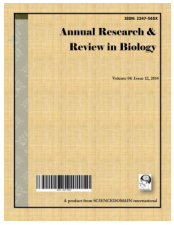

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Effect of Mutagenesis on Germination, Growth and Fertility in Sesame (*Sesamum indicum* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author VK designed the study, performed the statistical analysis, wrote the protocol, and supervised the whole work. Author HKC provided necessary instrumentation and managed the manuscript editing. Author RP helped in analyses of the study, read and edited the manuscript. Authors AK and AS managed manuscript editing and review. Author SJ belong to funding agency, reviewed the manuscript and approved the final version for publication. Author SS recorded field data, collected the literature and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In order to study the effect of mutagenesis on germination, growth and fertility in sesame, the seeds of a local variety 'LTK-4' were got irradiated with 6 gamma radiation doses *viz.,* 150Gy, 300Gy, 450Gy, 600Gy, 750Gy and 900Gy at BARC, Mumbai. The seeds were also treated with 0.5%, 1.0% and 1.5% EMS. About 100 seeds of each dose along with control 'LTK-4' were sown in pots at

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CSKHPKV, Palampur during *kharif,* 2013. The observations were recorded on percent germination, root length (cm), shoot length (cm) and plant survival. Besides, about 2000 treated seeds per dose/concentration were grown in randomized block design with three replications each at CSKHPKV, Palampur and RSS, Akrot (H.P.) during *kharif,* 2013. The observations on percent germination, plant survival, reduction in plant survival over control and survival till maturity were recorded at appropriate stages of crop growth. The analysis of variance indicated that all the 9 treatments differed significantly for seed germination and plant survival parameters indicating the presence of sufficient variability for these parameters. Under field conditions, the maximum reduction in survival over control was observed in 900Gy (87.7%) while lowest was observed in 150Gy (61.9%). Likewise, percent survival was highest in 0.5% EMS (39.3%) and reduced thereafter with increasing concentration of EMS. Percent pollen fertility reduction ranged from 3.1% in 150Gy to 28.5% in 900Gy dose while in chemical mutagen, it ranged from 7.1% in 0.5% EMS to 31.5% in 1.5% EMS at both locations. Maximum injury as percent of control was observed in 900Gy dose (59.6%) and 1.5% EMS (42.2%) in chemical mutagen. Overall, a dose dependent relationship was observed between biological damage (%) and type of mutagen used. Higher doses of gamma radiations and EMS both caused considerable reduction in all biological parameters. Based upon the sensitivity of mutagens, EMS treatments were highly effective for modifying majority of the traits in the crop.

Keywords: Sesame; gamma rays; ethyl methane sulphonate; mutagenesis; radio sensitivity; chemo sensitivity.

ABBREVIATIONS

Ethyl methane sulphonate (EMS); Gray (Gy); Bhabha Atomic Research Centre (BARC); Cobalt (Co).

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the ancient oilseed crops of India and belongs to family Pedaliaceae. It is called as 'queen of oilseeds' because of its high quality oil and protein. It is a tropical and subtropical crop cultivated for its seeds. Cultivated sesame (*Sesamum indicum* L.) is diploid with 2n=26. India is the world's second largest producer of sesame seeds [1]. In India, the crop is grown over an area of 1.86 million hectares with the annual production and productivity of 0.64 million tons and 344 kg/ha, respectively. It is cultivated in almost all parts of the country during different seasons of the year. In Himachal Pradesh, sesame is one of the most important *kharif* oilseed crops, grown over an area of 3.00 thousand hectares with production and productivity of 1.06 thousand metric tons and 355 kg/ha, respectively [2]. The productivity is relatively low compared to other oilseed crops. The major yield constraints are narrow genetic base, lack of shattering resistance, longer days to maturity, low harvest index and prevalence of various biotic (*Phytophthora* blight, leaf spot and *Phyllody*) and abiotic stresses.

Genetic variability is the basic requirement for genetic improvement of any crop. Induced mutagenesis is an effective and potential method to create variability in a crop [3]. The rate of spontaneous mutations in nature is very low. Both physical and chemical mutagens can be used to induce mutations in crop plants and subsequent improvement can be done through selection. Among physical mutagens, gamma rays directly penetrate the plant tissue and are partially ionizing. Depending upon the radiation level, they can damage or modify important components of plant cells and affect the morphology, anatomy, biochemistry and physiology of plants [4]. Chemical mutagens generally produce induced mutations that lead to substitution of base pairs especially GC to AT resulting in amino acid changes which change the function of proteins. EMS (Ethyl Methane Sulphonate) is reported as the highly effective and powerful mutagen compared to other mutagenic agents and typically produces only point mutations [5,6]. The mutagen is very efficient for creating genetic variability in the natural gene pool of *Sesamum indicum* L. [7]. Seed germination, seedling growth, pollen sterility and chromosomal aberration are some of the commonly used criteria for studying mutagenic sensitivity in plants [8,9].

Keeping this in view, the present study was conducted to understand the immediate effects

of mutagenesis on sesame in terms of germination, injury as percent of control, plant survival and pollen fertility in order to select most beneficial mutagen or dose/concentration.

2. MATERIALS AND METHODS

The experiment was conducted on a high yielding variety of sesame 'LTK-4' commonly known as 'Brajeshwari', developed at CSK HPKV, Palampur. For physical mutagenesis, about 5000 healthy, dry and uniform colored seeds were got irradiated with 6 gamma radiation doses *viz.,* 150Gy, 300Gy, 450Gy, 600Gy, 750Gy and 900Gy from 60cobalt source at Bhabha Atomic Research Centre, Mumbai, to create variability. For chemical mutagenesis, about 5000 healthy, uniform and dry seeds were selected and pre-soaked in the distilled water for 12 hrs. prior to treatment at room temp. EMS solution was prepared in phosphate buffer of pH 7.0. The pre-soaked seeds were treated with freshly prepared EMS solution for 6 hrs. with concentrations of 0.5%, 1.0% and 1.5%. The soaked seeds were washed under running water continuously for 2 hrs. in order to remove the excess mutagen. The seeds were dried with filter paper and immediately sown after treatment. Two control treatments were also maintained using non-irradiated seeds and seeds soaked in distilled water. In order to study the effect of different doses/concentrations of mutagens on germination and seedling growth, about 100 seeds of each dose along with control 'LTK-4' were sown as pot culture experiment in the experimental farm area of the Department of Crop Improvement, CSKHPKV, Palampur during *kharif*, 2013. Plants were raised in well-prepared growth media of farm yard manure, soil and sand in the ratio of 1:1:1. The observations were recorded on percent germination, root length (cm), shoot length (cm) and plant survival. About 30 days old seedlings were selected to record observations on root and shoot lengths. Ten seedlings were selected at random to record observations on seedling height and number of leaves per seedling.

In order to have sufficient number of M_1 plants, about 2000 treated seeds per dose/ concentration both in gamma rays and EMS each were grown in randomized block design with three replications each at experimental farm area of the Department of Crop Improvement, CSKHPKV, Palampur (H.P.) and CSKHPKV, RSS, Akrot during *kharif,* 2013. The row to row and plant to plant spacings were kept at 30 cm and 10 cm, respectively. Standard cultural and agronomic practices were followed to raise the crop and maintain good plant growth. The observations on percent germination, plant survival, reduction in plant survival over control and survival till maturity were recorded at appropriate stages of crop growth in the M_1 generation. The emergence of coleoptiles for percent germination was recorded up to 15 days after sowing. The survival percentage was computed as percent of plants surviving till maturity. The Biological damage was computed as injury expressed as percent of control, plant survival reduction (%)/ emergence reduction (%) and pollen fertility reduction (%) for each treatment. The percent injury or percent reduction was calculated as per the standard procedure [10].

Seed germination (%) = [(Number of seeds germinated/ Total number of seeds sown) x 100]

Plant survival (%) = [(Number of plants survived / Total number of seeds germinated) x 100]

Emergence reduction (%) =
100 − [(Average emergence in the dose × 100) /Average emergence in the control)]

2.1 Statistical Analysis

Collected data were subjected to Analysis of variance technique [11].

3. RESULTS AND DISCUSSION

The analysis of variance for seed germination and plant survival parameters in induced mutants indicated that all the 9 treatments (doses and EMS treatments) differed significantly for the parameters such as percent germination, M_1 plant survival, percent survival and M_1 plants survival till maturity indicating the presence of sufficient variability for these parameters (Table 1).

3.1 Pot Culture Experiment

3.1.1 Plant survival

The effects of mutagens on root length, shoot length, number of leaves per seedling and plant survival (30 days after sowing) in M_1 generation are presented in Table 2. The plant survival (30 days after sowing) ranged from 15 in 900Gy to 61 in 150Gy in physical mutagens while it ranged from 18 in 1.5% EMS to 77 in 0.5% EMS in

Table 1. Mean squares for germination and plant survival parameters in induced mutants of sesame

Source of variation	Degrees of freedom	Mean squares				
		M_1 plants	Percent germination	M_1 plant survival	Percent survival	M_1 plant survival till maturity
Replication	2	5226.3	29.20	3136.8	81.20	76.04
Treatments	8	136244.0*	766.5*	65835.3*	592.20*	1957.5*
Error	16	7857.1	44.26	5301.2	60.3	668.4
CD (5%)	-	153.4	11.5	126.0	13.4	44.8

**Significant at p≤0.05*

chemical mutagen. The control had 81 plants out of 100 seeds sown under pot culture experiment. The plant survival was maximum in lower doses of physical as well as chemical mutagens as compared to higher doses/concentrations. The reduction in plant survival is attributed to altered enzyme activities, cytogenetic damage and physiological disturbances caused by the mutagen [12].

3.1.2 Number of leaves per seedling

Number of leaves per seedling also exhibited a decreasing trend with the increase in dose/concentration of the mutagen used. In physical mutagens, 450Gy dose recorded highest number of leaves per seedling (3.3) while in chemical mutagen, 0.5% EMS exhibited maximum number of leaves per seedlings (3.2). Reduction in number of leaves per seedling was similar in EMS as well as gamma radiations. This reduction increased with increase in dose/concentration except 450Gy dose where no such relationship was observed.

3.1.3 Root length (cm)

The reduction in the root length was caused by the higher doses/concentrations of physical as well as chemical mutagens while lower doses of physical and chemical mutagens exhibited higher root length. Maximum root length was observed in 300Gy dose (6.0 cm) of physical mutagens and 0.5% EMS concentration (5.3 cm).

3.1.4 Shoot length (cm)

The shoot length also decreased causing seedling injury progressively with increase in dose/concentrations of the mutagen. Lower doses of physical as well as chemical mutagens exhibited higher shoot length. Maximum shoot length was observed in 300Gy dose (7.9 cm) of physical mutagens and 0.5% EMS concentration (6.3 cm) while the higher dose/concentrations retarded shoot length (Table 2). Similar results have also been recorded in cluster bean [13], cow pea [14] and tomato [15].

The reduction in lengths of root and shoot due to mutagenic treatments has been observed in sesame [16] and sunflower [17]. The increase in mutagenic doses lead to decrease in seedling vigor because of increase in physiological damage induced in the seeds and seedlings [18] and variations in stimulation due to cell division rates as well as an activation of growth hormones such as auxin [19,20]. The reduced seedling growth in mutagenically treated seeds might be due to injury caused at cellular level because of gene controlled biochemical processes or acute chromosomal aberrations or both [21].

3.2 Field Experiment

The effects of both physical and chemical mutagens on various parameters of plant growth such as germination, percent survival, reduction in survival and survival at maturity in sesame (total of two locations *i.e.* Palampur and Akrot) are presented in Table 3.

3.2.1 Germination

The seed germination decreased with the increase in radiation dose as well as EMS treatment. Germination ranged from 1434 in 900Gy to 2859 (300Gy) in physical mutagens. In chemical mutagen, germination ranged from 1128 in 1.5% EMS to 2607 in 0.5% EMS. The germination of seeds decreased with increase in dose/concentration both in physical and chemical mutagens except 300Gy. Similar trends were also observed in percent germination. Reduction/stimulation in seed germination might have been due to the effect of mutagens on meristematic tissues of the seed. The decrease in seed germination at higher doses/

concentration of the mutagens may be attributed to disturbances at cellular level (caused either at physiological or physical level) including chromosomal damages or altered enzyme activity [22,23]. Reduction in seed germination in mutagen treated population may be either due to the delay or inhibition in physiological and biological processes necessary for seed germination [24].

3.2.2 Percent survival

In physical mutagens, the maximum percent survival of M_1 plants (55.8%) was observed in 600Gy dose while the lowest percent survival was recorded in 900Gy (30.3%). The maximum reduction in survival over control was observed in 900Gy (87.7%) while lowest was observed in 150Gy (61.9%). In chemical mutagen, percent survival was highest in 0.5% EMS (39.3%) and reduced thereafter with increasing concentration of EMS. The highest reduction (95.1%) was recorded in 1.5% EMS while lowest was recorded in 0.5% EMS (69.7%). Earlier studies on the effect of mutagens on M_1 parameters have reported similar results in different crops including soybean [25], sesame [26] and pigeon pea [27]. Thus, the survival rates of treated seeds and M_1 plants are reduced probably due to genetic aberrations caused by higher doses/concentrations of the mutagen. However, the lower doses/concentrations of mutagens used in the present study can be successfully utilized for enhancing genetic variability in sesame.

3.2.3 Survival till maturity

The M_1 plant survival till maturity reduced progressively with increase in dose/concentration both in gamma radiations and EMS treated population. The maximum survival till maturity was observed in 150Gy (300 plants) dose of gamma radiations while the lowest survival was observed in 900Gy (117 plants) dose. In chemical mutagen, 0.5% EMS exhibited maximum survival till maturity (228 plants). Thus, higher doses of gamma radiations as well as EMS used in present study were more detrimental for plant survival.

3.2.4 Percent pollen fertility reduction

Variable degree of pollen fertility response was observed in different doses/concentrations. Percent pollen fertility reduction ranged from 3.1% in 150Gy to 28.5% in 900Gy dose while in chemical mutagen, it ranged from 7.1% in 0.5% EMS to 31.5% in 1.5% EMS at both locations. The negative effect of mutagens on pollen fertility percentage in mutagenic treatment plants may be due to meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains [28,29]. The pollen fertility reduction is also caused by the increase of free radical activities that trigger the death of seeds. The ionizing radiations of gamma rays interact with atoms or molecules to produce free radicals in cells that can damage or modify the important components of plant cells [30].

Table 2. Effect of mutagens on different parameters in M_1 generation (Pot culture)

Dose	No. of seeds sown	Root length (cm)	Shoot length (cm)	No. of leaves	Plant survival (30 days after sowing)
Physical mutagens					
Control	100	5.1	8.3	4.3	82
150Gy	100	5.2	7.2	3.1	61
300Gy	100	6.0	7.9	3.1	54
450Gy	100	4.4	7.0	3.3	39
600Gy	100	4.1	7.7	2.3	29
750Gy	100	4.5	6.3	2.0	23
900Gy	100	2.4	4.8	2.1	15
Mean		4.53	7.03	2.89	43.3
SE (±)		0.43	0.45	0.31	8.95
Chemical mutagen					
Control	100	6.2	7.4	4.9	79
0.5% EMS	100	5.3	6.3	3.2	77
1.0% EMS	100	3.0	4.2	2.4	43
1.5% EMS	100	2.7	4.0	2.0	18
Mean		4.3	5.5	3.1	54.3
SE (±)		0.86	0.83	0.64	14.64

Table 3. Effect of mutagenic treatments on various parameters in M_1 generation (total of two locations) during *kharif*, 2013

Dose	Total number of seeds sown (1)	M_1 plants germinated (2)	% germination (3)	M_1 plants Survival (4)	% survival (4/2)	Reduction in survival over control (%) (5)	M_1 plants survival till maturity (6)	% pollen fertility reduction (7)	Injury as % of control (8)
Physical mutagens									
Control	4000	3684	92.1	3540	96.1	-	3226	-	-
150Gy	4000	2796	69.9	1349	48.2	61.9	300	3.1	31.2
300Gy	4000	2859	71.5	1251	43.8	64.7	291	4.1	34.6
450Gy	4000	2302	57.6	1230	53.4	65.3	269	6.3	39.2
600Gy	4000	1957	48.9	1092	55.8	69.2	282	17.9	42.6
750Gy	4000	1734	43.4	741	42.7	79.1	194	21.3	50.2
900Gy	4000	1434	35.9	434	30.3	87.7	117	28.5	59.6
Mean	4000	2180	54.5	1016	45.7	71.3	242	13.5	42.9
Chemical mutagens									
Control	4000	3678	92.0	3387	92.1	-	3136	-	-
0.5% EMS	4000	2607	65.2	1025	39.3	69.7	228	7.1	23.2
1.0% EMS	4000	1458	36.5	317	21.7	90.6	116	16.2	36.1
1.5% EMS	4000	1128	28.2	166	14.7	95.1	134	31.5	42.2
Mean	4000	1731	43.3	503	25.3	85.1	159	18.3	33.8

Table 4. Radio and chemo sensitivity in sesame

Parameter	Gamma radiations	EMS	Sensitivity (%) in EMS over gamma rays	Sensitivity (times) in EMS over gamma rays
Injury as percent of control	42.9	33.8	(-)21.2	0.78
Reduction in pollen fertility over control (%)	13.5	18.3	(+)35.6	1.35
Reduction in survival over control (%)	52.4	72.6	(+)38.5	1.38

3.2.5 Injury as percent of control

Injury as percent of control also increased with increase in dose/concentration and maximum injury was observed in 900Gy dose (59.6%) and 1.5% EMS (42.2%) in chemical mutagen. The Injury as percent of control due to mutagens has also been observed in sesame [26], okra [31] and cluster bean [13]. For this character, EMS treatments were less sensitive (0.78 times) than gamma rays. For reduction in pollen fertility (%) and reduction in survival (%), EMS treatments were 1.35 and 1.38 times more sensitive than gamma rays, respectively (Table 4). Thus, EMS treatments are highly effective for modifying majority of the traits in the crop. Reduction in mean over control at higher doses/ concentrations reveals the highest mutagenic response of the variety.

Overall, a dose dependent relationship was observed between biological damage (%) and type of mutagen used. The higher dose of gamma radiations and EMS both caused considerable reduction in all biological parameters. These results are in confirmation with earlier findings [32] who also observed that the increase in dosage levels of both gamma rays and EMS reduced the biological parameters significantly such as seed germination, seedling survival, plant height and pollen fertility in sesame. The information from M_1 parameters may help in the initial rejection of less important mutagenized population which may save time and efforts in further mutation breeding programme. Irradiation at lower dose induces the growth stimulation by altering the hormonal signaling network in plant cells or by increasing anti oxidative capacity of the cells [33]. In contrast, growth inhibition due to high dose irradiation could be due to cell cycle arrest during somatic cell division at G_2/M phase and various damages to the entire genome [34].

4. CONCLUSION

In the present study, the survival rates of treated seeds and M_1 plants were reduced probably due to more genetic aberrations caused by higher doses/concentrations of the mutagen used. The seed germination, survival rate, seedling growth and root and shoot lengths were inhibited by increasing doses/concentrations of mutagens. However, the lower doses/concentrations of mutagens used in the present study can be successfully utilized for enhancing genetic variability. The information from M_1 parameters may help in the initial rejection of less important mutagenised populations which may save time and efforts in further mutation breeding programme. For reduction in pollen fertility (%) and reduction in survival (%), EMS treatments were 1.35 and 1.38 times more sensitive than gamma rays, respectively. Thus, EMS treatments are highly effective for modifying majority of the traits in the crop. Reduction in mean over control at higher doses/concentrations reveals the highest mutagenic response of the variety.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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