

ASSESSMENT OF THE GROWTH RESPONSES OF SESAME (*SESAMUM INDICUM* L.) AND FALSE SESAME (*CERATOTHECA SESAMOIDES* ENDL.) TO COLCHICINE TREATMENTS

ABSTRACT

Aim: The aim of this study is to assess the responses of sesame and false sesame to different colchicine concentration and determine the concentration that could be utilized in the genetic improvement of the plants.

Study design: The treated seeds were sown in a plot with three blocks in a Randomized Complete Block Design (RCBD) with three replications in a factorial arrangement for two mutant generations (M_0 and M_1).

Place and Duration of Study: The study was conducted in the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria in 2006 and 2007 growing seasons.

Methodology: The seeds of sesame and false sesame were treated with five different colchicine concentrations (0.1mM, 0.5mM, 1.0mM, 2.0mM and 0.0mM as control) for two mutant generations (M_0 and M_1). Data collected from germination percents (7 DAS), seedlings height, flowering period, root length, height at maturity, number of leaves/plant, leaf area and internodes length.

Results: The results obtained showed highly significant difference ($P \leq 0.01$) in the effects of the mutagen on the germination percents, seedlings height, flowering period, root length, height at maturity, leaf area and internodes length in almost all the mutant generations except in the number of leaves/plant of the M_1 where the effect is significant ($P = 0.05$). The effect of the mutagen is concentration dependent, decreases with increase in concentration (except on the flowering period, where the effect decreases with decrease in the mutagen concentration. The result shows that early maturing mutants with high rate of germination that are tall in stature are formed by the mutagen. More so, the mutants produced high number of leaves that are larger in size and which are far distant apart.

Conclusion: 0.1mM colchicine concentration was found to be more effective in the genetic improvement of sesame and false sesame growth parameters. The response of sesame to the mutagen was found to be the best.

Key Words: Colchicine, False sesame, Sesame, Mutation.

INTRODUCTION

Sesame (*Sesamum indicum* L.) commonly called beniseed [1] and false sesame (*Ceratotheca sesamoides* Endl.) known as Bungu [2] in Nigeria belong to the plant family *Pedaliacea* [3,4,5]. They are among the most ancient plants that are native to Africa [6,7] and were been cultivated in the African sub-region since 3050-3500 B.C [8,9]. The name sesame is used in literature worldwide. It is mentioned in the old Hebrew and Egyptian scripts and the ancient Sanskrit literature. This can be proved from the fact that a wall picture showing ancient Egyptians adding sesame seeds to bread was found in the tomb of Ramses, the third (thirteenth century B.C.) [10] and also ancient seeds of the crop were identified in excavations at Harappa, Pakistan dated 2000 B.C. [11]. The Hausa, Ibo and Yoruba major tribes of Nigeria called it "Ridi", "Ekuku" and "Isasa" respectively [12]. Other tribes in Nigeria also have names for it [1]. Oplinger *et al.* [13] opined sesame to be highly prized oilseed crop in Babylon and Assyria about 4,000 years ago. Sesame is one of

the oldest cultivated plants in the world [13] grown mainly for its seeds that contain approximately 50% oil and 25% protein [14] and sometimes for its edible leaves [15] used as vegetables. It is ranked as sixth most important oilseeds crops in the world [16].

False sesame is widely distributed in variable forms in Nigeria and consumed as a leaf vegetable in the savanna ecological zones. The leaves and flowers of false sesame are consumed as a vegetable [4] rich in protein, fat, carbohydrate and minerals such as Calcium, Phosphorus and Iron. In addition, the seeds are also rich in thiamin, riboflavin and niacin [17]. The oil extracted from the seeds is similar in composition to sesame oil. It contains the phenylpropanoid lignan sesamin which showed antioxidant, anti-inflammatory, antihypertensive, cytotoxic (including anti-tumor) and insecticidal activities [4]. *C.sesamoides* shows strong antibacterial activity [18]. Its decoction is used against diarrhea, while the leaves are stepped in water and the slimy liquid dropped into the eyes to treat *conjunctivitis*. Its leaf maceration also facilitates delivery in both humans and animals, while the leaves when ground with the rhizome of *Anchomanes difformis* can be applied topically in cases of leprosy [4]. False sesame is also reported to be used as an aphrodisiac, against jaundice, snakebites and skin diseases [19]. Ironically, despite all the benefits endowed within these plants, little attention was put on their improvement in Nigeria. While they are appreciated for nutritional qualities [7] and the sources of animal protein tend to be far from the reach of common man due to current world economic melt-down, they are the first items to be neglected from the diet. The vast reservoir of wealth of these leafy vegetables is deteriorating due to neglect by the scientific and development communities as reported by Attere [20]. Attention of the authorities concerned is therefore needed towards exploring the hidden treasures conserved within these species of plants thereby stimulate interest in their utilization beyond the traditional localities; as they are potent supplements to the starchy staple foods with which are traditionally consumed. Beside these, the prohibitive cost of animal protein in developing countries (of which Nigeria is one) calls for extensive exploitation of plant protein sources, which are often economically cheaper [21].

Mutation (a change in genetic material of an organism) induced both in seeds and vegetatively propagated crops are of scientific and commercial interest to improve both the growth and yield parameters of economic plants. It provides raw materials for the genetic improvement of economic crops [22]. 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 and reduced agronomic in-puts [23]. This research aim at using colchicine (a poisonous alkaloid from autumn crocus plant, *Colchicum autumnale*) to induce beneficial mutation in sesame and false sesame so as to determine the concentration that could be beneficial in the genetic improvement of these two plants.

MATERIALS AND METHODS

The research was conducted in the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University Zaria (Lat $11^{\circ} 11' N$; Long $7^{\circ} N 38^{\circ} E$) in 2006 and 2007 growing seasons. The seeds of sesame and false sesame were obtained from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. The seeds were pre-soaked at four different colchicine concentrations including control (0.1mM, 0.5mM, 1.0mM, 2.0mM and control) for four hours and then washed thoroughly in running water. The treated seeds were sown in a plot with three blocks in a Randomized Complete Block Design (RCBD) with three replications in a factorial arrangement for two mutant generations (M_0 and M_1). All cultural practices followed the protocols described by Kano State Agricultural and Rural Development Authority crop production guide [24] and Bedigian and Adetula [4]. Data were collected for the germination percents Seven Days After Sowing (7 DAS), seedlings height, flowering period, root length, height at maturity, number of leaves/plant, leaf area and internodes length. All the data obtained were analyzed using factorial analysis in a CRBD format with Duncan's Multiple Range Test to separate the means.

RESULTS

The results obtained from the M_0 analysis of variance following treatment of sesame and false sesame with colchicine showed highly significant difference ($P \leq 0.01$) in the effect of different colchicine concentrations on the selected traits of the two plants (Table 1). Similarly, the result (Table 1) showed highly significant difference ($P \leq 0.01$) in the response of the two plants to different colchicine concentrations; except ion the germination percents.

106 Table 1: M₀ Mean Squares at Different Concentrations of Colchicine on Some Traits of False Sesame and Sesame

Sources of Variation	Df	Germination Percent (7 DAS)	Seedlings Height (cm)	Flowering Period (days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves / Plant	Leaf Area (cm ²)	Internodes Length (cm)
Blocks	2	1.24**	992.65**	255.96**	11.85**	1753.19**	149.64**	10539.71**	8.58**
Concentration	4	0.63**	156.41**	583.74**	8.02**	344.45**	35.48**	1676.62**	48.66**
Variety	2	0.23 ^{ns}	986.75**	250.29**	33.34**	1565.48**	109.94**	10141.33**	47.23**
Error	208	0.12	0.69	1.93	0.80	17.67	3.49	34.94	0.14

107 Keys: ns= No significant difference * = Significant difference (P=.05) **= Highly significant difference (P≤0.01)

108 Furthermore, the result from the responses of sesame and false sesame to various colchicine
 109 concentrations (Table 2) showed higher response by sesame in all the selected traits except in the
 110 flowering period (where the response of false sesame is recorded higher) and germination percents.

111 Table 2: M₀ Mean Responses of Sesame and False Sesame to Different Colchicine Concentrations

Variety	Germination Percent (1WAP)	Seedlings Height (cm)	Flowering Period (Days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/plant	Leaf Area (cm ²)	Internodes Length (cm)
Sesame	86.00 ^{a*1}	45.46 ^a	42.93 ^b	4.87 ^a	56.04 ^a	24.28 ^a	57.43 ^a	7.15 ^a
False sesame	84.57 ^a	29.74 ^b	50.20 ^a	4.68 ^b	56.47 ^a	18.76 ^b	8.10 ^b	4.08 ^b
MEANS	85.29	36.46	47.64	4.71	55.65	21.52	28.97	5.11
S.E±	0.62	1.29	1.14	0.72	11.61	2.93	7.12	0.54

112 N.B:*1 Means within the columns with the same letter(s) are not significantly different (P=.05)

113 However, the result of the mean effect of different colchicine concentrations on the traits of
 114 sesame is presented in Table 3. The result showed that, 82.86-94.29% of the mutants germinated where
 115 as 77.14% of the controls germinated. It was also found that the mutants attained a maximum height of
 116 48.70 cm at seedlings stage which reached up to 75.00 cm at maturity with a root length of 6.00 cm. More
 117 so, the time required for flowering decreased by half as such early flowering mutants were found among
 118 the colchicine induced mutants of sesame. Similarly, the mutants produced 22-29 leaves that are 51.40-
 119 77.77 cm² in cross sectional area with internodes length of 6.67-8.53 cm in between.

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 122
 123 **Table 3: M₀ Means of some Traits at Different Colchicine Concentrations in Sesame**

Concentration	Germination Percent (7DAS)	Seedlings Height (cm)	Flowering Period (days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/Plant	Leaf Area (cm ²)	Internodes Length (cm)
0.0 mM	77.14 ^{d*1}	40.40 ^d	67.67 ^a	4.43 ^c	57.10 ^c	20.40 ^d	40.80 ^e	5.60 ^c
0.1 mM	94.29 ^a	48.70 ^a	35.00 ^b	6.00 ^a	75.00 ^a	28.80 ^a	77.77 ^a	8.53 ^a
0.5 mM	88.57 ^b	47.50 ^{ba}	35.00 ^b	5.23 ^a	70.30 ^a	25.60 ^b	65.27 ^b	7.73 ^{ba}
1.0 mM	87.14 ^b	45.80 ^b	35.00 ^b	5.17 ^{ba}	65.90 ^{ba}	24.40 ^b	55.73 ^c	7.20 ^b
2.0 mM	82.86 ^c	44.80 ^c	42.00 ^{ba}	4.77 ^b	63.50 ^b	22.20 ^c	51.40 ^d	6.67 ^b
MEANS	86.00	45.44	42.93	6.14	66.36	24.28	58.19	6.08
S.E±	2.87	1.43	6.32	2.49	3.03	1.44	6.27	0.49

124 N.B:*1 Means within the columns with the same letter(s) are not significantly different (P=.05)

125 Similarly, the result of the mean effect of various concentrations of colchicine on the traits of false
126 sesame (Table 4) showed higher rate of germination among the mutants (81.42-94.29%). The result
127 showed that, early maturing mutants that attained a maximum height of 52.20 cm were found among the
128 mutants. The mutants were also found to produce high number of leaves that are larger in size and which
129 are 3.97-4.53 cm apart.

130 **Table 4: M₀ Means of some Traits at Different Colchicine Concentrations in False Sesame**

Concentration	Germination Percent (7DAS)	Seedlings Height (cm)	Flowering Period (days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm ²)	Internodes Length (cm)
0.0mM	74.29 ^{c*1}	27.90 ^d	75.67 ^a	5.67 ^c	38.80 ^c	15.80 ^d	3.50 ^e	3.77 ^d
0.1mM	94.29 ^a	31.30 ^a	42.00 ^c	7.10 ^a	52.20 ^a	21.20 ^a	13.03 ^a	4.53 ^a
0.5mM	88.57 ^{ba}	30.60 ^b	42.00 ^c	7.00 ^a	47.80 ^{ab}	19.60 ^b	9.57 ^b	4.13 ^{ab}
1.0mM	84.29 ^b	29.60 ^c	45.00 ^{bc}	6.67 ^{ba}	46.00 ^{ba}	19.20 ^b	8.03 ^c	4.00 ^b
2.0mM	81.42 ^b	29.20 ^c	46.33 ^b	6.33 ^b	44.90 ^b	18.50 ^c	5.90 ^d	3.97 ^c
MEAN	84.57	29.72	50.20	6.55	45.94	18.86	8.01	4.08
.S.E±	2.37	1.32	6.41	0.26	2.17	0.88	1.62	0.13

131 N.B: *1 Means within the columns with the same letter(s) are not significantly different ($P=0.05$)

132 Furthermore, the result from the analysis of variance of the M₁ (Table 5) showed highly significant
133 difference ($P\leq 0.01$) in the effect of the mutagen on the two plants in all the selected traits; except in the
134 number of leaves produced per plant, where the effect is significant ($P=0.05$). Similar result is found in the
135 response of the plants to the mutagen; except in the root length (where no significant difference is found).

136 **Table 5: M₁ Mean Squares of the Effects of Different Concentrations of Colchicine on Some Traits of False Sesame and Sesame**

Sources of Variation	Df	Germination Percents (7DAS)	Seedlings Height (cm)	Flowering Periods (Days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm ²)	Internodes Length (cm)
Blocks	2	0.34**	992.97**	342.87**	71.00**	2422.55**	235.49**	10517.31**	64.93**
Concentration	4	0.55**	17.24**	586.24**	7.00**	106.79**	23.52*	161.97**	1.31**
Variety	2	1.14**	1113.33**	4460.60**	0.80 ^{ns}	3203.97**	415.59**	11881.32**	76.01**
Error	208	0.09	0.54	2.57	0.99	13.71	4.81	23.87	0.25

137 Keys: ns= No significant difference

138 *= Significant difference ($P=0.05$)

139 ** = Highly significant difference ($P\leq 0.01$)

140 However, the result of the response of the two plants to the mutagen in the M₁ generation (Table
141 6) showed that sesame has the highest response in almost all the selected traits (except in flowering
142 period).

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144 **Table 6: M₁ Responses of Sesame and False Sesame to Different Colchicine Concentrations**

Variety	Germination Percent (1WAP)	Seedlings Height (cm)	Flowering Period (Days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/plant	Leaf Area (cm ²)	Internodes Length (cm)
Sesame	85.23 ^a	45.69 ^a	42.07 ^b	5.41 ^a	69.34 ^a	27.01 ^a	60.75 ^a	7.73 ^a
False sesame	82.83 ^b	30.07 ^b	50.93 ^a	5.53 ^b	43.96 ^b	19.13 ^{ba}	9.63 ^b	4.27 ^b

MEANS	84.03	37.88	46.50	5.47	56.65	23.07	35.19	6.00
S.E±	0.85	5.52	3.13	0.05	8.64	2.79	18.07	1.22

N.B:*1 Means within the columns with the same letter(s) are not significantly different ($P=0.05$)

More so, the mean effects of different colchicine treatments on the traits of sesame (Table 7) showed that, 84.29-93.29% of the mutants germinated and attained a height of 44.97-48.93 cm at seedlings stage. The mutants are also found to be early maturing that flower 32-39 days with a root length of 5.07-5.31cm and a height of 67.43-79.27 cm at maturity. Similarly, the mutants produced 25-32 leaves per plant which are larger in size (57.40-75.50 cm²) and which are 7.53-8.87 cm apart.

Table 7: M₁ Generation Means of some Traits at Different Colchicine Concentrations in Sesame

Concentration	Germination Percent (7 DAS)	Seedlings Height (cm)	Flowering Period (days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm ²)	Internodes Length (cm)
0.0 mM	74.71 ^{c*1}	40.67 ^d	68.67 ^a	4.77 ^c	59.93 ^d	22.33 ^d	42.77 ^e	6.10 ^c
0.1 mM	93.29 ^a	48.93 ^a	31.67 ^c	6.31 ^a	79.27 ^a	32.00 ^a	75.50 ^a	8.87 ^a
0.5 mM	88.58 ^{ba}	47.80 ^{ba}	34.00 ^c	5.40 ^{ba}	71.23 ^b	30.03 ^b	67.80 ^b	8.33 ^{ba}
1.0 mM	85.29 ^b	46.10 ^b	36.67 ^b	5.24 ^{ba}	68.83 ^{cb}	25.73 ^c	60.10 ^c	7.83 ^b
2.0 mM	84.29 ^b	44.97 ^c	39.33 ^{ba}	5.07 ^b	67.43 ^c	24.97 ^c	57.60 ^d	7.53 ^b
MEAN	85.23	45.69	42.07	5.41	69.34	27.01	60.75	7.73
S.E±	3.07	1.46	6.76	0.99	3.10	1.76	5.48	0.48

N.B:*1 Means within the columns with the same letter(s) are not significantly different at $P=0.05$

Furthermore, Table 8 shows the means of the M₁ traits of false sesame. It showed that, the mutants germinated better than the controls and attained higher height at both seedlings and maturity stages. Their roots also grow deeper than that of the control, though their flowering periods decreased substantially. The result also showed that, the mutants produced high number of leaves that are larger in size and which are far distant apart.

Table 8: M₁ Generation Means of some Traits at Different Colchicine Concentrations in False Sesame

Concentration	Germination Percent (7 DAS)	Seedlings Height (cm)	Flowering Period (days)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm ²)	Internodes Length (cm)
0.0 mM	76.14 ^{e*1}	27.93 ^c	77.00 ^a	4.90 ^c	35.70 ^d	16.20 ^d	3.87 ^d	3.97 ^c
0.1 mM	91.43 ^a	31.90 ^a	43.33 ^c	5.70 ^a	50.07 ^a	21.60 ^a	14.00 ^a	5.10 ^a
0.5 mM	88.57 ^b	31.37 ^{ba}	43.33 ^c	5.95 ^a	46.27 ^b	19.97 ^b	11.43 ^b	4.30 ^{ba}
1.0 mM	80.43 ^c	29.97 ^b	45.33 ^b	5.60 ^{ba}	44.73 ^b	19.27 ^b	9.60 ^c	4.03 ^b
2.0 mM	77.57 ^d	29.17 ^b	45.67 ^b	5.48 ^b	43.03 ^c	18.60 ^c	9.27 ^c	3.93 ^b
MEAN	82.83	30.07	50.93	5.53	43.96	19.13	9.63	4.27
S.E±	2.70	0.70	6.53	0.17	2.37	0.61	1.67	0.19

N.B:*1 Means within the columns with the same letter(s) are not significantly different at $P=0.05$

DISCUSSION

In view of the excellent possibilities to improve the quality and quantity of plants, chemical mutagenesis was reported, which appears to fit well with the major role played by colchicine in the improvement of plants growth and yield parameters. The results obtained in this research implied that sesame and false sesame respond well with different colchicine concentration. The increased mean germination percent after Seven days of planting due to various colchicine concentrations revealed the effects of the mutagen in the germination process. The number of germinating seeds decreases with the increase in the concentration of colchicine, which was in agreement with the findings of Ulmalkar *et al.* [25] who reported high germination percentages in *Capsicum annum* due to chemical mutagenesis by Sodium Azide treatment but was in contrast to the work of Bird and Neuffer [26] who reported reduction in the germinating rates in plants treated with mutagen. Germination been one of the critical stages required by sesame for its optimal growth as reported by Uzo [11] was improved through the use of colchicine mutagenesis. Chemical mutagenesis through the application of colchicine increases the germination potentiality of sesame and false sesame seeds after Seven days of planting; probably by stimulating the rates of enzymatic activities, facilitating the water absorption capacity of the seeds, by turning-on the genes responsible for controlling the seeds germination or by a combination of both.

The mean increase in height and roots length of the plants induced by colchicine at both seedlings and matured stages were due to the alteration of their genome integrated by environmental signals as reported by Uno *et al.* [27]; probably by increasing the rates of cellular division and expansion at their meristematic regions. This was in agreement with the findings of Hoballah [28] who reported increased in plant heights of sesame due to mutagenesis; but was in contrast to the findings of Maluszynski *et al.* [29] who reported decreased in plant height due to induced mutation in rice. The root length increase due to colchicine concentration was in agreement with the findings of Adamu [30] who reported root length increase in popcorn as a result of gamma and Thermal Neutrons irradiations. Certain genes that function in stress tolerance and cause changes in the root morphology were recently been identified through genetic mutation in *Arabidopsis* by Maggio *et al.* [31] which might have consequently increased the roots absorption capacity. 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 and morphogenesis as suggested by Deikman and Ulrich [32].

The period of anthesis in sesame and false sesame were found to be increased with increase in the concentration of colchicine. This improved earliness was in agreement with the work of Hoballah [28] who reported improved earliness among the gamma irradiated mutants of sesame. 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.* [33]. Mutation induction for improved earliness is also in agreement with the findings of Rutger [34] who reported early flowering mutants in medium grain *Japonica* cultivar; but was in contrast to that of Archana *et al.* [35] who reported increase in the number of days required for maturity due to increased doses of mutagen treatments in *Glycine max* (soya beans).

Increased leaf area in sesame and false sesame mutants induced by different colchicine concentrations was in agreement with the findings of Maluszynski *et al.* [29] who reported an increase in leaf area among *Zea mays* mutants. The increase in leaf area provides an increase in the surface area for gaseous ex-change which has considerable effect on the process of photosynthesis as gene duplication has profound effect in the evolution of photosynthesis reaction center proteins as suggested by Lockhart *et al.* [36] thereby affecting plant productivity in terms of the dry matter contents and seeds weight. Similar findings were found in the work of Duranceau *et al.* [37] who reported a greater leaf mass per leaf area and enriched organic matter in the leaves of *Nicotiana sylvestris* mutants. This was also consistent with the assumption that mutation increases the genetic capacity for the utilization of photosynthates due to the ability of plants to sustain increased photosynthesis as suggested by Ainsworth *et al.* [38]. The mutagen stimulated growth of the cells of the lamina causing its remarkable expansion. Thus, increase in the leaf area of sesame of false sesame has a strong relationship with the yield components. Besides the leaf area, the number of leaves produced per plant and their proportionate distance from one another were increased under different concentrations of colchicine. This finding was in agreement with that of Hoballah [28] who reported increase in the number of branches among sesame mutants due to gamma irradiation.

CONCLUSION

Induction of mutation through the use of colchicine on sesame and false sesame was found to improve the growth responses of the two plants. Improvements in these traits were due to the ability of the plants to respond to the effects of the mutagen in inducing favorable mutation. Thus, the responses of the traits of these plants were best under 0.1mM colchicines concentration.

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