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INDUCED MUTATIONS IN SESAME (*Sesamum indicum* L.)

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Abstract:

The seeds of *Sesamum Indicum* L. were treated with various doses/ concentrations of gamma radiation (GR-300 to 500Gy), Ethyl Methane Sulphonate (EMS- 0.3 to 0.5%) and Sodium azide (SA-0.15, 0.20, 0.25%). The physiological effects on seed germination and seedling height on 7th day after sowing were investigated. Gradual reduction in seed germination and seedling height was recorded with increase in dose / concentration of mutagens. Almost all the mutagenic treatments caused decrease in seed germination, seedling height, seedling injury, pollen sterility and survival of plants at maturity.

Keywords: Sesame, seed germination, seedling injury, mutation.

Introduction

Sesame *(Sesamum indicum* L.*)* is also called as *Kala Til*. It plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (Khier *et al.*, 2008). It has ability to add nutty flavor, they were used for oil (Gandhi, 2009). Sesame seed is rich in fat, protein, carbohydrates, fibre and some minerals. The oil seed is renowned for its stability because it strongly resists oxidative rancidity even after long exposure to air (Global Agri Systems, 2010).

Sesame (2n = 26) is a self pollinated oilseed crop, belongs to the family Pedaliaceae. Brown or black seeded are valued more for oil whereas, white seeded are rich in iron. Sesame is an important oilseed crop, highly tolerant to both heat and drought (Weiss, 2000). Its seeds are a good source of proteins (23-30 g/100 g) oil (50 g /100 g), other minerals and vitamins.

In India, it is the fifth important edible oil seed crop after groundnut, rapeseed-mustard, sunflower and soybean with the annual average production around 6,80,000 metric tons. As a quality oil seed crop, it can reduce the shortage of other edible oil production not only in India but also in major sesame growing countries in the world, if the production and productivity are enhanced using modern breeding techniques like mutation breeding with irradiation.

The major sesame producing states in India are West Bengal, Madhya Pradesh, Rajasthan, Gujarat and Uttar Pradesh. Creation of variation for improvement of one or two traits becomes a necessity for this crop. Mutation breeding is an effective tool for crop improvement and an efficient mean to supplement existing germplasm for cultivar improvement in breeding programmes.

A major constraint in this approach was the lack of sufficient genetic variation within the existing germplasm collections, especially for traits such as resistance to various diseases and seed retention. This is where mutation techniques could offer a possible solution. The United Nations Food and Agriculture Organization (FAO) recommended the use of mutation induction for the enhancement of genetic variability in sesame with respect to modified plant architecture, seed retention, and resistance to diseases and pests (http://www.iaea.org/programmes/nafa).

Co-ordinated research project (CRP) focused on the induction of improved characters in sesame for the genetic improvement of sesame through mutation techniques (http://www.iaea.org/programmes/nafa). The success of this CRP is documented by the official release of 12 sesame varieties in Egypt, India, Republic of Korea, Sri Lanka, and the more than 140 agronomically useful sesame mutants developed by the participants (http://www.iaea.org/programmes/nafa).

Mutation breeding is one possible alternative to conventional breeding for crop improvement. Exposing plant genetic material to mutagens enhances the chance of isolating unique genetic material. In the past, induced mutations have effectively been utilized in development of new and valuable alterations in plant characteristics that have contributed to increased yield potential. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops (Maluszynski *et al.*, 1995; Muduli and Mishra, 2007). Mutation breeding not only creates variability in crop species, but also shortens the time taken for the development of cultivars via induced mutation compared to those via hybridizations.

In general, mutagenesis has been successfully used to induce genetic variability in many crops, allowing to isolate mutants with desirable characters such as increased seed yield, earliness (Wongyai *et al*., 2001), modified plant architecture, closed capsules, disease resistance (Cagirgan, 2001; Ashri 1998), seed retention, larger seed size, desirable seed colour and high oil content (Hoballah, 2001). Many new cultivars have been directly or indirectly released in the world through induced mutations.

Although many research has been made on through irradiation and chemical mutagen to improve: yield (Wongyai *et al.*, 2001), capsules number, leaves maturation, male sterility, plant architecture (Cagirgan, 2006), seed size and seed color (Hoballah, 2001), narrow leaf (Sengupta and Dutta, 2005), resistant to fusarium wilt (Silme, 2010) and early matured plant (Mensah *et al.*, 2007), however, studies on growth habit, maturity, shattering habit and plant architecture are still scanty.

Seed shattering is also a major issue to lose the production and productivity, early harvesting causes low seed yield, and poor quality due to immature seeds on the top of the plant. The late harvesting too reduces seed yield from the lower matured capsule because of seed shattering (Sruba Saha and Amitava Paul (2017).

MATERIALS AND METHODS

Seed material : The seeds of sesame (*Sesamum Indicum* L.) were procured from local market of Manchar, Tal. Ambegaon, Dist Pune-410503 (M.S.) India.

Mutagens used : Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were employed in present study for the treatments of seeds of sesame.

Gamma radiation from ^{60}Co source fixed in the gamma cell 200 installed at Department of Chemistry, Savitribai Phule, Pune University, Pune was used in the present work. Healthy, dry and uniform seeds of sesame with moisture content of 10-12 % were treated with 300, 400 and 500 Gy. Ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatment of sesame. Various concentrations of EMS (0.3% to 0.5%) were prepared in distilled water. Sodium Azide is inorganic compound. It is colour less salt, the gas forming component in many car airbag system. It is ionic compound, soluble in water and is highly toxic. Mol. Wt. is 65.0099g/mol. It is chemical mutagen and used for induction of mutations in the crop plants. Various concentrations of SA (0.3% to 0.5%) was prepared in distilled water.

Treatment details : To begin with, pilot experiments were conducted to determine the lethal dose (LD_{50}), suitable concentrations of EMS, SA and duration of seed treatment. The doses of gamma rays, 300, 400 and 500Gy, EMS 0.3, 0.4 and 0.5% while SA 0.15, 0.20 and 0.25% were finally selected for the seed treatment and the duration fixed was four hours.

Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS (0.3, 0.4 and 0.5%) and SA 0.15, 0.20 and 0.25% for four hours. The untreated seeds served as control. For each treatment 330 seeds were used.

The seeds treated with various concentrations of EMS and SA were washed thoroughly with tap water for two hours to terminate the reaction of chemical mutagen and to leach out the residual chemicals. A total of 30 seeds from each treatment was used for seed germination in laboratory. Three replications with 10 seeds / replication kept in petri dishes, containing seed germination paper, were used for recording seed germination percentage, root as well as shoot length, seedling growth on 7^{th} day. The remaining lot of treated seeds (300) from each treatment was used for raising M_1 generation in field.

Experimental site : Present investigation was carried out at Department of Botany and Ph. D. Research Center in Botany, Rayat Shikshan Sanstha's Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar, Tal. Ambegaon, Dist- Pune (410503) (M.S.). The soil type of the experimental field was slightly deep, fine and calcareous with good drainage. The average minimum temperature was recorded as 17.63^0C and maximum 32.73^0C with average annual rainfall 641.03mm.

Experimental design for field experiments: The field experiments were conducted on the experimental field at Department of Botany. The crop of sesame was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate following RBD design. Each plot had 100 plants. The distance between two rows and two plants was 45 X 30 cm and the distance between two adjacent plots was one meter.
A total of 10 treatment combinations in M_1 generation including untreated dry seeds were used as control. Treated and control seeds were sown in field in randomized block design replicated thrice.

Observations in M_1 generation
Germination percentage: The number of seeds showing emergence of the radical and plumule was counted from the seeds kept in petriplates lined on moist germination paper, data was used to calculate percent seed germination.

Root and shoot length: On 7^{th} day of sowing, 10 seedlings from control and each treatment were randomly selected for measuring the root and shoot length and the average values were recorded in table.

Seedling injury: Seedling height was recorded on 7^{th} day. Reduction in the mean seedling length as compared to the control was regarded as seedling injury and expressed as percentage. The seedling injury was calculated as follows

$$\% \text{ seedling injury} = \frac{\text{Control seedling height - Treatment seedling height X 100}}{\text{Control seedling height.}}$$

Pollen sterility : Pollen sterility was determined from 10 randomly selected plants per treatment, along with control. The pollen grains from freshly dehisced anthers were stained with 1% aceto-carmine. Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile.

Survival of plants at maturity : Survival percentage was calculated by scoring the total number of plants attaining maturity in each treatment and expressed as percentage over the control.

Quantitative traits (Micromutations) : The treated as well as control plants were screened for quantitative traits to study the induced variability. From each replication and treatment including control 10 plants were randomly selected for recording data on different quantitative characters in M_1 generations. Data on quantitative traits such as plant height (cm), primary branches/plant, DAS for first flowering, DAS for first capsule maturity, No. of capsule/plant, capsule length (cm), no. of seeds/capsule, 100 seed weight (g) and seed yield/plant (g) were recorded.

Harvesting of seeds from M_1 plants : All the surviving M_1 plants were harvested individually and seeds of single plant from each treatment were kept separately for raising M_2 generation.

Statistical analysis
The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.25).

RESULTS AND DISCUSSION

Results obtained in the present investigation on seed germination, seedling injury, pollen sterility and survival of plant at maturity in M_1 generation of sesame are illustrated in Table- 1. Data obtained on mean percent seed germination in control and mutagen treatments presented in Table-1 clearly indicated that the seed germination percent was decreased in all the treatments as compared to control. It has clearly indicated that the mutagens had exerted negative effects on seed germination. Percent seed germination was decreased with the increase in doses/ concentrations of the mutagens. The percent seed germination was 95.21% in control while in 100Gy, 0.2%EMS and 0.15%SA it was 78.39Gy, 81.51% and 77.26% respectively. The percent seed germination decreased from 78.39% to 52.16% in gamma radiation, 81.51% to 53.43% in EMS and 77.26% to 48.62% in SA. The maximum (50%) decrease in percent seed germination was observed with gamma radiation treatment 500Gy (52.16%), EMS

0.5% (53.43%) and in SA 0.25% (48.62%). Thus 0.25% SA treatment was very effective in reducing percent seed germination in sesame to almost 50%.

Table :1 Effect of mutagens on seed germination, seedling height, Seedling injury, pollen sterility and survival of plant in M_1 generation of sesame (*Sesamum indicum* L.).

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury %)	Pollen Sterility (%)	Survival of plant (%)
Control	95.21±0.53	5.03±0.45	4.05±0.36	9.08±0.82	00.00±0.00	1.69±0.15	79.15±7.57
300Gy	78.39±7.84	4.11±0.41	3.62±0.36	7.73±0.77	14.87±1.19	19.27±1.93	71.31±7.13
400Gy	67.23±8.07	4.07±0.49	3.03±0.36	7.10±0.85	21.81±2.40	24.14±2.90	69.40±8.33
500Gy	52.16±4.17	3.45±0.28	3.11±0.25	6.56±0.52	27.75±3.61	31.09±2.49	54.00±4.32
0.3 %EMS	81.51±8.97	4.09±0.45	4.01±0.44	8.10±0.89	10.79±1.51	17.21±1.89	74.02±8.14
0.4 %	65.32±5.88	3.04±0.27	3.01±0.27	6.05±0.54	33.37±2.34	22.67±2.13	71.11±6.40
0.5 %	53.43±6.41	2.81±0.58	2.32±0.64	5.13±1.22	43.50±3.48	23.59±2.71	70.55±8.47
0.15% SA	77.26±7.73	3.65±0.37	3.11±0.31	6.76±0.68	25.55±3.83	20.16±2.42	68.43±6.84
0.20 %	62.78±5.02	2.91±0.23	2.62±0.21	5.53±0.44	39.10±5.47	27.30±2.18	57.22±4.58
0.25 %	48.62±7.18	2.54±0.21	2.07±0.31	4.51±0.31	50.33±4.53	30.52±2.11	46.49±5.87
SEM±	7.90	0.42	0.44	0.65	2.65	4.11	8.44
F-value	12.38	9.62	9.23	18.57	68.79	12.89	6.42
P-value	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
$LSD_{0.05}$	17.21	0.92	0.96	1.42	5.77	8.96	18.39

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

The results of present study have clearly shown that the cv. sesame was sensitive to all the mutagens. Amongst the mutagens used, SA treatments were more effective in reducing percent seed germinations, followed by gamma radiation and EMS. The lethal dose (LD_{50}) values for gamma radiation (GR), EMS and SA treatments were calculated from the data on percent seed germination. From the data, 500Gy dose for GR, 0.5% concentration for EMS and 0.25% for SA were adjusted as values close to LD_{50} for the cultivar. These LD_{50} values were taken in to consideration while planning the experimental mutagenesis in sesame.

Seed germination was reduced with increasing doses / concentrations of gamma rays, EMS and SA was reported in horsegram (Bolbhat and Dhumal, 2009). Progressive increase in biological damage measured in terms of reduction in germination, seedling growth, pollen fertility and plant survival at maturity in M_1 generation with increasing doses/ concs. of mutagens was reported in groundnut (Venkatchalam and Jayabalan, 1997), *Lathyrus* (Waghmare and Mehra, 2001), lentil (Gaikwad and Kothekar, 2004), soybean (Kartika and Subba Laximi, 2006), in groundnut Badigannavar and Murty (2007). It clearly indicated that the mutagens have exerted an inhibitory effect on seed germination.

Results shown in Table-1 indicated that the shoot length was decreased in all the treatments as compared to the control (4.05cm). Maximum reduction in shoot length was recorded in 500Gy (3.11cm), EMS 0.5% (2.32cm) and SA 0.25% (2.07cm). Results presented in the same Table- 1 revealed that root length in gamma radiation single treatment was inhibited by all the treatments as compared to control (5.3cm). Amongst all the treatments the highest reduction in root length was noted at 500Gy (3.45cm). In all the EMS treatments root length was significantly decreased as compared to control. The root length was gradually decreased as the concentrations of SA increased. The highest reduction was recorded in 0.25%SA. Results recorded in Table-1 indicated that doses of gamma radiation and concentrations of EMS and SA treatments showed inhibitory effect on seedling height. Maximum decrease in seedling height (4.51cm) was noted in 0.25%SA, 0.5%EMS (5.13cm) and 500Gy (6.56cm). The overall results recorded in present investigation revealed that there was drastic decrease in seedling height due to all the combination treatments. Data on the effect of gamma radiation, EMS and SA on seedling injury at M_1 shown in Table-1 revealed that all mutagenic treatments were highly injurious to the seedlings. SA treatments had caused highest seedling injury, followed by the single treatments of EMS and gamma radiation. The seedling injury increased with the increase in doses/ concentrations of mutagenic treatments. Maximum seedling injury (50.33%) was observed in 25%SA, followed by EMS 0.5% and GR 500Gy.

The data recorded on pollen sterility induced by gamma radiation, EMS, and SA treatments recorded in Table- 1 indicated that there was liner increase in pollen sterility with increasing dose/ concentrations of mutagens. In gamma radiation, EMS and SA treatments, the range of pollen sterility % was 19.27 to 31.09%, 17.21 to 23.59% and 20.16 to 30.52%. The highest pollen sterility (31.09%) was recorded in 500Gy and lowest in 300Gy. On the other hand the highest pollen sterility (23.59%) was noted in 0.5%EMS, while lowest pollen sterility was found in

0.3%EMS (17.21%). In SA treatments the highest pollen sterility was observed in 0.25% (30.52%) and lowest (20.60%) in 0.25%SA.

The rate of pollen sterility increased with increase in concentration or dose. Induction of pollen sterility with gamma radiation was reported by Kharkwal (1998) in chickpea. Arora and Pahuja (2008) in guar reported pollen fertility also decreased with the increasing dose resulting in considerable loss in yield. Various types of chromosomal abnormalities such as translocation, anaphase bridges and laggards were found in the progenies obtained from treated seeds.

The results on the effects of gamma radiation, EMS and SA revealed that in all the mutagenic treatments, survival % was decreased than the control (Table- 1). There was linear decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS, and SA. The lowest survival % at the higher treatments was noted (54.00%) in 500Gy, 0.5%EMS (70.55%) and 0.25%SA (46.49%) as compared to control (79.15%). All mutagens reduced the rate of survival at maturity, Kavithamni *et al*., (2008) in soybean supported the above findings.

CONCLUSION

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. All mutagens (GR, EMS and SA) were effective in inducing pollen sterility in M_1 generation. The rate of pollen sterility increased with increase in dose/ concs. of the mutagens and the survival rate was highly reduced with increasing dose/concs. of mutagens.

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