Mutation breeding is an alternative to conventional method of breeding especially in plants. This method has been utilized extensively in developed nations, but the story is different in African countries especially in Nigeria. This book is an effort that bring to limelight the state of mutation breeding in Nigeria. It considered information on some important crops that have received attention in the area of mutation breeding.

Dr Muhammad Liman Muhammad is an academic staff and young researcher in Department of plant biology, Federal University of Technology,Minna, Nigeria. He is a plant geneticist and breeder. He has special interest in mutation breeding and currently searching for suitable place for his postdoctoral programme.

978-613-9-85286-4

Mutation Breeding in Nigeria

Liman (Ed.), Ahmed, Abdulazeez

Muhammad Liman (Ed.)
Olamide Ahmed
Oladipupo Abdulazeez

Status of mutation breeding of crop plants in Nigeria

LAP LAMBERT Academic Publishing

Muhammad Liman (Ed.)
Olamide Ahmed
Oladipupo Abdulazeez

Status of mutation breeding of crop plants in Nigeria

Muhammad Liman (Ed.)
Olamide Ahmed
Oladipupo Abdulazeez

Status of mutation breeding of crop plants in Nigeria

LAP LAMBERT Academic Publishing

Imprint

Cover image: www.ingimage.com

Publisher:
LAP LAMBERT Academic Publishing
is a trademark of
International Book Market Service Ltd., member of OmniScriptum Publishing Group
17 Meldrum Street, Beau Bassin 71504, Mauritius

Printed at: see last page
ISBN: 978-613-9-85286-4

STATUS OF MUTATION BREEDING OF CROP PLANTS IN NIGERIA

CONTENTS

OVERVIEW OF INDUCED MUTAGENESIS IN NIGERIA

Muhammad Liman MUHAMMAD[1], Olamide Ahmed FALUSI[1], Matthew Omoniyi ADEBOLA[1], Oladipupo Abdulazeez Yusuf DAUDU[1], Dorcas Ropo ABEJIDE[1], Aishatu Adamu GADO[1],

[1] Department of Plant Biology, Federal University of Technology, Minna, Nigeria

Corresponding author: ml.muhd@futminna.edu.ng, +2348060901051

Introduction

Mutagenesis is an induction of sudden heritable changes in the genetic makeup of an organism through the use of chemical, physical or biological agents (Roychowdhury and Tah 2013). According to Oladosu *et al*. (2016), three types of mutagenesis are adopted in mutation breeding. The first is induced type, in which mutations result due to application of physical mutagens (like gamma rays, X-rays, ion beam etc.) and or chemical mutagens (such as sodium azide, Ethyl methane sulphonate etc.). Site-directed is the second

type and it involves creating a mutation at a defined site in a DNA molecule (Oladosu *et al*., 2016). The third is insertion mutagenesis which involves insertion of DNA molecule. This can be achieved either through genetic transformation and insertion of T-DNA or activation of transposable elements (Kharkwal and Shu, 2009; Forster and Shu, 2012). Induced mutagenesis shall be our area of discussion as it is the most commonly used technique in Nigeria.

History of mutagenesis

The history of plant mutation could be dated back to 300 BC with reports of mutant crops in China (Kharkwal, 2012). Mutation induction by radiation advanced as a field of research after Stadler demonstrated the mutagenic action of X-rays in maize, barley and wheat (Oladosu *et al*., 2016). Tobacco plant was the first to have its mutant variety commercialized in 1934. Earlier to 1995, a total of 77 mutant cultivars was reported by Acquaah (2006)**.** However, in 1995, the number of mutant varieties commercialized and released rouse to 484. Since then this number has grown sharply with new cultivars being reported

continuously in different continents (Oladosu *et al*., 2016)**.** Some agronomic traits modified by mutation breeding include resistance to lodging, early maturity, hardiness to winter, quality of the plant products (such as protein and lysine content) and many ornamental mutants. According to IAEA (2015), China, Japan, India, Russia and Netherland are the first five ranked countries in terms of released mutant varieties. Although mutation induction as a field is old, it has gained very little attention in Africa and particularly in Nigeria. Nigeria (giant of Africa) is lagging behind in the area of induced mutagenesis. Internationally, Nigeria has record of only 3 released mutant varieties which were registered between 1980 and 1988 (Table 1).

Table 1: The List of top five rank countries and some African countries with released mutant varieties.

Country	Registration date	No. of released varieties
China	1957-2011	810
Japan	1961-2008	481
India	1950-2010	330
Russia	1965-2011	216
Netherlands	1954-1988	176
Cote D'Ivoire	1976-1987	25
Mali	1998-2000	15
Iraq	1992-1995	23
Egypt	1980-2011	9
Nigeria	1980-1988	3

Source (IAEA, 2015)

Kharkwal and Shu (2009) brought to limelight achievements in the area of food security in Africa through mutagenesis. Egypt, Sudan and Ghana made significant achievements while Nigeria was not mentioned due to her little or no achievements in the recent years. Egypt for example introduced two mutant varieties of rice; semi-dwarf, 'Giza 176' (1989) and 'Sakha 101' (1997). Similarly, in Sudan a banana mutant cultivar (Albeely) was released in the year 2003 which excelled the yield of the existing cultivars by 40% and has better crop stand and fruit quality (Table 2).

Table 2: Some African countries that have significant achievements in recent years

Country	Crop	Achievements	Source
Egypt	Rice	Increased yield from 3.8 t/ha to 8.9 t/ha	González *et al*., 2008
Sudan	Banana	Increments in yield by 40%	Ali, 2008
	Ground nut	Drought tolerant	Ali, 2008
	Tomato	Resistant to yellow leaf curl virus and good poundability	Ali, 2008
Ghana	Cassava	High dry matter content (40%)	Danso *et al*., 2008
	Cocoa	Resistant to cocoa swollen shoot virus	Danso *et al*., 2008

Mode of operation of induced mutagenesis and its products

All induced mutations (whether by physical or chemical method) occur at random across the whole genome and within any locus or gene (Forster and Shu, 2012). In addition to the probability of generating mutations for any gene of interest, induced mutagenesis, also enables in a predictive manner, the development of multiple mutations for any target gene (Oladosu *et al*., 2016). The source of genetic diversity for crop breeding as well as functional analysis of the targeted genes, are multiple mutant alleles. The bulk of gene mutations produced by radiation efficiently destroy gene function as the gene is either knocked out or the mutation product is nonfunctional. Therefore the bulk of mutated genes are recessive in nature.

Importance and Advantages of induced mutagenesis

Induced mutagenesis as plant breeding strategy, is a recognized, harmless, robust and low-cost technique (Mba, 2013). In some crops, many parents used in hybridization had their desired genes from induced

mutation (Maluszynski *et al*., 2000). Mutagenesis has over time become an important aspect and useful tool in plant breeding. It has helped in increasing variations in plants which is the raw material needed for plant breeding. These variations form the basis for the evolution of new and better forms, varieties or species.

Induced mutations has helped in supplementing natural genetic variability which may not occur as fast as needed by the plant breeders considering the rapid changing needs for food and fiber, there is need for a more rapid generation of new genetic forms which can only be supplied by induced mutations (Nilan *et al*., 1977). The effectiveness of mutagenesis in generating desired variation in crop plants has been widely proven and properly documented (Maluszynski *et al*., 2000). Genetic variation of desirable traits for crop improvement, is a core requirement in plant breeding. According to Maluszynski *et al*. (2000) mutation techniques are highly efficient especially in generating desirable variation in crop plants.

Induced mutations have been used to produce improved varieties of crop plants with desirable characters such

as higher yield, disease resistance, improved nutritional composition and abiotic stress resistance. Mutagenesis produces new phenotypic characters which increases the scope for selection and hybridization. Therefore artificial induction of mutation can be of help in hybridization work especially if the parents lack variability or are deficient in certain desirable characters (Aamir 2016).

Mutation breeding is also a faster way to produce crops with improved traits compared to the traditional hybridization method which may take up to seven years, but with induced mutation, one can arrive at crops with improved traits in the M_2 or M_3 mutants. Induced mutation is the ultimate source to alter the genetics of crop plants that may be difficult to achieve, through cross breeding and other breeding procedures (Khan and Wani, 2004). In addition, for some crops that are propagated by vegetative method, mutagenesis provides an alternative and useful tool in improving such crops in which hybridization cannot be used (Aamir, 2016). Rapid improvement of plant yield and quality could be ascribed to direct use of mutation

in the development of molecular maps in structural and functional genomics (Raina *et al*., 2016).

Conclusion

Literatures have shown many elementary works on plant mutagenesis in Nigeria. However, the number of registered mutant varieties by Nigeria as a nation is an indication that the country is lagging behind in the area of Plant mutagenesis. Achievements made in recent years by other African countries like Egypt, Sudan, Ghana implies that Nigeria has not given enough attention to this area of research.

References

Aamir R Rafiul A LShahnawaz K Rahul A Younes R T Kouser P (2016). Role of mutation breeding in crop improvement - past present and future Asian research journal of Agriculture (2)2:1-13.

Acquaah, G. (2006). Principles of plant genetics and breeding. Chichester: Wiley-Blackwell.

Ali, A.M. (2008). Overview of mutation breeding in Sudan. Abstract p.8. In: Book of Abstracts, FAO/IAEA International Symposium on Induced

Mutations in Plants. 12-15 Aug., 2008, Vienna, Austria.

Danso, K.E., Safo-Katanka, O., Adu-Ampomah, Y., Oduro, V., Amoatey, H.M., Asare, O.K., Ofori Ayeh, E., Amenorpe, G., Amiteye, S and Lokko, Y. (2008). Application of induced mutation techniques in Ghana: Impact, challenges and the future. Abstract p.108. In: Book of Abstracts, FAO/IAEA International Symposium on Induced Mutations in Plants. 12-15 Aug., 2008, Vienna, Austria.

Forster, B.P and Shu, Q.Y. (2012). *Plant mutagenesis in crop improvement: basic terms and applications*. In: Shu QY, Forster BP, Nakagawa H, editors. Plant mutation breeding and biotechnology. Wallingford: CABI; p. 9-20.

González, M.C., Pérez, N., Cristo, E and Mayra, R. (2008). Salinity tolerant mutant obtained from protons radiations. Abstract p.56. In: Book of Abstracts, FAO/IAEA International Symposium on Induced Mutations in Plants. 12-15 Aug., 2008, Vienna, Austria.

International Atomic Energy Agency (IAEA). (2015). IAEA mutant database. Vienna: International Atomic Energy Agency; c2015 [accessed July 2015]. Available from: http://mvd.iaea.org/

Khan, S and Wani M.R. Isolation of high yielding mutants in mungbean (*Vigna radiata* (L.) Wilczek). *Tropical Agriculturist*, 154:51-59.

Kharkwal, M. C and Shu, Q.Y. (2009). *The role of induced mutations in world food security*. In: Shu QY, editor. Induced plant mutations in the genomics era. Rome: Food and Agriculture Organization of the United Nations; 2009. p. 33-38.

Kharkwal, M.C. (2012). A brief history of plant mutagenesis. In: Shu QY, Forster BP, Nakagawa H, editors. Plant mutation breeding and biotechnology. Wallingford: CABI. p. 21-30.

Maluszynski, M., Nichterlein, K., Van Zanten, L. and Ahloowalia, B.S. (2000).Officially released mutant varieties- The FAO/IAEA Data.

Mba, C. (2013). Induced mutations unleash the potentials of plant genetic resources for food and agriculture. *Agronomy*, 3(1), 200-231.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. R., Miah, G and Usman, M. (2016) Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, 30(1), 1-16, doi: 10.1080/13102818.2015.1087333.

Raina, A., Laskar, R. A., Khursheed, S., Amin, R., Tantray, Y.R., Parveen, K and Khan, S. (2016). Role of Mutation Breeding in Crop Improvement-Past, Present and Future. *Asian Research Journal of Agriculture*, 2(2): 1-13.

Roychowdhury, R and Tah, J. (2013) Mutagenesisa potential approach for crop improvement. In: Hakeem KR, Ahmad P, Ozturk M, editors. Crop improvement: new approaches and modern techniques. New York (NY): Springer, p. 149-187.

IMPROVEMENTS OF SOME CROP PLANTS IN NIGERIA THROUGH GAMMA RAY

Muhammad Liman MUHAMMAD[1], Olamide Ahmed FALUSI[1], Oladipupo Abdulazeez Yusuf DAUDU [1]., Abdulhakeem ABUBAKAR [1], Aishatu Adamu GADO[1], Josephine Teni KOLO[1],

[1] Department of Plant Biology, Federal University of Technology, Minna, Nigeria

Corresponding author: ml.muhd@futminna.edu.ng, +2348060901051

1.0 Introduction

This chapter brings to limelight some outcomes of researches in induced mutagenesis using gamma ray, in selected crops in Nigeria. Most of the crops discussed are those that have shown positive responses toward improvement by gamma radiation. Gamma radiation is outstanding among other physical mutagens as it has been extensively used for mutation induction for both seed and vegetative propagated crops (Jain and Suprasanna, 2011). Gamma radiation is a heavy ion

beam (HIB) and are predominantly used for inducing mutations in plants (Jain, 2010).

2.0 Sesame (*Sesamum indicum* L.)

Sesame (*Sesamum indicum* L.) is a very ancient crop and one of the earliest domesticated oil crops in the world (Ashri, 2007). Some archeological findings have supported that sesame is one of the most important crops in the world (Kumar and Yadava, 2010). Sesame is locally called by various names in Nigeria; 'Ridi' (Hausa), 'Eeku' (Yoruba), Ekuku (Igbo), 'Esso' (Nupe). Sesame belong to family pedaliacae and genus *Sesamum*. The genus consists of about thirty six (36) species out of which the commonly recognized is *Sesamum indicum* L. (Falusi, 2006). According to Falusi and Salako (2003), the *S. indicum* and *S. radiatum* are indigenous to Nigeria and naturally self-pollinated (Falusi and Salako, 2003). Evolution and practical breeding both depend on genetic variation which is achievable through mutagenesis (FAO, 2011). Mutagenesis has been identified as one of the effective method of inducing genetic variability in many crops (Wongyai *et al*., 2001). Through induced mutation, a

large number of new cultivars have been released globally (Diouf *et al*., 2010). In Nigeria gamma radiation has been routinely in improvement of many crops including sesame.

2.1 Effects of gamma irradiation on vegetative parameters of sesame

Muhammad (2018) evaluated three varieties of sesame after exposure to different doses of gamma irradiation. He reported both positive and negative correlations between some vegetative parameters and irradiation doses. He also recommended the dose range 150- 550 Gy to be effective in improvement of vegetative parameters in sesame.

Table 4.6: Correlation coefficient of Vegetative parameters of M_1 plants and irradiations doses

Vegetative parameter	Variety		
	NCRIBEN-04E	NCRIBEN-01M	NCRIBEN-03L
Germination	-0.848	0.844	0.071
Emergence	0.207	0.925*	-0.276
Plant Height	-0.462	0.230	-0.121
No. of Branches	0.745	0.405	-0.491

*Significant at 0.05 Level of Significance
Muhammad (2018)

Figure 1: Sesame plant bearing flowers and fruits

2.2 Reduction of seed loss through capsule shattering in sesame

Shattering of capsules at maturity has posed serious problem in sesame production worldwide (Ashri, 1994) and the majority of the world's sesame (probably over 99%) have shattering capsule (Langham, 2001). Figure 2 shows a sesame plant with shattering capsule. In attempt to overcome seed loss due to shattering, Muhammad *et al.* (2017) exposed three varieties of Nigerian Sesame to four different doses (250, 350, 450 and 550 Gy) of gamma irradiation. The results of first mutant generation revealed higher seed retention capability in irradiated plants over their respective parental stocks (See Table 1)

Figure 2: A. Mutant with closed capsule; B. shattering capsule Source: Muhammad *et al.* (2017)

Table 1: Increments in Seed retention capability of irradiated plants over their parental stocks

Variety/Dose (Gy)	I (%)	I'
NCRIBEN-04E		
0	20.97	-
250	30.06	30.24
350	28.25	25.77
450	40.59	48.34
550	63.99	67.23
NCRIBEN-01M		
0	50.54	-
350	51.96	2.73
550	62.18	18.72
NCRIBEN-03L		
0	49.93	-
250	57.84	13.68

I = Seed retention in inverted position; I'= increment in retention capability of the irradiated seeds over their parental stocks

2.3 Creating genetic variability in sesame through gamma ray treatment

The success of any interbreeding population is dependent on its variability. Genetic variation of desirable traits for crop improvement, is a core requirement in plant breeding. Inadequate genetic variability has remained one of the setbacks of plant breeding programs, especially in sesame. According to Monpara (2016), the essence of sesame breeding for more yield can be attributed to its comparatively low seed yield and that cultivation of inherently low yielding varieties have been pointed as one of the factors responsible for the low average yield of sesame. According to Maluszynski *et al*. (2000) mutation techniques are highly efficient especially in generating desirable variation in crop plants. Gamma rays has been reported to be the effective and economical among all the physical mutagens for crop improvement. Muhammad *et al*. (2018) evaluated M_1 and M_2 generations of three varieties of sesame for spectrum and frequency of mutation induced by Gamma radiations. In M_1 plants a clear and significant alterations in capsule morphology and orientation were

reported. Four mutant with respect to capsules: multicarpellate capsule, multiple capsule per leaf axil, indehiscent capsule and terminal capsules were reported (See Figure 3, 4 and 5). The mutant capsule traits especially multicarpellate capsule and multiple capsule per leaf axil have been reported to have direct impact on yield of the crop. In selection for high yielding, mutants with higher number of carpels per capsule and capsule per leaf axil are inevitable.

Sesame is known to be indeterminate in growth habit which is responsible for non-synchronization of the capsules. Mutant with terminal capsules are indication of determinate growth and such can cater for non-synchronization of capsule.

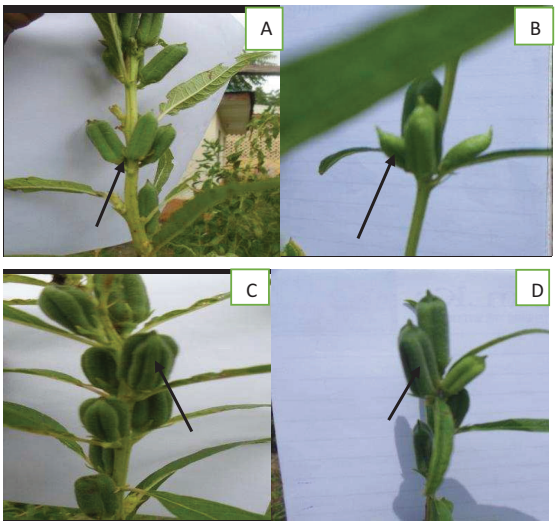

Figure 3: Mutants from NCRIBEN-04E and the control; A= Control with Multiple capsule at leaf axil and bicarpellate, B= a mutants with multiple capsules at leaf axil and indehiscent capsule, C = a mutants with tricarpellate capsule, D = a mutant with terminal capsule. **(Source Muhammad *et al*., 2018)**

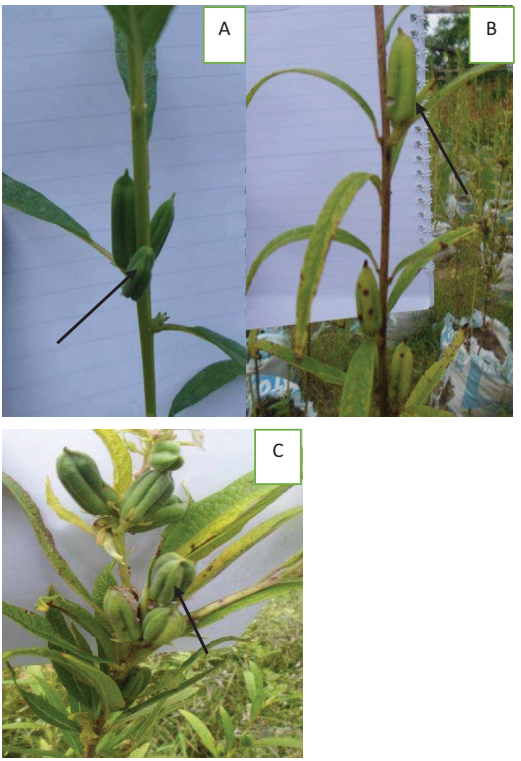

Figure 4: Mutants from NCRIBEN-01M exposed to 350 Gy and the control; A= a mutants with multiple capsules at leaf axil and bicarpellate, B = Control with Single capsule at leaf axil and bicarpellate, C = a mutants with multiple capsules at leaf axil and tricarpellate. **(Source Muhammad *et al*., 2018)**

Plate III: Mutants from NCRIBEN-03L at 450 Gy and the control; A= a mutants with multiple capsules at leaf axil, B = Control with Single capsule at leaf axil, C = a mutant with tricarpellate capsule. **(Source Muhammad *et al*., 2018)**

When you make a critical observation of features of M_2 lines, it will be noticed that some of the mutant lines acquired new desirable traits but also lost (an existing) desirable trait (Table 2). For example line 04E-550-G1-3 acquired tricarpellate capsule but now single (1) capsule per leaf axil instead of 2-3 in the parental stock. The two traits can be brought together through hybridization.

Table 2: The characteristics features of M_2 lines of sesame and their parental stock

Lines	Carpels or locules Per capsules	Capsule number per leaf axil
NCRIBEN-04E	2	2-3
04E-550-G1-3	3	1
04E-550-G2-3	2	2-3
04E-550-G3-3	2	2-3
NCRIBEN-01M	2	1
01M-350 -G1-2	3	1
01M-350 –G2-2	2	1
NCRIBEN-03L	2	1
03L-250-G1-1	2	1
03L-450-G1-2	2	1
03L-450-G2-2	3	1

(Source: Muhammad *et al*., 2018)

3.0 Cereals

3.1 *Digitaria exilis*

Digitaria exilis commonly known as Acha is often reffered to as 'hungry rice' by indigenous people of West Africa who consume this grain. It also belongs to grass family (Robert *et al*., 2013). Animasaun *et al*. (2013) evaluated M_1 generation of Acha for vegetative and yield parameters after gamma irradiation treatment. All the doses (20, 40, 80 and 100 Gy) had positive increments for all the parameters except days to maturity that were negative. In addition, the node per tiller and plant height had negative values for dose 100 Gy (See Table 3). The negative values implies decrease in those parameters which might be due to irradiation doses. The positive values implies increase in the parameters. In mutation breeding both positive and negative increment in quantitative and qualitative trait can be of interest depending on the character in question. For example in all cereal crops, number of tiller per plant can be directly linked to higher yield and thus in evaluating population for selection, individuals with higher number tillers will not be overlooked. From

Table 3, the dose 40 Gy with value 37.77 implies that plants treated with the dose performed better than the parent (control) by 37.77% for instance, if the parent produced 100 tillers per plant, then the irradiated ones (40Gy) will produce 137.77 per plant. However, increase in parameters like days to maturity will not be of interest because every will like crop with shorter life cycle and if possible he/she cultivate it 2-3 time within a growing season. From Table 3, the values of days to maturity were all negative which implies decrease in days to maturity as a result of irradiation. For example, if the parental stock takes 100days to mature, the irradiated (20 Gy) plants will take 85.55 days i.e. 100-14.45.

Table 3: Percentage Increments in quantitative characters of *Digitaria exilis* after gamma irradiation

Dose (Gy)	No. of tillers (%)	Leaf length (%)	No. of nodes/tiller (%)	Internode (%)	Plant height (%)	No. of days to maturity
20	21.26	7.86	8.33	9.51	-2.36	-14.45
40	37.77	8.11	0.00	9.18	3.05	-11.02
80	34.20	21.87	5.56	31.97	4.41	-5.40
100	12.00	4.05	-19.44	5.25	-5.38	-3.02

4.0 Rice (*Oryza sativa* L.)

Rice a member of grass family is of the staple food crop in the world. The crops is consumed daily by almost 3 billion people globally. Flash flood has been identified as one of the limiting factors of rice cultivation particularly the lowland rice in Nigeria. Mohammed *et al*. (2018) evaluated two improved varieties of rice 'FARO 44' and 'FARO 60' for submergence tolerance after exposure to different doses of gamma irradiation. The two varieties responded differently to doses of the irradiation. While low doses were effective for 'FARO 60' higher doses were much more effective for 'FARO 44' (see Table 4). The table showed percentage increment/decrement of the irradiated plant over their parental non-irradiated plants. Negative values imply decrease while positive values means increase. Both negative and positive values are appreciated depending on the character of interests. For examples in term of maturity period, early maturing individuals (fewer number of days to maturity i.e. decrease in days to maturity) are desirable. In this regard, dose 50 Gy and 100 Gy were the most effective for FARO 44 and

AFARO 60 decreasing days to maturity by 2.00 and 4.22 % respectively. In other parameters, survival, number of tillers, 1000 seed weight and number of panicles, higher values are often desirable. For FARO 44 all the doses had higher survival than the control. The dose 150 Gy showed 66.67% increment over the control followed by 200 and 50 Gy with percentage increment of 55.56 and 22.22 respectively (Table 4). This implies that if the control had 60% survival, then the plants treated with 150 Gy will have 100% (i.e. 40.00+ 60.00), where 40 is 66.67% of the control. The same thing applies to all other values in that table.

Table 4: Influence of gamma irradiation on some agromorphological parameters of rice

Variety/Dose (Gy)	SP	NTM	100 seed weight	NP	PDM
FARO 44					
0			-	-	-
50	22.22	4.81	76.36	4.81	-2.00
100	11.11	-9.52	131.81	-9.52	3.00
150	66.67	19.05	136.36	19.05	3.33
200	55.56	-23.81	94.54	-23.81	6.33
FARO 60					
0		-		-	
50	-31.20	5.26	286.36	5.26	-3.31
100	-40.00	5.26	331.82	5.26	-.4.22
150	-30.00	-26.31	177.27	-26.31	-2.41
200	-20.00	-5.26	45.45	-5.26	1.51

NTM= Number of tillers at maturity, SP= Survival percentage, NP=Number of panicles per plant PDM= Percentage decrease in Days to Maturity (Source Mohammed *et al*., 2018)

Conclusion

Gamma ray has been extensively used in mutation breeding. The outcomes from most of ongoing researches in this area of research have shown the possibilities of Nigeria registering in few years to come, a number of new mutant varieties, especially in sesame plant. This will be facilitated only when adequate research grants in this area is well available and accessible to the researchers.

References

Animasaun, D. A., Morakinyo, J. A. & Mustapha, O. T. (2014). Assessment of the effects of gamma irradiation on the growth and yield of *Digitaria exilis* [Haller]. *Journal of Applied Biosciences*, 75, 6164– 6172.

Ashri, A. (1994). Genetic resources of sesame: present and future perspectives. In: ARORA R. K. & RILEY K. W. (eds.) Sesame Biodiversity in Asia: Conservation, Evaluation and Improvement. IPGRI Office for South Asia, New Delhi, India: 25-39.

Ashri, A. (2007). Sesame (*Sesamum indicum* L.). In:R.J.singh, Ed.,*Genetic Resources, Chromosome Engineering, and Crop improvement*, 4, oilseed crop,.231-289, CRC Press,BocaRaton,FL,USA.

Diouf, M.; Boureima, S.; Diop, T. and Çağirgan, M.Ġ. (2010). Gamma Rays-Induced Mutant Spectrum and frequency in Sesame. *Turkish Journal of Field Crops,* 15(1): 99-105.

Falusi, O. A. & Salako, E.A. (2003). Inheritance studies in wild and cultivated *Sesamum* L. Species in Nigeria. *Journal of Sustainable Agriculture*, 22(3), 75-90.

Falusi, O. A. (2006). Estimation of natural cross pollination in two species of the genus. *Sesamum* (pedaliacea). *Production Agriculture and Technology,* 2(2), 61-6.

Food and Agricultural Organization of the United Nations (FAO) (2011). Statistical Database. http://faostat.fao.org/

Jain, S.M. (2010). Mutagenesis in crop improvement under the climate change. *Romania Biotech. Letter*, 15(2):88-106.

Jain, S. M. and Suprasanna, P. (2011). Induced mutations for enhancing nutrition and food production. *Geneconserve*, 40, 201 – 215.

Kumar, G. and Yadav, R. S. (2010). EMS induced genomic disorders in sesame (*Sesamum indicum* L.) Rome Journal Biol – PLANT BIOL. 55 (2): 97–104.

Langham, D.R. (2001).Shatter Resistance in Sesame. Joint FAO/IAEA Division of Nuclear Techniques, in Food and Agriculture, International Atomic Energy Agency, SESACO Corporation San Antonio, Texas, United States of America.

Maluszynski, M., Nichterlein, K., Van Zanten, L. and Ahloowalia, B.S. (2000).Officially released mutant varieties- The FAO/IAEA Data.

Monpara, B. A., Vora, M. D., Chovatiya, B. M. & Radadia, B.V. (2008). G. Til 3: A white and bold seeded sesame variety for Saurashtra region of Gujarat. *Journal of Oilseeds Resources*, 25(2), 186-187.

Muhammad, M. L., Falusi O. A., Adebola, M. O., Oyedum, O. D., Daudu, O. A. Y. and Aisha, G.

A. (2017). Preliminary studies on effects of gamma ray on seed retention indices of three Nigerian sesame (*Sesamum indicum* L.) varieties. *Journal of Plant Development*, 24: 67-72.

Muhammad, M. L. (2018). Induced mutagenesis by gamma irradiation in three varieties of Nigerian sesame (*Sesamum indicum* L.). A PhD thesis submitted to Federal University of Technology, Minna, Nigeria.

Muhammad, M. L., Falusi O. A., Adebola, M. O., Oyedum, O. D., Gado, A. A., Dangana, M.C.(2018). Spectrum and Frequency of Mutations Induced by Gamma Radiations in Three Varieties of Nigerian Sesame (*Sesamum indicum* L.). *Notulae Scientia Biologicae*, 10(1):87-91.

Wongyai W., Saengkaewsook W. & Veerawudh J. (2001). Sesame Mutation Induction: Improvement of Non-shattering Capsule by Using Gamma Rays and Ems. Report Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna (Austria): 71-78.

INDUCED MUTAGENESIS BY FAST NEUTRON IRRADIATION FOR CROP IMPROVEMENT IN NIGERIA

Oladipupo Abdulazeez Yusuf DAUDU[1], Olamide Ahmed FALUSI[1], Abdulhakeem ABUBAKAR [1], Muhammad Liman MUHAMMAD[1], Dorcas Ropo ABEJIDE[1],

[1]Department of Plant Biology, Federal University of Technology, Minna, Nigeria

1.0 Introduction

It a well-known fact that evolution and practical breeding highly depend on genetic variation. The variations that are present in nature do not represent the original spectra of spontaneous mutations. Rather, they are the result of genotypes recombining within populations and their continuous interaction with environmental factors (Oladosu *et al*., 2016). Mutagenesis is the process by which new alleles are created. It is the process whereby sudden heritable

changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents (Roychowdhury and Tah, 2013). The newly created mutants may be used as parents in future breeding programs, in which case mutagenesis is a breeding technique as a source of variation (Acquaah, 2009). However, an induced mutant can be systematically processed through conventional breeding steps to be released as a cultivar, hence making it a breeding method (mutation breeding). Mutations arise spontaneously in nature and are pivotal in natural evolution (Acquaah, 2009). Mutations are known to enhance the genetic variability of crop plants, it should be noted that the variability at species level has reached the ceiling due to high breeding intensity and rapid erosion of plant genetic resources (Poornananda and Hosakatte, 2009). Since spontaneous mutations occur at very low frequency, induced mutations facilitate the development of improved varieties at a swifter rate (Maluszynski, 1990). The FAO (2009) reported that 2008 marked the 80th anniversary of mutation induction in plants.

Application of gamma rays and other physical mutagens such as fast neutrons has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies (David, 2010). The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2700 plant mutant varieties (FAO, 2009) this number has increased worldwide to about 3222 officially released mutant varieties in the FAO/IAEA Mutant Varieties Database in 2015 (IAEA, 2015). Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield components of various crops like *Oryza sativa* (Awan *et al*., 1980; Singh *et al*., 1998), *Hordeum vulgare* (Ramesh *et al*., 2001), *Cicer arietinum* (Wani and Anis, 2001), *Vigna mungo* (Misra *et al*., 2001), *Helianthus annuus* (Elangovan, 2001) *Sesamum indicum*, *Triticum durum* (Sakin and Yildirim, 2004), (Mensah *et al*., 2007).

Among the ionising radiations which are known to produce excitation as well as ionisation in atoms located in their paths, Fast Neutron is less utilised.

Ventura *et al*. (2013) described Fast neutron as a form of ionizing radiation with a high linear energy density, can cause secondary ionization and gene mutations in plant cells, and the traits of the resulting mutants can be stably inherited Fast Neutron is produced during radioactive decay oh heavier elements. It is uncharged, particulate, highly penetrating and densely ionising radiations. Neutron radiation was discovered as a result of observing a beryllium nucleus reacting with an alpha particle thus transforming into a carbon nucleus and emitting a neutron, Be (α, n) C. The combination of an alpha particle emitter and an isotope with a large (α, n) nuclear reaction probability is still a common neutron source. Neutrons are hazardous and hence have less penetrating abilities, but they are known to cause serious damage to the chromosomes. They are best used for materials, such as dry seeds (Acquuah, 2006). Various forms of neutrons were also studied extensively for their use in mutagenesis in the 1960s and 1970s. Though it has been proved to be an effective mutagen, particularly for producing large DNA fragment deletions, the application of neutrons in induced mutagenesis is limited (Kharkwal, 2012).

It has been known for many years that exposure of crop plant cells under natural conditions of growth and development to physical radiations such as ionizing FNI resulted in excessive production of free radicals (Nabil *et al*., 2009; Agarwal *et al*., 2009). These free radicals are highly reactive due to the presence of unpaired valence shell electrons (Vishal-Tandon, 2005) and can result in non-controlled oxidation in cells, cellular macromolecules compartments such as enzymes, proteins, lipids and DNA (Gill and Tuteja, 2005). On the other hand, free radicals reactions and interactions induced genotoxic damage which can lead to structural changes in DNA, such as chromosomal rearrangement, strand breaks, base deletions, pyrimidine dimers, cross-links and base modifications, mutations, and other genotoxic effects (Ahmad *et al*., 2008). Despite the destructive activity of the free radicals, their production in plant tissues is controlled by the very efficient enzymatic and non-enzymatic antioxidant defense systems which serve to keep down the levels of the free radicals, permitting them to perform useful biological functions without too much damage and act as a cooperative network employing a

series of redox reactions (Karuppanapandian *et al*., 2011). These reports therefore aim at showing that Fast Neutron is a potential tool to be employed for crop improvement.

2. Review on Fast Neutron Irradiation and its Effects on Plant Morphology and Cytology

The study of the effects of irradiation on crops is a broad and complex field. FNI was found to cause increase in certain agro-morphological and yield attributes in plants such as pepper, Lagos Spinach, Sesame etc. by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues. Different doses of FNI irradiation tend to enhance different traits in crop plants. It is one of the important physical mutagens used to improve the characters and productivity of many plants (Acquua, 2009). However, FNI had adverse effect on some other traits of plants depending on plant species and the dose of irradiation (Daudu, 2011).These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in

photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds.

2.1 Effects of FNI on Morphology of Plant

2.1.1 Germination

According to a research conducted by Falusi *et al*. (2012) results showed that FNI can affect the germination of Nigerian pepper (*Capsicum annuum* L.) seeds. It was observed that different doses of FNI have various effects on the total number of germinated seeds and its respective germination rate. The general effects on the three genotypes of Nigerian pepper (*C. annuum* var. *accuminatum* i.e 'Ata Shombo'; *C. annuum* var. *abbreviatum* i.e. 'Ata Rodo' and *C. annuum* var. *grossum* i.e. 'Ata Tatase') in term of germination was phenomena. The control experiment which was not expose to FNI had 100 percent seeds germinated for all the genotypes, they also had germination rate of 100 percent. In the 30 minutes exposure to FNI, only 50 %, 30 % and 52 % seeds germinated for 'Shombo', 'Rodo' and 'Tatase' respectively when compared with the

control. In the 60 minutes exposure period, 51 %, 21 % and 30 % germinated respectively in Shombo, Rodo and Tatase. Ninety (90) minutes exposure period tend to enhance 67 % germination in Tatase but only 16 % of the seeds germinated in Rodo and 51 % in Shombo. 120 minutes irradiation exposure period only lead to just 16 % germination in Rodo but 37 and 66 % in Shombo and Tatase respectively. Similarly, Daudu and Falusi (2011) observed that exposure of African Chilli pepper (*Capsicum frutescens* var *baccatum*, i.e. 'Ata Wewe') for 30, 60, 90 and 120 minutes caused only 44 %, 13 %, 26 % and 40 % of the seeds to germinate whereas the control set up had 100 % of the seeds germinated. In *Celosia argentea* (Lagos Spinach), slight reductions in germination percentage with increased irradiation period of exposure were observed from 90 (86.6%) to 120 (80.00%) minutes exposure periods. Highest percentage survival among all the irradiated treatment plants were due 30 and 90 minutes exposure period with a value of 86.67% while the least of 80% was recorded in 120 minutes irradiated treatment. However, these values were less than the control value (96.00%) (Abubakar *et al*., 2017). This is

an indication that FNI had stimulatory effects in early growth of pepper and Lagos spinach plants.

2.2 Seedling height of Pepper

Study revealed that there is significant effect of low doses of FNI in the pepper accessions. The seedling height of the pepper tend to increase as the exposure periods increase from the lowest to highest. In Shombo, for example, 120 minutes exposure period produced significantly highest seedling (15.51 cm) when compared with the control (12.51 cm). Similar trend was also obtained in Rodo, Tatase and Wewe (Daudu and Falusi, 2011; Falusi *et al*., 2012). In Lagos Spinach (*C. argentea*), 90 minutes exposure period had the highest seedling height (19.54 cm) while the control had 18.56 cm ((Abubakar *et al*., 2017).

2.1.2 Plant height at maturity

According to Daudu (2011) on pepper, over a wide range of radiation doses, the frequency of mutation induced by radiation is proportional to the radiation dose. Thus, the higher the dosage of radiation, the more evident the expression of morphological changes is

observed in the samples. Thus, in Shombo, the 120 minutes irradiation exposure period tend to produce the highest plant height (59.93 cm) when compared with the control which produced 44.20 cm. in fact, the 30, 60 and 90 minutes also produced higher plant height at maturity than the control. Similar trend was also produced in Rodo where the 120 minutes irradiation produced significantly higher height than the control (33.70 cm and 21.70 cm respectively). However in Ata Tatase, despite having highest plant height at maturity (37.66 cm) is significantly the same as that of the control (36.32). In Ata Wewe, 120 minutes irradiation exposure period produced the highest plant height, although it is significantly the same with that of the control Table 1.

In Lagos Spinach, the 60 minutes irradiation exposure period produced the highest plant height (77.48 cm) than any of the other exposure periods and the control (55.18 cm). In fact, all the exposure periods tend to produce higher plant height than the control.

2.1.3 Number of Leaves per Plant

In the work of Falusi *et al*., 2013a and b, there is general increase in number of leaves per plant as the irradiation periods increase. In all the Nigerian pepper species studied 120 minutes irradiation period produced the highest number of leaves per plant while the control (0 minutes) produced the least (Table 1). In Lagos Spinach, the highest number of leaves per plant was found in 60 minutes irradiation exposure period while the control produced the lowest.

2.1.4 Leaf Area

According to the work of Abubakar *et al*. (2017) on Lagos Spinach, estimation of leaf areas for all the irradiated plants when compared with the control were significantly ($p \leq 0.05$) higher throughout the study periods. At maturity, the leaf areas of all the treated plants were higher than the control (25.96cm^2) set up. Significant highest mean leaf area (40.83cm^2) was recorded in 60 minutes irradiation exposure period, followed by 120 minutes irradiation exposure period with mean value of 36.76cm^2.

2.1.5 Leaf Shapes

Falusi *et al*. (2012), all the pepper produced from the non-irradiated seeds – which served as the control – produced normal leaves (Fig. 1A–C). However, this was not the case among plants whose seeds were irradiated. Leaf irregularities such as small leaves, chlorophyll-deficient leaves, or leaves with invaginated margins, with inverted margins, with blunt apices and with bifurcated apices (Fig. 1D–I) were observed. These leaf morphological abnormalities that were observed are indications that FNI caused certain physiological changes that lead to production of irregularities in pepper plants. Abubakar *et al*. (2017) also observed that some leaves of Lagos Spinach irradiated for 120 minutes irradiation exposure period had various irregularities. Some of the leaves exhibited chlorophyll aberration (Chlorina) (Figure 2E), however, normal ovate-shaped leaf with acute apex of *C. argentea* was observed in the control (Figure 2A). Others include dented leaves, leaves with irregular margin, leaves with invaginated apex as well as wrinkled-shaped leaves.

Table 1: Effects of Fast neutron irradiation on the mean performance of some morphological parameters of the four pepper genotypes

MN/SH/001	Seedling Height	Height at Maturity	Number of Leaves per Plant
control	12.51ab	44.20a	129.93a
30mins	11.87a	45.27a	132.70a
60mins	13.38a	52.86ab	173.00b
90mins	13.53ab	50.23ab	161.80b
120mins	15.51b	59. 93b	230.80c
MN/AR/002			
control	5.43a	21.70a	67.08a
30 mins	7.85b	29.94b	99.20b
60 mins	7.92b	32.79b	113.00b
90 mins	7.43b	29.32b	99.09b
120mins	7.95b	33.70b	120.10b
MN/AT/003			
control	15.78bc	36.22a	82.67a
30 mins	14.36ab	34.14a	77.33a
60 mins	12.52a	31.49a	68.67a
90 mins	15.48bc	36.32a	86.55a
120mins	16.40bc	37.66a	90.58ab
MN/AW/004			
control	9.58a	41.50ab	112.00a
30 mins	11.04b	37.77a	103.80a
60 mins	9.72a	38.84a	105.64a
90 mins	9.79a	34.84a	100.10a
120mins	11.34b	45.85ab	156.90b

Means followed by the same letter (s) within the same column do not statistically differ at 5% level tested by LSD. (Source: Daudu, 2011).

2.2 Yield parameters

According to the work of Falusi *et al*. (2013) on Sesame, 16 µSv tend to lead to increase in number of seeds per capsule in Ex-Sudan, whereas in E-8 variety, it was the 4 µSv that produced the highest number of seeds per fruit. For weight of capsule, 16 µSv produced the highest weight of capsule in Ex-Sudan whereas 4 µSv produced the highest weight in variety E-8. In Ex-Sudan, 12 µSv produced the highest number of capsules per plant but in E-8, it is the control that produced the highest number of capsule per plants. In *Capsicum* sp, FNI consistently lead to increase in number of fruit per plant, number of seeds per fruit, length of fruit, width of fruit and weight of fruit. 120 minutes irradiation exposure period continuously lead to highest yield parameters in Ata Shombo. In Ata Rodo, 90 minutes irradiation exposure period produced the highest number of fruit per plant and number of seeds per fruit; 60 minutes irradiation exposure period produced the highest width of fruit and length of fruit. Highest fruit weight in Ata Rodo was produced by 120 minutes irradiation exposure period. Yield parameters

in Ata Tatase tend to be affected by higher doses of FNI. The control produced the highest number of seeds per fruit, width of fruit, length of fruit and weight of fruit. For Ata Wewe, the highest number of fruit per plant and number of seeds per fruit were produced by the 90 minutes irradiation exposure period. For *Celocia argentea*, 90 and 120 minutes irradiation exposure periods produced the highest number of spike per plants while the control produced the smallest.

2.3 Pollen Sterility and Pollen Restitution

Abubakar *et al*. (2013), the result of pollen production, diameter, fertility and percentage pollen injury indicates that significant variations ($p \leq 0.05$) have been induced in some treatments by the FNI. A decrease in pollen production was recorded in all the treatment plants when compared with the control. The control plant had pollen productions of 92,750.00 per flower and 15458.00 per anther. Meanwhile, 4 μSv was the dose among the irradiated doses that produced relatively high number of pollen production per flower

(787.50) and pollen production per anther (135. 25). In addition, 4 μSv produced pollens with larger diameters than all other doses and even the control. The significant difference in the number of pollen produced per flower and anther observed in this study might be attributed to slight changes in the genetic composition of the plant due to mutagenic action. A reduction in percentage fertility was recorded for all the plants exposed to FNI when compared with the control, 16 μS FNI causes the least (8.53) damages on the pollen among the irradiated exposed plants. Pollen restitution was also observed in the 8 μSv and 12μSv, the pollens clumped-up into dyad, triad and tetrad. The observation of pollens clumping in dyads, triads and tetrads could be due to abnormal activities in meiotic cell division.

Table 2. Effects of FNI on some Agro-morphological Parameters of *Celosia argentea*

Exposure Periods (Minutes)	Plant Height (cm)	No. of Leaves	No. of Leaves/plant	Spike Length	Leaf Area (cm^2)
Control (0)	55.18 ± 3.45a	51.00 ± 5.59a	51.00 ± 5.59a	26.60 ± 3.28a	25.96 ± 3.80a
30	73.74 ± 1.93b	76.80 ± 6.32c	76.80 ± 6.32c	29.20 ± 3.57a	33.84 ± 2.65b
60	77.48 ± 5.49c	77.40 ± 3.10c	77.40 ± 3.10c	49.75 ± 6.51b	40.83 ± 2.96c
90	69.64 ± 5.61b	57.80 ± 4.75ab	57.80 ± 4.75a	71.40 ± 7.50c	32.89 ± 4.08b
120	70.08 ± 4.66b	62.20 ± 4.68b	62.20 ± 4.68b	63.50 ± 8.87c	36.76 ± 5.69bc

Values are means ± S. E, values followed by the same letter(s) along the column are not significantly different at $p>0.05$ as tested by DMRT. Source: Abubakar *et al*., 2017

Table 3: Yield Parameters of Sesame Varieties Treated with FNI

Treatment combination	Number of flower/plant	Number of capsule/plant	Length of capsule (cm)	Weight of capsule (g)	Number of seeds per capsule
Ex-Sudan					
0 µSv	3 0 ± 13^{a}	34 ± 17^{a}	2.35 ± 0.17^{bc}	0.28 ± 0.05^{bc}	52 ± 09^{b}
4 µSv	31 ± 11^{a}	36 ± 20^{a}	2.49 ± 0.27^{b}	0.23 ± 0.05^{c}	47 ± 02^{b}
8 µSv	27 ± 10^{a}	35 ± 17^{a}	2.53 ± 0.20^{a}	0.30 ± 0.08^{bc}	50 ± 08^{b}
12 µSv	34 ± 27^{a}	46 ± 22^{a}	2.42 ± 0.26^{b}	0.31 ± 0.09^{b}	53 ± 08^{ab}
16 µSv	24 ± 10^{a}	30 ± 11^{a}	2.55 ± 0.16^{a}	0.32 ± 0.06^{a}	59 ± 05^{a}
E-8					
0µ Sv	20 ± 04^{a}	26 ± 13^{a}	2.35 ± 0.19^{a}	0.44 ± 0.10^{b}	49 ± 06^{a}
4 µSv	21 ± 11^{a}	25 ± 16^{a}	2.19 ± 0.26^{a}	0.56 ± 0.09^{a}	53 ± 06^{a}
8 µSv	18 ± 05^{a}	22 ± 09^{a}	2.19 ± 0.34^{a}	0.33 ± 0.09^{c}	50 ± 08^{a}
12 µSv	21 ± 10^{a}	22 ± 11^{a}	2.32 ± 0.32^{a}	0.33 ± 0.12^{c}	49 ± 09^{a}
16 µSv	21 ± 10^{a}	21 ± 05^{a}	2.29 ± 0.45^{a}	0.15 ± 0.04^{d}	48 ± 13^{a}

Source: Falusi *et al*., 2013.

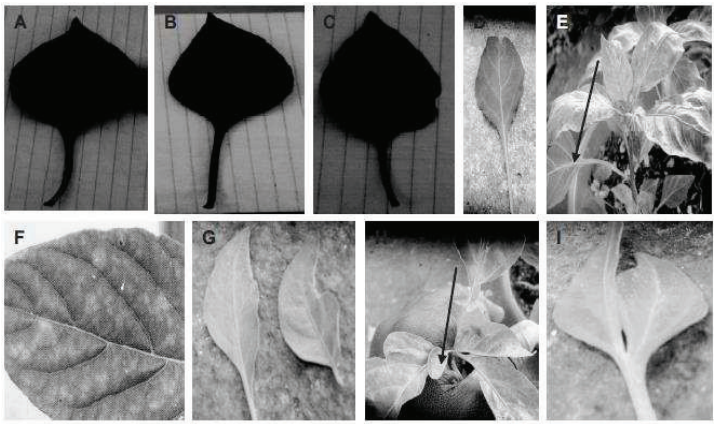

Figure 1. A, B, C. Normal leaves of *Capsicum annuum* var. *accuminatum* (Ata Shombo), *C. annuum* var. *abbreviatum* (Ata Rodo), and *C. annuum* var. *grossum* (Ata Tatase), respectively. D. Leaf with bifurcated apex in Shombo (60 min irradiation exposure). E. Fully mature Tatase plant with chlorophyll mosaic leaves. F. Leaf showing chlorophyll mosaic points in Ata Tatase (90 min irradiation exposure). G. Leaves showing invaginated margins in Shombo (120 min irradiation exposure). H. Crinkled leaves with dented margins in Tatase. The arrow also shows a small leaf with a blunt apex (30 min irradiation exposure). I. A leaf that became bi-foliate in Shombo (30 min irradiation exposure). Source: Falusi *et al*. 2012:

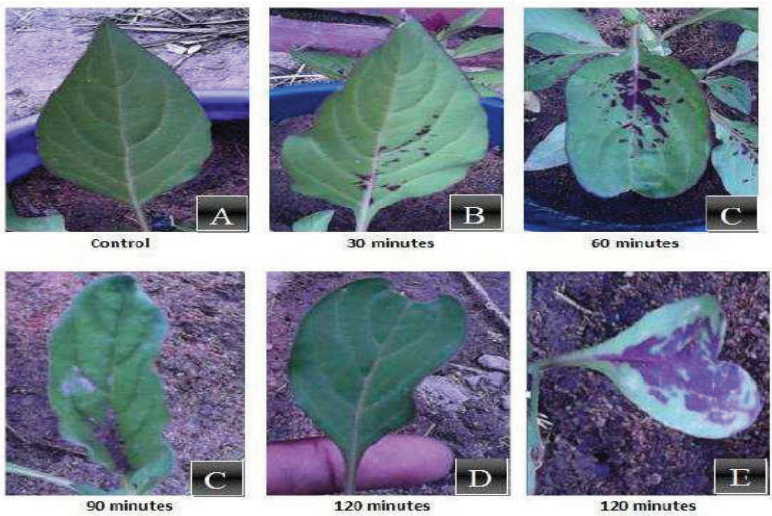

Figure 2. Phenotypic Effects of Fast Neutron Irradiation on *Celosia argentea* Leaves. A: normal ovate leaf of *C. argentea*, B: dented, C: irregular margin and invaginated apex D: wrinkle shapes E: Chlorina mutated leaf. Source: Abubakar *et al*., 2017,

2.9 Conclusion

This review revealed that Fast Neutron Irradiation is a potential tool in enhancing variability in different plants in Nigeria. In addition, there is variation in the sensitivity of different crops to different doses of FNI. *Capsicum annuum*, *C. frutescens*, *Celosia argentea*,

and *Sesamum indicum* showed different sensitivity to FNI. In pepper, 120 minutes irradiation exposure periods (16 μSv) was the most effective irradiation period to induce viable and useful mutations for yield parameters in pepper. Mutagenic treatments will generally increase genetic variability, which can be utilized for selection and improvement of plants. The mutagen (Fast neutron irradiation) used in this investigation could have been successful in broadening the genetic base and increasing genetic variability of pepper plants. Fast neutron irradiation therefore could be utilized to increase variability in peppers and this will ultimately increase the possibility of isolating beneficial mutants for the improvement of the crop. FNI at certain IEPs can be used to increase select growth and yield characters and induce variability in peppers through the isolation of beneficial mutants and can thus be used in pepper improvement programmes. Since both *C. annum* and *C. frutescens* were both responsive to FNI treatment, either species could serve as the parent plant in breeding and improvement programmes, or through mass propagation in vitro. FNI

therefore, could serve as a valuable tool for the improvement of yield of the crop.

For *C. argentea*, FNI had positive effects on pollen and cytological parameters of the plant. Significant valuable variability was induced in the pollen size and fertility. The occurrence of meiotic restitution mechanisms in 8.00 μSv is an evidence of the formation of balanced gametes which contribute to increase the pollen fertility. The study further affirm that pollen restitution in mutated plants might be an indication of disturbance at cellular level or physiological activities of the cell due to mutagenic action. It can therefore be concluded that Fast Neutron Irradiation is a potent physical mutagen capable of inducing beneficial mutation in Lagos Spinach. The magnitude of increase in variability differed for different traits, and inconsistent with different doses of the mutagen. The best irradiation period to induce this beneficial mutation is the 8 μSv. In Sesame, correlations between irradiation doses and the yield parameters also varied and they were generally higher in Ex-Sudan than in E-8, this suggests that Ex-Sudan was more sensitive to

Fast Neutron Irradiation. Therefore, FNI could serve as a valuable tool for the improvement of yield of the crop.

References

Abubakar, A., Falusi A. O., Daudu, O. A. Y., Oluwajobi A. O., Dangana M. C. and Abejide, D. R. (2015). Mutagenic Effects of Sodium Azide and Fast Neutron Irradiation on the Cytological Parameters of M2 Lagos Spinach (*Celosia argentea* var *cristata* L.). *World Journal of Agricultural Research*, 3 (3), 107-112. DOI:10.12691/wjar-3-3-3.

Abubakar, A., Falusi, A. O., Daudu, O. A. Y. (2017). Morphological and Phenotypic Effects of Fast Neutron Irradiation (FNI) on Lagos Spinach (*Celosia argentea* L.). *Radiation Science and Technology*, 3(5): 47-53 doi:10.11648/j.rst.20170305.12.

Acquaah G. (2006). *Principles of plant genetics and breeding.* Chichester: Wiley-Blackwell; 2006.

Acquaah, G. (2009). *Principles of Plant Genetics and Breeding*. Blackwell Publishing Main Street,

Malden, MA 02148-5020, USA 9600 Garsington Road, Oxford OX4 2DQ, UK. 569 pp.

Agrawal, S. B. Singh, S., and Agrawal, M. (2009). Chapter 3 ultraviolet-B induced changes in gene expression and antioxidants in plants, *Advances in Botanical Research*, 52, 47–86.

Ahmad, P., Sarwat, M., and Sharma, S. (2008). Reactive oxygen species, antioxidants and signaling in plants, *Journal of Plant Biology*, 51(3), 167–173.

Awan, M. A., Konzak, C. F., Rutger, J. N. and Nilan, N. (1980). Mutagenic effects of sodium azide in rice. *Crop Science*, 20, 663-668.

Daudu, O.A.Y. (2011). *The mutagenic effects of fast-neutron irradiation on selected morphological and yield traits of* C*apsicum annuum and Capsicum frutescens*. A Thesis submitted to the Postgraduate school, Federal University of Technology, Minna, Nigeria. In partial fulfilment of the requirements for the award of

degree of Master of Technology in Botany (Applied Plant Genetics and Breeding). 62 pp.

Daudu, O.A., and Falusi, O.A. (2011). Induced Genetic variability for morphological and Yield parameters in *Capsicum annum* and *Capsicum frutescens*. *West Management and Bioresource Technology*, 1, 31-37.

David, T. (2010). All you wanted to know about induced mutations in crop breeding. *Bulletin of Biofortified*, http://www.webster@ext.msstate.edu

Elangovan, M. (2001). Gamma radiation induced mutant for improved yield components in sunflower. Mutat. Breed. Newslett. 45, 28-29.

Falusi, O.A., Daudu, O.A., and Teixeira, da Silva, J.A. (2012a). Effects of fast neutron irradiation on agronomic traits of Nigerian pepper (*Capsicum annuum* L.). *European Journal Horticultural Science*, 77(1), 41-45.

Falusi, O.A., Daudu, O.A.Y., and Teixeira da Silva, J. A. (2012b). Effect of exposure time of fast

neutron irradiation on growth and yield parameters of *Capsicum annum* and *Capsicum frutescens. African Journal of Plant Science*, 6(9), 251-255.

Falusi, O.A., Liman, M.M., Lateef, A.A., Adamu, G.A., Daudu, O.Y., and Abejide, D.R. (2013). Effects of Fast Neutron irradiation on Yield Parameters of Two Nigerian Sesame Cultivars. *Academia Journal of Biotechnology*, 1(7), 105-108.

Food and Agricultural Organization (FAO) 2009: Induced plant mutations in the genomic era. Publication of the Food and Agricultural Organization of the United Nations (FAO) Rome.

IAEA. IAEA mutant database. Vienna: International Atomic Energy Agency; c2015 [Accessed April 2018]. Available from: http://mvd.iaea.org/.

Karuppanapandian, T., .Moon, J., Kim, C., Manoharan, K. and Kim, W. (2011). Reactive oxygen

species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science*, 5(6): 709–725.

Kharkwal, M.C. (2012). *A brief history of plant mutagenesis*. In: Shu QY, Forster BP, Nakagawa H, editors. Plant mutation breeding and biotechnology. Wallingford: CABI; 2012. p. 21-30.

Maluszynski, M. (1990). Gene manipulation in plant improvement. Vol. 2. Gustafsson JP (eds) Plenum press, New York.

Mensah, J. K., Obadoni, B. O., Akomeah, P. A, Lkhajiagbe, B., and Ajibolu J. (2007). The effect of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). *African Journal of Biotechnology*, 6(5), 534-538.

Misra, R. C., Mohapatra, B. D., and Panda, B. S. (2001). High yielding mutants of blackgram

variety PH-25. *Mutation Breeding Newsletter*, 45, 39.

Nabil, G. M., Attia, A. M. M., and Elhag, M. A. (2009). "Radioprotective effects of dietary ginger (*Zingiber officinales* Rosc.) Against fast neutron-induced oxidative stress in rats," *World Applied Sciences Journal*, 6(4), 494–498.

Oladosu, Y., Rafii, Y.M., Abdullah, N., Hussin, G, Ramli, A., Rahim, H.A., Miaha, G. and Usmana, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review *Biotechnology & Biotechnological Equipment*, 30(1), 1-16, DOI: 10.1080/13102818.2015.1087333.

Poornananda, M. N. and Hosakatte, N. M. (2009). The effect of gamma and ethylmethyl sulphonate treatments on agronomical traits of Niger (*Guizotia abyssinica* Cass.). African J Biotech 8(18), 4459-4464.

Ramesh, B., Prasad, B. K., and Singh, V. P. (2001). Semi dwarf, high yielding and high protein mutants in barley. *Mutation Breeding Newsletter*, 45, 26-27.

Roychowdhury, R., and Tah, J. (2013). *Mutagenesis*: a potential approach for crop improvement. In: Hakeem KR, Ahmad P, Ozturk M, editors. Crop Improvement: new approaches and modern techniques. New York (NY): Springer; p. 149-187.

Sakin, M. A., Yildirim, A. (2004). Induced mutations for yield and its component in durum wheat (*Triticum durum* Defs.). *Food Agriculture and Environment*, 2(1), 285-290.

Singh, S., Richharia, A. K., and Joshi, A. K. (1998). An assessment of gamma ray induced mutations in rice (*Oryza sativa* L.). *Indian Journal of Genetics*, 58(4), 455-463.

Ventura, L, Giovannini, A., and Savio, M, (2013). Single cell gel electrophoresis (comet) assay with plants: Research on DNA repair and

ecogenotoxicity testing. *Chemosphere*, 13, 383–4

Vishal-Tandon, M. D., Gupta, B. M., and Tandon, R. (2005). Free radicals/reactive oxygen species, JK Practitioner, vol. 12(3), 143–148.

Wani, A. A. and Anis, M. (2001). Gamma rays induced bold seeded high yielding mutant in chickpea. *Mutation Breeding Newsletter*, 45: 20-21.

THE ROLE OF CHEMICAL MUTAGENS IN CROP IMPROVEMENT IN NIGERIA.

Aishatu Adamu GADO[1], Olamide Ahmed FALUSI[1], Josephine Teni KOLO[1], Oladipupo Abdulazeez Yusuf DAUDU[1], Abdulhakeem ABUBAKAR[1], Muhammad Liman MUHAMMAD[1],

[1]Department of Plant Biology, Federal University of Technology, Minna, Nigeria

Introduction

A mutation is a sudden heritable change in the DNA in a living cell, not caused by genetic segregation or genetic recombination. Mutation breeding is the purposeful application of mutations in plant breeding. Unlike hybridization and selection, mutation breeding has the advantage of improving a defect in an otherwise elite cultivar, without losing its agronomic and quality characteristics. Mutation breeding is the only straightforward alternative for improving seedless crops. Since the first release of mutant cultivars that resulted from basic mutation research in Europe,

mutation breeding has found a niche in plant breeding because of these advantages. Methodologies for mutation induction have been improved in main crops for both physical and chemical mutagens, and selection methodologies for mutant populations have been described.

Chemical Mutagenesis

Chemical mutagenesis (the non-GMO approach) is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutations are the tools and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu *et al*., 2007). Mutation methodology has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena (Van, Den-Bulk *et al*., 1990; Bertagne- Sagnard *et al*., 1996). It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutations and its practical value in plant improvement

programs has been well established. The main advantage of mutational breeding is the possibility of improving one or two characters without changing the rest of the genotype. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al.,* 2001). Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated. Various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahloowalia and Maluszynski, 2001).

Chemical mutagens are the one cause of mutations in living organism. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals (Yuan and Zhang, 1993). These effects can occur both spontaneously and artificially following induction by mutagens. Chemical mutagen generally produce induced mutations which

lead to base pair substitutions, especially GC→AT resulting in amino acid changes, which change the function of proteins but do not abolish their functions as deletions or frame shift mutations mostly do. These chemomutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants. Many researchers compared the mutagenic efficiencies of different mutagens on different crops and their results seem to be entirely specific for particular species and even varieties. While many researchers found chemical mutagens are to be more effective than physical ones (Dhanayanth and Reddy, 2000; Bhat *et al.,* 2005a), and many others researchers found the reverse case (Zeerak, 1991). A number of workers (Mashenkov, 1986; Ricardo and Ando, 1998) have reported the role of chemical mutagens in enhancing genetic variability in higher plants because it is the fundamental characteristics to successful breeding programs in vegetatively and sexually propagated plants (Kleinhofs *et al.,* 1978). This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the

interest of plant breeders for many decades. The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yield and quality traits (Ahloowalia and Maluszynski, 2001).

High rates of chromosome aberrations resulting from ionizing radiation and the accompanied detrimental effects made researchers look for alternate sources for inducing mutations. As a result an array of chemical mutagens has been discovered. A wide variety of chemical mutagens, however, make it difficult to establish common rules and conditions for treatment. Since the reporting of mutagenic effects of sodium azide, no new chemical mutagens of widespread use in plant breeding have been discovered. The most widely used chemical mutagens are alkylating agents, with EMS being the most popular because of its effectiveness and ease of handling, especially its detoxification through hydrolysis agents widely used, but they are light-sensitive and more precautions need to be taken because of their higher volatility. Chemical mutagens are also popular in in vitro mutation

induction, although irradiation can also be applied at low doses. EMS has become the mutagen of choice for developing mutant populations for high throughput screening such as in developing tilling populations.

Many people attempted induction of mutations by chemical agents over a long period, but there were no clear or convincing positive results until 1939 when Thom and Steinberger found that nitrous acid was effective in causing mutations in Aspergillus. Auerbach (1941) was the first to report that mustard gas (1,5-dichloro-3- thiapentane) had a mutagenic effect on fruit fly, which was similar to that of X-rays on plants. Auerbach and Robson (1946) later obtained clear evidence to show that mustard gas is mutagenic. Chemical mutagens were found to be highly effective in inducing true gene mutations and the specificity of action could be investigated through analysis of their reaction with different DNA bases. However, the question, whether chemical agents do indeed produce mutations with the same frequency as the physical mutagens (like the ionizing radiations) was settled after the first paper published by Auerbach and Robson

(1946). They used the standard technique devised by Muller to score recessive and visible gene mutations in Drosophila following exposures of flies to a predetermined dose of the gas. Their most important observation was that mustard gas is highly mutagenic and capable of producing lethal and visible mutations at rates comparable with the effect of X-rays. Besides gene mutations, chromosomal aberrations in the form of deletions, inversions and translocations were produced. Through such extensive investigations mustard gas was found to be a very potent mutagen in Drosophila. Oehlker (1943), and Gustafsson and Mackey (1948) proved that mustard gas was mutagenic in barley.

Rapoport (1946, 1948) and others in Russia also discovered and demonstrated mutagenic effects of mustard gas and several other chemicals such as formaldehyde, diethylsulphate, diazomethane, and other compounds and established that alkylating agents are the most important group of chemical mutagens. Since then hundreds of chemical agents belonging to several groups such as alkylating agents, nitroso

compounds, base analogues, azide, acridine dyes etc., have been found to produce mutagenic activity in a range of organisms.

Principles and applications of chemical mutagenesis in plants

Chemical mutagens are defined as those compounds that increase the frequency of some types of mutations. They vary in their potency since this term reflects their ability to enter the cell, their reactivity with DNA, their general toxicity and the likelihood that the type of chemical change they introduce into the DNA will be corrected by a repair system.

The effect of chemical mutagens on plant materials is generally considered milder (Acquaaty 2006). An advantage of chemical mutagenic agents is that they can be applied without complicated equipment or facilities. Using chemical mutagens, the ratio of mutational to undesirable modifications is generally higher compared to physical mutagens (Acquaaty, 2006).

Chemical mutagens are generally carcinogenic and therefore extra care must be taken for health protection during the procedure. Material and safety data sheets for the specific chemical mutagen chosen should be carefully read and the agent should be appropriately inactivated before disposal. In spite of the large number of mutagenic compounds only a small number of mutagenic compounds, only a small number has been tested in plants (Wani *et al* 2014). Among them, only a very restricted group of alkylating agents has found large application in plant experimental mutagenesis and plant breeding. One of the most effective chemical mutagenic group is the group of alkylating agents (these react with the DNA by alkylating the phosphate groups as well as the purines and pyrimides (Acquaaty 2006)

Alkylating agents can be found among a large array classes of compounds including sulphur mustards, nitrogen mustards, exposides,ethyleneimines, ethyleneimides, alkyl methane sulphonates, alkyi halides, alky sulphates, alkyl phosphates, chloroethyl sulphides etc. base analogues are another group of

chemical mutagenic groups that are closely related to the DNA bases and can be wrongly be incorporated during replication. Examples of these base analogues are 5-bromouracil and maleic hydrazides. A strong advantage of the point mutations created by chemical mutagens is their potential to generate not only loss of function but also gain of function (Bradshaw *et al*., 1997).

Important factors influencing the outcome of mutagenesis using chemical mutagens include the condition of the mutagenic solution; inherent characteristics of the target issue; the environment; concentration of mutagen; treatment volume; treatment duration; temperature; pre-soaking of seeds; pH(7.0); catalyst agents (cu^{2+} and zn^{2+}) and post treatment handling (Mba *et al*., 2010).

In general, the steps required for inducing and detecting mutations vary among sexually and asexually propagated plants crops but there are some basic principles that they share in common. The common practical considerations that need to be taken into

account in induction and detection of mutations summarized by (Mba, 2013) include the following:

1. A perfect understanding of the genetic make-up of the traits to be improved is very important.
2. Understanding of the mode of reproduction of the target crop is also a prerequisite whether sexually or asexually propagated.
3. The determination of the material that is to be used for the propagation prior to treatment i.e gametes or seeds for sexually propagated crops, and stem cuttings, buds, nodal segment or twigs for asexual propagated ones.
4. Knowledge of the number of sets of chromosomes in the nucleus of the cell of the target crop, especially when it relates to how hybridisation barriers could impact on the predicted effectiveness of the induced mutants.
5. Determination of the genetic pedregree of the target crop for inducing mutations i.e selecting homologous plants and the best genotypes that is deficient in a single trait.
6. Selection of an appropriate mutagen and dose.

7. Identification of infrastructure (irradiation house, laboratories, screen/glass house fields etc) for successful selection of desired mutants.
8. Screening techniques for dissociation of chimeras from stable mutants.

Review on the Effect of Sodium Azide and Colchicine on Morphology and Yield of Crop Plant

According to a research conducted on the effects of Chemical mutagens on Sesame and Tomatoes shows increase in certain agro-morphological and yield parameters by inducing changes in cells and tissues. Different doses tend to enhance different traits in the crop. However, Chemical mutagens had adverse effect on some other traits of plants depending on the dose of the chemical (Gado *et al.,* 2017). Artificial induction of mutation is of scientific and commercial interest as it is one of the methods used in improving the growth and yield of economic plants. It provides raw materials for the genetic improvement of economic crops (Adamu *et al.,* 2004). Although various mutagens were known to

induce mutation in plants, colchicines, a poisonous alkaloid derived from the autumn crocus (Colchicum autumnale) is effective in inducing genetic variability through mutagenesis to improve both the quality and quantity of crops.

Effects of Sodium Azide on Morphology of Plant

According to a research conducted by Adamu and Aliyu (2007) on dry seeds of three varieties of tomato (*Lycopersicon esculentum*), varieties T106, T244 and T420 obtained from the Institute of Agriculture Research, Ahmadu Bello University Zaria, Nigeria were treated with sodium azide at concentrations of 1.0, 2.0 and 4.0 mM.. Highly significant differences ($P<0.01$) were observed in the varieties and treatments with respect to the studied traits (seed germination, seedling survival, seedling height, root length, number of leaves per seedling, height at maturity, number of branches per plant and fruits per plant)(Table 1) . Treatment and variety interactions were similarly highly significant ($P<0.01$) with respect to all traits except height at maturity. Variety T106 showed better

performance when compared to T244 and T420. There was general decrease in germination percentage, seedling height, root length, number of leaves per seedling, seedling survival, height at maturity and fruit yield per plant, with increase in mutagen concentration. Variety and treatment interactions were also highly significant for traits studied except height at maturity. There was a highly significant difference in the performance of the 3 varieties in response to sodium azide treatment with T106 performing better in all the traits studied. Seedling survival in T244 and T420 was nill, therefore other characters such as height at maturity, number of branches per plant and number of fruits were not obtained since the plants could not survive to those stages. There were highly significant differences ($P<0.01$) between the concentration of sodium azide and all the traits studied, with 4.0mM being the most effective for germination percentage, seedling height, root length, number of leaves per seedling and number of fruits per plant (Table 1)

TABLE 1: MORPHOLOGICAL EFFECT OF SODIUM AZIDE ON TOMATO [*Lycopersicon esculatum* (Mill)]

Variety		Germination %		Seedling height (cm)	Root length (cm)	Number of leaves per seedling	Percentage seedling survival	Height at maturity	No. of branches/plant	50% flowering (Days)	No of fruits/plant
		5days	8days								
T106	0mM	8.00[a]	89.60[a]	10.00[b]	8.12[c]	7.00[c]	80.00[a]	49.00[a]	1000[a]	85.80 [b]	30[a]
	1mM	44.60[c]	76.40[b]	10.38 [b]	10.92 [b]	9.00 [b]	62.80 [b]	39.38 [b]	600 [b]	89.80 a	12 [d]
	2mM	53.60[b]	71.80[b]	12.80 [a]	14.22 [a]	9.00 [b]	58.20 [b]	32.44 [b]	600 [b]	82.80 a	7 [e]
	4mM	39.60[d]	47.00[c]	6.32 [c]	7.44 [c]	7.00 [c]	61.20 [b]	24.40 [b]	400 [b]	89.20 [a]	4 [f]
									900 [a]	88.40 [a]	20 [b]
T224	0mM	19.00 [e]	68.40 [b]	13.10 [a]	5.50 [c]	11.00 [a]	33.40 [c]	23.40 [b]			
	1mM	6.80 [h]	47.20 [c]	5.22 [c]	3.36 [d]	4.00 [d]	0.00	-	-	-	-
	2mM	0.00 [i]	43.80 [c]	3.74 [d]	3.24 [d]	3.00 [d]	0.00	-	-	-	-
	4mM	2.00 [h]	24.40 [d]	3.44 [d]	2.36 [d]	3.00 [d]	0.00	-	-	-	-
T420	0mM	20.20 [e]	87.00 [a]	9.12 [b]	6.52 [c]	4.00 [d]	38.80 [c]	23.28 [b]	900 [a]	85.80	16[c]
	1mM	11.20 [f]	22.20 [d]	7.36 [c]	2.46 [d]	5.00 [d]	0.00	-	-	-	-
	2mM	13.20 [f]	20.20 [d]	3.38 [d]	2.18 [d]	4.00 [d]	0.00	-	-	-	-
	4Mm	6.60 [h]	21.80 [d]	3.10 [d]	2.50 [d]	3.00 [d]	0.00	-	-	-	-

Means with the same letter within the same column are not significant different.(Source: Adamu and Aliyu, 2007)

Effects of Sodium Azide on Yield of Some Crops

The effect of Sodium azide on the yield traits of two varieties of sesame (*Sesamum indicum*) viz; Kenana-4 and Ex-Sudan were studied by Gado, *et al.* (2017) . Three hundred seeds of each variety were treated with Sodium azide at five different concentrations (0.00%, 0.02%, 0.04%, 0.06% and 0.08%) before they were sown and grown to maturity in order to assess the effects of the different concentrations of the chemical on the plants. The results showed that 0.02% Sodium azide concentration had significantly higher values ($p<0.05$) when compared with its control in most of the characters (Table 2). It was observed that one of the variety Kenana-4 had higher value ($p<0.05$) for oil content at 0.08% sodium azide concentration. The results suggest that while 0.02% Sodium azide concentrations is the most effective to induce valuable and useful mutants for many of the yield parameters, 0.08% is effective with respect to Oil contents of sesame. Therefore, sodium azide can be an important tool for enhancing yield traits in sesame.

According to a research conducted by Gado, *et al.*(2017) on Sesame, the number of flowers in the control (0.00%) was lower than the treated with a mean of 14.60 while the dose 0.20% gave the highest yield with 29.80 lower concentrations gave higher yield. Higher concentrations gave lower yield (0.40%= 21.80, 0.06%=14.70, 0.60%=14.70 and 0.80%=15.70. The same trend was observed in Ex- sudan. Both Kenena and Ex-sudan had highest yield at 0.20% with 29.80 and 29.90 respectively. Higher concentration treatments gave lower yields in both varieties. The result on number of capsule per plant showed that the control (0.00%) had the lowest yield in the two varieties of Sesame. The highest in kenena -4 was 0.40% with 19.90, and 0.20% in Ex -Sudan with 22.10. The capsule length was highest at 0.20% in both varieties and lowest in the control. The two varieties treated with sodium azide showed significant differences with respect to capsule Weight, (Table 2). The weight of capsule was at its peak in both varieties at 0.02%. Similarly Kenana and Ex-Sudan showed significant differences with respect to number of seed per capsule. In terms of number of seed

per capsule in both varieties 0.20% has the highest yield (Table 2).

Table 2: The yield parameters of the two varieties at different concentration of sodium azide.

Treatment combination	No. of flower per plant	No. of fruit per Plant	Length of capsule	No. of seed per capsule	Weight of capsule
KENENA -4					
0.00%	14.60±2.05a	10.10±6.69[b]	2.10±0.12ab	49.50±3.40b	0.21±0.02b
0.20%	29.80±5.14d	12.90±6.20[a]	2.49±0.07c	54.70±3.64c	0.50±0.06c
0.40%	21.80±4.69c	19.90±14.21[a]	2.35±0.14bc	50.70±1.43c	0.23±0.01b
0.60%	14.70±2.62a	12.70±6.81[a]	2.22±0.10b	48.00±3.04a	0.23±0.03b
0.80%	15.70±2.28b	14.30±7.42[a]	1.91±0.10a	46.00±1.61a	0.11±0.01a
EX-SUDAN					
0.00%	20.70±2.98a	14.90±16.46[a]	2.08±0.09a	43.10±3.55a	0.21±0.01b
0.20%	29.90±3.99c	22.10±9.09[d]	2.37±0.06a	52.20±2.18b	0.28±0.02c
0.40%	23.40±3.24b	19.40±8.04[c]	2.21±0.11a	51.70±2.84b	0.24±0.01b
0.60%	21.00±3.32a	16.70±9.40[b]	2.06±0.18a	49.40±1.55b	0.16±0.01a
0.80%	18.10±2.92a	16.70±9.40[b]	2.12±0.06a	45.90±1.66a	0.20±0.01b

*Values are mean ± SD. Values followed by the same letter (s) within the same column do not statistically differ at the 5% level according to DMRT, analysed for the Treatment combination. Source: Gado *et al*. (2017)

Effects of Colchicine on Morphology of Plant

Chemical mutagenesis through the use of colchicine on the seeds of two varieties of sesame (*Sesamum indicum* L. Var. Ex-Sudan and E-8) with the aim of inducing variability that could be exploited in the genetic improvement of its growth and yield was carried out. The sesame seeds were treated with colchicines at four different concentrations (0.1mM, 0.5mM, 1.0mM, 2.0mM and control) for two mutant generations (M_1 and M_2). Highly significant variation ($P \leq 0.01$) was observed in such quantitative traits like the germination percents, height at maturity, number of leaves produced per plant, internodes length, leaf area, number of pods/plant, number of seeds/pod and 1000 seeds weight which decreased with increase in colchicines concentrations (Table 3 and 4). Besides these, a segment of chlorophyll deficient mutants such as: Chlorina, Xantha, Striata, Virescents and Lustescents were found among the mutant generations, with their frequency decreasing with increase in colchicines concentrations. Lower concentrations of

colchicines were recommended for inducing genetic variability in sesame (*Sesamum indicum* L.) to improve the yield of such economic plant. Five categories of chlorophyll deficient mutants in form of Chlorina, Xantha, Striata, Virescent and Lustescent were also observed among the mutants with Xantha and Lustescent being lethal, while Chlorina, Striata and Virescent as viable. The percentages of chlorophyll deficient mutants found in the M_1 and M_2 generations were presented in Table 4. Even though the Lustescents have the highest percentage of occurrence, the occurrence of viable chlorophyll mutants propensities and percentages (Chlorina, Striata and Virescents) were higher than that of the lethal types (Xantha and Lustescents) in both the two mutant generations.

TABLE 3 Mean Effects of Different Colchicines Concentrations on *Sesamum indicum Var. Ex- Sudan*

Mutant seeds Generation	Conc.	Germination (7 DAS)	Height at Maturity(cm)	Number of leaves/Plants	Internodes Length (cm)	Leaf Area (cm^2)	Number of Pods/Plants	Length of Pods	Number of seed/Pod	100 seeds Weight (g)
	0.0 mM	86.67[C*1]	60.33 [c]	17.40 [d]	2.75 [d]	39.67 [e]	3.00 [e]	2.20 c	50.67 [c]	3.00 [e]
	0.1 mM	100.00 [a]	82.67 [a]	25.40 [a]	4.85 [a]	56.00 [a]	5.40 [a]	3.03 [a]	66.67 [a]	3.70 [a]
M_1	0.5 mM	100.00 [a]	81.33 [a]	23.00 [b]	4.43 [b]	53.33 [b]	4.27 [b]	2.77 [b]	60.00 [ba]	3.50 [b]
Generation	1.0 mM	95.50 [b]	78.00 [ba]	21.80 [b]	4.30 [c]	51.00 [c]	3.73 [c]	2.60 [b]	58.33 [b]	3.40 [c]
	2.0 mM	95.50 [b]	76.73	20.00 [c]	4.20 [c]	49.00 [d]	3.40 [d]	2.50 [b]	57.33 [b]	3.20 [d]
	MEAN.	95.53	75.73	21.52	4.11	49.80	3.96	2.62	58.60	3.36
	S.E -+	2.47	4.01	1.35	0.35	5.57	0.42	0.14	2.56	0.12
	0.0 mM	84.50 [c]	65.73 [d]	41.13 [c]	11.13 [c]	41.07 [d]	13.33 [c]	1.89 [c]	40.47 [b]	3.04 [e]
	0.1mM	97.83 [a]	82.93 [a]	72.60 [a]	14.53 [a]	66.47 [a]	26.40 [a]	2.30 [a]	45.53 [a]	3.63 [e]
M_2	0.5 mM	95.50 [ba]	75.60 [b]	68.27 [a]	14.20 [ba]	52.13 [cb]	25.80 [a]	2.09 [b]	45.87 [a]	3.51 [b]
Generation	1.0 mM	95.50 [ba]	73.27 [cb]	61.47 [b]	13.00 [b]	53.27 [b]	19.67 [b]	2.10 [b]	45.47 [a]	3.35 [c]
	2.0 mM	93.33 [b]	72.87 [c]	58.53 [b]	12.87 [b]	50.47 [c]	18.67 [b]	2.10 [b]	48.53 [a]	3.28 [d]
	MEAN	93.33	74.08	60.40	13.15	52.48	20.77	2.09	45.17	3.36
	S.E -+	2.33	2.76	5.41	0.59	4.66	2.42	0.13	1.30	0.09

N.B *1 Means within the columns with the same letter (s) are not significantly different at $P \leq 0.05$ (Source: Nura *et al.*, 2011)

TABLE 4 Mean Effects of Different Colchicines Concentration of *Sesamum indicum Var. E-8*

Mutant seeds Generation	Conc.	Germination (7 DAS)	Height at Maturity(cm)	Number of leaves/Plants	Internodes Length (cm)	Leaf Area (cm^2)	Number of Pods/Plants	Length of Pods	Number of seed/Pod	100 seeds Weight (g)
	0.0 mM	93.33 ^{c*1}	60.67 ^{c}	20.40 ^{d}	11.27 ^{c}	39.33 ^{e}	12.13 ^{c}	1.93 ^{c}	42.33 ^{c}	2.80 ^{e}
	0.1 mM	100.00 ^{a}	77.00 ^{a}	28.80 ^{a}	15.13 ^{a}	55.67 ^{a}	29.40 ^{a}	2.39 ^{a}	64.33 ^{a}	3.60 ^{a}
M_1	0.5 mM	100.00 ^{a}	74.00 ^{a}	25.60 ^{b}	14.33 ^{ba}	51.67 ^{b}	23.67 ^{a}	2.19 ^{b}	63.00 ^{ba}	3.50 ^{b}
Generation	1.0 mM	96.67 ^{b}	73.00 ^{ba}	24.40 ^{b}	13.73 ^{b}	4933 ^{c}	21.47 ^{b}	2.15 ^{b}	61.33 ^{b}	3.40 ^{c}
	2.0 mM	96.67 ^{b}	71.33 ^{b}	22.20 ^{c}	13.33 ^{b}	48.00 ^{d}	20.73 ^{b}	2.17 ^{b}	60.33 ^{b}	3.10 ^{d}
	MEAN	97.33	73.14	21.52	13.34	48.49	21.15	2.12	59.11	3.31
	S.E -+	1.23	34.48	2.93	2.53	19.63	7.14	0.29	23.79	1.33
	0.0 mM	83.33 ^{c}	66.80 ^{d}	42.53 ^{c}	11.27c	38.27 ^{d}	12.13 ^{c}	1.93 ^{c}	37.80 ^{b}	3.03 ^{e}
M_2	0.1 mM	97.83 ^{a}	79.60 ^{a}	74.60 ^{a}	15.13 ^{a}	71.73 ^{a}	29.40 ^{a}	2.39 ^{b}	42.47 ^{a}	3.59 ^{a}
Generation	0.5 mM	95.50 ^{ba}	77.60 ^{b}	74.93 ^{a}	14.33 ^{ba}	57.47 ^{cb}	23.67 ^{a}	2.19 ^{b}	46.60 ^{a}	3.48 ^{b}
	1.0 mM	91.17 ^{b}	76.40 ^{cb}	64.33 ^{b}	13.73 ^{b}	59.87 ^{b}	21.47 ^{b}	2.15 ^{b}	40.20 ^{a}	3.33 ^{c}
	2.0 mM	93.33 ^{ba}	74.47 ^{c}	60.47 ^{b}	13.33 ^{b}	47.00 ^{c}	20.73 ^{b}	2.17 ^{b}	40.67 ^{a}	3.25 ^{d}
	MEAN	92.23	75.03	61.99	13.34	52.71	21.15	2.12	43.12	3.35
	S.E -+	2.49	7.45	11.64	2.53	17.14	7.14	0.29	9.37	0.12

N.B.: ^{*1} Means within the columns with the same letter(s) are not significantly different at $P \leq 0.05$ (Source: Nura *et al*., 2011)

Table 5 Percentages of Chlorophyll Deficient Mutants Induced by Colchicines in Sesame (*S.indicum Var. Ex-Sudan* and *E*-8)

Mutant M_1 Generation (*E* - 8)	**Concentration M_2 Generation (*Ex – Sudan*)**		**M_1 Generation M_2 Generation (*Ex - Sudan*) (*E* - 8)**		
Chlorina	0.0mM	10.09%	11.36%	6.45%	9.09%
	0.1mM	10.09%	8.03%	9.68%	9.09%
	0.5mM	8.03%	5.01%	6.45%	9.09%
	1.0mM	1.15%		3.23%	3.03%
	2.0mM	-	-	-	-
Xantha	0.0mM	-	-	-	3.03%
	0.01mM	-	-	-	-
	0.0mM	-	-	3.23%	-
	1.mM	-	-	-	-
	2.0mM	-	-	-	-
Striata	0.0mM	-	-	-	6.06%
	0.01mM	4.03%	4.03%	-	-
	0.5mM	4.03%	1.15%	3.23%	3.03%
	1.0mM	1.15%	1.15%	3.23%	3.03%
	2.0mM	1.15%	-	3.23%	-
Virescent	0.0mM	-	-	-	-
	0.01mM	11.36%	11.36%	9.68%	9.09%
	0.05mM	10.09%	11.36%	9.68%	9.09%
	0.0mM	8.03%	8.03%	6.45%	6.06%
	2.0mM	6.76%	4.03%	6.45%	6.06%
Lustescent	0.0mM	-	-	-	-
	0.1mM	10.09%	11.36%	12.90%	9.09%
	0.5mM	8.03%	11.36%	6.45%	9.09%
	1.0mM	4.03%	6.76%	6.45%	6.06%
	2.0mM	1.89%	5.01%	3.23%	-

Nura *et al*., 2011

Effects of Sodium Azide and Fast Neutron Irradiation on the Cytological Parameters of M_2 Lagos Spinach (*Celosia argentea* var *cristata* L.)

The effects of fast neutron irradiation (FNI) and sodium azide (SA) on the pollen and cytological parameters of *Celosia argentea* was carried out. M_1 seeds of treated *C. argentea* plant with fast neutron and sodium azide were collected from the seed bank of Department of Biological Sciences, Federal University of Technology, Minna, Nigeria and raised on the field to maturity stage. Young flower buds were collected from the plants for cytological studies. Cytological analysis of the plants revealed heterogeneous size of pollen grains, with three distinct variant of 29.12, 34.31 and 39.21 μm. The least average pollen diameters (32.66 μm) was recorded in 6.00 mM SA and the highest (37.58 μm) in 4.00 μS FNI. Significant variation ($p \geq 0.05$) in the numbers of pollen production per flower and anther were obtained. Lower percentage pollen fertilities were recorded in all the treated plants when compared with the control (94.15 %). However, these values were insignificance ($p \leq 0.05$), except for 8.00 mM which had the least of percentage pollen fertility of 71.62

%. The phenomenon of pollen restitution caused by abnormal meiotic division resulted in the formation of dyad, triad and tetrad in higher irradiated doses plants. Cytological analysis of the plant indicated that 8.00 μS had the highest mitotic index with metaphase (56.56) being the most frequent stage followed by telophase (28.40). Meiotic chromosomal counts revealed n = 18 at metaphase, with the formation of dyad and tetrad in most of the treated plants and the control. Abnormal meiotic division in 4.00 mM and 12.00 μS resulted in triad division. Observation from this study therefore, revealed that pollen restitution coupled with high mitotic index in 8 μS confer greater reproductive advantages to the plant.

Table 6. Effects of Mutagen on Pollen Production and Diameter of *Celosia argentea*

Treatments	Pollen Production /flower($x10^2$)	Pollen Production /anther($x10^2$)	Pollen Diameter (µm)
Control	927.50±3.49^{cd}	154.58±2.63^{cd}	35.95±1.14^{bc}
Buffer	777.50 ±1.50^{c}	129.58±0.42^{c}	35.62±0.58^{ab}
2.0mM	380.00±4.19^{ab}	63.33±2.64^{ab}	34.30±0.49^{ab}
4.0mM	467.50±4.69^{ab}	77.92±2.78^{ab}	35.29±0.52^{ab}
6.0mM	1110.00±4.11^{d}	185.00±1.85^{d}	32.66±7.78^{c}
8.mM	660.00±1.49^{bc}	110.00±0.51^{bc}	35.95±1.14^{bc}
4.0 µS	787.50±5.11^{c}	131.25±3.16^{c}	37.58±0.92^{c}
8.0 µS	400.003.54^{ab}	66.67±2.27^{ab}	35.29±1.28^{ab}
12 µS	330.00±4.16^{a}	55.00±2.53^{a}	34.97±1.25^{ab}
16 µS	470.00±2.12^{ab}	78.33±1.24^{ab}	37.26±1.96^{bc}

Values are means ± Standard Error, followed by the same letter(s) along the column are not significant different at p >0.05 as tested by DMRT. (Abubakar *et al*., 2015

Table 7. Effects of Mutagens on Pollen Restitution, Fertility and injury of *Celosia argentea*

	Actual Pollen	Pollen Restitution (%)	Pollen Fertility (%)	Pollen Injury (%)
Control	140	-	94.15±2.92[b]	-
Buffer	69	-	90.11±3.32[ab]	4.27±1.52ab
2.0mM	112	-	75.82±4.20[ab]	19.44±3.65cd
4.0mM	88	-	89.90±1.15[ab]	4.52±0.22ab
6.0mM	117	-	92.99±2.89[b]	1.23±0.07a
8.0mM	93	-	71.62±3.12[a]	23.93±4.62[d]
4.0 µS	118	-	84.51±4.57[ab]	10.28±4.02[ab]
8.0 µS	128	24.56±3.12	76.88±4.65[ab]	18.49±5.11[c]
12 µS	467	32.56±4.26	79.02±3.02[ab]	16.06±3.33[c]
16 µS	65	19.56±3.32	85.72±2.57[ab]	8.53±1.80[b]

Values are Means ± Standard Error, followed by the same letter (s) along the column are not significant different at $p > 0.05$ as tested by DMRT. (Source: Abubakar *et al*., 2015)

Table 8. Effects of Sodium Azide and Fast Neutron Irradiation on Mitotic Activities of *Celosia Argentea*

Conc.	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cell	Total Cell	Mitotic Index
Control	12	2	59	3	24	100	120	83.33
Buffer	-	5	39	2	36	82	108	75.93
2Mm	37	4	42	5	23	111	154	72.08
4Mm	-	2	82	-	9	93	123	75.61
6Mm	9	1	17	4	13	44	68	64.71
8Mm	-	2	32	4	39	77	97	79.39
4.0 µS	14	-	43	-	51	108	134	80.60
8.0 µS	32	3	139	20	29	223	297	75.09
12 µS	52	-	76	10	17	155	162	95.68
16 µS	7	3	36	2	43	91	118	77.12
Mean	23.29	2.75	56.50	6.25	28.40	108.40	138.10	77.95

Source: Abubakar *et al*., 2015

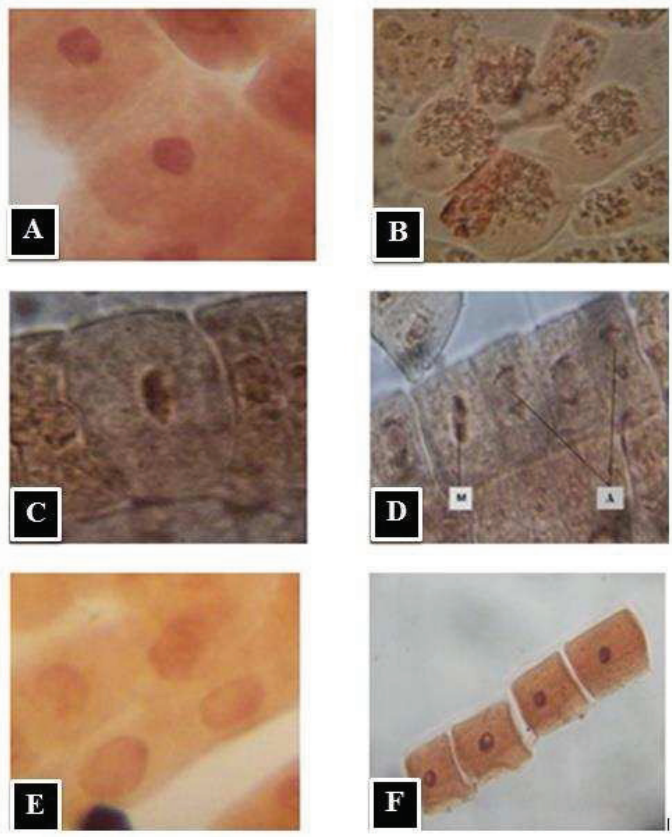

Figure 1. Mitotic Division Stages of *Celosia argentea* (A) Interphase, (B) Restitution Prophase, (C) Metaphase, (D) Late Metaphase and Anaphase stages, (E) Early Telophase, (F) Late Telophase

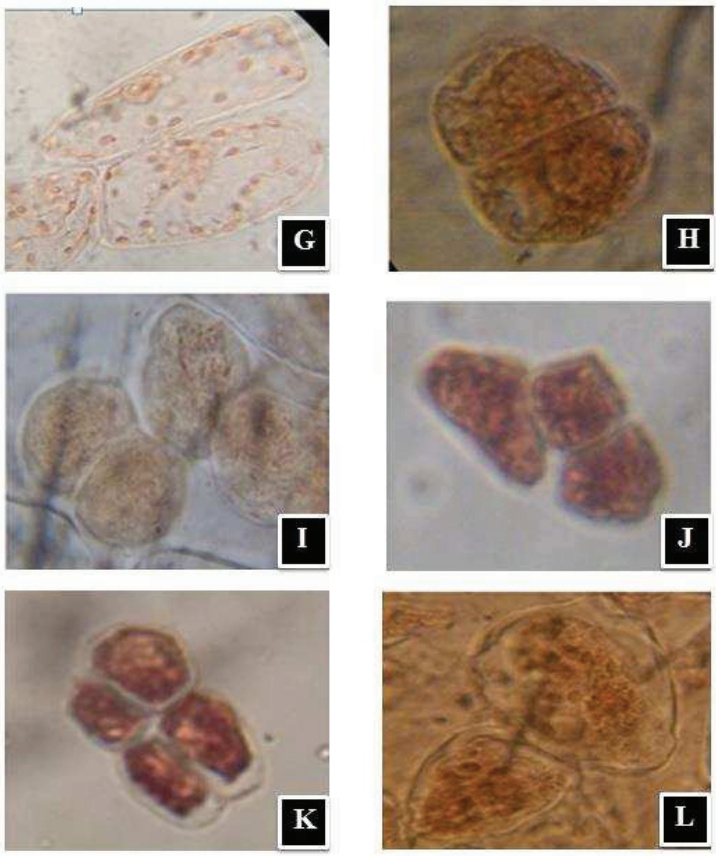

Figure 2. Meiotic Division of *Celosia argentea* (G) Meiotic Chromosomes n = 18 at metaphase stage, (H) Telophase I stage, (I) Dyad chromosome, (J) Triad chromosomal division due to abnormal meiotic division at 4 mM and 12 µS FNI, (K) Tetrad chromosome and (L) Nuclear estitution at 12 µS

Conclusion

This review has revealed that Sodium azide and Colchicine can serve as useful tool for creating variability in different plants in Nigeria. Genetic diversity is of great significance for breeding programmes as well as for taxonomic studies. Sodium Azide can serve as useful tool for creating variability in sesame. Artificial induction of mutation through the use of sodium azide proves vital in the improvement of genetic variability in sesame. Certain concentrations of Sodium azide (0.2% through 0.4% sodium azide concentration) have the potentiality of inducing variability that could be used in the improvement of the yield of sesame. The application of sodium azide on crop is easy and inexpensive. It creates mutation to improve their traits. The mutagenic effect of sodium azide appears after sowing the seeds observed by naked eyes. Sodium azide has been used in various crops to improve their yield and quality traits and create resistance to them against harmful pathogen. Different concentration of sodium azide 0.5%, 1% and 2% are used with control in chilli seeds. The best growth is obtained in 0.5% NaN3 concentration and beneficial growth is obtained in control and reduced growth was obtained in 1% NaN3

concentration. It was observed that these concentrations increases or decreases the growth rate of plant. It is concluded that sodium azide could be utilized to induce variability for the improvement of tomato.

References

Adamu, A. K. and Aliyu, H. (2007). Morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill.). *Science World Journal*, 2(4), 9-12.

Ahloowalia, B. S and Maluszynski, M. (2001). Induced Mutation. A new paradigm in plant breeding. *Euphytica*, 118(2), 167-173.

Auerbach, C. and Robson, J.M. (1944). Production of mutations by allyl isothiocyanate. *Nature.* 154, 81.

Auerbach, C. and Robson, J.M. (1946). Chemical production of mutations. *Nature*. 157, 302.

Bertagne-Sagnard, B., Fouilloux, G. and Chupeau, Y. (1996). Induced albino mutations as a tool for genetic analysis and cell biology in flax (*Linum usitatssimum*). *Journal of Experimental Botany* 47, 189–194.

Bhat, T.A., Khan, A.H. and Parveen, S. (2005a). Comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in *Vicia faba* L. *Journal of Indian Botanical society* 84, 45-58.

Dahot, M.U., Rafiq, M., Ambreem, M. A. and Naviq,S.H.A.(2012). Effect of Sodium azide on the growth of *Capsicum annum* (Chilli). *Pakistan Journal of Biotechnology* 9(1)13-20.

Dhanayanth, K. P.M. and Reddy, V. (2000). Cytogenetic effect of gamma rays and ethyle methane sulphonate in chilli piper (*Capsicum annum*). *Cytologia* 65, 129-133.

Gado, A. A., Falusi,O.A., Muhammad, L. M., Daudu, O.A.Y., Yahaya,S.A., Abejide,D.R.and Garba, Y.(2017). Effect of Sodium azide on two varieties of sesame. *International Journal of Applied Biological Research*, 8(1), 85-94.

Gustafsson, A. and Mackey, J. (1948). The genetic effects of mustard gas substances and neutrons. *Hereditas*. 34, 371-386.

Kleinhofs, A., Sander, C. and Nilan, R.A. (1974). Azide mutagenicity – mechanism and nature of mutants produced. In: Polyploidy and Induced Mutations in Plant Breeding. IAEA, Vienna. pp. 195-199.

Mahandjiev, A., Kosturkova, G. and Mihov, M. (2001). Enrichment of Pisum sativum gene resources through combined use of physical and chemical mutagens. *Israel Journal of plant sciences*, 49(4), 279-284.

Mashenkov, A. (1986). Induced mutation process as a source of new Mutants. Maize Genetics Cooperation newsletter, 60, 70-71.

Nura, S., Adamu, A. K., Mu'azu, S. and Dangora, D. B. (2011a). Effect of colchicine induced mutagenesis on growth and yield of sesame (Sesamum indicum L.). Bayero Journal of Pure and Applied Sciences, 4 (1): 121 – 125.

Nura, S., Adamu, A. K., Mu'azu, S., Dangora, D. B. and Mahamud, F. M. (2011b). Mutational Rectification Induced Via Colchicine in Sesame (Sesamum indicum L.). *International Journal of Emerging Technologies in Science and Engineering*, 5 (2): 119-122.

Oehlker, F. (1943). Chromosome mutation in meiosis by chemicals. In: Mutation Research - Problems, Results and Prospects. C. Auerbach (ed.) (1976), Chapman and Hall, U.K.

Rapoport, I.A. (1946). Carbonyl compounds and the chemical mechanism of mutation. C.R. Doklady Academic. Science. USSR. 54, 65.

Rapoport, I.A. (1948). Alkylation of gene molecule. C.R. Doklady Acad. Sci. USSR. 59: 1183-86.

Ricardo, M. and Ando, A. (1998). Effects of gamma-radiation and Sodium azide on quantitative characters in rice (Oryza sativa L.). *Genetics Molecular Biology* 21(1), 244-251

Van, Den-Bulk RW., Loffer, H.J.M., Lindhout, W.H. and Koornneef, M. (1990). Somaclonal variation in tomato: effect of explants source and a comparison with chemical mutagenesis. *Theoretical Applied Genetics* 80, 817–825.

Yuan, H.Y. and Zhang, Z., (1993). Effect of free radicals and temperature on sister chromatid exchanges in *Hordeum vulgare* L. *Acta Botanica Sinica* 35, 20-26.

Zeerak, N.A., 1991. Cytogenetical effect of gamma rays and ethylmethane sulphonate in brinjal (*Solanum melongena* L.). *Cytologia* 56, 639-643.