**RESEARCH COMMUNICATION** 

# The mutagenic effect of hydroxylamine hydrochloride on the agronomic traits of Sesame (*Sesamum indicum* L.)

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## Abstract

The effects of mutation induction through the use of a chemical mutagen as a method of improving few agronomic traits in sesame (Sesamum indicum L.) were investigated. Healthy and dry seeds of sesame varieties (Abasena and Kelafo 74) were treated with hydroxylamine hydrochloride (HA) at six different concentrations (0.01, 0.02, 0.03, 0.04, 0.05 % (w/v) and control) with the aim of improving the growth and yield parameters of the plant. Bioassay studies showed highly significant difference in germination percentage of the two varieties under the treatment of the mutagen compared to the control. The results obtained from the quantitative parameters also revealed highly significant increase ( $P \le 0.01$ ) in the plant heights, number of seeds/pod, number of capsules/plant, internode length and capsule length with decrease in the concentration of the mutagen. In addition, days to maturity have shown a negative mean shift in all the treatments and days to flowering showed a significant positive mean shift only at 0.02% concentration of HA. The chemical mutagen was therefore found to improve the quantitative traits associated with growth and yield of sesame. The induced variation can be exploited in the evolution of new varieties of sesame with improved agronomic traits.

**Keywords:** Effectiveness; Hydroxylamine hydrochloride; Mutation; Sesame.

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#### Introduction

Sesame (*Sesamum indicum* L., 2n = 26), which belongs to the Pedaliaceae family, is one of the most ancient oilseed crops and is cultivated in tropical and subtropical regions of Asia, Africa and South America (Brar and Ahuja, 1979; Ashri, 1998; Anilakumar *et al.*, 2010). It is cultivated for its edible leaves used as vegetables (Mann *et al.*, 2003) or oily seeds (Burkill, 1997). It is an important oilseed crop used to generate high quality edible oil and protein for low income peasants of major sesame growing countries including Sudan, Ethiopia, Uganda, Nigeria, Mexico, Venezuela, India, China, Pakistan, Turkey and Myanmar (Oplinger *et al.*, 1990).

Ethiopia is known to be the origin of diversity for cultivated sesame (Bedigian, 2003). The sesame seeds harbors 50-60% oil and 25% protein with antioxidant lignans such as sesamolin, sesamin and have been used as active ingredients in antiseptics, bactericides, vermicides, disinfectants, moth repellants, anti-tubercular agents and are considerable source of calcium, tryptophan, methionine and many minerals (Bedigian *et al.*, 1985). Attention has recently been focused on this crop in Ethiopia because of its great potential as an export commodity for its seeds. Worldwide, sesame oil is in high demand due to the importance in the confectionary industry.

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 & Aliyu, 2007) and also used to create genetic variability in quantitative traits in various crop plants (Mahla *et al.*, 1990; Shah *et al.*, 1990; Mahandjiev *et al.*, 2001). Generally mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement. The successful utilization of hydroxylamine to generate genetic variability in plant breeding has been reported in cowpea (Mensah & Akomeah, 1992), rice (Mensah *et al.*, 2003) soyabean (Mensah *et al.*, 2013). To increase production of the crop there is a need to have a better understanding of its genetic background. However, there is no information on the locally cultivated varieties, which lack variability because of their self-pollination status. The present study was therefore undertaken to fill the gap in knowledge of the genetic background of the crop and assess the effect of the chemical on the two varieties of the plant with the aim of inducing mutation in the seeds of sesame for the improvement of its growth and yield parameters.

## Materials and methods

The research was conducted in the genetics and biotechnology laboratory of the Department of Biological Sciences and at Rare agricultural research field, Haramaya University (Lat. 9°26'N and Long. 42°3'E), Dire Dawa, Ethiopia. The seeds of sesame (Sesamum indicum L.) were obtained from the Werer Agricultural Research Institute. Four hundred and forty dry and normal uniform seeds of each variety were used and these seeds were surface sterilized with 0.1% mercuric chloride for 1 minute to remove the fungal spores on the surface of the seeds. These seeds were treated at six different hydroxylamine hydrochloride (HA) concentrations (0.01, 0.02, 0.03, 0.04, 0.05 % (w/v) and control) through presoaking. The treated seeds were sown in a Completely Randomized Design (CRD) with three replications in a factorial arrangement. The design of the study involved both laboratory and greenhouse experiments. Data from the laboratory based bioassay study on seed germination percentage and seedling growth and morphological traits representing the growth and yield attributes from the greenhouse study on the plant height, number of capsules/plant, capsule length, internodes length, days to 50% flowering, days to 90% maturity, number of seeds/pod and 100 seed weight were collected. All the data obtained were analyzed using Analysis of Variance (ANOVA) with least significant difference (LSD) at 1% and 5% level of significance to separate the means. In addition, the effectiveness of mutation was determined according to the formula suggested by Konzak et al. (1965).

Mutagenic effectiveness = 
$$\frac{MF}{C \times t}$$

where

MF: Mutation frequency (%)

C: Concentration of chemical mutagen

t: Period of treatment with chemical mutagen

## Results

The results obtained following the treatment of sesame seeds with different HA concentrations on seed

germination, length of root, and shoot is presented in Table 1. The highest concentration (0.05%) showed a highly significant difference ( $P \le 0.01$ ) in comparison to the control and other treatments in reducing seed germination in both varieties, i.e. Abasena and Kelafo 74. The rate of germination showed a negative correlation with the mutagen concentration. HA inhibited root growth in all the concentrations, but the effect was highly significant at 0.04 and 0.05% concentrations (Table 1). Significant increase was also observed in shoot length at 0.01% concentration of HA in comparison with other treatments and the control, in addition the stimulatory effect was significant over the control plants. The other treatments displayed a negative mean shift in shoot length. Generally the inhibitory effect of HA on seed germination and seedling growth showed a similar trend on both sesame varieties.

Effects of HA on the phenological parameters of sesame varieties are shown on Table 2. The results indicated that plant height and internodes length was significantly increased (P < 0.01) at 0.01 and 0.02 % concentration of HA in Abasena and Kelafo 74 varieties respectively. The maximum plant height (Table 2) at maturity of 64 cm was attained in 0.01 % concentration while minimum plant height (56 cm) was observed in 0.05 % HA for Abasena whereas maximum plant height of 90 cm was recorded for Kelafo 74 at 0.02% concentration of HA. In the present study, HA treatment in the lower concentrations has shown stimulatory effect as compared to control and the rest of the treatments. In addition, the lower doses of mutagenic treatments decreased significantly the days required for flowering and maturity. The significant changes in terms of days that are needed for flowering and maturation were observed at 0.01% concentration of HA for both varieties. Significant increase in capsule number (P<0.05) was observed at 0.01%, 0.02% and 0.03% concentration of HA treatments when compared to the control and the rest of treated plants for Abasena variety while Kelafo 74 showed a reduction in capsule number for all concentrations of HA. The HA treatment did not show any significant increase on both varieties of sesame on seed number. In general number of capsules per plant, number of seeds per pod, capsule length and hundred seed weight exhibited totally a negative mean shift in Kelafo 74 variety.

The mutagenic effectiveness was found to be the highest at lower concentration with all the mutagenic treatments in germination percentage as well as the phenological parameters of both sesame varieties (Figure 1 and 2). At 0.01% concentration of HA did not show any significant increase on both varieties of sesame, maximum effectiveness was observed in germination percentage (1335) for both Abasena and Kelafo 74 varieties. Similarly, days to maturity (1333, 1450) and plant height (1067, 1433) showed a highest value at 0.01% concentration of HA and showed a significant increase on both Abasena and Kelafo 74 sesame varieties respectively.

# Discussion

The results for the M1 generation revealed that lower doses of mutagens were effective and efficient in causing

mungbean (Roychowdhury *et al.*, 2004), in black gram (Sharma *et al.*, 2005; in cowpea (Dhanavel *et al.*, 2008), in little millet (Ganapathy *et al.*, 2008; Lal *et al.*, 2009; Thilagavathi & Mullainathan, 2009) and in sesame

Table 1. Effects of hydroxylamine hydrochloride on seed germination and growth related parameters.

Treatment	Seed germination (%)	Shoot length (cm)	Root length (cm)		
Variety Abasena					
control	93.87ª	6.60ª	4.53ª		
0.01 %	80.07°	6.89 <sup>b</sup>	3.66 <sup>b</sup>		
0.02 %	80.00 <sup>d</sup>	3.66 <sup>c</sup>	3.30 <sup>cd</sup>		
0.03 %	63.90 <sup>f</sup>	3.24 <sup>d</sup>	3.30 <sup>cd</sup>		
0.04 %	61.30 <sup>i</sup>	3.18 <sup>d</sup>	1.92 <sup>cg</sup>		
0.05 %	52.03 <sup>k</sup>	3.00 <sup>e</sup>	1.89 <sup>dg</sup>		
CV (%)	0.64	1.02	1.02		
LSD	0.03	0.06	0.06		
Variety Kelafo 74					
control	94.90ª	5.91 <sup>b</sup>	3.96ª		
0.01 %	80.07 <sup>b</sup>	6.50 <sup>c</sup>	3.84 <sup>b</sup>		
0.02 %	79.80°	5.61ª	3.64 <sup>c</sup>		
0.03 %	63.00 <sup>d</sup>	4.83 <sup>d</sup>	3.60 <sup>d</sup>		
0.04 %	60.00 <sup>e</sup>	4.59e	3.54 <sup>e</sup>		
0.05 %	52.90 <sup>f</sup>	3.63 <sup>f</sup>	3.20 <sup>f</sup>		
CV (%)	0.1	0.21	0.51		
LSD	0.09	0.01	0.02		

Table 2. Effects of hydroxylamine hydrochloride on the phenological parameters of sesame varieties.

Treatment	РН	IL	DM	CL	NCPP	HSW	NSPP	DF		
Abasena										
control	60.00 <sup>a</sup>	11.00 <sup>c</sup>	85.00 <sup>b</sup>	2.00 <sup>c</sup>	15.00 <sup>d</sup>	3.00 <sup>d</sup>	50.00 <sup>d</sup>	44.00 <sup>b</sup>		
0.01 %	64.00 <sup>b</sup>	14.00 <sup>a</sup>	80.00 <sup>c</sup>	2.00 <sup>c</sup>	17.00 <sup>b</sup>	3.80 <sup>c</sup>	50.00 <sup>d</sup>	40.00 <sup>c</sup>		
0.02 %	61.00 <sup>a</sup>	12.00 <sup>bc</sup>	85.00 <sup>b</sup>	1.90°	16.00ª	2.50ª	48.00 <sup>c</sup>	44.00 <sup>b</sup>		
0.03 %	60.00 <sup>a</sup>	11.00c	90.00 <sup>d</sup>	1.80 <sup>b</sup>	16.00ª	2.40 <sup>b</sup>	46.00 <sup>c</sup>	44.00 <sup>b</sup>		
0.04 %	58.00 <sup>c</sup>	10.00 <sup>c</sup>	92.00 <sup>cd</sup>	1.50 <sup>d</sup>	15.00 <sup>d</sup>	2.40 <sup>b</sup>	44.00 <sup>a</sup>	45.00 <sup>a</sup>		
0.05 %	56.00 <sup>bc</sup>	9.00 <sup>c</sup>	95.00 <sup>e</sup>	1.50 <sup>d</sup>	14.00 <sup>d</sup>	2.20 <sup>e</sup>	42.00 <sup>e</sup>	45.00 <sup>a</sup>		
CV	0.06	0.32	0.53	11.23	0.07	0.99	0.58	0.69		
LSD	0.08	0.046	0.60	0.26	0.014	0.032	0.035	0.39		
Kelafo 74										
control	86.00 <sup>a</sup>	9.00 <sup>c</sup>	85.00 <sup>d</sup>	1.80 <sup>a</sup>	12.00ª	2.50ª	45.00 <sup>a</sup>	40.00 <sup>c</sup>		
0.01 %	86.00 <sup>a</sup>	9.00 <sup>c</sup>	87.00 <sup>e</sup>	1.60 <sup>b</sup>	10.00 <sup>ab</sup>	2.40 <sup>ab</sup>	45.00ª	40.00 <sup>c</sup>		
0.02 %	90.00 <sup>c</sup>	9.50 <sup>b</sup>	90.00ª	1.60 <sup>b</sup>	10.00 <sup>ab</sup>	2.40 <sup>ab</sup>	45.00ª	36.00 <sup>d</sup>		
0.03 %	85.00 <sup>b</sup>	9.00 <sup>c</sup>	90.00ª	1.40e	8.00 <sup>c</sup>	2.20 <sup>bc</sup>	39.00 <sup>b</sup>	41.00 <sup>c</sup>		
0.04 %	83.00 <sup>e</sup>	8.70 <sup>a</sup>	92.00 <sup>b</sup>	1.40e	7.00 <sup>cd</sup>	2.20 <sup>bc</sup>	39.00 <sup>b</sup>	43.00 <sup>bc</sup>		
0.05%	80.00 <sup>d</sup>	8.50 <sup>ab</sup>	93.00 <sup>bc</sup>	1.20c	7.00 <sup>cd</sup>	2.00 <sup>d</sup>	35.00 <sup>e</sup>	45.00 <sup>a</sup>		
CV	0.24	3.85	0.18	5.12	3.38	2.02	0.48	0.50		
LSD	0.27	0.45	0.20	0.10	0.40	0.06	0.26	0.27		

PH= Plant Height (cm), IL= Internodes length (cm), DM= Days to Maturity, CL = Capsule Length (cm), NCPP = Number of Capsule per plants, HSW= Hundred Seed Weigh (gram), DF= Days of Flowering, NSPP = Number of seeds per pod.

polygenic variability in various quantitative characters, with a negative relationship between effectiveness and mutagen dose. These findings agreed with those in (Ajibolu, 2005; Aliero, 2006; Begum & Dasgupta, 2010). The lowest concentration of the chemicals (0.01%) was the most effective in causing mutations.

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Chemical mutagenesis induced by the application of different concentrations of HA in the seeds of *S. indicum* has proved to be beneficial in the improvement of some of its selected growth and yield traits. Generally, the decrease

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),



Figure 1. The mutagenic effectiveness of hydroxylamine hydrochloride on selected biometrical traits of Abasena variety at different concentration, (G % = Germination percent, DF = days to flowering, DM = Days to maturity, PH = Plant height, NCPP = Number of capsules per pod, HSW = Hundred seed weight, IL = Intenode length).



Figure 2. The mutagenic effectiveness of hydroxylamine hydrochloride on selected biometrical traits of Kelafo 74 variety at different concentration, (G % = Germination percent, DF = days to flowering, DM = Days to maturity, PH = Plant height, NCPP = Number of capsules per pod, HSW = Hundred seed weight, IL = Intenode length).

in germination showed a negative correlation with the increased mutagen concentration in both varieties of sesame for the mutagen. The reduction in seed hormonal imbalance (Chrispeels & Varner, 1967; Adamu & Aliyu, 2007) and inhibition of mitotic activity (Ananthaswamy *et al.*, 1971, Kulkarni, 2011).

Root length and shoot length showed a similar pattern of growth *i.e.*, with the increase of concentration of chemical mutagen there was adverse effect on the growth of the root length and shoot length of sesame seedlings in the laboratory conditions. The reduction in seedling survival is attributed to cytogenetic damage and physiological disturbances (Sato & Gaul, 1967). Thus, the probable reason of this may be the hindrance caused by the HA on different metabolic pathway of the cells. Similar findings have also been reported by (Rachovska & Dimova, 2000) in wheat, (Akhaury *et al.*, 1996; Ilbas *et al.*, 2005) in barley, (Adamu & Aliyu, 2007) in tomato, (Khan *et al.*, 2004) in mungbean, (Al-Qurainy, 2009) in *Eruca sativa* and (Mostafa, 2011) in sunflower.

The mean increase in plant height at maturity of the two sesame varieties induced by the chemical 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. Significant increase in capsule number, seed weight, capsule length and number of seeds/pod was also observed at a relatively lower concentration of the mutagen. Since capsules are seed bearing structures their increase has a positive correlation with the yield of a seed (Sureja & Sharma, 2000). Hoballah (1999) also reported that there was an increase in the number of capsules per plant among sesame mutants.

Many workers have reported the efficiency values to be higher at lower dose of mutagens such as sodium azide (Roychowdhury et al., 2012; Khan & Goyal, 2009), gamma rays, EMS and HZ (Siddiq & Swaminathan, 1968; Nerker, 1976) for different agronomic and growth related parameters. Khan et al. (2004) proposed that the relative higher efficiency at lower concentration/dose of the mutagen could be ascribed to the lesser percentage of injury at such doses. General decrease in effectiveness with increasing doses of Gamma rays has been reported in foxtail millet (Gupta & Yashvir, 1975), lentil (Sharma, 1990; Solanki & Sharma, 1999), soybean (Menash et al., 2013). In the present study, it was also observed that effectiveness reduced with an increase in concentration in both the varieties of sesame for the different phenological parameters.

Generally, the greater sensitivity at higher mutagenic level has been attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens and to the disturbance of balance between promoter and inhibitors of growth regulators (Krishna *et al.*, 1984). Adegoke (1984) reported that sodium azide induces chromosomal damages leading to bridge formation during mitotic division and hence increased phenotypic aberration.

Hence it can be concluded that utilizing this chemo mutagen it is possible to foster genetic variability in a tangible way. Generally speaking the mutagenic efficiency is inversely proportional to the concentration gradient of the mutagen. Overall results indicated the possibility of obtaining sesame varieties which display more agrometrical traits variation 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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