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Mutagenic Influence of EMS and SA on Germination, Root–Shoot Growth and Pollen Viability in Sesame (*Sesamum Indicum* L.)

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An experiment was carried out from July to October 2021 at CRC Farm, SoAG, ITM University, Gwalior (MP) to induce genetic variability in sesame genotype TKG-55. Various concentrations of EMS and SA (0.1%, 0.2%, 0.3% and 0.4%) as well as combination treatments (EMS + SA at 0.1% + 0.1% and 0.2% + 0.2%) were evaluated in Randomized Block Design with three replications. A significant reduction in seed germination percentage, shoot & root length, pollen viability, seedling height & vigour of seedlings, and other yield-related traits was observed for all the mutagenic treatments. Dose depended linear trend in reduction was appeared for most of the traits. The mutagens alter the chemical nature of genetic material causing disturbance in physiology of cell therefore, both the mutagens showed a detrimental effect on the expression of all the traits. Furthermore, all the treatments caused a delay in flowering and maturity. Among the treatments, 0.2% SA was found to be the optimum dose for most of the quantitative traits. Higher concentrations of EMS and SA (0.4%) had harmful effects on most traits, whereas lower concentrations were more effective and beneficial for inducing mutations in sesame. The combination treatments (EMS + SA at 0.1% + 0.1% and 0.2% + 0.2%) were particularly beneficial for improving plant height and pollen viability.

Keywords: EMS; Mutation; Quantitative Traits; SA; Sesame.

Among oilseed crops, sesame (*Sesamum indicum* L.) holds significant importance as one of the oldest oil-yielding species, with a history of cultivation spanning more than 3,000 years. Belonging to the family Pedaliaceae and the genus *Sesamum*, up to 38 species have been identified through morphological and cytogenetic characterization. Sesame is known by different vernacular names across regions, including Til, Gingelly, Benniseed, Sim-Sim, Gergelim, Tilli, Nuvrula, Vellvor, and Rasi. Among the nine major oilseed crops in India, sesame occupies a prominent place and is often referred to as the "Queen of Oilseeds" due to its high-quality oil, pleasant aroma, and remarkable resistance to oxidation and rancidity.[1] Despite its importance,

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sesame has long been considered an orphan crop owing to its neglect in breeding programs and its relatively low productivity. Currently, the global area, production, and productivity of sesame are estimated at 12,507,504 hectares, 6,741,479 tonnes, and 539 kg/ha, respectively.[2]

Sesamum is primarily a diploid species (2n = 2x = 26) with a basic chromosome number of x = 8 or 13, although some species have been reported as tetraploids or octoploids.[3] Sesame seeds are nutritionally rich, containing 44–47% edible oil, 18–25% protein, 13.5% carbohydrates, 5% ash, 6–8% fiber, and a wide range of antioxidants, including sesamol, sesamin, sesamolin, and sesaminal.[4] They also serve as a dietary alternative for individuals with breast milk allergies. Most sesame seeds are utilized for oil extraction, while a smaller proportion is consumed directly as food.[5] Sesame oil, in particular, is valued for its preservative properties due to its strong resistance to oxidative rancidity, even after prolonged exposure to air.[6]

Numerous mutant lines have been developed in sesame using chemical mutagens such as ethyl methane sulfonate (EMS) and sodium azide (SA). Mutation breeding has proven to be an effective strategy for developing promising lines with improved yield, plant architecture, oil quality, and tolerance to biotic and abiotic stresses. It has been successfully applied to generate variability in both qualitative and quantitative traits in several crops, including rice and sesame.[3,7,23]

The present investigation was carried out to evaluate the effects of chemical mutagens—ethyl methane sulfonate (EMS) and sodium azide (SA) on the sesame variety TKG-55. The findings aim to support future breeding efforts focused on the genetic improvement of sesame.

MATERIALS AND METHODS

The experimental material consisted sesame variety TKG-55 procured from RSKVV, Gwalior. The dry seeds of sesame were soaked in distilled water for six hours to initiate imbibition and metabolic activities. Subsequently, they were transferred into solutions of EMS and SA with concentrations of 0.1%, 0.2%, 0.3%, and 0.4%, respectively, for four hours. For each treatment, the volume of solution was kept 50ml and 27^0C temperature for 400 seeds. Multiple washings of treated seeds were carried out using ordinary tap water to remove the residual effect of chemical mutagens. The seeds were further used in laboratory as well as in field exercise, which are mentioned below in brief:

Laboratory Exercise

To assess seed germination percentage, root & shoot length, seedling height, and injury to the seedling, 50 seeds from each treatment, including control, were placed on moist triple-layered blotting paper in petri dishes. Each treatment was maintained with two replications, providing a controlled environment in a seed germinator for germination maintaining at 27^0C temperature and 75% humidity. The observations were recorded on the 7th day of sowing.

Field Exercise

The treated seeds, along with an untreated control, were sown in the field keeping proper distance in a randomized block design (RBD) with three replications to raise M1generation. All the agronomic practices were carried out for proper growth and development. The observations were recorded from the field on plant height, branches per plant, survival at maturity, pollen viability at blooming stage, no of capsules per plant, length of capsule, seeds per capsule, 1000 seeds weight, and seed yield per plant at harvest stage in the M1 generation.

Statistical analysis

The experiment was conducted in 3 replications and the statistical analysis of the data was done by computer software namely SPSS for RBD design.[27]

Laboratory observation

The highest germination percentage was noted in the control (91.33%), which was significantly higher than all mutagenic treatments ($p < 0.05$). Among treated sets, EMS 0.1% (85.67%) and SA 0.1% (81.67%) showed comparatively higher germination, whereas higher concentrations exhibited a progressive decline. Maximum shoot length was also observed in the control (4.19 ± SE cm), which was statistically at par with SA 0.1% (4.15 ± SE cm), but significantly higher than EMS 0.1% (3.74 ± SE cm). The greatest reduction in shoot length was noticed at EMS 0.4% (1.40 ± SE cm) and SA 0.4% (1.50 ± SE cm), both of which differed significantly from the control. A similar

trend was observed for root length, where the control (3.38 ± SE cm) recorded the maximum, followed by SA 0.1% (3.31 ± SE cm) and EMS 0.1% (2.72 ± SE cm). The minimum root lengths were obtained at EMS 0.4% (0.50 ± SE cm) and SA 0.4% (0.62 ± SE cm), showing statistically significant reductions. Seedling height followed the same trend, with the control exhibiting the maximum height (7.63 ± SE cm), statistically similar to SA 0.1% (7.27 ± SE cm), while the lowest values were recorded in EMS 0.4% (1.98 ± SE cm) and SA 0.4% (2.12 ± SE cm). Seedling injury increased significantly with higher concentrations of mutagens, being maximum in EMS 0.4% (74.17%) and SA 0.4% (72.78%), while the lowest was found in SA 0.1% (4.41%) and EMS 0.1% (16.55%). Pollen viability data indicated the highest value in the control (93.37%), which was significantly higher than EMS 0.4% (77.26%) and SA 0.4% (75.30%). Intermediate values were recorded in combination treatments (89.21%), SA 0.1% (87.35%) and EMS 0.1% (86.92%).

Field observation

The highest germination percentage was reported in the control (93.50%), which was statistically at par with 0.1% EMS and 0.1% SA (92.00%), but significantly higher than higher concentrations of mutagens ($p < 0.05$). The minimum number of days to 50% flowering was observed in the control (31.33 ± SE days), followed by 0.1% EMS, 0.1% SA, and 0.2% SA, whereas the maximum delay was noted with 0.4% SA (39.33 ± SE days), showing a significant difference from the control. Similarly, the shortest time to maturity occurred in the control (85.33 ± SE days), statistically similar to 0.1% EMS and 0.1% SA (87.33 ± SE days), while the longest maturity duration was recorded in 0.4% SA (93.33 ± SE days). Survival percentage at maturity was maximum in the control (90.46%), which was statistically superior to 0.4% EMS (66.86%) and 0.4% SA (77.50%). Among treated sets, 0.1% EMS (86.38%) and 0.4% SA (82.50%) showed comparatively higher survival. Maximum plant height was obtained under the combination treatment (EMS + SA) at 118.50 cm, which was significantly higher than all other treatments, followed by the control (114.25 cm), 0.1% SA (112.61 cm), and 0.1% EMS (106.54 cm). The lowest plant height was recorded in 0.3% EMS (101.97 cm). The highest number of branches per plant was noticed in 0.1% SA (6.40), which was significantly higher than the control (5.40) and comparable to 0.2% EMS (5.80). The maximum number of capsules per plant was produced by 0.4% SA (46.60), followed by 0.1% EMS (45.20), while the control exhibited significantly fewer capsules (35.43). Capsule length was greatest in the control (2.61 ± SE cm), which was statistically similar to 0.2% SA (2.48 cm), 0.1% SA (2.46 cm), and 0.1% EMS (2.45 cm). The number of seeds per capsule was significantly highest in 0.2% SA (52.53), followed by 0.3% SA (51.97) and 0.1% EMS (50.32). The maximum 1000-seed weight was noted in 0.2% SA (3.72 g), which was statistically at par with 0.3% SA (3.62 g) and 0.2% EMS (3.61 g). Seed yield per plant was significantly highest in 0.1% SA (8.44 g), followed by 0.3% SA (8.11 g), while the control found 6.17 g. Overall, seed yield per plant showed an inverse relationship with the intensity of mutagen dose.

RESULTS

Result obtained in the present investigation in laboratory conditions on 7th days seed germination, shoot length, root length, seedling height, seedling injury, and pollen viability in M_1 generation of sesame variety TKG-55 are as under.

DISCUSSION

A progressive reduction in seed germination was observed with increasing concentrations of EMS and SA, which is consistent with earlier reports.[9-11] Similar dose-dependent effects on germination have been documented in other crops,[12] and reductions with higher doses of gamma rays, EMS, and SA were also reported in horse gram.[13] The decline in germination is generally attributed to cellular, physiological, and molecular disturbances caused by mutagens.[14]

Regarding vegetative traits, significant reductions in shoot length, root length, and seedling height, along with increased seedling injury at higher concentrations of mutagens, were consistent with previous studies.[15-17] These effects can be

Mean performance for all the seventeen characters under investigation: -

S. No.	Treatments	Germination%	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Seedlings injury %	Pollen viability %	Germination % in field	DAS to 50% flowering	DAS to maturity
1	T1 (control)	91.33	4.19	3.38	7.63	0.00	93.37	93.50	31.33	85.33
2	T2(EMS0.1)	85.67	3.74	2.72	6.45	16.55	86.92	92.00	33.33	87.33
3	T3(EMS0.2)	81.67	3.42	2.17	5.25	34.23	84.27	89.50	35.33	89.33
4	T4(EMS0.3)	73.67	2.98	2.12	5.10	35.54	80.11	86.50	37.33	91.33
5	T5(EMS0.4)	63.67	1.48	0.50	1.98	74.17	77.26	84.17	38.33	92.00
6	T6(SA0.1)	80.67	4.15	3.13	7.27	4.41	75.30	92.00	33.33	87.33
7	T7(SA0.2)	80.61	3.32	1.73	5.05	32.19	86.30	90.67	34.33	88.33
8	T8(SA0.3)	69.33	3.41	1.84	5.34	29.97	85.27	87.00	37.33	91.33
9	T9(SA0.4)	60.67	1.50	0.62	2.12	72.78	87.35	85.00	39.33	93.33
10	T10(EMS+SA, 0.1+0.1,0.2+0.2)	84.33	3.37	2.63	5.99	18.78	89.21	87.00	36.33	90.33
	Mean	77.16	3.16	2.08	5.22	31.86	84.54	88.73	35.63	89.60
	Min	60.67	1.48	0.50	1.98	0.00	75.30	84.17	31.33	85.33
	Max	91.33	4.19	3.38	7.63	74.17	93.37	93.50	39.33	93.33
	SE(m) ±	1.41	0.09	0.06	0.14	0.66	1.52	0.60	0.72	1.47
	SE(d) ±	1.99	0.12	0.08	0.19	0.93	2.15	0.85	1.01	2.08
	C.D. at 5%	4.21	0.26	0.17	0.41	1.97	4.54	1.79	2.15	4.39
	C.V. (%)	3.15	4.83	4.68	4.55	3.58	3.11	1.17	3.48	2.84

S. No.	Treatments	Survival at maturity	Plant height (cm)	Branches /plant	No. of capsule/ plants	Length of capsule/ plant	No. of seed/ capsule	1000 seed weight (g)	Seed yield / Plant (g)
1	T1 (control)	90.46	114.25	5.40	35.43	2.61	51.20	3.40	6.17
2	T2(EMS0.1)	86.38	106.32	5.00	40.62	2.45	50.32	3.50	6.99
3	T3(EMS0.2)	80.44	106.54	5.80	45.20	2.40	47.17	3.61	7.42
4	T4(EMS0.3)	73.55	101.97	4.33	38.63	2.32	44.97	3.50	6.08
5	T5(EMS0.4)	66.86	103.42	6.00	33.20	1.89	45.68	3.54	5.39
6	T6(SA0.1)	82.50	112.61	6.40	46.60	2.46	50.67	3.58	8.44
7	T7(SA0.2)	80.50	104.12	5.27	35.27	2.48	52.53	3.61	6.68
8	T8(SA0.3)	82.00	110.39	4.80	42.00	2.36	51.97	3.72	8.11
9	T9(SA0.4)	77.50	103.55	3.20	34.80	2.20	48.63	3.62	6.12
10	T10(EMS+SA, 0.1+0.1,0.2+0.2)	82.50	118.50	4.80	42.92	2.47	51.17	3.52	7.73
	Mean	80.27	108.17	5.10	39.47	2.36	49.43	3.56	6.91
	Min	66.86	101.97	3.20	33.20	1.89	44.97	3.40	5.39
	Max	90.46	118.50	6.40	46.60	2.61	52.53	3.72	8.44
	SE(m) ±	2.01	2.60	0.13	1.05	0.06	1.28	0.05	0.17
	SE(d) ±	2.84	3.68	0.18	1.48	0.09	1.81	0.07	0.24
	C.D. at 5%	6.01	7.80	0.38	3.14	0.18	3.84	0.15	0.51
	C.V. (%)	4.33	4.17	4.35	4.61	4.45	4.49	2.45	4.26

linked to mutagen-induced damage in actively dividing cells, which interferes with normal growth and development.

For reproductive traits, a linear decline in pollen viability was evident with increasing mutagen doses, in agreement with findings in groundnut,[18-20] lentil, and soybean.[21-23] The reduction in viability is likely due to physiological and chromosomal damage, resulting in the production of non-viable pollen grains and subsequent pollen sterility, as also noted by earlier researchers.[24]

With respect to yield-related traits, mutagenic treatments generally reduced plant height, capsule length, 1000-seed weight, and seed yield per plant, showing a clear dose-dependent negative trend. However, the number of branches per plant and the number of capsules per plant did not exhibit a consistent linear pattern. These irregular responses may be explained by compensatory growth mechanisms, where reduced growth in one organ is offset by increased growth in another, as well as underlying genetic variability and genotype × environment interactions that modulate the plant's response to mutagenic stress. Similar complex trends have been reported in earlier studies.[25-26]

Therefore, both mutagens altered the chemical composition of the genetic material, which disrupted normal cellular physiology and ultimately had adverse effects on the expression of all evaluated traits.

CONCLUSION

The optimum dose of EMS was found to be 0.1% for traits such as germination percentage and survival at maturity. For SA, 0.1% was most effective in improving root length, shoot length, seedling height, reducing seedling injury, and enhancing the number of branches per plant, number of capsules per plant and seed yield per plant. A concentration of 0.2% SA was optimal for improving capsule length and the number of seeds per capsule, while 0.3% SA showed the best results for 1000-seed weight. The combination treatments of EMS + SA (0.1% + 0.1% and 0.2% + 0.2%) were found to be most effective for improving plant height and pollen viability. Additionally, early flowering and early maturity were associated with the 0.1% doses of both EMS and SA. Based on the findings of the present study, it can be concluded that a 0.1% concentration of both EMS and SA may be considered optimal and beneficial for further crop improvement in sesame.

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Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

_____This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

Pratima Jaiswal- Data collection; Priyanka Gupta-Analysis the data and aided as major advisor; Lakshman Singh- Wrote the research article (Introduction, Material & Methods, Tables, Results and discussion and aided as co-advisor; Harendra- Made all possible corrections before and after sending the manuscripts.

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