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Assessments of Effectiveness and Efficiency of Gamma Rays, EMS and their Combined Treatment in Soybean (*Glycine max* L.)

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Abstract The effects of gamma rays, EMS and their combinations were studied in the field conditions for spectrum and frequency of chlorophyll as well as viable mutations in M_3 generation under 11 treatments along with two controls (Dry seed PK 1029 and Presoaked seed PK 1029). In M_3 generation the highest frequency of chlorophyll mutation (1.29) was observed in combined treatments followed by EMS (1.25) whereas the highest frequency of viable mutation (2.92) was recorded in gamma rays followed by combined treatment (1.79). Among the chlorophyll mutation, frequencies of xantha mutants (0.59) were higher in EMS treated populations followed by xantha (0.41) in conbination and albino (0.39) in gamma treated populations in M_3 generation. Among the viable mutation, the frequencies of early maturing mutants (0.64) were higher in gamma treated populations followed by early maturing mutants (0.48) in combination and broad leaf mutants (0.26) in EMS treated populations. These can be further utilized in recombination breeding with other mutants of cultivars to derive distinct lines with improved agronomic traits.

Keywords Soybean, Ethyl methane sulfonate, Gamma rays, Effectiveness, Efficiency.

Introduction

Soybean is one of the most important oil seed crop with multi utility and widely grown in Indian subcontinent in *kharif* season. Induced mutagenesis plays an important role in the improvement of soybean because hybridization process is very complicated due to its small and cleistogamous nature of flower. The usefulness of any mutagenic agent in plant breeding not only depends on the ability to induce mutation but also on its desirable changes as compared to the undesirable ones. Alternatively, the induced mutation is the best way to enhance genetic variability within a short period of time with many useful genetic changes including high yield, flower color, disease resistance and early maturity. Many physical and chemical mutagens have been used for induction of useful mutations in a number of crops. The practical utility of induced mutation for improvement of quantitatively inherited characters in soybean or in other crop was reported earlier [1—4]. Selection of effective and efficient mutagens is very essential to recover high frequency of desirable mutations in mutation breeding program. Chlorophyll mutations are one among the few dependable param-

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Table 1. Mutation frequency of various doses of GR, EMS and their combinations in M_3 generation of soybean.

Mutagen	No. of M_3 plants studied	Chlorophyll mutants		Viable mutants		Total	
		No.	%	No.	%	No.	%
20 kR	6525	42	0.64	190	2.91	232	3.55
30 kR	2105	25	1.19	65	3.09	90	4.27
40 kR	504	8	1.59	12	2.38	20	3.96
Total	9123	75	0.82	267	2.92	342	3.74
0.05% EMS	16532	195	1.18	162	0.98	357	2.16
0.10% EMS	12204	188	1.54	148	1.21	336	2.75
0.15% EMS	15244	165	1.08	168	1.10	333	2.18
Total	43980	548	1.25	478	1.09	1026	2.33
20kR + 0.05% EMS	2014	25	1.24	35	1.74	60	2.98
20kR + 0.10% EMS	1533	22	1.43	26	1.69	48	3.13
20kR + 0.15% EMS	1032	12	1.16	21	2.03	33	3.19
Total	4579	59	1.29	82	1.79	141	3.08
Dry control	1750	0		0		0	0
Presoaked control	1836	0		0		0	0
Total	3586	0		0		0	0
Grand total	61279	682	1.11	827	1.35	1509	2.46

eters for evaluation of genetic effects of various mutagens and are widely used as genetic markers in basic and applied research. The main objective of the present investigation was to assess the mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments of mutagens in soybean.

Materials and Methods

The present investigation was carried out at the N. E. Borlaug Crop Research Center of Govind Ballabh Pant University of Agriculture and Technology, Uttrakhand. One thousand dry seeds of the cultivar PK 1029 of soybean were used for each treatment. Seeds were treated with three different doses (20 kR, 30 kR and 40 kR) of gamma rays and the EMS (Ethyl Methane Sulfonate) of three different doses (0.05%, 0.10% and 0.15% of EMS) was used for chemical treatment and seeds treated with 20 kR, 30 kR and 40 kR gamma-rays were immersed, respectively in 0.05%, 0.10% and 0.15% EMS in different combination treatments. Untreated seeds were used as control. The seeds obtained from lower doses particularly 9 treatments of M_1 generation were harvested, separately. Individual plant progenies of 11 treatments along with both the control were evaluated in M_2 generation. This experimental material was comprised of 11 treatments along with two controls (Dry seed PK 1029 and Pre-soaked seed PK 1029) in M_3 generation. Seeds from each fertile normal looking 500 M_2 plants were harvested individually in each treatment and M_3 progeny raised in separate rows of 5 m length with spacing of 45 cm between row and 10 cm between plants in randomized complete block design with three replications during 2011–2012. The treated as well as control populations were carefully screened for viable chlorophyll mutations (albino, xantha, chlorina and viridis types) and other desirable viable mutations (Leaf mutants, Growth habit mutants, Seed type mutants, Maturing type mutants, Purple flower mutants, High pubescence mutants and High yielding mutants) were scored throughout the life period of the plants in the seed beds in the field. The mutation frequency in per cent for mutant was calculated using the formula suggested by Gaul [5]. The efficiency and effectiveness of mutagen in inducing mutations were estimated by adopting the formula suggested by Konzak et al. [6].

Results and Discussion

The mutation rate of various doses of gamma rays, ethyl methane sulfonate and their combinations for chlorophyll and viable mutations in M_3 generation of

Table 2. Spectrum and frequency of induced chlorophyll mutations in M_3 generation of soybean.

Mutagen and dose	Albina No.	Albina %	Xantha No.	Xantha %	Chlorina No.	Chlorina %	Viridis No.	Viridis %	Total No.	Total %	No. of M_3 plants	Mutant/ 1000 M_3 plants
20 kR	22	0.34	13	0.20	0	0.00	7	0.11	42	0.64	6525	6.43
30 kR	10	0.47	7	0.33	8	0.38	0	0.00	25	1.19	2105	11.88
40 kR	4	0.79	0	0.00	2	0.39	2	0.39	8	1.59	504	15.87
Total	36	0.39	20	0.22	10	0.10	9	0.09	75	0.82	9123	8.21
0.05% EMS	75	0.45	88	0.53	0	0.00	32	0.19	195	1.18	16532	11.79
0.10% EMS	69	0.56	76	0.62	43	0.35	0	0.00	188	1.54	12204	15.40
0.15% EMS	0	0.00	98	0.64	45	0.29	22	0.14	165	1.08	15244	10.82
Total	144	0.32	262	0.59	88	0.20	54	0.12	548	1.25	43980	12.46
20 kR + 0.05% EMS	8	0.39	10	0.49	7	0.34	0	0.00	25	1.24	2014	12.41
20 kR + 0.10% EMS	5	0.32	0	0.00	6	0.39	11	0.72	22	1.43	1533	14.35
20 kR + 0.15% EMS	0	0.00	9	0.87	0	0.00	3	0.29	12	1.16	1032	11.63
Total	13	0.28	19	0.41	13	0.28	14	0.30	59	1.29	4579	12.88
Dry control	0		0		0		0		0		1750	
Presoaked control	0		0		0		0		0		1836	
Total	0		0		0		0		0		3586	
Grand total	193	0.31	301	0.49	111	0.18	77	0.12	682	1.11	61279	11.13

soybean are presented in (Table 1). In M_3 generation, the less number of chlorophyll mutants (682) were observed than the viable mutants (827). The overall highest mutagenic frequency was observed in gamma rays (3.74) treated population followed by combination (3.08) and EMS (2.33) treated populations. The high frequency of gamma rays than EMS was also reported by Usharani and Kumar [4] in urdbean. More specifically highest mutagenic frequency was observed by 30 kR dose of gamma rays (4.27) followed by 40 kR (3.96) and 20 kR (3.55). The highest frequency of chlorophyll mutations (1.29) induced by combinations treatments followed by EMS (1.25) and gamma rays (0.82) whereas highest frequency of viable mutations (2.92) induced by gamma rays than their combinations (1.79) and EMS (1.09). Higher frequency of viable mutations by gamma rays was also reported earlier [7, 8]. While specifically higher frequency of chlorophyll mutation (1.59) was observed by 40 kR dose of gamma rays followed by 0.10% EMS (1.54) and 20 kR + 0.10% EMS whereas specifically higher frequency of viable mutation (3.09) was recorded by 30 kR dose of gamma rays than 20 kR (2.91) and 40 kR (2.38) (Table 1). Almost all the mutagenic treatments showed different degree of mutants with respective dose of mutagen.

Chlorophyll mutations in soybean consisted of albina, chlorina, xantha and viridis (Table 2). In the present investigation, the overall mutation spectrum showed that xantha (0.49) occurred with the highest frequency followed by albina (0.31), chlorina (0.18) and viridis (0.12). These result showed that xantha type of chlorophyll mutation occurred more frequently than albina. The higher frequency of xantha than albina mutant was also reported earlier [2, 4, 9]. Among all the mutation, viridis mutant occurred with lowest frequency whereas, highest frequency of viridis mutation among all type of mutation was also given earlier [3, 9]. The spectrum and frequency of different types of chlorophyll mutations were induced by gamma rays, EMS and their combinations in M_3

Table 3. Efficiency and effectiveness of chlorophyll mutations in M_3 generation of soybean. kR=Dose of gamama rays in kilo Roentgen, t=Duration of treatment in hrs, c=Concentration of chemical in percent.

Mutagen	Lethality %	Mutant 100 M_3 plants	Mutagenic efficiency	Mutagenic effectiveness
Gamma rays				
20 kR	7.80	0.64	0.08	0.032
30 kR	14.52	1.19	0.08	0.039
40 kR	28.70	1.59	0.05	0.039
EMS				
0.05% EMS	24.36	1.18	0.05	0.009
0.10% EMS	20.40	1.54	0.07	0.026
0.15% EMS	22.36	1.08	0.05	0.027
Combination				
20 kR + 0.05% EMS	18.41	1.24	0.07	0.21
20 kR + 0.10% EMS	25.62	1.43	0.05	0.24
20 kR + 0.15% EMS	35.68	1.16	0.03	0.19
Control				
Dry control	5.56			
Presoaked control	5.60			

generation of soybean, in which xantha type of chlorophyll mutation occurred more frequently (0.59) in EMS treated population and albina type of chlorophyll mutation occurred more frequently (0.39) in gamma rays treated population (Table 3). Similar type of result on mutation frequency was also reported by Ahirwar et al. [3]. The chlorophyll mutations might occur in mutagen treated population due to failure of synthesis of enzyme responsible for chlorophyll synthesis. The occurrence of chlorophyll mutation after treatment with physical and chemical mutagen has been reported in crops earlier [1—3]. The differential response of treatments to induced chlorophyll mutants is possible due to differences in the genetic make up of variety used for mutagenesis.

The mutagenic efficiency was found to be highest in sole treatment of 20 kR (0.08) and 30 kR (0.08) of gamma rays followed by 0.10% EMS (0.07) and combined treatment of 20 kR +0.05% EMS (0.07). So that it's noted that gamma rays are more effective than EMS in inducing chlorophyll mutation. More specifically, it was found to be maximum (0.24) in combined treatment of 20 kR+0.10% EMS (Table 2). Selection of effective and efficient mutagens is very essential to recover the high frequency of desirable mutation in a mutation breeding studies.

The spectrum and frequency of viable mutations identified in M_3 generation of soybean are described in (Table 4). The data revealed that highest frequency of viable mutations (2.92) was recorded in gamma rays treated population followed by their combinations (1.79) and EMS (1.09). The spectrum of viable mutation observed in the present investigation consisted of different types of mutants namely, leaf mutants, growth habit mutants, seed type mutants, maturing type mutants, purple flower mutants, high pubescence mutants and high yielding mutants. Various type of viable mutation as like growth habit, leaf type and high yielding type was also reported by Nair and Mehta [9]. In the present investigation, the overall mutation spectrum showed that leaf mutants (0.39) and maturing type mutants (0.39) occurred with the highest frequency followed by growth habit mutants (0.32), seed type mutants (0.12) and high pubescence mutants (0.07). Among the viable mutation, the frequencies of early maturing mutants (0.64) were higher in gamma treated populations followed by early maturing mutants (0.48) in combination and broad leaf mutants (0.26) in EMS treated populations. In gamma rays treated population, maturity type mutants occurred more frequently (0.91) and more specifically in 30 kR doses of gamma rays with the frequency of (1.14). In EMS treated population, leaf type mutants occurred more frequently (0.35) in all the categories of viable mutants with more frequently (0.43) in 0.10% dose of EMS treatment, while in combined treatment population, maturity type occurred more frequently (1.05) and more specifically in 20 kR + 0.15% EMS dose of combine treatment with the frequently (1.16). The spectrum and frequency of different types of viable mutations were induced by gamma rays, EMS and their combinations in M_3 generation of soybean, in which leaf type mutants occurred more frequently

Table 4. Spectrum and frequency of induced viable mutations in M_3 generation of soybean.

Mutagen	Leaf mutants						Growth habit mutants					
	Broad leaf		Narrow leaf		Total		Tall erect		Dwarf bushy		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20 kR	31	0.47	15	0.23	46	0.70	13	0.19	28	0.43	41	0.63
30 kR	8	0.38	0	0.00	8	0.38	0	0.00	15	0.71	15	0.71
40 kR	3	0.59	0	0.00	3	0.29	2	0.39	2	0.39	4	0.79
Total	42	0.46	15	0.16	57	0.62	15	0.16	45	0.49	60	0.66
0.05% EMS	39	0.23	15	0.09	54	0.33	6	0.04	38	0.23	44	0.27
0.10% EMS	35	0.29	18	0.15	53	0.43	0	0.00	35	0.29	35	0.29
0.15% EMS	41	0.27	8	0.05	49	0.32	12	0.08	28	0.18	40	0.26
Total	115	0.26	41	0.09	156	0.35	18	0.04	101	0.23	119	0.27
20 kR + 0.05% EMS	0	0.00	17	0.84	17	0.84	0	0.00	10	0.49	10	0.49
20 kR + 0.10% EMS	4	0.26	0	0.00	4	0.26	0	0.00	8	0.52	8	0.52
20 kR + 0.15% EMS	6	0.58	3	0.29	9	0.87	0	0.00	0	0.00	0	0.00
Total	10	0.22	20	0.44	30	0.65	0	0.00	18	0.39	18	0.39
Dry control	0		0		0		0		0		0	
Presoaked control	0		0		0		0		0		0	
Total	0		0		0		0		0		0	
Grand total	167	0.27	76	0.12	243	0.39	33	0.05	164	0.27	197	0.32

Table 4. Continued.

Mutagen	Seed type mutants					
	Bold seeded		Small seeded		Total	
	No.	%	No.	%	No.	%
20 kR	15	0.22	9	0.13	24	0.37
30 kR	7	0.33	6	0.28	13	0.62
40 kR	0	0.00	0	0.00	0	0.00
Total	22	0.24	15	0.16	37	0.40
0.05% EMS	0	0.00	0	0.00	0	0.00
0.10% EMS	15	0.12	0	0.00	15	0.12
0.15% EMS	22	0.14	0	0.00	22	0.14
Total	37	0.08	0	0.00	37	0.08
20 kR + 0.05% EMS	0	0.00	0	0.00	0	0.00
20 kR + 0.10% EMS	2	0.13	0	0.00	2	0.13
20 kR + 0.15% EMS	0	0.00	0	0.00	0	0.00
Total	2	0.04	0	0.00	2	0.04
Dry control	0		0		0	
Presoaked control	0		0		0	
Total	0		0		0	
Grand total	61	0.09	15	0.02	76	0.12

Table 4. Continued.

Mutagen	Maturing type mutants: Early maturing No.	%	Late maturing No.	%	Total No.	%
20 kR	38	0.58	16	0.24	54	0.83
30 kR	18	0.85	6	0.28	24	1.14
40 kR	3	0.59	2	0.39	5	0.99
Total	59	0.64	24	0.26	83	0.91
0.05% EMS	48	0.29	11	0.07	59	0.36
0.10% EMS	25	0.20	4	0.03	29	0.23
0.15% EMS	32	0.21	8	0.05	40	0.26
Total	105	0.24	23	0.05	128	0.29
20 kR + 0.05% EMS	8	0.39	0	0.00	8	0.39
20 kR + 0.10% EMS	6	0.39	0	0.00	6	0.39
20 kR + 0.15% EMS	8	0.77	4	0.39	12	1.16
Total	22	0.48	4	0.09	26	1.05
Dry control	0		0		0	
Presoaked control	0		0		0	
Total	0		0		0	
Grand total	186	0.30	51	0.08	237	0.39

Table 4. Continued.

Mutagen	Purple flower mutants No.	%	High pubescence mutants No.	%	High yielding mutants No.	%	Total viable mutants No.	%	No. of M_3 plants	Mu-tants/ 1000 M_3 plants
20 kR	11	0.17	8	0.12	6	0.09	190	2.91	6525	29.12
30 kR	0	0.00	3	0.14	2	0.09	65	3.09	2105	30.88
40 kR	0	0.00	0	0.00	0	0.00	12	2.38	504	23.81
Total	11	0.12	11	0.12	8	0.09	267	2.92	9134	29.23
0.05% EMS	0	0.00	5	0.03	0	0.00	162	0.98	16532	9.79
0.10% EMS	6	0.05	8	0.06	2	0.02	148	1.21	12204	12.13
0.15% EMS	0	0.00	15	0.09	2	0.01	168	1.10	15244	11.02
Total	6	0.01	28	0.06	4	0.09	478	1.09	43980	10.87
20 kR + 0.05% EMS	0	0.00	0	0.0	0	0.0	35	1.74	2014	17.38
20 kR + 0.10% EMS	0	0.00	4	0.26	2	0.13	26	1.69	1533	16.96
20 kR + 0.15% EMS	0	0.00	0	0.00	0	0.00	21	2.03	1032	20.34
Total	0	0.00	4	0.09	2	0.04	82	1.79	4579	17.90
Dry control	0		0		0		0		1750	
Presoaked control	0		0		0		0		1836	
Total	0		0		0		0		3586	
Grand total	17	0.03	43	0.07	14	0.02	827	1.35	61279	13.49

(0.87) in 20 kR + 0.15% EMS treated population, growth habit mutants occurred more frequently (0.79) in 40 kR gamma rays treated population, seed type mutants occurred more frequently (0.62) in 30 kR gamma rays treated population (Table 4). Similar results on leaf mutants were also reported by Nairand Mehta [9] in cowpea. Among the viable mutations in gamma irradiated populations, early and late maturing type mutants occurred more predominantly followed by growth habit mutants. These types of mutations were reported earlier [7, 10, 11]. EMS and gamma rays are potent mutagens for their action in inducing point mutation and chromosomal change.

Conclusion

Based on the above results it may be concluded that, highest frequency of chlorophyll mutations were recorded in EMS treated population whereas highest frequency of viable mutations were recorded in gamma rays treated population in M_3 generation. Among the chlorophyll mutation, in gamma irradiated population, the frequencies of albino type mutants were higher, whereas in EMS treated population, the frequencies of xantha type mutants were higher in M_3 generation. Among the viable mutation, in gamma irradiated populations, maturing mutants occurred more predominantly whereas in EMS treated population, leaf type mutant occurred more frequently. The usefulness of any mutagenic agent depends on its ability to induced high frequency of desirable mutation as compared to undesirable ones. Therefore, it is necessary to assess the efficiency and effectiveness of mutagens for efficient and effective use in future for improvement in crop plant.

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