES

M. Usman, A. Usman and R. A. Sami

Department of Plant Science, Faculty of Agriculture/Institute for Agricultural Research, Ahmadu Bello University, Zaria

Corresponding author: muhammadu709@gmail.com

Abstract

Jatropha curcas (Linnaeus) belongs to the family Euphorbiaceae and is closely related to other important cultivated plants like rubber tree and castor. Nigeria being a tropical country has wide variations in climatic and soil conditions and therefore has a wide variety of oil crops such as Jatropha. But the paucity of information on the proximate composition and utilization of its seeds in Nigeria is a problem when it comes to the genetic improvement of the crop. Information about nature and extent of genetic variability present in the Jatropha germplasm and association of various proximate compositions is a pre-requisite in planning successful breeding programme. The objectives of this study were to determine the variation in the proximate composition of some Jatropha curcas L. genotype seeds, determine the correlation among the proximate compositions in the Jatropha and suggest the best genotypes in terms of the proximate composition for further improvement of the crop. The experiment was conducted in the laboratory of Product Development Research Programme of the Institute for Agricultural Research, Ahmadu Bello University, Zaria. Data were collected on the proximate compositions of the seeds: moisture, ash, protein, lipid, fibre and carbohydrate content and analyzed. Phenotypic correlations were computed for all the proximate compositions. Rank summation index was generated to identify the best genotype in terms of the proximate compositions. Significant differences were observed for all proximate compositions studied except the lipid which showed highly significant variation for all the genotypes. Highly significant correlations were observed between protein and lipid content (r = 0.67). Sokoto3 and Kebbi10 ranked first and last with rank summation indices of 14 and 118 respectively. Finally Sokoto3 was found to be the best genotype in terms of the proximate compositions.

***Keyword:* Jatropha, germplasm, proximate composition**

Introduction

Jatropha curcas L. is a perennial, monoecious shrub of the Euphorbiaceae family, native to Central America but distributed widely in the tropical and subtropical areas (Cano-Asseleih *et al.,* 1989). The crop has attracted a great deal of attention worldwide, regarding its potential as a new energy plant. It is stress tolerant, drought resistant, grows in semi-arid and marginal lands and more interestingly, does not compete with conventional food or feed crops for land and water, which makes it as an ideal choice to make use of vast presently underutilized land resources (Heller, 1996). The growing interest in *Jatropha curcas* as a biodiesel to help alleviate the energy crisis and generate income in rural areas of developing countries make people call it "miracle tree" (Mike, 2008). Jatropha has several industrial, pharmaceutical, environmental and other uses (Abubakar, 2010). The plant, because of its numerous uses has potential to generate rural employment, reclaim wasteland, earn foreign exchange, facilitate the establishment of rural based agro-industries, improve the socio-economy of rural dwellers and lead to overall development of the country (Abubakar, 2010). The seeds of Jatropha contain averagely 34.4% oil that can be processed to produce a high

quality biodiesel fuel usable in a standard diesel engine (Achten, 2008). Seeds yield under cultivation can range from 1,500 to 2,000 kilograms per hectare corresponding to extractable oil yields of 540 to 680 liters per hectare (Dar, 2007). There is paucity in the availability of information on the existence of variation in terms of the proximate composition in the seeds of *Jatropha curcas* L. in Nigeria. Comprehensive knowledge of germplasm diversity and variation in the proximate composition of the crop is invaluable aid in its genetic improvement. Information about nature and extent of genetic variability present in the Jatropha germplasm and association of various proximate analyses is a pre-requisite in planning successful breeding programme (Nabiswa, 2012). The progress in developing a superior variety depends largely on the genetic basis of selection of diverse parents and the breeding approach followed. For a rational approach towards the improvement of seed content, selection has to be made through proximate analysis also. This work was carried out to determine the variation in the proximate composition of some *Jatropha curcas* L. genotype seeds, determine the correlation among the proximate compositions in the Jatropha and suggest the best genotypes in terms of the proximate composition for further improvement of the crop.

MATERIALS AND METHODS

Genetic Materials

The genetic materials used in this study were obtained from Sokoto and Kebbi States (Table 1).

Data collection

Data on proximate composition were determined in the laboratory of the Product Development Research Programme of the Institute for Agricultural Research, Ahmadu Bello University, Zaria on moisture, ash, lipid, protein, fibre and carbohydrate.

Analysis of variance

The data collected were analysed using Statistical Analysis Software (SAS) version 9.0 to compute for Analysis of Variance (ANOVA). The statistical model used for analysis variance and determination of expected mean square was based on the linear model for randomized complete block design according to Kaps and Lamderson, (2004). The form of ANOVA is presented in Table 2. The model used for analysis was:

$$Y_{ijk} = \mu + g_i + \varepsilon_{ijk}$$

Where; Y_{ijk} = Seedling in treatment combination, μ = Grand mean, g_i= Genotype, ε_{ijk} = Residual error.

Correlation analysis

Phenotypic correlation analysis was undertaken according to Singh and Chaudhary (1985) using Statistical Analysis Software (SAS) version 9.0.

$$Phenotypic\ (r) = \frac{Cov_{p12}}{\sqrt{(\sigma^2 p_1).\sigma^2 p_2}}$$

Where;

Cov_{p12} = Phenotypic covariance of the progeny means between two traits,

$\sigma^2 p_1$ = Phenotypic variance of the first trait,

$\sigma^2 p_2$ = Phenotypic variance of the second trait.

Rank Summation Index (RSI)

For the purpose of selection, an index, Rank Summation Index (RSI) according to Malumba and Mock (1978) was generated from the 6 proximate compositions namely moisture, ash, protein, lipid, fibre and carbohydrate. Entry with high values in terms of the six proximate compositions ranked first while the reverse ranked last. Rank Summation Index as computed by Malumba and Mock (1978) was summarized as follows; $RSI = \sum_{i=1}^{n} R_i s$ Where; *RSI* =

Aggregate performance of a genotype using the ranking of each of the desired traits. R_i= Rank of the mean of each of the desired traits.

RESULTS AND DISCUSSIONS

Analysis of variance

All the genotypes showed significant variation ($P \leq 0.05$) for all the proximate compositions except lipid which indicated highly significant variation ($P \leq 0.01$) among the 20 *Jatropha curcas* L. genotypes (Tables 3).

Mean performance

The mean performance for the proximate composition in the 20 *Jatropha curcas* L. genotypes studied is presented (Table 4). The mean for moisture content was 8.29%and ranged from 8.2%for most of the genotypes to 8.5% for Sokoto 3. Ash content has a mean of 2.63%and ranged from 2.3%for Kebbi 3 to 2.9% for Sokoto 3. The protein content ranged from 10.6% for Kebbi 10 to 16.9% for Sokoto 1 and Sokoto 2 with a mean of 16.03%. On the other hand, lipid has a mean of 37.21%ranging from 29.8% for Kebbi 10 to 40.7% for Sokoto 1. Fibre content however, has a mean of 22.2% and ranged from 20.8% for Kebbi 10 to 26.5% for Sokoto 1. Finally, Carbohydrate has a mean of 34.9% and ranged from 31.8% for Kebbi 10 to 38.1% for Sokoto 1.

Correlation coefficients

Phenotypic correlation coefficients were computed for the proximate composition in the 20 *J. curcas* L. genotypes (Table 5). Highly significant correlation was observed between protein content and lipid content (r = 0.67). Highly significant and negative correlation was observed between protein content and fibre content (r = -0.81). Significant and negative correlation was also observed between lipid content and fibre content (r = -0.55). Significant correlation was observed between protein content and carbohydrate content(r = 0.46). Finally, significant correlation was observed between fibre content and carbohydrate content (r = 0.49).

Rank Summation Index (RSI)

The rank summation indices on the six proximate compositions, moisture, ash, protein, lipid, fibre and carbohydrate content as computed according to Malumba and Mock (1978), is presented in Table 4.1.4. Sokoto3, Sokoto2 and Sokoto1 ranked first, second and third with rank summation indices of 14, 15 and 30 respectively, while Kebbi10 ranked last with rank summation index of 118.

DISCUSSIONS

The result of the analysis revealed that Sokoto 3 had the the highest moisture content. This is followed by Sokoto 2 and Sokoto 4 while Kebbi 10 had the lowest moisture content. This indicates that Sokoto 3 will have the shortest shelf life in comparison among the genotypes studied. However, Kebbi 10 will have a longer storage life. This is because the moisture content of genotypes determines their shelf life. The higher the moisture content, the more susceptible the genotypes will be to microbial attack and this reduces the shelf life. This is in line with the observations made by Magu *et al.,* (2018). High moisture content accelerates all types of food deterioration like chemical, enzymatic and microbial actions. The result of this study shows that Sokoto 3 which had the highest ash content in comparison with the other genotypes followed by Kebbi 1 and Kebbi 5 while Kebbi 3 had the lowest ash content. This implies that Sokoto 3 followed by Kebbi 1 and Kebbi 5 have high mineral content while Kebbi 3 has the least mineral content. Ash content of a plant based food is a function of mineral elements present. The results obtained from this study also revealed that Sokoto 1 which had the highest protein content. This shows that Sokoto 1 can be used as alternative source of nitrogen. This indicates that Sokoto 1 has the potential to appreciable amount of protein. According to the

result obtained from this study, Sokoto 1 had the highest lipid content in among genotypes studied. This was followed by Sokoto 2 and Sokoto 3 which ranked 2nd and 3rd respectively while Kebbi 10 had the lowest lipid content which ranked last. This shows that Sokoto 1, 2 and 3 can be used as alternative sources of lipid for both industrial and commercial purpose (Ayodele *et al.,* 2000). Similarly, Sokoto 1 had the highest fibre content. This was followed by Sokoto 2 and Sokoto 3 which ranked 2nd and 3rd respectively while Kebbi 10 had the lowest fibre content which ranked last. Sokoto 1, 2 and 3 could be important sources of fibre. Highest carbohydrate content was also observed in Sokoto 1. This was followed by Sokoto 2 and Sokoto 3. This revealed that Sokoto 1, 2 and 3 are good sources of carbohydrate. Thus, Sokoto 1 can be a good source of carbohydrate.

CONCLUSIONS AND RECOMMENDATIONS

Significant variation ($P \leq 0.05$) has been observed for all the proximate compositions except lipid which indicated highly significant variation ($P \leq 0.01$) among the 20 *J. curcas* L. genotypes. Highly significant correlation was observed between protein content and lipid content (r = 0.67). Highly significant and negative correlation was observed between protein content and fibre content (r = -0.81). Significant and negative correlation was also observed between lipid content and fibre content (r = -0.55). Significant correlation was observed between protein content and carbohydrate content(r = 0.46). Finally, significant correlation was observed between fibre content and carbohydrate content (r = 0.49). Based on RSI, Sokoto 1, Sokoto 2 and Sokoto 3 are the best in terms of proximate composition.

Recommendations

Further studies should be carried out on how Jatropha seeds and their oil can be more useful in human nutrition in form of dietary supplements and possibly livestock feed production. Further research should therefore be carried out on how to detoxify Jatropha in order to take full advantage of the potential it offers.

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Table 1: Genetic Materials for the study

S/No.	Accession	Source	S/No.	Accession	Source
1	Sokoto 1	Sokoto State	11	Kebbi 1	Kebbi State
2	Sokoto 2	Sokoto State	12	Kebbi 2	Kebbi State
3	Sokoto 3	Sokoto State	13	Kebbi 3	Kebbi State
4	Sokoto 4	Sokoto State	14	Kebbi 4	Kebbi State
5	Sokoto 5	Sokoto State	15	Kebbi 5	Kebbi State
6	Sokoto 6	Sokoto State	16	Kebbi 6	Kebbi State
7	Sokoto 7	Sokoto State	17	Kebbi 7	Kebbi State
8	Sokoto 8	Sokoto State	18	Kebbi 8	Kebbi State
9	Kebbi 1	Kebbi State	19	Kebbi 9	Kebbi State
10	Kebbi 2	Kebbi State	20	Kebbi10	Kebbi State

Table 2: Form of Analysis of variance

Source of Variance	DF	MS	EMS
Genotype	$(g\ -1)$	MS_g	$\sigma_e^2 + r\sigma_g^2$
Error	$(g-1)(r\ -1)$	MS_e	σ_e^2

DF = Degree of Freedom, MS = Mean Square, EMS = Expected Mean Squares, r = number of replication, g = number of genotype, MS_r = Mean Square due to Replication, MS_g = Mean Square due to Genotype, MS_e = Mean Square due to Error, σ_e^2 = Error Variance,σ_g^2 = Genetic Variance.

Table 3: Analysis of variance for the proximate composition of 20 *Jatropha curcas* L. genotypes

Source	DF	Moisture	Ash	Protein	Lipid	Fibre	CHO
Genotype	19	0.03*	0.06*	5.22*	17.97**	3.86*	9.49*
Error	38						

DF = Degree of Freedom, CHO = Carbohydrate. *Significant at ($P \leq 0.05$), **highly significant at($P \leq 0.01$).

Table 4. Mean performance for the proximate composition of 20 *Jatropha curcas* L. genotypes

Entry	Moisture	Ash	Protein	Lipid	Fibre	Carbohydrate
Sokoto1	8.2	2.5	16.9	40.7	26.5	38.1
Sokoto2	8.4	2.7	16.9	39.9	22.8	37.7
Sokoto3	8.5	2.9	16.6	39.6	22.7	37.3
Sokoto4	8.4	2.6	16.5	39.5	22.6	36.9
Sokoto5	8.3	2.7	16.5	39.2	22.5	36.7
Sokoto6	8.4	2.7	16.5	38.6	22.5	36.0
Sokoto7	8.4	2.5	16.3	38.5	22.3	35.7
Sokoto8	8.3	2.7	16.3	38.5	22.3	35.4
Sokoto9	8.3	2.6	16.3	38.0	22.3	35.1
Sokoto10	8.2	2.6	16.2	37.7	22.0	34.8
Kebbi1	8.3	2.8	16.2	37.5	21.9	34.4
Kebbi2	8.3	2.5	16.2	36.8	21.9	34.3
Kebbi3	8.3	2.3	16.2	36.5	21.8	34.1
Kebbi4	8.2	2.4	16.2	36.4	21.8	33.8
Kebbi5	8.2	2.8	16.2	36.3	21.7	33.8
Kebbi6	8.2	2.7	16.0	35.9	21.6	33.8
Kebbi7	8.2	2.8	16.0	35.4	21.5	33.4
Kebbi8	8.2	2.7	16.0	34.8	21.5	33.2
Kebbi9	8.2	2.5	15.9	34.7	21.3	32.1
Kebbi10	8.2	2.5	10.6	29.8	20.8	31.8
LSD	0.2	0.4	3.4	6.4	2.8	5.8

LSD = Least Significant Difference.

Table 5: Correlation coefficients between proximate composition traits in 20 *Jatropha curcas* L. genotypes

	Ash	Protein	Lipid	Fibre	Carbohydrate
Moisture	0.01	-0.19	-0.19	0.07	-0.048
Ash		-0.18	-0.11	0.11	-0.18
Protein			0.67**	-0.18*	0.46*
Lipid				-0.55*	-0.22
Fibre					0.49*

*Significant at ($P \leq 0.05$), **highly significant at ($P \leq 0.01$).

Table 6: Rank Summation Index (RSI)

Entry	Moisture	Ash	Protein	Lipid	Fibre	CHO	Rank
Sokoto3	1	1	3	3	3	3	14
Sokoto2	2	5	2	2	2	2	15
Sokoto1	12	14	1	1	1	1	30
Sokoto4	3	11	4	4	4	4	30
Sokoto5	6	6	5	5	5	5	32
Sokoto6	4	7	6	6	6	6	35
Sokoto8	7	8	8	8	8	8	47
Sokoto7	5	15	7	7	7	7	48
Kebbi1	9	2	11	11	11	11	55
Sokoto9	8	12	9	9	9	9	56
Sokoto10	13	13	10	10	10	10	66
Kebbi2	10	16	12	12	12	12	74
Kebbi5	15	3	15	15	15	15	78
Kebbi3	11	20	13	13	13	13	83
Kebbi4	14	19	14	14	14	14	89
Kebbi6	16	9	16	16	16	16	89
Kebbi7	17	4	17	17	17	17	89
Kebbi8	18	10	18	18	18	18	100
Kebbi9	19	17	19	19	19	19	112
Kebbi10	20	18	20	20	20	20	118

CHO = Carbohydrate

CGBPB 057

Genotypic variability and trait profile of jute mallow (*Corchorus olitorius*) genotypes

Oyetunde, O.A.[1*], Olayiwola, M.O.[2] and Ariyo, O.J.[3]

[1]School of Agriculture, Lagos State Polytechnic, Ikorodu, Nigeria
[2]College of Agricultural Sciences, Olabisi Onabanjo University, Ogun State, Nigeria
3College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

[*]Correspondence to: biyi.oyetunde@gmail.com

Abstract

Evidence of genotypic variability among crop genotypes will provide basis for selection for improvement of desired traits. Nineteen genotypes of Corchorus olitorius were grown during the rainy season of 2018 to investigate the extent and pattern of genotypic variability within the germplasm. Data on plant height (PHT), number of leaves per plant (NOL), stem girth (STG), number of branches per plant (NOB), root fresh weight (RFW), leaf fresh weight (LFW), and harvest index (HID) were subjected to analysis of variance and principal component analysis. Leaf fresh weight was taken as yield to construct the genotype × yield-trait combination (GYT) biplot to visualize the trait profiles of the genotypes. There were significant ($p \leq 0.05$) genotype mean squares for PHT, NOL, RFW, LFW, TBW, and HID. Phenotypic variance was higher than genotypic variance for all measured traits except LFW. The first two principal component axes, which were characterized by all the measured traits except STG and HID, controlled 64.90% of observed variation. Only genotype NGB01261 belonged to the sector containing all the yield-trait combinations, and was considered resourceful for simultaneous improvement of leaf yield with other traits. Further studies should determine the extent of interrelationship among the traits considered, and the trait profiles of the remaining genotypes.

Keywords: *biplot, jute mallow, improvement, selection, variability*

Introduction

Jute mallow (*Corchorus olitorius* L.) is an annual shrub belonging to the family malvaceae. The tender or dried leaves are nutritious and are popularly cooked into a thick viscous soup in many households in Africa (Adjatin et al., 2019) since they are rich in vitamins and minerals (Branda et al., 2004). It has been classified as an underutilized indigenous vegetable in Nigeria, and like several others, can be cultivated at low costs, on poor soils with little or no management, and can better tolerate the vagaries of weather than the exotic vegetables (Tanimonure, 2021). Thus, the vegetable is often seen growing freely as a weed and is often regarded as 'poor man's vegetable' (Gowthami et al., 2019).

Jute mallow is under-researched (Tanimonure, 2021). Most studies involving the vegetable have focused on improving agronomic practices and nutritional quality while paying little or no attention to genetic differences among germplams lines. Thus, there is limited information on the extent and pattern of genetic and morphological diversity among available genetic materials. Evidence of genotypic variability among crop genotypes will provide basis for selection for improvement of desired traits. Genetic superiority among germplam lines is

determinable through observable differences in plant characters (Oyetunde et al., 2021; Akin-Idowu, et al., 2016). Selection is often based on multiple traits. This implies that a superior genotype should have attained desirable levels for several important traits. Identification of superior genotypes among available genotypes is mostly often encumbered by unfavourable interrelationships among characters. Thus, knowledge of the pattern of trait variation among genotypes and the trait profile of available jute mallow genotypes will aid the exploitation of existing genotypic diversity for improvement of the crop for desired traits. Yan and Fregeau-Reid (2018) proposed the genotype x yield-trait (GYT) biplot analysis approach to combat the challenges posed by evaluation of genotypes based on multiple traits. They described the worth of a genotype by its value for yield in combination with other traits (Y-T) rather than its levels for individual traits. The approach is gaining popularity in discriminating among crop genotypes, and has been successfully applied to selection in amaranthus (Oyetunde et al., 2021), barley (Karahan and Akgun, 2019), cowpea (Oliveira et al., 2019), and wheat (Kendal, 2019; Mohammadi, 2019). This study investigated the extent and pattern of genotypic variability within *C. olitorius* genotypes, as well as the trait profiles of the genotypes with a view to providing information for, and identifying genetic materials for improvement of the crop.

Materials and method

Experimental field

The study was conducted at the Teaching and Research Farm of the Department of Crop Production and Horticulture, Lagos State Polytechnic, Ikorodu, Nigeria during the mains cropping season of 2018.

Genetic materials

A number of eighteen (18) genotypes of *C. olitorius* (Table 1) were evaluated with a local check. Seeds of the 18 genotypes were obtained from the National Agency for Crop Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria while seeds of the local check were obtained locally from a known farmer.

Field evaluation and phenotyping

The experimental area was tilled mechanically by ploughing twice and then harrowing. Raised beds 2 × 1 m were made manually, and cured poultry manure was applied at the rate of 10 tons/ha. The accessions were laid out in randomized Complete Block Design with two replications. Tow-row plots were used. The rows were of length of 2 m, spaced 0.4 m apart in each block. Borders were established on either side of each block to take care of border effects. Seeds were planted at the rate of 1.5 kg/ha, by drilling. Dry fine sand was mixed with the seeds to enhance even distribution within the drills. Weed control was done manually subject to field inspection. Harvesting was done by uprooting at 42 days after planting, when the plants had reached marketable stage. The soil around the base of each plant was soaked to minimize loss of root biological matter.

Ten plants were chosen randomly per accession; at the rate of five per row per replicate for data collection. Plant height (PHT) (cm), number of leaves per plant (NOL), and stem girth (STG) (mm), and number of branches per plant were measured. To measure root fresh weight (RFW) (g), leaf fresh weight (LFW) (g), and total biomass weight (TBW) (kg), the plants in each plot were all uprooted, and the roots were washed carefully in water. Leaves were plucked and expressed as percentage of the total biomass weight to obtain the harvest index. Observations were recorded according to the *Chocorus olitorius* descriptors of IPGRI (1999). Values obtained for RFW, LFW, and TBW were converted to kg/ha.

Data analysis

Data obtained were subjected to analysis of variance and principal component analysis in

SAS version 9.4 (SAS Inst., 2011). Estimates of genetic parameters were also via 'proc varcomp' in SAS. Leaf fresh weight was taken as yield to construct the genotype × yield-trait combination (GYT) biplot to visualize the trait profiles of the genotypes. Ten genotypes were selected for the biplot analysis using the procedure of Yan and Fregeau-Reid (2018) described by Oyetunde et al. (2021). The GYT biplot was constructed using the GGEBiplotGUI package in R.

Results and Discussion

Significant ($p \leq 0.05$) genotype mean squares were observed for plant height, number of leaves per plant, root fresh weight, leaf fresh weight, total biomass weight, and harvest index (Table 1). The only variable factor in the experiment was the genotype. Thus, the significant genotype mean square indicated the presence of substantial genetic variability for possible selection for improvement of these traits. Mia et al. (2020) reported comparable results among 12 tossa jute genotypes.

Estimates of genetic parameters are shown in Table 2. Phenotypic estimates (variance and coefficient of variation) were consistently higher than the corresponding genotypic estimates except for leaf fresh weight for which the values were equal suggesting the influence of the environment in the expression of other characters but leaf fresh weight. Both genotypic and phenotypic variances were highest for number of leaves per plant and were observed to be 68.98 and 70.47% respectively while the lowest estimates of 0.0001 and 0.0002 respectively were obtained for root fresh weight. Estimates of environmental (ECV), genotypic (GCV), and phenotypic coefficients of variation (PCV), broad-sence heritability (Hb), genetic advance (GA), and genetic advance (% of mean) (GAM) were highest for stem girth (76.50), leaf fresh weight (102.22), total biomass weight (117.41), leaf fresh weight (1.0), stem girth (-0.74), and leaf fresh weight respectively (210.57). for the respectively parameters, the lowest estimates were obtained for number of leaves per plant (7.19), number of branches per plant (12.05), harvest index (19.08), number of branches per plant (0.12), root fresh weight (0.01), and number of branches per plant (8.61). The higher PCV than GCV values with low ECV values for plant height, number of leaves per plant, leaf fresh weight, total fresh biomass weight, and harvest index implied high contribution of the genotypic component for the expression of the characters. These observations compare favourably with those of Mia et al. (2020) for plant height, stem girth, and fresh biomass weights of *C. olitorius*. High GCV and PCV values are those > 20% while values < 10% are low while values between the two extremes are medium. Medium to high estimates of GCV and PCV for a character is an indication of sufficient variability for effective selection for improvement of such character. Yadeta et al. (2011) reported comparable findings for plant height, stem base diameter, and green weight per plant of *C. olitorius*.

High broad-sense heritability for a character is an indication of little influence of the environment on expression of the character. Heritability estimates have been classified as high, moderately high, medium, and low for values >80%, 60-79%, 40-59%, and <40% respectively. Thus, plant height, number of leaves per plant, leaf fresh weight, total fresh biomass weight, and harvest index with high or moderately high Hb estimates were showed a true reflection of genotype potentials. Additionally, high GCV and PCV estimates were observed for the same traits, implying the possibility of considerable advance from selection based on the traits. These observations are comparable with those of Sreelathakumary and Rajamory (2004).

Principal component analysis of measured traits revealed that two of the seven possible principal components (PCs 1 and 2) jointly accounted for 64.90% of the observed variation among the *C. olitorius* genotypes

(Table 3). The PC1 had Eigen value of 3.36 and singly explained 42% of the total variation while PC2 with Eigen value of 1.84 controlled 22.9% of the total variation. High character loadings (≥ 0.30) were obtained in PC1 for plant height (0.46), leaf fresh weight (0.51), root fresh weight (0.46), and total biomass weight (0.524) while the PC2 was characterized by numbers of leaves and branches per plant both with loadings of 0.61. The Eigen value signifies the relative discriminating capacity of the PC. The PC1 had the highest discriminating power (>2) and would therefore be more reliable in identifying important sources of variability within the germplasm. The high loadings of plant height and root, leaf and total biomass weights in PC1 is an indication that the characters were the major contributors to variation among the *C. olitorius* genotypes. These characters will likely be crucial in maintaining variability within the breeding population and they could be the focus of selection in jute mallow improvement programme. Denton and Nwangburuka (2012) earlier reported the significant contributions of plant height, number of leaf per plant, and fresh and total plant weights to variability within 15 *C. olitorius*a accessions.

Figure 2 shows the polygon view of the GYT biplot for possible trait profiles of 10 selected jute mallow genotypes. The polygon view revealed three sectors with the NGB01261, and NGB00187 as the vertex genotypes in their respective sectors while NGB00230, NGB01264, and NGB00201 were joint vertex genotypes in their sector. The GYT biplot adequately dispersed the genotypes based on their level of superiority. Only genotype NGB01261, which was ranked as the most superior belonged to sector 1 which contains all the yield-trait combinations considered. Sector 2 contained genotypes NGB00193, NGB00187, NGB00196, NGB00219, and the local check which were next in rank. In the same vein, sector 3 comprised NGB00230, NGB01264, and NGB00201 which were the lowest-ranking genotypes. The fact that NGB01261 was associated with all the yield-trait combinations in this study suggests superior trait profile of the genotype.

Substantial genetic variability existed among the jute mallow genotypes for effective selection using plant height, leaf and root fresh weight, and total fresh biomass weight as selection criteria. Genotype NGB01261 which is associated with all the yield-trait combinations considered would be resourceful for simultaneous improvement of leaf yield with other traits. Further studies should determine the extent of interrelationship among the traits considered, and the trait profiles of the remaining genotypes.

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Table 1: Mean squares of some important characters of *Corchorus olitorius* grown in 2018

SOV	DF	PHT	NOL	STG	NOB	RFW	LFW	TBW	HID
Block	1	0.10ns	2.53ns	0.76ns	2.63ns	2.60*	4.90**	60.8**	4.01*
Genotype	18	60.97**	139.45**	5.70ns	2.53ns	2.80**	32.80**	34.83**	5.15**
Error	18	11.81	1.49	7.57	1.99	0.53	0.49	5.59	0.88

* and **, significant at 5 and 1% probability respectively; DF, degrees of freedom; PHT, plant height; NOL, number of leaves per plant; STG, stem girth; NOB, number of branches per plant; RFW, root fresh weight; LFW, leaf fresh weight; TBW, total fresh biomass weight; HID, harvest index.

Table 2: Estimates of genetic parameters of selected characters of *Corchorus olitorius*

Variability parameter	Plant height	Number of leaves per plant	Stem girth	Number of branches per plant	Root fresh weight	Leaf fresh weight	Total biomass weight	Harvest index
Environmental variance	11.8124	1.4924	7.5662	1.9871	0.0001	0.0000	0.0056	0.0009
Genotypic variance	24.5797	68.9788	-0.9312	0.2718	0.0001	0.0016	0.0146	0.0021
Phenotypic variance	36.3921	70.4712	6.6350	2.2589	0.0002	0.0016	0.0202	0.0030
Environmental coefficient of variance	17.1880	7.1927	76.4971	32.5833	60.3209	17.8841	61.7573	10.3541
Genotypic coefficient of variance	24.7939	48.9004	26.8366	12.0505	82.7887	102.2192	99.8165	15.9593
Phenotypic coefficient of variance	30.1689	49.4266	71.6352	34.7400	117.0809	102.2192	117.409	19.0750
Heritability (broad sense)	0.6754	0.9788	-0.1403	0.1203	0.5000	1.0000	0.7228	0.7000
Genetic advance	8.3934	16.9269	-0.7447	0.3725	0.0146	0.0824	0.2116	0.0790
Genetic advance as percentage of mean	41.9753	99.6626	-20.7103	8.6101	120.8715	210.5716	174.8000	27.5126

Table 3: Summary of principal component analysis of measured traits of *C. olitorius*

Trait	Principal component 1	Principal component 2
Leaf fresh weight	0.508	-0.153
Plant height	0.461	0.197
Number of leaves per plant	0.112	0.610
Stem girth	-0.033	-0.260
Number of branches per plant	0.113	0.609
Root fresh weight	0.463	-0.222
Harvest index	-0.117	-0.231
Total biomass weight	0.524	-0.159
Eigenvalue	3.357	1.835
Percent (%) variation controlled	0.420	0.229
Cumulative (%) variation controlled	0.420	0.649

Table 4: Estimates of yield-trait combination and superiority indices of 19 *C. olitorius* genotypes grown in 2018

Genotype	ID on biplot	Y*PHT	Y*NOL	Y*STG	Y*NOB	Y*RFW	Y*HID	Y*TBW	Superiority Index
NGB 01261	1	3.781	2.120	3.385	2.345	4.046	2.789	4.044	3.216
NGB 00193	2	0.368	1.159	0.910	1.193	0.338	1.593	0.292	0.836
NGB 00187	3	0.561	1.637	0.986	1.178	0.070	1.098	0.118	0.807
NGB 00196	4	0.284	1.982	0.356	0.754	-0.208	0.028	-0.114	0.440
NGB 00219	5	0.404	0.122	0.671	0.842	0.028	0.865	0.058	0.427
Local Check	6	0.149	0.052	0.247	1.186	-0.039	0.639	-0.037	0.314
NGB 00199	7	-0.148	0.065	-0.115	-0.062	-0.134	0.118	-0.211	-0.069
NGB 00194	Not selected	-0.082	0.000	-0.167	0.327	-0.197	-0.092	-0.276	-0.070
NGB 00195	Not selected	-0.080	-0.376	-0.094	0.034	-0.251	-0.058	-0.188	-0.145
NGB 00192	Not selected	-0.242	-0.367	-0.399	-0.217	-0.333	-0.365	-0.312	-0.319
NGB 00207	Not selected	-0.364	-0.100	-0.405	-0.450	-0.311	-0.420	-0.305	-0.336
NGB 00197	Not selected	-0.387	-0.476	-0.451	-0.548	-0.340	-0.593	-0.333	-0.447
NGB 00198	Not selected	-0.474	-0.528	-0.555	-0.653	-0.371	-0.484	-0.368	-0.490
NGB 00188	Not selected	-0.524	-0.647	-0.633	-0.773	-0.358	-0.711	-0.378	-0.575
NGB 00224	Not selected	-0.617	-0.949	-0.762	-1.002	-0.403	-0.805	-0.400	-0.706
NGB 00235	Not selected	-0.664	-0.972	-0.621	-1.069	-0.406	-0.877	-0.403	-0.716
NGB 00230	8	-0.658	-0.899	-0.769	-1.018	-0.403	-0.881	-0.399	-0.718
NGB 01264	9	-0.653	-0.894	-0.782	-1.035	-0.392	-0.919	-0.388	-0.723
NGB 00201	10	-0.655	-0.931	-0.804	-1.032	-0.336	-0.926	-0.401	-0.726

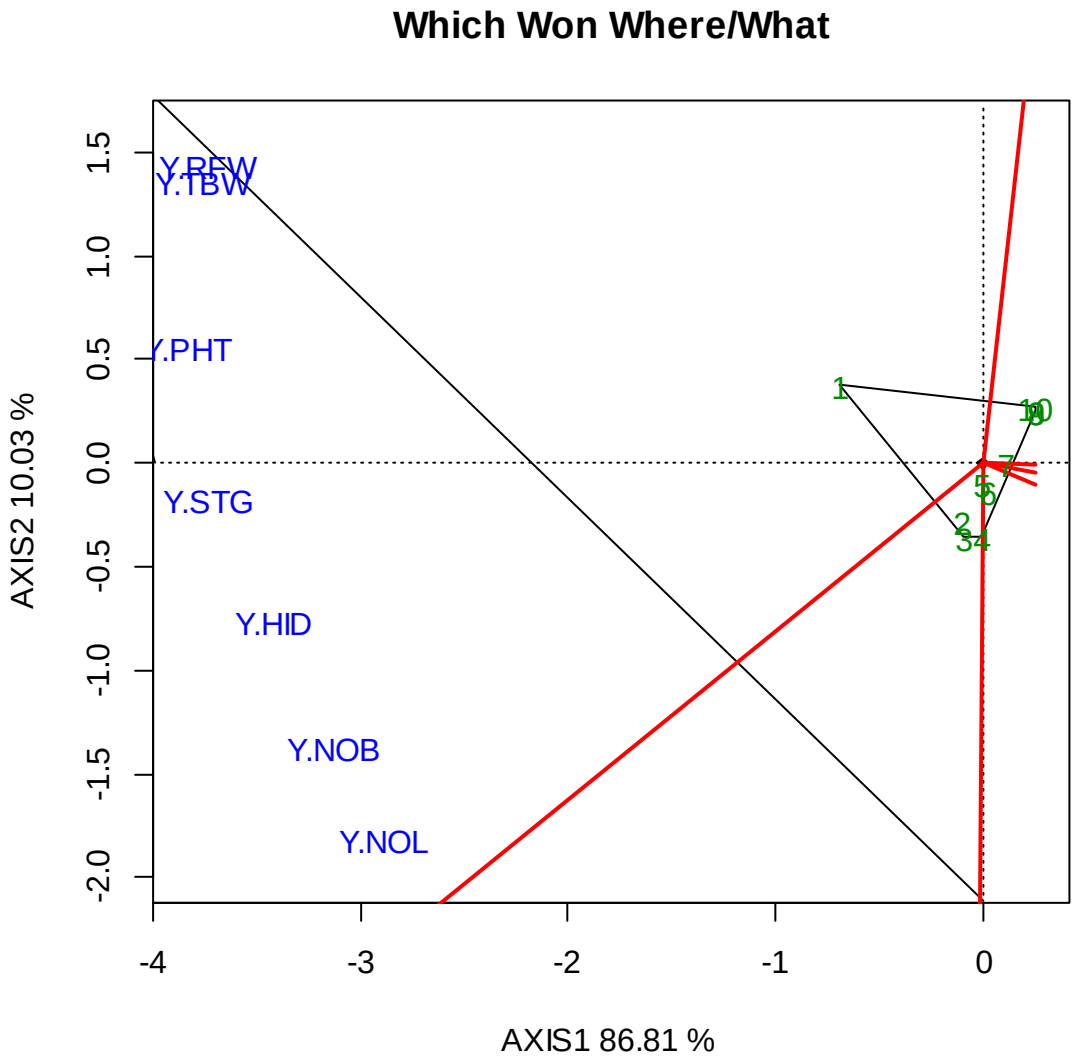

Fig. 1: The genotype × yield-trait biplot of 'which won where' of selected seven best and 3 worst *Corchorus olitorius* genotypes (names in Table 4).

Y.PHT, Y.NOL, Y.NOB, Y.STG, Y.TBW, Y.RFW, Y.HID = Leaf fresh weight combination with plant height, number of leaves per plant, number of branches per plant, stem girth, total fresh biomass weight, root fresh weight, and harvest index respectively.

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Repeatability and Representativeness of Testing Sites for Maize (*Zea mays* L.) Hybrids

*M. Oyekunle, R.S Adamu, A. Usman and H. Mani

Institute for Agricultural Research Samaru, Ahmadu Bello University, PMB 1044, Zaria, Nigeria.
*Corresponding author: Email: moyekunle@abu.edu.ng; +2348059884805

Abstract

Assessment of testing sites for selection of superior genotypes is crucial in maize hybrid development and commercialization. Twenty-five maize hybrids were evaluated at nine sites in Nigeria in 2020 to identify ideal test sites for selection of superior maize hybrids in Nigeria. Genotype, site and genotype × site interactive effects were significant (P<0·01) for grain yield. Mean grain yield of the hybrids ranged from 3950 kg ha^{-1} for P4063W to 6660 kg ha^{-1} for WE9216. The highest yielding hybrid, WE9216 out-yielded the best commercial check (Oba Super 13) by 13.8%. The biplot analysis revealed that WE9216 was the highest-yielding and stable hybrid across sites. Kadawa, Mokwa, Birnin Kudu and Ibadan were the most discriminating sites. Mokwa and Birnin Kudu were the most repeatable sites with repeatability of 82.0 and 78.6%, respectively. Kadawa was discriminating and representative site and, thus, were identified as the ideal testing site in Nigeria. The ideal testing site would facilitate the identification of high-yielding maize hybrids for commercialization in Nigeria.

Keywords: *Ideal hybrid, multiple environments, GGE biplot, stability analysis*

Introduction

Maize (*Zea mays* L.) is an important staple food crop and source of calories for rural and urban dwellers of Nigeria. Savanna agro-ecological zones of the country have the highest potential for increased maize productivity because of the suitability of climatic factors required for its production. Its production has steadily increased over years replacing other cereal crops such as sorghum and millet (Macauley, 2015). Despite the suitability of the savannas of the country for maize production and productivity, its production is still constrained by a number of biotic and abiotic stress factors such as drought, low soil nitrogen, poor adaptation to test environments, and vulnerability to diseases and pests (Badu-Apraku *et al.*, 2010). Contrasting environments in the country are caused by different factors such as insufficient and erratic rainfall, varying soil fertility, temperatures and cultural practices, cation exchange capacity of the soil, parent material and agent of weathering, field management, all of which act and interact to influence performance of the maize crop (Abdulai *et al.*, 2007; Badu-Apraku *et al.*, 2011a). As a result, assessment of diverse testing sites to determine their suitability in selecting superior maize hybrids for commercialization is crucial for sustainability of maize production and productivity in Nigeria.

The existence of genotype × environment interactions (Badu-Apraku et al. 2011b; Oyekunle *et al.* 2017) usually complicates the selection of outstanding maize hybrids and the suitable testing sites that could be used to identify high-yielding and stable genotypes for commercialization. This challenge necessitates the need for extensive testing of hybrids in multiple environments over a period of time before an appropriate genotypes could be selected for commercialization. Over years, maize hybrids were developed from different breeding programmes and extensively tested at different sites for identification of superior hybrids and were registered and released in

Nigeria. However, with the changes in climatic and other factors, there is need to re-examining the reliability of the testing sites in identifying outstanding products for commercialization. Therefore, the objective of this study was to assess the repeatability of the testing sites and identify ideal testing sites for selection of superior maize hybrids in Nigeria.

MATERIALS AND METHODS

Twenty-five maize hybrids were evaluated at nine sites in Nigeria in 2020 using 5 × 5 lattice design with three replications. A plot consisted of two rows 5-m long, with inter-row spacing of 0·75 m and intra-row spacing of 0·5 m. Three seeds were planted per hill and later thinned to two plants/hill, giving a final population density of 53,333 plants/ha. A compound fertilizer NPK 20:10:10) was applied at the rate of 60 kg N, at two weeks after planting (WAP) in all experimental sites. An additional 60 kg N ha^{-1} urea was top-dressed at 6 WAP. The experiments were kept weed-free by applying 5 l ha^{-1} each of a mixture of gramoxone as a foliar contact herbicide and atrazine as a pre-emergence herbicide. Subsequently, manual weeding was done as necessary to maintain the trials weed-free.

Data collected on grain yield was subjected to analysis of variance (ANOVA) for each site and across sites using PROC GLM of SAS (SAS 2002). Repeatability of grain yield was computed for each site. The grain yield of the hybrids was subjected to GGE biplot analysis (Yan, 2001). The data were not transformed (Transform=0), not standardized (Scale=0), and were environment-centred (Centring=2).

RESULTS AND DISCUSSION

Genotype, site and genotype × site interactive effects were significant ($P<0·01$) for grain yield (Table 1). Site had the highest impact on grain yield, accounting for 53.7% of the total variation in grain yield followed by genotype × site interaction that accounted for 34.7% of the variation in grain yield whereas genotype accounted for 11.6% of the total variation in grain yield. The large contribution of the site and genotype × site interaction effects and low genotypic effect in the present study were not surprising because the hybrids evaluated were the elite hybrids emanating from different breeding programmes. This result is in agreement with the findings of Mohammadi *et al.* (2009), Badu-Apraku *et al.* (2010, 2011b), Oyekunle *et al.* (2017) who reported that the largest contribution of the total variation was due to environments and the least to genotypes. Mean grain yield of the hybrids ranged from 3950 kg ha^{-1} for P4063W to 6660 kg ha^{-1} for WE9216 (Table 2). The highest yielding hybrid, WE9216 out-yielded the best commercial check (Oba Super 13) by 13.8%. In the genotype main effects plus genotype × environment interaction (GGE), PC1 and PC2 accounted for 57.9% of the G+G×E variation for the grain yield of the maize hybrids evaluated at nine sites in Nigeria (Fig 1). The GGE biplot analysis revealed that WE9216 was the highest-yielding and stable hybrid across sites. Kadawa, Mokwa, Birnin Kudu and Ibadan were the most discriminating sites. However, Mokwa and Birnin Kudu were the most repeatable sites with repeatability estimates of 82.0 and 78.6% respectively (Table 3), indicating that expression of maize genotype at these sites would be stable over time. Kadawa was the most discriminating and representative site and, thus, was identified as the ideal testing site in Nigeria. This results is in disagreement with the findings of Oyekunle *et al.* (2017) who identified Minjibir as the most discriminating and representative location in Nigeria.

In conclusion, Kadawa was identified as the ideal testing site and would facilitate the identification of high-yielding maize hybrids for commercialization in Nigeria. The hybrid, WE9216 was the highest-yielding and stable hybrid across sites and should be promoted for

adoption and commercialization to alleviate food insecurity in Nigeria.

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Table 1. Mean squares for grain yield of maize hybrids evaluated at nine sites across Nigeria in 2020.

Source	df	Grain yield kg ha^{-1}	% Contribution
Site	8	58872501**	53.74536
Block (Site x Rep)	108	2613838**	
Rep (Site)	18	2730363**	
Genotype	24	4225986**	11.57385
Site x Genotype	192	1582885**	34.68079
Error	324	789324	

**, Significant at 0.01 probability level.

Table 2. Grain yield and other agronomic traits of maize hybrids evaluated at nine sites in 2020.

Code	Hybrid	Grain yield, kg ha^{-1}	Yield advantages over best check, %
1	WE1254	5752	0.2
2	WE1259	5845	1.8
3	WE3205	5626	-2.0
4	WE3210	5210	-10.2
5	WE4207	5978	4.0
6	WE4208	6089	5.7
7	WE5202	5876	2.3
8	WE5210	5524	-3.9
9	WE5215	5860	2.0
10	WE5229	6260	8.3
11	WE6204	6150	6.7
12	WE6205	6028	4.8
13	WE7202	6015	4.6
14	WE7208	6008	4.5
15	WE7211	5268	-9.0
16	WE8204	5459	-5.1
17	WE8206	6050	5.1
18	WE8216	5986	4.1
19	WE9202	5981	4.0
20	WE9214	6195	7.3
21	WE9216	6660	13.8
22	Oba Super 9	5250	-9.3
23	Oba Super 13	5740	0.0
24	SC719	5607	-2.4
25	P4063W	3950	-45.3
	Mean	5775	
	LSD	745	
	CV	14	
	Repeatability	0.7	

Table 3. Repeatability of grain yield of maize hybrids evaluated at nine sites in Nigeria in 2020.

Site	Repeatability, %
Abuja	46.6
Birnin kudu	78.6
Ibadan	68.3
Kachia	52.0
Kadawa	57.1
Lere	67.7
Minjibir	69.5
Mokwa	82.0
Zaria	52.1

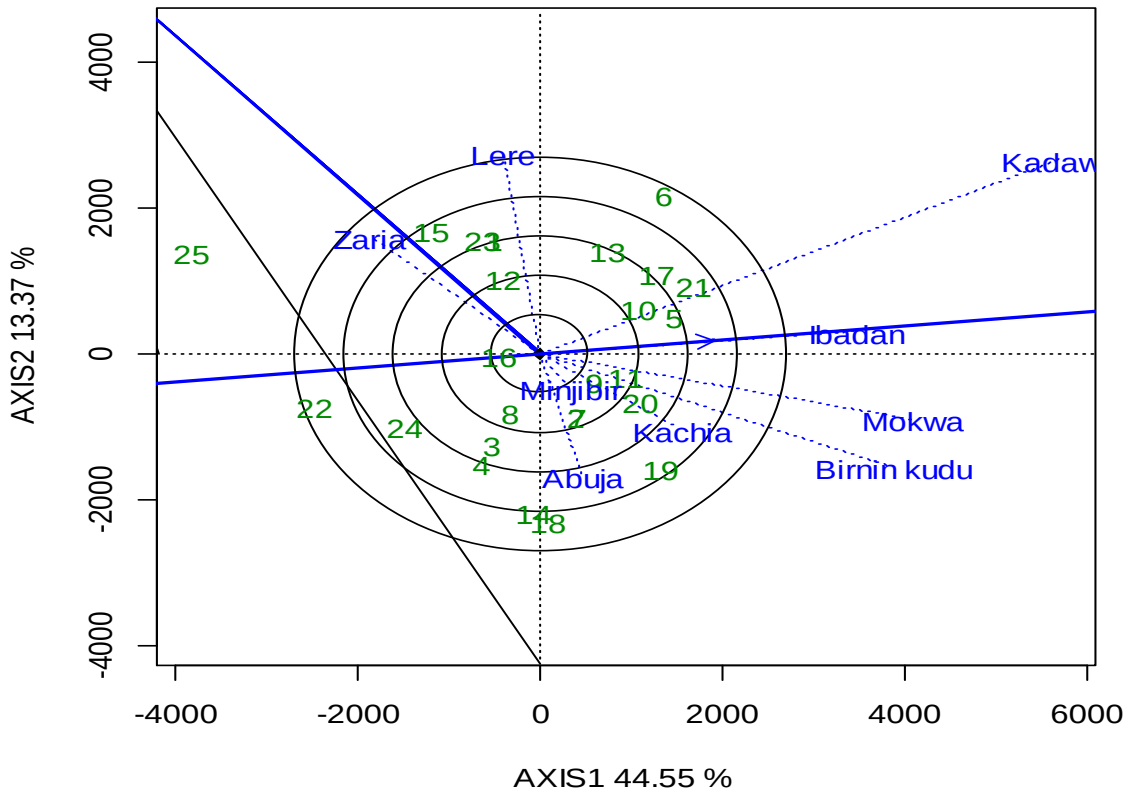

Fig 1. The biplot view showing the ranking of the nine sites in Nigeria based on both the discriminating ability and representativeness of the locations. See legend for hybrids in Table 2.

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ESTIMATION OF GENETIC DIVERSITY IN SOME COTTON (*Gossypium hirsutum*) GENOTYPES

[1*]A.M. Iyad., [1]Echekwu, C.A., [1]Ado, S.G., [1]Katung, M.D., U.U. Umar[2] and [1]Jibrin M.S.
[1]Plant Science Department, Institute for Agricultural Research Ahmadu Bello University, Zaria
[2]Department of Biological Sciences, Kaduna State University, Kaduna

*Corresponding author's email: amiyad0803@gmail.com, +2348033818281

ABSTRACT

Forty nine cotton genotypes were evaluated at Samaru, Northern Guinea Savanna Zone of Nigeria during 2013 cropping season. The experimental design used was 7×7 lattice design with three replications. Cluster analysis grouped 49 cotton genotypes into five major clusters with (PC1, PC2, PC3, PC4 and PC5) having Eigen values greater than one accounted for 63.60% of the total variability and account values of 17.25%, 14.34%, 12.02%, 10.66% and 9.32%, respectively, with each cluster having distinct features. The present investigation provided considerable information useful in genetic improvement of cotton. Genotypes PIMA S4, RSA (79) 4A, ASA (78) 34A, SAMCOT 12 and SAMCOT 13 obtained from the USA, Mexico and Nigeria were the best materials for all the traits studied. These genotypes should therefore be considered the best potential parents for use in hybridization programs for the improvement of cotton in Nigeria. Important morphological and fiber quality traits like greater seed cotton yield potential, plant height, fibre length, fibre fineness, number of sympodia per plant and number of bolls per plant etc. served as selection criterion to produce promising cotton genotypes. The low level of genetic diversity observed among the genotypes studied indicate the need for breeders to search for novel and diverse material in order to explore the unutilized genetic diversity for future cotton breeding program of Nigeria.

Keywords: *Genetic diversity, Cotton, Phenotypic, Genotypic*

Introduction

The word "cotton" is derived from the Arabic word "al qatan" (Chaudhary and Guitchounts, 2003) used to describe fine textile. Cotton is the leading non-food agricultural and industrial fiber crop grown in more than 80 countries (Dutt *et al.*, 2004; Shakeel *et al.*, 2011). There are four domesticated species of the cotton, two tetraploid cultivars from Americas, *Gossypium hirsutum* (L.) and *G. barbadense*, and two diploid cultivars from Africa and Asia, namely *G. arboretum* and *G. herbaceum* (Wendel and Cronn, 2003). Of the four species, *G. hirsutum* (upland cotton) has dominated world cotton commerce, being responsible for 95% of the annual cotton, with approximate annual plantation of 35 million ha worldwide and grown in over 50 countries (Wilkins *et al.*, 2000). Cotton is the world's most important natural textile fiber and an important source of feed, foodstuff, and oil, with approximate world consumption put at 27 million metric tons per year (Chen *et al.*, 2007). The seed has approximately 25,000 cotton fibres', which are specialized single-celled trichomes that occur on the epidermal layer of the ovule (Wendel and Cronn, 2003; Chen *et al.*, 2007). Cotton production provides income for approximately 180 million people, with the fibre industry producing $30 billion worth of raw cotton and its economic impact estimated to be approximately $500 billion/year worldwide (Chen *et al.*, 2007). China is the largest producer of cotton followed by the United States, which grows cotton worth $6 billion/year for fiber and approximately $1 billion/year for cottonseed oil and meal, on

12 million acres (Chen *et al.*, 2007). Additionally, cotton is a major economic driver for some developing countries like India, Pakistan and Uzbekistan, among others (Wendel and Cronn, 2003). Cotton crop, mainly grown for fibre purpose, is also an important source of vegetable oil among its many other valuable uses.

Worldwide cotton breeders and producers have expressed concern over the narrow genetic basis of cultivated cotton germplasm that has caused a decline in yield and quality. Globally cotton breeding programmes are working with a narrow germplasm pool thus resulting in genetic bottleneck through historic domestication events and selection (Iqbal *et al.,* 2001). Breeders used only a fraction of the available germplasm for cultivar breeding and most of the modern cultivars were developed by reselection rendering a drastic reduction in genetic variability (Guang and XiongMing, 2006). Therefore, the genetic potential of cotton yields has not reached its full potential and current cotton yields are static if not declining.

Genetic variability and heterozygosity within populations existed in both natural and cultivated populations (Jain and Workman 1966). Assessment of the genetic diversity of cotton cultivars is essential to breeding strategies, such as the characterization of individuals, accessions, and for the choice of parental genotypes in breeding programs. For any meaningful plant-breeding programme, accurate determination of genetic diversity is an essential step for an effective utilization of germplasm resources. An accurate estimation of genetic diversity can be invaluable in the selection of diverse parental combinations to generate progenies with maximum genetic variability and heterosis. The foregoing hence form the basis for employing multivariate statistical tools approaches to estimate genetic variability of some cotton genotypes. This study was carried out to determine the variation pattern in collection of cotton germplasm and identify characters that differentiate genotype into different groups.

MATERIALS AND METHOD

The experimental materials for this study comprised of forty nine cotton genotypes selected from the germplasm pool of the Institute for Agricultural Research Samaru. The collections include 19 genotypes each from Nigeria and the USA, 8 from Sudan and 3 from Egypt. Evaluation was carried out in 2013 cropping season at the Institute for Agricultural Research Farm Samaru. The experiment was laid out in a 7 × 7 simple lattice design with three replications. Each replication consisted of seven sub-blocks with seven genotypes in each sub-block. Each genotype was grown in one row of 5m length. A spacing of 90cm between rows and 40cm between plants was maintained. All cultural practices according to IAR recommendations for cotton were adhered to. Agronomic and fibre quality traits were recorded on 5 randomly selected plants in each entry within each replication for twelve characters as follows; plant height (cm), number of days to 50% flowering, number of monopodia per plant, number of sympodia per plant, number of bolls per plant, boll weight (g), seed cotton yield (kg/ha), seed index (g), lint index (g), ginning outturn, per cent span length (mm), micronaire (µg/inch). The genetic divergence of the 49 cotton genotypes was estimated based on Ward's method using a squared euclidian distance (Kumar *et al.*, 2000). Divergence analysis is a technique used to categorize genotypes that are similar into one group and others into different groups. D-square statistics (D^2) developed by Mahalanobis (1936) has been used to classify the divergent genotypes into different groups. Principal component analysis was performed using statistical software SAS version 9.0. The data obtained from the 49 genotypes were analyzed using principal component analysis multivariate technique. With 12 traits and 49 cotton genotypes, a data matrix of 12 × 49 was prepared for the analysis. In principal component analysis, one of the most commonly used criteria for solving the number-of-components problem is the Eigen value one criterion, also known as the Kaiser criterion (Kaiser, 1960). With this

approach, you retain and interpret any component with an Eigen value greater than 1.0. Thus, principal components with eigenvalue > 1 were used for further analyses. Important traits in each principal component were determined from the associated Eigen vectors.

RESULTS AND DISCUSSION

Genetic Divergence Analysis

The D^2 statistic values which is based on the pooled mean of genotypes resulted in classifying the 49 genotypes into five distinct clusters (Table 1). This reveals the presence of wide genetic diversity among the experimental materials. The genetic distance among the 49 genotypes is presented in a dendrogram (Figure 1) which classified the genotypes into five clusters. Cluster 2 had one genotype accounting for 2% of total genotypes characterized by highest plant height, highest number of days to 50% flowering, highest fiber length, high micronaire, high ginning outturn, moderate seed index. Cluster 2 had also one genotype accounting for 2% of total genotypes and is characterized by high plant height, highest seed cotton yield, moderate number of sympodia, highest number of bolls, highest boll weight, highest micronaire, and low number of monopodia. Cluster 3 consisted of two genotypes accounting for 4% of total genotypes characterized by high days to 50% flowering, lowest seed cotton yield, high number of sympodia, highest seed index, highest ginning outturn, lowest plant height, moderate number of bolls, low fiber fineness, and low number of monopodia. Cluster 4 consisted of seventeen genotypes accounting for 34% of the total genotypes characterized by high days to 50% flowering, highest lint index, high fiber length, moderate number of sympodia, high number of bolls, low seed cotton yield and plant height. Cluster 5 consisted of twenty eight genotypes accounting for 57% of total genotypes characterized by moderate seed cotton yield, low fiber length, high days to 50% flowering, moderate plant height, high number of monopodia, high ginning outturn, lowest seed index, and high number of sympodia.

When dissimilarity between a pair of variety is defined on a multivariate criterion, it is useful to determine the plant characters responsible for the dissimilarity and the relative contributions that the various characters make to the total variability in the germplasm (Albert, 2014). The range of dissimilarity obtained between the 49 genotypes was 1.376 (cluster 1) to 1.778 (cluster 5). Cluster 5 was more divergent than the others since it recorded high for seed cotton yield and other traits that contributed indirectly to improve yield. Therefore, hybridization between genotypes belonging to clusters 5 and especially cluster 1 is expected to produce genotypes with high heterotic values. The selection of parents should also consider the special advantage of each cluster and each genotype within a cluster depending on specific objective of hybridization (Chahal and Gasal, 2002). Thus, crosses involving genotypes of cluster 5, with any other cluster are suggested to exhibit high heterosis and could result in segregates with higher cotton seed yield.

Principal Components Analysis

Results from the principal component analysis for morphological and fiber quality traits are presented in Table 2. Eigen values and variances associated with each principal axis were extracted by principal component analysis. Five out of the twelve principal components (PC) extracted had Eigen values greater than one and altogether explained 63.60% of the total variation among the 49 cotton genotypes and were thus considered for further analyses. Eigen vectors of Principal Components for 12 Characters in 49 cotton genotypes are presented in Table 3. Principal component 1, contributed 17.25%, to the total variability. The variation on principal component 1 was mainly attributed to number of sympodia, followed by number of bolls, and ginning outturn. The PC 2 contributed 14.34% to the total variability and was depicted mainly in, plant height, followed by seed cotton yield, days to 50% flowering and number of monopodia. The PC 3 contributed 12.02% to the total variability and was mainly attributed to boll

weight and seed index. Principal component 4 contributed 10.66% to the total variability and was mainly attributed to days to 50% flowering and fibre length. The PC 5 described additional contribution of 9.32% to the total variability, illustrated primarily the divergence in lint index, fiber length and boll weight. Results from PCA revealed that only first five principal components of the twelve principal components contributed more than one Eigen values and jointly accounted for (63.60%) to the total variance among the genotypes, indicating that the total variation was fairly distributed across all of the studied traits and the level of variability exhibited by this population is low, substantiating the results from the analysis of variance, that revealed non-significant differences for most of the studied traits. To avoid a status quo whereby all the germplasm look alike, breeders must search for novel and diverse material in order to explore the unutilized genetic diversity for future generations. This result corroborates the findings of Makinde *et al.* (2010).

The principal component scores of the 49 nine cotton genotypes based on the first five principal components are presented in Table 4. On principal component 1, the highest score (-4.83) was recorded by RSA (79)4^{A}, followed by SAMCOT 6, Y 1422(79)4^{G}, BAR 14/25(81)24, and SAMCOT 11. On principal component 2, SAMCOT 11 has the highest score PIMA S-1, BAR 14/25(81)39, TX-CABS-1-83 and BAR XL 7(79)8. SAMCOT 11, Y 1422(79)5 and ACSA (79)13^{A} had the highest score on principal components 3. On principal components 4, SAMCOT 11 had the highest score, followed by BAR-XL 7(79)8, Giza 69, ASA (74)80, and TAMCOT SP 37. For principal components 5, ACSA (79)5, SAMCOT 13, TAMCOT SP 27, SAMCOT 8, and G.C.A-NH-1-83 contributed highest to the total variability.

CONCLUSION

Information on the genetics of economic values controlling traits of economic values in species is very important for breeding purposes. Based on the results of the current work, parental materials have been identified and classified in cotton germplasm. Important morphological and fiber quality traits like greater seed cotton yield potential, plant height, fibre length, fibre fineness, number of sympodia per plant and number of bolls per plant etc. served as selection criterion to produce promising cotton genotypes. The present investigation provided considerable information useful in genetic improvement of cotton Genotypes belonging to cluster 5 showed maximum inter cluster diversity. From cluster mean values, genotypes in cluster 1, 2, 4, and 5 deserve consideration for their direct use as parents in hybridization programs to develop high yielding cotton varieties. The genotypes in cluster 5 may be used for improvement and other desirable characters other than seed cotton yield. Presence of low genetic variability among tested genotypes emphasized the need for breeders to search for novel and diverse materials and explore the unutilized genetic diversity for future generations.

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Table 1: The 49 cotton genotypes grouped into clusters

Cluster	Number of genotypes	Genotype
1	1	BAR14/25(81)24
2	2	TAMCOT SP 21
3	2	GIZA 69, BJA 592(79)4
4 A	7	TXCABS1-83, ACSA (79)19, SAMCOT 13, TXCABS-1-83, TAMCOT SP 21S, SAMCOT 9, Y 1422 (79)5
4 B	10	BJA 592(79)28, SAMCOT 11, BAR 1425(81)39, SAMCOT 8, PIMA S-1, ACSA (79)13A, SAMCOT 10, TAMCOT CAMD-E, BAR 14/25A, BAR XL 7(79)6
5 A	8	GCA-NH-1-83, Y 1422(79)198, ACALA 91517C, Y 1422(79)80, TX-CDP37-HH-1-83, TAMCOT SP 37, BAR XL7(79)35, ASA(78)17F
5 B	20	Y1422(79)4A, TAMCOT CAMD-E, RSA(79)4A, TAMCOT CAMD-E, RSA(79)A, ACSA(79)5, TAMCOT SP374, BAR 14/25(81)23, BAR 14/25(81)43, ACSA(79)5, BAR 14/25(81)18, TX-L382BCH-1-85, ACSA(78)34A, PIMAS4, SAMCOT 8, BAR XL7(79)8, TAMCOT CABCS1-83, TAMCOT SP 37H, ASA(75)13, SAMCOT 12

Figure 1: Dendrogram of genotype clusters based on twelve morphological and fibre quality traits of 49 cotton genotypes

Table 2: Eigen values of the correlation matrix and the proportion and total variance

Principal components	Eigen values	Contribution (%)	Cumulative (%)
PC1	2.07	17.25	17.25
PC2	1.72	14.34	31.58
PC3	1.44	12.02	43.61
PC4	1.28	10.67	54.27
PC5	1.11	9.32	63.60

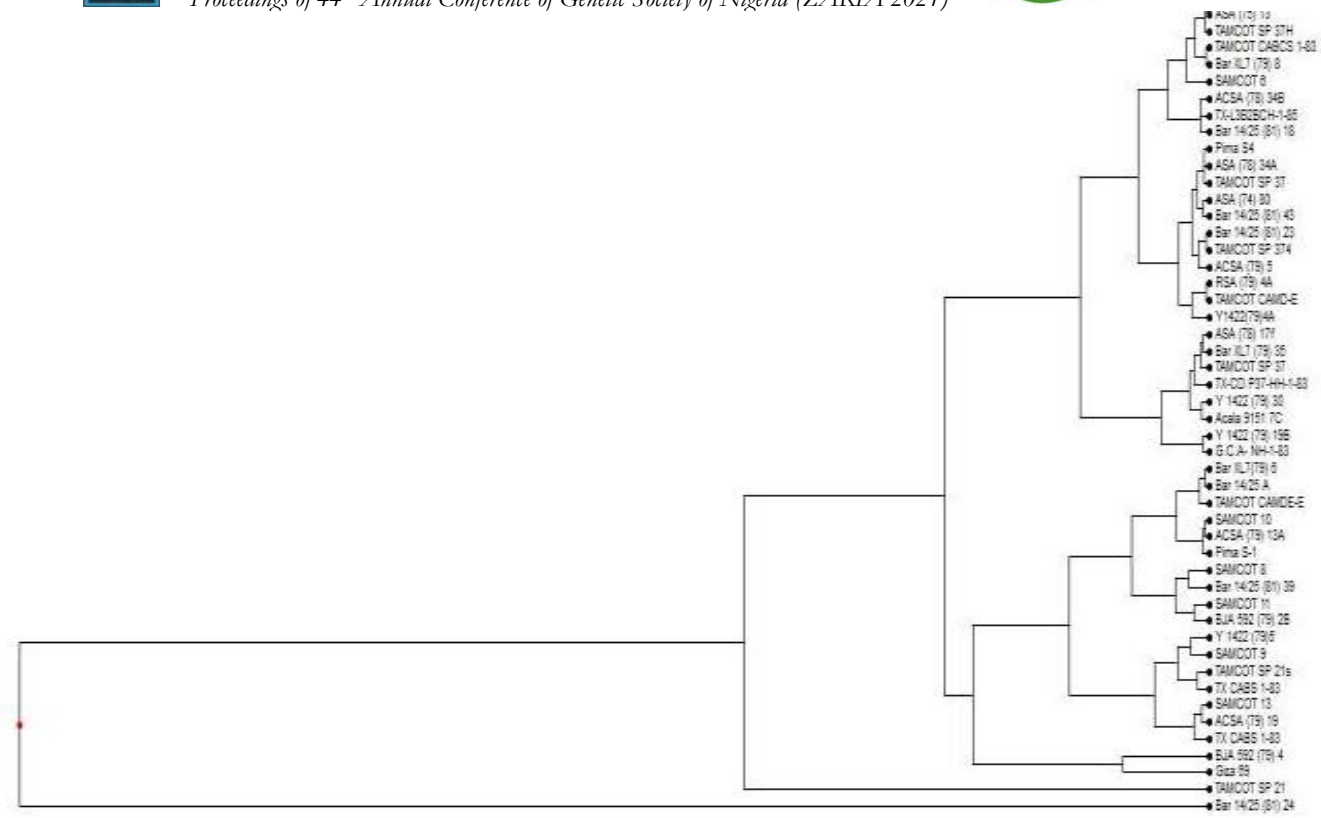

Table 3 Eigen vectors of Principal Components for 12 Characters in 49 cotton genotypes

Traits	PC1	PC2	PC3	PC4	PC5
DDF	-0.190	-0.335	-0.289	-0.505	0.147
NM	0.324	-0.355	-0.006	0.208	-0.300
NS	0.476	0.251	0.025	-0.332	-0.002
NB	-0.416	-0.287	0.001	-0.055	-0.186
BW	0.177	-0.223	0.565	0.031	-0.330
SI	-0.051	0.009	0.434	0.197	0.222
LI	0.092	-0.257	0.193	-0.280	0.624
GO	-0.422	-0.144	0.227	0.007	0.103
SCY	-0.284	0.391	-0.255	-0.064	-0.276
PH	-0.069	0.556	0.351	-0.248	0.110
FL	-0.169	0.128	-0.074	0.603	0.368
FF	-0.354	0.039	0.353	-0.202	-0.269

DFF=Days to 50% flowering, NM=Number of monopodia, NS=Number of sympodia, NB=Number of bolls, BW=Boll Weight, SI=Seed Index, LI=Lint Index, GO=Ginning outturn, SCY=Seed Cotton Yield, PHT=Plant Height, FLT=Fiber Length, FF=Fiber Fineness

Table 4: Principal component scores for 12 traits in 49 cotton genotypes

S/N	Genotypes	PC1	PC2	PC3	PC4	PC5	S/N	Genotypes	PC1	PC2	PC3	PC4	PC5
1	SAMCOT 6	3.75	0.78	-2.11	0.11	-0.04	26	Y 1422 (79) 30	1.26	0.25	0.40	-0.54	0.17
2	TX-CD P37-HH-1-83	0.57	0.75	-0.21	-0.12	1.02	27	ASA (75) 13	1.57	-0.01	0.50	0.09	-1.07
3	TX-L3B2BCH-1-85	0.17	0.92	-0.36	-0.63	0.72	28	TAMCOT SP 374	1.05	1.00	0.68	0.49	1.25
4	TAMCOT CAMD-E	-0.39	1.50	0.48	-1.36	-0.86	29	TAMCOT SP 37	1.10	-0.98	0.06	1.69	-0.37
5	TAMCOT SP 37H	0.03	0.08	-2.16	0.07	-0.41	30	TX CABS 1-83	-0.32	-0.05	0.11	1.13	0.05
6	TAMCOT CABCS 1-83	-0.39	-0.38	-0.09	-0.37	-0.84	31	Bar XL7 (79) 8	-0.85	-2.12	-0.47	2.11	-0.22
7	TAMCOT SP 37	0.41	0.06	-1.21	-0.01	-1.87	32	Acala 9151 7C	1.58	-0.57	-0.04	0.74	1.25
8	TAMCOT SP 21	0.66	0.16	0.76	1.01	-0.07	33	Bar XL7 (79) 35	0.29	-0.04	1.59	-0.74	-0.31
9	G.C.A- NH-1-83	-0.22	1.45	-0.34	0.34	1.71	34	Bar 14/25 (81) 18	0.35	-0.14	-1.15	0.97	-1.01
10	ASA (78) 17^{f}	1.10	0.67	0.42	-0.83	-0.85	35	Bar 14/25 (81) 23	0.60	-0.76	-0.72	-0.39	1.27
11	ASA (78) 34^{A}	-0.70	0.42	-1.18	-0.05	0.13	36	Bar 14/25 (81) 24	2.82	-0.91	-0.05	0.10	-0.01
12	BJA 592 (79) 2^{B}	1.40	1.20	-1.53	-0.58	0.02	37	Bar 14/25 (81) 39	1.76	-2.35	-0.25	-0.26	0.67
13	Y 1422 (79) 19^{B}	-1.23	2.11	0.66	-0.72	-0.65	38	Bar 14/25 (81) 43	-0.54	-1.16	2.07	-1.03	-1.66
14	Y 1422(79)4^{G}	-3.03	0.65	-0.64	1.29	-0.36	39	Pima S-1	-0.55	-2.62	1.23	1.57	-0.66
15	RSA (79) 4^{A}	-4.83	-0.16	-0.87	1.34	-0.36	40	Pima S4	1.38	-1.43	-0.77	0.92	-0.70
16	ACSA (78) 34^{B}	-1.61	1.59	-0.61	0.87	0.03	41	Bar 14/25 A	0.63	-1.14	1.17	0.26	-0.54
17	ACSA (79) 13^{A}	-0.53	0.96	-2.18	0.43	-0.24	42	Giza 69	-0.14	-0.50	-1.52	1.82	-0.53
18	TX CABS 1-83	0.16	2.21	-0.67	-0.49	1.80	43	Bar XL7(79) 6	0.32	-1.90	0.71	-0.11	-0.20
19	TAMCOT SP 21^{s}	-1.38	-0.48	1.29	-0.14	-0.87	44	SAMCOT 8	-0.22	0.52	-1.14	0.08	1.87
20	TAMCOT CAMDE^{-E}	-1.73	2.59	-0.91	-0.78	-2.14	45	SAMCOT 9	1.87	-0.39	0.52	-1.07	-0.59
21	BJA 592 (79) 4	-0.32	1.59	1.38	-1.16	0.41	46	SAMCOT 10	-1.10	-1.39	1.88	-1.60	1.29
22	ACSA (79) 19	-1.19	-0.71	0.99	0.57	1.31	47	SAMCOT 11	-2.72	-4.00	-2.89	-4.48	1.15
23	Y 1422 (79)5	-1.31	0.09	2.74	0.49	1.51	48	SAMCOT 12	-0.15	-0.86	0.73	0.51	-1.17

24	ACSA (79) 5	-0.45	1.09	1.31	1.54	2.55	49	SAMCOT13	0.29	1.54
25	ASA (74) 80	0.77	0.90	0.76	-1.79	0.47				

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Comparative Analysis of The Efficacy of Selected Gametocide Agents in Sorghum (*Sorghum bicolor* [L.] Moench)

Muhammad Ahmad Yahaya[1,2*], Hussein Shimelis[1], Mark Laing and[1], [1]Isack Mathew

[1]African Centre for Crop Improvement, School of Agricultural, Earth and Environmental Sciences, College of Agriculture, Engineering and Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa
[2]Department of Plant Science, Institute for Agricultural Research Samaru, Ahmadu Bello University Zaria, PMB 1044, Kaduna, Nigeria

ABSTRACT

A new generation of chemical hybridization agents (CHAs) or gametocides has shown potential to induce male sterility in predominantly self-fertilizing crops, including sorghum (Sorghum bicolor [L.] Moench). There is a lack of information on the relative efficacy of the various available CHAs for large-scale application in plant breeding programs. Therefore, the objective of the present study was to compare the relative effectiveness of three selected CHAs to induce male sterility in sorghum under a controlled environment for hybridization. Foliar applications of three CHAs and a control (ethrel, trifluoromethanesulfonamide [TFMSA], ethyl 4-fluorooxanilate [E_4FO] and distilled water [control]) were tested using three grain sorghum genotypes (ICS-1, ICS-2 and ICS-3) in two seasons. The 24 treatment combinations consisting of 4 levels of CHAs, 3 sorghum varieties and two seasons were laid out using a randomized complete block design with three replications. Data on pollen sterility, pollen diameter, plant height, and panicle height were collected and analyzed. Results showed that the CHAs had significant ($p<0.05$) differences for efficacy of inducing male sterility in sorghum. Ethrel at a dose of 1 gl^{1} induced the highest pollen sterility (98% in both seasons) but was highly phytotoxic with at least 60% mortality in the test population in both seasons, making it unsuitable for practical application. TFMSA (2 mg per plant) and E_4FO (1 gl^{1}) d induced 93% male sterility with minimal phytotoxic effects (20 to 30%). Application of either TFMSA at 2mg per plant after flag leaf emergence or 1gl^{1} of E_4FO at panicle initiation can be used to successfully induce male sterility in sorghum under greenhouse conditions.

Keywords: *Chemical hybridizing agent; emasculation, hybrid seed production; male sterility; sorghum*

Introduction

Crop genetic improvement has led to enhanced productivity and yield gains globally. Genetic gain in self-pollinated crops, including sorghum, can be enhanced through population improvement and hybrid breeding. Heterosis breeding has the potential to improve sorghum productivity by 40%, which will reduce the yield gap between potential and actual yield

(Atokple 2003). However, genetic gain in sorghum has stagnated because of limited genetic variation due to the autogamous nature of the crop, and a lack of effective and reliably cheap systems to produce hybrid seed. Sorghum has perfect flowers that are highly autogamous, with a low level of outcrossing (<20%) (Reddy et al., 2008). The flowers are small-sized and numerous, necessitating the use of new technologies for selective sterilization of the pollen grain to facilitate artificial cross-pollination for hybridization.

There are several methods, broadly categorized as physical, genetic and chemical, used to circumvent autogamy to allow cross-fertilization in sorghum. Physical or mechanical emasculation is the most widely used method, but it is more applicable where a limited amount of hybrid seed is required for breeding or genetic analyses. (Yahaya et al., 2019). Thus, the physical method is not appropriate for producing large amounts of hybrid seed. The most common genetic approach to control autogamy in sorghum is the use of a cytoplasmic male sterility (CMS) technique (Cisar and Cooper, 2003). The CMS system has been used sparingly in Africa because it is difficult to develop, maintain and use the three lines required for hybrid seed production using the CMS technique (Reddy et al., 2008). Also, there is a limit to germplasm accessions that can be used for generating all possible heterotic cross combinations due to strict requirements for maintaining the three-line CMS system. Chemical hybridization agents, also called gametocides, are synthetic chemicals that can induce temporary male sterility for developing breeding populations or producing large quantities of hybrid seed in sorghum (Sleper and Poehlman, 2006). Chemical hybridization agents (CHAs) can readily overcome the limitations posed by the physical and genetic techniques.

The use of CHAs is time and labor effective and allows for the generation of more hybrid seed for developing test populations for genetic studies, combining ability analyses and backcross breeding programs. The CHAs induce physiological abnormalities in the male gamete that prevent healthy pollen development and shedding and, ultimately, reduce pollen viability (Sleper and Poehlman, 2006). CHAs prevent the flower from self-pollination, allowing for effective cross-pollination if the female stigma is receptive and compatible with foreign pollen. Various CHAs have been used for hybrid seed production in wheat (Parodi and Gaju 2009), sorghum (Boerman et al., 2019), coriander (Kalidasu et al., 2009), rice (Efisue et al., 2010) and sunflower (Razaq et al., 2016).

In sorghum, Amelework et al., (2016) and Mangena et al., (2018) reported that E_4FO induced more than 95% male sterility when applied at a rate of 2 gl^{-1} at the heading stage. Ghebrehiwot et al., (2015) used between 1.0 and 1.5 gl^{-1} of E_4FO on tef once at the booting stage and reported 96% to 99% male sterility, without a significant reduction in female fertility or seed yield. Ethrel has been successful in inducing male sterility in different crop species. Amelework et al., (2016) applied 3.0 gl^{-1} of ethrel on sorghum and recorded 100% male sterility, while Ghebrehiwot et al., (2015) used 5.0 g l^{-1} to achieve a similar success rate in tef. Recently, trifluoromethanesulfonamide (TFMSA) has been successfully used under greenhouse and field conditions to induce temporary male sterility in sorghum without any apparent phytotoxicity in the treated plants (Hodnett and Rooney 2018; Boerman et al., 2019). The CHAs have different modes of action, efficacy and application methods, which should be considered to achieve the highest possible rate of male sterility while preserving the integrity of the female flower. However, CHAs have some disadvantages that include high costs and a high risk of phytotoxicity in treated plants when applied at higher dosages. The relative effectiveness of CHAs varies among different test populations and environments. The variation has been attributed to factors such as genotypic differences, differences in application dosages and environmental conditions, and their interactions. There is a need to assess the impact of these factors on the efficacy of the CHAs to determine their practical use in plant breeding and programs for hybrid seed production. Therefore, it is a pre-requisite to evaluate the relative efficacy of CHAs on a given test population and under the prevailing environmental condition before large-scale use in sorghum hybridization. Thus, the objective of the present study was to compare the effectiveness of three selected CHAs in inducing male sterility in sorghum under a controlled environment.

Materials and methods

Experimental site

Two experiments were performed during 2018 (rainy season) and 2019 (dry season) at the Controlled Environment Facility, University of KwaZulu-Natal Pietermaritzburg campus (29° 37' 35.20" S 30° 24' 18.60" E), South Africa.

Chemical formulation and application

The TFMSA (Sigma-Aldrich, United Kingdom) was dissolved in an aqueous solution containing glycerol (5%) and Tween 20 (0.25%) and applied at a rate of 2mg per

plant. The E_4FO (Industricord, Beijing, China) was dissolved in dimethyl sulfoxide (DMSO) (1:6 w/v), and 2% Tween 80 was added to make an E_4FO concentrate. An E_4FO solution of $1gl^{-1}$ concentration was prepared by diluting with distilled water. An aqueous solution of ethrel (Farmers Agri-Care, Pietermaritzburg, South Africa) was made by dissolving 1ml of ethrel per liter of distilled water and adding 2% Tween 80. The TFMSA was applied with a 3 ml disposable plastic pipette onto the youngest fully expanded leaves of each plant at the flag leaf emergence stage. The TFMSA was spread on the blade with a paintbrush that was previously saturated with the TFMSA solution to ensure an even and thorough distribution of the chemical. The E_4FO and ethrel were sprayed onto the panicles of the respective test plants at the heading stage using an atomizer, until runoff. Approximately, TFMSA was applied at a rate of 2mg per plant, while E_4FO and ethrel were each used at a rate of 8 to 10 ml per plant. All chemical applications were carried out early in the morning to ensure stronger adherence and to avoid chemical drift. Controlled plants were sprayed with distilled water.

Plant materials

Three grain sorghum genotypes (12ICKNSV297-4, 12KNICSV-260, and ICNSL 2014-027-2, designated as ICS-1, ICS-2, and ICS-3, in that order), were sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Kano Station, Nigeria. The genotypes are medium or tall in plant height, medium-late maturing, and drought tolerant.

Planting and trial establishment

The first experiment was established in the last week of November in 2018 (rainy season) and the second was conducted in last week of May in 2019 (dry season). Each genotype was planted in a plastic pot (300 mm diameter and 280 mm depth) filled with Gromor potting media (http://www.gromor.co.za). Four seeds were sown per pot and thinned to two plants per pot two weeks after sowing. Water was supplied adequately to maintain 75% field capacity, and the plants were fertilized with Agchem hydroponic water-soluble fertilizer (http://www.agchem.co.za). The plants received optimum fertigation using four three-minute cycles a day. Weeds were manually controlled. Appropriate isolation distance was observed for the control plots to avoid chemical drift and pollen contamination.

Experimental design

The two experiments were conducted using similar experimental designs and data

collection protocols. The study involved 24 treatment combinations as follows: three sorghum genotypes, three CHAs and a control, and two seasons making 3 x 4 x 2 factorial arrangement. Treatments were laid out in a randomized complete block design with three replications per season. Ten pots consisting of 20 plants were allocated to each treatment. In each treatment, ten plants were randomly selected and tagged, and the panicles bagged to avoid self-pollination while the rest were left uncovered for open pollination after chemical treatment. Data for each replication was collected based on average measurements of four plants.

Data collection

Male sterility was estimated as the difference between the total number of seeds in the control and the number of seeds in the treated plants per plot. Ten panicles from each treatment, including control plants, were harvested at maturity. The number of fertile (filled) and sterile (unfilled) grains per spike were counted after manual threshing and percent male sterility was computed using the formulae adapted from Chakraborty and Devakumar (2006):

$$Percent\ male\ sterility = \left(\frac{S_c - S_f}{S_c}\right) x100$$

Where, S_c= seeds per panicles in control plants

S_f= seeds per panicles in bagged and treated plants

Female fertility was recorded as a proportion of fertilized seeds in a plot relative to the total number of seeds in the control and treated plants. The number of seeds per panicle was counted for treated and untreated plants. Female fertility was determined as follows:

$$Percent\ female\ fertility = \left(\frac{S_p - S_f}{S_c - S_f}\right) x100$$

Where, S_c= seeds per panicles in control plants

S_f= seeds per panicles in bagged and treated plants

S_p= seeds per panicles in pollinated plants

Pollen sterility was determined by observation of the anthers from treated and control plants under a light microscope. Spikelets from three to four florets were randomly sampled per treatment three to five days after treatment and fixing in 70% ethanol to avoid pollen desiccation. Anthers were extracted from the spikelets, smeared on a glass slide over a drop of acetocarmine (1 %), and KI-1_2 (2%) and

examined under a light microscope. Four microscopic fields were used to count sterile and fertile pollen grains. Fertile pollen grains were considered to be well-filled pollen grains of normal size and shape that were wholly or partially stained while sterile pollen grains did not stain and were malformed and shriveled. Pollen sterility was quantified as follows (Amelework et al., 2016):

$$Percent\ pollen\ sterility = \left(\frac{P_s}{P_s + P_f}\right) x100$$

Where, P_s=number of sterile pollens

P_f= number of fertile pollens

Pollen diameter was measured under an electron microscope. Fresh pollen grains from treated and control plants were collected on dry Petri dishes and sieved through a 250-mm mesh to remove large floral particles. The fine pollen grains were mounted onto clean stubs using double-sided adhesive. The samples were coated with a 30 nm layer of gold using an EIKO IB3 Ion Coater (EIKO Engineering CO., Ltd, Osaka, Japan) at an accelerated voltage of 15 kV Electron at the Microscopy and Microanalysis Unit (MMU) at UKZN. The coated pollen grains were viewed and photographed with a ZEISS EVO LS 15 scanning electron microscope (SEM) (Carl Zeiss, München, Germany).

Plant height (cm) was measured from the soil surface to the tip of the panicle. The panicle length (cm) was recorded from the base to the tip of a panicle. Fours randomly selected plants were used to measure the plant height and panicle length and the average recorded per plot.

Data analysis

The data were analyzed for variability using a factorial randomized block design with three replications using the General Linear Model procedure in the Statistical Analysis System (SAS) software package (SAS, 2010). The genotypes, gametocides and seasons were considered as three factors. The mean values of the genotypes in each replication and each treatment were used for the analysis of variance. The Tukey's honestly significant difference test (Tukey's HSD) was used to test differences among sample means for significance at one present and five per cent levels of probability. The differences between season means were separated by a Student's t-test.

Results

Analysis of variance on genotype, gametocide and season effects

The combined analysis of variance revealed that the genotype by gametocide by season interaction effects were significant ($P < 0.05$) for plant height (Table 1). Gametocide effects were significant ($P < 0.01$) for all measured traits except plant height and panicle length. Similarly, there was significant ($P < 0.05$) seasonal variability for all measured traits except pollen sterility and pollen diameter. In contrast, the genotype differences were not significant for all measured traits except plant height.

Mean performance of test genotypes under different gametocides

Gametocides reduced plant height among the tested genotypes when compared to the control treatment. TFMSA reduced plant height of the genotype ICS-1 to 80.0 and 89.3 cm in 2018 and 2019 compared to the control, which had plant height of 87.3 and 104.7 cm in the respective seasons. Similarly, plant height for genotype ICS-2 decreased to 105.0 and 90.3 cm after E_4FO treatment in the 2018 and 2019 seasons, respectively. Among the CHAs, ethrel caused 98% male sterility in genotype ISC-2, which was the highest in both seasons (Table 2 and Figure 1). Ethrel caused the lowest level of female fertility of 34.2% in genotype ICS-1 in 2018, while genotype ICS-3 had the least female fertility value (26.7 %) in 2019. The application of TFMSA and E_4FO maintained high levels of female fertility (Table 2 and Figure 2).

Significant differences were recorded for pollen sterility. Ethrel induced the highest level of pollen sterility at 95.6% for genotype ICS-2 while the TFMSA caused the least pollen sterility (78.6 %) in genotype ICS-3 in 2018. Similarly, in 2019, the highest level of pollen sterility (96.4%) was caused by application of ethrel in genotype ICS-1 (Figure 3), while TFMSA caused a the lowest level of pollen sterility (77.6%) in genotype ICS-2 (Table 2).

Scanning electron microscopy analysis of pollen grains

The SEM analysis revealed that pollen grain from treated plots were shrunken or disfigured pollen grains with smaller mean diameter in contrast to the healthy pollen from the control plants (Figure 4). Pollen grains in the control plants were ellipsoidal with significantly larger pollen diameter (mean = 117.7μm in 2018 and 118.0 μm for ICS-1) when compared with treated plants. The smallest pollen diameter of 19.7 μm was recorded in ICS-2 after E4FO treatment. In comparison, the same genotype attained the widest diameter of 38.1 μm when treated with TFMSA.

Discussion

Chemical induction of male sterility in sorghum is potentially valuable for hybridization programs to enable genetic analyses, population development and heterosis breeding. Inducing temporary male infertility shortens the amount of time required for successful heterosis breeding and circumvents the need for developing suitable male-sterile and maintaining restorer lines for hybrid breeding, which are critical limiting factors during conventional hybrid breeding of sorghum. Chemical hybridization agents that selectively induce male sterility while maintaining female fertility can bypass these limitations.

The current study evaluated the efficacy of three CHAs to induce male sterility in sorghum. The differential effects of CHAs on genotypes due to environmental conditions constitute a considerable challenge because a stable and consistent CHA would be required for effective induction of male sterility. This suggested that the evaluated CHAs work within a narrow range of conditions, which must be ascertained before embarking on large-scale male sterility induction. The differential response is most likely attributable to variations in temperature and humidity, which are some of the factors that govern biological processes in a plant. Lack of consistency of gametocides across seasons has also been reported on rice genotypes (Efisue et al., 2010). These findings are similar to those of Hodnett and Rooney (2018) and Mangena et al., (2019), who reported that male sterility can be induced in sorghum with appropriate timing for application of CHAs. The significant differences among the gametocides in causing male sterility indicated that the chemical hybridizing agents used in this study had variable efficacies.

In the present study, plants treated with gametocides at panicle initiation incurred a significant reduction in plant height compared to control plants. Gametocides such as ethrel can be phytotoxic and cause significant growth impairment and poor vigor in plants leading to stunted growth, which sometimes manifests as reduced plant height. The reduction in plant height can be as a result of death or temporary inhibition of terminal buds, narrowing of leaves, chlorosis and interference with water absorption potential to support optimal plant growth. The potential reduction in plant height was also previously reported by Kalidasu et al., (2009) in coriander and Chakraborty and Devakumar (2006) in wheat, which was attributed to the effects of ethrel and E_4FO. In contrast, Loussaert (2004) reported no significant reduction in plant height when TFMSA was applied on maize.

Male sterility and female fertility are essential aspects when measuring the efficacy of any CHA. A desirable CHA is one that can induce high levels of male sterility without a significant reduction in the fertility of the female gamete (Loussaert 2004). The induction of male sterility facilitates the production of large amounts of hybrid seed and has the potential for commercial application in seed production. TFMSA and E_4FO induced high levels of male sterility with minimal adverse impact on female fertility, showing that these two CHAs could be used in large-scale hybrid seed production. Male sterility values ≥90 % were recorded in all genotypes, which suggested that the CHAs were highly effective as male gametocides. Their usefulness in hybrid seed production will, therefore, be determined by their impact on the fertility of the female flower. Although ethrel recorded the highest value for male sterility (98.5 % in 2018 and 98.4 % in 2019) among the three gametocides, it significantly reduced female fertility (60.9 % in 2018 and 73.3 % in 2019). Ethrel reduced the viability of both anthers and stigma, making it less useful as a gametocide for hybrid seed production. Amelework et al., (2016) asserted that an effective CHA for hybrid seed production must induce more than 90% male sterility in sorghum. However, the CHA must have minimal adverse effects on the female flower to facilitate cross-pollination.

From the staining test, pollen sterility was confirmed under the microscope based on the extent of staining using potassium iodide iodine (KI-I_2), which reacts with starch. During healthy pollen development, the conversion of carbohydrate substrates to starch for energy occurs in preparation to support fruit development (Amelework et al., 2016). When the CHA interferes with pollen development, the starch conversion process does not take place, and the KI-I_2 staining reaction would test negative. The lack of staining and the visible appearance of a transparent pollen grain when viewed under a light microscope is due to the absence of starch, which would indicate that the CHA successfully interfered with pollen development. In contrast, pollen grains from the control plots were deeply stained, showing the presence of starch as a result of uninterrupted pollen development. According to Loussaert (2004), male sterility was induced by TFMSA by interfering with proline and starch transportation, resulting in the disruption of the feedback loop between proline synthesis and accumulation at the respective sites. Loussaert (2004) further suggested that the osmotic potential of the developing microspores during starch accumulation is modulated by proline, and

blockage of its transport may cause microspore abortion. Amelework et al., (2016) and Chakraborty and Devakumar (2006) suggested that male sterility is induced by ethrel and E_4FO by blockage of the processes leading to starch synthesis or its metabolism. There was a moderately low level of male sterility (19.0 %) in the control treatment. Natural male sterility can occur due to fluctuations in environmental conditions such as temperature (Prasad et al., 2006). High temperatures experienced in the greenhouse resulted in the abortion of pollens in the controlled plot. Interference by the CHAs during pollen development significantly reduced pollen diameter in comparison to the control treatment. The smaller diameter observed in CHA treated pollen further showed that CHAs reduced the size of the pollen grain, which has negative implications on the fertility of the male gametes. The size of the pollen grain is correlated to food and energy reserves required during fertilization (Chakraborty and Devakumar 2006). The sterile pollen grains appeared compressed and irregular in shape due to a delay in the degeneration of the tapetum of the anther. A short delay in tapetum degeneration hampers the possibility of developing healthy pollen grains and partial or complete abortion can occur as a result depending on the extent of the degeneration. Significant changes in pollen morphology due to application of CHAs have been reported by Amelework et al., (2016) on sorghum, Chauhan et al., (2009) on tree cotton (*Gossypium arboretum* L.) and Chakraborty and Devakumar (2006) on wheat.

Conclusion

The study sought to assess the efficacy of three CHAs before embarking on a large-scale CHA-mediated hybridization program. The results showed that the CHAs had variable effects and successfully induced variable levels of male sterility in sorghum. Ethrel is an effective male fertility suppressant but its applicability in a hybrid seed development programs will be limited by its negative impact on the female gamete that reduces chances of cross-pollination. TFMSA and E_4FO were considered more applicable in a hybrid development program as they suppressed male gamete development while maintaining the integrity of the female gamete to allow controlled pollination. TFMSA should be applied twice at a rate of 2mg per plant; the first application after the emergence of the flag leaf and a second application one week later for effective induction of male sterility to facilitate outcrossing in sorghum. Alternatively, applying E_4FO at $1gl^{-1}$ at panicle initiation and another application a week later will be equally effective.

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Table1: Mean squares and significant tests for plant height, panicle length, male sterility, female fertility, pollen sterility and pollen diameter of three grain sorghum genotypes when evaluated with three chemical hybridizing agents in two seasons.

Source of variation	Degree of freedom	Plant height (cm)	Panicle Length (cm)	Male Sterility (%)	Female fertility (%)	Pollen sterility (%)	Pollen diameter (µm)
Replication	2	55.43	9.11	89.31	332.98	74.84	488.53
Genotype (V)	2	64963.35***	8.93	18.30	565.30*	122.40	231.68
Gametocide (G)	3	232.33	23.71	35520.07***	9397.69***	21157.49***	29962.97***
Season (S)	1	589.39*	85.59**	343.96***	1101.81***	141.87	276.95
VxG	6	479.63***	17.33	16.43	264.01	44.97	105.21
VxS	2	1098.76***	0.18	0.50	54.06	32.10	20.01
VxGxS	8	1006.14***	0.79	10.74	90.04	62.38	31.70
Error	46	94.23	8.47	25.40	141.57	63.39	130.26

*, **, *** denote significant effect at P = 0.05, P = 0.01 and P = 0.001, respectively.

Table 2: Mean values for plant height, panicle length, male sterility, female fertility, pollen sterility and pollen diameter of three grain sorghum genotypes when tested with three chemical hybridizing agents

Chemical hybridizing agents	Genotype	Plant height (cm)		Panicle length (cm)		Male sterility (%)		Female fertility (%)		Pollen sterility (%)		Pollen diameter (µm)	
		2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
TFMSA (2 mg)	ICS-1	87.3	89.3	14.3	15.5	94.2	89.9	80.2	74.1	83.0	85.0	23.0	28.4
	ICS-2	110.7	111.3	13.2	13.7	93.8	94.9	75.6	78.3	80.3	77.6	22.3	38.1
	ICS-3	200.0	143.0	18.0	18.0	93.1	92.8	75.1	74.0	78.6	81.6	26.4	33.9
	Mean	135.7	143.5	15.2	15.7	93.7	92.5	76.9	75.5	80.6	81.4	23.9	33.5
	HSD	27.4	60.0	5.3	5.6	9.0	14.6	46.6	11.6	14.5	15.1	28.7	37.9
E_4FO (1 gl^{-1})	ICS-1	87.3	78.0	13.7	16.3	93.1	95.3	77.2	72.6	84.7	84.3	28.0	27.9
	ICS-2	105.0	90.3	16.2	18.3	92.7	91.6	68.4	76.8	90.0	80.3	19.7	23.0
	ICS-3	187.3	191.7	12.0	15.3	94.1	93.1	77.3	71.7	82.8	83.0	23.5	27.5
	Mean	126.6	120.0	13.9	16.6	93.3	93.3	74.3	73.7	85.8	82.5	23.7	26.1
	HSD	18.9	21.5	7.0	14.2	13.4	11.3	25.5	34.0	11.9	14.6	16.2	14.5
Ethrel (1 ml/l)	ICS-1	87.3	92.0	11.7	13.0	95.9	98.4	34.2	45.4	94.9	96.4	25.2	33.9
	ICS-2	107.7	103.3	13.5	14.3	98.5	97.6	39.1	28.8	95.6	93.9	28.3	27.0
	ICS-3	194.0	184.0	13.0	15.7	98.1	97.4	40.1	26.7	91.5	96.4	22.7	29.8
	Mean	129.7	126.4	12.7	14.3	97.5	97.8	37.8	33.6	94.0	95.6	25.4	30.2
	HSD	24.6	10.9	5.4	8.3	2.8	4.4	41.8	19.5	6.8	7.7	13.8	31.4
Control	ICS-1	87.3	104.7	14.0	17.3	0.0	0.0	100.0	100.0	19.0	8.2	117.7	118.0
	ICS-2	120.0	130.7	14.0	18.7	0.0	0.0	100.0	100.0	3.2	14.6	105.1	99.5
	ICS-3	209.0	188.7	15.0	18.5	0.0	0.0	100.0	100.0	13.8	15.8	104.9	106.9
	Mean	138.8	141.4	14.3	18.2	0.0	0.0	100.0	100.0	12.0	12.9	109.2	108.1
	HSD	5.5	6.8	7.0	8.2	0.0	0.0	0.0	0.0	10.0	12.3	45.5	56.3

Tukey's HSD = Tukey's honestly significant difference test

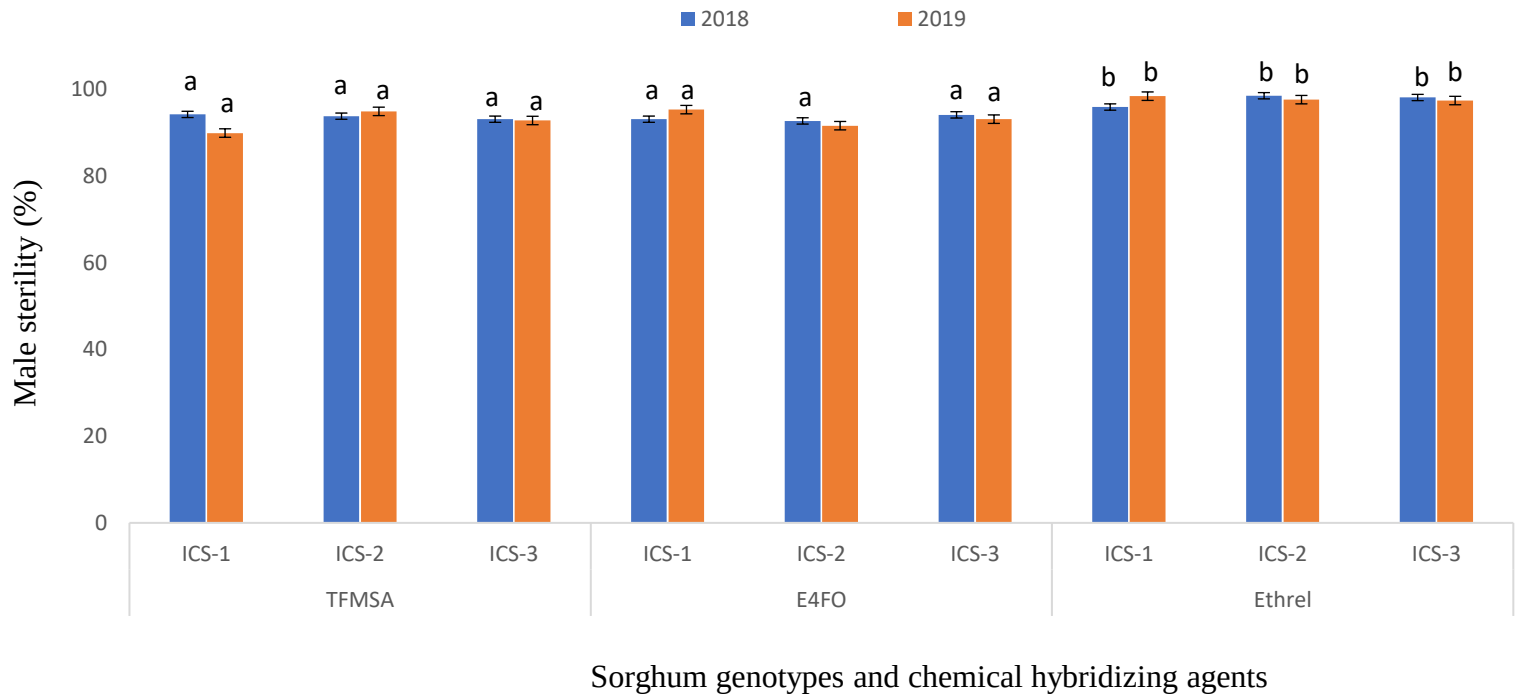

Fig 1. Male sterility (%) of three sorghum genotypes (ICS-1, ICS-2 and ICS-3) induced by three chemical hybridizing agents (trifluoromethanesulfonamide [TFMSA], ethyl 4-fluorooxanilate [E4FO] and ethrel) in two seasons. Columns with the same letter do not significantly differ ($p \leq 0.05$) according to the Tukey's test.

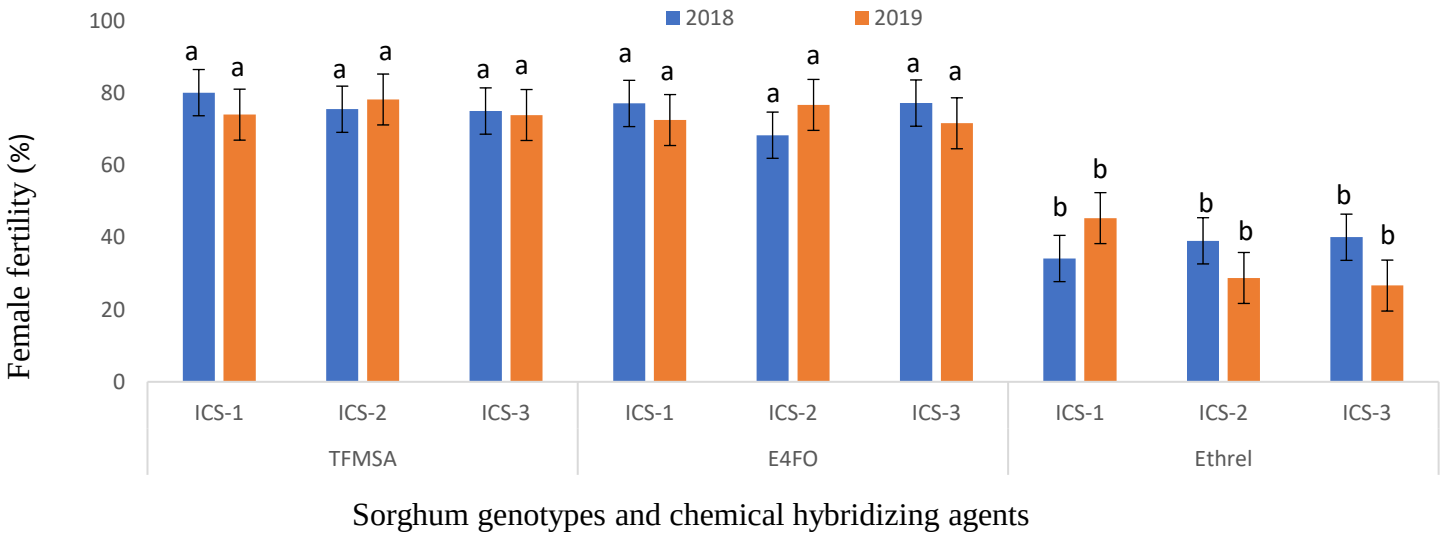

Figure 2: Female fertility (%) after application of CHAs (trifluoromethanesulfonamide [TFMSA], ethyl 4-fluorooxanilate [E4FO) and ethrel) on three sorghum genotypes (ICS-1, ICS-2 and ICS-3). Columns with the same letter do not significantly differ ($p \leq 0.05$) according to the Tukey's test.

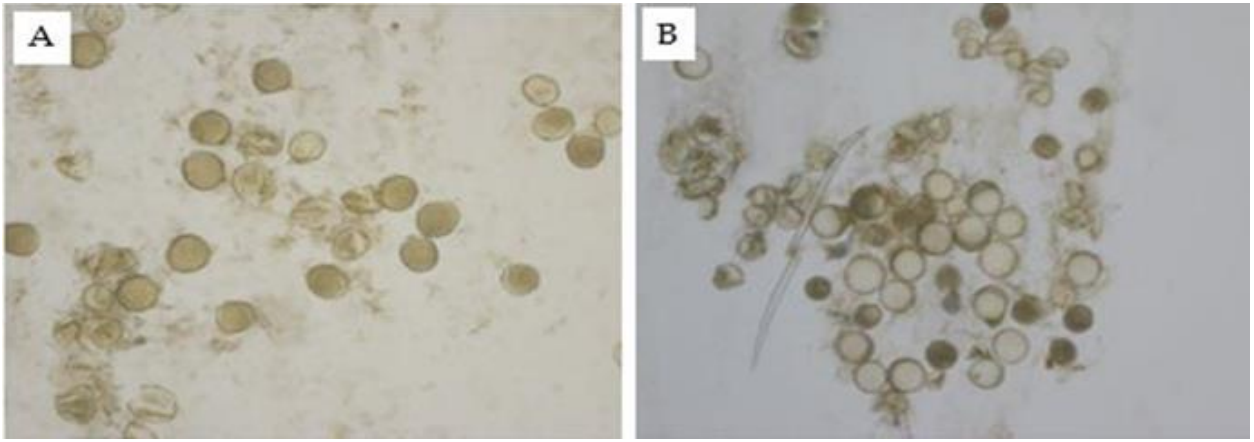

Figure 3: Sterile pollen of ICS-1 genotype following treatment with ethrel *vis- à-vis* fertile pollen from the control plot as revealed by the KI-I_2 staining test using a light microscope . Note: (A)= fertile pollen grains and (B)= sterile pollen grains. Bars: A and B = 100 µm

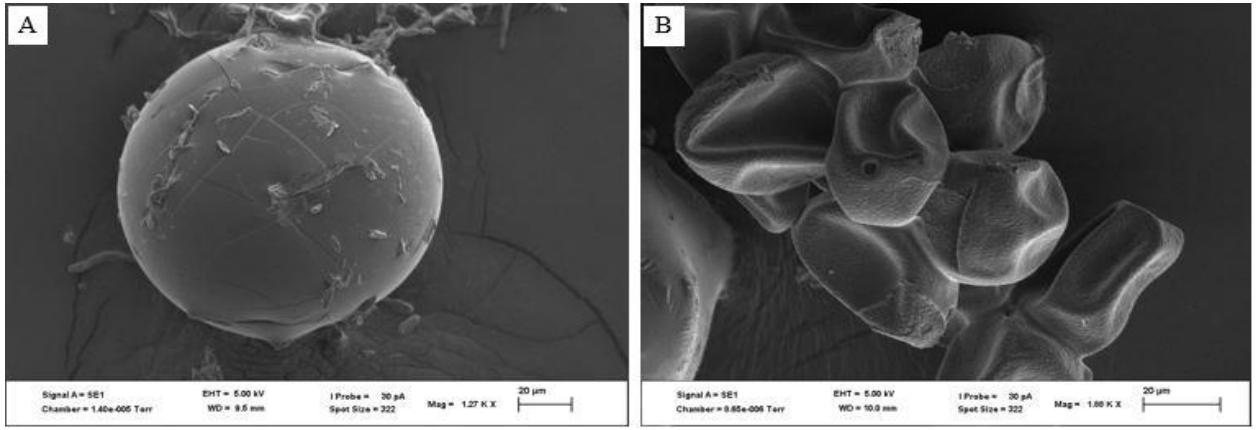

Figure 4: Sterile pollen of ICS-1 genotype following treatment with trifluoromethanesulfonamide (TFMSA) *vis- à-vis* fertile pollen from the control plot as revealed by scanning electron microscope (SEM). Note: (A)= fertile pollen grain and (B)= sterile pollen grains. Bars: A and B = 20 µm

CGBPB 061

Comparative Estimates of Mid Parent and Better Parent Heterosis of Two Crosses In Cowpea

Buba U. M

Department of Crop Production, Abubakar Tafawa Balewa University, Bauchi

ABSTRACT

Mid parent and better parent heterosis were estimated in two pair of crosses involving: two advanced breeding lines (IT98K-205-8 x IT00K-1263) and two local accessions (Tvu16514 x Aloka).The results indicated that hybrid vigour in the F_1 generations in both crosses for most of the characters under investigation. However, pod length, plant biomass and number of days to fifty percent flowering recorded negative heterosis in the first cross. All three flowering namely ; number of days to flower initiation, days to fifty percent flowering and days to pod maturity in the second cross (IT98K-205-8 x IT00K-1263) recorded shorter in F1 generation compared to their parents. This study suggest that yield can be improve by exploiting hybrid vigour inherent in these traits by manipulating these reproductive characters in cowpea provided In addition, reduced number of days to flowering and maturity offer a good prospect of breeding for early maturity or drought escape.

Keywords: *Cowpea, drought, heterosis*

Introduction

Heterosis or hybrid vigour expresses the superiority of F_1 hybrid over its parents in term of yield and other traits. Heterosis has been widely exploited in many crops, especially in cross-pollinated species like maize and watermelon. Although it has limited utilization in autogamous species like cowpea, it has been exploited in some species like tomato and sorghum (Longin *et al.,* 2012). Genetic divergence among parents has been shown to be essential requirement in the manifestation of heterosis.

In cowpea, heterosis has been demonstrated to have great potential in vegetable types, as heterotic crosses may give rise to transgressive segregates for economic traits (Sharma *et al.*, 2010).. Heterosis may also indicate some degree of genetic diversity between parents; hence, with increased genetic diversity, high levels of heterosis would be expected (Bernnet-Lartey and Ofori, 1997). On the other hand, inbreeding depression indicate a reduction or loss in vigour, fertility and or yield as a result of inbreeding. When the proportion of F_1's for mid parent and better parent are almost equal it may indicate equal involvement of additive and non -additive gene action. They further postulated that preponderance (>60%) of both types of heterosis in F_1's may infer that dominance and over-dominance could be responsible for expression of such a character. Ajibade and Ilori (2016) reported significant positive heterosis over better parent for the characters;

plant height, number of leaves per plant, 100 seed weight, number of pods per plant, number of seeds per plant, as well as, seed weight per plant. This study was establish with the objective of estimating mid parent and better parent heterosis among two crosses in cowpea

Materials and Methods

Mid parent heterosis (MPH) and better parent heterosis (BPH) were estimated, as suggested by Patel *et al.* (2014) follows;

MPH = F1 –Mid parent /Mid parent x 100, while

Better parent heterosis was estimated as

BPH is = F1- better parent/ better parent x 100

Where MP = Mid parent value, BP is the mean of better parent. F_1= mean value of the first filial generation

Results

Result obtained in this study indicated that plant height, number of leaves at 8 weeks after sowing cowpea, number of pods per plant, pod weight, seed weight, number of seed per plant and seed yield recorded positive heterosis in the F_1 generation in the cross involving TVu16514 x Aloka. However, negative heterosis (inbreeding depression) was observed for plant biomass, pod length as well as for the three flowering traits (days flower initiation days to 50% flowering and days to pod maturity).Similar trend was observed in the second cross (IT98K-205-8 x IT00K-1263).

A similar trend was observed in the second cross involving IT98K-205-8 x IT00K-1263. Most of characters under investigation recorded increase in performance in the F_1 generation compared to their parents, except for characters mentioned above days to fifty percent flowering, pod length and number of seed per pods.

In plants, heterosis is a phenomenon in which the F1 progeny exhibit values that exceed its parents. Long-standing theories attempted to explain the basis of heterosis as either due to the sum of dominant alleles across multiple loci or over-dominant interactions between alleles within the genome. Many have argued that the phenomena is a multi-genic complex trait and can be inferred as the sum total of many physiological and phenotypic traits, including extent and rate of vegetative growth, flowering time, yield and even resistance to biotic and abiotic stress (Lippman and Zamir, 2007). Crosses between inbred lines normally show increase in vigour in the F1 generation but under increase in homozygosity (selfing,) vigour and productiveness decrease by 50% in every generation due to inbreeding depression. In breeding depression estimated using the performance of F2 generation has not been considered in this preliminary study. However results obtained in this study on heterosis revealed high percent MPH and BPH for most of the characters, namely; number of pods per plant, number of seeds per plant, pod weight and seed yield, indicating their potential for future exploitation. On the other hand, flowering trait (DTI, DTFF and DTMY) show negative heterosis or decrease in number of days to flowering and maturity. This also portend prospect for early maturity. Estimates of mid parent heterosis was 80% for number of pods per plant, while better parent heterosis was 2.94% for number of leaves per plant to 86.5% in the case of number of pods per plant in the cross between TVu16514 x Aloka. Up to 80.10% mid- parent heterosis and 76.89 % for better parent heterosis were recorded for number of pods per plant (NPPL). Two of these characters number of pods per plant, pod weight per plant have been found to contribute significantly to yield This result is in agreement with the work of Rashwan, (2002) and Perthe *et al.* (2017). Similarly, 92.92 % MPH and 86.52 % BPH were recorded for pod weight per plant in this study.

Arunachalam, (2000) suggested that when performance of hybrid lines exceed that of mid parent and better parents it may indicate the occurrence of dominance type of gene action which emphasize hybridization. Many crop scientists have often opined that it would be difficult to exploit heterosis on a commercial scale in a self-pollinating crop like cowpea Perthe *et al.* (2017). However, depending on gene action that is involved, magnitude and direction of heterosis and biological feasibility of exploiting heterosis, it occurrence can be used to isolate a high frequency of its derivatives in latter generations.

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Table 1: Estimates of Mid-parental and Better-parent heterosis (%) and mid-parent (%) heterosis in cross involving Tvu16514 and Aloka.

Trait	Parent 1: (Tvu 16514)	Parent 2 (Aloka Local)	Mid Parent Value	F_1 Mean Value	Mid parent heterosis (%)	Better Parent heterosis (%)
Growth Characters						
Plant height at 8wks (cm)	21.78	19.92	20.85	**23.96**	14.91	10.00
No of leaves plant^{-1} at 8WAS	16.44	20.72	18.58	**21.33**	14.80	2.94
Phenological Traits						
Days to flower initiation	71.39	**80.61**	76.00	69.71	-8.28	-2.35
Days to 50% flowering	75.00	**79.44**	77.22	73.58	-4.69	-1.89
Days to 50% maturity	88.00	**97.94**	92.97	85.21	-8.35	-3.17
Reproductive traits						
Number of pods plant^{-1}	3.27	3.39	3.33	**6.00**	80.10	76.99
Pod length (cm)	**14.16**	13.15	13.65	12.94	-5.20	-8.61
Pod weight Plant-^{1}(g)	4.79	5.12	4.95	**9.55**	92.92	86.52
Seed weight plant-^{1}(g)	4.52	4.29	4.41	**7.36**	66.89	62.83
Number of seed plant-^{1}	28.78	28.62	28.70	**49.35**	71.95	71.47
Yield Related Traits						
Plant biomass	11.09	**17.25**	14.17	16.58	17.00	-3.88
Grain yield (Kg/ha)	314.80	287.8	301.3	**510.8**	69.6	62.26

Table 2: Estimates of Mid-parent and Better-parent heterosis (%) and mid-parent (%) heterosis in cross involving IT98K-205-8 and IT00K-1263.

Trait	Parent 1: IT98K-205-8)	Parent 2 (IT00K-1263)	Mid Parent Value	F_1 Mean Value	Mid parent heterosis (%)	Better Parent heterosis (%)
Growth Characters						
Plant height at 8wks (cm)	47.28	46.39	20.85	**54.92**	17.26	16.16
No of leaves plant^{-1} at 8 WAS	85.43	79.20	18.58	**106.17**	7.86	24.27
Phenological Traits						
Days to flower initiation	-	**-**	-	-	-	
Days to 50% flowering	75.00	**79.44**	77.22	73.58	-4.69	-1.89
Reproductive traits						
Number of pods plant^{-1}	6.63	9.77	11.80	**6.00**	80.10	76.99
Pod length (cm)	**14.16**	13.15	13.65	12.94	-5.20	-8.61
Pod weight Plant-^{1}(g)	4.79	5.12	4.95	**9.55**	92.92	86.52
Seed weight plant-^{1}(g)	4.52	4.29	4.41	**7.36**	66.89	62.83
Number of pods peduncle-1	4.33	5.80	5.06	**8.40**	62.63	44.82
Number of peduncle plant	3.10	4.80	3.95	**6.93**	75.44	44.37
Hundred seed weight (g)	52.8	44.7	48.75	**53.7**	10.15	1.70
Number of seed per pod	7.95	5.70	6.82	**4.72**	-30.7	-40.6
Grain yield (Kg/ha)	710	906.7	1463	**510.8**	69.6	62.26

SUB-THEME: Human Health and Food Security

HFS 001

A REVIEW ON MICROBIAL CONTAMINATION IN HERBAL MEDICINE; A SERIOUS HEALTH HAZARD TO CONSUMERS.

Zigau Z.A[1], Aliyu B.S[2] and Muhammad U.A[3]

[1]Department of Biological sciences, Faculty of sciences Bauchi State University, Gadau Bauchi State, Nigeria
Corresponding Author; zainabauwalzigau87@gmail.com, +2348036638487

ABSTRACT

Many medicinal plant species worldwide are used in traditional medicine for treating different diseases, about 70% of the population of Nigeria depend on traditional medicine for their primary health care needs. Safety issues associated with herbal medicines may have an exacerbated impact on consumers especially the elderly because this population has an increased susceptibility and sensitivity to health complications. Among the main safety risks related to herbal medicine is contamination by microorganisms of various kinds that may be adherent to leaves, stems flowers, seeds and roots from which herbal medicines are prepared. Most of these preparations are used in the form of concoctions mainly in form of liquid to drink after boiling or by infusion method (soaking for a particular period of time). The medicinal plants used carry a large number of microbes originating from the soil, some other contamination may occur during the periods of harvesting, handling, preparation and storage of the herbal remedies. According to several studies conducted, microorganisms such as bacteria and fungi are responsible for making herbal remedies unsafe for use. Bacteria such as Staphylococcus aureus, Salmonella typhi, Enterococcus faecailis, Microcorcus luteus and Bacillus subtilis are found to be present in isolation from herbal remedies (Braide et al., 2013). Several species of fungi such as Aspergillus flavus, Penicillum notatum, Rhizopus stolonifer, mucor and Saccharomyces species are found to be present in herbal remedies administered orally after being subjected to a series of laboratory tests (Braide et al., 2013). Most of the isolates are residents in the soil, water, air and vegetation. The high incidence of bacteria and fungi fall short of international standard and portends danger to consumers. Contamination may result from inadequate sanitary measures employed during production, packaging and storage.

Keywords: *microorganisms, contamination, bacteria, fungi and Staphyloccocus aureus.*

Introduction

It is estimated that approximately 80% of the population in developing countries uses traditional herbal medicines as part of their primary health care (WHO, 2002).

Among the main safety risks related to herbal medicines is contamination by microorganisms of various kinds that may be adherent to leaves, stems, flowers, seeds, and roots from which herbal medicines are prepared. Alternatively, microorganisms can be introduced during

harvesting, handling, open-air drying, preserving, and manufacturing (Kosalec and Tomic, 2009). Because of gradual devaluation of the knowledge associated with traditional health care-related practices (Medeiros et al., 2013) health surveys conducted in several countries have demonstrated the use of herbal medicines as a mainstream practice among elderly people compared with that among young adults (Bardia, 2007). According to the World Health Organization (WHO, 2002.) the definition of "elderly people" is established according to the socioeconomic level of each nation, with elderly individuals defined as being 60 years of age or greater in developing countries, while in developed countries, the age limit extends to 65 years. The risk of microbial contamination may have an exacerbated impact in the elderly population because this population has increased susceptibility to the consumption of herbal medicines and sensitivity to health complications due to the aging process (Almeida *et al.,* 2004).

Medicinal Plants

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseas es, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain un assessed by rigorous scientific research to define efficacy and safety (Bauer, 1998).

The compounds found in medicinal plants are of many kinds, but most are in four major biochemical
classes: alkaloids, glycosides, polyphenols, and terpenes (Bauer, 1998).

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of the thousands of types of plants with suspected medicinal properties was estimated to be US$2.2 billion in 2012 (Adeleye *et al.,* 2005). In 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars (Desmert, 1999). In many countries, there is little regulation of traditional medicine, but the World Health Organization coordinates a network to encourage safe and rational usage. Medicinal plants face both general threats, such as climate change and habitat destruction, and the specific threat of over-collection to meet market demand (Desmert, 1999).

Microorganism

A microorganism, or microbe, is a micrscopic organism, which may exist in its single-celled form or a colony of cells (Schopf *et al.,* 2017). Microorganisms include all unicellular organisms and so are extremely diverse. Of the three domains of life identified by Carl Woese, all of the Archaea and Bacteria are microorganisms. These were previously grouped in the two domain system as Prokaryotes, the other being the eukaryotes. The third domain Eukaryota includes all multicellular organisms and many unicellular protists and protozoans. Some

protists are related to animals and some to green plants. Many of the multicellular organisms are microscopic, namely micro-animals, some fungi,

Microbes are important in human culture and health in many ways, serving to ferment foods and treat sewage, and to produce fuel, enzymes, and other bioactive compounds. Microbes are essential tools in biology as model organisms and have been put to use in biological warfare and bioterrorism. Microbes are a vital component of fertile soil (Adeleye, 2005).

Bacteria

Bacteria like archaea are prokaryotic – unicellular, and having no cell nucleus or other membrane-bound organelle. Bacteria are microscopic, with a few extremely rare exceptions, such as *Thiomargarita namibiensis* (Danladi, 2009). Bacteria functions and reproduces as individual cells, but they can often aggregate in multicellular colonies, some species such as myxobacteria can aggregate into complex swarming structures, operating as multicellular groups as part of their life cycle (Danladi, 2009) or form clusters in bacterial colonies such as *Escherichia coli.*

Fungi

The fungi have several unicellular species, such as baker's yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*). Some fungi, such as the pathogenic yeast *Candida albicans,* can undergo phenotypic switching and grow as single cells in some environments, and filamentous hyphae in others (Bamnet, 1987).

Common microbial contaminants associated with medicinal plants.

The growing, harvesting and manipulation methods usually applied cannot avoid microbial contamination of the plant material which therefore reflects the environmental conditions as well as the specific hygiene during the diverse treatments (Kneifel *et al.,* 2002). Biological contamination refers to impurities in medicinal herbs and their preparations and products, and may involve living microbes such as bacteria and their spores, yeasts moulds, viruses, protozoa, insects (their eggs and larvae), and other organisms. However, products of microbial metabolism such as toxic, low-molecular-weight metabolites from moulds are important chemical contaminants (Kosalec and Tomic, 2009). The main microbial contamination of plant materials, in general, are attributed to total aerobic mesophilic, enterobacterial yeast and mould. The presence of higher numbers of spores bacteria could be explained by the fact that some of these organisms (e.g. *Bacillus* and *Clostridium* spp.) produce spores which are resistant to harsh processing, elevated heat and dry conditions. Therefore, they can survive for a long time on the product in a dormant state. *Bacillus cereus* and *Clostridium perfringens* are recognized as having potential pathogenicity and have been incriminated in food poisoning (Kunene *et al.,* 1999).

Herbal medications are likely to be contaminated with a wide variety of other potentially pathogenic bacteria. In a study where evaluation of the bacterial contamination of powdered herbal medicinal preparations sourced from identified herbal retail outlets in different parts of Kaduna, Nigeria was carried out, the results showed that a number of herbal remedies were

contaminated with *Salmonella typhi* and *Shigella* spp., *Eschericia coli* and *Staphyloccocus. aureus* (Abba *et al*., 2009). In addition, the presence of pathogenic bacteria like *B. cereus*, *Aeromonas hydrophila, Shigella* spp., *Enterobacter agglomerans*, *E. cloacae*, *Vibrio fluvialis*, *Pasteurella multocida*, *S. epidermidis*, *Acinetobacteria woffii*, *Klebsiella* spp., *B. subtilis* and *Pseudomonas aeruginosa*, and fungi *Rhizopus stolonifer* also were observed to be present in plant samples analyzed (Abba *et a*l.,2009).

Because they are widespread in the atmosphere, moulds are common natural contaminants of medicinal herbs. It is known that, under favorable conditions, some fungi can synthesize toxical metabolites – mycotoxins. Among the known mycotoxins, the most toxic one is aflatoxin synthesised by species of *A. flavus* and *A. parasiticus*, and a minor number of other fungi (Kulshresta and Katiyar, 2008). In a study of 91 medicinal herb samples in Brazil, were found that 50 % of aerial part samples were contaminated with fungi. Samples of medicinal plants were evaluated by Martin *et al.,* 2001 for the fungal contamination, and results indicated that predominant mycoflora (89.9% of the isolates) corresponded to genera *Aspergillus* and *Penicillium*, which are extremely important from the mycotoxicological standpoint. The fungal contamination of powdered herbal medicinal preparations sourced from some herbal retail outlets in some parts of Nigeria was evaluated by (Anyawu, 2010) and the results showed that all of the herbal preparations had the presence of fungal contaminants with predominance of *Aspergillus* spp. and *Penicillium* spp., but *Mucor* spp., *Candida* spp., *Trichosporium* spp., also were found. The fungal deterioration adversely affects the chemical composition of the raw materials and thereby decreases the medicinal potency of herbal drugs (Kumar *et al*., 2009).

Influence of different preparation techniques on the microbiological quality

The production of an herbal medicine generally involves the steps in which the plant part is subjected to unfavorable conditions to survival of microorganisms. Some of these processes and their influence on the microbial load are mentioned.

Drying process

Drying is basically defined as the decreasing of plant moisture content, aimed at preventing enzymatic and microbial activity, and consequently preserving the product for extended shelf life (Rocha, 2011). This process may also contribute to facilitate the marketing of plants, because drying results in reduction of the weight and volume of the plant with positive consequences for transport and storage (Rocha, 2011).

The optimization of the drying process contributes to physical, chemical and microbiological stability of the medicinal herbs. The choice of drying conditions depend on the moisture content of tissue at harvest, the plant parts used, and the temperature best suited for preservation of the requested ingredients. For this reason, adequate dryers are needed, using temperature, velocity and humidity values for drying air that provides a rapid reduction in the moisture content without affecting the quality of the active ingredients of medicinal plants (Rocha, 2011).

Other methods such as freeze-drying, oven drying and tray drying have been previously used to preserve medicinal herbs but to date there is little information in the literature on the effect of these drying conditions on the

decrease of microbial loads (Harbourne *et al.*, 2009)

Extraction methods

Water is almost universally the solvent used to extract activity. At home, dried plants can be ingested as teas (plants steeped in hot water) or, rarely, tinctures (plants in alcoholic solutions) or inhaled via steam from boiling suspensions of the parts. Dried plant parts can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures (Cowann, 1999). These kinds of preparations are usually called medicinal teas and are prepared using natural plants collected, dried and packaged without an effective hygienic and sanitary control. In addition, there can be microbiological contamination and controlling microbial contamination can be difficult in aqueous extracts (Martin *et al.*, 2001).

However, bacterial spores of the Bacillaceae family are resistant to thermal treatment usually applied in infusion preparation, and this thermal shock may stimulate spore germination. Some of these bacteria like *B. cereus* and *C. perfringens* are recognized as having potential pathogenicity and have been incriminated in food poisoning (Kunene *et al.*, 1999).

pH influence

The pH value is one of the main factors influencing the quality of medicine. It always controls many chemical and microbiological reactions (Liu, 2011). When the pH value is low (presence of acidic substances), the bacterial count could be low, but at neutral or higher pH the level of contamination of the herbal preparations could be observed to be higher. This suggests that a neutral or alkaline pH favoured high contamination levels of the herbal preparations. This agrees with the observation that bacterial growth is optimal at more or less neutral pH, around pH 5-8.5 (Abba *et al.,* 2009).

Storage

Most pre-storage processing of plant material, such as that involving drying, heat, cooling and packaging, can prevent the degradation of plant material during storage (Fennel *et al.*, 2004). Prolonged storage in poorly ventilated storehouse usually increases sample moisture content in the bulk due to heat exchange capacity, rendering herbs more susceptible to molds growth and toxin production.

Fungi are the predominant contaminants of herbs, but most such microbial populations are probably regarded as commensal residents on the plant that survived drying and storage.

Most fungi are present on plants, which develop after harvest if relative humidity is not controlled during storage (Azeez *et al.,* 1998). Moulds are responsible for biodeterioration of a number of substrates including raw materials of some medicinal plants. Samples of herbal parts stored for sale in markets located in Ibadan, Nigeria were analysed for mycoflora associated with their storage and twenty eight fungal species were isolated, showing that herbal drug plant pieces are hazardous for human health (Efunyoye, 1996). Some samples of herbal raw materials have been reported to contain aflatoxin. The reference, Riozzo *et al.*, 2004 determined the incidence of toxigenic fungi and their mycotoxins on 152 dried medicinal and aromatic herbs from Argentina, which are used as raw material for drugs. *Aspergillus flavus* and *A. parasiticus* were the predominant species isolated, and high aflatoxin concentrations were detected. The

reduction of plant enzyme activity and inactivation of microorganisms is achieved by drying. Dried plant materials tend to be hygroscopic (readily absorbing moisture) and must be stored under controlled humidity. Rehydration can lead to the decomposition of the bioactive metabolites by enzymes from microorganisms or the plant itself. Significant contamination by bacteria and fungi suggest inadequate storage facilities and poor hygienic practice during preparation of these medicinal plants. The storage processes of such products are stages during which it is important to avoid even further contamination (Gonda and Vesas, 2012).

Studies on long-term stability of dried herbal teas and preparations are rare. In a study Kumar *et al.*, 2009 examined the deterioration of herbal drug samples which were stored for 6-9 months by traders after collection. Some of the contaminated materials were found to be deteriorated by toxigenic strains of *A. flavus* and contain aflatoxin B1 which was above the permissible limit. In a study of 38, dried *P. lanceolata* leaves were exposed to atmospheres of different relative humidity (75, 45 and 0%) for 24 weeks and was evaluated for the chemical changes of the compounds of interest. It was shown that exposure to water results in loss of bioactive molecules of *P. lanceolata* dried leaves, and that colonising fungi are the key contributors to this loss. The fungal deterioration adversely affects the chemical composition of the raw materials and thereby decreases the medicinal potency of herbal drugs. Biodeterioration of herbal products samples by associated fungi during storage has drawn attention regarding quality maintenance of these products (Shukla *et al.*, 2008).

The World Health Organization (WHO) recommends that whenever required and when possible, fresh medicinal plant materials should be stored at appropriate low temperatures, ideally at 2-8°C; frozen products should be stored at less than -20°C.

Processed medicinal plant materials should be packaged as quickly as possible to prevent deterioration of the product and to protect against unnecessary exposure to potential pest attacks and other sources of contamination.

Decontamination of plant materials

Attempts have always been made to decontaminate and preserve these medicinal plants so as to get more safe, natural and potent medicines. The number of methods has been tried for decontamination such as heat treatment, UV irradiation and fumigation. However, volatility and heat sensitivity of the delicate flavor and aroma components of the medicinal plants do not permit the use of heat treatment (Gupta, 2011).

Low penetration power of UV radiations makes this irradiation method unsuitable (Gupta, 2011). Fumigation with gaseous ethylene oxide brings down the microbial burden but this method is now prohibited or restricted in many countries due to the carcinogenic nature of one of its residue in treated medicinal plants (Satomi *et al.,* 2005). Various disinfectant technologies have been suggested which include electromagnetic radiations, photodynamic pulsing, ultra high pressure and CO_2 treatment (Gupta, 2011). Gamma irradiation is now getting recognition throughout the world as a phytosanitary treatment of herbal materials. It improves the hygienic quality of various herbal materials and reduces the losses due to microbial contamination and insect damage (Farkas, 1998). Besides, it is a fast, safe, convenient, eco-friendly method which reduces the reliance on

chemical fumigants and preservatives currently used by industries. The chances of recontamination are also reduced, as it can be done after packaging (Khattak and Simpson, 2009). Some studies showed that the exposition of plant samples to different doses of gamma radiation can result in reduction in total bacterial counts and also indicated that the microbial load could be decreased by increasing the radiation-absorbed dose. These studies indicate that gamma irradiation is an effective treatment for microbial decontamination of medicinal plants (Farkas, 1998).

Microbial quality parameters

The most widely accepted and used technique is that recommended by WHO for total count of microorganisms in plant materials. According to the methodology of WHO, 10 g of sample should be suspended in 90 ml of buffer sodium chloride-peptone, adjusting the pH to 7.0. To count total aerobic bacteria, sample should be plated in duplicate, using the official technique of sowing depth on casein-soybean digest agar, and then incubated at 30-35°C for 48h. To count yeast and mold, the technique employed is the sowing depth in Sabouraud dextrose plus a solution of 10% tartaric acid to obtain pH 3.0 to 3.5. The dilution is plated in duplicate and incubated at 20-25°C for 5 days (WHO, 1998). Analysis of specific pathogens, Enterobacteriaceae and other Gram negative bacteria (*E. coli*, *Salmonella* sp., *P. aeruginosa* and *S.aureus*) consists of specific methods of cultivation and through biochemical and serological tests. The specification of WHO for total aerobic microorganisms is not more than 107 CFU/g for the plant material for use as teas and infusions and at most 105 CFU/g for internal use. The specification of WHO for yeasts and molds are at most 104 CFU/g for the plant material for use as teas and infusions and at most 103 UFC/g for internal use. High counts of fungi are a risk because of the possibility to produce mycotoxin, such as aflatoxin, which is a carcinogen toxin. The WHO also recommends a test to detect the possible presence of aflatoxins, which are highly dangerous contaminants in any material of plant origin.

In Nigeria, despite the large consumption of products derived from plants, products sold and consumed are not subject to any kind of quality control. In 1995, the Ministry of Health instituted the ordinance MS/SNVS No. 6, January 31, 1995 (WHO, 1998). In Africa the evaluation of microbial contamination of medicinal plants has increasingly become an integral part of Good Agricultural Practice (GAP) and Hazard Analysis and Critical Control Point (HACCP) concepts (Efunyoye, 1996).

Summary

The use of herbal remedies in preventive and curative medicine dates back to the primitive era and progressively gave birth to the modern day chemotherapy and medicine. Safety issues associated with herbal medicines may have an exacerbated impact on consumers especially the elderly because this population has an increased susceptibility and sensitivity to health complications.

Investigation into microbial quality of medicinal plants consumed in Northern Nigeria, after a series of antibiotic susceptibility test undergone by researchers shows that a number of microorganisms such as bacteria and fungi are responsible for making herbal remedies un safe for use. Bacteria such as

Staphylococcus aureus, Salmonella typhi, Enterococcus faecailis, Microcorcus luteus and *Bacillus subtilis* are found to be present in isolation from herbal remedies (Braide *et al*., 2013).

Several species of fungi such as *Aspergillus flavus, Penicillum notatum, Rhizopus stolonifer*, mucor and *Saccharomyces species* are found to be present in herbal remedies administered orally

After being subjected to a series of laboratory tests. Most of the isolates are residents in the soil, water, air and vegetation. The high incidence of bacteria and fungi fall short of international standard and portends danger to consumers. Contamination may result from inadequate sanitary measures employed during production, packaging and storage. Good manufacturing practices (GMP) are recommended to ensure products with wholesome quality that meets international safety standards.

Conclusion

The use of homemade and commercial herbal medicines is a major risk to the health of consumers who use these therapies due to the lack of microbial quality standards. It was observed in several studies that the levels of viable bacteria and fungi were above safety limits. Presently, herbal medicines are used along with synthetic medicines to reduce health care costs for those individuals who have limited access to modern health care facilities (Umar and Altaf, 2017) because these individuals do not have health insurance coverage and do not have much education (Kosalec and Tomic, 2009). Herbal medicines are inexpensive treatment options because they are easy to prepare or purchase in street markets that are common in this region. Herbal medicines are extensively used in Nigeria due to the country's diverse plant population, great sociodiversity, and conventional wisdom originating from ethnic backgrounds..

Because of gradual devaluation of the knowledge associated with traditional health care-related practices (Khattak, 2012), health surveys conducted in several countries have demonstrated greater use of herbal medicines as a mainstream practice among elderly people compared with that among young adults.

Several studies demonstrated the presence of aerobic bacteria and fungi above the acceptable limits as well as the presence of pathogenic bacteria in samples of herbal medicines used by consumers in Nigeria. Findings demonstrate important risks for elderly individuals associated with the use of herbal medicines and the need for surveillance and the establishment of stricter control procedures in the production/ preparation and marketing of these herbal medicines

to guarantee quality. The need for constant monitoring and control of the standard of herbal remedies available in the Nigerian market is strongly advocated to curb the menace and maintain correct quality, safety and efficacy of the final herbal preparation The steps will help to avoid additional risks to the population in the consumption of herbal medicines, which is a common cultural health care habit. Control programs for the sale of these herbal medicines should be implemented by national regulatory agencies to prevent or reduce the consumption of products outside the minimum standards of quality.

In addition, campaigns linked to primary health care units or family health programs with the aim of guiding the proper preparation of herbal medicines (avoiding microbial contamination) should be initiated.

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HFS 002

REVIEW ON GENETICS OF XY CHROMOSOMES IN RESPONSE TO IMMUNE SYSTEM AND COVID 19 PANDEMIC

Odunlade, A.K[1]., Taiwo, I.A.[2], Ajayi B.E[3] & Hameed, I.O.[4]

[1]Department of Science Laboratory Technology, Yaba College of Technology, P.M.B 2001 Yaba, Lagos;
[2]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos;
[3]Department of Biochemistry, Hallmark University;
[4]Obafemi Awolowo University, Ile-Ife.
Corresponding Author: *akodunlade@gmail.com, +2348033360197*

ABSTRACT

The COVID-19 pandemic is a global crisis which is presently affecting every sector of the entire world. Genetics is the study of hereditary and variation. The sex of an individual is determined at conception. and cytological examination of the chromosomes structures of a range of animals revealed that males and females showed certain chromosomonal differences pair of chromosomes. The sex chromosomes in male are Heterogametes (XY) while females are Homogametes (XX). The Y chromosome is one of the two sex chromosomes in humans and this chromosome has more than 59 million of building block of DNA base pairs. It contains sex-determining region Y (SRY) genes which facilitate the production of testosterone that promotes immunosuppressive effects on the immune system, leading to short life span in men. The X chromosome contain 10% of all microRNAs in the human genome which assist in human response to diseases and this is absent in Y chromosome. Gene diversity is one of the factors that can explain the sex-bias in immune responses and female predominance of autoimmune diseases. The presence of two X chromosomes XX in females provides a buffer incase the gene is mutated. The X chromosomes have a lot of genes which regulates metabolic activities, thereby giving women advantages over men in terms of susceptibility to virus. This acclaimed while men die at higher rate than women at every age from birth even during this COVID-19 pandemic in developed and developing countries.

Keywords: *Genetics, XY Chromosomes, Immune system, COVID 19 Pandemic.*

Introduction

The X chromosome is known to harbor majority of the immune-related genes. The human X chromosome encodes for a number of critical genes involved in the regulation of immunity. The X chromosome also codes for crucial microRNAs (MiR) that regulate immunity. Differential immunological responses observed in women and men are attributed to the biased response from X chromosome (Markle and Fish, 2014). Among the X-linked microRNAs, miR-233 is widely studied and has been shown to regulate neutrophil differentiation. Similarly, miR-106A, miR-424, miR542, and miR-503 have been shown to negatively regulate monocyte differentiation (Pinheiro et al., 2011). This is considered to be a cause for prevalent autoimmune disorders observed in women (Hewagama et al., 2013).

Men and women differ in their susceptibility to infectious diseases, response to vaccines, and autoimmune diseases. Though behavioural differences partly explain sex bias in infection

susceptibility, sex differences in the immune response in animal models under controlled laboratory conditions indicate the role of biological differences. Thus, a sex bias in the immune system seems at least as important. In general, females are more immune-competent and have a higher leucocyte count than males. Furthermore, type 1 helper T cells (Th1) and the cellular immune response predominate in men, whereas the Th2-controlled antibody-mediated immune response predominates in women. Sex hormones may have a role in regulating the immune response, but hormonal intervention treatment in the clinic does not always yield the results observed in preclinical animal studies. Furthermore, a sex bias in susceptibility to certain autoimmune disease is observed in pre-puberty children, which suggests that other factors play a role (Amur *et al.,* 2012).

Sex Differences in Diseases Management

Females harbor two X chromosomes, whereas males carry one X and one Y chromosome. In order to prevent excessive responses from the X chromosome, the female mammals have evolved a complex mechanism termed as X-inactivation (Garenne, 2015). Through this process one of the X chromosomes is transcriptionally silenced during the development process of a female. This leads to cellular mosaicism; which means that either the X chromosome from paternal or maternal origin is expressed in different cell populations. As a result, gene mutation in X-linked chromosome is expressed in part of the cells in females, whereas all the cells in males will exhibit the mutation (Brooks *et al.,* 1990). Cellular mosaicism has proven to provide immunological advantage for the females. Diseases such as X-linked severe combined immunodeficiency (XSCID) and immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) harbor mutations in genes that are linked to the X chromosome. X-inactivation allows part of the cells to express the wild type genes in females compared to none of the cells in males. Therefore, these genetically inherited diseases are more prevalent in males (van der Vliet and Nieuwenhuis, 2007).

In human embryos, the SRY gene encodes a unique transcription factor that activates a testis-forming pathway at about week seven of development. Before this time, the embryonic gonad is "indifferent," meaning that it is capable of developing into either a testis or an ovary. Likewise, the early embryo has two systems of ducts. Once the SRY gene product stimulates the indifferent gonad to develop into a testis, the testis begins producing two hormones, testosterone and anti-Müllerian hormone, or AMH (Koopman, 1991). Testosterone and one of its derivatives, dihydrotestosterone, induce formation of other organs in the male reproductive system, while AMH causes the degeneration of the Müllerian duct. In females, who do not contain the SRY protein, the ovary-forming pathway is activated by a different set of proteins. The fully developed ovary then produces estrogen, which triggers development of the uterus, oviducts, and cervix from the Müllerian duct (Libert *et al.,* 2010).

The recent emergence of a novel coronavirus with an outbreak of unusual viral pneumonia in Wuhan, China and then pandemic outbreak is 2019-nCoV or COVID-19. Based on its phylogenetic relationships and genomic structures the COVID-19 belongs to genera Betacoronavirus which has a close similarity of the sequences of COVID19 to that of severe acute respiratory syndrome-related coronaviruses (SARSr-CoV) and the virus uses ACE2 as the entry receptor-like SARS-CoV (Seah and Agrawal, 2020). These similarities of the SARSCoV-2 to the one that caused the SARS outbreak (SARSCoVs) the Coronavirus Study Group of the International Committee on Taxonomy of Viruses termed the virus as SARS-CoV-2 (Cascella *et al.,* 2020). The understanding of the genetic and phenotypic structure of COVID-19 in pathogenesis is important for the production of drugs and vaccines.

Sex Chromosome Abnormalities

Two general types of chromosomal abnormalities occur, which are; numerical and structural abnormalities. Numerical aberrations result from non-disjunction; that is, from the failure of a pair of homologous chromosomes or pair of sister chromatids to separate during cell division. While Structural aberrations result from chromosome breakages. Chromosomes may break spontaneously, or they may be broken by such environmental agents as radiation, viruses, and toxic chemicals.

Coronavirus genome structure and life cycle

COVID-19 is a spherical or pleomorphic enveloped particles containing single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid comprised of matrix protein. The envelope bears club-shaped glycoprotein projections. Some coronaviruses also contain a hemagglutinin-esterase protein (HE) (Onder *et al.,* 2020). Coronaviruses possess the largest genomes among all known RNA viruses, with GþC contents varying from 32% to 43%. Variable numbers of small ORFs are present between the various conserved genes and, downstream to the nucleocapsid gene indifferent coronavirus lineages.

The viral genome contains distinctive features, including a unique N-terminal fragment within the spike protein. These polypeptides are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro) and one or two papain-like protease into 16nsps. All the structural and accessory proteins are translated from the sg RNAs of CoVs (Lu *et al.,* 2020).

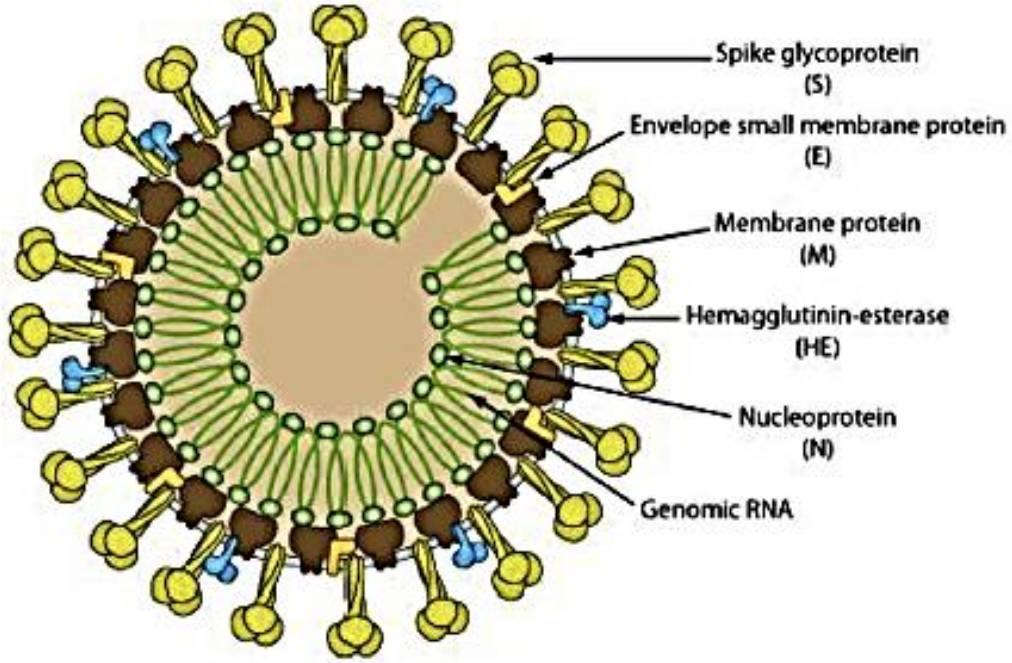

Fig 1: Schematic of a coronavirus

Four main structural proteins contain spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins are encoded by ORFs on the one-third of the genome near the 30-terminus. Besides these four main structural proteins, different CoVs encode special structural and accessory proteins. These mature proteins are responsible for several important functions in genome maintenance and virus replication. There are three or four viral proteins in the corona virus membrane. The most abundant structural protein is the membrane (M) glycoprotein; it spans the membrane bilayer three times, leaving a short

NH2-terminal domain outside the virus and a long COOH terminus
(cytoplasmic domain) inside the virion (MacArthur *et al.,* 2017). The spike protein (S) as a type I membrane glycoprotein constitutes the peplomers. In fact, the main inducer of neutralizing antibodies is S protein. Be-tween the envelope proteins with exist a molecular interaction that probably determines the formation and composition of the corona viral membrane. M plays a pre-dominant role in the intracellular formation of virus particles without requiring S. In the presence of tunicamycin coronavirus grows and produces spike-less, noninfectious virions that contain M but devoid of S (Ching *et al.,* 2010).

Challenges of COVID-19

Economic Challenge of COVID-19

The global COVID-19 pandemic has led to an unprecedented economic crisis. Governments everywhere are faced with innumerable, pressing policy questions around how they can protect their most vulnerable citizens during the crisis and deal with its economic impacts (Nguyen *et al.,* 2020). The COVID-19 pandemic has spread with alarming speed, infecting millions and bringing economic activity to a near-standstill as countries imposed tight restrictions on movement to halt the spread of the virus. As the health and human toll grows, the economic damage is already evident and represents the largest economic shock the world has experienced in decades.

The June 2020 Global Economic Prospects describes both the immediate and near-term outlook for the impact of the pandemic and the long-term damage it has dealt to prospects for growth. The baseline forecast envisions a 5.2 percent contraction in global GDP in 2020 (WHO, 2020), using market exchange rate weights—the deepest global recession in decades, despite the extraordinary efforts of governments to counter the downturn with fiscal and monetary policy support. Over the longer horizon, the deep recessions triggered by the pandemic are expected to leave lasting scars through lower investment, an erosion of human capital through lost work and schooling, and fragmentation of global trade and supply linkages (Heymann and Shindo, 2020).

The crisis highlights the need for urgent action to cushion the pandemic's health and economic consequences, protect vulnerable populations, and set the stage for a lasting recovery. For emerging market and developing countries, many of which face daunting vulnerabilities, it is critical to strengthen public health systems, address the challenges posed by informality, and implement reforms that will support strong and sustainable growth once the health crisis abates (Deng *et al.,* 2019).

Health Challenges of COVID-19

Health workers are at a high risk of catching the viral infection [COVID-19] and they need appropriate Personal Protective Equipment (PPE). In addition, and varying between countries, they also face threats and are exposed to violence. For example, people suspected of carrying the virus may violently object to screening or quarantining. In some countries, health workers experience assaults on their way to work as security forces attempt to enforce lockdowns. There are reports of health workers having lost their rental contracts or being denied access to shops or transport, or being physically assaulted because people fear they may spread the virus (Dong *et al.,* 2020).

The pandemic has brought some long-standing concerns about violence against health workers to the forefront. In some countries, like India and Mexico, patients, their families and other community members are known to have been violent towards health workers. Now, during the pandemic, we are seeing a sharp rise in reported incidents (Zhou *et al.,* 2020).

Treatment / Management of COVID-19

There is no specific antiviral treatment recommended for COVID-19, and no vaccine is currently available. The treatment is symptomatic, and oxygen therapy represents the first step for addressing respiratory impairment. Non-invasive (NIV) and invasive mechanical ventilation (IMV) may be necessary in cases of respiratory failure refractory to oxygen therapy (Prompetchara *et al.,* 2020). Again, intensive care is needed to deal with complicated forms of the disease. Concerning Acute Respiratory Distress Syndrome (ARDS) treatment, accumulating knowledge on the pathophysiology of lung damage, has gradually induced clinicians to review strategies for dealing with respiratory failure. This aspect of the disease is of fundamental importance and has probably negatively affected the therapeutic approach in the early stages of the pandemic. Indeed, despite at beginning of the pandemic, early IMV was postulated as the better strategy for addressing CARDS, in COVID-19 pneumonia the typical ARDS respiratory mechanics featuring reduced lung compliance (i.e., ability to stretch and expand lungs) cannot be found. On the contrary, in CARDS, good pulmonary compliance can be demonstrated. As a consequence, and in contrast to what was initially believed, NIV can have a key role in CARDS therapy (Ahmed *et al.,* 2020).

Other Therapies

Corticosteroids

Among other therapeutic strategies, although systemic corticosteroids for the treatment of viral pneumonia or Acute Respiratory Distress Syndrome (ARDS) were not recommended, in severe CARDS these drugs are usually used (e.g., methylprednisolone 1 mg/Kg/day). Of note, a recent large-size RCT (the RECOVERY trial) demonstrated that dexamethasone reduces deaths by one-third among critically ill COVID-19 patients. In the intervention group, 2,100 patients received dexamethasone (6 mg/day for 10 days) whereas in the control group patients (n=4,300) received standard care for the disease (Li *et al.,* 2020).

Antiviral agents

Although no antiviral treatments have been approved, several approaches have been proposed such as lopinavir/ritonavir (400/100 mg orally every 12 hours). Nevertheless, a recent randomized, controlled, open-label trial demonstrated no benefit with lopinavir/ritonavir treatment compared to standard care. Several anti-flu drugs such as oseltamivir have been used for the treatment of COVID-19 patients. Another anti-flu medication, favipiravir demonstrated a certain efficacy against SARS-CoV-2 in vitro. Again, a retrospective investigation showed that the broad-spectrum antiviral arbidol can improve the discharging rate and decrease the mortality rate of COVID-19 patients (Li *et al.,* 2020).

Antiviral/immunomodulatory drugs

Chloroquine (500 mg every 12 hours), and hydroxychloroquine (200 mg every 12 hours) were proposed as immunomodulatory therapy. In vitro and in vivo studies, indeed, have shown that macrolides may mitigate inflammation and modulate the immune system. In particular, these drugs may induce the downregulation of the adhesion molecules of the cell surface, reducing the production of proinflammatory cytokines, stimulating phagocytosis by alveolar macrophages, and inhibiting the activation and mobilization of neutrophils (Huang *et al.,* 2020). However, further studies are needed for recommending the use of azithromycin, alone or associated with other drugs such as hydroxychloroquine, outside of any bacterial overlaps. Again, attention must be paid with the concomitant use of hydroxychloroquine with azithromycin as the association can lead to a higher risk of QT interval prolongation and cardiac

arrhythmias (Zhou *et al.,* 2020). Chloroquine can also induce QT prolongation.

Serotherapy

Antibodies taken from the blood of healed individuals represent a therapeutic option currently under study. It is calculated that the dose of antibodies necessary for the treatment of a single patient with SARS-CoV-2, requires the removal of antibodies carried out by at least three patients recovered from the SARS-CoV-2 infection (Wu *et al.,* 2020).

Anticoagulant

Because COVID-19 patients have a higher incidence of venous thromboembolism and anticoagulant therapy is associated with reduced ICU mortality, it is suggested that patients should receive thromboprophylaxis. Moreover, in the case of known thrombophilia or thrombosis, full therapeutic-intensity anticoagulation (e.g., enoxaparin 1 mg/kg twice daily) is indicated (Rabi *et al.,* 2020).

Inflammation inhibitors

Anakinra is a recombinant IL-1 receptor antagonist used to treat autoinflammatory disorders such as adult-onset Still's disease, systemic-onset juvenile idiopathic arthritis, and familial Mediterranean fever. Targeting excessive host inflammation can be also

addressed in another way. Acalabrutinib is a selective Bruton tyrosine kinase inhibitor, which regulates macrophage signaling and activation (Zheng *et al.,* 2020).

Prevention of COVID-19

Preventive measures are the current strategy to limit the spread of cases. Because an epidemic will increase as long as R_0 is greater than 1 (COVID-19 is 2.2), control measures must focus on reducing the value to less than 1.

Preventive strategies are focused on the isolation of patients and careful infection control, including appropriate measures to be adopted during the diagnosis and the provision of clinical care to an infected patient. For instance, droplet, contact, and airborne precautions should be adopted during specimen collection, and sputum induction should be avoided.

The WHO and other organizations have issued the following general recommendations:

Avoid close contact with subjects suffering from acute respiratory infections.

Wash your hands frequently, especially after contact with infected people or their environment.

Avoid unprotected contact with farm or wild animals.

People with symptoms of acute airway infection should keep their distance, cover coughs or sneezes with disposable tissues or clothes and wash their hands.

Strengthen, in particular, in emergency medicine departments, the application of strict hygiene measures for the prevention and control of infections.

Individuals that are immunocompromised should avoid public gatherings.

The most important strategy is to frequently wash the hands and use portable hand sanitizer and avoid contact with their face and mouth after interacting with a possibly contaminated environment.

Isolation and contact tracing alone represent insufficient measures to control the spread of the disease. Nevertheless, their efficacy increases with the distancing. To this regard, a modeling study with data from over 40,000

participants in the UK, demonstrated that the combination of isolation and contact tracing with physical distancing measures can be effective for reducing the accounting of cases that would need to self-isolate and of contacts that would need to be traced, controlling in turn, the disease transmission.

Healthcare workers caring for infected individuals should utilize contact and airborne precautions to include PPE such as N95 or FFP3 masks, eye protection, gowns, and gloves to prevent transmission of the pathogen.

Conclusion

It is clear that sex-specific effects contribute to infectious disease susceptibility and females have a major immunological advantage over males. Understanding the origin of sex bias could guide treatment by allowing sex-specific diagnostic and treatment regimes, thereby decreasing time to initiation of treatment as well as increasing treatment success of diseases with sex differences. The X chromosome may contribute to the missing heritability or contain biomarkers that could be used as diagnostic tools. As analytical tools are now available to fully include the X chromosome in genetic analyses, it is clear that the X chromosome should not be ignored. Importantly, due to the haploid nature of males, the power to detect a significant association will be halved when compared to a female cohort of similar size and this could have an effect on the results of sex-stratified analysis. Thus, care must be taken when analysing results, and a non-significant association in one sex does not imply that that specific sex is not affected by the variant, but could simply be as a result of insufficient power to detect a sex-specific association.

While socioeconomic and behavioural factors as well as sex hormones do influence sex bias, these factors do not fully account for it, which leads to the conclusion that the X chromosome itself is likely to greatly influence the immune response and sex bias in disease susceptibility. The X chromosome contains multiple immune-related genes and immune regulatory elements as well as the XIC that regulates X chromosome inactivation.

It is therefore clear that the X chromosome is involved in the immune response and genes that escape inactivation or are preferentially inactivated could influence the dosage of X-linked gene expression between the sexes and as such could further influence the sex bias in disease. It is thus of vital importance that the XCI mechanisms be further investigated to understand all the regulatory elements involved and the contribution to sex bias. Furthermore, the role of the X chromosome in the innate and adaptive immune response should be extensively investigated to determine how it contributes and differs between the sexes.

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HFS 003

GENOTOXICITY EVALUATION OF *Azadirachta indica* (NEEM) ON THE REPRODUCTIVE INDEX OF MALE AFRICAN CATFISH (*Clarius gariepinus*)

Odunlade A.K[1*], Faremi, O.E.[2] Arojojoye L.A.[3] and Hameed I.O[4]

[1&2]Department of Science Laboratory Technology, Yaba College of Technology, Yaba, Lagos;
[3]Department of Biochemistry, Lead City University, Ibadan;
[4]Obafemi Awolowo University, Ile-Ife.
*Corresponding Author:akodunlade@gmail.com

ABSTRACT

The high cost of fish feed has caused a lot of problems in Aquaculture industry, and there is need to search a local protein that can be moderately available by fish farmers. Genotoxic evaluation of the effect of Neem (Azadirachta indica) was studied as a detrimental alternative source of protein feed meal component. Twelve weeks feeding trial was conducted to evaluate the effect Neem leaf on reproductive index, growth performance, mitotic index and chromosomal aberration of the male catfish (Clarius gariepinus) fed with varying inclusion levels of Neem Leaf Powder (NLP). A total of 200 fingerlings were divided into five groups comprising; control group (I) and four groups (II, III, IV, V) of varying treatment of NLP (5%, 10%, 15%, 20%) with three replicates. At the end of the feeding trial, reproductive efficiency were determined through gonadosomatic index, reproductive indices and chromosomal studies of the reproductive tissues. Catfish treated with NLP showed significantly decreased reproductive performance over the controlled fish, with increased mitotic index thereby enhancing chromosomal abnormalities of the reproductive tissue. Gonadosomatic index were low in fish fed diet of NLP 10-20% when compared to the control group. Histological results on the reproductive tissues showed that there were epithelial vaculation and degeneration of germ cell. The results indicated that supplement diet with Neem reduced; growth, gonadosomatic index, reproductive indices of male catfish and enhanced chromosomal abnormalities. Neem has a potential anti-fertility property and thereby cannot be exploited in fish fingerling production by hatchery operators.

Keywords: *Neem, catfish, gonadosomatic index, chromosomal aberration.*

Introduction

Many medicinal plants have been reported to have genotoxic effects on gonadosomatic index of *Claria gariepinus*. The continent of Africa is rich in biodiversity of various types of plants which can serve as an alternative source of protein in catfish meal production. However, in search for alternative source of protein, there is need to evaluate the genotoxicity of every plant based meal as an alternative source in catfish meal to enhance productivity and to prevent genotoxic effects on reproductive performance.

Genotoxicity tests are designed to detect substances that induce genetic damage directly or indirectly and these can be seen in form of gene mutations, chromosome damage, recombination and numerical chromosomal changes (ICH, 1997). Sperm morphology

serves as an indicator on the reproductive index of animals and can be used to assess spermatogenic damage, fertility and heritable changes.

Clarias gariepinus are freshwater fish found in the tropical regions of West Africa and their sources of feeds are mainly phytoplankton and zooplankton. Akah *et al.,* 1997 reported how papaya seed can be used to control excessive breeding of Tilapia in aquaculture in Nigeria because of its antiproliferative properties due to its piscidial components.

Materials and Methods

Fresh leaves of Neem (*Azadirachta indica*) were obtained from Yaba College of Technology, Yaba, Lagos, Nigeria. The leaves were collected during two seasonal periods i.e. (January - February and July - August).

The fresh leaves were air dried and milled to a fine powder using Maulinex electric blender and mixed with basal feed (40% crude protein), comprising standard amounts of fish meal, yellow maize, soya bean meal, blood meal, fish oil, vegetable oil, vitamin, premix and starch.

C. gariepinus fingerlings used in this study were collected from Fish Farm Estate, Odogunyan, Ikorodu, Lagos State, Nigeria. The brood stocks were transported to Animal Breeding unit of the department of Biological Science, Yaba College of Technology, Lagos State, Nigeria in plastic bowls. The fish were distributed into outdoor concrete tanks (1 x 1 x 0.6m), filed with well water and acclimatized for 2 weeks, during which they were fed the basal diet. The concrete tanks were cleaned weekly, and temperatures were monitored daily.

Experimental Design

The fingerlings were stocked into concrete tanks (1 x 1 x 0.6m) at a density of 30 (thirty) fish per tank with three replicates except the control. Four isonitrogenous diet were formulated from practical ingredients (Table 1) where the control was without Neem (*Azadirachta indica*) leaf powder (NLP) and other diets were supplemented by 5%, 10%, 15% and 20% *Azadirachta indica* leaf powder. All dietary ingredients were weighed with a weighing top load balance (Metler Toledo, PB 8001 London). The ingredients were milled to a 3mm particle size. Ingredients including vitamin, premix and *Azadirachta indica* leaf powder were thoroughly mixed in a Hobart A – 2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with 0.9 mm diameter size. The pellets were dried at ambient temperature (27 – 30°C) and stored at -20°C in a refrigerator until the start of the experiment. The diets were analyzed for proximate composition, which includes crude protein, crude lipid, crude fibre, ash and moisture content as described by AOAC (2005).

The diets were manually fed to the brood stocks at a daily rate of 3% body weight (bw), twice a day (09:00 and 16:00h) for 12 weeks. Fingerlings were weighed collectively at weekly intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly. At the end of the 12-wk feeding trial, fifteen male fish were randomly selected from each dietary treatment, euthanized, and the testes removed to determine milt quality indices (milt volume, motility duration, percentage motility and spermatozoa concentration). Milt volume was determined by making small incision into the lobes of the testes, the milt was squeezed out into a Petri dish and this was measured with plastic syringe in ml while motility durations were determined by placing 1µ of milt from each male on Neubauer improve

haemocytometer (model MC 1300) and a drop of distilled water was added and covered with a slip. The milt activity was viewed under Olympus microscopic at 100x magnification to see when all the sperm got stopped (Mims, 1991). Percentage motility was estimated using light microscope at 400x magnification immediately after addition of 20 µL distilled water as an activating solution. During spermatozoa activation, immotile sperm cell was counted, and when the activation stopped, whole sperm cells were counted as described by Canyurt and Akhan (2008). The motile sperm cells were calculated as;

Motile sperm cells (%) =Whole sperm cells - Immotile sperm cell × 100

Whole sperm cells Concentration (milt count) of sperm was determined by counting the number of spermatozoa in sample dilutes with distilled water (100× magnifications) in a Burker haemocytometer, under 400× magnification (Rainis *et al.,* 2003). One female African catfish broodstock (900 g) was induced to spawn with an injection of 0.45 mL Ovaprim®. After 12 h, the female was strip-spawned and 30 eggs were placed into each of fifteen 2 L plastic bowls. Eggs were fertilized with 1 mL of milt from males from each dietary treatment. The number of fertilized and unfertilized eggs were counted under a microscope (40× magnification) and used to make the following calculations:

Egg fertilization (%) = Number of eggs incubated - number of opaque eggs × 100
Total number of eggs

Hatchability (%) = Number of eggs hatched × 100
Total number of eggs

Survival (%) = Total number of hatchings × 100
Total number of eggs

Gonado-somatic Index (GSI)

GSI was computed according to the King (2007) as (wet weight of gonad/wet weight of fish) x 100.

Histological examination of testes

Histological sections of 8 µm thicknesses were prepared as described by Bancroft and Cook (1994). Photomicrographs were taken with Leitz (Ortholux) microscope and camera, development and printing of negative were done as described by Bancroft and Cook (1994).

Chromosomal aberration

Chromosomal aberration was measured by the method of Nagpure *et al.,* (2007). Take healthy fish (60-100g). 0.05% Colchicine was injected intramuscularly @ 1 ml per 100g of body weight. Fish was kept for 1.5-2 hours after injection of colchicines and then anesthetized with ethylene glycol. The kidney tissue was dissected in a Petri dish, and cut into small pieces. Tissue was then homogenize in 8 ml hypotonic solution in homogenizer. The cell suspension was poured in 15 ml centrifuge tube and incubated for 2025 minutes at room temperature. The hypotonic action was stopped by adding 1 ml freshly prepared Conroy's fixative and left for 30 minutes and was gently mixed with pasture pipette. Centrifuged cell was suspended at 1200-1500 rpm for 10 minutes at room temperature and the supernatant was removed with a pipette and 68 ml freshly prepared chilled fixative was slowly over layered. The tube was kept in refrigerator for half an hour and the contents were mixed after which the cell suspension was centrifuged at 1200-1500 rpm for 10 minutes at room temperature. Supernatant was removed without disturbing cell pellet at the bottom. Fresh fixative was added and the tube was kept in refrigerator for half an hour. This step was repeated 3-5 times until transparent cell suspension is obtained. The cell suspension was taken in pipette and dropped onto grease

free, pre cleaned slide. The slide was allowed to flame dry and the slides were kept for ageing (1-3 days) and then stained with 4-5% Giemsa in phosphate buffer (pH 6.8) for 15-20 minutes. The slide was washed with DDW and air-dried and a permanent preparation was made by mounting in DPX.

Oil immersion objective (100X) was used for screening mounted slides and chromosomal aberrations were observed under microscope (OLYMPUS CX21i) with Camera (Magnus MIPS USB 5MP).

Mitotic Index Determination

The Mitotic Index (MI) mean± SE was calculated for each group of experiments. Student's t-test was applied for significant difference between control and experimental groups (Sokal and Rohlf, 1973).

Water quality parameters

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored daily throughout the study period using mercury-in-glass thermometer, pH meter (Hanna H198106 model) and dissolved oxygen meter (JPP- 607 model).

Statistical Analysis

A one-way analysis of variance was conducted to test the effect of Neem leaf powder (NLP) on the growth, GSI and reproductive indices of male *C. gariepinus* broodstock using SPSS Version 11.0. Least significant difference was used to compare means at $P < 0.05$ (Zar, 1984).

Table 1: Dietary formulations of experimental diets.

Ingredients	Dietary treatment				
	Control	5%	10%	15%	20%
Fish meal (%)	25	25	25	25	25
Yellow maize (%)	15	15	15	15	15
Soy bean meal (%)	40	40	40	40	40
Fish oil (%)	4	4	4	4	4
Vitamin premix*(%)	3	3	3	3	3
Blood meal (%)	5	5	5	5	5
Binder (starch) (%)	2	2	2	2	2
Vegetable oil (%)	6	6	6	6	6
NLP (g/100g feed)	0	0.005	0.01	0.015	0.02

Vitamin premix**: An Animal Care® Optimix Aqua product for catfish, containing the following per 5 kg of premix: A = 20 000 000 IU, C = 2 000 000 IU, E = 200 000 mg, K3 = 10 000 mg, B2 =12 000 mg, B12 = 9 mg, B1 = 6 000 mg, B6 = 11 000 mg, C = 50 000 mg, Folic acid = 2 000 mg, Niacin =80 000 mg, Calpan = 25 000 mg, Biotin = 100 mg, x Zinc = 30 000 mg, Copper = 5 000 mg, Iron

= 30 000 mg, Manganese = 50 000 mg, Iodine = 1 000 mg, Selenium = 100 mg, Antioxidant = 125 000 mg; *NLP: Neem Leaf Powder.

Results

Supplementation of *Azadirachta indica* leaf powder resulted in reduced reproductive performance of male African catfish (Table 3). The greatest effect of *Azadirachta indica* leaf powder on reproductive parameters was measured on the duration of sperm motility. The duration of sperm motility of fish fed *Azadirachta indica* leaf powder at any level

was lower than that of fish fed the control diet. Sperm motility of fish fed *Azadirachta indica* leaf powder was significantly low than that of control fish. Sperm density was lowest in fish fed *Azadirachta indica* leaf powder at 20% and was greatest in fish fed the control diet but was only significantly different from the control at the highest level of dietary *Azadirachta indica* leaf powder. Egg hatchability and survival were significantly lower in treatments with dietary *Azadirachta indica* leaf powder as compared to the control. In general, dietary *Azadirachta indica* leaf powder had a negative effect on the measured reproductive performance indices such as motility duration, hatchability, fertilization. The results of the histology of the transverse sections of the testes of *C. gariepinus* in fish fed with 5% feed of *Azadirachta indica* leaf powder showing wide necrotized seminiferous tubules and empty vacuolization. While that of fish fed with 10%-20% showing decrease in seminiferous tubules and with well disorganized lobules and with scanty spermatozoa as shown in fig 2-5. The results are presented in Figures 1-5 respectively.

Table 2: Proximate composition (% DM) of experimental diets.

Composition	Control	5%	10%	15%	20%
Moisture	14.19 ± 0.02	14.43 ± 0.01	13.25 ± 0.03	12.75 ± 0.01	12.51 ± 0.01
Ash	10.00 ± 0.03	9.30 ± 0.08	9.00 ± 0.06	10.00 ± 0.01	10.00 ± 0.03
Fat	15.09 ± 0.03	18.46 ± 0.04	18.18 ± 0.03	19.36 ± 0.03	20.80 ± 0.04
Crude fibre	3.75 ± 0.03	4.04 ± 0.03	3.63 ± 0.04	3.47 ± 0.03	3.28 ± 0.01
Crude protein	40.23 ± 0.01	40.28 ± 0.03	40.35 ± 0.10	40.40 ± 0.07	40.45 ± 0.07
NFE	16.74 ± 0.08	13.49 ± 0.06	15.59 ± 0.03	14.02 ± 0.06	12.96 ± 0.03

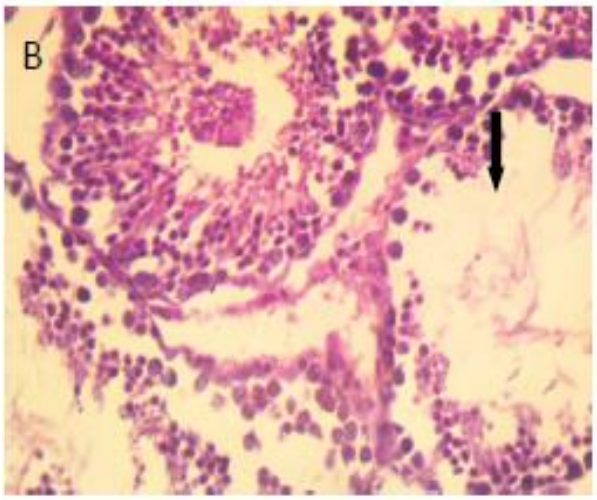


Figure1. A transverse section through the testes of *C. gariepinus* fed diet A, (0% feed of *Azadirachta indica* leaf powder) showing densely filled lumen and a well differentiated seminiferous tubule of the lobule with ripe spermatozoa ready to be released through the sperm duct. 160×.

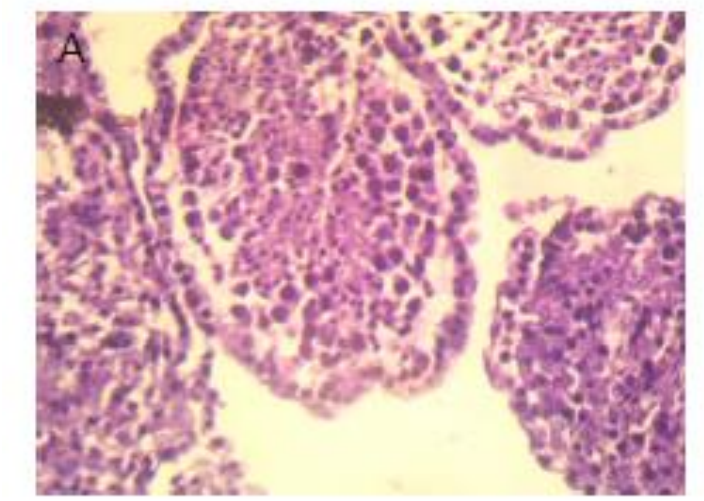


Figure2. A transverse section through the testis of *Azadirachta indica* leaf powder fed B (5% feed), showing necrotized seminiferous tubule, lobules are empty with few vacuolization. The spermatozoa are equally scanty. 160×. NS: Necrotized seminiferous tubule; AP: Apoptosi

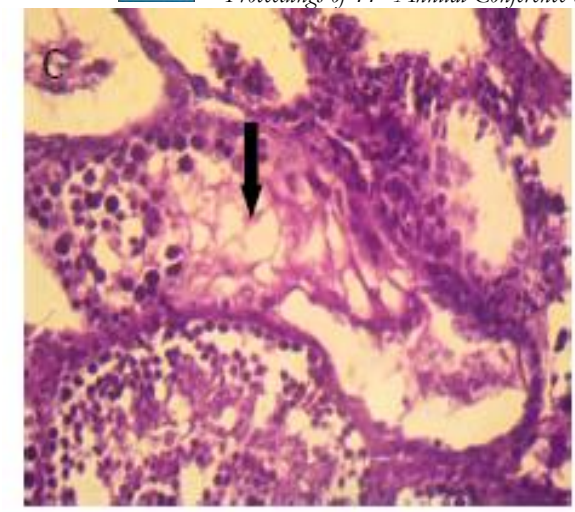


Figure 3. A transverse section through the testes of *C. gariepinus* fed diet B, (10% feed of *Azadirachta indica* leaf powder) showing increased shrinked seminiferous tubules. The spermatozoa are equally dispersed with few lumen filled with matured spermatozoa. 160×.

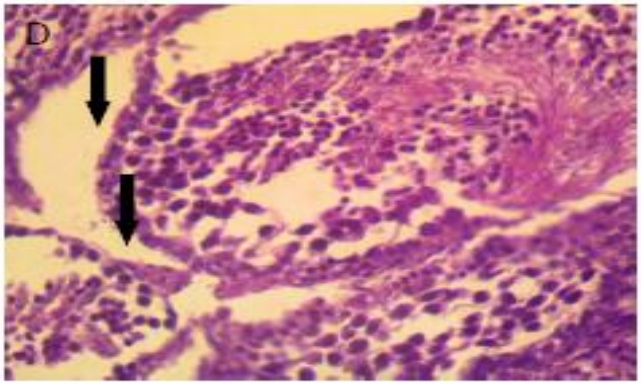
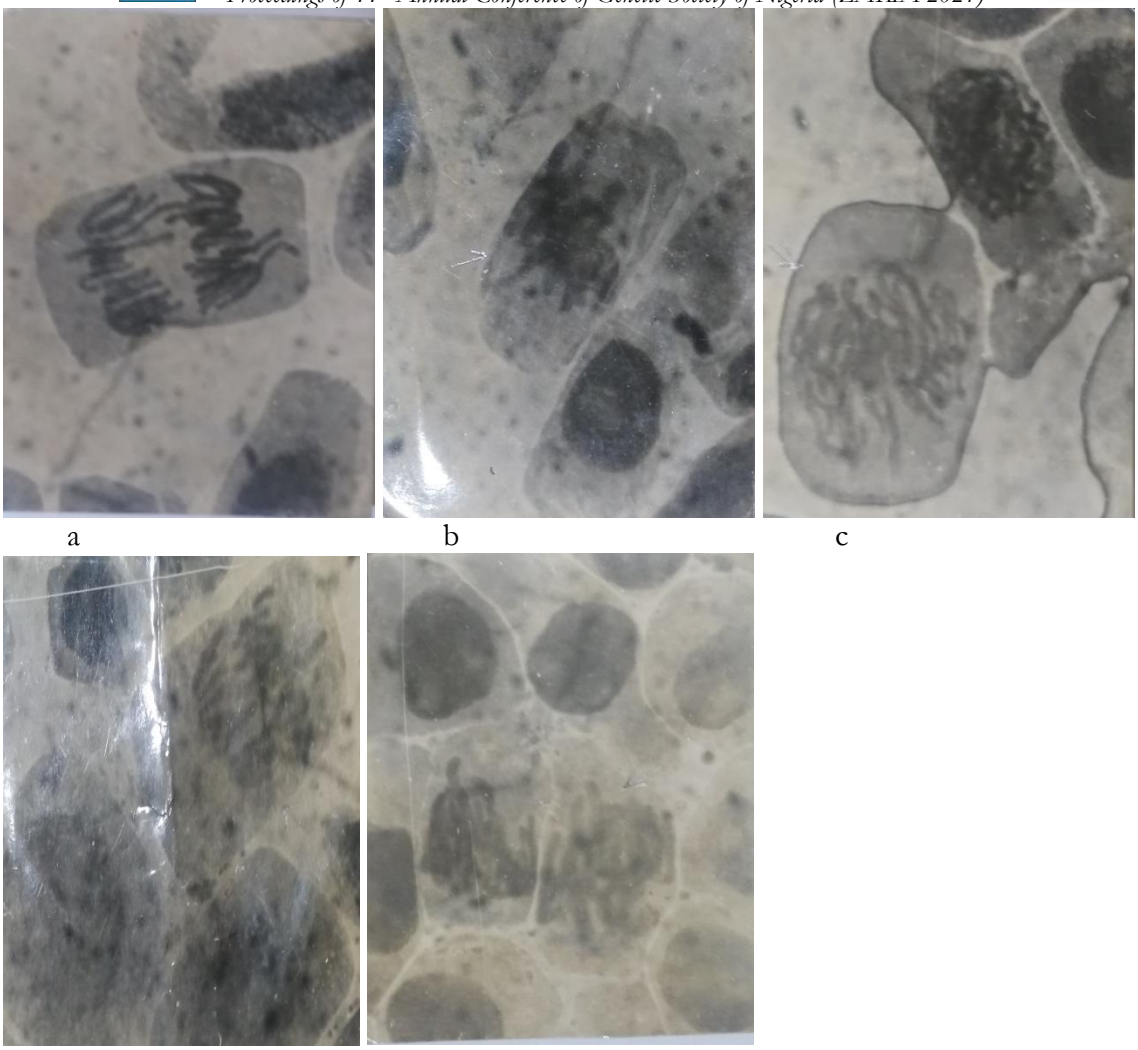


Figure 4. A transverse section through the testes of *C. gariepinus* fed diet C, (15% feed of *Azadirachta indica* leaf powder) showing disorganized lobules with populated lumen. 160×



a b c

d e

Figure 5: A= Conrol, B= 5% disturbed pole to pole arrangement of chromosomes at metaphase C= 10% vagrant chromosome at metaphase D= 15% laggard chromosome E= 20% sticky anaphase.

Table 3: Reproductive performance of male *C. gariepinus* fed dietary supplementation of *Azadirachta indica* leaf powder

Parameter	Experimental diets				
	A (control)	5%	10%	15%	20%
Initial fish weight (g)	304.10± 2.26	305.89±0.10	303.04±1.73	302.39±1.80	304.08 ± 0.54
Final mean weight (g)	587.1±67.32a	383.80±80.33a	320.80±22.91 a	312.00± 15.84a	307.00 ± 112.29a
Weight gain (g)	283.00±6.58a	77.9 ± 8.29a	17.4 ± 0.33a	9.61 ± 0.64a	2.2 ± 1.03a
Weight of testes (g)	3.12 ± 1.30a	1.03 ± 1.25a	1.04 ± 2.27a	1.02 ± 2.67a	0.05 ± 0.33a
GSI (%)	0.45 ± 0.13a	0.68 ± 0.12a	0.66 ± 0.26a	0.94 ± 0.39a	0.72 ± 0.07a
Milt volume (mL)	2.04 ± 0.65b	0.89 ± 0.52a	0.65 ± 0.28a	0.45 ± 0.21a	0.25 ± 0.42a
Milt count (× 104 spz/mL)	20.95±0.35b	2.45 ± 3.32b	1.30 ± 1.41b	0.98 ± 3.25b	0.955 ± 9.97a
Motility duration(seconds)	14.50± 2.12c	6.0 ± 1.41b	0.5 ± 1.6ab	0.3 ± 1.41a	0.25 ± 1.41a
Motility (%)	30.50± 4.95c	5.50 ± 4.95b	4.00 ± 2.83ab	2.50 ± 2.12a	1.20±11.43ab
Fertilization (%)	88.43± 1.12c	0.37±0.25bc	0.25 ± 0.09b	0.15 ± 0.35a	0.01 ± 0.03a
Hatchability (%)	70.45± 0.63b	4.04±0.20bc	1.01 ± 0.21b	0.05±0.03ac	0.02 ± 0.02
Survival (%)	38.42± 1.15c	0.19 ± 1.40b	0.08 ± 0.74b	0.05 ± 1.10a	0.03 ± 1.49a

Mean in a given row with the same letter were not significant different at $P < 0.05$

Table 4: Frequency of chromosomal aberrations induced by *Azadirachta indica* leaf powder in tissues of *Clarius gariepinus*

Group	Time of exposure in hours	Total no of metaphase analyzed (n1)	Metaphase with CA	Types of CA (Chromosomal aberrations)									% aberration (n2)	Mitotic index (MI)* (n2/n1)
				A	B	C	D	E	F	G	H	I		
Control	24h	200	15	1	2	1	3	2	1	2	3	0	7.5	3.75
	48h	200	15	1	2	1	3	2	1	2	3	0	7.5	3.75
	72h	200	15	1	2	1	3	2	1	2	3	0	7.5	3.75
	96	200	15	1	2	1	3	2	1	2	3	0	7.5	3.75
5%	24h	200	30	1	3	8	6	5	3	2	1	1	15	7.50
	48h	200	42	2	2	15	9	6	3	2	1	2	21	1.05
	72h	200	51	2	2	18	11	8	4	2	1	3	25.5	1.28
	96	200	44	1	2	13	8	7	6	4	1	2	22	1.10
10%	24h	200	75	2	2	23	21	12	6	2	5	2	37.5	1.88
	48h	200	90	1	3	28	22	13	6	8	6	3	45	2.25
	72h	200	88	2	2	23	18	13	9	9	7	5	44	2.20
	96	200	86	2	3	19	16	12	7	11	9	7	43	2.15
15%	24h	200	120	2	3	35	28	16	13	9	6	8	60	3.00
	48h	200	102	1	3	26	23	18	12	8	5	6	51	2.55
	72h	200	114	1	2	34	26	12	12	9	8	10	57	2.85
	96	200	82	2	2	21	15	15	10	4	7	6	41	2.05
20%	24h	200	133	2	3	35	20	23	17	8	12	13	66.5	3.33
	48h	200	130	3	3	46	32	13	10	6	9	8	65	3.25
	72h	200	125	1	2	38	27	15	10	13	12	7	62.5	3.13
	96	200	119	2	3	34	23	19	14	7	9	8	59.5	2.98

A: Chromatid gap, B: Chromosome gap, C: Chromatid break, D: Chromosome break, E: Chromatid deletion, F: Fragment, G: Acentric fragment, H: Ring chromosome, I: Dicentric chromosome
*, Significant (P<0.05) when Student's't' test was applied between treated and control groups.

Discussion

The results suggested that *Azadirachta indica* leaf powder supplementation reduced the reproductive indices of African catfish, *C. gariepinus*. These results showed that the *Azadirachta indica* leaf powder treatment inhibits reproductive performance, which is reflected in reduced milt density, milt volume, motility duration, percentage motility, GSI and histological parameters. Reduced reproductive performance values were obtained in the *Azadirachta indica* leaf powder treatments compared to the control and there were significant (P > 0.05) differences among the fish fed the supplementation of *Azadirachta indica* leaf powder in diets.

Dada and Ogunduyile (2011) reported that male catfish *C. gariepinus* brood stocks fed on diets supplemented by medicinal plants (*Mucuna pruriens*) exhibited improved reproductive performance than those fed with the control diet. The decrease in the milt density of *C. gariepinus* obtained in these studies could be as a result of the presence of azadirachtin, a phytochemical compound in the Neem leaf known as anti-fertility agent in the plants.

The significant decrease in percentage of fertilization, percentage of hatchability and percentage of survival in the fry of fish fed dietary. This is contrary to the report of Adeparusi *et al.,* (2010) reported that *C. gariepinus* broodstock fed dietary *Kigelia africana* seed meal had higher sperm density, higher percentage hatching and larval survivals than the control fish.

The results obtained from the photomicrographs of the transverse section through the testes of the fish fed on dietary *Azadirachta indica* leaf powder showed that the seed powder has negative effect on the histology of the testes. The testes of *C. gariepinus* fed on control diet composed mainly of densely filled lumen and a well differentiated seminiferous tubule of the lobule with ripe spermatozoa ready to be released through the sperm duct. The histological transverse section of the fish fed dietary *Azadirachta indica* leaf powder seed powder had deleterious effects of

Azadirachta indica leaf powder on the testicular structure with the spermatozoa dispersed and less matured seminiferous lumen in fish fed diet 5% of NLP, but apparently more string semiferous tubules in fish fed diet 20% while the photomicrograph shows organized tubules. CA is small fraction of a huge amount of changes in chromosomal DNA and reflects an enormous plasticity of the genome, which has far-reaching consequences for evolutions (Caporale, 1999). *Azadirachta indica* leaf powder can induced the formation of Chromosomal break, chromatid breaks, ring chromosomes, fragments, acentric fragments and deletions. Experimental analysis has shown that CA, in fish and other organisms, induced by DNA strand breaks (DSBs) (Bryant *et al.* 1998). DSBs arise spontaneously or through a variety of cellular processes. Sources of spontaneously induced DSB are DNA replication and DNA excision repair. Error in both mechanisms induced CA. The majority of chemical mutagens are not able to induce DSB directly but leads to other lesions in chromosomal DNA, which during repair, or DNA synthesis, may give, and rise to DSB and eventually to CA. DSBs, in which both strands in the double helix are severed, are particularly hazardous to the cell because they can lead to genome rearrangements. DNA double strand breaks are potentially lethal to cells.

Conclusion

The use of Neem leaf powder (NLP) is not productive as a feed additive for reproductive performance of male African catfish (*Clarias gariepinus*). Future researchers should look for other plants that can ameliorate this effect and which can also be a good benefit in aquaculture industries.

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