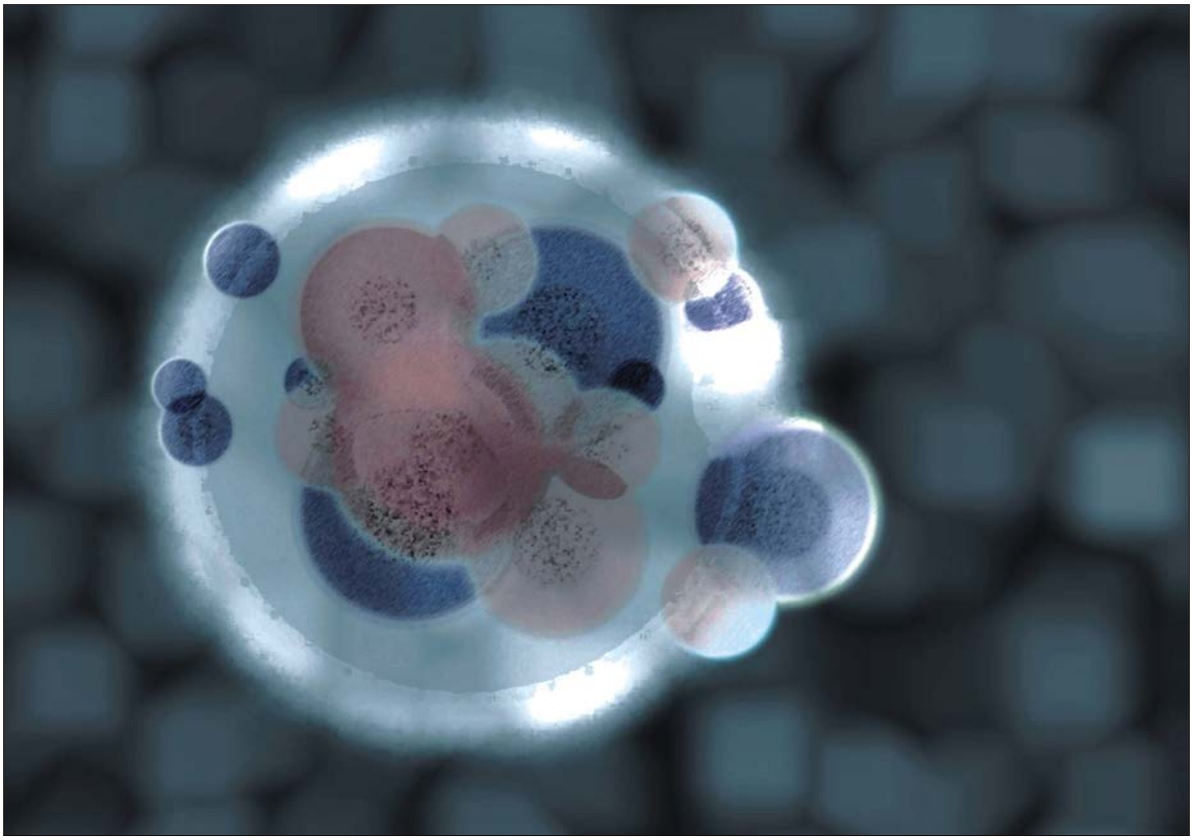

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Morphological Characterization of Colchicine-induced Mutants in Sesame (*Sesamum indicum* L.)

[1]S. Nura, [2]A.K. Adamu, [2]S. Mu'Azu, [2]D.B. Dangora and [3]L.D. Fagwalawa
[1]School of Basic and Remedial Studies, Ahmadu Bello University Funtua, Nigeria
[2]Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria
[3]Department of Biology, Kano University of Science and Technology, Wudil, Nigeria

Abstract: The mutagenic effect of colchicine to improve leaf and seed yield in two varieties of sesame (*Sesamum indicum* L. var. Yandev and Ex-Sudan) was investigated. Seeds of the two sesame varieties were presoaked in five different colchicine concentrations (0.1, 0.5, 1.0, 1.5 and 0.0 mM as control). The seeds were planted in a completely randomized block design for two mutant generations (M_1 and M_2). The M_2 mutants were characterized on the basis of morphological traits such as height at maturity, anthesis period, number of leaves/plant, leaf area, internodes length, number of capsules/plant, length of capsules, number of seeds/capsule and 1000 seeds weights. The results obtained revealed highly significant difference ($p \leq 0.01$) in the morphological traits of the mutants when compared with the controls, except in the number of seeds where the effect of the mutagen is significant ($p \leq 0.05$). More so, with the exception of internodes length and 1000 seeds weight, the leaf yield parameters of the M_2 mutants were found to correlate significantly with the seed yield. Similarly, colchicine was found to induce changes in the chlorophylls and carotenoids bio-syntheses among the M_2 mutants, leading to the formation of five forms of chimeras as chlorinas, xanthas, striatas, virescents and lustescents. It was therefore, inferred that, colchicine-induce mutants were characterized as early flowering with tall stature having larger leaves and which produced large number of capsules with numerous seeds. The improvement of the mutants' traits is concentration dependent, increases with decrease in colchicine concentration. Thus, we therefore suggested that, 0.1 mM concentration should be employed in improving sesame growth and yield related traits.

Key words: Colchicine, ex-Sudan, mutants, yandev

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most important oil seeds crops in the world according to some archaeological findings (Bedigian and Harlan, 1986) as ancient seeds of the crop were identified in excavations at Harappa, Pakistan dated 2000 B.C. (Uzo, 1998). It originated from Africa (Purseglove, 1968; Zeven and de Wet, 1982; Mabberly, 1997; Ashri, 1998) with the Indian sub-region serving as the secondary center of its diversity (Bedigian, 1981). A member of the family Pedaliaceae, sesame is grown all over the world for its oily seeds (Burkill, 1997) and edible leaves (Mann *et al*., 2003) used as vegetable. Sesame is a plant of high nutraceutical, pharmaceutical and economic importance. Sesame seeds are important source of high quality edible oil, minerals, vitamins (FAO, 1988; Pamplona-Roger, 1999; Katung and Asiribo, 2002) and proteins for poor farmers of major sesame growing countries such as Sudan, Nigeria, Ethiopia, Uganda, Mexico, Venezuela, India, China, Pakistan and Turkey (Kumar and Yadav, 2010). Besides being used in fish and beef canning, sesame oil is an important edible oil in Europe (Busari *et al*., 2005). The oil extracted from sesame accounts for about 2% of the world edible vegetable oil market in Europe (Busari *et al*., 2005). The oil substitutes olive oil and other oils in cosmetic creams and in Oleo-margarine and iodized oil (Budavari, 1996) because of its high quality for resisting oxidative rancidity due to the presence of phenylpropanoid lignans sesamin and sesamolin (Anonymous, 2005).

Even though, Nigeria is characterized by Weiss (1971) among the major sesame exporting countries, ranking second to Sudan in production and export of sesame seed (FAO, 2008); with an annual earning of over $US878 million in foreign exchange; its intensive cultivation and uses in Nigeria are not beyond subsistence level. This is attributed to the lack of high yielding varieties in conjunction with high agronomic in-puts needed for its cultivation. There is therefore, the

Corresponding Author: S. Nura, School of Basic and Remedial Studies, Ahmadu Bello University Funtua, Nigeria

need for improving the available germ-plasm to meet the demand of both subsistence and commercial production of the plant. Moreover, Begum and Dasgupta (2010) stressed that, several sesame genotypes are generally unimproved and many collections have been made of land races, with little or no genetic information that can lead to sesame utilization in breeding programmes. These brought about the lack of high yielding varieties compared to the high agronomic in-puts needed for sesame cultivation. Therefore, efforts need to be concerted in the improvement of sesame germplasm (Van Rheenen, 1973; Ashri, 1998). This will pave way for the genetic improvement of the crop to meet the needs of the world's growing population.

Induced mutation both in seeds and vegetatively propagated crops is one of the techniques employed in the improvement of traits of economic plants. It facilitates the isolation, identification and cloning of genes which would ultimately help in designing crops with improved yield, increased stressed tolerance and longer life span as well as reduced agronomic in-puts usage (Ahloowalia and Maluszynski, 2001). Over the years, man relied upon spontaneously occurring variants, arising from mutations to improve the yield and quality of crop plants (Herper, 1999). Effectiveness and efficiency are two distinct properties of mutagens that have been extensively discussed elsewhere (Kawai, 1986; Shah *et al*., 2008; Girija and Dhanavel, 2009). A number of chemicals have been found to be equally and even many times more effective and efficient mutagens (Thakur and Sethi, 1995; Kharkwal, 1998; Solanki, 2005; Rekha and Langer, 2007; Basu *et al*., 2008; Ganapathy *et al*., 2008; Wani, 2009). Therefore, this study aimed at investigating the effect of colchicine (an alkaloid derivative of *Colchicum autumnale*) on yield of sesame as a strategy for its improvement through chemical mutagenesis.

MATERIALS AND METHODS

The study was conducted in the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria (Lat11°11[1] N; Long 7°N 38[1]E) in 2007 and 2008 growing season. The seeds of sesame (*Sesamum indicum* L. *var*. Yandev and Ex-Sudan) were obtained from the Jigawa State Agricultural and Rural Development Authority (JARDA) Ringim. The seeds were treated by soaking for four hours at five different treatments of colchicine concentrations including a control (0.1, 0.5, 1.0, 2.0 mM and control) and thoroughly washed in running water. The treated seeds were sown in a Completely Randomized Block Design (CRBD) with three replications for two mutant generations (M_1 and M_2). All cultural practices followed the protocols described in the Kano State Agricultural and Rural Development Authority (KNARDA, 2005) crop production guide. All the data obtained were subjected to analysis of variance using SAS (1988). Duncan's Multiple Range Test was used to separate the means. The test of relationship was done using Pearson's product moment correlation.

RESULTS

The result obtained from the M_2 generation analysis of variance (Table 1) indicated highly significant improvement ($p \leq 0.01$) in the effect of the mutagen on all the selected traits of sesame except in the number of seeds/capsule, where the effect is significant.

Furthermore, the result of the mean effect of colchicine on Ex-Sudan (Table 2) revealed the emergence of early flowering mutants with tall stature and substantial number of leaves (58-73 leaves/plant). The mutants' leaves are larger in size (50.47-66.47 cm^2) and

Table 1: M_2 generation mean squares on the effects of colchicine on some traits of sesame

Sources of variation	Df	Anthesis period (days)	Height at maturity (cm)	No. of leaves/plant	Leaf area (cm^2)	Internodes Length (cm)	No. of capsules/plant	Length of capsules (cm)	No. of seeds/capsule	1000 seeds weight (g)
Blocks	2	2.11^{ns}	85.96**	39918.10**	73765.40**	2430.40**	13442.00**	7.62**	8615.10**	0.90**
Concentration	4	9942.50**	528.00**	7342.80**	4416.30**	72.09**	1200.00**	0.90**	287.90*	2.20**
Error	208	1.94	9.88	135.37	293.80	6.42	51.04	0.08	87.85	0.02

ns: No significant difference *Significant difference ($p \leq 0.05$) **Highly significant difference ($p \leq 0.01$)

Table 2: M_2 generation mean effects of colchicine on some traits of ex-Sudan

Concentration (mM)	Anthesis period (days)	Height at maturity (cm)	No. of leaves/plant	Leaf area (cm^2)	Internodes length (cm)	No. of capsules/plant	Length of capsules (cm)	No. of seeds/capsule	1000 seeds weight (g)
0.0	69.00^{a*1}	65.73^{d}	41.13^{c}	41.07^{d}	11.13^{c}	13.33^{c}	1.89^{c}	40.47^{b}	3.04^{e}
0.1	35.00^{c}	82.93^{a}	72.60^{a}	66.47^{a}	14.53^{a}	26.40^{a}	2.30^{a}	45.53^{a}	3.63^{a}
0.5	35.07^{c}	75.60^{b}	68.27^{a}	52.13^{cb}	14.20^{ba}	25.80^{a}	2.09^{b}	45.87^{a}	3.51^{b}
1.0	35.00^{c}	73.27^{cb}	61.47^{b}	53.27^{b}	13.00^{b}	19.67^{b}	2.10^{b}	45.47^{a}	3.35^{c}
2.0	36.93^{b}	72.87^{c}	58.53^{b}	50.47^{c}	12.87^{b}	18.67^{b}	2.10^{b}	48.53^{a}	3.28^{d}
Mean	42.20	74.08	60.40	52.48	13.15	20.77	2.09	45.17	3.36
SE±	6.69	2.76	5.41	4.66	0.59	2.42	0.13	1.30	0.09

*[1]Means within the columns with the same letter(s) are not significantly different at $p \leq 0.05$

Table 3: M_2 generation mean effects of colchicine on some traits of Yandev

Concentration (mM)	Anthesis period (days)	Height at maturity (cm)	No. of leaves/plant	Leaf area (cm^2)	Internodes length (cm)	No. of capsules/plant	Length of capsules (cm)	No. of seeds/capsule	1000 seeds weight (g)
0.0	68.60^{a*1}	68.07^{d}	40.20^{c}	38.53^{d}	11.87^{c}	14.27^{c}	1.89^{c}	38.60^{b}	3.03^{e}
0.1	35.00^{c}	82.60^{a}	72.27^{a}	59.93^{a}	14.60^{a}	24.07^{a}	2.26^{a}	43.67^{a}	3.61^{a}
0.5	35.13^{c}	78.67^{b}	71.87^{a}	54.93^{cb}	13.93^{ba}	24.87^{a}	2.14^{b}	44.60^{a}	3.51^{b}
1.0	35.00^{c}	78.47^{cb}	64.87^{b}	55.00^{b}	13.00^{b}	21.87^{b}	2.11^{b}	43.13^{a}	3.35^{c}
2.0	36.60^{b}	72.33^{c}	61.80^{b}	47.53^{c}	13.13^{b}	20.93^{b}	2.14^{b}	43.20^{a}	3.27^{d}
Mean	42.07	76.03	62.20	51.18	13.31	21.20	2.11	42.64	3.35
SE±	6.63	2.58	5.85	3.73	0.46	1.87	0.06	1.04	0.09

*[1]Means within the columns with the same letter(s) are not significantly different at $p\leq 0.05$

Table 4: Correlation co-efficients for the various traits of M_2 sesame varieties treated with colchicine

Parameters	Anthesis period (days)	Height at maturity (cm)	Leaves number/ plant	Leaf area (cm^2)	Internodes length	No. of capsules/ plant	Length of capsules (cm)	Seeds number/ capsule	1000 seeds weight (g)
Anthesis period	1.000								
Height at maturity	-0.137^{ns}	1.000*							
Leaves number/plant	-0.438^{ns}	0.819**	1.000						
Leaf area	-0.228^{ns}	0.774**	0.708*	1.000					
Internodes length	-0.193^{ns}	0.849**	0.793**	0.774**	1.000				
Capsules numberplant	-0.304^{ns}	0.774**	0.761**	0.728*	0.817**	1.000			
Length of Capsules/plant	-0.281^{ns}	0.640*	0.608*	0.532*	0.703*	0.651*	1.000		
Seeds number/capsule	-0.169^{ns}	0.629*	0.556*	0.597*	0.625*	0.612*	0.579*	1.000	
1000 seeds weight	-0.669*	0.398^{ns}	0.586*	0.508*	0.413^{ns}	0.517*	0.440^{ns}	0.383^{ns}	1.000

Table 5: Percentages of chlorophyll deficient mutants induced by colchicine in the M_2 generation of sesame

Mutant	Concentration (mM)	Ex-Sudan (%)	Yandev (%)
Chlorina	0.0	-	-
	0.1	6.45	7.41
	0.5	9.68	11.11
	1.0	6.45	7.41
	2.0	3.23	3.70
Xantha	0.0	-	-
	0.1	-	-
	0.5	-	-
	1.0	3.23	-
	2.0	-	-
Striata	0.0	-	-
	0.1	-	7.41
	0.5	3.23	-
	1.0	3.23	-
	2.0	3.23	3.70
Virescent	0.0	-	-
	0.1	9.68	11.11
	0.5	9.68	7.41
	1.0	6.45	7.41
	2.0	6.45	7.41
Lustescent	0.0	-	-
	0.1	12.90	11.11
	0.5	6.45	7.41
	1.0	6.45	3.70
	2.0	3.23	3.70

which have longer internodes (12.87-14.53 cm). Similarly, the mutants produced high number of capsules (19-26 capsules/plant) which are larger (2.10-2.30 cm) and which produced 46-48 seeds/capsule. More so, the 1000 seeds weights of the mutants are higher than that of the control.

Similarly, the result of the mean effects of colchicine on the traits of Yandev (Table 3) showed the emergence of early flowering mutants with tall stature. The mutants produced 62-72 leaves/plant that are 47.53-59.93 cm^2 in cross sectional area with a internodes length of 13.13-14.60 cm. The mutants also produced 21-24 capsules/plant that are 2.14-2.26 cm in length and which produced 43-45 seeds/capsule. More so, the mutants produced seeds that weigh higher than that of the control.

Furthermore, the leaf yield parameters of the M_2 generation were found to correlate significantly with the seed yield (except between the internodes length and 1000 seeds weight where no significant correlation was found) (Table 4).

More so, the mutants were also characterized by the deficiency in chloropylls and carotenoids forming chimeras in form of chlorine, xantha, striata, virescent and lustescent as presented in Table 5.

DISCUSSION

Chemical mutagenesis has proved vital in the improvement of crop plants. The use of chemical mutagen in crop improvements initiatives have been reported in a number of species by Sander and Muchlbever (1977) and Biswas and Datta (1988). The emergence of early flowering mutants in sesame was in agreement with the work of n Rutger (1982) who reported early flowering mutants in medium grain *Japonica* cultivar; but was in contrast to that of Archana *et al.* (2004) who reported increase in the number of days required for maturity due to increased doses of mutagen treatments in *Glycine max* (soya beans). This may probably be due to the tendency of the mutagen to turn-on the gene responsible for inducing flowering, by making the plant to respond to environmental signals such as photoperiodism and hormonal actions as suggested by Lewis *et al.* (2002). Artificial induction of mutation by colchicine leads to the alteration of plant genome integrated by environmental signals as reported by Uno *et al.* (2001); probably by increasing the rates of cellular division and expansion at their meristematic regions. This on the other hand can be viewed as the mechanism through which colchicine-induced tall mutants emerged. This was in agreement with the findings of Hoballah (1999) who reported increased in plant heights of sesame due to irradiation mutagenesis; but was in contrast to the findings of Maluszynski *et al.* (2001) who reported decreased in plant height due to induced mutation in rice. The mutagen might have probably influenced the activities of cytokinin which is of paramount importance in the fundamental processes of plant development including cell division, morphogenesis as suggested by Deikman and Ulrich (1995). Sesame mutants with increased leaf number and size were induced by different colchicine concentrations. This was in agreement with the findings of Nura *et al.* (2011) who reported increase in leaf number and area among mutants of jute. The increased leaf number and area provides an increase in the surface area for gaseous ex-change which considerable affect the process of photosynthesis as reported by Lockhart *et al.* (1996). However, the increased in the internodes length among the sesame mutants is contrary to the findings of Mensah *et al.* (2007) who discovered internodes among colchicine-induced mutants of sesame.

Furthermore, the increase in the number of capsules and size among sesame mutants was in agreement with the work of Hoballah (1999) who reported increase in the number of capsules per plant among sesame mutants due to gamma irradiation; which considerably facilitate improvement in the sesame seeds yield. The increase in number and size of the capsules permit a substantial increase in the number of seeds produced; thereby facilitating the production of mutants producing large number of seeds. Moreover, the increase in 1000 seeds weight of sesame mutants due to colchicine treatment was in line with the work of Shen *et al.* (1995) who reported increase in grain weight of rice due to gamma rays in *in vitro* mutagenesis. This was also in conformity with the work of Pathirana *et al.* (2000) who reported increased in seed yield of sesame due to gamma irradiation. The results obtained in this study were therefore in agreement with those of Antoun (1980), Gautam *et al.* (1998); Asmahan (2000), Rascio *et al.* (2001) and Osama (2002), who individually reported that the improvement of yield components in various plants such as tomato, maize, rice and wheat was induced after various mutagenic treatments such as E.M.S, sodium azide and gamma rays. Moreover, the correlation between the yield and yield components of the mutants is in conformity with the findings of Akram *et al.* (1982) and Brar and Saini (1976) among the irradiated mutants of rice. This indicated that selection should be based on the height, leaves number and size. Beside this, induced mutation was reported by Bartley *et al.* (1994) to be useful in the genetic control of *carotenoid* bio-synthesis in certain plants like *Arabidopsis thaliana*, *Lycopersicon esculentum*, *Zea mays* and other species of plants. The whole range of chlorophyll mutation occurred due to the deficiency in either: *chlorophylls*, *carotenoids* or combination of both in plastid genes causing variegation as reported by Kirk and Tilney-Bassett (1978).

CONCLUSION

In conclusion, the colchicine-induced mutants of sesame were found to be characterized with advantageous traits such as increased height, leaf, capsules and seeds number as well as increased size of leaves internodes which are needed for sesame improvement. Lower colchicine concentration (0.1 mM) was found to be more beneficial and effective in improving the yield traits of sesame. We therefore, recommend the use of lower colchicine concentration in the genetic improvement of sesame.

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