SECTION ONE: PLANT GENETICS, BREEDING AND BIOTECHNOLOGY

COLLECTION AND EVALUATION OF GROUNDNUT (*Arachis hypogaea* L.) GERMPLASM IN NIGER STATE

Kolo, J., Falusi, O. A., Daudu, O. A. Y., Abubakar A., Ahiwe, A. U., Adebimpe, Y. A., Onobo, I. Y., Abdullahi, A. S. and Abdulmumin, A
Department of Plant Biology, Federal University of Technology, Minna , Niger State. Nigeria
Email: jobtimothyjob@gmail.com Tel +2348034265639

ABSTRACT

Genetic diversity of groundnut (Arachis hypogaea L.) gerplasm from Niger state was evaluated for qualitative relationship. A survey mission was undertaken to all major groundnut producing areas of the State. The survey covered 8 towns and 21 villages in 13 Local Government Areas. A total number of thirty-seven (37) accessions of groundnut were collected from forty-five (45) farmers and assessed for qualitative parameters using groundnut descriptor. Result showed that 59.46% of these accessions were having deep pod constriction while 40.54% have moderate constriction. A 62.16% of these accessions were having prominent pod beak, 29.73% have moderate pod beak, 5.41% of the accessions have slight pod beak while only 2.70% of the accessions have very prominent pod beak. The highest number of groundnut accessions was collected from Lapai Local government (4 accessions) followed by 9 Local Governments (Gbako, Bida, Lavun, Paikoro, Agaie,Shiroro, Bosso, Kontagora and Katcha Local Government) each with 3 accessions while, 2 accessions each were collected from Borgu, Rijau and Agwara Local Government. The major limitation pointed out by the farmers was the non-availability of improved varieties of the groundnut accession. Hence, there is need for scientific evaluation of these accessions to identify promising genotype for further improvement.

Key Words: Groundnut, Genetic diversity, Germplasm, Groundnut accessions

INTRODUCTION

Groundnut (*Arachis hypogaea L*) is also known as wonder nut, peanut, earth nut, monkey nut, goobers, pinder, panda, manila nut and poor men's cashew nut (Gadhiya *et al* ., 2014). It is an important annual legume, one of the world's most important oilseed crops (Upadhyaya *et al*., 2006; Mukhtar *et al*., 2013). It is thirteenth in the world food crops ranking, it ranks fourth in oil production or edible oil after soyabean, rapeseed, and cottonseed and third vegetable most principal protein (FAO, 2007; FAO, 2017).

Groundnut has been stated to have originated in South America and later spread to Brazil (Zhao *et al*., 2012). Groundnut was introduced by Portuguese from Brazil to West Africa and then to South Western India in the 16th Century; in the present day, the groundnut is grown in almost all the countries of the world (Anjana *et al.*, 2016). Groundnut is grown on 23.4 million ha worldwide with a total production of 34.9 million metric tons and an average productivity of 1.4 metric t/ha (FAO, 2007). Developing countries amount to 97% of the global area and 94% of the global production of this crop. The production of groundnut is concentrated in Asia and Africa (56% and 40% of the global area and 68% and 25% of the global production, respectively (FAO, 2007).

Groundnut can reach the height of 30-50 cm tall, leaves are opposite, and pinnate with four leaflet; each leaflet is 1-7cm long and 1-3cm across (wide), the flowers are yellowish orange with reddish veining, it developed underground to produced "pegs" which later develops to a matured groundnut pod; the pods are 3-7cm long containing 1-4 seeds (Krapovickas *et al*., 2007). Light, sandy loam soil is preferred for the production of groundnut, the temperature of 30°C is considered to be the optimum for rapid germination

and development of pods (Nautiyal, 2012).

The significance of this crop cannot be overemphasis; it is used for diverse purposes. It is a good source of cooking oil, salad, margarine and groundnut butter, it is a cash crop and widely grown in all the tropical and sub tropical regions of the world for direct use as food, for oil, and for the high protein meal produced after oil

extraction. The seeds have palmitic, oleic and linoleic acids accounting for about 90% of total fatty acids at seed maturity (Engin *et al.*, 2018). Groundnut is important source of vitamins E, K, and B (the richest source of thiamine and niacin) and other essential minerals (Kassa *et al*., 2009). Groundnut cake after oil extraction, and with its high protein content is particularly used for feeding animals (Savage and Keenan 1994). It was reported that eating of groundnut at least four times a week showed a 37% reduced risk of coronary heart disease (Suchoszek-Lukaniuk *et al*., 2011). Studies also indicated that groundnut contain anticancer activity with 50% inhibition of the proliferation of related leukemia cells (Hwang *et al*. 2008).

It was reported that groundnut oil contained 47% fat, 38.6% protein, 1.8% carbohydrate, 3.7% crude fibre, 5.8% moisture and 3.1% ash (Atasie *et al*., 2009).
Abdulrahaman *et al.* (2014) reported that groundnut as a legume plays an enormous function in feeding the world's people and animals, mostly in the third world countries, where they meet as much as two thirds of human nutritional needs. Furthermore, due to the fact that they can pull nitrogen out of air, they do not need much chemical fertilizers. Thus, make it a better good deal for poor farmers who cannot afford fertilizers and boon to richer ones (Khan *et al*., 2004).
In Niger state, groundnut is widely used and very popular in parts of the Local Governments where it is usually grown. Groundnut oil which is called "maiengeda (Hausa) "emi kuli" (Nupe) is a byproduct from preparation of "kulikuli and "donkwa , however "Kulikuli" and "donkwa" are important food from groundnut, groundnuts are also roasted, cooked or boiled as food in Niger state (Abdulrahaman *et al.*, 2014).

The popular uses to which a crop has been assigned usually fueled increasing demand for the crop (Daudu *et al*., 2015). For that reason, there is a need for corresponding increased supply of the important crops. Groundnut production has not increased as expected in Nigeria (Niger state inclusive) and the eras of groundnut pyramids has vanished or remains redundancy (Nahanga, 2017). Nevertheless, attempts has been made to achieve increased supply through increased farming of the diverse landlances, the successes of such attempts has been restricted by challenges ranging from poor environmental conditions, as well as reduced man-power. As the crop continues to play vital horticultural function in Niger State, its development will surely improve agricultural yield, reduce poverty, and aid food security. But unfortunately very little attention has been given to the development or improvement of the crop in Niger State. This background has made it essential to collect and evaluate the germplasm of the crop as a foundation for research into its improvement and back-up as a major crop in Niger State.

MATERIALS AND METHODS

A survey mission was undertaken to all major groundnut producing areas of the State. The survey was conducted when the farmers were expected to be harvesting the crop. The local governments visited were Gbako, Lapai, Bida, Lavun, Paikoro, Agaie,Shiroro, Bosso, Kontagora, Katcha Borgu, Rijau and Agwara Local Government. Questionnaires were administered through an interpreter in some cases where language was a barrier and samples of groundnut accessions under husbandry were collected. The questions asked include local name of accession, source of seed supply, yield, groundnut seed preferences, constraints to cultivation and economic importance. Groundnut descriptor was also used to identify the phenotypic traits of the pods and the seeds as well prior to planting.

Measurement of Seed Length

Ten seeds at random were selected from each of the accessions for the seed length. The seed lengths were measured using meter rule and the mean value was recorded as the average length. Phenotypic character of the pod such as pod constriction and pod beak were determined using groundnut descriptor guideline.

RESULTS AND DISCUSSION

The survey covered 8 towns and 21 villages in 13 Local Government Areas. A total of 45 farmers were interviewed and 37 accessions of groundnut were collected (Table 1). It was observed that some of these accessions that were collected were duplicated in most towns and villages.

Table 1. Sources and description of Groundnut Germplasm in Niger State

S/N	Accession Number	Local Name	Local Government Area	Pod Constriction	Pod Beak	Seed Length (mm)
1	NG-AGA-NUT-001	Kusha Bologi	Agaie	Deep	Prominent	10.20
2	NG-AGA-NUT-002	Yekerigi	Agaie	Moderate	Moderate	12.80
3	NG-AGA-NUT-003	Etwagutagi	Agaie	Deep	Prominent	10.20
4	NG-BOS-NUT-004	Barna	Bosso	Deep	Prominent	10.20
5	NG-BOS-NUT-005	Wata Uku	Bosso	Deep	Moderate	14.70
6	NG-BOS-NUT-006	Wata Uku	Bosso	Deep	Prominent	10.20
7	NG-BOR-NUT-007	Etwagutagi	Borgu	Deep	Prominent	10.20
8	NG-BOR-NUT-008	Kampala	Borgu	Moderate	Prominent	14.30
9	NG-AGW-NUT-009	Wata Uku	Agwara	Deep	Prominent	10.20
10	NG-AGW-NUT-010	Kampala	Agwara	Moderate	Prominent	14.20
11	NG-BDA-NUT-011	Gusha Bologi	Bida	Deep	Prominent	10.20
12	NG-BDA-NUT-012	Kusha Guba	Bida	Deep	Prominent	10.20
13	NG-BDA-NUT-013	Kusha Eyeko	Bida	Moderate	Slight	17.20
14	NG-GBA-NUT-014	Patigici	Gbako	Deep	Prominent	10.20
15	NG-GBA-NUT-015	Makwaci	Gbako	Moderate	Prominent	12.80
16	NG-GBA-NUT-016	Kusha Eyeko	Gbako	Moderate	Slight	17.20
17	NG-KAT-NUT-017	Kusha Bologi	Katcha	Deep	Prominent	10.20
18	NG-KAT-NUT-018	Yekiregi	Katcha	Moderate	Moderate	12.80
19	NG-KAT-NUT-019	Kusha Eyeko	Katcha	Moderate	Slight	17.20
20	NG-KON-NUT-020	Kampala	Kontagora	Moderate	Prominent	14.30
21	NG-KON-NUT-021	Wata Uku	Kontagora	Deep	Prominent	10.20
22	NG-KON-NUT-022	Etwagutagi	Kontagora	Deep	Prominent	10.20
23	NG-LAV-NUT-023	Kusha Bologi	Lavun	Deep	Prominent	10.20
24	NG-LAV-NUT-024	Wawagi	Lavun	Deep	Prominent	10.20
25	NG-LAV-NUT-025	Gushako	Lavun	Moderate	Moderate	12.80
26	NG-LAP-NUT-026	Kwaso	Lapai	Moderate	Moderate	14.16
27	NG-LAP-NUT-027	Wata Uku	Lapai	Deep	Prominent	10.26
28	NG-LAP-NUT-028	Kadala	Lapai	Deep	Moderate	15.30
29	NG-LAP-NUT-029	Yekiregi	Lapai	Moderate	Moderate	12.80
30	NG-PAK-NUT-030	Kampala	Paikoro	Moderate	Prominent	14.30
31	NG-PAK-NUT-031	Yekiregi	Paikoro	Moderate	Moderate	12.80
32	NG-PAK-NUT-032	Wata Uku	Paikoro	Deep	Prominent	10.20
33	NG-RIJ-NUT-033	kampala	Rijau	Moderate	Prominent	14.30
34	NG-RIJ-NUT-034	Wata uku	Rijau	Deep	Prominent	10.20
35	NG-SHI-NUT-035	Kwaso	Shiroro	Moderate	Moderate	15.30
36	NG-SHI-NUT-036	Wata Uku	Shiroro	Deep	Very Prominent	10.20
37	NG-SHI-NUT-037	Tasagutagi	Shiroro	Deep	Prominent	13.90

Values are means of the seed measured in millimeter.

It was observed using groundnut descriptor that 59.46% of these accessions were having deep pod constriction while 40.54% have moderate constriction. A 62.16% of these accessions were having prominent pod beak, 29.73% have moderate pod beak, 5.41% of the accessions have slight pod beak while only 2.70% of the accessions have very prominent pod beak. The highest number of groundnut accessions was collected from

Lapai Local government (4 accessions) followed by 9 Local Governments (Gbako, Bida, Lavun, Paikoro, Agaie,Shiroro, Bosso, Kontagora and Katcha Local Government) each with 3 accessions while, 2 accessions each were collected from Borgu, Rijau and Agwara Local Government (Table 1). It is an indication these local governments had the greatest diversity of groundnut genetic resources.

Some of the accessions collected

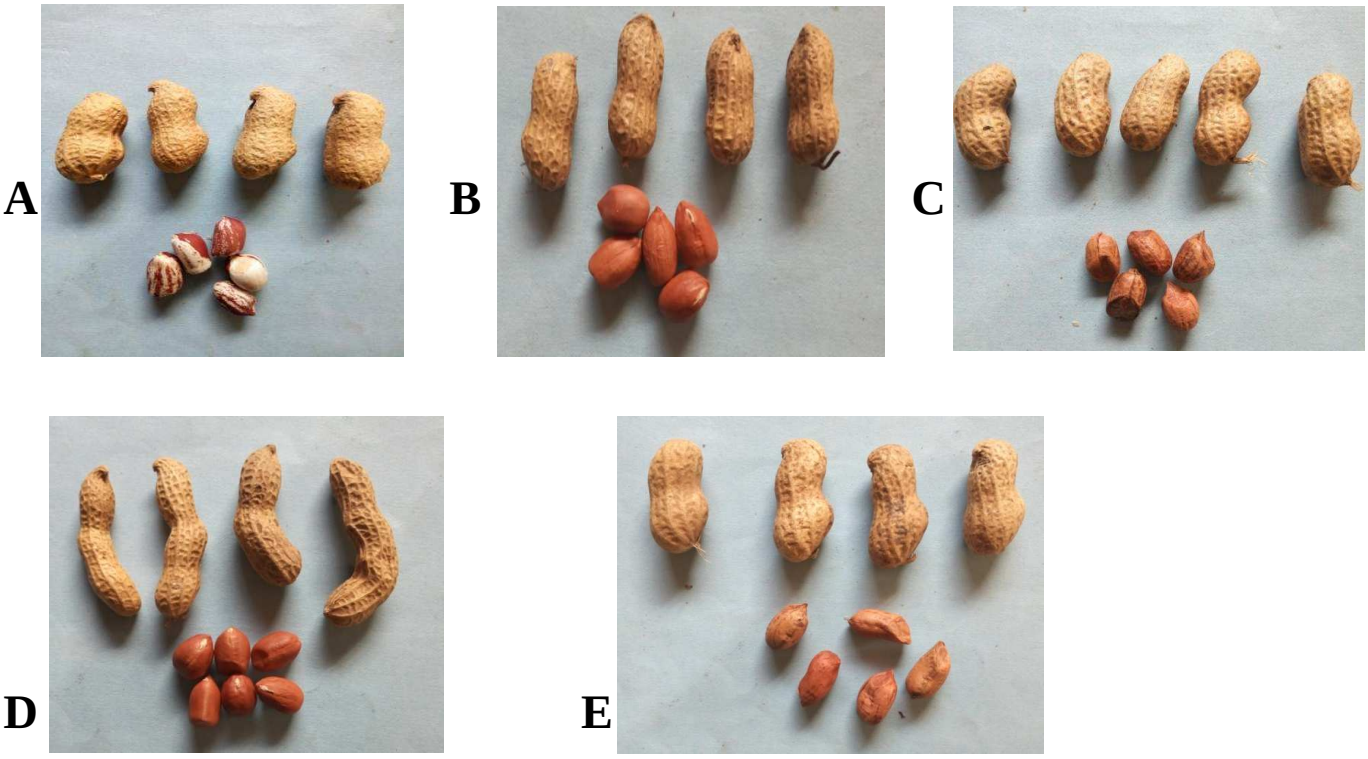

Plate 1: Variation in Fruit
"A" moderate pod constriction and prominent beak
"C" moderate pod constriction and moderate pod beak
"D" deep pod constriction and very prominent pod beak
"E" moderate pod constriction and slight pod beak

It was also observed that about 75% of farmers prefer accessions with moderate pod constriction and moderate pod beak, the reason given by then was that such accessions can stay for a long period of time even after maturity without germinating as compare with accessions having deep pod constriction and prominent pod beak which according to them germinate after maturity when not harvested; Anjana *et al*. (2016) had earlier reported that some accessions of groundnut germinates after maturity when not harvested. However, 100% of the farmers in Gbako and Lavun Local Governments preferred accessions with moderate pod constriction and moderate pod beak with the reason being that it can stay for long period of time and also have high seed oil content. In view of the popularity of groundnut as a crop of considerably economic importance in Nigeria and

Niger sate in particular, there is a need to retain the diversity of the indigenous germplasm. A scientific morphological, cytological and molecular evaluation of the accessions collected is therefore important to ascertain the genetic diversity existing within the species in Niger State.

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EFFECTS OF SEED DRESSING CHEMICALS ON IMPROVED AND LOCAL VARIETY OF PEARL MILLET INFECTED WITH *Sclerospora graminicola*

Shuaibu, A.M. *[1], Ahmad, F. S. [1], Zakari, S. M.[1], Abdullahi, S.[1], Tijjani, A.[2] and Mohammed, U. A.[3]

[1]Department of Plant Biology, Faculty of Life Sciences, Bayero University Kano, P. M .B 3011, Kano, Nigeria.
[2]Deparment of Biological Science, Faculty of science, Federal University Gashua, Gashua, Yobe, Nigeria
[3]Department of Biological Sciences, Faculty of Sciences, Bauchi State University Gadau, Bauchi, P.M.B. 065, Bauchi State, Nigeria.
GSM: +2348134412110, E-mail: musashuaibuabubakar@gmail.com

Abstract

Pearl millet is an important staple food the world over. One of the constraints of its production is the downy mildew disease caused by Sclerospora graminicola which is a very destructive disease of pearl millet. The disease is common in places where pearl millet is cultivated for food and fodder and these includes some Asian and African countries. A field experiment was conducted at Minjibir, International Crop Research Institute of Semi-Arid Tropic (ICRISAT) station, Kano state situated within the Sudan savanna of North West Nigeria between longitude 08.66485^{o}N and latitude 12.1459^{o}E at an altitude of 440m to test the effect of seed dressing chemicals on yield and growth of pearl millet infected with Sclerospora graminicola on improved and local variety. The experiment include ten treatment (Apron star, Agrolyser, Apama plus, Boost extra, Dress force, MOP, SSP, All-star, Apron star + Boost extra, and control) and replicated three times in a split plot design. The improved variety is super sosat while the local variety is Jirani. The experiment was investigated during the 2017 rainy season. The result obtained showed that seeds dressed with SSP, Apron star + Boost extra lower the incidence of downy mildew disease caused by Sclerospora graminicola on both Jirani and Super sosat variety. The super sosat variety is more tolerant to disease downy mildew than the Jirani variety. Seed dressing chemicals increased the yield and growth of improved pearl millet variety while there is no significant difference with the local variety compared to the control.

Key words: Pearl millet, downy mildew, varieties and Seed dressing chemicals.

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br] is an important staple food the world over most especially in the semi-arid and warmer parts of the world that are close to the equator. It is grown yearly on 26 million ha or there about (Raj and Wati, 2014; Jidda and Anasa, 2017). In some parts of Nigeria, pearl millet is the second most consumed staple food crop after sorghum. Millet is a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food (D'Andrea *et al.,* 2001). Pearl millet is the only cereal that reliably provides grain and fodder under dry land conditions. It is rich in nutrition compared with other cereals and adapts well to low-fertility soils in areas receiving less than 400mm of annual precipitation (Bhatnagar *et al.*, 2002). Despite the fact that the crop possess a huge potential in Nigeria, it is prone to a lot of pathogenic diseases. One of the most important pathogenic diseases is downy mildew which is caused by *Sclerospora graminicola* (Sacc.) (Jidda and Anasa, 2017). Seeds of crops play an important role in the transmission of plant pathogens causing plant diseases. The pathogen may be externally or internally seed-borne or associated with seed as contaminant. Many important diseases of plants caused by fungi spread through seeds. (Neergard, 1977). Healthy seeds plays an important role for increasing successful cultivation and yield of crops.

Fungicidal seed treatment may kill or inhibit seed-borne pathogens and may form a protective zone around seeds that can reduce seed decay and seedling blight caused by soil-borne pathogens, resulting and vigorous seedlings (Singh *et al.,* 1995). However, downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet is a major biotic constraint, causing an annual economic loss of $US 270million in the major pearl millet producing

countries (Shetty *et al.,* 1995). It is highly destructive and widespread (Singh *et al.,* 1995). Epidemics have also been reported from Asia and Africa (Wilson *et al.,* 2000) and this disease has been the major biotic factor affecting grain yield for the last decades (Singh *et al.,* 1998). The pathogen can be transmitted to new areas by wind and infected seeds (Sundaram *et al.,* 1973). *S. graminicola* can be controlled efficiently with systemic fungicide metalaxyl (Dang *et al.,* 1983, Muthusamy *et al.,* 1981 and William *et al.,* 1981). Seed dressing with Apron 35SD (6 kg/t seeds) in pearl millet growing regions of India and foliar application of Ridomil MZ72 (2 kg/ha) for seed crop were recommended (Singh *et al.,* 1990).

MATERIALS AND METHOD

Experimental site (Location)

The research was conducted at International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) station, Wasai town Minjibir Station. Minjibir Local Government, Kano State in the Northern Sudan Savanna of Nigeria between longitude 08.66485^{o}N and latitude 12.1459^{o}E at an altitude of 440m (IITA, Kano).

Varieties

Two millet varieties: improved (Super sosat) and local (Jirani) were used for the experiment.

Experimental layout and Procedure

The field experiment was carried out during the rainy season 2016, the trial was laid out in a split plot design. The measurement of the plot are as follows:

Main plot = 40 rows, 5m long (40×5×0.75) = 150m^2. Sub plot = 40 rows, 5m long (4×5×0.75) = 15m2. Gross plot = 40 rows (30m) × 35.5m = 1080m^2. Net plot = 2 rows × 5m long = 7.5m^2

The super sosat and Jirani varieties of pearl millet was sown in the main plot, while the variable seed dressing chemicals of Apron star, Dress force, SSP, Agrolyser, MOP, Apron star + Boost extra, Apama plus, All-star and the control of the test was sown in the sub plot and replicated three times. There was 70cm and 30cm inter and intra spacing between plants stands and rows respectively. There was 0.75m and 1.5m as spacing between plots and replicates respectively. The total land area used = 30m × 33m.

The seeds were put in ten (10) bowls labeled with each treatment, leaving the control unlabeled. The powdered chemicals such as Apron star, Dress force, SSP, All + star and Apama plus was sprinkled on each bowl of seed respectively and little amount of water was added, after which it was mixed thoroughly. Agrolyser which is in a solid state was first been dissolved in water before applying to the seed while MOP was applied as solid, little water was added to help mixing. The control bowl is left untreated and served as reference.

The treated seed and control was sown 3cm deep per hill. Each plot has a different treatment as the design is split plot. First weeding was done manually after 2 weeks of sowing, this was done as at when due to keep the experimental plots weed free throughout the experiment. Nitrogen fertilizer at 272 kg ha-1 was used in the form of urea as per agronomic practices. No other conventional fertilizers were used during the experiment and it was applied after four (4) weeks of sowing. The millet was thinned to three (3) plants per stand.

Pathogen inoculation

Conidial suspension of the cultured pathogens (106 spores' ml^{-1}) was sprayed on the plants at 7 days old (Singh *et al.,* 1997) to ensure that maximum infection took place during the trial (Williams *et al.,* 1981)

DATA COLLECTION

The data was collected on emergence percentage, based on the following parameters:

Growth parameters

SPAD (Chlorophyll content) 3, 6 and 9 weeks after sowing: This measures the chlorophyll content of the plants and was taken at 3, 6 and 9 week after sowing from the net plots, 5 plants were randomly selected, measured and the average taken.

LAI (Leaf Area Index): This was determined at 6, and 9 weeks after sowing: Septometer was used to measure the leaf area index of the plants. Five (5) locations were randomly selected and the average taken at 3, 6 and 9 weeks after sowing.

Vegetative parameters

50% flower: this was calculated by observing individual plot from the first appearance of flower until 50% of the plants have flowered. Plant height: net plot plant height was measured using measuring tape and recorded.

Yield parameters

Panicle number: net plot panicle number was counted and recorded, one day before harvesting.

Panicle weight: net plot panicle weight was measured using weighing scale after harvesting and recorded. Panicle length: the length of the panicle was recorded by measuring the net plot using a meter rule after harvesting. Grain weight: net plot grains were weighed using a scale after harvesting and the value was taken. 1,000 seeds: the seeds were first counted using a counting machine for a 1000 seed. Then weighed and the value was recorded.

Disease incidence

Downy mildew. D.I% = $\frac{\text{number of infected plant}}{\text{total number of plant}} \times 100$

Data Analysis

The data was subjected to General linear model, Analysis of Variance (ANOVA) using Minitab sixteenth edition. Where there is significant difference the means were separated using the Tukey method.

RESULTS AND DISCUSSION

Results recorded in Table 1 indicated that Apron star + Boost extra had the mean highest value of chlorophyll content, leaf area index, plant weight (kg) overall while Apron star recorded the highest panicle number. The variety Super sosat also had the highest value of chlorophyll content, leaf area index, plant weight (kg) overall while Jirani recorded the highest panicle number.

In Table 2 Agrolyser was the treatment that produced the tallest plants while Boost extra had the tallest panicle. In terms of disease incidence the highest value recorded was observed in plants treated with Agrolyser and MOP. On the other hand plants treated with MOP had the heaviest 1000 seed weight and 50% flower while Boost extra had the highest total grain weight. Super sosat performed better in terms of plant height, panicle length, 1000 seed weight, grain weigh and 50% flower while Jirani had the highest value of disease incidence.

Table 3 provides information on the interaction between the varieties and treatments. Here Apron star + Boost extra treatment had the higher chlorophyll content in Super sosat variety and Dress force in Jirani variety, although Dress force had the highest mean chlorophyll content. Boost extra has the highest leaf area index and plant weight on the other hand Apron star recorded the highest panicle number in Super sosat variety. While in Jirani variety Apron star + Boost extra, Apama plus and Apron star recorded the higher leaf area index, panicle number and plant weight respectively.

All-star, Boost extra, MOP, had the highest mean values for plant height, panicle length and disease incidence in the variety super sosat. While Apron star, Agrolyser and MOP had the highest values in terms of plant height, panicle length and disease incidence in Jirani. In relation to 1,000 seed weight and grain weight comparison MOP and Boost extra recorded the highest values for the variety super sosat while MOP and Apron star had the highest values in Jirani. Agrolyser comes on top in terms of 50% flowering in super sosat while MOP was on top for the variety Jirani.

Table 1: Comparison of chlorophyll content (SPAD), Leaf Area Index (LAI), panicle number and plant weight to improved (Super sosat) and local (Jirani) varieties of pearl millet in response to various seed dressing chemicals.

Treatment	SPAD 3	SPAD6	SPAD 9	LAI 6	LAI 9	Panicle number	Plant weight (kg)
Agrolyser	39.17[a]	49.65[a]	45.88[a]	3.0[a]	2.04[a]	74.5[a]	2.68[a]
All star	42.50[a]	51.81[a]	47.84[a]	2.77[a]	2.0[a]	74.5[a]	2.83[a]
Apama plus	40.45[a]	52.36[a]	48.12[a]	3.05[a]	2.08[a]	92.5[a]	2.86[a]
Apron star	39.62[a]	50.50[a]	45.23[a]	2.91[a]	2.06[a]	98.0[a]	3.17[a]
Boost extra	41.78[a]	53.15[a]	47.70[a]	3.09[a]	2.16[a]	75.5[a]	3.09[a]
Dress force	41.65[a]	53.00[a]	50.15[a]	2.92[a]	2.09[a]	78.5[a]	2.96[a]
MOP	39.87[a]	51.05[a]	50.30[a]	2.94[a]	2.1[a]	59.0[a]	2.69[a]
SSP	38.72[a]	51.50[a]	49.85[a]	2.85[a]	2.22[a]	76.5[a]	2.86[a]
Apron star + Boost extra	41.47[a]	51.77[a]	47.63[a]	3.02[a]	2.2[a]	88.5[a]	3.17[a]
Control	41.87[a]	48.77[a]	48.08[a]	2.72[a]	1.93[a]	66.0[a]	2.88[a]
Variety							
Super sosat	41.86[a]	50.79[a]	49.45[a]	3.30[a]	2.31[a]	68.70[ab]	3.35[a]
Jirani	39.56[ab]	51.93[a]	46.71[a]	2.56[ab]	1.87[ab]	88.00[a]	2.49[ab]
S.E. ±	3.2	4.6	4.1	0.3	0.21	7.2	0.42

Means followed by the same letter(s) in each column are not significantly different by Tukey at $P \leq 0.05$.

Table 2: Comparison of plant height, panicle length, disease incidence, 1,000 seeds, grain weight, and 50% flower to improved (Super sosat) and local (Jirani) varieties of pearl millet in response to various seed dressing chemicals.

Treatment	Plant Height (cm)	Panicle length (cm)	Disease Incidence	1,000 seed	Grain weight	50% flower
Agrolyser	198.62[a]	22.98[a]	1.67[a]	9.63[a]	1978.33[a]	50.0[a]
All star	199.55[a]	23.09[a]	1.0[a]	10.27[a]	2243.3[a]	50.0[a]
Apama plus	185.84[a]	22.75[a]	1.33[a]	9.54[a]	2058.6[a]	49.0[a]
Apron star	185.28[a]	23.09[a]	1.0[a]	9.28[a]	2286.03[a]	50.0[a]
Boost extra	192.78[a]	24.05[a]	1.0[a]	9.57[a]	2329.11[a]	49.0[a]
Dress force	191.11[a]	23.61[a]	0.67[a]	9.7[a]	2094.93[a]	49.5[a]
MOP	187.22[a]	22.97[a]	1.67[a]	10.79[a]	1906.04[a]	51.0[a]
SSP	170.83[a]	22.56[a]	0.84[a]	9.98[a]	2056.47[a]	50.33[a]
Apron star + Boost extra	185.0[a]	24.28[a]	1.17[a]	9.65[a]	2254.77[a]	49.5[a]
Control	188.61[a]	22.65[a]	1.17[a]	10.0[a]	2030.37[a]	50.5[a]
Variety						
Super sosat	209.13[a]	25.68[a]	0.3[ab]	10.02[a]	2423.57[a]	54.0[a]
Jirani	167.83[ab]	20.72[ab]	2.0[a]	9.66[a]	1824.02[ab]	45.77[a]
S.E. ±	11.2	2.8	0.12	0.7	24.5	4.7

Means followed by the same letter(s) in each column are not significantly different by Tukey at $P \leq 0.05$

Table 3: Comparison of chlorophyll content (SPAD), Leaf Area Index (LAI), panicle number and plant weight to the interaction between improved (Super sosat) and local (Jirani) varieties and treatments of various seed dressing chemicals.

Interaction (Variety*Treatment)	SPAD 3	SPAD6	SPAD 9	LAI 6	LAI 9	Panicle number	Plant weight (kg)
Super sosat * Agrolyser	38.77[ab]	47.23[ab]	43.97[ab]	3.33[a]	2.15[a]	60.0b	3.07a
Super sosat *All star	44.80[a]	52.13[a]	45.57[ab]	2.77[ab]	2.18[a]	44.0c	2.83ab
Super sosat *Apama plus	43.57[a]	49.83[ab]	43.53[ab]	3.52[a]	2.37[a]	74.0[ab]	3.2[a]
Super sosat *Apron star	39.0[ab]	50.13[ab]	44.03[ab]	3.27[a]	2.28[a]	94.0[a]	3.47[a]
Super sosat *Boost extra	43.63[a]	54.27[a]	48.4[ab]	3.65[a]	2.51[a]	76.0[ab]	3.87[a]
Super sosat *Dress force	44.63[a]	53.27[a]	47.23[ab]	3.22[a]	2.23[a]	66.0[b]	3.2[a]
Super sosat *MOP	42.13[a]	53.53[a]	47.97[ab]	3.69[a]	2.39[a]	53.0[ab]	3.2[a]
Super sosat *SSP	38.9[ab]	49.67[ab]	47.97[ab]	3.24[a]	2.38[a]	90.0[a]	3.63[a]
Super sosat *Apron star + Boost extra	42.3a	49.97[ab]	49.97[ab]	3.27[a]	2.49[a]	71.0[ab]	3.77[a]
Super sosat *Control	40.83[ab]	47.83[ab]	48.43[ab]	3.02[ab]	2.16[a]	59.0[b]	3.23[a]
Jirani * Agrolyser	39.57[ab]	52.07[a]	47.80[ab]	2.67[ab]	1.94[ab]	89.0[a]	2.3[b]
Jirani * All star	40.20[ab]	51.50[a]	50.10[a]	2.78[ab]	1.82[ab]	105.0[a]	2.83[ab]
Jirani * Apama plus	37.33[ab]	54.90[a]	52.70[a]	2.58[b]	1.79[ab]	111.0[a]	2.53[ab]
Jirani * Apron star	40.23[ab]	50.87[ab]	46.43[ab]	2.54[b]	1.85[ab]	102.0[a]	2.87[ab]
Jirani * Boost extra	39.93[ab]	52.03[a]	47.00[ab]	2.52[b]	1.82[ab]	75.0[ab]	2.30[b]
Jirani * Dress force	38.67[ab]	52.73[a]	53.07[a]	2.62[ab]	1.95[ab]	91.0[a]	2.73[ab]
Jirani * MOP	37.60[ab]	48.57[ab]	52.63[a]	2.2[b]	1.81[ab]	65.0[b]	2.17[b]
Jirani * SSP	38.53[ab]	53.33[a]	51.73[a]	2.46[ab]	2.07[ab]	63.0[b]	2.1[b]
Jirani *Apron star + Boost extra	40.63[ab]	53.57[a]	45.30[ab]	2.77[ab]	1.91[ab]	106.0[ab]	2.57[ab]
Jirani * Control	42.90[a]	49.70[ab]	47.73[ab]	2.42[ab]	1.7[ab]	73.0[ab]	2.53[ab]
S.E. ±	6.4	6.7	6.66	0.33	0.23	7.2	0.31

Means followed by the same letter(s) in each column are not significantly different by Tukey at $P \leq 0.05$.

Table 4: Comparison of plant height, panicle length, disease incidence, 1,000 seeds, grain weight, and 50% flower to the interaction between improved (Super sosat) and local (Jirani) varieties and treatments of various seed dressing chemicals.

Interaction (Variety*Treatment)	Plant H (cm)	Panicle length (cm)	Disease Incidence	1,000 seed	Grain weight	50% flower
Super sosat * Agrolyser	215.56[a]	23.47[a]	0.33[b]	10.13[a]	2201.5[a]	55.0[a]
Super sosat *All star	232.44[a]	25.71[a]	0.33[c]	10.9[a]	2420.23[a]	54.0[a]
Super sosat *Apama plus	213.89[a]	25.8[a]	0.33[c]	9.8[a]	2289.03[a]	53.0[a]
Super sosat *Apron star	187.78[ab]	26.1[a]	0[d]	9.23[ab]	2430.97[a]	54.0[a]
Super sosat *Boost extra	214.45[a]	27.37[a]	0.33[c]	9.97[a]	2955.43[a]	54.0[a]
Super sosat *Dress force	211.11[a]	26.63[a]	0.33[c]	9.87[a]	2210.30[a]	54.0[a]
Super sosat *MOP	219.44[a]	25.97[a]	0.67[bc]	10.6[a]	2301.9[a]	54.0[a]
Super sosat *SSP	176.67[b]	23.9[a]	0[d]	9.3[ab]	2614.17[a]	54.0[a]
Super sosat *Apron star + Boost extra	201.11[a]	27.25[a]	0[d]	9.93[a]	2619.57[a]	54.0[a]
Super sosat *Control	218.89[a]	24.57[b]	0.67[bc]	10.47[a]	2192.63[a]	54.0[a]

Jirani * Agrolyser	181.67 ab	22.48 a	3.0a	9.13 ab	1755.17 b	45.0 ab
Jirani * All star	166.66 b	20.47 ab	1.67 ab	9.63 ab	2066.37 a	46.0 ab
Jirani * Apama plus	157.78 b	19.7 ab	2.33 a	9.27 ab	1828.17 b	45.0 ab
Jirani * Apron star	182.78 ab	20.07 ab	2.0 a	9.33 ab	2141.1 a	46.0 ab
Jirani * Boost extra	171.11 b	20.73 ab	1.67 ab	9.17 ab	1702.8 b	44.0 ab
Jirani * Dress force	171.11 b	20.58 ab	1.0bc	9.53 ab	1979.57 a	45.0 ab
Jirani * MOP	155.0 b	19.97 ab	2.67 a	10.97 a	1510.17 b	48.0 ab
Jirani * SSP	165.0 b	21.22 ab	1.67 ab	10.67 ab	1498.77 b	46.67 ab
Jirani *Apron star + Boost extra	168.89 b	21.30 ab	2.33 a	9.37 ab	1889.97 b	45.0 ab
Jirani * Control	158.33b	20.73ab	1.67ab	9.53ab	1868.1b	47.0ab
S.E. ±	15.6	5.6	0.1	0.34	32.5	8.6

Means followed by the same letter(s) in each column are not significantly different by Tukey at $P \leq 0.05$

DISCUSSION

The tested seed dressing chemicals exerted different impacts on the yield and growth of pearl millet (*pennisetum glaucum*) and reduced the incidence of downy mildew disease caused by *sclerospora graminicola*. The research shows that an appropriate SSP, Apron star and Apron star + Boost extra lower the incidence of downy mildew disease caused by *Sclerospora graminicola* on super sosat 100% while in the local variety (Jirani) Dress force lower the incidence most (1.0). And also improved (Super sosat) variety is more tolerant to downy mildew disease caused by *Sclerospora graminicola* than the local (Jirani) variety.

Seed treatments increase the growth of both the improved and local variety of pearl millet (Table 1 and 2). With exception of Apron star and Apron star + Boost extra in local variety treatment. While in the improved variety all the treatments except Apron star + Boost extra lower its growth. Seed treatments influenced radically the grain yield of both the improved and local variety. All the treatment except Agrolyser, SSP, Apron-star + Boost extra, Apron star, All- star, Dress force, lower the yield of the Jirani variety. While in the Super sosat variety MOP, Apama plus, Agrolyser, Dress force, SSP, lower its yield.

The seed dressing chemicals have the capacity to disrupt infection cycles of downy mildews either by killing their asexual spores or by preventing of growth of the parasite within its host. Germling infection and basal tillers of pearl millet is very detrimental, while secondary tillers infection does not contribute much in terms of yield reduction (Singh, 1981; Deepak and Shekar, 2005). Similar result was obtained by Singh (1990) by Seed dressing with Apron 35SD (6 kg/t seeds) in pearl millet growing regions of India. And also by Williams and Singh (1981), by dressing the seed with metalaxyl. Pandya *et al*., (2007) reported that the seed treatment with metalxyl (Apron 35 WS) seed controlled downy mildew up to 20-22 days after sowing.

CONCLUSION

Seed dressing chemicals have great impact on growth of both improved (Super sosat) and local (Jirani) varieties of pearl millet.

Also, seed dressing chemicals have positive impact on the yield of both improved and local varieties of pearl millet.

It also lowers the incidence of downy mildew disease caused by *S. graminicola* up to 100% in improved variety (Super sosat) and 70-80% in the local variety (Jirani)

Therefore, this result will immensely serve as first-hand vital information to the farmers for the effective management of downy mildew disease caused by *Sclerospora graminicola* in Nigerian Savannah and other semi-arid regions.

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MORPHOLOGICAL AND CYTOLOGICAL CHARACTERIZATION OF SOME PEPPER ACCESSIONS (*Capsicum* spp*)* IN NIGER STATE, NIGERIA

Ishaya*, E. B., Gana, A. S. and Oladiran, J. A.

Department of Crop Production, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria.
***Corresponding author:** elishabahuishaya@gmail.com

ABSTRACT

Morphological and cytological evaluation of pepper accessions is important for the crop breeding and improvement programme. The lack of understanding on the genetic variation of pepper accessions has led to little progress in breeding and improvement of the crop. Therefore, the objectives of this study were to characterize some available pepper accessions based on morphological, agronomic and cytological characters and their suitability for subsequent use in breeding programme. The field experiment was conducted at Gidan Mangoro, Bosso, Niger State, in a randomized complete block design with three replications. The cytological investigation was carried out at the laboratory of the Department of Crop Production, Federal University of Technology, Minna, Niger State. Data were collected on plant height, leaf length, leaf width, days to first flowering, days to 50 % flowering, fruit length, fruit girth, number of fruits, average fruit weight average seed weight and plant growth habit. From Analysis of Variance, genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance were estimated. There were significant differences in the accessions studied. Analysis of variance for genetic diversity revealed that plant height, leaf width, and all yield components studied contributed to genetic diversity. The highest heritability value was observed in average fruit weight per plant (98 %), and the accessions with the best performance are DKD-RD and DG-SB which are characterized by early days to first flowering, number of fruits per plant, number of branches and fruit girth. The variance component of variation result revealed that PCV values were greater than those of GVC. Also, genetic advance (GA) was higher than heritability. The cytological study showed that at meiosis, two daughter cells divides and formed four daughter cells thereby contributing to genetic variation. Based on the findings of this research work, it is recommended that breeders should collect germplasm from DKD-RD and DG-SB, for further breeding and improvement programme of the crop.

Keywords: genetic variation, agro-morphological traits, cytological behaviours, heritability; pepper (*Capsicum* spp).

INTRODUCTION

Peppers belong to the genus *Capsicum* and member of the nightshade family *Solanaceae*. This genus also called the chilli pepper (Joshi, 2012) originated from Central and South America (Grubben and El-Tahir, 2004). There are about thirty species in the genus *Capsicum* and several species have been domesticated to produce many cultivated types which range from mild to hot (Bosland and Votavas, 2000). Fruit characters have been extensively used in the taxonomy of the family Solanaceae (Pabon-Mora and Litt, 2011). Most natural populations of pepper are diploid and have the same chromosome number, 2n=24 (Grubben and El- Tahir, 2004; Okoli and Osuji, 2008). Nigeria is known to be one of the major producers of pepper in the world accounting for about 17 % with 78,462 t from 90,000 ha of the world production (Food and Agriculture Organization, 2013). Chillies are largely grown in many parts of Nigeria and the major area of production is the northern region between latitude 10^{o}N and $12^{o}30'$N (Adetula and Olakojo, 2006). The chilli peppers are excellent source of nutrition for humans, a rich source of most B vitamins and vitamin B_6 in particular (Deepa *et al.*, 2007; United SDA Nutrient Database, 2007). They can be eaten raw, cooked or processed into powder form for culinary uses (Collingham, 2006).

Estimating the amount of genetic variability and determining the nature of the association between variables are very important steps in selection and improvement programme. Furthermore, the success of any selection programme depends largely on the amount of genetic variability existing in the population. Cytological and agro-morphological traits have been found to provide a good assessment of variability in *capsicum* species (Del *et al.,* 2007; Bozokalfa *et al.,* 2009).

MATERIALS AND METHODS

Location of Experimental Site

The research was conducted at Gidan Mangoro village, Bosso Local Government Area of Minna, located at an elevation of 482 metres above sea level in the Southern Guinea Savanna zone of Nigeria and lying between latitude 9° 33′57.69″N, longitude 6° 29′19.896″E. The elevation of the site was tracked using GPS (Geographical Positioning System, Garmin Taiwan). The Cytological investigation was carried-out at the Laboratory of the Department of Crop Production, Federal University of Technology Gidan Kwano Campus, Minna, Niger State of Nigeria.

Treatment and Experimental Design

Ten accessions of chilli pepper were collected from local farmers in different locations of Northern Guinea Savanna and Sudan savanna of Nigeria. Seeds of four accessions were collected from Kaduna State [Dan-BirninGwari (DBG), Dan-Zaria 'Tatashe (DZ-TSH), Dan-Kaduna (DKD), Dan-Gada' Shombo' (DG-SB)], three accessions Sokoto State [Dan-Sokoto 'Rodo' (DSKT-RD), Dan-Sokoto 'Tatashe' (DSKT-TSH) and Dan Sokoto 'Shombo' (DSKT-SB)], one from Kano State [Dan-Kano (DKN)], one from Adamawa State [Dan-Adamawa (DADAM)], and one from Katsina State [Dan-Katsina (DKST)].The planting was done using Randomized Complete Block Design with three replications.

Data Collection and Statistical Analysis

Data were collected on plant height, leaf length, leaf width, days to first flowering, days to 50 % flowering, fruit length, fruit girth, number of branches per plant, number of fruit per plant, average fruit weight per plant and average seed weight per plant. All data collected were subjected to analysis of variance (ANOVA) using SAS statistical package (SAS, 2008). The means were separated by Duncan's Multiple Range Test (DMRT, 1955) at 5 % level of probability. The photomicrographs of the cytological observation were taken using the Olympus microscope with a Sony digital camera (16.1 Mega pixels).

RESULTS

The agronomic performance of the ten pepper accessions studied revealed that the accessions differed significantly ($p<0.05$) in all the study traits (Table 1). The tallest plant was accession DSKT-SB which differed significantly with the other accessions except for DG-SB which showed no significant difference. Accession with the highest leaf length was DSKT-TSH which showed no significance difference with all the other nine accessions except for DADAM which differed significantly. The highest leaf weight was observed in DKD-RD, early days to first flowering and days to 50 % flowering were noted in accessions DG-SB and DBG respectively. DKST recorded the highest number of branches and number of fruit. DSKT-TSH had the highest average fruit weight per plant and differed significantly with all the other accessions except for DZ-TSH which showed no significant difference. For seed weight per plant, accession DZ-TSH recorded the average seed weight and showed no significant difference with DSKT-TSH but differed significantly with all the other remaining eight accessions

Table 1: Mean values for agronomic traits (quantitative) of the ten pepper accessions studied

ACCESSIONS

	PHT	LL	LW	DFF	DF50%FL	FG	NOB	NOF	AFWT	ASDWT
DKST	47.39^{bc}	11.85^{ab}	4.44^{c}	86.00^{ab}	106.00^{ab}	4.71^{de}	1.13^{fg}	8.00^{a}	54.33^{a}	14.66^{d}	2.32^{e}
DSKT-TSH	38.12^{d}	12.87^{a}	4.93^{bc}	86.67^{ab}	106.00^{ab}	8.55^{b}	4.44^{a}	4.67^{b-d}	12.00^{c}	261.01a	7.87^{a}
DG-SB	52.91^{ab}	12.02^{ab}	4.61^{c}	93.67^{a}	108.67^{ab}	9.77^{a}	2.29^{c}	4.00^{c-e}	33.33^{b}	93.42^{b}	5.98^{b}
DADAM	47.63^{bc}	10.62^{b}	4.26^{c}	91.67^{ab}	105.57^{b}	7.34^{c}	1.33^{ef}	5.00bc	37.33^{b}	33.31^{d}	5.18^{bc}
DZ-TSH	38.77^{d}	12.72^{a}	4.54^{c}	89.00^{ab}	107.67^{ab}	6.39^{c}	4.51^{a}	3.67^{de}	12.33^{c}	253.84^{a}	8.070^{a}
DKN	49.12^{bc}	11.77^{ab}	4.51^{c}	86.67^{ab}	107.67^{ab}	6.79^{c}	0.91^{g}	7.33^{a}	30.67^{b}	23.27^{d}	3.09^{de}
DSKT-SB	56.56^{a}	12.28^{ab}	5.88^{ab}	87.00^{ab}	107.67^{ab}	8.84^{ab}	1.90^{cd}	4.00^{c-e}	27.67^{b}	59.18^{c}	3.00^{de}
DBG	46.48^{c}	11.63^{ab}	4.33^{c}	87.00^{ab}	109.00^{ab}	5.23^{d}	1.58^{ed}	5.67^{b}	31.00^{b}	31.80^{d}	4.07^{cd}
DKD-RD	38.67^{d}	11.30^{ab}	6.56^{a}	88.00^{ab}	110.33^{a}	1.85^{f}	2.13^{c}	3.33^{e}	37.00^{b}	33.20^{d}	0.71^{f}
DSKT-RD	40.28^{d}	11.73^{ab}	6.35^{a}	83.33^{b}	108.67^{ab}	3.65^{e}	3.70^{b}	4.00^{c-e}	30.33^{b}	105.19^{b}	3.39^{de}
Mean	**45.593**	**11.879**	**5.041**	**86.0**	**106.0**	**6.312**	**2.392**	**4.967**	**30.599**	**90.888**	**4.368**
±SE	**1.90**	**0.57**	**0.33**	**2.85**	**1.29**	**0.37**	**0.13**	**0.34**	**5.01**	**7.10**	**0.39**
CV	**7.202**	**8.2734**	**11.3506**	**5.6192**	**2.0779**	**10.0508**	**9.6180**	**11.6889**	**28.3650**	**13.5384**	**15.4804**

Means followed by the same letter(s) within the column are not significantly different (p = 0.05) by Duncan Multiple Range Test.

PHT= plant height, LL= leaf length, LW= leaf width, DFF= days to first flowering, D50%F= days to 50% flowering, FL= fruit length, FG= fruit girth, NOB= number of branches, NOF= number of fruits/plant, AFWT= average fruit weight/plant, ASDWT= average seed weight/plant. DKST= Dan Katsina, DSKT-TSH = Dan Sokoto 'Tatashe', DG-SB= Dan Gada'Shombo', DADAM= Dan Adamawa, DZ-TSH= Dan Zaria 'Tatashe' DKN= Dan Kano, DSKT-SB= Dan Sokoto 'Shombo', DBG= Dan BirninGwari, DKD- RD= Dan Kaduna 'Rodo', DSKT-RD= Dan Sokoto 'Rodo'.

Variance Component for Genetic Diversity among the Ten Pepper Accessions

The highest genotypic variance were observed in number of fruit per plant, average fruit weight per plant and plant height, while leaf length, days to first flowering, days to 50 % flowering and fruit girth are among the traits that showed low genotypic variance (Table 2). Phenotypic variance result showed that average fruit weight per plant had the highest phenotypic value next was number of fruits per plant, while the least values were observed in plant height and days to first flowering, but the remaining seven traits showed a very low phenotypic influence. Phenotypic coefficients of variation were observed to be higher than genotypic coefficient of variation. Similarly, the results of genetic advance were higher than that of heritability with an exception on fruit length per plant, fruit girth, number of branches per plant, number of fruit per plant, average fruit eight and average seed weight which showed a lower genetic advance in relation to heritability.

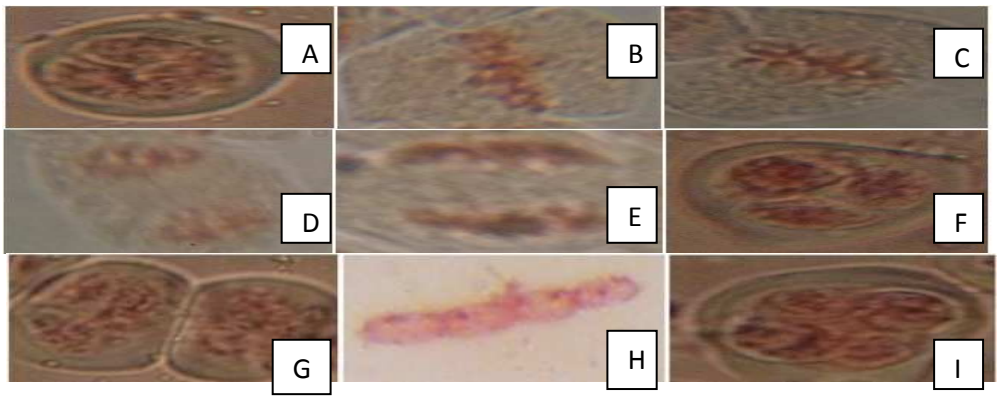
Table 2: Variance component for genetic diversity among the accessions studied

Traits	GV	PV	GCV	PCV	H^2 (%)	GA
PHT	37.83	48.61	13.49	15.29	78.0	78.22
LL	0.11	1.08	2.79	8.75	10.0	22.28
LW	6.00	6.33	48.5	49.92	9.50	12.40
DFF	0.51	24.91	0.83	5.80	2.00	10.27
D50%F	0.60	5.61	0.73	2.23	12.0	13.88
FL	6.01	6.41	38.85	40.12	94.0	12.43
FG	1.79	1.84	55.98	56.76	97.0	36.82
NOB	2.39	2.73	31.11	33.18	88.0	49.56
NOF	124.00	199.8	36.45	46.19	62.0	25.55
AFWT	85.31	86.82	101.60	102.50	98.0	17.55
ASDWT	5.56	6.02	53.96	56.15	92.0	11.42

PHT= plant height, LL= leaf length, LW= leaf width, DFF= days to first flowering, D50%F= days to 50 % flowering, FL= fruit length, FG= fruit girth, NOB= number of branches, NOF= number of fruits/plant, AFWT= average fruit weight per plant, ASDWT= average seed weight per plant. GV= genotypic variance, PV= phenotypic variance, GCV= genotypic coefficient of variation, PCV= Phenotypic coefficient of Variation H^2_b= heritability (%). GA= genetic Advance.

Cytological Observation

The result of the cytological study under a light microscope of the accession DKN revealed that at diakinenssis, chromosomes are mostly associated to bivalent from the first phase to the last phase of meiosis (Plate 1). At the first stage of meiosis, accession DKST was observed to be at the resting phase (Interphase I). In this stage no chromosomes were observed except a thread-like structure. Accession DSKT-SB showed a normal segregation pattern of chromosomes at metaphase I, with bivalent aligned at the spindle equator. While at the early prophase chromosomes were observed forming a bivalent as in accession DK-RD and DBG. At early anaphase, two bipolar spindles were observed in accession DKN with chromosomes migrating to the poles while at late metaphase, chromosomes were observed in separate spindle dyad cell. At late telophase, accession DADAM showed a complete separation of the two sister's cell from each other. During the process of meiotic division accession DSKT-SB showed an abnormal disjoint resulting into a triad or trivalent. On the contrary, accession DKN and DG-SB showed a linear and a regular tetrad after the disjoint at telophase II.



DISCUSSION AND CONCLUSION

This research revealed that variations existed among the accessions in respect to agro-morphological traits studied. The variability that existed among the accessions in traits such as plant height, days to first flowering, fruit girth and numbers of fruit per plant, fruits shape, average seed weight per plant, average fruit weight per plant, number of branches per plant might be due to genetic make of the plant as well as soil type. This agrees with the work of Del *et al.* (2007); Maga *et al.* (2010) who reported that, the variation observed in pepper genotypes are due to differences in plants, soil type and the effect of environmental factors of the experimental site. The phenotypic variations that existed among the ten pepper accessions could importantly be utilized in the crop breeding and improvement programme. Chromosomes behaviours during meiotic investigation explained the phases involving inheritance in the ten pepper accessions studied and how the observed traits are transmitted from one generation to another, while maintaining their gene combination which interacts in a functional adaptive manner. Based on the findings in this research work it is recommended that breeders should collect Germplasm from DKD-RD, DG-SB, DSKT-RD and DADAM for further breeding and improvement programme due to their outstanding performances which are related to high valued traitssuch as days to first flowering, number of branches per plant, number of fruits per plant and average fruit weight.

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STUDIES ON HERITABILITY AND CHARACTER ASSOCIATION IN MAIZE (*Zea mays* L.) UNDER NON-STRESS AND DROUGHT STRESS CONDITIONS

UMAR, U.U.[1], ADO, S.G.[2], MIJINYAWA, A.*[3], JUNAIDU, H.[4] AND BASHIR, Y.R.[5]

[1]Department of Biological Sciences, Kaduna State University, Kaduna
[2] Department of Plant Science, Ahmadu Bello University, Zaria
[3]Department of Botany, Ahmadu Bello University, Zaria
[4]Samaru College of Agriculture, Division of Agricultural Colleges, A.B.U Zaria
Department of Biological Sciences, Federal University, Gusau, Gusau
*Corresponding author: ahmijinyawa@yahoo.com 08069237924

ABSTRACT

Fifty-six maize genotypes consisting of fourty-two F_1 hybrids, thirteen inbred lines and a commercial check were laid-out in a in a simple lattice design with two replications at Kadawa (11°39'N, 08°027`E) in the Sudan Savanna ecological zone of Nigeria during 2012/13 dry season. Evaluations were done to estimate broad-sense and narrow sense heritability as well as correlations between grain yields and other traits under non-stress, intermediate stress and severe drought stress conditions. The results showed high (>60%) broad sense heritability estimates for all the traits studied under the three conditions except anthesis-silking interval (58.33%), plant height (53.71%) and ears per plant (50.00%) which were moderate (30-60%) under non-stress condition. These estimates revealed that variations were transmitted to the progenies and indicated the potential for developing high yielding drought tolerance varieties through selection of desirable plants in succeeding generations. Narrow sense heritability was low (<30%) for all the traits under the different conditions which indicate that the studied traits are mainly controlled by non-additive genes. The correlation analysis revealed that plant height, ear height and ears per plant expressed positive and significant genotypic and phenotypic correlations with grain yield under the three conditions. These means that any improvement of these traits will induce increase in grain yield. Traits that had high heritability and positive correlations with grain yield may be considered as important traits in selection programmes aiming to maize yield improvement and the breeder may consider these traits as main selection criteria under non-stress and drought stress conditions.

Key Words: Drought, Narrow sense, Broad sense, Heritability, and Correlation

INTRODUCTION

Global climate change is now generally considered to be underway (Hillel and Rosenzweig, 2002), and is expected to result in a long-term trend towards higher temperatures, greater evapo-transpiration, and an increased incidence of drought in specific regions. Drought is one of the most important abiotic stresses threatening maize production, food security and economic growth in sub-Saharan Africa (Kamara *et al*., 2004). The risk of drought stress is severe particularly in the Sudan savanna zone due to unreliable and uneven distribution of rainfall (Eckebil, 1991). In maize, grain yield reduction caused by drought ranges from 10 to 76% depending on the severity and stage of occurrence (Bolaños *et al*., 1993). The use of genetics to improve drought tolerance and provide yield stability is an important part of the solution to stabilizing global maize production. For this to happen, yield improvement through genetic approaches would become essential. Since yield is a complex character, which is the product of multiplicative interactions of a number of its component characters (Grafius, 1959); yield cannot be improved to a greater extent on its own. Hence, a clear picture of contribution of each component in final expression of complex character is essential. In order to achieve the goal of increased production by increasing the yield potential of the crop, knowledge on the magnitude of association between yield and yield related traits is essential for a plant breeder.

Heritability on the other hand provides an idea to the extent of genetic control for expression of a particular trait and the reliability of phenotype in predicting its breeding value (Tazeen *et al*., 2009). High heritability indicates less environmental influence in the observed variation (Songsri *et al*., 2008). High heritability for a given trait

also indicates that it is governed by additive gene action and, therefore, provides the most effective condition for selection (Tazeen *et al*., 2009). The present investigation was therefore, carried out to understand the association of grain yield with yield components and also to estimates the heritability of traits in maize under non-stress and drought stress conditions.

MATERIALS AND METHODS

The research was conducted at Kadawa Irrigation Research Sub-station (11°39'N, 08^{0}027`E) of the Institute for Agricultural Research, Ahmadu Bello University, Zaria during 2013 dry season. Fifty six maize genotypes were used for this study comprising six drought tolerant male inbred lines; seven drought susceptible female inbred lines, forty two single cross hybrids and a commercial check. The single cross hybrids were generated in the year 2012 rainy season using North Carolina mating design II at the Institute for Agricultural Research Farm at Samaru-Zaria (11^{o}11'N; 07^{o}38'E). The genotypes were grown in a simple lattice replicated two times under three conditions resulting in non-stress, intermediate stress and severe drought stress conditions. Apart from the targeted stress, the management of the trials was the same in all the three conditions.

The non-stress condition continued to receive irrigation water once every week until the end of physiological maturity. In the intermediate stress condition, water stress was imposed by withdrawing irrigation water as from 6 weeks after planting until the end of the growing season to achieve drought stress at grain filling stage. The crop was allowed to mature only on stored soil water. In the severe stress condition, water stress was imposed by withdrawing irrigation water as from 5 weeks after planting to achieve drought stress at flowering stage. The crop was allowed to mature only on stored soil water. Each entry was planted in a 3 m row plot spaced 0.75 m apart with 0.25 m spacing between plants within each row. Two seeds were planted in a hill and thinned to one plant after emergence to obtain a population density of approximately 53,333 plants per hectare. Data were taken on the following traits: 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). Broad sense heritability, genotypic and phenotypic correlation estimates were worked out according to Singh and Chaudhary (1985) while narrow sense heritability was calculated according to Grafius *et al*. (1952).

RESULTS AND DISCUSSION

The results from the estimates for broad-sense and narrow sense heritability of maize traits under non-stress, intermediate stress and severe stress drought conditions at Kadawa are presented in Table 1. Drought conditions played significant role in modifying the broad sense heritability estimates for different traits. Under non-stress conditions, high broad heritability (>60%) estimates were observed for days to 50% tasseling (85.14%) followed by days to 50% silking (84.75%), grain yield (73.77%) and ear height (68.80%). However, moderate broad sense heritability (30-60%) estimates were observed for ASI (58.33%) followed by plant height (53.71%) and ears per plant (50.00%). Under intermediate stress condition, high broad sense heritability (>60%) estimates were observed for plant height (77.20%) followed by days to 50% tasseling (75.16%), ears per plant (75.00%), ear height (74.35%), days to 50% silking (72.04%), grain yield (67.25%) and ASI (63.86%). Under severe stress condition, high broad sense heritability (>60%) estimates were observed for ears per plant (90.91%), plant height (85.40%), ear height (77.28%), ASI (69.07%), days to 50% silking (67.50%), days to 50% tasseling (64.20%) and grain yield (62.36%).

The high broad-sense heritability obtained for most traits revealed that variations were transmitted to the progeny and indicated potential for developing high yielding varieties through selection of desirable plants in succeeding generations. These results were in agreement with those of Aminu and Izge (2013) and Umar *et al*. (2014). Traits that had high broad sense heritability estimates indicate the preponderance of additive gene action. These results are in line with earlier results reported by Aminu *et al*. (2013) and Tazeen *et al*. (2009).

The broad sense heritability of ASI, plant height, ear height and ears per plant increased with increasing drought stress, whereas, that of days to 50% tasseling, days to 50% silking and grain yield decreased with increasing drought stress. Bolaños and Edmeades, (1996) reported that under stress conditions, the broad sense heritability of ASI and ears per plant increased while that of grain yield falls mainly because there is a decrease in genotypic variance. Decreased broad sense heritability for grain yield, days to 50% tasseling and days to 50% silking under stress conditions were reported earlier by Umar *et al*. (2014). The decreased broad sense heritability for traits under stress indicates the need for selection of genotypes under specific conditions for rapid genetic improvement.

Table 1 Estimates for broad-sense and narrow heritability (%) of maize traits under non-stress, intermediate stress and severe drought stress conditions at Kadawa in 2013 dry session

	Broad sense heritability (%)			Narrow sense heritability (%)		
Traits	NS	IS	SS	NS	IS	SS
DYTS	85.14	75.16	64.20	2.49	10.98	5.11
DYSK	84.75	72.04	67.50	5.05	3.66	1.50
ASI	58.33	63.86	69.07	13.95	6.07	9.63
PLHT	53.71	77.20	85.40	1.44	15.46	3.48
EHT	68.80	74.35	77.28	10.41	4.23	9.86
EPP	50.00	75.00	90.91	6.25	4.55	47.61
GY	73.77	67.25	62.36	4.06	21.21	4.09
Range	50.00-85.14	62.16-77.20	62.36-93.73	1.44-13.95	3.66-21.21	1.50-47.61

NS=non-stress; IS=intermediate stress; SS=severe stress; 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

However, for narrow sense heritability the trend was generally low (<30%) for all the traits studied except number of ear per plant (47.61%) under severe stress which was moderate (30-60%). The narrow sense heritability ranged from 1.44% to 13.95%, 3.66% to 21.21% and 1.44% to 47.61% under non-stress, intermediate stress and severe stress conditions, respectively. The relatively low narrow sense heritability estimates for most of the traits in this study under the different conditions are an indication that the studied traits are mainly controlled by non-additive genes. The best exploitation of this type of gene action would be in F_1 hybrids implying that breeding gains can be made through inbreeding then crossbreeding, with selection being made in later generations.

Results of the genotypic and phenotypic correlation coefficients of maize traits under non-stress, intermediate stress and severe drought stress conditions at Kadawa are presented in Table 2. Grain yield showed positive and significant correlations with plant height, ear height and ears per plant at genotypic and phenotypic levels under the three conditions except ear height under non-stress at phenotypic level which was positive but non-significant (r_g = 0.121). This shows that selection for any of these traits could result in corresponding increase in grain yield. Similar results were also reported for plant height and ear height by Bello *et al*. (2010). However, this contradicts the reports of Olakojo and Olaoye (2011), who reported negative correlation between grain yield and plant height and ear height. The positive correlation between ears per plant and grain yield was expected because grain yield is a primary dependent variable of ears per plant. This was supported with the report of Ngugi *et al*. (2013) on ears per plant.

Contrarily, grain yield had negative and significant correlations with ASI at genotypic and phenotypic levels under the three conditions. Days to 50 % tasseling showed negative and significant genotypic correlation with grain yield under non-stress and severe stress conditions while at phenotypic level were negative but not significant. Under intermediate stress condition, the correlations between days to 50 % tasseling and grain yield were negative but non-significant at both levels. Under non-stress condition, days to 50% silking showed negative and significant correlation with grain yield at phenotypic level; in contrast at genotypic level it showed negative but non-significant correlation. Under intermediate stress condition, the correlations between grain

yield and days to 50% silking were negative but non-significant at both levels. Days to 50% silking correlated negatively and significantly with grain under severe stress condition at genotypic level while at phenotypic level was negative but non-significant. This indicates that an increase in any of these traits could 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 as well as short ASI. These findings were consistent with findings of Kumar *et al.* (2011).

Besides the correlation studies, *inter se* association studies also provide an opportunity to select only those characters which are favourably associated among themselves as well as with yield. In the present investigation, studies on *inter se* associations among yield components revealed that the trait plant height was positive associated with days 50% tasseling, days to 50% silking and ear height under the three conditions at both levels. Bello *et al*. (2010) observed that plant height was positively correlated with days to 50% tasseling and silking, as internodes' formation stops at floral initiation, and that early flowering maize varieties are usually shorter in height. Highly significant favourable correlation among yield attributes indicates that, the unit increase in one trait will cause a unit increase in the associated trait, which in turn will cause an increase in the yield.

Table 2 Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficients of maize traits under non-stress, intermediate stress and severe drought stress conditions at Kadawa in 2013 dry session

Traits	DYTS	DYSK	ASI	PLHT	EHT	EPP	GY
Non-stress							
DYTS	1	0.964**	0.751**	0.493*	0.060	-0.968**	-0.908**
DYSK	0.976**	1	0.815**	0.348*	0.186	-0.925**	-0.099
ASI	0.004	0.220	1	0.409*	0.979**	-0.933**	-0. 710**
PLHT	0.178	0.151	-0.094	1	0.748**	0.124	0.791**
EHT	0.512*	-0.465*	0.152	0.091	1	0.012	0.121
EPP	0.131	0.123	-0.015	0.057	-0.298	1	0.306*
GY	-0.163	-0.614**	-0.615**	0.416*	0.812**	0.614**	1
Intermediate stress							
Traits	DYTS	DYSK	ASI	PLHT	EHT	EPP	GY
DYTS	1	0.998**	-0.042	0.217	0.961**	-0.034	-0.250
DYSK	0.978**	1	-0.033	0.133	-0.961**	-0.028	-0.250
ASI	0.720**	0.226	1	-0.965**	0.053	-0.006	-0.623**
PLHT	0.020	0.036	0.077	1	0.066	0.092	0.344*
EHT	0.423*	-0.443*	-0.165	0.092	1	-0.198	0.320*
EPP	-0.188	-0.208	-0.119	0.045	0.165	1	0.622**
GY	-0.273	-0.275	-0.620**	0.296	0.628**	0.623**	1
Severe stress							
Traits	DYTS	DYSK	ASI	PLHT	EHT	EPP	GY
DYTS	1	0.956**	-0.980**	0.598*	0.055	-0.634**	-0.639**
DYSK	0.979**	1	-0.977**	0.716**	0.161	-0.666**	-0.998**
ASI	0.022	0.217	1	0.967**	0.975**	-0.965**	-0.491*
PLHT	0.317*	0.304*	0.009	1	0.500*	-0.606**	0.230
EHT	0.590*	-0.574*	-0.019	0.775**	1	-0.302*	0.523*
EPP	-0.086	-0.070	-0.077	0.203	0.214		0.699**
GY	-0.080	-0.097	-0.305*	0.312*	0.421*	0. 310*	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

CONCLUSIONS

The results showed that days to 50% tasseling, days to 50% silking, ear height and grain yield had high broad-sense heritability under three conditions. Anthesis-silking interval, plant height and ears per plant showed higher values under intermediate stress and severe stress while under non-stress were moderate. High to moderate broad sense heritability indicated considerable potential for development of drought tolerance and high yielding varieties through selection of desirable plants in succeeding generation. The decreased broad sense heritability

of days to 50% tasseling, days to 50% silking and grain yield remind breeders for selection of genotypes under specific conditions for rapid genetic improvement. The relatively low narrow sense heritability estimates for most of the traits in this study under the different conditions are an indication that the studied traits are mainly controlled by non-additive genes and so imply that breeding gains can be made through inbreeding then crossbreeding, with selection being made in later generations. Plant height, ear height and ears per plant were positively correlated with grain yield under drought stress. Traits that had moderate to high heritability and positive correlations with grain yield may be considered as important traits in selection programme aiming to maize yield improvement under non-stress and drought stress conditions and the breeder may consider these traits as main selection criteria.

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GENETIC DIVERSITY OF *CHRYSOPHYLLUM ALBIDUM* G. (AFRICAN STAR APPLE) POPULATIONS IN NORTH CENTRAL NIGERIA, TROPICAL WEST AFRICA

Aguoru, C. U., Olasan, J. O. and Ameh, E.O.

Department of Biological Sciences, University of Agriculture, Makurdi. Nigeria
Corresponding author: Prof Aguoru C.U (celeaguoru@yahoo.com); Dr. Olasan J.O. (lekanolasan@yahoo.com)

Abstract

Diversity of Chrysophyllum albidum populations was studied. Forty (40) trees were randomly sampled across fifteen (15) rural communities of Benue State, Nigeria. Twenty five (25) quantitative characters and twelve (12) qualitative features were assessed per tree. Agronomic characterization, epidermal studies and biochemical analysis were carried out. Due to the low variability observed, benchmark of 8% was used to identify characters with high CV as displayed using scatterplots. Trees were classified through cluster analysis using the Average Linkage (Between Group) methods to generate a dendrogram. No variation was recorded in most of the qualitative features. Stomatal type was anomocytic and paracytic with irregular shape. Among the quantitative characters, number of seeds produced per fruit, seed length, stomatal indices and nutritional components recorded very low variability (CV<8%) across the trees. The traits with CV values above the benchmark are: leaf sizes (L=8.93%, B=8.75%), leaf weight (20.88%), fruit length (10.26%), fruit weight (11.46%), seed weight (8.35%), seed width (17.70%), wet mesocarp weight (14.38%), fruit moisture (8.98%), stomatal sizes (L=10.08%, B=11.27%) and the fruit ash content (10.33%). On the average, the fruit contains 7.13% carbohydrate, 1.68% protein, 2.28% fat and 66.49% moisture. The seed contain 60.29% carbohydrate, 5.2% protein, 5.8%fat and 9.88% moisture. Thus, the nutritional relevance of C.albidum is substantiated. Trees with the tastiest/sweetest or moderately sweet fruits were also selected. Dendrogram classified all trees into four main clusters grouped on the basis of observed similarities and differences. Grouping is location independent. Unique trees are noted. The study has described C.albidum trees in Benue State as a population with moderate gene flow not strong enough to cause speciation as there are fewer traits accounting for the observed intraspecific variability. Pieces of information provided in this report are indispensable in the management, improvements, utilization and systematics of the indigenous fruit crop.

Key words: Characterization, *Chrysophyllum albidum*, Improvement, Variability, Utilisation

Introduction

Chrysophyllum albidum G., Family Sapotaceae, is indigenous to Africa hence popularly called the African star apple (Okigbo, 2007). It is rarely cultivated but grows in the wild in most tropical areas especially in Nigeria, Cameroon, Cote d'Ivoire, Uganda and Niger Republic (Ayuk *et al*., 2012). The tree has been classified in Okigbo (2007) as a wild uncultivated but neglected fruit tree which occurs naturally in the forest and bushes and seldom planted as a fruit tree. Many authors have opined that the tree crop deserves some level of attention, management and improvement as witnessed in exotic fruit trees. Simon and Leakey (2003) and Shreckneiberg *et al*. (2006) advocated proper domestication of all indigenous fruit trees as the way forward in poverty reduction as well as eradication of malnutrition in Africa. This is because the tropical rain forests have recently witnessed massive habitat destruction and unsustainable use of resources leading to loss of biodiversity and genetic erosion (Onyekwelu *et al*., 2008; Aguoru *et al*., 2015a).

Chrysophyllum albidum is commonly called "agbalumo" in Yoruba and "udara" in Igbo speaking parts of Nigeria. The edible fruit has been reported to contain more vitamin C, calcium, iron and fibre than most foreign fruits (Amusa *et al*., 2003; Nwadingwe, 2011; Oboh *et al*., 2009; Udofia *et al*., 2012). The unparalleled quality of nutritional component is proven through the affinity of fruit maggots occupying the exposed parts of the fruit within 2-5 days of harvesting. The fruit can be exploited to produce jam, jellies, stewed fruit, syrup and juicy drinks to overcome malnutrition in Africa (FAO, 2015). The seed contains high volume of useable oil (Oyelade

et al., 2005) while the pulp in the fruit as well as the leaf extracts are highly medicinal (Adewusi, 2012; Atangana, 2002). The high amount of antioxidant in the fruit is a good chelating agent to combat oxidative stress related diseases such as diabetics, cancer, fibroid and heart problems (Burits and Bucar, 2002).
Table 1: Tree samples and their locations

Location	Local Government Area	Tree code
Igwu Akor	Ogbadibo	T1, T2
Owukpa	Ogbadibo	T3, T4, T5
Otukpa-Olabochai	Ogbadibo	T6, T7
Adupi	Ogbadibo	T8, T9, T10
Orokam	Ogbadibo	T11, T12, T13
Iduobe	Okpokwu	T14, T15
Ugwu	Okpokwu	T16, T17
Ameju	Okpokwu	T18, T19, T20
Ichama	Okpokwu	T21, T22
Okpodu	Okpokwu	T23, T24, T25
Aliade	Gwer-East	T26, T27, T28
Mbalom	Gwer-East	T29, T30
Mbachir	Gwer-East	T31, T32, T33
Mbayom	Gwer-East	T34, T35, T36
Mbaiase	Gwer-East	T37, T38, T39, T40

Results and Discussion

Table 2 presents some unvaried characters observed among the forty trees. The alternately arranged leaf is simple with entire margin and net venation. No variation was recorded in fruit and seed colour and their shapes. Stomatal type was anomocytic and paracytic with irregular shape. These are unifying characters that remain unchanged as evolution progresses and they do not contribute to variation among the trees (Taylor *et al*., 2007). Among the quantitative characters evaluated, the petiole length, number of seeds produced per fruit, seed length, stomatal indices and nutritional components recorded very low variability across the trees (Table 3). The traits that recorded high CV values are: leaf sizes (L=8.93%, B=8.75%), leaf weight (20.88%), fruit length (10.26%), fruit weight (11.46%), seed weight (8.35%), seed width (17.70%), wet mesocarp weight (14.38%), fruit moisture (8.98%), stomatal sizes (L=10.08%, B=11.27%) and the fruit ash content (10.33%). These quantitative traits have accounted for the variation recorded and thus important in the evolution of the tree crop (Oboh *et al*., 2008; Aguoru *et al*., 2015d). As reported in the work of Oboh *et al*. (2008) variation in stomatal sizes within *Terminalia catappa* population is physiologically and systematically relevant.

The various patterns of variability among distinguishing characters are displayed in Figure 1-4. The leaf sizes (Figure 1), fruit weight (Figure 2), mesocarp weight and fruit moisture (Figure 3) and stomatal sizes (Figure 4) have uneven distributions. Although biochemical analysis yielded little variation in the fruit and seed, the results have proven the nutritional relevance of *C.albidum*. For instance, the fruit averagely contain 7.13% carbohydrate, 1.68% protein, 2.28% fat and 66.49% moisture. The seed also contain 60.29% carbohydrate, 5.2% protein, 5.8%fat and 9.88% moisture. The fruit and seed are rich in dietary compositions for healthy living and therapeutic purposes (Amusa *et al*., 2003). This agrees with previous suggestions that *C.albidum* as an indigenous tree could serve as a potential source of addressing food malnutrition in Africa (FAO,2016) and that it deserves some levels of attention even more than the much embraced exotic fruit crops (Simon and Leakey, 2003; Shreckneiberg *et al*.,2006). Table 4 shows the variation in the tastiness of ripe fruit samples. Tree samples T2, T8, T18, T20, T21, T27, T29 T33 and T40 produced the tastiest/sweetest fruits. These are fruits with the lowest amount of moisture. Fruits of T1, T3, T4, T5, T11, T23, T24, T31 and T39 were moderately sweet.

Others are classified as neither sweet nor sour though their moisture content was relatively high. Plant breeders may capitalize on the first two groups for selection and breeding.

Dendrogram (Figure 5) has classified the trees into four main clusters grouped on the basis of observed similarities and differences. Grouping is location independent. Cluster 1 for instance consists of trees (T14, T22, T30, T38, T21, T13, T29 and T37) sampled from different localities. Trees 9, 25, 6 and 3 are quite distinguished from others. Generally, the *C.albidum* trees studied can be described as a population with moderate gene flow not strong enough to cause speciation as there are fewer traits accounting for the observed intraspecific variability. However, these characters are crucial in the improvement of the crop. DNA molecular marker may be employed in future studies to reveal the actual level of genetic polymorphism (Aguoru *et al*.,2015e) or the population genetic structure of *C.albidum* in the entire North Central Nigeria (Aguoru *et al*.,2015f). Marker assisted selection of trees with desirable traits can then be applied on the crop even from the young stages.

Table 2: Unvaried characters

Characters	Observation	Variation
Leaf apex	Acuminate	None
Leaf base	Cuneate	None
Leaf margin	Smooth entire edge	None
Leaf arrangement	Alternately arranged along the branches	None
Leaf venation	Pinnately veined	None
Fruit shape	Spherical with slight points	None
Seed colour	Brown in yellowidh/milky acid pulp	None
Seed shape	Beanlike shiny, compressed with a sharp edge	None
Fruit colour	Dark green when unripe. Yellow when ripe	None
Leaf epidermal cell shape	Irregular	None
Trichome	Absent	None
Stomatal type	Anomocytic and Paracytic	None
Tree sizes	All tall	*Not measured

Table 3: Descriptive Statistics of quantitative assessment

	Range	Minimum	Maximum	Mean		Std. Deviation	Coefficient of variation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	CV (%)
Leaf length (cm)	9.150	21.090	30.240	26.55350	.374920	2.371203	**8.93***
Leaf width(cm)	2.320	7.100	9.420	8.11250	.112942	.714307	**8.75***
Petiole length (cm)	.530	2.080	2.610	2.31225	.025764	.162945	6.93
Leaf weight (g)	1.930	2.040	3.970	2.96650	.098346	.621997	**20.88***
Fruit length (cm)	1.490	4.800	6.290	5.55700	.090271	.570925	**10.26***
Fruit width(cm)	1.090	3.310	4.400	3.99175	.049920	.315724	7.92
Fruit weight (g)	14.340	38.310	52.650	44.60075	.808444	5.113047	**11.46***
Number of seeds	.500	4.500	5.000	4.91250	.030422	.192404	3.91
Seed weight (g)	1.720	6.810	8.530	7.75650	.102387	.647554	**8.35***
Seed length(cm)	.380	1.860	2.240	2.06225	.021675	.137085	6.65
Seed width (cm)	.830	1.410	2.240	1.79325	.050097	.316838	**17.70***
Wet mesocarp weight (g)	14.800	30.500	45.300	37.09400	.843518	5.334877	**14.38***
Fruit moisture content (%)	16.780	59.450	76.230	66.48500	.944292	5.972226	**8.98***
Stomatal length(µm)	9.390	22.110	31.500	27.17925	.433245	2.740083	**10.08***
Stomatal width(µm)	8.540	12.600	21.140	18.54550	.330778	2.092026	**11.27***
Stomatal index (%)	18.100	53.700	71.800	60.92000	.659999	4.174200	6.85
Fruit ash content (%)	.350	1.030	1.380	1.20325	.019632	.124167	**10.33***
Fruit carbohydrate (%)	1.170	6.660	7.830	7.13475	.060149	.380418	5.33
Fruit protein (%)	.530	1.350	1.880	1.68350	.021257	.134442	7.98
Fruit fat (%)	.480	1.950	2.430	2.27725	.026307	.166379	7.29
Seed moisture content (%)	1.520	9.010	10.530	9.87800	.083800	.529999	5.36
Seed ash content (%)	.360	1.950	2.310	2.13275	.018987	.120085	5.63
Seed carbohydrate (%)	4.200	58.400	62.600	60.28850	.183350	1.159608	1.92
Seed protein (%)	1.140	4.850	5.990	5.19875	.039962	.252741	4.81
Seed fat (%)	.510	5.550	6.060	5.80150	.039511	.249888	4.31
							CV cut off=8%

Table 4: Ripe fruit tastiness

Level of Tastiness/Sweetness	Tree samples
3= Very sweet	T2, T8,T18, T20, T21, T27, T29 T33, T40
2= Moderately sweet	T1, T3, T4, T5, T11, T23, T24, T31, T39
1= Neither sweet not sour	T6, T7, T9, T10, T12, T13, T14, T15, T16, T17, T19, T22, T25, T26, T28, T30, T32, T34, T35, T36, T37, T38.

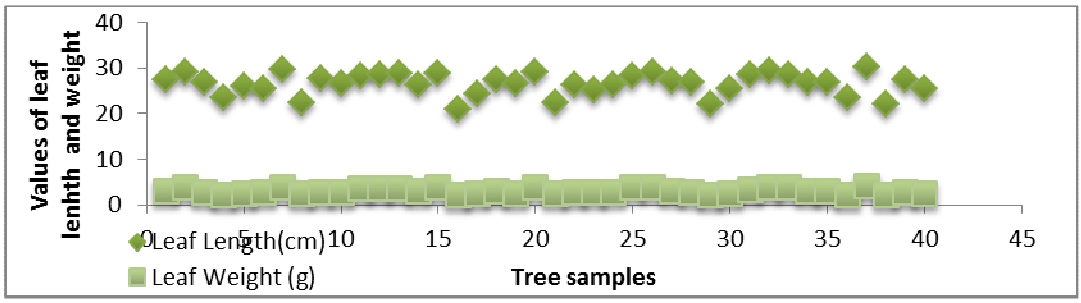

Figure 1: Variation in leaf size and weight across 40 tree samples

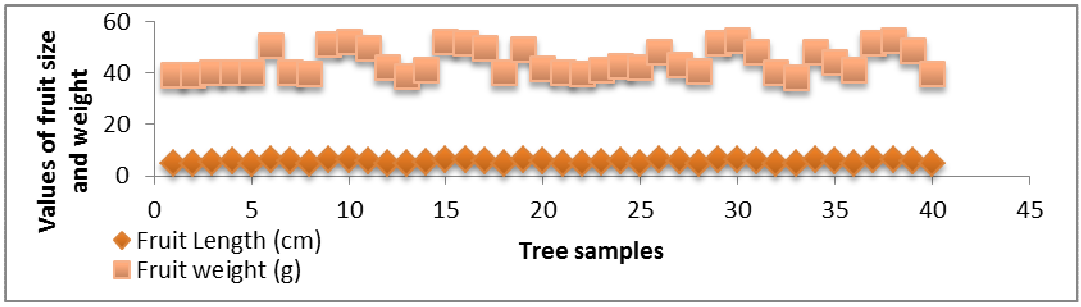

Figure 2: Variation in fruit size and weight across 40 tree samples

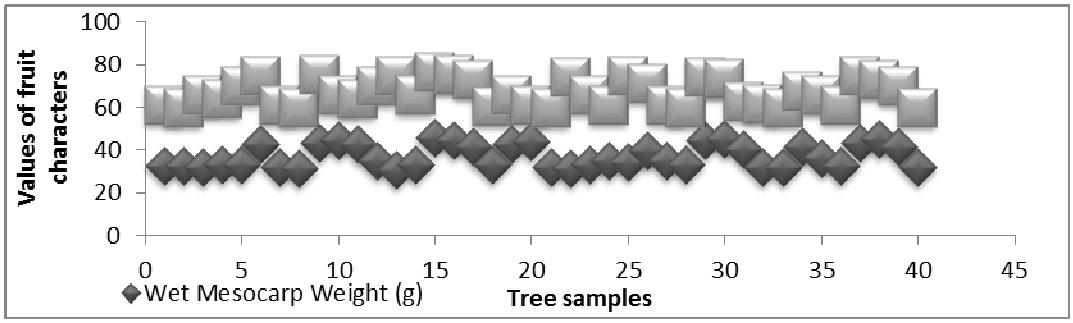

Figure 3: Variation in fruit moisture and wet mesocarp weight across 40 tree samples

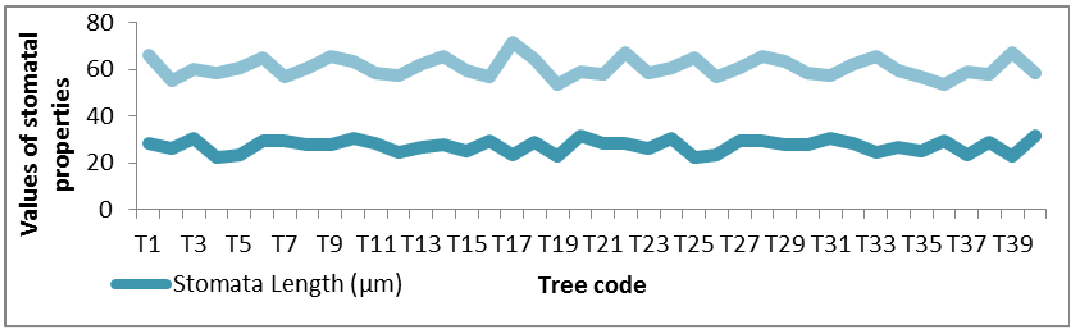

Figure 4: Variation in stomatal length and stomatal index across 40 tree samples

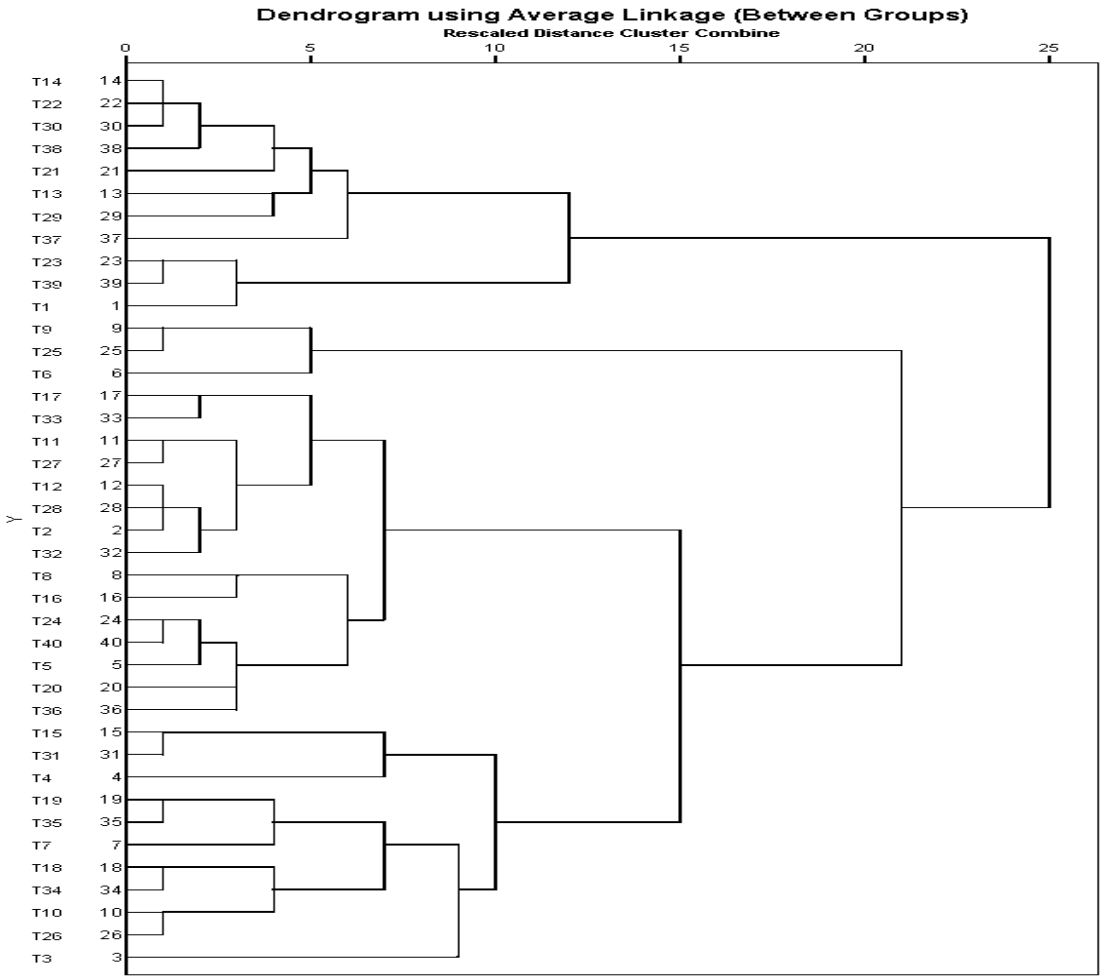

Figure 5: Dendrogram of 40 tree samples

Conclusion

Variability was low in this crop. However, some accessions were outstanding. Traits that recorded high CV above 8% cut off values are fruit/seed sizes, fruit weight and fruit biochemical content including moisture and ash. These traits are of importance in the crop agronomy and breeding.Trees with the tastiest fruit juices have been selected. Grouping is location independent. The study has described *C.albidum* trees in Benue State as a population with moderate gene flow not strong enough to cause speciation as there are fewer traits accounting for the observed intraspecific variability. Pieces of information provided in this report are indispensable in the management, improvements, utilization and systematics of the indigenous fruit crop.

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IN VITRO MULTIPLICATION OF DATE PALM'S (*PHOENIX DACTYLIFERA* L.) SOMATIC EMBRYO USING PROEMBRYOS AS STARTING MATERIAL

Aghimien, E.G.[1], Shittu, H.O.[2], Eke, C.R. [1], Emoghene, B.O. [1]

[1]Physiology Division, Nigerian Institute for Oilpalm Research, P.M.B 1030, Benin-City, Edo State, Nigeria.
[2] Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin- City, Edo State.
*Correspondence author: aghimieneseosa1@gmail.com, Mobile: +2348067756848.

Abstract

The study was aimed at investigating the effect of some plant growth regulators on in vitro somatic embryo induction and multiplication of date palm (Phoenix dactylifera L.). Proembryos of date palm was used as starting materials. Murashige and Skoog (MS) medium was prepared with various combinations of plant growth regulators such as cytokinins (Kinetin and 2iP) each at 0.0, 0.05, 0.1 and 0.2mg/l and auxins (NAA and 2, 4-D) each at concentrations of 0, 0.025, 0.05, 0.1 and 0.2mg/l. The in vitro proembryos were aseptically transferred into media. The resulting cultures were incubated in a partially lighted growth room at $25^0C \pm 2^0C$ with 70% relative humidity. Data on the number of embryos formed were collected at two week's interval for a period of two months. The result showed that plant growth regulator-free cultures were which the most effective for embryo induction and multiplication. This was followed by very low concentrations of combinations of and 0.025mg/l of 2ip and 0.05mg/l of 2, 4-D. The findings from this study indicated that embryos can be induced and multiplied from date palm proembryos without the presence of plant growth regulators in the culture medium.

Keywords: Somatic embryo, *Phoenix dactilifera* and Proembryos.

Introduction

Date palm (*Phoenix dactylifera* L.) is considered as one of the most important cash crop in the world, as well as an export item for date palm growing countries (Jain, 2011). Its rich fruit is important in nutrition of human population and animals (Al-Baker, 1972). Moreover, this crop has a great potential as a source of renewable energy, an alternate source to the fossil energy, by producing bio-fuel since its fruits high in carbohydrates, 44-88% total sugars (Jain, 2012). It plays an important role towards the creation of an equable microclimate around oasis ecosystems and in turn provides an enabling sustainable agricultural development in draught and saline areas. Many of its products are made to generate employment and influence socioeconomic aspect of people. It creates reasonable opportunities for rural employment, provides a main source of income for farmers and helps food security and livelihood of rural areas (Jain *et al*, 2011 and Rajmohan, 2011). It has well acknowledged sustainability value socio-economic and ecological terms.

It is a known fact that date palm is usually propagated sexually by seeds and vegetatively by offshoot (Alkhateeb and Alidnar, 2002). Propagation by seed is not suitable as a result of heterozygozity and dioecious nature of date palm (Alkhateeb, 2006). Seedlings produce approximately 50% male. Male and female seedlings are not known until flowering stage. Only few male palms are required in plantations to provide pollen for fruit development

Offshoot propagation is a slow technique with high mortality rate (Alkhateeb *et al*, 2008). It is affected by restricted number of offshoots produced by a single date palm tree with the risk of disease transmission and low survival rate (Al-khalifah and Askari, 2011). It is used to obtain identifiable female planting materials; offshoots are usually taken from mother palms for planting. The average sucker production is low and restricted mainly to juvenile years and suckers are difficult to root. Some genotypes do not produce suckers (Eke *et al*, 2005).

Plant tissue culture is a very important modern technology, which concentrates on planting various tissues of plants by *in vitro*. This is in order to obtain many plants that are genetically identical to mother plants (Almauru

and Alghamdi, 1998). This technology gives advantages over conventional methods of propagation for fast and large scale production of important plants under *in vitro* conditions irrespective of season, time and space conservation (Nehra and Kartha, 1994). It can be done either through somatic embryogenesis or direct organogenesis.

Somatic embryogenesis is characterized by the development of a somatic cell into an embryo with shoot and root formation. Somatic embryos can be obtained from the meristemic regions in the leaves or from shoot tips by callus formation. For many plant species, somatic embryos are employed for mass propagation, genetic transformation, *in vitro* preservation and usually used in most commercial laboratories. It is depicted with giving *in vitro* plants in a comparatively shorter time as well as high propagation ranges. But the most disadvantage of this method is the possibility of mutation and abnormalities occurrence during growing *in vitro* which appear in the field later on (Bekheet, 2013).
Date palm propagation by seeds produces 50% females and 50% males. Meanwhile a male date palm can fertilize up to at least 50 females. The difficulties of conventional methods can be solved through propagation by tissue culture which helps in mass production of palms.
The aim of this research was to investigate the effect of plant growth regulators on *in vitro* multiplication of somatic embryos from proembryos using standard tissue culture techniques with well-defined media.

Materials and methods

Starting material: Proembryos which are initial or immature embryos of *Phoneix dactilyfera* were used as starting materials for embryo induction and multiplication. The starting materials were collected from the Physiology Division of the Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Edo State.

Plate 1:Culture of proembryos.

Equipment used: The major equipment used in this study include: laminar flow chamber, scalpel, sterile blade, Petri dish, aluminum foil paper, test tubes, test tube racks, shelves, autoclave, drying oven, weighing balance, microwave, ethanol lamp, pipette, beaker, magnetic stirrer, refrigerator, masking tape, funnel, measuring cylinders, conical flask, other plastic and glass wares.

Culture media preparation: Three-quarter strength of Murashaige and Skoog (1962) media with stock solutions of MS (Murashaige and Skoog) macro, micro, vitamin and iron were prepared containing 3% sucrose supplemented with cytokinins (Kinetin, 2iP and BAP), each at concentrations of (0, 0.025, 0.05, 0.1 and 0.2mg/l) and auxins (NAA, 2,4-D), each at concentrations (0.0, 0.05, 0.1 and 0.2mg/ml) were used for this

experiment. The pH of the medium adjusted to 5.7±0.02 with 1 N NaOH or 1N HCl and solidified by addition of agar. The medium was dispensed into test tubes and autoclaved at 121°C for 30 minutes and allowed to cool before transfer.

Preparation of source material: Proembryos of *Phoenix dactylifera* were collected from partially lighted growth room and transferred to the laminar flow chamber

Culture of source material: Forceps with the aid of inoculation needles were used to transfer source material to culture on solidified MS media with various levels of plant growth regulators for morphogenetic responses. The test tubes containing the media and source material were placed in a partially lighted growth room at $25^0C \pm 2^0C$ with 70% relative humidity for incubation. The result were observed and taken at two weeks interval for a period of two months. The cultures gave rise to matured embryos.

Results and Discussion

Generally, researchers have found that exogenous plant growth regulators such as gibberilins, auxins and cytokinins induce and help in the multiplication of somatic embryos (Sidky, 2014). This current study majorly concentrated on the effect of a combination of auxins and cytokinins on embryo multiplication. Development of somatic embryo has been successful with no plant growth regulator at certain stages and with minute amount of it at another stage of embryogenesis.

The results obtained in this study of embryo multiplication of initial somatic embryo of *Phoneix dactylifera* using various growth regulators Kinetin,2iP, BAP each at concentrations of (0, 0.025, 0.05, 0.1 and 0.2mg/l) and NAA, 2,4-D each at concentrations (0.0, 0.05, 0.1 and 0.2mg/ml) respectively to supplement MS medium for embryogenesis. The morphogenetic responses were observed from two weeks after inoculation of culture and observations were taken for a period of two months to yield the following results.

From this findings as shown in table1 and figure 1, a gradual decrease in the concentration of the quantity of cytokinin (2iP, Kinetin, BAP)in combination with auxins (NAA, 2,4-D) led to increase in the average no. of embryos in the decreasing order; 2iP + 2,4-D > 2iP +NAA>Kinetin +2,4-D>Kinetin +NAA. 2iP and 2,4-D was the most effective combination, followed by 2iP and NAA and then Kinetin and 2,4-D and so on as indicated in the order. The range of concentration of cytokinins used was 0.0, 0.025, 0.05, 0.1mg/l against auxins 0.0, 0.05, 0.1, 0.2mg/l. From this result, it could be deduced that the type of cytokinin used in the combination played a major role in the average quantity of embryos produced. 2iP was shown to be the stronger cytokinin in this experiment when used in combination with different concentrations of auxins.

In this experiment controls were set up without plant growth regulator and it was observed that it yielded more embryos within the period of two months. This could be as a result of endogenous phytohormone in the proembryos. About 15ml of MS media in a test tube of phytohormone free medium gave an average of 9 to 10 embryos. It thus appears that large scale production can be obtained, if subsequent work is carried out. The result obtained shows the importance of Plant growth regulator concentration balance and of balance between auxins and cytokinins in optimization of embryo multiplication protocol.

Table 1: Average no. of embryos produced in an ascending concentration of Plant Growth regulators (PGRS).

Concentration(s) (mg/l)	Kinetin/ NAA	Kinetin/ 2,4-D	2iP/ NAA	2iP/2,4-D
0.0 /0.0	9.25± 2.20	9.25 ± 2.65	10.00 ± 2.94	10.00 ± 2.94
0.025/0.05	6.25± 1.71	6.50 ± 2.65	7.50 ± 2.08	7.50 ± 2.89
0.05/0.1	5.75 ± 1.71	6.00 ± 1.83	6.00 ± 2.16	7.00 ± 2.94
0.1/0.2	5.00 ± 1.92	5.50 ± 2.38	5.50 ± 2.65	6.75 ± 1.25

Key: Average no. of embryos (Mean ± S.D)
Abbreviations:
NAA – Naphthalene acetic acid

2, 4-D – 2, 4- dichlorophenoxyacetic acid
2ip – 2 isopentyladenine

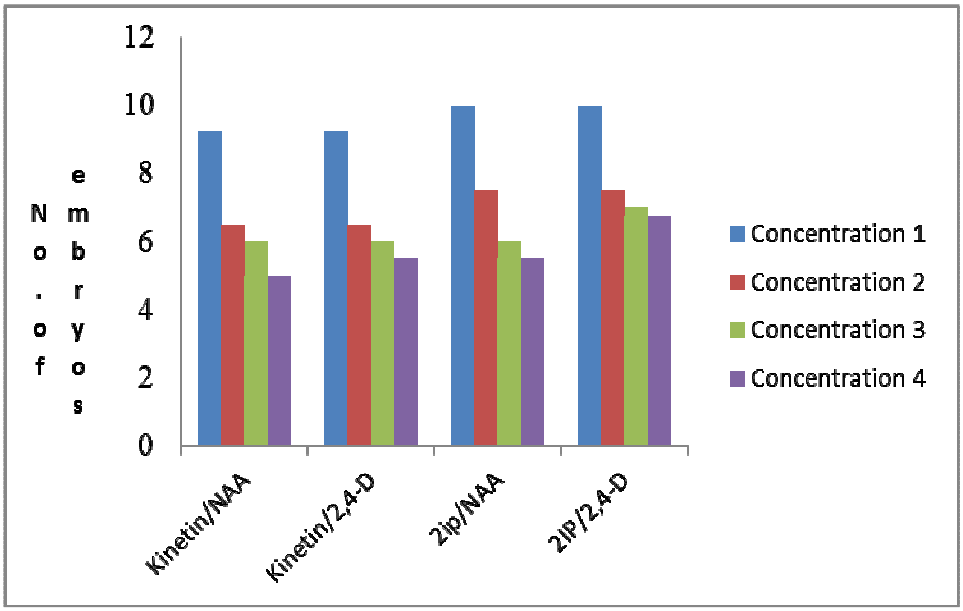

Fig 1: Effect of different combinations of Plant Growth Regulators on no. of matured embryos produced.
Concentration1: 0.0mg/l and 0.0mg/l of cytokinin and auxin
Concentration 2: 0.025mg/l and 0.05mg/l of cytokinin and auxin
Concentration 3: 0.05mg/l and 0.1mg/l of cytokinin and auxin
Concentration 4: 0.1mg/l and 0.2mg/l of cytokinin and auxin

Plate 4: somatic embryos obtained from 2ip+2, 4-D.

Conclusion

Results obtained from this research have revealed increase in embryo multiplication in *Phoenix dactylifera* as plant growth regulator (PGR) concentration decreased. When different auxins and cytokinins were used in combination to supplement Murashaige and Skoog (MS) media.. The PGR-free media yielded most embryos ranging from a mean no. of 8.25 to 10.00. While PGR combinations which were also very effective were at concentrations of 0.025 and 0.05mg/l which include combinations of 2iP and 2, 4-D, 2iP and NAA ranging with a mean of 5.5 to 7.5 embryos.

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HALF DIALLEL TO ESTIMATE COMBINING ABILITY VARIANCE COMPONENTS OF MAIZE (*Zea mays* L.) INBRED LINES

[*]Dawaki K. D[1]., Mohammed S.M[2], A.K Ibrahim[3], GarkoM.S[1], Jibrin M.S[2] and Hamisu H.S[4]

[1]Department of Crop Science Kano University of Science and Technology, Wudil P.M.B 3244, Kano-Nigeria
[2]Department of Plant Science, Ahmadu Bello University, Zaria. Kaduna State, Nigeria
[3]Department of Agronomy, Bayero University. Kano. P. M.B. 3011, Kano-Nigeria
[4]National Institute of Horticultural Research, Bagauda Station, P.M.B 3390, Kano-Nigeria
[*]Corresponding author: kabirudawaki@gmail.com +2348024794501

Abstract

The experiment was conducted to study combining ability and kernel quality of white maize (Zea mays L.) populations. A half diallel cross comprising of six parental inbred lines were developed and studied for combining ability variance components of some agronomic and kernel quality traits to determine the type of gene action of kernel quality traits. The parental materials consisted of six genotypes of white maize inbred lines which were crossed in a partial diallel pattern, which generated 15F1's crosses. Twenty five entries comprising of 15F1's, six parents, and four checks were evaluated at Samaru- Zaria, Kaduna State, Nigeria (496 m above sea level, $11^0 36^1$ N, $0.8^0 26^1$ E). The genotypes were arranged in 5×5 lattice design with three replications. The GCA /SCA values were less than unity for most of kernel quality and Agronomic traits depicting the importance of non-additive genetic action over additive type of gene action. Based on the findings of this study the parents P1, P2, P5 and P6 can be considered for hybridization to develop genotype with good agronomic and kernel quality traits.

Key Words: Diallel, Combining ability, Variance Components, Maize and Inbred Lines.

INTRODUCTION

Maize or corn (*Zea mays* L.), belong to the grass family Poaceae and tribe Maydeae, originated 5000 to 10,000 years ago (Hallauer, 1997; Paliwal and Smith, 2002). The origin of maize is controversial; however, it is believed to have originated in the mid-altitude regions of Mexico and Guatemala or Mesoamerica (Paliwal and Smith, 2002). It is one of the three most important cereal crops in the world together with wheat and rice. In industrialized countries, it is largely used as livestock feed and as a raw material for industrial products, while in developing countries, it is mainly used for human consumption. Africans consume maize as a starchy base in a wide variety of porridges, pastes, grits, and beer. In sub-Saharan Africa, it is a staple food for an estimated 50% of the population. It is an important source of carbohydrate, protein, iron, vitamin B, and minerals. It is fast becoming a very important commodity in animal feed, food and beverage industries (USAID, 2010).

Worldwide maize is cultivated in an area of 159 million hectares with a production of 796.46 million metric tons (USDA, 2010). Maize production in Africa in 2004 was estimated to be 41.6 million metric tons of which 27.7 million metric tons was produced in sub-Saharan Africa. The United States is the world's largest producer and exporter of maize in 2003-2004, maize production in the U.S was 256 million metric tons (USDA-FAS, 2005). Other top producing countries include China, Brazil, Mexico, Argentina, India and France. Nigeria is the 10th largest producer of maize in the world, and the main producing country in tropical Africa (USAID, 2010). It is cultivated both as rain fed and under irrigation on more than 5 million hectares, spread through the six agro-ecological zones and maize production is put at about 26 million tons from 3,845,000 hectares (FAO, 2009).
In Africa, maize is grown by small- and medium-scale farmers who cultivate 10 ha or less (DeVries and Toenniessen, 2001) under extremely low-input systems where average yields are 1.3 tons ha^{-1} (Bänziger and Diallo, 2004). The primary objective of most maize breeding programs is the evolution of high yielding and well

adapted cultivars. Breeding for improving varieties is a continuous process and requires primarily a thorough knowledge of the genetic mechanism governing yield and yield components. Diallel cross technique developed

by Hayman (1954) provides information on the inheritance mechanism in the early generations and help the breeder to make effective selection.

There are few publications on white maize breeding because it is mainly performed by private companies. White maize breeding programs generally use well established white maize populations and inbred as base germplasm because the development of new varieties is complicated because of the strict quality requirement and the complex genetic regulation of white endosperm. Crosses among white and yellow varieties often produce new white varieties with undesirable kernel quality traits and there is therefore the need to study the combining ability of some agronomic and kernel quality traits for their improvement.

Although plant breeding has been extremely successful at improving the yield of maize, but kernel quality traits has received less attention. However, advances were made by breeders in this area as well, resulting in maize kernels with a wide range of structures and compositions.

By exploiting genetic variation, the composition of the kernel was altered for both the quantity and quality of starch, proteins, and oil throughout kernel development. Furthermore, the ability of maize breeders to use existing genetic variation and to identify and manipulate economically important genes will open new avenues for the design of novel variation in grain composition, thus providing the basis for the development of the next generation of specialty in maize and of new products to meet future needs. (Leford and Russel, 1985).The objectives of this study are as follows:

i. To determine the type of gene action for agronomic and kernel quality traits in white maize.
ii. To determine the best hybrid with better agronomic and kernel quality traits in white maize.

Materials and methods

The material consists of six genotypes of white maize inbred lines which were crossed in a half diallel pattern and generated $15F_{1'S}$. The six (6) parentals, $15F_{1'S}$ and four (4) checks (25 genotypes table 1) were evaluated at Samaru during rainy season, it is located at an altitude of 686 m above sea level, $11^{0}11^{1}$N, $07^{0}38^{1}$E in the Northern guinea savanna zone of Nigeria, with a mean annual rainfall of 1050 mm distributed within five months, the soil type is loamy. The 21-entries and four (4) checks were arranged in 5×5 lattice with three replications. One row plot of 5m long spaced 0.75m x 0.25m inter and intra row spacing were used. Sowing was done manually two seeds per hill were planted, and at about two weeks after planting, it was thinned to one stand per hill. All IAR recommended agronomic activities were carried out accordingly. Data were collected for: days to 50% flowering, days to 50% silking, anthesis silking interval, plant height, days to maturity, ear height, ear length, ear diameter, percent whole kernel, percent kernel without pericarp damage, milling test, moisture content at harvest, 100-Grain weight, density and grain yield

Statistical Analysis was computed using Statistical Analysis System (SAS, 2004)

GENOTYPES EVALUATED

S/N	GENOTYPES	STATUS
1.	P1 (P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B)	Parent
2.	P2 (1368× *HI* ×4269-1368-7-2-B-B-B-B-B)	Parent
3.	P3 (9071-B-B-B)	parent
4.	P4 ((TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B)	parent
5.	P5 (1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B-B)	parent
6.	P6 (TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B)	parent
7.	P1 × P2 (P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B × 1368× *HI* ×4269-1368-7-2-B-B-B-B-B)	Hybrid
8.	P1 × P3 (P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B × 9071-B-B-B)	Hybrid
9.	P1×P4(P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B× (TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B)	Hybrid
10.	P1×P5(P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B × 1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B-B)	Hybrid
11.	P1 × P6 (P43SRC9FS100-1-1-8-#1-B1-13-B1-B-B-B-B-B-B-B-B × TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B)	Hybrid
12.	P2 × P3 (1368× *HI* ×4269-1368-7-2-B-B-B-B-B × 9071-B-B-B)	Hybrid
13.	P2 × P4 (1368× *HI* ×4269-1368-7-2-B-B-B-B-B × (TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B)	Hybrid
14.	P2 × P5 (1368× *HI* ×4269-1368-7-2-B-B-B-B-B × 1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B)	Hybrid
15.	P2 × P6 (1368× *HI* ×4269-1368-7-2-B-B-B-B-B × TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B)	Hybrid
16.	P3 × P4 (9071-B-B-B × (TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B)	Hybrid
17.	P3 ×P5 (9071-B-B-B × 1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B-B)	Hybrid
18.	P3 × P6 (9071-B-B-B × TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B	Hybrid
19.	P4 × P5 (TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B × 1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B-B)	Hybrid
20.	P4 × P6 (TZMI501×KU1414×501)-1-4-3-1-B-B-B-B-B-B-B-B × TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B)	Hybrid
21.	P5 × P6 (1368×ICAL224-1×1368-3-1-B-B-B-B-B-B-B-B-B-B-B × TZL-COMP3-C2-S2-34-4-1-2-B-B-B-B-B-B-B)	Hybrid
22.	Oba-98	Check
23.	SAMMAZ-15	Check
24.	JO-F	Check
25.	Nasara (QPM)	Check

RESULTS AND DISCUSSIONS

Table 2 Estimates of combining ability variance components of nineteen traits of maize at Samaru evaluated in 2014

Characters	Days to 50% Flowering	Days to 50% silking	Anthesis Silking Interval	Plant Height (cm)	Days maturity	Ear Height (cm)	Ear Length (cm)	Ear Diameter (cm)	Field Weight (kg)	% whole kernel
GCA	3.13	0.18	-14	118.98	1.53	0.93	0.4	-12.02	-1.5563	38.08
SCA	2.49	-12.21	-112.13	-506.05	0.52	-201.25	3.47	-94.01	-13.02	-51.78
GCA/SCA Ratio	1.26	-0.02	0.12	0.24	0.94	0.01	0.12	0.13	0.12	0.74

Cont. Table

Characters	% kernel Without Pericarp damage	Milling test	Moisture content at harvest (%)	100 Grain Weight (g)	Volume (m^3)	Density (kgpm^3)	Yield (kgpha)
GCA	9.45	0.42	0.91	-1806691.4	-0.34	-8419.7	560726.3
SCA	4.99	4.73	3.2	-14296624	12.03	-46897	810277.2
GCA/SCA Ratio	1.89	0.09	0.28	0.13	-0.03	0.18	0.69

*, ** significant at 5% and 1% levels respectively.

Table 2 revealed that the magnitude of GCA/ SCA variance components ratio were less than unity for days to 50% silking, anthesis silking interval, plant height, days to maturity, ear height, ear length, ear diameter, percent whole kernel, milling test, moisture content at harvest, 100-Grain weight, volume, density and grain yield implying that the inheritance of these traits were due to SCA and mostly controlled by dominant and/ or epistatic gene action (Griffing, 1956). The higher magnitude of SCA for the traits affected was as a result of the departure of the heterozygote (Aa) from the mean of two homozygote (AA or aa) at a locus reflecting the extent of dominance. The response of non-additive gene action for the conditioning of days to fifty percent flowering, fruit weight and fruit yield per plant has been reported in tomato breeding (Mahendrakar *et al.*2005). Based on the findings of this study the parents P1, P2, P5 and P6 can be considered for hybridization to develop genotype with good agronomic and kernel quality traits.

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THE MUTAGENIC EFFECTS OF A LOCALLY MADE PERFUME (UMRA) ON THE GROWTH AND YIELD PARAMETERS OF TOMATOES.

[1]Mshelmbula, BP., [2]Galadima, Y., [3]Yusuf, CS, [4]Thomas, GM and [1]Bello, S., [5]Ikhajiagbe, B

[1]Department of Botany, Federal University, Lafia, Nasarawa State, Nigeria.
[2,3]Department of Biological Sciences, Adamawa State University, Mubi, Nigeria
[4]Department of Biological Sciences, Taraba State University, Jalingo
[5]Department of Plant biology and Biotechnology, University of Benin
Corresponding author: *Email: barkapeter5@gmail.com Phone number: 07068115440

Abstract

The effects of a locally made perfume on the growth and yield parameters of tomato were studied. Four (4) different concentrations were prepared viz: 0.038%, 0.0150%, 0.30% and 0.600% based on weight basis. A complete Randomized design with 5 replicates each was adopted and various parameters were recorded which include leaf area, plant height, leaf length, number of flowers and number of fruits while other cultural practices were observed throughout the study. ANOVA was used to analyze the date. It was discovered that at 90 days after planting, the least concentrations (0.038 and 0.150) recorded the highest agronomical characters compared to the control. Also, there was increase in number of flowers compared to the control and also increase in the number of fruits was generally observed in lower doses of the local perfume used in the study.

Key words: Umra, mutagen, yield, parameters

Introduction

Tomato is one of the important fruits crops in the world (Parray *et. al*., 2007). Tomato is highly versatile and is used in thousands of recipes right across Europe, from Ketchup chowder to bloody mares, (Parray *et al*., 2007). Tomatoes have both nutritional and medicine values .It is important for neutralizing the acids produced during the digestion of meat and other fatty acids (Smith, 1994). It is a valuable roughage which promotes digestion and helps to alleviate constipation (Parray *et*. *al*., 2007).Tomato is a source of carbohydrate, fats, proteins, vitamins and minerals which when eaten makes the eye brighter than using cosmetics on it (Gojale, 2002). The plant produce the tomato fruits which for purely culinary purpose, is often included among vegetables (Clerk, 2008).It probably originated in the highlands of the world. The tomato plant is an annual or a short –lived perennial dicot, although it is always cultivated as crop (Smith ,1994) .After planting, it takes tomato between 50 to 95 days to fruits ripen, depending on the cultivar (Peet *et. al*.,2009). According to Clerk (2008), the tomato plant is a deep rooted crop and a heavy feeder.

Induce mutation are used to generate genetic and component of various crops like *Triticum* durum (Sarkin yildirim, 2004). When no gene for resistance to a particular diseases, Plants breeders have no obvious alternative but to attempt mutation induction. Mutation breeding supplement conventional plants breeders as a source of increasing variability and could improve without significant altering in phenotype (Mshembula B.P *et. al*., 2012. The effect of different concentration of sodium azide on survival percentage, mutation frequency and mutagenic effectiveness is presented. The survival percentages decreased progressively as the dosage increased. (Mensah *et. al*., 1992). Cheng and Gao (2003) treated barley seeds with sodium azide and found a significant decrease in the germination percentage. We detected insignificant differences in the shoot length at 0.001 and 0.003 M of NaN_3 treatment, implying that these doses have similar effects on plants. According to Sachs *et. al*., (1997) x- rays was to be

used for biological research including the induction of genetic changes, the germination test performs according to the role of international seeds associating , or the American Association of official Analysis these percentage of seed are capable of germinating normally, these performed under ideal condition of seed germination .

Selecting the best yield for subsequent harvest every season takes several years to achieve the desired characters or traits for higher agronomic and yield parameters. The aim of this study therefore, is to determine the effect of local perfume ‘’umra’’on the growth and yield of tomato plant.

Materials and methods

Seed collection

A variety of reddish tomatoes (U T C) were obtained from the Mubi Main Market seed and stored in petri dishes under room temperature until required for use.

Soil collection

Top soil was collected and dried to constant weight, and there after 5kg of the soil was measured in to palm nursery polybags 30 cm in height and 23 cm in diameter. These polythene bags were perforated at the bottom and sides.

The bags were irrigated with 200ml water daily at dusk, and also cleared of any unwanted weeds during the period under fallow bags was ready for cultivation on the 7th day .The bags was placed on the field spacing of 60 cm x30 cm, as proposed by Ikhajiagbe (2004).

Preparation of mutagenic solution

The local perfume (umra) was used as a mutagenic agent in this study. Four (4) different concentrations were prepared on weight basis, viz: 0.038%, 0.150%, 0.300%, and 0.600%. These were labeled in the field accordingly. The control used was distilled water (0 %), designated.

Pre-treatment of seeds with mutagenic solution

Viable tomato seeds were subjected to varying ‘’umra’’ concentrations for six (6) hours. The treated seeds was washed in running water to remove excess chemical and exudates from the seeds and sown in petri dishes containing soaked cotton wool. Germination was observed for 6 days.

Field study

The other set of pre-soaked seeds were sown directly in to polythene bags containing soil. Planting was done in the evening, just beyond sunset following the methods Ikhajiagbe, (2004). The Seeds were sown at the rate of 4 seed per hole and at a depth of 3cm. When seedlings attained 2-leaf stage (8-12cm long), It was thinned down to 2 seedlings per bag. All other cultural practices were followed till harvest period. When the plants were long enough and branched, the plants were staked on poles.

Experimental design

The experimental design adopted in this study was Completely Randomized Design (CRD). The treatments was randomized over the whole plot, with each treatment consist of 4 replicates. In other to avoid bias and misidentification, each treatment was properly labeled according to a given treatment name and replicate number.

Parameters measured

The following parameters were measured:

Weight of 10 dried fruits, Weight of 10 fresh fruits, Number of seeds germinated, Shoots length (seedling height), Stem diameter (girth), Number of leaves, Number of days to flower, Number of flower per plants , Number of fruits per plant, Number of day early ripening, Weights of 10 fruits and `Total fruits yield per plant

Data analysis

The data were collected and analyzed based on Analysis of Variance (ANOVA).

Data analysis

FIG.1: Effect of local perfume on plant height

Plant height at the 90th day following sowing was 44.4 cm in 0.38% treatment, compared to 55.9 cm in 0.3%. Plant height in the control plant was 40.1 cm (Fig. 1).at the 120th day after sowing, plant height ranged from 44.2 – 68.1 cm

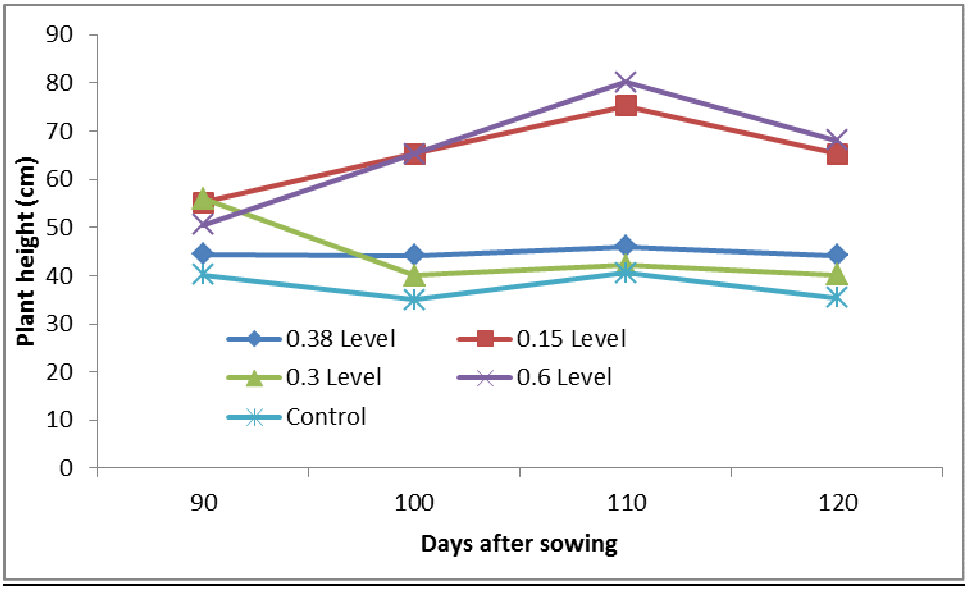

Fig. 1: Plant height of test plant under experimental condition

The breadth of leaf of test plant has been presented. In the 0.38% level treatment, leaf breadth was better within the 2nd and 3rd week after planting, compared to the 4th week. This was similar to observations in 0.15% level treatment as well as the control. In 0.3% level treatment, leaf breadth incrased to 10.5 cm in the 0.3% level treatment.

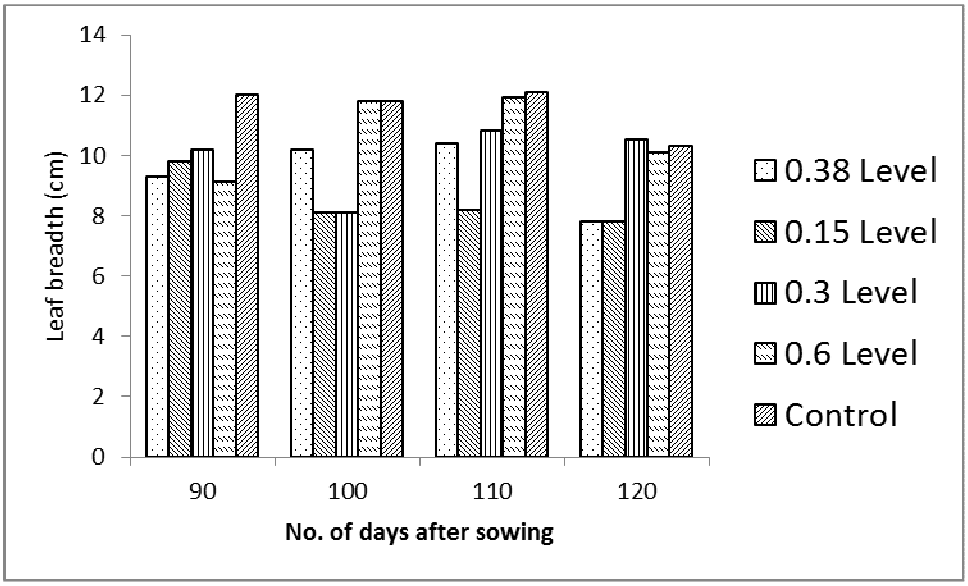

Fig. 2: Leaf breadth of test plant under experimental condition

Effect of local perfume on leaf length (cm) of tomato

Leaf length ranged from 10.2 – 11.8 cm at day 7 (Fig. 3). These values increased at the 28th day to 10.3 – 11.8 cm respectively. Stem girth of the test plant (Fig. 4) ranged from 1.3 – 2.8 cm from the 7th to 28th week.

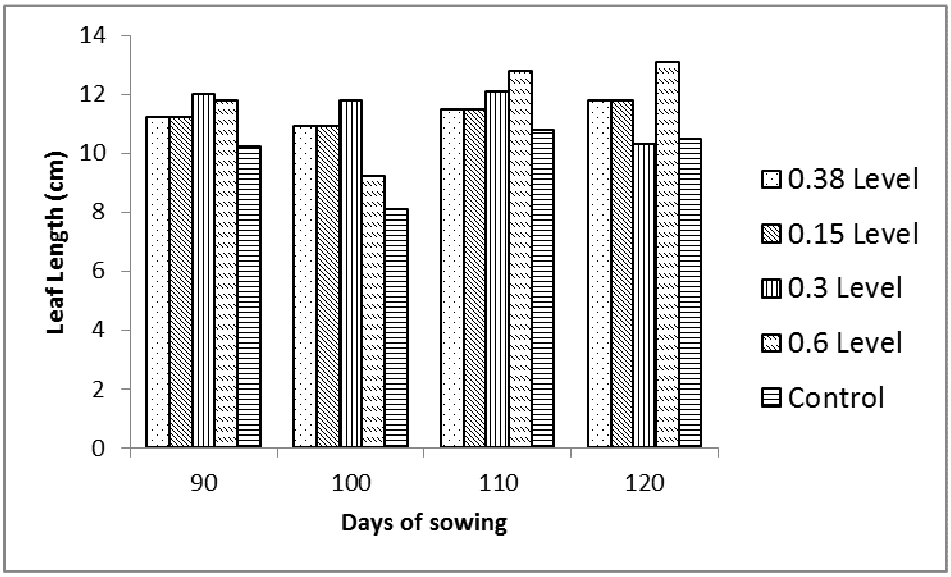

Fig. 3: Leaf length of test plant under experimental condition

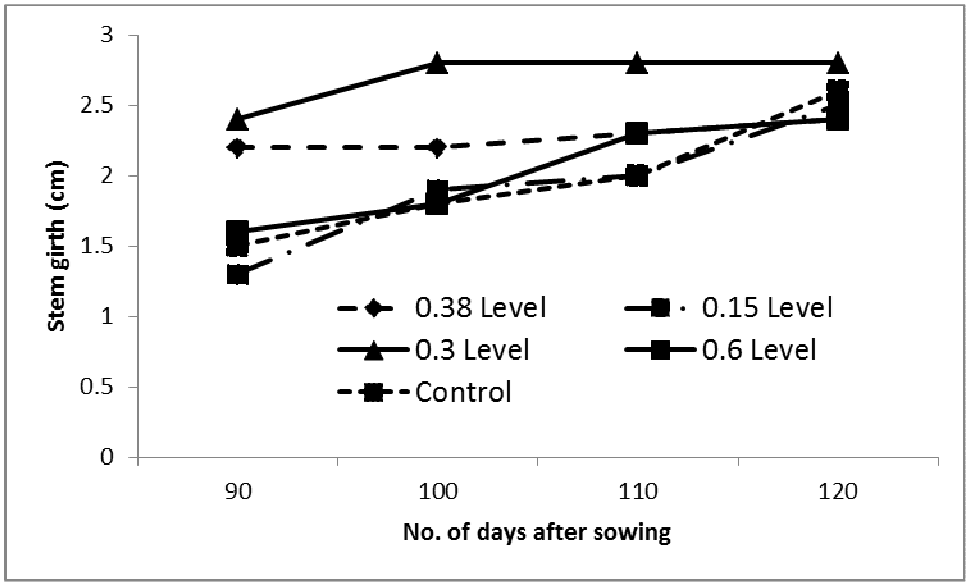

Fig. 4: stem girth of test plant under experimental condition.

Fig. 5: Number of flowers of test plant under experimental condition

The plant exposed to 0.38% level of the treatment had the highest number of flower per plant (8), compared to the control, which had 3 flower (Fig. 5). Similarly, there were generally 2 fruits per plant in all treatments but 0.6% level, which had only one fruit.

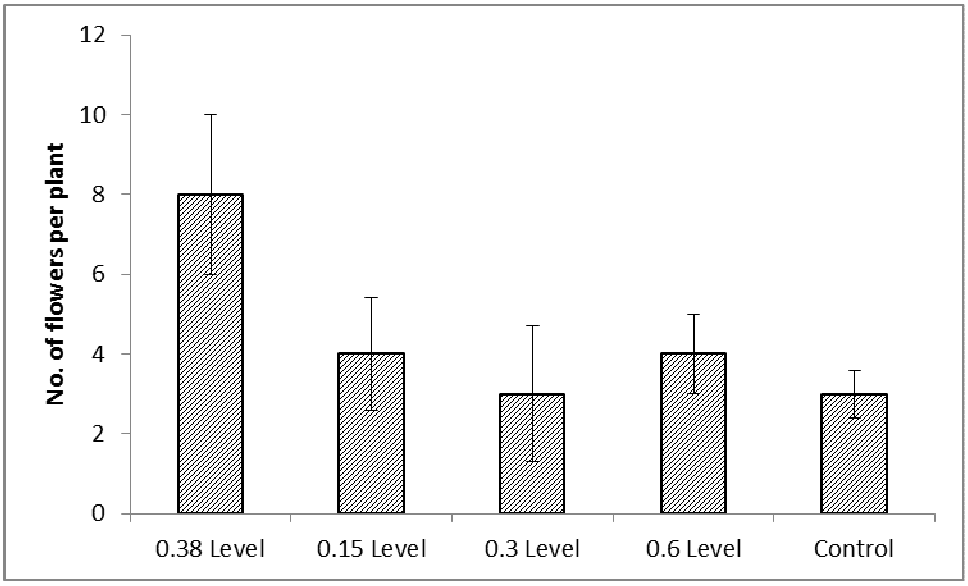

Fig. 5: Number of flowers of test plant under experimental condition

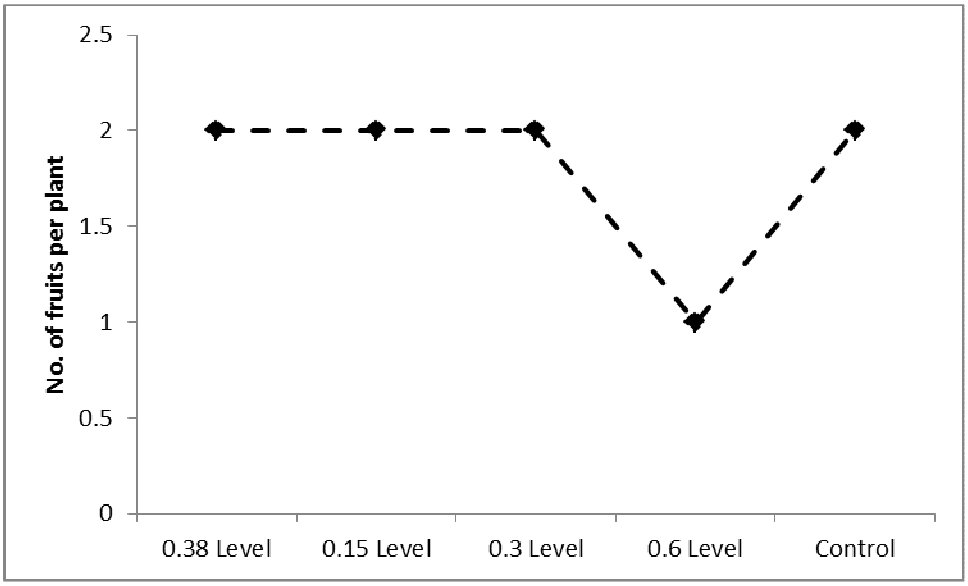

Fig. 6: Number of fruits of test plant under experimental condition

Discussion

From the results obtained, plant height treated with higher dose of the Umra concentration (0.3%) recorded significant increase in plant height (55.9cm) compared to the control (10cm) at 90 days after planting (fig.1). This finding disagrees with Mensah *et al*., (1992) working on the mutagenic effects of Hyroxylamine and streptomycin on growth and seed yield of cowpea (Vigna unguiculata (L.) Who reported that, the lower concentration had higher shoot length than higher concentration had higher shoot length. This suggests that in the case of the local perfume studied, higher concentrations triggered increase in plant height in tomato.

From fig. 2, lower umra concentration (0.38% and 0.150%) had the larger leaf breath at the 2nd and 3rd week after planting compared to other concentration. This could be as result of more mitotic division and physiological activities at that concentration which triggered increased the leaf breadth. This agrees with work done by Ajayi *et al*., (2010) who reported that leaf breadth increases with reduced concentrations of chemical mutagens. Mshelmbula *et.al*.,2015 working with Indole-3 acetic acid on Sesame also affirmed that lower doses of IAA brings about reduction in leaf breadth.

Higher concentration (0.30%) of the chemical brought about increase in leaf length from 10.2cm-11.8cm at 7 days after sowing this however disagrees with Mensah *et.al*., 2007 who reported that increase in sodium azide concentration leads to decrease in leaf length of groundnuts. It is worthy of note to state that there was no significant difference in the stem girth of the test plant at all levels of concentration.

Reduced umra concentration (0.038) brought about increase in the number of flowers per plant (8) compared to the control (fig.5). This means that higher mutagenic concentrations suppress the fruitions of tomato in this study which agrees with Mensah *et al*., (2006) who reported that higher concentrations of mutagenic agent or solution caused delay in flowering compared with lower concentrations and control. Succinctly, lower concentration of umra triggered increase in the number of fruits in tomato (fig.6) which agrees with Ajayi *et al*., 2010 who reported that lower concentration usually give more yield than highest concentrations of mutagens.

Conclusion

From the present findings, lower concentration of Umra (0.038 and 0.150%) could be said to be very important mutagenic agent in the improvement of agronomical characters of tomato. This is evident in the increase in the number of flowers and the fruits also.

Recommendations

Molecular analysis of the F1 generation needs to be carried out to determine the effect at the molecular level of treatment. Also, subsequent generations need to be raised to develop a pure line of tomato which could be resistant to diseases and pests, and which could lead to general crop improvement.

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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, *In vitro* ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF THE LEAF EXTRACTS OF *Ocimum basilicum* (L)

Adamu Aisha Isa[1]*, ThankGod Ositadinma Ndibe[2] and Benthai Benjamin[3]
[1]Department of Biochemistry, Ahmadu Bello University, Zaria.
[2,3]Department of Biological Sciences, Nigerian Defence Academy, Kaduna.
***Email address: aishadamuisa1141@gmail.com**
Phone Number: +2348060468355, +2348034282642, +2348061166247

ABSTRACT

Since time immemorial, humans depend on plant resources for their benefit, from the recollection, as food and medicine. Medicinal plants contribute to the raw materials used for both traditional and modern systems of medicine. Among plants known for its medicinal importance, is Ocimum basilicum. This plant is widely used in Northern Nigeria, with little knowledge to its phytochemicals and antimicrobial properties. Need arises for an evaluation of the phytochemicals, antioxidant properties and antimicrobial activity of this indigenous Ocimum basilicum, with a view to access their therapeutic values. Standard methods were employed for the analyses. The result of phytochemical screening confirmed the presence of flavonoids, cardiac glycosides, tannins, saponins and phenols in both aqueous and methanolic extracts of the leaf. The quantitative estimation of chemical constituents of the leaf of the plant showed high percentage of flavonoids of 94.00% and 51.29% for aqueous and methanolic extracts respectively. The in vitro antioxidant activity of the plant extracts is due by its high concentration of flavonoids. The leaf extracts showed significant effect on Staphylococcus aureus, Bacillus subtilis and Shigella dysentriae. Salmonella typhi showed complete resistance to the leaf extracts. Methanolic extract of the leaf showed wide zone of inhibition of about 30mm against Staphylococcus aureus, with MIC and MBC of 1.56mg/ml and 3.125mg/ml respectively. The phytochemicals such as flavonoids and alkaloids obtained from the plant are appreciable. These are responsible for the ethno-medical, pharmacological, therapeutic and traditional uses of the plant.

Keywords: *Ocimum bacilicum*, phytochemicals, antimicrobial and antioxidant.

INTRODUCTION

Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature, dates from far past (Ahmet *et al.*, 2005). Awareness of medicinal plant usage is a result of many years of struggle against illnesses, in which case, many people learnt to pursue drugs from the barks, seeds, fruits bodies and other parts of plants. The knowledge of development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life (Liu and Scagel, 2009).

The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance (Meera *et al.*, 2009). In Nigeria, many indigenous plants are widely consumed as food or home remedies especially in the treatment or management of common diseases. Among plants known for its medicinal value, is the plants *Ocimum basilicum* Linn of genus Ocimum, which is rich in phenolic compounds and are very useful for their therapeutic potential (Muralidharan and Dhananjayan, 2004). Methanolic extract of *ocimum basilicum* has been evaluated for its analgesic activity (Choudhury *et al*., 2010), antimicrobial activity against *Pseudomonas aerogenosa* (Harsh *et al*., 2002). Aqueous *Ocimum basilicum* has also been evaluated to have lipid lowering effect in Trinitron WR-1339-induced hyperlipidaemic rats (Ramesh and

Satakopan, 2010) and anti-hyperglycemic activity in diabetic rats without affecting the basal plasma insulin concentrations (Zeggwagh *et al*., 2007).

In Northern Nigeria, indigenous *Ocimum basilicum* is commonly used as traditional medicine. In the light of this, need arises to evaluate the phytochemical, antioxidant and antimicrobial activity of this indigenous *Ocimum basilicum*, with a view to access their therapeutic values. Therefore, the main objective of this study was to investigate the phytochemical, antioxidant activity and antimicrobial effect of aqueous and methanolic leaf extracts of *Ocimum basilicum* (L).

MATERIALS AND METHODS

1.1.1.1.1.1 Preparation of the Leaf Extracts

Leaves of *Ocimum basilicum* were collected from Ahmadu Bello University Dam, Zaria, Nigeria. The leaf samples were identified appropriately and were dried at room temperature under shade and finely ground before extraction. A known amount of sample was extracted by percolation method using methanol/water (70:30) and percolation method using 100% water. The resulting extract was concentrated over a rotary vacuum until a crude extract was obtained, and the aqueous extracts were dried using water bath.

Qualitative Phytochemical Screening

Adopting the method of Evans and Trease (1996) for phytochemical screening of *Ocimum basilicum*, the plant extracts were tested for tannins, flavonoids, cardiac glycosides and saponins,

Test for tannins

To a portion of the extracts, 3-5 drops of ferric chloride solution was added. A greenish- black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate (Evans and Trease, 1996).

Test for Flavonoids

A portion of the extract was dissolved in 1-2ml of methanol (50%) in the heat. Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates the presence of flavonoids (Evans and Trease, 1996).

Test for cardiac glycosides

To a portion of the extract, 1ml of 2% solution of 3,5-Dinitrobenzoic acid was added in 95% alcohol. The solution was made alkaline with 5% sodium hydroxide, appearance of purple-blue colour, indicates the presence of cardenolides (Evans and Trease, 1996).

Test for Saponins

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates presence of saponins (Evans and Trease, 1996).

Quantitative Phytochemical Screening

Alkaloid was determined using Rijke *et al*. (2006) method. Portion of the sample was weighed into a 250ml beaker and 80ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original

volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue (alkaloid) was dried and weighed.

Flavonoid was quantified by the method of Rijke *et al*. (2006). Portion of the plant sample was extracted with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Cyanogenic Glycoside was quantified according to Wang and filled method as described by Schoner and Scheiner-Bobis (2007). A portion of the sample was made into paste and the paste was dissolved into 50ml distilled water. The extract was filtered and the filtrate was used for Cyanide determination. To 1ml of sample filtrate, 4ml of alkaline picrate was added and absorbance was recorded at 490nm and cyanide content was extrapolated from a cyanide standard curve.

Determination of Antioxidant Activity
DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using the method of Armani *et al*. (2006). Each extract (0.1g) was weighed and added to 10ml of methanol. Varying concentrations (20-100µg/ml) were prepared from each sample solution using methanol as solvent and 1000µl (0.1mM) DPPH solution (in methanol) was added giving a total of 2.0ml per sample concentration. Varying concentrations of the standard, vitamin C, were also prepared at similar concentrations while the blank (control) was prepared using methanol and DPPH without the extract. The mixtures were shaken vigorously and left to stand for 30 min in the dark at 37^{o}C, and the absorbance was then measured at 517 nm against a blank. Lower absorbance of the mixture indicated higher free radical scavenging activity. The percentage inhibition was calculated as: % inhibition = $(A_0 - A_1/A_0) \times 100$

Where A_0 was the absorbance of the blank, A_1 was the absorbance in the presence of the extract or the standard, vitamin C.

Phosphomolybdenum assay for antioxidant activity

Each extract (0.1g) was weighed and added to 10ml of distilled water. Varying concentrations (20–100µg/ml) were prepared from each sample solution using distilled water as solvent. 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) was added to each aliquot of different concentrations (20-100µg/ml) of sample solution. Tubes were capped with silver foil and incubated at 95ºC for 90 minutes. The tubes were allowed to cool at room temperature. Absorbance was taken at 695nm against a blank (Methanol). Ascorbic acid was used as the standard (Armani *et al*., 2006).

Test Organisms

The test organisms used for this analysis were clinical isolates of bacteria and a fungus obtained from the Microbiology Laboratory, Ahmadu Bello University Zaria. The isolates were *Bacillus subtilis*, *Staphylococcus aureus*, *Shigella dysentriae* and *Salmonella typhi*. The culture media used for the analysis include Mueller Hinton agar (MHA), Mueller Hinton broth (MHB) and Nutrient agar (NA). These media were used for sensitivity test, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All media were prepared according to manufacturer's instruction and sterilized by autoclaving at 121^{0}C for 15 minutes.

Sensitivity Test

The standardized inocula of the bacterial isolates were streaked on a sterilized Mueller Hinton agar plates. Four wells were punched on each inoculated agar plate with a sterile cork borer. The wells were properly labelled according to different concentrations of the extract prepared which were 100, 50, 25 and 12.5 mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract. The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract diffuse on the agar. The plates were then incubated at 37^0C for 24hrs. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured using a transparent ruler calibrated in millimeter and the results were recorded.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using tube dilution method with the Mueller Hinton broth used as diluents. The lowest concentration of the extracts showing inhibition for each organism when the extract was tested during sensitivity test was serially diluted in the test tubes containing Mueller Hinton broth. The organisms were inoculated into each tube containing the broth and the extract. The inoculated tubes were then incubated at 37°C for 24hrs. At the end of the inoculation period, the tubes were examined for growth using turbidity as a criterion, the lowest concentration in the series without visible sign of growth (turbidity) was considered to be the minimum inhibitory concentration.

Minimum Bactericidal Concentration (MBC)

The result from the MIC was used to determine the MBC of the extract. A sterilized wire loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test and a loopful was taken and streaked on a sterile nutrient agar plates. The plates were incubated at 37°C for 18- 24 hrs. At the end of the incubation period, the plates were observed for the presence of bacterial growth. This was to determine whether the antimicrobial effect of the plant extract is bacteriostatic or bactericidal.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The result of phytochemical screening confirmed the presence of flavonoids, cardiac glycosides, tannins, saponins and phenols in both aqueous and methanolic extracts of the leaf. Phytochemicals are non-nutritive plant chemicals possessing varying degrees of disease-preventive properties. They are invaluable sources of raw materials for both traditional and orthodox medicine (Oikeh *et al.*, 2013). However, the abundance of these phytochemicals varied from plant to plant. These phytochemicals reduce oxidation (Zeggwagh *et al*., 2007), stimulate the immune system against viruses, bacteria and other disease causing agents (Cushnie and Lamb, 2005), slow growth of cancer cells (Havsteen, 2002) and reduce inflammation that provides a setting for cancer growth (Cushnie and Lamb, 2005). In plants, they promote physiological survival of plant by protecting it from fungal infections and UV radiations. In addition, flavonoids are involved in photosensitisation, energy transfer, respiration and photosynthesis control, morphogenesis, sex determination, energy transfer (Cushnie and Lamb, 2005). Esquenazi *et al*. (2002), Harsh *et al*. (2002), Havsteen (2002) and Rauf *et al.* (2014) extracted various phytochemicals from various medicinal plants. Ocimum basilicum can be regarded as a medicinal plant because of the presence of essential phytochemicals.

Table 1. Qualitative phytochemical analysis of aqueous and methanolic extracts of the leaf of *Ocimum basilicum*

Phytochemicals	Aqueous extract	Methanolic erxtract
Flavonoids	+	+
Cardiac glycosides	+	+
Tannins	+	+
Saponins	+	+
Phenols	+	+

Quantitative

Phytochemical Analysis

The quantitative estimation of chemical constituents of the leaf of the plant showed high percentage of flavonoids of 94.00% and 51.29% for aqueous and methanolic extracts respectively. Flavonoids are favorable, effective and usually innocuous substituents for the classical therapeutic agents. Similar to aspirin, actylated flavonoids may transfer their acyl group to the side chain of hydroxyl group of serine in the active site of cyclo-oxygenases (Havsteen 2002). Cardiac glycosides showed little concentration for both aqueous and methanolic extracts. These little concentrations may play a role in cardio protective effects that can cause opening of the mitochondrial ATP-sensitive potassium channels (KATP) which have therapeutic potential for the protection of ischaemic heart tissue (Pierre, 2007).

Table 2. Quantitative phytochemical content of aqueous and methanolic extracts of the leaf of *Ocimum basilicum*

Phytochemicals	Concentration (g/100g)	
	Aqueous extract	Methanolic extract
Flavonoids	94.00	51.29
Alkaloids	13.88	9.42
Cardiac glycosides	0.23	0.36

***In Vitro* Antioxidant Activity (DPPH)**

The *in vitro* antioxidant activity of the plant extracts is followed by its high concentration of flavonoids. Flavonoids are powerful antioxidants against (Esquenazi *et al*., 2002). They inhibit lipid peroxidation *in vitro* at an early stage by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate chain radical reaction by donating hydrogen atom to superoxy radical thus forming flavonoids radical which further react with free radical thus terminating propagating chain (Havsteen 2002).

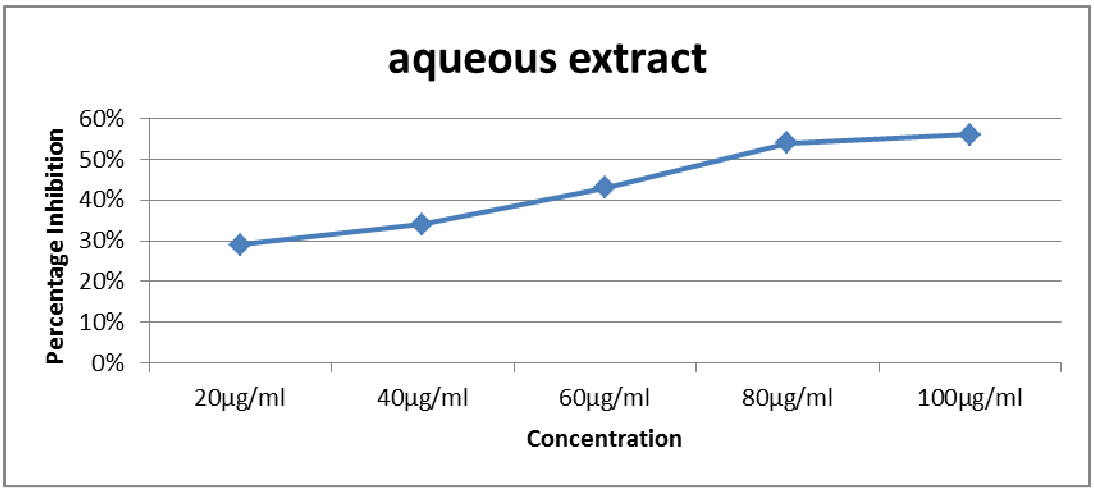

Fig. 1. *In vitro* antioxidant activity of aqueous extract of the leaf of *Ocimum basilicum*

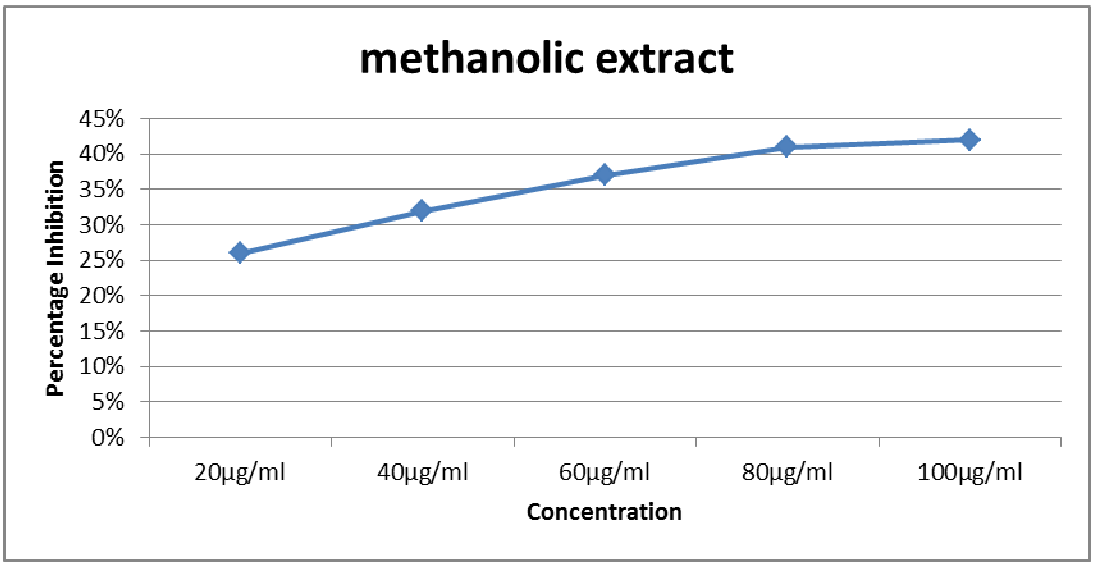

Fig. 2. *In vitro* antioxidant activity of methanolic extract of the leaf of *Ocimum basilicum*

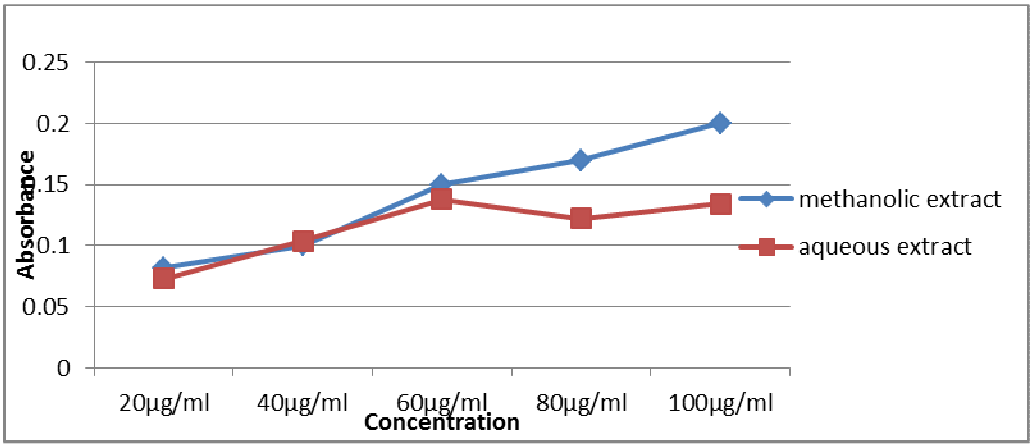

Fig 3. Phosphomolybdenum assay for methanolic and aqueous extracts of the leaf *ocimum basilicum*

Antimicrobial Activity

The leaf extracts showed significant effect on *Staphylococcus aureus*, *Bacillus subtilis* which are Gram positive bacteria and also the Gram negative *Shigella dysentriae*. The Gram negative *Salmonella typhi* showed complete resistance to the leaf extracts. Methanolic extract of the leaf showed wide zone of inhibition of about 30 millimetres against *Staphylococcus aureus*, with MIC and MBC of 1.56mg/ml and 3.125mg/ml respectively. The antimicrobial activity may be attributed to its high flavonoids content and small amount of cardiac glycoside. Ahmet *et al.* (2005) documented that flavonoids having sugar moiety showed antimicrobial activity while flavonoids with no cardiac glycosides showed no inhibitory activity on microorganisms. Cushnie and Lamb, 2005) reported the inhibitory effect of flavonoid-rich extracts against some microorganisms like *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

Table 3. Antimicrobial Activity of aqueous extract of *Ocimum basilicum*

Microorganism	**Extract concentration (mg/ml)**	**Zone of inhibition (mm)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Salmonella typhi	100 50 25 12.5	- - - -	-	-
Bacillus subtilis	100 50 25 12.5	- - - -	-	-
Staphylococcus aureus	100 50 25 12.5	20 15 - -	50	-
Shigella sp.	100 50 25 12.5	- - - -	-	-

TABLE4.4 antimicrobial activity of methanolic extract of *Ocimum basilicum*

Microorganism	Extract concentration (mg/ml)	Zone of inhibition (mm)	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	100 50 25 12.5	- - - -	-	-
Bacillus subtilis	100 50 25 12.5	15 12 - -	25	50
Staphylococcus aureus	100 50 25 12.5	30 25 20 18	1.56	3.125
Shigella sp.	100 50 25 12.5	20 16 12 10	12.5	25

CONCLUSION

The concentrations of secondary metabolites (phytochemicals) such as flavonoids and alkaloids obtained from the plant are appreciable. These are responsible for the ethno-medical, pharmacological, therapeutic and traditional uses of the plant. Traditionally, *Ocimum basilicum* are used as whole herb to treat a good number of diseases in Northern Nigeria. The wide range of and the diversities in the action of *Ocimum basilicum* can be a result of synergistic effect of its phytochemical constituents which cannot be fully duplicated with the isolated extracts or constituents. The present study showed quantitative estimation of the phytochemicals, antimicrobial potential as well as moderate antioxidant activity of the leaf of *Ocimum basilicum*. There is need for further research on extraction and purification of active component of the plant, including mineral and proximate analysis to be carried out.

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A HIGH DENSITY GENETIC LINKAGE MAP OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA L.*)

Kendabie, P[1,2] and Mayes, S[1,3]

[1]University of Nottingham, Sutton Bonington Campus, Loughborough UK
[2] Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[3]Crops for the Future Research Centre, Semenyih, Malaysia
Email Address: drkens2015@gmail.com Phone number: +234-803-380-9954
Postal Address: Department of Biological Sciences, Niger Delta University, Wilberforce Island, PMB 007, Bayelsa State, Nigeria.

Bambara groundnut (BGN) is an African native legume, rich in protein, able to fix nitrogen, highly drought tolerant and with reasonably good disease resistance that bears a rich food, nutritional and cultural history for the poor resource-base farmers in sub-Saharan Africa. This study was to construct a high density genetic linkage map and to enhance understanding of the Bambara groundnut genome. A dense genetic map was constructed in an F_2 population (263 lines) derived from quantitative long day and qualitative short-day parents (IITA-686 and Ankpa4, respectively) based on SNP and DArT markers. The linkage map consisted of 1238 marker loci (859 SNPs and 379 DArTs), with good coverage (1185 cM spanning 11 linkage groups; one marker per 1 cM, on average). This genetic map is an invaluable resource for QTL analysis and represent qualitative advances in the genetic improvement of Bambara groundnut. We will present the current state of genetic mapping in Bambara groundnut, and describe future directions for the genetic analysis and marker assisted breeding of this important legume species.

Key words: Bambara groundnut, Markers, Genetic mapping

INTRODUCTION

A genetic linkage map defines the relative positions of, and distances between, genes or DNA-based markers along linkage groups based on recombination frequencies obtained in mapping pedigrees (Paterson *et al*., 1996a; Collard *et al*., 2005). Genetic linkage maps are an essential tool for marker assisted selection, map-based cloning, comparative genomics, targeted genome sequencing and QTL studies (Lucas *et al*., 2011; Young and Bharti, 2012). The availability of molecular markers, mapping populations, genetic maps and sequence information provided by marker-trait associations would enhance the breeding process through the application of marker assisted selection (MAS) of favorable alleles in Bambara groundnut. Bambara groundnut is an African native legume, rich in protein, able to fix nitrogen, highly drought tolerant and with reasonably good disease resistance that bears a rich food, nutritional and cultural history for the poor resource-base farmers in sub-Saharan Africa.

Genetic linkage mapping has rapidly moved onto under-researched legumes (Varshney *et al*., 2010; Bohra *et al*., 2014). For example, linkage mapping in cowpeas, an important member of the legume family and perhaps the closest relative to Bambara groundnut, has advanced with marker technology to yield informative and increasingly dense genetic maps (Quedraego *et al*., 2002; Muchero *et al*., 2009; Lucas *et al*., 2011). However, in comparison to other legume crops of equal or less economic importance to farmers in the developing world, platforms (viz., high resolution genetic maps, mapping populations, and the whole genome sequence) for map-based cloning in Bambara groundnut are lagging in development (Ahmad *et al*., 2013; Ho *et al*., 2017). A major challenge, therefore, before the Bambara groundnut community has been the development of saturated genetic maps from large mapping populations that will facilitate the identification of important QTLs.

A recent advancement in genotyping technology is genotyping-by-sequencing (GBS), which involves the adaptation of next generation sequencing (NGS) protocols to simultaneously discover and score

segregating single nucleotide polymorphism (SNP) markers in mapping populations of interest (Deschamps *et al*., 2012; Spindle *et al*., 2013). This remarkable progress has resulted in thousands of DNA markers being discovered and mapped in a one-step procedure using NGS-based methods (Chen *et al*., 2013; Davey *et al*., 2011), thereby facilitating construction of high and ultrahigh density genetic maps not only for major crop species with reference genome sequence but also for the crops where no reference genome is available (Poland *et al*., 2012). The DArT-based method combined genome complexity reduction with massive Illumina short tag sequencing to generate two types of data: 1) scores for "presence/absence" (dominant) markers, called SilicoDArT as they are analogous to microarray DArTs, but extracted "in silico" from sequences obtained from genomic representations, and 2) SNPs in fragments present in the representation (Sansolina *et al*., 2011). Details of the DArT-based GBS technology are available at http://www.diversityarrays.com/dart-application-dartseq. High throughput genotyping of DArTs and SNPs using NGS technologies have afforded us the opportunity to generate large number of sequence-derived markers for even non-model species. The availability of these markers should facilitate genetic mapping and QTL analysis in Bambara groundnut. The aim of this study was to construct a high density genetic linkage map and to enhance our understanding of the Bambara groundnut genome.

MATERIALS AND METHODS

An F_2 segregating mapping population (n=268) derived from Bambara groundnut genotypic landraces (Ankpa 4 and IITA-686) were used in this study. These parental lines have been well characterised previously in daylength experiments, and are divergent in their response to extreme day length conditions (>12 h). From July – December, 2012, 6 plants of each parent and 263 F_2 lines of the cross IITA × Ankpa 4 were grown for photoperiod evaluation: 158 lines in 16 h and 105 lines in 12 h. Plant materials were grown in soil beds in a climate-controlled glasshouse at the FutureCrops Glasshouses, Sutton Bonington Campus of the University of Nottingham. Glasshouse conditions were: 12 h day/night using an automatic blackout screen, 28^{o}C daytime temperature and 24^{o}C during the night. Planting distance of 25 cm x 25 cm between and within rows was maintained. Irrigation was automatic using trickle tape, twice per day for 10 min water flow in the morning and 15 min evening throughout the experiment period. Lighting was complemented in all glasshouses with artificial lights, when light levels were below 20,000 lux during the non-blackout period.

Genomic DNA was isolated from fresh young leaves of both parental genotypes, and each individual of the F_2 population grown using the Qiagen Plant genomic DNA kit (Qiagen), following the manufacturers instruction with slight modifications. Approximately 100 mg of leaf tissue was ground in a mortar with liquid nitrogen, for better yield of DNA. All DNA concentrations were estimated by electrophoresis of samples in a 1 % agarose gel alongside standard lambda DNA. Microsatellite markers previously reported (Ahmad *et al.*, 2013) were tested on parents and polymorphic markers were used to analyse 94 individuals of the F_2 segregating population of IITA-686 × Ankpa 4. Polymerase chain reaction (PCR) was done as previously reported (Ahmad *et al*., 2013). After confirming the segregation in the F_2 population, this cross was later sent off to DArT Pty Ltd (Canberra, Australia) for genotyping by sequencing. This also generated 64 bp sequence tags which were associated with each marker (DArT and SNPs) used for linkage mapping. The JoinMap4 software (van Ooijen, 2006) was used to construct the linkage map, comprising both sequence-derived DArT and SNP marker data.

RESULTS AND DISCUSSION

The DArT-based GBS of the F_2 mapping population (IITA-686 x Ankpa4), and a diversity panel comprising the parents of the F_2 cross and 111 genotypic landraces returned 8,872 (3092 SNPs and 5,780 SilicoDArTs) potential markers. After filtering this data set, a total of 3852 markers (1,312 SNPs and 2540 SilicoDArTs) were identified as high quality data and are polymorphic between the two parents used to generate the mapping population. This accounts for 43.4% of sequence derived markers that could

be used for linkage analysis and map construction. However, in an attempt to produce a linkage map with minimal missing data, 1,238 (859 SNPs and 379 SilicoDArTs) markers were used to construct a genetic map for 263 individuals. The segregation data assembled for polymorphic markers tested for goodness of fit, and the proportion of segregation distortion was automatically detected by JoinMap at ($p < 0.05$ for significance). The locus genotype frequency suggested 1117 (772 SNPs and 345 SilicoDArTs, 90.2%) markers showed a goodness of fit of 1:2:1 and 3:1 for both SNP and SilicoDArT marker types, respectively, while the remaining 121 (87 SNP and 34 SilicoDArTs, 9.2%) showed significant deviation from Mendelian ratios in the map for all 263 F_2 lines. Segregation distortion is a common phenomenon observed in actual genetic mapping projects that utilize bi-parental crosses, and chromosomal regions responsible for distorted segregation ratios have been mapped (Vogl and Xu, 2000). Markers linked to segregation distorted locus shows distorted segregation, and thereby contributes to a deviation in locus genotype frequency from the expected Mendelian ratios (Vogl and Xu, 2000). In the present study, GBS (SNPs and SilicoDArTs) markers showed distorted segregation of 9.2% for the map involving all 263 F_2 lines. Markers that showed obvious distortion were excluded from the linkage analysis. In contrast to earlier studies that reported a high proportion of distorted marker segregation for SSRs, DArTs and AFLPs in two mapping populations (32 and 27%, respectively) used for map construction and QTL analysis in Bambara groundnut (Ahmad, 2013), the influence of segregation distortion on QTL analysis will be negligible. Segregation distortion may result from a number of factors, such as residual heterozygosity, gametic or zygotic selections, embryo lethal genes, genotyping errors, non-homologous recombination, transposable elements and environmental agents (Xian-Liang *et al*., 2006).

A genetic map was produced for all 263 F_2 individuals, with total map lengths of 1185 cM (Figures 1-1, 1-2). In this map, mapped markers were precisely assigned to 11 linkage groups (LGs) using an LOD threshold of 6.0, which is in agreement with the haploid chromosome number (n=x=11) in Bambara groundnut (Uguru *et al*., 2006). On average, the distances between adjacent markers were 1.2 cM (Table 1). Linkage groups were arranged in order of magnitude, with LG1 being the largest to LG11 as the shortest, in agreement with the chromosome lengths of Bambara groundnut genotypes (Uguru *et al*., 2006). The length of LGs ranged from 78 (LG 11) to 132 cM (LG 1) in the genetic map.

Table1: Marker distribution across 11 linkage groups (263 F_2 lines): linkage groups and map lengths, number of mapped markers, and marker intervals

Linkage groups	Map length, cM	Number of loci	Average inter-marker distance, cM
LG1	132	150	0.88
LG2	128.67	113	1.14
LG3	125.49	57	2.2
LG4	123.97	73	1.7
LG5	114.8	102	1.13
LG6	113.51	91	1.25
LG7	109.54	59	1.86
LG8	89.58	112	0.8
LG9	87.17	67	1.3
LG10	80.63	73	1.1
LG11	78.31	112	0.7
Total	1184	1009	1.2

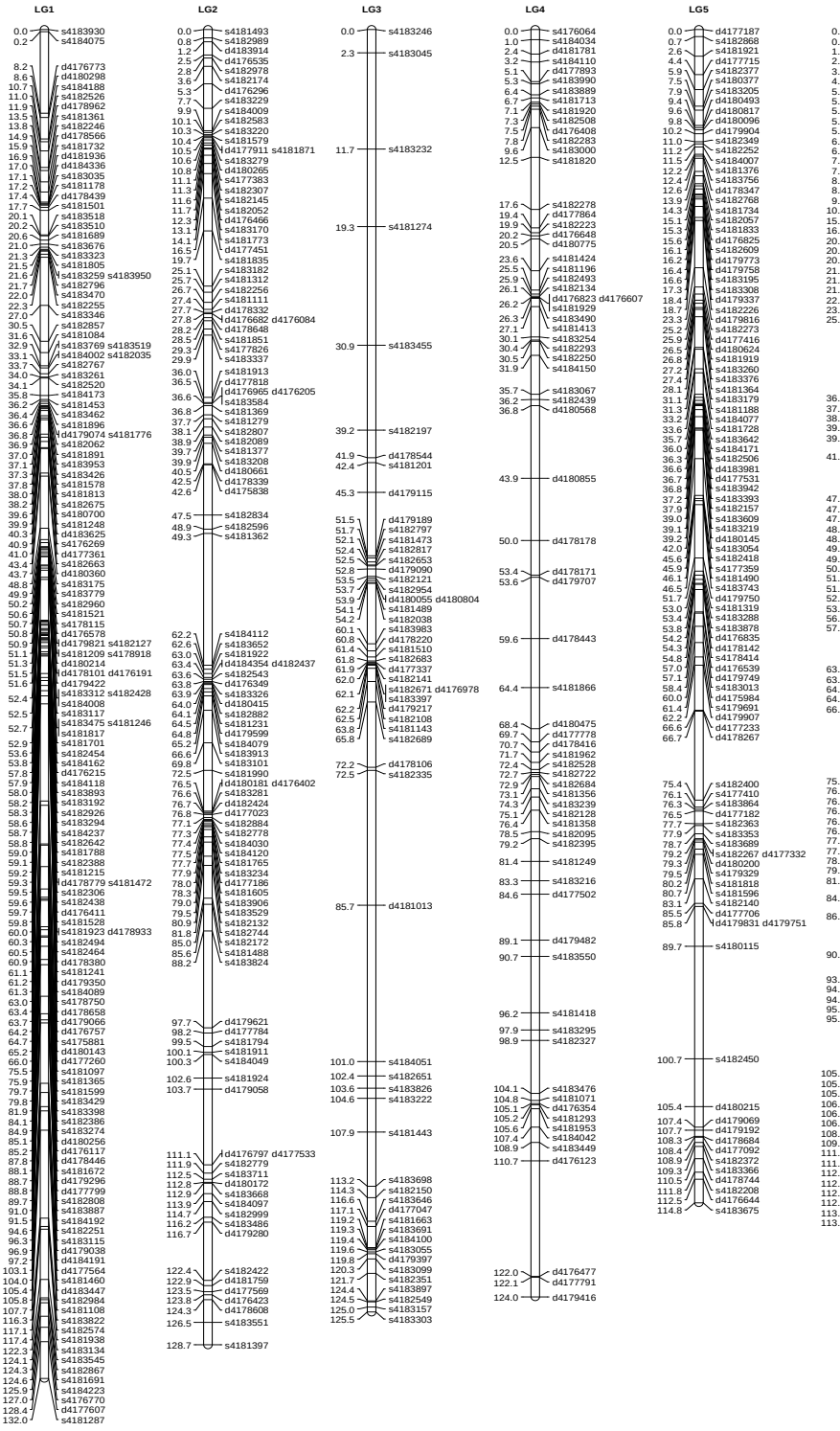

Figure 1-1: Genetic linkage map of 11 linkage groups (LGs 1, 2, 3, 4, 5 and 6). This map was constructed using 263 F_2 lines derived from a IITA-686 × Ankpa4 cross. Positions are given in centimorgan (Kosambi units) to the left of the linkage groups and the name of the marker the right. A total coverage of 1184 cM was obtained with 1009 markers (683 SNPs, 326 DArTs).

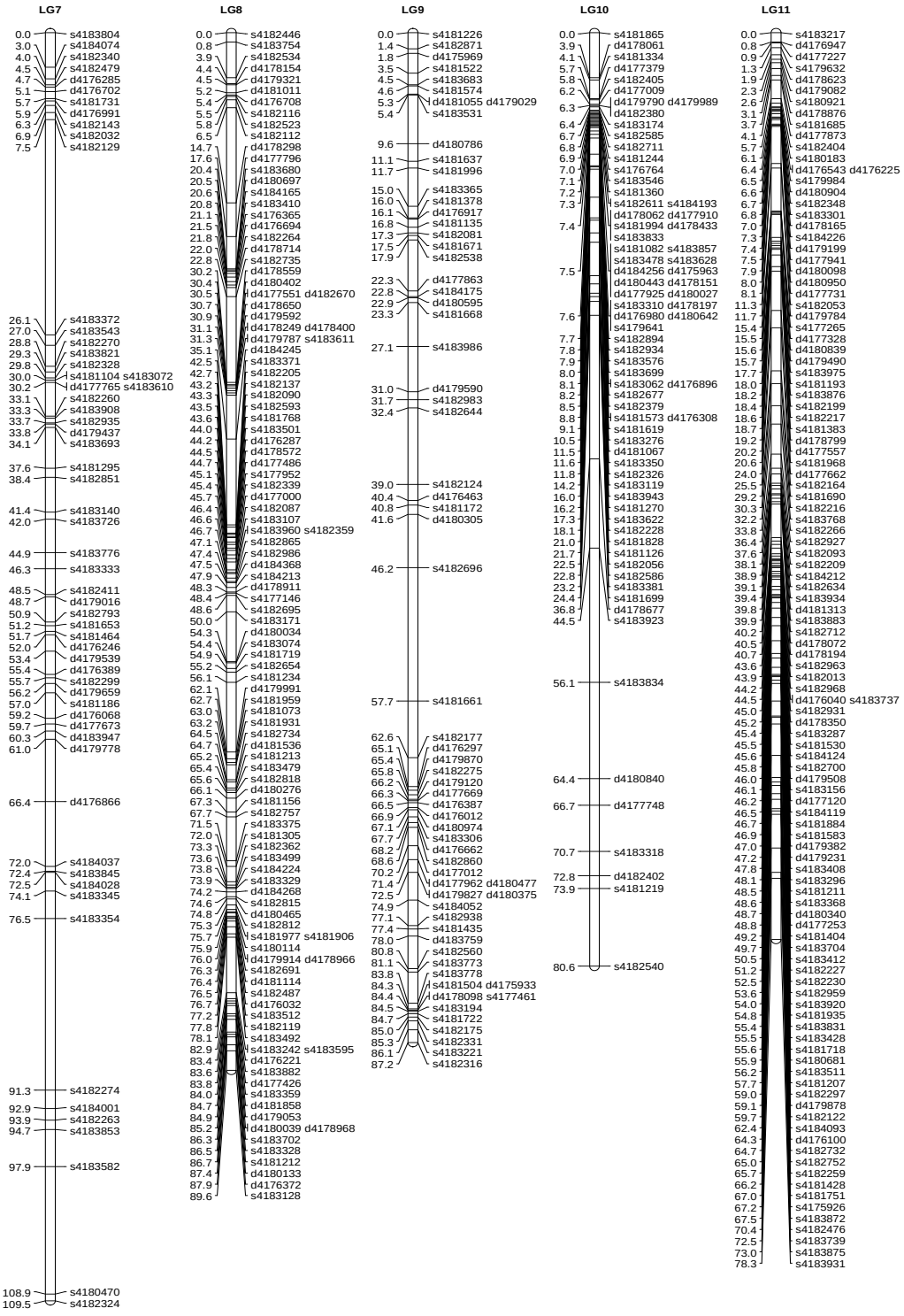

Figure 1-2: Genetic linkage map of 11 linkage groups (LGs 7, 8, 9, 10 and 11). This map was constructed using 263 F_2 lines derived from a IITA-686 × Ankpa4 cross. Positions are given in centimorgan (Kosambi units) to the left of the linkage groups and the name of the marker the right. A total coverage of 1184 cM was obtained with 1009 markers (683 SNPs, 326 DArTs).

This high density map provides a full coverage of the Bambara groundnut nuclear genome, and the number of linkage groups corresponds to the 11 chromosome numbers and lengths reported for the Bambara groundnut genome (Uguru *et al*., 2006). The current Bambara groundnut linkage map is a considerable improvement over the previous Bambara groundnut genetic linkage maps built on SSR, AFLP and DArT markers (Ahmad, 2013). The broad genome coverage achieved in this study was due to the capacity of GBS markers to detect a high level of genetic polymorphism between the genetic cross IITA-686 (from Tanzania) x Ankpa4 (Nigeria, West Africa), large F_2 population size and maximum recombination events between the two parents of the same species. Such a high density intra-specific molecular linkage map based on genic-SNP and genic-SSR markers is also available for pigeon pea, covering a genome map length of 1520.22 cM (Kumawat *et al*., 2012). While the number of markers assigned to each linkage group could reflect the relative amount of genetic variation present among the linkage groups, their map distances on the other hand reveal the similarity in chromosome lengths (Osuji *et al*., 2005; Uguru *et al*., 2006). Furthermore, this map was constructed using 64-bps sequence derived markers of the Bambara groundnut genome; therefore it will be highly useful for comparative genome mapping and synteny studies with other legume genomes that has reference genetic maps and a draft sequenced genome.

Construction of a detailed genetic map and QTL analysis relies on the identification of sufficient number of markers revealing polymorphism among parents used in a genetic cross, and the availability of relevant mapping populations. In the present study, the mapping population was based on a pair of genetically diverse genotypic landraces (IITA-686 and Ankpa4), for which a high percentage of polymorphic markers (43.4% of SNPs and SilicoDArTs) with wide genome coverage were identified. The large genetic distance between the parental lines of the mapping population in the present study provided a high degree of polymorphism for markers across most of the linkage groups (Table 1). This finding is at variance to the report (Ahmad *et al.*, 2013) that observed lower polymorphism for DArTs in a narrow cross, as evident from the reported in the identification of 236 (3.1%) of polymorphic DArTs out of 7680 DArTs screened between the parents of the narrow cross. However, a polymorphic rate of 36.3 and 33.1% for SSRs was also reported in a narrow and wide cross populations of Bambara groundnut, respectively (Ahmad *et al*., 2013). The level of polymorphism obtained in this study is consistent with previous research, and it demonstrates that a greater level of genetic diversity exists in Bambara groundnut than in a number of other legume crops. In pigeon pea for instance, even after using large number of SSR markers (3,072) a polymorphism level of 4.65% and 2.5% was obtained for the parental alleles in ICP 8863 x ICPL 20097 and TTB 7 x ICP 7035 crosses respectively, confirming the narrow genetic base present in cultivated pigeon pea genepool (Saxena *et al*., 2010a; Bohra *et al*., 2012). Furthermore, a total of 296 SSR and SNP markers (10.17%) showed polymorphism between parents in the pigeon pea genetic cross Pusa Dwarf x HDM04-1 and were used to genotype 186 F2 plants (Kumawat *et al* 2012).

CONCLUSION

A high resolution genetic map was constructed in an F_2 mapping population derived from the cross IITA-686 x Ankpa4, based on GBS (SNP and SilicoDArT) markers. The linkage map consisted of marker loci (1281 SNPs and DArTs), with good coverage (1196 cM spanning 11 linkage groups); and average marker intervals (1.2 cM). The marker information obtained demonstrates the power of GBS in understanding plant genomes. Based on this current genetic map, it is feasible to conduct comparative genome mapping to discover synteny between Bambara groundnut and other sequenced legume genomes.

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SECTION TWO: ANIMAL GENETICS, BREEDING AND BIOTECHNOLOGY

CRYOPRESERVATION OF RABBIT SEMEN IN FREEZING DILUENT CONTAINING 20% EGG YOLK PLASMA FROM 3 DOMESTICATED AVIAN SPECIES.

Popoola, M. A.[1], Ola, S.I.[2] , Adeyosoye, O. I [2] and Shoyombo A.J.[3]

1. Animal Genetic Resources Lab, National Biotechnology Development Agency, Abuja, Nigeria.
2. Department of Animal Sciences, Obafemi Awolowo University, Ile Ife, Nigeria.
3. College of Agricultural Sciences, Landmark University,Omu-Aran,Kwara State,Nigeria.

Corresponding author: Dr. Popoola M.A, honmusty@gmail.com, +234 8066301780.

Abstract

The outcomes of the various rabbit semen cryopreservation protocols are still varied. Our present study is aimed at evaluating the cryoprotective potentials of bio-fortifying basic buck semen freezing extenders with 20% egg yolk plasma(EYP) from 3 avian species; Chicken(Control), Turkey and Muscovy duck. Using artificial vagina, heterospermic pool of semen was collected from 20 rabbit bucks and samples aliquoted for the 3 treatments. Spermatological parameters (%) evaluated were Mass motility (MM), Forward Progressive Motility (FPM), Liveability (LV), Acrosome integrity (ACI) and Total abnormality (TA). Treated semen samples were analyzed; Post dilution and chilling (5°C, I hour), Post equilibration (-120°C, 5minutes) and Post thaw(-196 °C). Muscovy duck EYP treated sample gave the best result regarding all other studied spermatological parameter post chilling except mass motility of 76.67% (Turkey EYP). After equilibration, semen in diluents containing Muscovy duck EYP was the best in all the parameters studied followed by Turkey EYP treated semen samples. All the treatments were significantly different from each other across the spermatological parameters (P> 0.05).Post thaw evaluation of semen samples showed best combination of results with Muscovy duck EYP treated samples which was significantly different from other two treatments. Chicken EYP and Turkey EYP diluents were statistically similar in all evaluated spermatological parameter except total abnormality. Total abnormality in Turkey EYP bio-fortified samples was the worst 28.00% post thaw. In conclusion, Muscovy duck EYP containing diluent could provide better cryoprotection to rabbit semen than conventional Chicken (EYP) at 20 % inclusion in rabbit buck semen freezing diluents.

Key Words: rabbit semen, egg yolk plasma (EYP), cryopreservation, avian species, diluents.

Introduction

The multi- purpose benefits and usage of rabbits in human food chain, biopharmaceutical researches including industrial inputs associated with its other bye products positioned this species as very important in the animal agricultural value chain. Rabbit farming can also provide a very valuable additional source of income in the rural areas and expand the value chain of livestock sub sector of many developing countries in search of sustainable protein of animal origin. Yearly, biomedical and innovative researchers will need some thousands of rabbit to carry out studies relating to product discovery, development and testing. On a small scale, it involves small number (a few dozen or less) of animals fed on a variety of green forage, vegetable, household waste and agricultural by products (FAO, 1997). Biotechnological tools of artificial insemination commonly known as (AI) facilitate the revolution the business of animal reproduction, breeding and genetics.

The AI technology provide a veritable platform for the advancement of other Assisted Reproductive Techniques (ARTs) to support sustainable use of animal genetic resources for livestock precision breeding, genetic resources conservation and development of new value chains in animal agriculture including semen banking. Nowadays, semen freezing is not a reliable or repeatable method yet, and

results after AI with rabbit frozen semen are still too inconstant to plan a routine utilization of this technique (Moce, Lavara and Vincente, 2003). Extensive and commercial use of AI in rabbit breeding is limited by availability and affordability of cryopreserved semen. In practice, the use of fresh and diluted semen for AI operations is only feasible provided the buck from which the semen is collected is resident on the farm or at short inter-farm distances between and within farms.

Many livestock semen cryopreservation diluents have egg yolk a part of the constituents because of its documented preservative factors including the cryoprotection of the spermatozoan's plasma membranes and maintenance of the acrosomes against possible cryo injury. It is believed that the phospholipids, cholesterol and low density lipoproteins in egg yolk may be the factors that provide protection to sperm against cold shock during the freeze–thaw process (Amirat *et al*., 2005).There have also been some reports that egg yolk from avian species such as the duck, quail, pigeon or chicken have different combinations of fatty acids, phospholipids and cholesterol, which could result in different cryopreservation effects on the sperm (Bahtgate *et al*., 2006; Humes and Webb, 2006; Andrabi and Maxwell, 2007; Clulow *et al*., 2007; Su *et al*., 2008).

For many years, hen egg yolk has routinely been used in extenders to cryopreserve the semen of different animals; however, this practice entails several drawbacks (Huopalahti et al., 2007). Yolk plasma has been reported to be as efficient as whole egg yolk for freezing stallion semen, thereby avoiding many of these negative factors. It is a well-known fact that, with equal dry matter, plasma exhibit better properties than yolk. In fact, plasma proteins have been shown to play a prominent role in egg yolk gelation (Guilmineau et al, 2005). Egg yolk plasma is an intermediate product of raw whole egg yolk and LDL. The extraction of EYP is faster and more cost effective compared to LDL hence the new trend in its utilization in semen cryopreservation. There species specific difference in the composition of egg yolk from different avian species thereby affecting the cryoprotective effects of EYP from different birds. Therefore, our aim in this study is to evaluate the cryoprotective effects of bio fortifying freezing diluents with 20% inclusion of Egg yolk plasma from Chicken (Control), turkey and Muscovy duck on the spermatological parameters of rabbit buck semen

Materials and Methods

Rabbit Buck and their Managements

Twenty sexually matured mixed breed of rabbit (*Oryctolagus cuniculus)* bucks under the intensive management system were used in this study. The animals were fed with both commercial concentrates. Experimental animals were exposed to half a day of natural lightening condition during the experiment, kept securely inside metabolic cages for easily daily husbandry services and semen collection. Daily, hygienic water (*ad libitum*) and feed were served to the rabbit bucks uniformly during this study at the Teaching and Research Farm, Obafemi Awolowo University, Ile Ife, Nigeria.

Semen Collection

Pre warmed (40 °C) rabbit artificial vagina (AV) was used for the collection of semen from the experimental bucks. The artificial vagina (AV) used was designed and constructed at the experimental station. The experienced bucks were introduced to teaser does to ensure natural stimulation for ejaculation. The penis of the bucks was located to ensure penetration into the artificial vagina for onward ejaculation. Semen was collected two times a week and only ejaculates with minimum of 0.5 ml volume were used in this research work. Micropepette was used to remove gel plugs from ejaculates immediately and collected semen transferred to the laboratory at 40°C inside flask for assessment, processing and storage. Semen was collected on a fixed day twice weekly. The average temperature of rabbit does vagina was 39^0C at the time of collection.

Semen Evaluation

Phase contrast microscopy was used for the semen evaluation. The semen arrived laboratory within 30 minutes for assessment. The parameters assessed and recorded in percentage (%) were the sperm concentration, mass motility (MM), forward progressive motility (FPM), live ability (live: dead),acrosome integrity and total abnormalities.

The volume of semen used for the assessment of each parameter evaluated was measured with eppendorf pipette. The concentration of spermatozoa greater than or equal to 50 million cells ml^{-1} and the proportion of motile cells greater than or equal to 80 % were used. The concentration was estimated using a haemocytometer.

Mass motility and FPM percentages were evaluated by aliquoting samples (10 micro litres) of the fresh spermatozoa at 37 °C on a pre-warmed slide and then covered with cover slips (22 mm x 22 mm) and then transferred to a heated microscope stage set at 37 °C. Semen samples were assessed by phase contrast microscopy (x100 and x 200 magnifications). The proportion of motile sperm cells and spermatozoa with progressive motility were estimated.

Liveability and proportion of morphologically abnormal spermatozoa (Total Abnormality, abnormal head, tail and mid piece) were assessed by staining the aliquots of the sperm suspension with eosin- nigrosin and Giemsa (Cassinello, Abigar, Comendio and Roldan, 1998). Acrosome integrity of the semen samples were examined by phase-contrast microscopy using x100 oil immersion objective. The aliquot was stained using Congo red and the slides were fixed with ethanol. The stained and fixed samples on the slide were soaked in the Trypan blue stain for 3 hours before estimation of spermatozoa with intact acrosomes (i.e. with normal apical ridges in percentage) using the oil immersion microscope.

Semen Processing, Equilibration and Cryopreservation

The acceptable ejaculates for the analyses were characterized by volume ≥0.5 ml, concentration of 250 Million per ml, mass motility ≥ 85 %, Forward progressive motility (FPM) ≥80%, liveability of ≥75 %, acrosome integrity ≥ 70 % and total abnormality less than or equal to 10 %.This was based on the results of the preliminary studies using heterospermic pool from the bucks' ejaculates used. The semen was initially diluted with Tris Citric acid fructose cooling extender the at dilution ratio of 1:5 in a sterile bottle. The compositions of the cooling extender include Tris 0.302g, citric acid 0.167g, fructose 0.125g, streptomycin 0.05g and 20% egg yolk plasma each from Chicken, Turkey, Muscovy duck whole yolk samples.

The tubes containing the diluted semen were chilled in the refrigerator at 5 °C for 1 hour. The aliquot of the chilled extended semen was measured using eppendorf pipette into cryo tubes (0.5 ml). Cryodiluents was subsequently added to the chilled semen. The composition of the cryodiluents include Tris 0.302g, citric acid 0.167g, fructose 0.125g, streptomycin 0.05g, Sucrose (%) , DMSO (8%) and 20% egg yolk plasma each from Chicken, Turkey, Muscovy duck whole yolk samples. The samples were then exposed to liquid nitrogen vapour at -120 °C held at 4 cm above the surface for a period of 10 minutes and was then plunged into the liquid nitrogen(-196 °C) for the cryopreservation. Frozen semen inside cryo tubes were thawed at 50 °C for 12 seconds (Rosato,DiIorio,Manchisi, Petrosino, Centoducati …….Iaffaldano,2013) in a water bath and contents were assessed.

Experimental Design

The experimental design used in the experiments is completely randomized design (CRD).Three treatments using egg yolk plasma from the 3 selected avian species. The procedure was replicated thrice for all the 5 parameters evaluated per treatment.

Data Collection
Spermatological parameters evaluated in percentages (%) are mass motility, forward progressive motility (FPM), Liveability (LV), Acrosome integrity (ACI) and total abnormality were assessed post dilution and chilling, post equilibration/pre freezing and post thaw.

Statistical Analysis
Data obtained were recorded and then subjected to analysis of variance using Statistical analysis software (SAS, 2004). Means ± S.E.M were compared using Duncan's multiple range test (Duncan, 1955). Mean values were considered to be statistically different at $P \leq 0.05$

Results

TABLE 1: Spermatological Parameters of Extended Rabbit Semen with 20 % Egg Yolk Plasma diluents at 5°C, 1hour

SPECIES	MASS MOTILITY (%)	FPM (%)	LIVEABILITY (%)	ACROSOME INTEGRITY (%)	TOTALABNORMALITY (%)
CHICKEN	71.33^{c}±5.92	42.50^{c}±4.44	65.33^{b}±4.33	63.33^{b}±4.91	13.67^{b}±1.20
TURKEY	76.67^{a}±0.67	51.50^{b}±0.50	68.33^{b}±3.17	65.00^{b}±2.77	10.33^{ab}±0.67
MUSCOVY DUCK	73.33^{b}±3.75	56.08^{a}±2.97	75.00^{a}±2.51	69.00^{a}±3.05	9.67^{a}±0.89

Means on the same column with different superscript are significantly different ($P \leq 0.05$).
FPM- Forward Progressive Motility

TABLE 2: Spermatological Parameters of Extended Rabbit Semen with 20% Egg Yolk Plasma Cryodiluents at -120°C, 5minutes (Post Equilibration, Pre Freeze)

SPECIES	MASS MOTILITY (%)	FPM (%)	LIVEABILITY (%)	ACROSOME INTEGRITY (%)	TOTAL ABNORMALITY (%)
CHICKEN	54.00^{b}±5.00	50.50^{c}±3.50	55.00^{c}±9.00	54.50^{c}±2.50	19.00^{b}±3.00
TURKEY	56.50^{b}±4.50	55.75^{b}±2.75	59.50^{b}±3.50	58.50^{b}±0.50	16.00^{b}±1.00
MUSCOVY DUCK	70.00^{a}±5.00	60.00^{a}±3.50	67.50^{a}±2.50	66.50^{a}±3.50	9.50^{a}±2.50

Means on the same column with different superscript are significantly different ($P \leq 0.05$).
FPM- Forward Progressive Motility

TABLE 3: Post Thaw Spermatological Parameters of Cryopreserved Rabbit Semen with 20% Egg Yolk Plasma Cryodiluents (-196 °C)

SPECIES	MASS MOTILITY (%)	FPM (%)	LIVEABILITY (%)	ACROSOME INTEGRITY (%)	TOTAL ABNORMALITY (%)
CHICKEN	50.00^{b}±2.00	48.75^{b}±2.75	56.00^{b}±2.00	54.00^{b}±10.00	22.00^{b}±1.00
TURKEY	52.50^{b}±4.50	49.75^{b}±3.15	58.50^{b}±3.50	54.00^{b}±4.00	28.00^{a}±3.00
MUSCOVY DUCK	66.00^{a}±3.00	57.20^{a}±9.10	67.50^{a}±8.50	60.50^{a}±2.50	10.00^{c}±2.00

Means on the same column with different superscript are significantly different ($P \leq 0.05$).
FPM- Forward Progressive Motility

Discussion

The need for rabbit semen cryopreservation like semen from other livestock species cannot be underestimated. Chicken egg yolk has been conventionally used in the cryopreservation of livestock semen however due to its bulkiness and sanitary condition, its replacement with either egg yolk plasma EYP or low density lipoprotein LDL is now trendy. Studies revealed that Whole Chicken Egg Yolk contains substances like high-density lipoproteins (HDLs) and minerals, which have the potential to affect sperm functionality (Manjunath *et al*, 2002).

The present study was carried out to evaluate the use of alternative source of egg yolk from other avian species apart from chicken because of many economic pressure on chicken egg compared to other poultry occasioned by human needs for food and industrial uses. Egg yolk from different avian species such as duck, quail, pigeon, chicken, and turkey has different combinations of fatty acids, phospholipids, and cholesterol (Kulaksiz *et al*, 2010). Interestingly, the sperm membranes of different species also vary in their cholesterol and phospholipid content that influences their susceptibility to cold shock. Therefore, the differences in sperm membrane composition and the components of the egg yolk from different avian species may culminate in species-specific interactions (Moreno *et al*, 2008).

Post chilling dilution results of best sperm motility in Turkey EYP extender was indicative of its better performance when compared with the EYP from conventional Chicken egg yolk. Egg yolk has been reported to have stimulatory effect on sperm motility (Chinsomboon *et al*, 1990). The best performance in the studied parameters by Muscovy duck EYP containing diluents was consistent with previous studies in other livestock species. Shah et al 2017 reported that Whole Chicken Egg Yolk (20%, v:v) can be replaced with UV-C–irradiated chicken EYP (20%, v:v) in tris-citric acid extender to improve the post-thaw *in vitro* quality, and *in vivo* fertility of water buffalo spermatozoa.

Previous studies reported the substitution of chicken with duck egg yolk to improve the post thawing motility in stallion and bull sperm. Burris and Webb (2009)), reported that although the values were not significant, the inclusion of duck egg yolk in the diluent resulted in the second highest progressive sperm motility while the inclusion of turkey egg yolk provided a higher ($p < 0.05$) post thawing progressive sperm motility than any of the three extenders studied – which included chicken egg yolk. Turkey egg yolk contained the highest levels of cholesterol. Our results in this present study using 20% EYP showed the best mass motility and forward progressive motility of 66.00% and 57.00% respectively in diluent

containing Muscovy duck EYP. This results was significantly different from Turkey EYP and Chicken EYP treated samples which were not significantly different from each other except worst total abnormality of 28% reported in Turkey EYP sample post thaw. Andrabi *et al*, (2007)) and Clulow *et al*, (2007)) reported that duck egg yolk could compare favourably with other avian egg yolks in extenders used to improve the frozen–thawed quality of buffalo bull and stallion sperm. Egg yolk from duck was reported to have better protection on sperm quality considering parameters like motility, viability, abnormal sperm and membrane integrity than other avian egg yolks – except chucker egg yolk. This may be attributed to the higher levels of protein, lipid and cholesterol present in the duck egg in comparison with chicken egg yolk (Choi *et al*., 2002).

Andrabi *et al*, 2008 reported that DEY protects buffalo bull sperm tail from damage during cryopreservation is of enormous significance. The author concluded that DEY compared to other avian yolks in extender improves the frozen-thawed quality of buffalo bull spermatozoa.

The results from the present study agree with some previous works using other species of livestock. The use of EYP as against EY commonly used by other authors was innovative in rabbit semen cryopreservation and provide a better alternative to the use of chicken EY for EYP production in semen freezing protocol of rabbit buck. Total abnormality of 10% and acrosome integrity of 60.00% in Muscovy duck EYP is quite significant and indicative of better performance over Turkey EYP and Chicken EYP containing diluents.

Conclusion

Muscovy duck EYP (20%) provides a better cryoprotection for rabbit semen during cryopreservation in comparison to Turkey EYP and Chicken. Muscovy duck EYP (20%) can therefore replace Chicken EYP in the cryodiluents used in freezing rabbit buck semen.

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PHENOTYPIC CORRELATIONS BETWEEN BODYWEIGHT AND LINEAR BODY MEASUREMENTS OF CROSS BETWEEN NEW ZEALAND WHITE × DUTCH AND CHINCHILLA × DUTCH RABBITS (*Oryctolagus cuniculus*)

[1]Mallam, I., [2]Nwagu, B.I., [1]Kabir, M., [3]Musa, J. and [1]John, P.A.
[1]Department of Animal Science, Ahmadu Bello University, Zaria.
[2]National Animal Production Research Institute/Ahmadu Bello University, Shika-Zaria.
[3]Livestock Section Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Shika-Zaria
Corresponding Author; mallamiliya2011@gmail.com. +2348188146452

ABSTRACT
A study was carried out at the Skills Acquisition and Entrepreneurship Development Centre of National Agricultural Extension Research and Liaison Services (NAERLS), Ahmadu Bello University, Zaria-Nigeria to identify the level of phenotypic correlations that exists between the linear body measurements and body weight of cross between New Zealand White × Dutch and Chinchilla× Dutch rabbits at post-weaning.
Data on 16 weaner rabbits were obtained from the cross between New Zealand White × Dutch (NZ×DU) and Chinchilla × Dutch (CH×DU). Data on body weight and linear body measurements (LBMs) namely: body length (BL), chest girth (CG), head-to-shoulder(HS), shoulder-to-tail drop (ST), length of hind leg (LHL), ear length (EL), height at withers (HTW) ear length (EL), heart girth (HG), body length (BL), head to shoulder (HS), leg length (LL) and tail length (TL) were collected after weaning (6 to 14 weeks of age). The relationships among the measured traits were determined using Linear Correlation Procedure of SAS (version 8.0, 2004).
The value of the Pearson's linear correlation coefficients determines the level of relationship between the LBMs. Correlation coefficients ranged from 0.33-0.99 and 0.12-0.93 in NZ×DU and CH×DU respectively. The correlation coefficients varied from positive to negative in NZ×DU while CH×DU had positive correlation coefficients. In all the genotypes, low to moderate and high correlation coefficients were observed among body weight and LBMs. In conclusion, offspring from cross between NZ×DU had both positive and negative correlation coefficients while all the correlation coefficients observed in CH×DU were positively correlated.

Keywords: New Zealand White, Dutch**,** Chinchilla, Linear body Measurements, Phenotypic correlations.

Introduction
Estimation of genetic parameters for productive and reproductive traits is very vital to the use of man-made selection which will lead to maximum genetic improvement when suitable breeding programs are used. Estimates of correlations between rabbit body weights and morphometric traits are scarce in available scientific literature all around the world (Akanno and Ibe, 2005). Moreover, rabbit producers and breeders are interested in the relationship that exists between body weight and linear traits because this information would tell something about rabbit feed efficiency and production performance (Okoro *et al*., 2010).
This in turn will make the work of breeders easier and faster as its effects can then be concentrated on traits that are easier to measure.

MATERIALS AND METHODS
Description of Experimental Site
The study was conducted at the Skills Acquisition and Entrepreneurship Development Centre of National Agricultural Extension Research and Liaison Services (NAERLS), Ahmadu Bello University, Zaria,

Kaduna State, Nigeria. Zaria is located within the Northern Guinea Savannah Zone of Nigeria between latitude 11^0 33' N and longitude 12^0 33′E (Ovimaps, 2016).

Experimental Animals and Management

A total of 16 weaner rabbits consisting of 8 from each crosses with the main treatment effect being the genotype were housed in individual row cages of metal and wire-gauze of 60×44×50cm^3. The weaner rabbits were fed concentrate ration (16% crude protein and 2504 Kcal/kg metabolizable energy) and forage legume. Forage legume (*Digitaria smutssi*) was chopped and mixed with the formulated feed before feeding. Routine management operations such as regular cleaning of the cages and feeders were carried out throughout the research period

Data Collection

The traits measured were body weights (BW) and linear body measurements (LBMs) namely: body length (BL), chest girth (CG), head-to-shoulder (HS), shoulder-to-tail (ST), length of hind limb (LHL), ear length (EL) and height at withers (HTW). Body weight was taken in grams using a weighing scale (Dimensions: 56 x 47 x 37cm, Model Number: KFC, Manufacturer: Yongkang Huaying weighing apparatus company limited, China) and height at withers with a ruler in centimeters. Measurements were done after weaning on a bi-weekly basis for 5 weeks (6, 8, 10, 12 and 14 weeks). All the traits, except for body weight and height at withers were measured using measuring tape in centimeters.

Statistical Analysis

The relationships among the measured traits were determined using Linear Correlation and Analysis Procedure of SAS (version 8.0, 2004).

RESULTS AND DISCUSSION

Table 1 shows phenotypic correlations among body weight and LBMs of New Zealand White × Dutch and Chinchilla× Dutch weaner rabbits. Results obtained indicated that the growth traits measured showed varying degrees of relationships. The phenotypic correlation were positive and negative, low to high ranging between 0.12 to 0.99 and were significant ($p<0.05$). For New Zealand White x Dutch, a very highly significant ($p<0.001$) correlation were obtained between BW and BL (0.99), BW and HS (0.93), BW and LHL (0.99), BW and ST (0.98), BL and HS (0.92), BL and LHL (0.98), BL and ST (0.94), HS and LHL (0.89), HS and ST (0.88), LHL and ST (0.98). A significant ($p<0.05$) correlation were observed between other traits except for BW and HG (-0.52), BL and CG (-0.53), HG and HS (-0.79), CG and LHL (-0.43), CG and EL (0.33), CG and ST (-0.41), CG and HTW (-0.72), HS and EL (0.31), EL and HTW (0.23) where there were non-significant ($p>0.05$) difference. For Chinchilla x Dutch a very high ($p<0.001$) correlation were obtained between BL and ST (0.89), BL and HTW (0.88), HG and LHL(0.98), HG and EL(0.89), HG and ST (0.85), CG and HTW (0.89), LHL and ST (0.94), LHL and HTW (0.93), ST and HTW (0.92) while other correlated traits are significant ($p<0.05$) with the exception of BW and EL (0.23), BL and HS (0.12), BL and EL (0.32). The highest correlation coefficient was obtained between LHL and ST (0.94) while the lowest correlation coefficient was between BL and HS (0.12).

In the weaner rabbits obtained from the crosses, both positive and negative correlation coefficients were obtained in all the growth traits measured in NZ×DU while in CH×DU only positive correlation coefficients were found. Phenotypic correlation between body weight and other linear parts ranged from low to high (0.33-0.99) in NZ×DU while CH×DU had 0.12-0.93. The high coefficients of correlation suggest possible strong relationship between the traits, and the likelihood of pleiotropic effect of genes operating on them. Therefore, any attempt to select for one trait in a breeding programme will automatically result to improvement on those other correlated traits. Previous studies have indicated positive and significant correlations between live weight and body dimensions in farm animals, body dimensions are good indicators and can be used to predict the body weight of rabbits. The positive and negative phenotypic correlations obtained in NZ×DU disagreed with the findings of Okoro *et al.* (2010) while the positive correlation coefficients in CH×DU agreed with the findings of the authors. The authors

observed positive relationship between body weight and LBMs such as EL, BL, HS, LL, HG and TL in Chinchilla breed at week 3, 6 and 8 weeks of age. The possible reason for this variation may be due to breed or genotype differences, age of the animals and other environmental factors like climate, temperature and feeds. The positive correlation coefficients obtained in CH×DU simply means that as any one LBMs or BW is increasing a corresponding increase is expressed in the other while the negatively correlated traits are the reverse. The moderate to high correlation coefficients obtained corroborates the work of (Akano and Ibe 2005) in various breeds of rabbits. The results obtained in NZ×DU are similar with the findings of Tiamiyu *et al.* (2000) who reported both positive and negative correlation coefficients among medium breed rabbits.

CONCLUSION

The result indicates that offspring from cross between NZ×DU had both positive and negative correlation coefficients while all the correlation coefficients observed in CH×DU were positively correlated between the LBMs and body weight.

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Table 1: Phenotypic correlations among Body weight and LBMs of New Zealand White × Dutch and Chinchilla× Dutch weaner rabbits

Genotype	Traits	BW(g)	BL(cm)	CG(cm)	HS(cm)	LHL(cm)	EL(cm)	ST(cm)	HTW(cm)
NZ×DU	BW(g)	-							
	BL(cm)	0.99***	-						
	CG(cm)	-0.52^{NS}	-0.53^{NS}	-					
	HS(cm)	0.93***	0.92***	-0.79^{NS}	-				
	LHL(cm)	0.99***	0.98***	-0.43^{NS}	0.89***	-			
	EL(cm)	0.61*	0.54*	0.33^{NS}	0.31^{NS}	0.67*	-		
	ST(cm)	0.98***	0.94***	-0.41^{NS}	0.88***	0.98***	0.72**	-	
	HTW(cm)	0.66*	0.57*	-0.72^{NS}	0.81**	0.60*	0.23^{NS}	0.70**	-
CH×DU	BW(g)	-							
	BL(cm)	0.41*	-						
	CG(cm)	0.49*	0.61*	-					
	HS(cm)	0.48*	0.12^{NS}	0.82**	-				
	LHL(cm)	0.54*	0.74**	0.98***	0.75**	-			
	EL(cm)	0.23^{NS}	0.32^{NS}	0.89***	0.76**	0.81**	-		
	ST(cm)	0.58*	0.89***	0.85**	0.54*	0.94***	0.56*	-	
	HWT(cm)	0.40*	0.88***	0.89***	0.46*	0.93***	0.74**	0.92***	-

NZ=New Zealand White, DU= Dutch, CH=Chinchilla, BW=Body weight, BL=Body length, CG=Chest girth, HS= Head-to-shoulder, LHL=Length of hind leg, HTW=Height at wither, EL=Ear length, ST=Shoulder-to-tail drop, NS= Not significant ($p>0.05$), *=$p<0.05$, **=$p<0.01$, ***= $p<0.001$.

SECTION THREE: GENETICS AND DISEASE CONTROL

ENDEMICITY OF *S. haematobium* AND CO-INFECTION WITH SALMONELLA BACTERIA AMONG SCHOOL PUPILS IN TALATA MAFARA LOCAL GOVERNMENT AREA, ZAMFARA STATE, NIGERIA.

Mudassir, I., [1] Suleiman, A.B.,[2] Dibal, D.M.,[1] Abdulhamid, Y.,[3] Abba, A. M.[1] and Omenesa, R. L.[1]

[1]Department of Biological Sciences, Nigeria Defence Academy, P. M. B. 2219 Kaduna Nigeria
[2]Department of Microbiology, Ahmadu Bello University Zaria Kaduna, Nigeria
[3]Biology Department, SPRS, Umaru Musa Yar'adua University, P. M. B. 2218, Katsina, Nigeria
Author's email address/phone numbers: imudassir63@gmail.com, 08061524257

Abstract
There is high incidence of chronic Salmonella bacterial infections and carriers in schistosomiasis endemic areas. A study was carried out to determine the endemicity of S. haematobium and co-infection with salmonella bacteria among school children in three selected Primary Schools from T/mafara Local Government area, Zamfara State. A total of 360 urine samples were analyzed by sedimentation technique and presence of S. haematobium eggs were determined microscopically. Infected urine samples were cultured for Salmonella bacteria and identified/confirmed by standard Biochemical/Serology tests. The overall incidence of S. haematobium was 37.2% and co-infection with Salmonella spp. was 20.0% with Gurbi Primary School having the highest incidence rate than Yalwa and Abubakar Tunau Primary Schools. In both cases of S. haematobium and co-infection with Salmonella spp, Male pupils had the highest rate of infections than the female pupils. Age group 8-11 had highest incidence rate of 44.2% while the lowest (20.0%) was in age group 12-15. Co-infection rate was high among the age group 4-7 with 69.4% than age group 12-15 with 12.5%. Chi-square analysis revealed no significant difference (P>0.01) in the rate of infections with all the tested variables. Salmonella bacteria isolated was Salmonella typhi 39.0%, S. paratyphi A 30.0% and S. paratyphi B 21.4%. Cases of co-Infection with enteric fever bacteria was found in the urine of tested pupils hence could complicate treatment. Therefore there is need for public health enlightenment among pupils to adapt preventive measures with control programs for snails' intermediate host.

Key words: Co-infection, Schistosomiasis, *S. typhi, S. haematobium*

INTRODUCTION

Schistomiasis haematobium is a helminthes parasite that causes urinary schistosomiasis, a disease that is characterized by bloody urine, lesion of bladder, kidney failure and bladder cancer in children (Butterworth, 2007), Though the disease kills few people, its clinical effects, incidence and association with other diseases and expansion of agriculture and water development projects, movement of population and increase in population density and some social habits like passing urine and faeces near water bodies makes it a problem of great health importance (WHO, 2010). In other hand *Salmonella* infections of humans and animals continue to be a major public health problem worldwide and also have a large negative economic impact on food production (Chiang, 2008). The main sources of Salmonellosis in humans are food, animals and their products such as raw eggs, poultry meat and pork (Chaunchom, 2003). The true incidence of *Salmonella*-associated diseases (SADs) in humans is difficult to evaluate because of lack of an epidemiological surveillance systems, especially in developing countries. The annual incidence of typhoid is estimated to be about 17 million cases worldwide, and is highest in those between the ages of 5 and 12 years (WHO, 2014).

In the study area, Sokoto Rima River Basin Development Authority (SRRBDA) has been executed numbers of development irrigation project for rice farming in progress. This situation provide suitable environment for the survival of the intermediate host of the causal agent of schistosomiasis. Though there are reports of schistosomiasis in Zamfara states and other neighboring states (Adamu, *et al*., 2001; ladan *et al*., 2011; Bala, *et al*., 2012),

They predominantly depend on bakalori dam for their daily water needs and other water activities. Hence, this study reported endemicity of *S. haematobium* and co-infection with Salmonella bacteria among school pupils in Talata Mafara LGA area Zamfara State, Nigeria.

MATERIALS AND METHODS

Study area

Zamfara state is located in the Sudan savannah zone in the extreme North-west part of Nigeria, between longitude 5°44'30'E and 6° 0'0" E and latitude 12° 33'30"N and 12°49'0"N. Rainfall in this area is between May/June to early October, when the natural water bodies are often flooded (Adamu *et al*., 2001). Annual rainfall in that area ranges between 500 and 1300 mm, while the dry season last for 7 to 8 month that is November to May. It shared common boarders with sokoto state to the North, Niger state to the west and Katsina state to the East. The total land area is about 1892 square kilometers. The settlement areas in the district are mostly low lying with various types of fresh water bodies such as dams and rivers. This area has Bakalori dam. The vegetation is mainly grassland with trees. T/mafara district has mainly farmers and fishermen. People around the area are very poor and dependent on fish, irrigation farming and other animals for food and nutrition and they use water from Bakalori dam for their domestic need.

Sampled schools

Three (3) schools were randomly selected from different area of Talata Mafara. The primary schools selected were: Yalwa, Abubakar Tunau and Gurbi primary schools.

Sample Collection

A total of 360 urine samples 120 each from school were collected among school age children by stratified random sampling method. Structured questionnaires were used to collect some information on age, and sex; from the children during samples collection. Each child was given a cleaned, dried screw capped urine specimen bottle which were appropriately labeled and instructed by demonstration on how to collect the urine samples used for this study. The samples were collected from 10 am-12 pm, placed in black polyethylene bag and transported to the Parasitology and Microbiology research laboratories of Usmanu Danfodio University Sokoto for parasitological and bacteriological analysis.

Laboratory (Parasitological) Analysis

Sedimentation technique for identification of *S. heamatobium* eggs

Urine samples collected were examined macroscopically for the evidence of haematuria. A drop was placed on a slide which was examined under the microscope at x10 and x40 magnifications. Eggs were confirmed by the position of spine (at terminal end) (Cheesbrough, 2006).

Bacteriological Analysis for the Isolation of *Salmonella* Bacteria

About 2ml of positive urine samples (those infected with *S. heamatobium* eggs) were inoculated into sterile culture tubes containing about 20ml of thioglycolate broth agar and incubated for 24-48 hours at 37ºC. At the end of the incubation period, growth was observed by change of color of the medium from pink to milk and or by formation of turbidity. Positive tubes were sub-cultured by streak plate method of inoculation into pettri dish plates containing Salmonella-Shigella agar. Negative tubes were discarded and considered as negative result for co-infection with bacteria after sub-culturing for 10days. The Salmonella-Shigella agar plates were incubated overnight at 37ºC and were examined after 24-48 hours for growth. Suspected colonies of *Salmonella* bacteria (fine semitransparent colonies with black centers) were sub cultured on a pettri dish plate containing fresh MacConkey agar to obtain a pure culture. Semitransparent (colorless) colonies were seen. The pure culture obtained were inoculated and stored in agar slant bottles containing nutrient agar for storage and further characterization (Bayeh *et al.,* 2010).

Gram Staining was carried out according to (Eugene *et al.*, 2007).
Immersion oil was added on stained slides and viewed under x100 objective lens, rod bacilli colonies with pink/red color was considered as gram negative bacteria and were further taken for biochemical test.

Biochemical Identification of the Bacterial Isolates:

Gram-negative Suspected colonies were identified using biochemical test which include Citrate Utilization, Triple Sugar Iron (TSI), Urease, Indole, Methyl Red (MR), Voges Proskaur and Oxidase tests (Brown, 2012).

Serological Test for *Salmonella* spp.

Isolates whose biochemical characteristics conformed to those of salmonellae were further subjected to serological analysis. *Salmonella* serogroup kit obtained from Statens Serum Institute, Copenhagen 8, Denmark was used to determine the serogroup of the isolates. The serogroup kit came with 7 rabbit antiserum (D, B, C, E, G, F, and A group) and 1 monoclonal antibody (Vi). The test was carried out according to manufacturer's specification (Ochei and Kolhatkar, 2008).

Data Analysis

The data obtained were analyzed by using simple percentage while Chi-square test was
Used to compare differences at P<0.01 considered significant.

RESULTS AND DISCUSSION

Results obtained from this study showed 37.2% (134) over rall incidence rate of *S.haematobium* among the school children with Gurbi primary school having the highest rate of 42.5% (51), followed by Abubakar Tunau school with 40.0% (48) while Yalwa Primary School had the lowest incidence rate of 30.8% (37). However the study showed 20.0% (72) a total incidence rate of co-infection with Gurbi primary school having the highest rate of 64.7% (33), followed by Yalwa school with 48.6% (18) while Abubakar Tunau school had the least rate of 43.7% (21). Chi-square analysis showed no significant differences in the incidence rate of infection with selected schools at P<0.01 considered significant (**Table 1).**

Table 2: Showed the incidence of *S. haematbium* and co-infection with salmonella bacteria by Sex and Age of pupils: The incidence rate by Sex was observed with Male pupils having the highest rate (*S. haematobium*) of 44.7% (97) than female pupils with the lowest rate of 25.8% (37), In other hand Male children also had highest incidence rate of co-infection of 73.6% (53), than Female pupils with lowest rate of 26.4% (19). Among the age groups, age 4-7 years had the highest incidence rate of infection 44.2% (61) followed by 8-11 age group with 34.2% (40) and the lowest rate of 20.0% (33) was observed among the age group of 12-15 years. The incidence rate of co-infection was: Age group 4-7 showed highest rate of 69.4% (50) followed by 8-11years had 15.3% (11) and Age group 12-15 had the lowest rate of 12.5% (09)**.** Chi square analysis showed no significant difference in the incidence rate of infection with the age groups and sex of pupils **(**Table 2).

Table 3. Showed the Biochemical and cultural characterization for the identification of bacterial species isolated. The bacterial species isolated were *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B.

Table 4: Showed the frequency of occurrence of *Salmonella* spp. isolated from pupils urine with *Salmonella typhi* having the highest frequency of 39.0% (28) followed by *Salmonella paratyphi* A having 30.0% (22) and *Salmonella paratyphi* B had the lowest frequency of 21.4% (15).

Table 5: showed the serological reaction of the test isolates with 3 isolates reacted positively on both antisera D and monoclonal antibody.

Table 1: Incidence of *S. haematobium* and Co-infection with Salmonella bacteria by the selected schools sampled in Talata Mafara local government area Zamfara State.

SCHOOLS	NE	NI/P	I (%)	NI/C	I (%)
Gurbi	120.0	51.0	42.5	33.0	64.7
A/Tunau	120.0	37.0	30.8	18.0	48.6
Yalwa	120.0	48.0	40.0	21.0	43.7
TOTAL	**360.0**	**134.0**	**37.2**	**72.0**	**20.0**

KEYS: NE= Numbers examined, **NI/P** = Numbers infected with eggs of parasite**, I (%)** = Percentage Incidence**, NI/C** = Numbers infected with co-infection.

Table 2: Incidence of *S. haematobium* and Co-infection with Salmonella bacteria by Sex and Age group of the pupils

Variables	NE	NI/P	I (%)	NI/C	I (%)
Sex/Gender					
Male	217.0	97.0	44.7	53.0	73.6
Female	143.0	37.0	25.9	19.0	26.4
Total	**360.0**	**134.0**	**37.2**	**72.0**	**20.0**
Age Group					
4 -7	138.0	40.0	34.2	50.0	69.4
8-11	117.0	61.0	44.2	11.0	15.3
12-15	165.0	33.0	20.0	09.0	12.5
Total	**360.0**	**134.0**	**37.2**	**72.0**	**20.0**

KEYS: NE= Numbers examined, **NI/P** = Numbers infected with eggs of parasite**, I (%)** = Percentage Incidence**, NI/C** = Numbers infected with co-infection.

Table 3: Biochemical and cultural characterization of bacteria isolated from pupils urine

Biochemical and cultural characteristics																	**Probable Isolates**
Isolates no.	**Gram reaction**	**Urease**	**Methyle red**	**Voges Proskauer**	**Lactose**	**Manitol**	**Glucose**	**Sucrose**	**Oxidase**	**Citrate**	**Motility**	**Indole**	**Slope**	**Butt**	**H_2s**	**Gas**	
1.	GNB	-ve	+	-ve	-ve	+	+	-ve	-ve	-ve	+	-ve	R	Y	+	-ve	*Salmonella typhi*
2.	GNB	-ve	+	-ve	-ve	+	+	-ve	-ve	-ve	+	-ve	R	Y	+	+	*S. paratyphi* A
3.	GNB	-ve	+	-ve	-ve	+	+	-ve	-ve	+	+	-ve	R	Y	+	+	*S. paratyphi* B

Keys: GNB = Gram Negative Bacilli, **-ve** = Negative reaction, **+** = Positive reaction, **R** = Red and **Y** = Yellow

Table 4: Frequency of isolation of bacteria isolated from infected urine samples of pupils

Organisms (probable Isolates)	Number of occurrence	Frequency (%)
Salmonella typhi	28	39.0
Salmonella paratyphi A	22	30.0
Salmonella paratyphi B	15	21.4
Total =	**72**	**100**

Table 5: Serogrouping of the isolates in co-infected Patients.

Antisera	No. of isolates tested	Positive agglutination (+)	Negative agglutination (-)
Group D	16	3	13
Monoclonal ATB (VI)	21	3	19

Key; ATB = Antibody

DISCUSSION

The result of this investigation has revealed that *S. haematobium* and associated cases of co-infection with salmonella bacteria is highly prevalent in the study area. The observation of a relationship between *Salmonella spp.* and *S. haematobium* is in agreement with several other previous works though with varied rates of 6.4% in Jos and 5.4% in Kaduna (Igwe and Agbo, 2014). The total incidence of 20.0% (co-infection) is high compared to other works such as Modebe *et al*., 2014 reported on the prevalence of co-infection (13.6%) but it is however low results when compared with report by Mazin *et al.,* 2017, *Salmonella* spp. were positive in 64 schistosomiasis patients (30.9%), but is in agreement in sex incidence rate, where it was highest among the males with 22 (28.6%) than in females 6 (14.3%), on a dual infection study of enteric *Salmonella* spp. in Jos, Nigeria. Sample size and sampling type could have contributed to the slight differences in the results (Modebe *et al*., 2014).

Of the 134 infected pupils tested who were positive for *S. haematobium* eggs, only (20.0%) had co-infection with *Salmonella* spp. present in their urine culture. Compared to the results reported by Mazin *et al.,* 2017, *Salmonella* spp. were positive in 64 schistosomiasis patients (30.9%) and Salih *et al.,* 43 (29.4), however this can be attributed to the fact that Mazine *et al.,* 2017 used both stool and urine culture method while Mogasale *et a.,* 2014 used Widal test in their study whereas the current one applied urine culture method alone.

In the present study, *Salmonella* species were isolated from *S. hematobium* positive subjects. These findings out distance those reported by Modebe *et al.,* 2014 who had stated that *Salmonella* bacteraemia has been described in association with *S. mansoni* (Hathout *et al*., 2006). In this study the association of *Salmonella* species was found with *S. haematobium*, this is in agreement with Mazin *et al.,* 2017 who found *Salmonella* species more with urinary schistosomiasis compared to intestinal schistosomiasis. In contrast to this findings, other studies detected more association with *S. mansoni*, *S. intercalatum* and *S. japonicum* as against *S. haematobium* (Muniz *et al*., 2009).

This study is in agreement with the findings of (Ukaegbu *et al*., 2014) in Jos, Nigeria who observed 68(42%) for Widal test against 9(5.6%) for urine culture and suggested that the use of Widal test alone in the diagnosis of typhoid fever is unreliable, misleading and should be discouraged; and that culture technique still remains the gold standard in the diagnosis of typhoid fever and should be embraced.
Three (3) species of *Salmonella* were isolated from pupil's urine infected with eggs of *S. haematobium* they are *Salmonella typhi*, *Salmonella paratyphi* A and S*almonella paratyphi* B among which *S. typhi* was found to be the most frequent 28 (39.0%). It is low when compared with the findings of Mazin *et al.,* 2017 with *S. typhi* having the highest frequency of (70.7%) on the association between schistosomiasis and enteric fever in a single *Schistosoma* endemic area in Sudan, where urinary schistosomiasis was found in 95 patients (46%) respectively.

CONCLUSION

High rate of infection with *S. haematobium* has been recorded and the study areas are therefore, considered to be endemic. Cases of co-infection of *S. haematobium* with (*Salmonella typhi*, *S. paratyphi* A and *S. paratyphi* B) were also established and a relationship between *S. haematobium* and *Salmonella* bacteria exist supporting the fact that their co-infection could complicate treatment. Incidence of infection with *S. haematobium* and *Salmonella spp* has been found to be associated with age and sex factors of the sampled subjects.

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