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Effect of Chemical Mutation by Sodium Azide on Quantitative Traits Variations in *Sesamum indicum* L.

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ABSTRACT

In this experiment healthy and dry seeds of sesame (*Sesamum indicum* L.), varieties of Abasena and Kelafo 74, were treated with sodium azide at ascending mutagen concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05% targeted at determining the effects of the chemical mutagen to promote genetic variability in terms of the quantitative traits parameters of sesame. Highly significant differences ($p<0.01$) were noticed in the varieties and treatments with respect to the traits under consideration (germination percentage, root length,shoot length, plant height, days to flowering, days to maturity, internodes length, capsule length, No. of capsules plant^{-1}, No. of seeds pod^{-1}, hundred seed weight). Similarly Treatment and variety interactions were also highly significant ($p<0.01$) with respect to all traits. The variety Abasena exhibited better performance in comparison to Kelafo 74. Therefore, sodium azide could be utilized to induce genetic variability for the improvement of sesame and it is more effective. Since the produced mutants from first generation are not adequate for studying the genetic stability these traits should be investigated for the desired traits in subsequent generations and in the field conditions.

Key words: Chemical mutagen, sesame, sodium azide, quantitative traits variations

INTRODUCTION

The chromosome No. of sesame is 2n = 26 diploid and it is usually self-pollinated (Beckstrom-Sternberg *et al*., 1994). Sesame belongs to the Pedaliaceae family. The most important genus of this family is *Sesamum*. Sesame is typically an annual species. There are lots of varieties of *Sesamum indicum* L. according to the size, form and color of flowers, seed size, color and composition. Variation can also be manifested in such a way that some varieties are highly branched whereas others are unbranched (Peter, 2004).

African continent is naturally gifted with suitable weather conditions that can enhance sesame production. The crop requires only 500-650 mm of rainfall annum^{-1}. Unfortunately, average world yield of sesame is still low at 0.46 ton ha^{-1}. Low yield had been attributed to cultivation of low yielding dehiscent varieties with low harvest index values, significant yield loss during threshing and shortage of agricultural inputs such as improved varieties, fertilizers and other agro-chemicals (Ashri, 1998; Weiss, 2000; Uzun and Cairgan, 2006). However, non-dehiscent sesame varieties with yield potential of over 1 ton ha^{-1} and appropriate for mechanical combine harvest have been developed by Sesame Coordinators (SESACO) in USA.

Ethiopia is known to be the origin of diversity for cultivated sesame. Its seed harbors 50-60% oil and 25% protein with antioxidants lignans such as sesamolin, sesamin and has been used as active ingredients in antiseptics, bactericides, viricides, disinfectants, moth repellants, anti-tubercular agents and considerable source of calcium, tryptophan, methionine and many minerals.

In general terms Sesame is unimproved and variety of collections have been generated of land races, with little or no genetic information that can lead to its application in breeding programs. A No. of factors affecting sesame improvement programs have been identified. Firstly, the germplasm of sesame is not as large as in other crops. Secondly, the genetic architecture of sesame is poorly adapted to mechanized farming system due to its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Ashri, 1982).

Mutations are the tools at hand exploited by the geneticist to study the nature and function of genes which are the building blocks and basis of plant growth and development, hence producing raw materials for genetic improvement of economic important crops. Primarily the advantage of mutational breeding is the probability of improving one or two characters without amending the rest of the genotype. Induced mutations have great potencies and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al.*, 2001).

Chemical mutagenesis is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutations are the tools 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 and Aliyu, 2007). Mutation breeding methodology has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena (Beckstrom-Sternberg *et al.*, 1994). The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in groundnut (Mensah and Obadoni, 2007), barley (Kleinhofs and Sander, 1975) and other crops (Routaray *et al.*, 1995). Therefore, this study will try to intentionally broaden the gene pool of sesame hence, leaving ample genetic resource for breeding foundation, increasing the productivity and selection for desirable yield components.

MATERIALS AND METHODS

The selection of the crop plant was based on economic importance as well as availability in Haramaya University Ethiopia. Accordingly, Abasena and Kelafo 74 variety of sesame was selected.

Preparation of chemical mutagen: The induction of chemical mutation was achieved by using sodium azide. The different concentrations of sodium azide (0.01, 0.02, 0.03, 0.04 and 0.05% v/v) were prepared in plastic beakers with distilled water.

Method of mutagenesis: Seeds of sesame variety of Abasena and Kelafo 74 were used for inducing chemical mutation by sodium azide. The seeds of the selected varieties were surface sterilized with 0.1% mercuric chloride for 1 min to remove the fungal spores on the surface of the seeds. Then the seeds were washed with distilled water several times to remove the mercuric chloride. After this the seeds were pre soaked in plastic beaker which contains distilled water for six hours and then treated with sodium azide at different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05%) for 6 h (Dhanavel *et al.*, 2008).

Bioassay studies: The bioassay studies were carried out following the method of Heisey (1990) in Department of Biology, Haramaya University, Ethiopia. Five seeds from each of the treatments were placed on whatman filter paper in petriplates (9×2 cm). Each petriplate was moistened with 2 mL $plate^{-1}$ of distilled water and the control were normal seeds (untreated) and incubated at room temperature. The germination percentage, root and shoot length were measured after 8 days. The germination percentage was calculated by using the following equation:

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds placed for germination}} \times 100$$

Green house experiments: The seeds that were treated with chemical mutagenic agent in the laboratory were sown in earthen pots (24×24 cm) in green house by CRD factorial design with three replications along with their respective control. Then the experimental plants were grown under the green house condition in Haramaya University, Ethiopia. The control plants were grown from the untreated seeds. The agronomic traits such as plant height, No. of branches on the main axis, seed length, internode length, days to 50% flowering, days to 90% maturity, pods per plants and above ground biomass were measured and determined.

RESULTS

Table 1 shows the germination percentage, length of root and shoot of sodium azide treated plants. The decrease in germination showed a negative correlation with the increased mutagen concentration in both varieties of sesame. In Abasena variety maximum reduction in seed germination was 47.37% and it was observed in 0.05% concentration in comparison to the control and other treatments and highly significant difference was observed at ($p<0.01$) level. Root growth was stimulated in 0.02% concentration, where as the maximum reduction in root growth was observed in 0.05% concentration. Highly significant increase was observed in shoot length in 0.02% concentration in comparison with other treatments and the control. The rest treatments displayed a negative mean shift in shoot length.

The inhibitory effect on the seed germination by sodium azide on kelafo 74 was also in a similar trend with that of abasena and the effect was drastical at 0.05% concentration. In both varieties negative correlation was observed between germination percentage and ascending mutagen concentration. The root and shoot length was stimulated in 0.02 and 0.03% concentrations and the effect was highly significant in comparison to the control and the remaining mutagen concentrations.

Table 1: Bioassay study of sodium azide on seed germination and seedling growth of sesame (Abasena and kelafo 74 variety)

	Germination (%)		Root length(cm)		Shoot length(cm)	
Treatment	Abasen	Kelafo 74	Abasen	Kelafo 74	Abasena	Kelafo 74
Control	95.00^{a}	93.90^{a}	4.530^{b}	3.960^{c}	6.60^{b}	5.910^{c}
0.01%	82.20^{b}	80.00^{b}	3.030^{d}	3.500^{d}	4.47^{c}	5.500^{d}
0.02%	80.07^{bc}	67.90^{c}	5.030^{a}	4.500^{a}	7.00^{a}	6.500^{a}
0.03%	72.50^{d}	67.80^{c}	3.090^{cd}	4.000^{b}	3.87^{c}	6.000^{b}
0.04%	59.70^{e}	65.90^{d}	2.250f	3.500^{d}	3.90^{d}	4.500^{e}
0.05%	52.63^{f}	59.20^{e}	1.890^{g}	2.500^{e}	3.72^{f}	3.500^{f}
CV (%)	0.200	0.120	0.370	0.430	7.11	0.300
LSD	0.190	0.110	0.016	0.020	0.45	0.020

Table 2: Effects of sodium azide on the phenological parameters of sesame (Abasena variety)

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	60.00ᵃ	11.000ᵉ	85.000ᵇ	2.000ᶜ	15.000ᵈ	3.000ᵈ	50.00ᵈ	44.000ᵇ
0.01%	61.00ᵃ	12.000ᵈ	85.000ᵇ	2.100ᵇ	18.000ᵇ	3.200ᶜ	55.00ᶜ	44.000ᵇ
0.02%	66.00ᵇ	15.000ᵃ	80.000ᶜ	2.500ᵃ	20.000ᵃ	3.700ᵃ	68.00ᵃ	40.000ᶜ
0.03%	61.00ᵃ	14.000ᵇ	85.000ᵇ	2.000ᶜ	17.000ᶜ	3.500ᵇ	60.00ᵇ	44.000ᵇ
0.04%	60.00ᵃ	13.000ᶜ	90.000ᵃ	1.800ᵈ	15.000ᵈ	3.000ᵈ	50.00ᵈ	45.000ᵃ
0.05%	55.00ᵃ	12.000ᵈ	90.000ᵃ	1.800ᵈ	13.000ᵉ	2.800ᵉ	45.00ᵉ	45.000ᵃ
(CV%)	22.15	0.340	0.023	0.400	0.013	0.021	0.021	0.043
LSD	16.39	0.056	0.024	0.011	0.027	0.015	0.015	0.024

PH: Plant height (cm), IL: Internodes length (cm), DM: Days to maturity, CL: Capsule length, (cm), NCPP: No. of Capsule plants^{-1}, HSW: Hundred seed weigh (gram)t, DF: Days of flowering, NSPP: No. of seeds pod^{-1}

Effects of Sodium Azide on the quantitative traits parameters of Sesame (Abasena variety). The results indicated that plant height and internodes length was significantly increased at 0.02% concentration of sodium azide treated plants (Table 2). The effect was highly significant at $p<0.01$ level, when compared with the control and other treatments. The maximum plant height (Table 2) at maturity of 66 cm was recorded in 0.02% of sodium azide treatment while minimum plant height was observed in 0.05 m% sodium azide (55 cm). In the present study, sodium azide treatment in the lower concentrations has shown stimulatory effect as compared to control and the rest of the treatments. Again in the lower doses of mutagenic treatments, days required for flowering and days required for maturity were found to be significantly decreased. The significant changes in terms of days were observed in 0.02% concentration of sodium azide at $p<0.01$ level than the control and the rest of treatments plants. Days to first flowering, ranged from 44, 44, 40, 44, 45 and 45, respectively, among control and treatments. A minimum decrease in days to first flowering (40) was recorded in 0.02% when compared to control and other treatments of sodium azide. The plants germinated from seeds which were treated with 0.04 and 0.05% sodium azide took the longest duration for corresponding traits of abasena variety (Table 2).

The No. of capsule plant^{-1} was observed. Significant increase in capsule No. ($p<0.05$) was observed at 0.01, 0.02 and 0.03% concentration of sodium azide treatments when compared to the control and the rest of treated plants. Since capsules are seed bearing structures their increase has a positive correlation with the yield of a seed. Similar result was cited by Sureja and Sharma (2000). The hundred seed weight was increased significantly at (0.02% concentration) at $p<0.01$ level in comparison to the control and other treatments.

The No. of seeds pod^{-1} of treated and untreated plants was counted. Maximum No. of seeds pod^{-1} (68) was observed in 0.02% sodium azide among. A minimum No. of seeds pod^{-1} were (45) and recorded in 0.05% sodium azide, respectively. A low concentration of sodium aside (0.02%) was shown to increase seed No. when compared to other mutagen treatments.

Effects of Sodium Azide on the quantitative traits parameters of Kelafo 74 variety. The effects of sodium azide on the plant height of Kelafo 74 variety was observed at 0.01, 0.02 and 0.03% concentrations and highly significant difference at ($p<0.01$) was observed when compared with other concentrations and control plants. The inhibitory effects were concentration dependent (Table 3).

Table 3: Effects of sodium azide on the phenological parameters of kelafo 74 variety

Treatment	PH	IL	DM	CL	NCPP	HSW	NSPP	DF
Control	86.00^{d}	9.00^{c}	85.00^{e}	1.80^{a}	12.00^{a}	$2.5.0^{a}$	45.00^{a}	40.00^{c}
0.01%	88.00^{c}	9.00^{cd}	87.00^{d}	1.60^{b}	10.00^{ab}	$2.4.0^{ab}$	45.00^{a}	40.00^{c}
0.02%	90.00^{b}	9.50^{b}	90.00^{c}	1.60^{b}	10.00^{ab}	$2.4.0^{ab}$	43.00^{b}	38.00^{d}
0.03%	95.00^{a}	9.00^{a}	93.00^{b}	1.50^{c}	9.00^{bc}	$2.3.0^{bc}$	40.00^{c}	40.00^{c}
0.04%	85.00^{d}	9.00^{c}	95.00^{a}	1.40^{d}	8.00^{c}	$2.2.0^{c}$	39.00^{d}	42.00^{b}
0.05%	80.00^{e}	8.50^{d}	95.00^{a}	1.30^{e}	6.00^{d}	2.00^{d}	35.00^{e}	44.00^{a}
CV	0.70	2.95	0.38	2.99	10.54	3.46	1.79	0.90
LSD	0.78	0.35	0.45	0.06	1.23	0.10	0.94	0.47

PH: Plant height, IL: Internodes length, DM: Days to maturity, CL: Capsule length, NCPP: No. of Capsule plants^{-1}, HSW: Hundred seed weight, DF: Days of flowering, NSPP: No. of seeds pod^{-1}

There was no significant difference observed in internodes length of different concentration mutagenic treatments and control and that of No. of capsules plant^{-1}, No. of seeds per pod, capsule length and hundred seed weight exhibited totally a negative mean shift. The experimental results showed favorable effects on days to flowering and days to maturity was decreased in 0.02 and 0.01%, respectively and highly significant changes was observed at ($p<0.01$). It gives a clue for selecting early maturing mutants (Kelafo 74 variety).

DISCUSSION

In bioassay studies the seed germination percentage was concentration dependent. Similar result was suggested by Siddiqui *et al.* (2007). Mutagens such as sodium azide decrease the germination percentage and increase chromosomal aberrations in root tip mitotic cells of plants in a dose-dependent fashion. The reduction in seed germination in mutagenic treatments has been explained due to the delay or inhibition of physiological and biological processes necessary for seed germination including enzyme activity (Kurobane *et al.*, 1979), hormonal imbalance (Jacobsen and Varner, 1967) and inhibition of mitotic process (Ananthaswamy *et al.*, 1971). Ussuf and Nair (1974) also inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation existing enzymes involved in the formation of auxins and thus reduce the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). These findings are in close agreement with the earlier reports of Kumar (1988) in Faba bean.

Effects of sodium azide on the quantitative traits characters of Sesame (Abasena variety). The results indicated that plant height and internodes length was significantly increased at 0.02% concentration of sodium azide treated plants. Such a similar result on plant height has been reported by Khan and Al-Qurainy (2009). The No. of capsule plant^{-1} was observed. The significant increase in capsule No. ($p<0.05$) was observed at 0.01, 0.02 and 0.03% concentration of sodium azide treatments when compared to the control and the rest of treated plants. Since capsules are seed bearing structures their increase has a positive correlation with the yield of a seed (Abasena variety). Similar result was cited by Sureja and Sharma (2000). In addition to this Singh and Kole (2005) reported increase in variability for No. of pods plant^{-1} following mutagenic treatments of sodium azide in *Vigna mungo*.

CONCLUSION

Analysis of variance in Bioassay studies showed highly significant difference in germination percentage of abasena and kelafo 74 varieties under the treatment of sodium azide. In Abasena variety the root and shoot growth was stimulated in 0.02 and 0.01% of sodium azide. On the other side statistical inference done on the quantitative traits in a green house study reflected that in the variety Abasena plant height, No. of seeds per pod, internode length, capsule length and No. of capsules plant^{-1} were stimulated at 0.02% of sodium azide and the days to maturity and flowering were reduced at this same concentration. In the variety kelafo 74 internode lengths and plant height were stimulated at 0.02 and 0.03% of sodium azide, respectively and the days to flowering was reduced at 0.02%. Hence, it can be concluded that utilizing the chemical mutagen it is possible to foster genetic variability in a tangible way and here sodium azide is more positive effective on Abasena variety than kelafo 74. Overall results indicated the possibility of obtaining sesame varieties which display more quantitative traits variations than their parents. But the produced mutants from first generation are not adequate for studying the genetic stability, so these traits should be investigated for the desired traits in subsequent generations and in field conditions.

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