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EFFECT OF COLCHICINE INDUCED MUTAGENESIS ON GROWTH AND YIELD OF SESAME (*SESAMUM INDICUM* L.)

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ABSTRACT
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. 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.

Key words: Concentrations, Colchicines, Sesame, Ex-Sudan and E-8

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the most ancient oil seeds crops (Purseglove, 1968) cultivated for its edible leaves used as vegetables (Mann *et al*., 2003) or oily seeds (Burkill, 1997). It is an erect annual herb with mucilaginous stem, the leaves are opposite at the lower side and alternate at the upper side, simple, ex-stipulated and petiolated with lanceolated lamina. The flowers are solitary on leaf axils, zygomorphic, hermaphroditic and pentamerous. Stamens are 4 or 2, epipetalous with superior ovary. The fruit is a capsule lacking lateral horns and many seeded with smooth testa. The seeds are mostly white and show epigeal germination (Saunders, 1985; Mann *et al.,* 2003 and Grubben *et al.,* 2004).

The plant is rich in vitamins, proteins, minerals and lipids that is free from oxidative rancidity, thereby increasing the quality of the oil (Anonymous, 2005). Beside these, its oil contains *Lecithin* (a *Phospho-lipid*) that decreases the level of *cholesterol* in the body thereby preventing *Arteriosclerosis*, *angina pectoris* and *arterial thrombosis* (Pamplona-Roger, 1999). About 6.6 million hectares of the total world crop area are under sesame cultivation (Thangavelu, 1992) with Africa ranking second with 25% of the total hecterage planted (Busari *et al*., 2005). Nigeria alone exports about 50,000 tons annually (Anonymous, 2010), which if properly managed can reduce the pressure of Nigerian economy on crude oil exportation. But ironically, despite the tremendous benefits of sesame, its diversity and uses are under threat due to neglect by the authorities. The reservoir of wealth of this plant is deteriorating; efforts are therefore needed in the exploration of the hidden treasures conserved within this plant.

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, this work has made use of colchicines, a poisonous alkaloid derived from the autumn crocus (*Colchicum autumnale*) in inducing genetic variability through mutagenesis to improve both the quality and quantity of sesame. The aim of the present study is to induce mutation through the use of various colchicines concentrations in two varieties of sesame (*S. indicum* L. *Var*. *Ex-Sudan* and *E-8*) to improve the quality and quantity of the plants.

MATERIALS AND METHODS

The research was conducted in the botanical garden of the Department of Biological Sciences, Ahmadu Bello University Zaria (Lat11^{0}N, Long 7^{0}N 42^{1}E) The seeds of two varieties of sesame (*Sesamum indicum* L. *Var*. *Ex-Sudan* and *E-8*) were obtained from the Jigawa State Agricultural and Rural Development Authority (JARDA) Ringim. The seeds were treated through soaking at five different colchicines concentrations (0.1mM, 0.5mM, 1.0mM, 2.0mM and 0.0mM) for two mutant generations (M_1 and M_2). The treated seeds were sown in a plot with three blocks in a Completely Randomized Block Design (CRBD) with three replications in a factorial arrangement.

All cultural practices followed the protocols described in the Kano State Agricultural and Rural Development Authority (KNARDA) crop production guide (2005). Data were collected from the germination percents, height at maturity, number of leaves, internodes length, leaf area, number of pods/plant, number of seeds/pod and 1000 seeds weights. All the data obtained were analyzed using factorial analysis in a CRBD format. Means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

The results obtained following treatment of sesame seeds with five different colchicines concentrations revealed the presence of highly significant variation ($P \leq 0.01$) in the germination percents, heights at maturity, number of leaves produced per plant, internodes length, leaf area and number of pods/plant in both the mutant generations (M_1 and M_2 generations). While significant variations ($P \leq 0.05$) were found in the effect of the mutagen on 1000 seeds weights in M_1 and number of seeds/pods in M_2 (Table, 1).

The results of the mean effects of different concentrations of the mutagen on some selected traits of *Ex-Sudan* were presented in Table, 2. The results in the M_1 generation showed that significant variation exist between the mutants and the controls. For each traits, the least significant difference (LSD) was calculated and used to compare the mutants against the controls. Higher germination percents were found among the mutants treated with 0.1mM concentration (100.00% and 97.83% in M_1 and M_2 respectively). The lowest germination percent was found in 2.0mM treated plants with an average percentage of 94.50% in M_1 and 93.33% in M_2. Germination percent decreases with increase in the concentrations of colchicines. However, greater variability in heights at maturity from 76.33 cm under 2.0mM to as higher as 82.67 cm in M_1 and 74.08 cm to 82.93 cm were found among the mutants in the M_2. More so, increase in the mean number of leaves produced per plant and their internodes lengths in the M_1 were higher in 0.1mM treated plants (producing 25 leaves/plant with a corresponding internodes length of 4.85 cm) and lowest under 2.0mM concentrations (20 leaves/plant with corresponding internodes length of 4.20 cm).

Similarly, in the M_2 generation the mutants greater variability exist with a difference of 32 leaves/plant under 0.1mM concentrations and 17 leaves/plant under 2.0mM concentrations higher than the controls in the number of leaves produced per plant More so, the corresponding internodes lengths varied between the mutants and the controls with an average internodes length of 14.53 cm under 0.1mM concentration which decreased to 12.87 cm under 2.0mM concentrations. Furthermore the leaf area of the mutants varied significantly from the controls with a mean increase of 49.00cm^2-56.00cm^2 in M_1 and 50.47cm^2-66.47cm^2 in M_2 under 2.0mM and 0.1mM concentrations respectively . On the other hand, the number of pods produced per plant varied significantly with the colchicines concentrations (5pods/plant in M_1 and 26pods/plant in M_2) under 0.1mM concentrations but decreased to 19pods/plant in 2.0mM treated plants. These values are greater than the values obtained from the controls.

Similarly, a greater variation in the number of seeds/pod and the 1000 seeds weights were found among the mutants (with a difference of 7-16 seeds and 10-13 seeds higher than the controls in M_1 and M_2 respectively). However, the mutants showed an increase in the weights of 1000 seeds ranging from 3.20g-3.70g in M_1 and 3.28g-3.63g in M_2. The variability decreased with increase in the concentrations of colchicines.

Highly significant variations also exist among the germination percents of *E-8* mutants (Table, 3) in the M_1 with 100% germination in 0.1mM treated plants to 96.67% in 2.0mM treated plants. However, in the M_2 generation 97.83% germination was found under 0.1mM concentrations which decreases to 93.33% under 2.0mM. Variations in the plants heights at maturity from71.33 cm-77.00 cm in M_1 and 74.47 cm to 79.60 cm in the M_2 were found among the mutants. Similarly, leaves number at maturity produced/plant decreased with increase in colchicines concentrations (22-28 leaves/plant in M_1 and 61-75 leaves/plant in M_2). But the corresponding internodes lengths showed no marked variation among the generations except that they decreased with increase in colchicines concentrations. More so, variation in leaves size from 48.00cm^2 to 55.67 cm^2 in M_1 and from 47.00 cm^2 to 71.73 cm^2 in M_2 through 2.0mM to 0.1mM concentrations respectively. Therefore, variability in the number of pods produced /plant was found among the mutants, decreasing with increase in colchicines concentrations in all the generations. Variability in seed number and weight exist among the mutants' generations of *E*-8. In the M_1 the seeds number increased with decrease in colchicines concentration from 60 seeds/pod to 64 seeds/pod. Also, the weights of 1000 seeds decreased with increase in colchicines concentrations from 3.10g to 3.60g. Similarly, in the M_2 the seeds number and the 1000 seeds weights decreased with increase in colchicines concentrations.

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 1 Mean Square Estimates of the Effects of Different Colchicines Concentrations on Sesamum *indicum* (*Var. Ex-Sudan and E-8)*

Mutant Generation	Sources of Variation	Df	Germination Percent (%)	Height at Maturity (cm)	No. of leaves per plants	Leaf area (cm^2)	Internodes length (cm)	No. of pods per plant	Length of pods /plant (cm)	No. of seeds per pod	1000 seeds weight (g)
	Blocks	2	4.01**	85.96**	18.70^{ns}	33.36^{ns}	1780.30**	5.85**	0.58**	21.10^{ns}	0.04^{ns}
M_1	Concentration	4	9.70**	528.00**	334.34**	391.53**	2864.50**	61.73**	0.71**	473.49**	0.72*
	Error	208	0.38	9.88	8.60	40.01	50.71	0.56	0.06	29.23	0.90
	Blocks	2	1.07^{ns}	112453.00**	39918.10**	73765.40**	2430.40**	13442.00**	7.62**	8615.10**	0.90**
M_2	Concentration	4	4.81**	1357.60**	7342.80**	4416.30**	72.09**	1200.00**	0.90**	287.90*	2.20**
	Error	208	0.32	55.51	135.37	293.80	6.42	51.04	0.08	87.85	0.02

Keys: ns= No significant difference * = Significant difference (P≤0.05) **= Highly significant difference (P≤0.01)

Table 2 Mean Effects of Different Colchicines Concentrations on *Sesamum indicum Var. Ex-Sudan*

Mutant Generation	Concentration	Germination Percent (7 DAS)	Height at Maturity (cm)	Number of Leaves/ Plant	Internodes Length (cm)	Leaf Area (cm^2)	Number of Pods/ Plant	Length of Pods (cm)	Number of Seeds/ Pod	1000 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 Generation	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}
	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}	76.33^{ab}	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
M_2 Generation	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}
	0.1 mM	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^{a}
	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}
	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≤0.05

Table 3 Mean Effects of Different Colchicines Concentrations on *Sesamum indicum Var. E*-8

Mutant Generation	Concentration	Germination Percent (%)	Height at Maturity (cm)	Number of Leaves/ Plant	Internodes Length (cm)	Leaf Area (cm^2)	Number of Pods/ Plant	Length of Pods (cm)	Number of Seeds/ Pod	1000 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 Generation	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}
	1.0 mM	96.67^{b}	73.00^{ba}	24.40^{b}	13.73^{b}	49.33^{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
M_2 Generation	0.0 mM	83.33^{c}	66.80^{d}	42.53^{c}	11.27^{c}	38.27^{d}	12.13^{c}	1.93^{c}	37.80^{b}	3.03^{e}
	0.1 mM	97.83^{a}	79.60^{a}	74.60^{a}	15.13^{a}	71.73^{a}	29.40^{a}	2.39^{a}	42.47^{a}	3.59^{a}
	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	52.91	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≤0.05

Table 4 Percentages of Chlorophyll Deficient Mutants Induced by Colchicines in Sesame (*S.indicum* Var. *Ex-Sudan* and *E-8*)

Mutant	Concentrations	M_1 Generation (*Ex-Sudan*)	M_1 Generation (*E-8*)	M_2 Generation (*Ex-Sudan*)	M_2 Generation (*E-8*)
Chlorina	0.0mM	_	_	-	-
	0.1mM	10.09%	11.36%	6.45%	9.09%
	0.5mM	10.09%	8.03%	9.68%	9.09%
	1.0mM	8.03%	5.01%	6.45%	9.09%
	2.0mM	1.15%	_	3.23%	3.03%
Xantha	0.0mM	_	_	-	-
	0.1mM	_	_	-	3.03%
	0.5mM	_	_	-	-
	1.0mM	_	_	3.23%	-
	2.0mM	_	_	-	-
Striata	0.0mM	_	_	-	-
	0.1mM	4.03%	4.03%	-	6.06%
	0.5mM	4.03%	1.15%	3.23%	-
	1.0mM	1.15%	1.15%	3.23%	3.03%
	2.0mM	1.15%	_	3.23%	3.03%
Virescent	0.0mM	_	_	-	-
	0.1mM	11.36%	11.36%	9.68%	9.09%
	0.5mM	10.09%	11.36%	9.68%	9.09%
	1.0mM	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%	-

DISCUSSION

Mutation induction through the use of different concentrations of colchicines has proved vital in inducing variability that could be exploited in the improvements of sesame growth and yields. It is therefore the origin of genetic variability as suggested by Tamarin (1999). The increased mean germination percent due to various colchicines concentrations revealed the effects of the mutagen in the germination process. The number of germinating seeds decreases with the increase in the concentration of colchicines, which is in agreement with the findings of Ulmalkar *et al.* (1998) who reported high germination percentages in *Capsicum annum* due to Sodium Azide treatment but is in contrast to the work of Bird and Neuffer (1988) who reported reduction in the germinating rates in plants treated with mutagen. The mean increase in plants heights at maturity of the two sesame varieties induced by colchicines was due to the alteration of their 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 is also in agreement with the findings of Hoballah (1999) who reported increased in plant heights of sesame due to radiation mutagenesis; but is in contrast to the findings of Anandakumar and Sree-Rangasamy (1995) and Maluszynski *et al.* (2001) who independently reported decrease in plant height due to induced mutation in rice and other cereals. The increase in leaf number and internodes length with decrease in the concentrations of colchicines was in agreement with the findings of Hoballah (1999) who reported increased in leaf number and internodes length among sesame mutants due to gamma irradiation. The increase in the leaf area of sesame due to colchicines means an increase in the surface area for gaseous ex-change which consequently affects the photosynthetic process. This agrees with the work of Maluszynski *et al.* (2001) who reported increase in the leaf area among *Zea mays* mutants due to irradiation. The increase in the number of pods facilitates increase in the number of seeds produced /pod due to colchicines concentrations. This is in conformity with the findings of Pathak (1991) in M_2 cowpea mutants and Lonnig (1982) in the X-ray induced mutants of Pea. The presence of some segments of chimeras among the mutants was in agreement with the findings of Ranchyalis *et al.* (1988) who reported the occurrence of chlorophylls mutation in plants due to mutagenesis.

Artificial induction of mutation through the use of colchicines proves vital in the improvement of genetic variability in sesame. Certain concentrations of colchicines (0.1mM through 2.0mM colchicines concentration) have the potentiality of inducing variability that could be used in the improvement of the yield of sesame.

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