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Effect of mutagens on germination, seedling height and survival in M_1 generation of soybean (*Glycine max* L. Merill.)

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Abstract
Gamma rays and EMS treated seeds of three soybean genotypes (TAMS 38, AMS 1001 and RSC 10-46) was evaluated for germination, seedling height and survival percentage during M_1 generation. Mutagenic treatment with four doses of gamma rays (150 Gy, 200 Gy, 250 Gy and 300 Gy) and three concentration of ethyl methane sulphonate (0.1%, 0.15% and 0.2%) showed reduced germination as compared to corresponding control. Decrease in seedling height and survival percentage with increase in dose / concentration of mutagen was also observed in all three soybean genotypes. Maximum reduction in three parameters was noticed at 300 Gy gamma rays treatment except seedling height in RSC 10-46 whereas as regards to soybean genotypes, AMS 1001 found more sensitive to mutagens as compared to TAMS 38 and RSC 10-46.

Keywords: Soybean, mutagen, gamma rays, EMS, germination, seedling height, survival

Introduction
In India, at present soybean plays pivotal role in edible oil economy and has become most economically important crop having several uses in feed, food, and industrial applications. Hence in last decade soybean has gained tremendous popularity among the farmers as well as industrialist. As a result the demand of soybean is increasing but the productivity is still low. The different factors identified that are responsible for low soybean productivity in India are inadequate genetic diversity, the narrow genetic base and stagnated yield potential of Indian soybean varieties and the short growing period available in Indian latitude (Tiwari, 2003) [19]. Many new high yielding varieties were developed with the use of similar parental source which results in yield plateau. Soybean (*Glycine max* L. Merill.) being a self-pollinated crop, has narrow genetic base and naturally existing variability in this crop is very less. Induced mutagenesis is important tool for plant breeders to create variability in such crops. Physical (gamma rays) and chemical (ethyl methane sulphonate (EMS)) mutagen has found great potential to induce the genetic variability in crop plants (Dhulgande *et al.* 2011) [2]. Irrespective of the crop, the mutation breeding program starts with study on effect of mutagens on germination, growth reduction and survival percentage to obtain optimum population size in M_2 generation. Therefore, the present investigation was planned to generate the information on sensitivity of different soybean genotypes to physical and chemical mutagen.

Materials and Method
The experimental material was comprised for three soybean genotypes namely TAMS 38, AMS 1001 and RSC 10-46. The seeds of these genotypes were obtained from Regional Research Centre (Dr. PDKV), Amravati (MS) and AICRP on Soybean, Department of Genetics and Plant Breeding, IGKV, Raipur (CG). The gamma rays treatment to pure and healthy dried seed of three soybean genotypes was obtained from Bhabha Atomic Research Center, Trombay in Mumbai. The seeds were exposed to four doses of gamma rays (CO^{60}) *viz*. 150 Gy, 200 Gy, 250Gy and 300 Gy with a dose rate of 2.39kR per minute. Similarly, the pre-soaked seeds (in doubled distilled water for 6 hrs.) of three genotypes were also treated with three concentrations of EMS *viz*. 0.1%, 0.15% and 0.2% using phosphate buffer solution. Mechanical shaker is used for uniform application of EMS treatment. The EMS treatment was given for 8 hrs. at room temperature. The EMS treated seeds were thoroughly washed under tap water to remove the residuals of mutagenic chemical, then surface dried and immediately

used for sowing.

The gamma rays and EMS treated seeds of three genotypes along with respective control was sown in the field at Regional Research Centre, (Dr. Panjabrao Deshmukh Krishi Vidyapeeth), Amravti during *kharif*, 2021. The experiment was laid in randomized complete block design with three replications. 150 seeds per treatment per replication were dibbled with 45 x 10 cm spacing. The plot size of each treatment in each replication was 2.25 x 3 m^2 comprised of 5 rows of 3 m length. Recommended package of practices were followed during the crop growth period. In M_1 generation data on germination of each treatment along with control was recorded 10 days after sowing. Seedling height of 10 randomly selected plants from each treatment was measured in centimeter from soil surface to tip of the main stem at 30 DAS and the survival percentage was determined by counting the numbers seeds germinated and number of plants at maturity.

Results and Discussion

Germination percentage (%)

In M_1 generation the observed germination percentage in all gamma radiation and ethyl methane sulphonate (EMS) treatments was lower than that of the corresponding control (Table 1). Among all gamma rays treatment, 300 Gy recorded lowest germination percentage in all soybean genotypes under study *viz*. AMS 1001 (41.11%), followed by RSC 10-46 (44.22%) and TAMS 38 (48.67%). whereas, in case of ethyl methane sulphonate (EMS) treatments, 0.2 percent mutagenic treatment exhibited lowest germination percentage in RSC 10-46 (47.78%) followed by AMS 1001 (48.22%) and TAMS 38 (51.78%).

Relatively higher reduction in germination percentage was observed in 300 Gy gamma rays irradiated AMS 1001 (53.17%) followed by RSC 10-46 (51.82%) and TAMS 38 (45.38%). Among all ethyl methane sulphonate (EMS) treatment, 0.2 percent had reported maximum reduction in germination percentage in all three soybean genotypes *viz*. AMS 1001 (53.84%), followed by RSC 10-46 (49.96%) and TAMS 38 (43.58%).

In all three soybean genotypes under study, it was observed that higher dose or concentration of mutagen leads to gradual increase in reduction of germination percentage. The results obtained in the present study indicated that, as dose or concentration of mutagen increased there was decrease in germination percentage. Similar trend in germination percentage was also reported by Khan and Tyagi, (2009) [9], Satpute and Fultambkar, (2012) [14] and Hussain, *et al*. (2020) [6]. According to Singh *et al.* (1997) [15] the reduction in germination percentage might be due to acute chromosomal damage and physiological disturbances. Earlier, Strickberger, (1976) [17] reported that the reason for reduction in germination may be because of the disturbed base pair relationship due to radiation. Kurobane, *et al*. (1979) [10] found that the decrease in the percentage seed germination was linked with the disruption of physiological and biological processes, such as enzyme activity, affected by the mutagens.

Seedling height (cm)

The data presented in Table 2 revealed that all the genotypes showed decreasing trend in seedling height with increasing gamma radiation doses and EMS concentration.

Minimum seedling height reduction was observed in lower dose/ concentration of mutagenic treatments. In gamma radiation treatments under study, maximum reduction in seedling height was exhibited at 300 Gy gamma radiation in AMS 1001 (30.50%) followed by TAMS 38 (23.64%) and RSC 10-46 (20.50%). Similarly, amongst EMS treatments, 0.2 percent showed maximum seedling height reduction in three genotypes viz. RSC 10-46 (36.99%) followed by AMS 1001 (32.09%) and TAMS 38 (20.78%). Differential response of soybean genotypes was noticed in various mutagenic treatments. In terms of seedling height, AMS 1001 found more sensitive to gamma rays while RSC 10-46 was found more sensitive to EMS treatments.

In soybean, Kalpande, *et al.* (2020) [7], Geetha and Vaidyanathan, (2000) [5] and Wakode, *et al.* (2000) [20] reported results on seedling height that are close agreement with the findings of present study. The gamma rays inhibited the growth of the seedling by interfering with the cell cycle when it was in the somatic cells and by destroying the complete genome (Khadimi *et al*., 2016) [8]. Several research workers in other crops reported that probable reason for seedling growth reduction might be due to changes in hormonal levels, certain enzymatic activity or mitotic irregularities (Suganthi, *et al*. 1994) [18] or even restriction of DNA synthesis.

Survival percentage (%)

The mutagenic treatments adversely affect the survival percentage in TAMS 38 AMS 1001 and RSC 10-46 genotypes during M_1 generation (Table 3). The survival percentage was maximum in lower dose/concentration of both the mutagens and decreasing trend was observed as the dose/concentration of mutagens was increased. Survival percentage was effectively influenced by both the mutagens.

In case of gamma rays treatment, 150 Gy dose exhibited maximum survival percentage in TAMS 38 (72.85%) followed by AMS 1001 (71.60%) and RSC 10-46 (70.97%) while in ethyl methane sulphonate (EMS) treatments, maximum survival percentage was observed in 0.1 percent concentration of TAMS 38 (70.51%) followed by RSC 10-46 (70.68%) and AMS 1001 (68.84%).

The percent reduction in survival percentage was determined and it was observed that in higher doses/concentration of mutagen recorded maximum percent reduction as compared to lower dose / concentration of mutagen. In case of survival percentage AMS 1001 found more sensitive to mutagenic treatments than other two genotypes. Maximum percent reduction in survival percentage was recorded by 300 Gy gamma treatment in AMS 1001 (43.90%) followed by RSC 10-46 (42.85%) and TAMS 38 (40.13%) whereas, it was maximum in 0.2 percent ethyl methane sulphonate treatments (EMS) treatment The percent reduction in survival percentage in AMS 1001 was (41.00%) followed by RSC 10-46 (36.15%) and TAMS 38 (35.72%). Narayana and Konzak, (1969) [12] reported that the main reason for the decline in the survival percentage of M_1 plants may the imbalance in physiological processes as well as chromosomal aberration which has lethal effect in different growth stages.

Although there was a dose-dependent relationship between survival percentage and percent reduction, the genotypes showed variation in their responses. Such result also reported by Balakrishnan, (1991) [1], Mehetre, *et al.* (1994) [11], Pavadai and Dhanavel, (2004) [13], Singh and Kole, (2005) [16] and Geetha and Wakode, (2011) [4]. According to Gaul, *et al.*

(1972) [3], a decrease in the percentage of plants survival as a result of mutagenic treatments in the M_1 generation may have been caused by physiological harm in addition to chromosomal and point mutations.

Table 1: Effect of mutagen on germination percentage (%) in M_1 generation of soybean under field condition

Treatment	TAMS 38		AMS 1001		RSC 10-46	
	Germination (%)	Percent reduction	Germination (%)	Percent reduction	Germination (%)	Percent reduction
Gamma rays						
Control	89.11	--	87.78	--	91.78	--
150 Gy	68.44	23.20	65.78	25.06	65.56	28.57
200 Gy	57.56	35.41	54.22	38.23	59.56	35.11
250 Gy	54.67	38.65	44.89	48.86	52.22	43.10
300 Gy	48.67	45.38	41.11	53.17	44.22	51.82
Ethyl methane sulphonate (EMS)						
Control	91.78	--	89.56	--	94.54	--
0.1%	66.44	27.61	63.56	29.03	62.44	33.95
0.15%	60.22	34.39	57.33	35.99	55.33	41.47
0.2%	51.78	43.58	48.22	46.16	47.78	49.46

Table 2: Effect of mutagen on seedling height (cm) in M_1 generation of soybean under field condition

Treatments	TAMS 38		AMS 1001		RSC 10-46	
	Seedling height (cm)	Percent reduction	Seedling height (cm)	Percent reduction	Seedling height (cm)	Percent reduction
Gamma rays						
Control	18.21	--	17.40	--	17.74	--
150 Gy	17.26	5.22	15.71	9.73	16.94	4.51
200 Gy	16.22	10.91	15.44	11.28	16.50	7.01
250 Gy	14.96	17.83	14.37	17.41	15.59	12.10
300 Gy	13.91	23.64	12.09	30.50	14.10	20.50
Ethyl methane sulphonate (EMS)						
Control	17.75	--	17.05	--	17.58	--
0.1%	16.63	6.31	15.01	11.97	15.76	10.33
0.15%	14.89	16.13	12.99	23.82	13.75	21.78
0.2%	14.06	20.78	11.58	32.09	11.08	36.99

Table 3: Effect of mutagen on survival percentage (%) in M_1 generation of soybean under field condition

Treatment	TAMS 38		AMS 1001		RSC 10-46	
	Survival (%)	Percent reduction	Survival (%)	Percent reduction	Survival (%)	Percent reduction
Gamma rays						
Control	93.7	--	91.59	--	94.86	--
150 Gy	72.85	22.25	71.6	23.59	70.97	24.26
200 Gy	67.26	28.22	65.87	28.08	64.67	31.83
250 Gy	63.85	31.86	58.04	36.63	61.65	35.01
300 Gy	56.1	40.13	51.38	43.90	54.21	42.85
Ethyl methane sulphonate (EMS)						
Control	95.44	--	90.44	--	93.94	--
0.1%	70.51	26.12	68.64	24.10	70.68	24.76
0.15%	65.65	31.21	62.37	31.04	65.95	29.80
0.2%	61.35	35.72	53.36	41.00	59.98	36.15

Conclusion

The present investigation was planned with the aim to generate the information on sensitivity of three soybean genotypes for gamma rays and EMS in terms of germination, seedling height and survival during M_1 generation. The finding of the present study showed significant effect of both the mutagens on all three parameters under field condition. The germination percentage, seedling height and survival percentage was reduced with increase in dose/concentration of both the mutagens. The ideal dose of mutagens can be estimated from the data generated from the present study to optimize the M_2 population.

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