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Assessment of the Growth Responses of Sesame (*Sesamum indicum* l.) and False Sesame (*Ceratotheca sesamoides* Endl.) to Colchicine Treatments

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Authors' contributions

This work was carried out in collaboration between all authors. Author SN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AKA, SM, DBD and KS managed the analyses of the study. Author sFMM and KM managed the literature searches and language setting. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of this study was to assess the responses of sesame and false sesame to different colchicine concentrations in order to determine the concentration(s) that could be best 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 at 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

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mutant generations (M_0 and M_1). Data collected included germination percents (14 DAS), root length, height at maturity, number of leaves/plant, leaf area, internodes length, 1000 seeds weight and dry weight.

Results: The results obtained showed highly significant difference ($P \leq 0.01$) in the effects of the mutagen on the germination percents, root length, height at maturity, leaf area, internodes length, 1000 seeds weight and dry weight in almost all the mutant generations except in the number of leaves/plant of the M_1 where the effect is significant (P=.05). The effect of the mutagen is concentration dependent, decreases with increase in concentration).The result shows that 0.1mM colchicines concentration produced mutants with high rate of germination that are tall in stature. More so, the mutants produced high number of leaves that are larger in size and which are far distant apart; with higher seed and dry weight.

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.

Keywords: Colchicine; false sesame; sesame; mutation.

1. 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 *Pedaliaceae* [3,4,5]. They are among the most ancient plants that are native to Africa [6,7] and were cultivated in the African sub-region since 3050-3500 B.C [8,9]. Sesame seeds contained high level of PUFAs used in reducing blood cholesterol, high blood pressure and play an important role in preventing atherosclerosis, heart diseases and cancers [10,11,12]. 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 crop 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 effort has been made on their improvement in Nigeria. 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]. An effort is needed to explore the benefits of using these plants and to stimulate interest in their utilization beyond the traditional localities as they are potent supplements to the starchy staple foods which are typically consumed. Also, 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], and 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]. The aim of this research is to use colchicine (a poisonous alkaloid from autumn crocus plant, *Colchicum autumnale*) to induce beneficial mutation in sesame and false sesame to determine the effectiveness of different concentrations of colchicine that could be beneficial to the genetic improvement of these two plants.

2. MATERIALS AND METHODS

The research was conducted at the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University Zaria (Lat11^{0} 11^{1} N; Long 7^{0}N 38^{1}E) during the 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 five different colchicine concentrations (0.1mM, 0.5mM, 1.0mM, 2.0mM and untreated soaked in distilled water) 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 collected included: Percents germination 14 (DAS), root length, height at maturity, number of leaves/plant, leaf area, internodes length, 1000 seeds weight and dry weight. All the data were analyzed using factorial analysis in a CRBD format with Duncan's Multiple Range Test to separate the means.

3. RESULTS

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, results showed a significant difference ($P \leq 0.01$) in the response of the two plants to different colchicine concentrations with the exception of percent germination (Table 1).

Furthermore, the result from the responses of sesame and false sesame to various colchicine concentrations (Table 2) in M_0 generation showed a greater response by sesame in all the selected traits with the exception of germination percents.

Results of the mean effect of different colchicine concentrations on the traits of sesame are presented in Table 3. The results showed high germination (90%) was found among the mutants treated with 0.1mM concentration. It was also found that the 0.1mM treated mutants' roots grow to a depth of 6.00 cm and 75 cm above the ground. More so, the mutants produced 22 leaves that are 78 cm^2 in cross sectional area with internodes length of 9 cm in between. Similarly, the 1000 seeds weight and dry weight of the mutants treated by 0.1 mM colchicines concentration weigh 3.4g and 233g respectively.

Similarly, the result of the mean effect of various concentrations of colchicine on the traits of false sesame (Table 4) showed higher rate of germination among 0.1mM treated mutants (86%). The result showed that, 0.1mM have roots that grow to a depth of 7.1 cm and height of 52 cm. The mutants were also found to produce high number of leaves that are larger in size and which are 4.5 cm apart; having 1000 seeds weight of 3g and dry weight of 19.6g.

The result from the analysis of variance of the M_1 (Table 5) showed high significant difference ($P \leq 0.01$) in the effect of the mutagen on the two plants in all the selected traits; except in the number of leaves produced per plant, where the effect is significant (P=.05). Similar result is found in the response of the plants to the mutagen; except in the root length (where no significant difference is found).

Result of the response of the two plants to the mutagen in the M_1 generation (Table 6) showed that sesame has the highest response in almost all the selected traits.

More so, the mean effects of different colchicine treatments on the traits of sesame (Table 7) showed that, 91% of the 0.1mM treated mutants germinated produced roots that are 6 cm in length and attained a height of 79 cm at maturity. The mutants are also found to produce 32 leaves per plant which are larger in size (76 cm^2) and which are 9 cm apart. Similarly, 1000 seeds of the mutants weigh 3.6g with 3.2g dry weight.

Furthermore, Table 8 shows the results of the M_1 traits of false sesame. It showed that, 0.1mM treated mutants germinated better than all the others. They showed taller stature, with their roots growing deeper. The result also showed that, the mutants produced high number of leaves that are larger in size and which are far distant apart. Similarly, the 1000 seeds and dry weight of the mutants weigh higher.

Table 1. M_0 mean squares at different concentrations of colchicine on some traits of false sesame and sesame

Sources of Variation	Df	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves / Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
Blocks	2	0.9*	12**	1753**	150**	10540**	9**	0.04**	0.3*
Concentration	4	82**	8**	345**	36**	1677**	49**	0.1**	24**
Variety	1	0.3^{ns}	33**	1566**	110**	10141**	47**	0.1**	1.4**
Error	208	0.3	0.8	18	3.5	35	0.1	0.003	0.1

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

Table 2. M_0 mean responses of sesame and false sesame to different colchicine concentrations

Variety	Germination Percent (14DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
Sesame	84^{a1}	4.9^{a}	56^{a}	24.3^{a}	57^{a}	7^{a}	3.2^{a}	21^{a}
False sesame	81^{a}	4.7^{b}	57^{a}	18.8^{b}	8^{b}	4^{b}	2.8^{b}	17^{b}
S.E±	1.3	0.7	11.6	2.9	7.1	0.5	0.1	1.4

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

Table 3. M_0 means of some traits at different colchicine concentrations in sesame

Concentration	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
0.0 mM	78^{d1}	4^{b}	57^{c}	20^{d}	41^{e}	6^{c}	2.9 ^{c}	16^{c}
0.1 mM	90^{a}	6^{a}	75^{a}	29^{a}	78^{a}	9^{a}	3.4 ^{a}	23^{a}
0.5 mM	88^{b}	5^{ba}	70^{ba}	26^{b}	65^{b}	8^{ba}	3.2 ^{b}	22^{b}
1.0 mM	86^{b}	5^{ba}	66^{b}	24^{b}	56^{c}	7^{b}	3.2 ^{b}	22^{b}
2.0 mM	82^{c}	5^{ba}	64^{b}	22^{c}	51^{d}	7^{b}	3.1^{ba}	22^{b}
S.E±	1.9	2.5	3.0	1.4	6.3	0.5	0.1	1.1

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

Table 4. M_0 means of some traits at different colchicine concentrations in false sesame

Concentration	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
0.0mM	73^{c1}	6^{c}	39^{c}	16^{d}	3.5^{e}	3.8^{d}	2.6^{d}	12^{c}
0.1mM	86^{a}	7^{a}	52^{a}	21^{a}	13^{a}	5^{a}	3.0^{a}	20^{a}
0.5mM	85^{ba}	7^{a}	48^{ab}	20^{b}	9.6^{b}	4^{b}	2.9^{b}	19^{ba}
1.0mM	83^{b}	7^{a}	46^{ba}	19^{b}	8^{c}	4^{b}	2.8^{c}	19^{ba}
2.0mM	78^{b}	6^{b}	45^{b}	19^{b}	5.9^{d}	3.9^{c}	2.8^{c}	18^{b}
.S.E±	2.2	0.3	2.2	0.9	1.6	0.1	0.1	1.3

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

Table 5. M_1 mean squares of the effects of different concentrations of colchicine on some traits of false sesame and sesame

Sources of Variation	Df	Germination Percents (14DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
Blocks	2	0.4^{ns}	71**	2423**	236**	10517**	65**	0.01**	2.1*
Concentration	4	33**	7**	107**	24*	162**	1.3**	0.18**	41**
Variety	1	16**	0.8^{ns}	3204**	416**	11881**	76**	0.01**	6**
Error	208	0.3	0.9	13.7	4.8	23.9	0.3	0.003	0.6

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

Table 6. M_1 responses of sesame and false sesame to different colchicine concentrations

Variety	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/plant	Leaf Area (cm^{2})	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
Sesame	86[a]	5.4[a]	69[a]	27[a]	61[a]	8[a]	3.2[a]	21[a]
False sesame	76[b]	5.5[b]	44[b]	19[ba]	10[b]	4[b]	2.9[b]	17[b]
S.E±	3.5	0.05	8.6	2.8	18	1.2	0.1	1.4

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

Table 7. M_1 generation means of some traits at different colchicine concentrations in sesame

Concentration	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
0.0 mM	82[c1]	4.8[c]	60 [d]	22[d]	43[e]	6[b]	3.1[e]	19[d]
0.1 mM	91[a]	6[a]	79[a]	32[a]	76[a]	9[a]	3.6[a]	32[a]
0.5 mM	87[ba]	5[ba]	71[b]	30[b]	68[b]	8[ba]	3.5[b]	30[b]
1.0 mM	86[b]	5[ba]	69[cb]	26[c]	60[c]	8[ba]	3.4[c]	26[c]
2.0 mM	84[b]	5[ba]	67[c]	25[c]	56[d]	8[ba]	3.2[d]	26[c]
S.E±	1.4	0.9	3.1	1.8	5.5	0.5	0.08	1.9

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

Table 8. M_1 generation means of some traits at different colchicine concentrations in false sesame

Concentration	Germination Percent (14 DAS)	Root Length (cm)	Height at Maturity (cm)	Number of Leaves/ Plant	Leaf Area (cm^2)	Internodes Length (cm)	1000 Seeds Weight (g)	Dry Weight (g)
0.0 mM	72[d1]	5[b]	36[d]	16[d]	3.9[d]	3.9[c]	2.4[c]	16[c]
0.1 mM	79[a]	6[a]	50[a]	22[a]	14[a]	5[a]	3.2[a]	19[a]
0.5 mM	76[b]	6[a]	46[b]	20[b]	11[b]	4[b]	3.1[b]	18[b]
1.0 mM	76[b]	6[a]	45[b]	20[b]	10[c]	4[b]	3.2[a]	18[b]
2.0 mM	75[c]	6[a]	43[c]	19[c]	10[c]	4[b]	3.1[b]	18[b]
S.E±	1.0	0.17	2.4	0.6	1.7	0.2	0.1	0.4

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

4. DISCUSSION

The results obtained in this research implied that sesame and false sesame responds well to different colchicine concentrations. The increased percent germination 14 days of planting due to various colchicine concentrations revealed the effects of the mutagen in the germination process. This was in agreement with the findings of Waghmare and Mehra [25] in grass pea due to increase in the concentration of Ethyl Methane Sulphonate (EMS). But, this finding was in contrast to the work of Bird and Neuffer [26] who reported reduction in the germinating rates in plants treated with mutagen. Germination being 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 fourteen days of planting.

The increase in height and roots length of the plants induced by colchicine 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].

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. [33] thereby affecting plant productivity in terms of the dry matter content and seed weight. Similar findings were found in the work of Duranceau et al. [34] 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. [35]. The mutagen stimulated growth of the cells of the lamina causing its remarkable expansion. Thus, increase in the leaf area of sesame and 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. The increase of 1000 seeds weight of sesame and false sesame due to colchicine treatment was in line with the work of Shen et al. [36] who reported increase in grain weight of rice due to gamma rays in in-*vitro* mutagenesis. Increase in the dry weights of sesame and false sesame with decrease in colchicine concentration was in conformity with the work of Kolesnikova and Maksimova [37] who reported an increase in the dry matter contents of potato tuber mutants. It also tallies with the work of Stelling [38] who reported improvements in biomass besides other traits in faba bean mutants.

5. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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