Induced variation in quantitative traits due to physical (gamma rays), chemical (EMS) and combined mutagen treatments in soybean [*Glycine max* L.)Merrill]

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Abstract

Seeds of these two soybean genotypes viz., Pusa-16 and PK-1042 were exposed to various doses of gamma rays, ethyl methane sulphonate (EMS) and combination of both. Observations on germination %, lethality % (30 days after sowing and at flowering) and pollen sterility were recorded on M_1 generation. The results indicated that a reduction in the germination percent over control was noticed in all the mutagenic treatments in both the cultivars. In general, reduction in germination percent was associated with the increase in the dose/concentration of the mutagen. The LD-50 for Pusa-16 was found to lie between 30KR + 0.2% EMS and 45 kR + 0.2% EMS, while in PK-1042, it was found to lie between 30 kR and 45 kR gamma rays and in between the higher doses/concentrations of combined treatment. In case of pollen sterility combined treatment exhibited high percentage of pollen sterility followed by EMS and gamma-rays. In Pusa-16, the increase in the dose/concentration of gamma rays and EMS was associated with the increase in the pollen sterility percent, while in combined treatment, the increase was noticed upto the intermediate dose level followed by decrease at higher level. On the other hand in PK-1042, increase in EMS concentration was associated with increase in sterility percent, while no definite trend was noticed in gamma rays and combined treatment.

Key words: Gamma rays, EMS, germination, lethality, pollen sterility, soybean.

Introduction

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding work in soybean crop has yielded in identification of many mutant lines with desirable traits like high germination and survival % (Rehman *et al*. 1994). Variations in M_1 generation, though less important in view of obtaining stable gene

mutations, are often considered as indicators in measuring efficiency of mutagen treatments (Plesnik, 1993). Also, pollen sterility is used for calculating the mutation index which is a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors. The present investigation was, therefore, initiated to study the effect of physical (gamma rays), chemical (EMS) and combined mutagenic treatments on some quantitative traits at different doses of two cultivars of soybean [*Glycine max* (L.)Merrill].

Material and Methods

Dry (9-12 % moisture) and healthy seeds of Pusa-16 and PK-1042 cultivars of soybean obtained from Division of Genetics/Pulses, IARI, New Delhi were used in the present investigation. The experiment was categorized into three treatment groups i.e. gamma rays, ethyl methane sulphonate (EMS) and combination treatment. In first category seeds were exposed to 15, 30 and 45 KR gamma rays at NRL, IARI, New Delhi. In second category the seeds were subjected to 0.1, 0.2and 0.3% EMS for eight hours followed by four hours washing of seeds under running water. In the third category each gamma rays treatment was followed by 0.2% EMS treatment for eight hours. The experiment was conducted at Research Farm of Kisan (P.G) College, Simbhaoli. Twenty treatments including sole treatment of gamma rays, EMS, combination and untreated control were sown in the field in a single plot design. Each plot consisted of 4 rows in which 20 seeds per row were sown with 30 and 10 cm distance between rows and plants, respectively. Data were recorded on germination %, lethality %(30 DAS and at flowering) and pollen sterility. Likewise percentage reductions due to mutagen treatments over untreated control were estimated.

For analysis of pollen sterility, flower buds were collected from five plants of each treatment and were fixed in Carnoy's fluid (absolute alcohol: chloroform: acetic acid, 6:3:1 v/v) for 24 hours after which they were stored at 4^{o}C. Slides were prepared by the squash technique and stained with 1% acetic carmine. Ten slides per treatment were evaluated and sterile pollens were counted and averaged.

Results and Discussion

Germination (%)

The data on per cent germination in M_1 generation for various mutagenic treatments in Pusa-16 and PK-1042 are given in Table 1. In both the cultivars, in comparison to the control, the per cent germination was low in all the treatments. Similar results were also reported by

Padavai and Dhanavel (2004) and Singh & Kole (2005). In general, the reduction in germination percentage was associated with the increase in the dose/concentration of mutagens; however, there was one exception to this i.e. 0.2 % EMS in PK-1042, which showed a marginal increase over its lower concentration of treatment. Reduction in germination over control in Pusa-16 ranged from 13.92% (30 kR) to 60.76% (45 kR + 02% EMS), while in PK-1042 it ranged from 3.84% (15 kR) to 59.34% (45 kR + 0.2% EMS).

Survival (at flowering) due to the different mutagenic treatments in Cv. Pusa-16 ranged from 47.92% (45 kR) to 83.87% (45 kR +0.2% EMS), while in PK-1042 it ranged from 66.32% (30 kR + 0.2% EMS) to 93.14% (15 kR) (Table 1). The decrease in the survival percentage was associated with the increase in the dose/concentration of the mutagens in both cultivars except in combined treatment of Pusa-16 and EMS treatment of PK-1042, where a reverse trend and increase after the intermediate concentration was noticed, respectively. Further, perusal of Table 2 revealed that the response of gamma rays were negatively linear in both the cultivars in which PK-1042 showed higher slope as compared to Pusa-16. In case of EMS, higher slope was exhibited by Pusa-16, while combined treatment exhibited the higher slope for PK-1042.

Among the mutagens used, EMS alone or in combination with different doses of gamma rays exerted more influence on these parameters. The above results could be attributed to the effect of mutagens on the meristematic tissues of the seed. The decrease in germination at higher doses of the mutagens may also be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens like EMS leading to decrease in germination. Reduced growth due to higher doses was also explained differently by different workers. It may be attributed to one or more of the following reasons (i) the increase in destruction of growth inhibitors (ii) the increase in growth promoters (iii) the sudden increase in metabolic status of seeds at certain levels of dose or (iv) it may be due to the induced chromosomal aberrations. These findings are in close agreement with the earlier reports of Wang and Yu (1988), Solanki and Sharma (1999), Solanki and Sharma (2002), Kumar and Selvaraj (2003) Solanki and Phogat (2005).

Lethal Dose 50 (LD 50)

Mutagens are known to induce lethality at the seedling stage and more so in M_1 generation because of high residual toxicity. Perusal of Table 1 revealed that there was high survival of seedlings (50.65%) for PK-1042 as compared to Pusa-16 (43.6%). The behaviour in terms of lethality of Pusa-16 at highest doses of gamma rays (45 kR) was more than two times (52.08%) as compared to PK-1042 (25.29%), whereas at the lower doses, the behaviour of two cultivars was nearly similar. The existence of such varietal differences in sensitivity to ionizing radiations and chemical mutagens is now well known and has also been reported in several other crops like lentil (Malik, 1998) and pigeonpea (Venkateshwarlu *et al.,* 1978). In case of EMS treatment no definite trend of lethality was noticed in both the cultivars, while in combined treatment the increase in the dose/concentration of mutagens was associated with the increase in the lethality per cent. In Pusa-16, LD50 was found to lie between 30 kR + 0.2% EMS and 45 kR + 0.2% EMS, whereas in PK-1042, it was found to lie between 30 kR and 45 kR of gamma rays and in between the higher doses of combined treatment.

Pollen Sterility (%)

The pollen sterility among different mutagenic treatments in Pusa-16 ranged from 16.81% (15 kR gamma rays) to 58.23% (0.3% EMS), while in Pk-1042, it ranged from 19.42% (15 kR gamma rays) to 52.20% (45 kR + 0.2% EMS) combined treatment (Table 3). In both the cultivars, combined treatment exhibited high percentage of pollen sterility followed by EMS and gamma rays. The high percentage of pollen sterility in EMS treatments as compared to gamma rays is in conformity with the earlier reports of Dixit and Dubey (1986). The above results are in strong contrast with the general hypothesis that physical mutagens produce more meiotic abnormalties than chemical mutagens. In Pusa-16, the percentage of pollen sterility increased with the increase in the dose/concentration of the gamma rays and EMS, while in combination treatment the pollen sterility increased upto the intermediate dose level followed by decrease at highest level of the mutagen. On the other hand, in PK-1042, the gamma rays exhibited increased pollen sterility at the intermediate dose level, while the combined treatment showed the reverse trend of gamma rays. In case of EMS treatment, the increase in the dose of mutagen was associated with the increase in the pollen sterility. Earlier the dose dependent increase was also reported by Ignacimuthu and Babu (1992) in mungbean and Kumar and Dubey (1998) in Khesari. In general, during the present study the sterility was more at the intermediate or higher

dose/concentration levels, which may be due to the fact that the cultivars were more sensitive at the higher doses/concentration and therefore, the genic, chromosomal and physiological disturbances were more at the higher doses/concentration which has also been shown by Ekberg (1969).

Conclusion

Based on the results obtained in the present study, it is advocated that mutagenesis with EMS treatment is much more beneficial as compared to gamma rays and combination of EMS and gamma rays in developing the cultivars with desirable alleles for further improvement in seed yield and its component characters in soybean [*Glycine max* (L.) Merrill].

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Table 1. Effect of mutagen (chemical, physical and combined) treatments on germination in M_1 generation in two cultivars of soybean

S. No.	Doses/ cultivars	No. of seeds sown	No. of seedling	Germination (%)	% of lethality over control	% Survival (at flowering)
Pusa-16						
1.	Control	200	158	79.00	0.00	-
2.	15 kR	200	133	66.50	15.82	83.46 (111)
3.	30 kR	200	136	68.00	13.92	79.41 (108)
4.	45 kR	200	96	48.00	39.24	47.92 (46)
5.	0.1%	200	121	60.50	23.41	79.34 (86)
6.	0.2%	200	131	65.50	17.09	78.63 (103)
7.	0.3%	200	108	54.00	31.64	62.04 (67)
8.	15 + 0.2%	200	117	58.50	25.94	66.67 (78)
9.	30 + 0.2%	200	86	43.00	45.57	73.26 (63)
10.	45+ 0.2%	200	62	31.00	60.76	83.87 (52)
	Total		**872**	**43.6**		**81.88 (714)**
PK-1042						
1.	Control	200	182	91.00	0.00	
2.	15 kR	200	175	87.50	3.84	93.14 (163)
3.	30 kR	200	150	75.00	17.58	85.33 (128)
4.	45 kR	200	87	43.50	52.19	74.71 (65)
5.	0.1%	200	119	59.50	34.61	89.92 (107)
6.	0.2%	200	125	62.50	31.31	73.60 (92)
7.	0.3%	200	92	46.00	49.46	79.35 (73)
8.	15 + 0.2%	200	109	54.50	40.10	80.73 (88)
9.	30 + 0.2%	200	95	47.50	47.80	66.32 (63)
10.	45+ 0.2%	200	74	37.00	59.34	70.21 (52)
	Total		**1013**	**50.65**		**82.03 (831)**

() figures in parenthesis indicate number of plants

Table 2. Genotypic radio-sensitivity mutability in terms of germination per cent and survival as affected by Gamma rays, EMS and their combination in two cultivars of soybean

Doses/ cultivars	Per cent survival			
	a	**b**	**SE_b**	**LD-50**
Gamma rays				
Pusa-16	5.393	-0.086	0.003	3.770
PK-1042	5.604	-0.146	0.004	4.674
EMS				
Pusa-16	5.125	-0.171	0.011	5.403
PK-1042	5.360	-0.022	0.005	7.773
Combined				
Pusa-16	5.167	-0.226	0.006	5.472
PK-1042	5.278	-0.269	0.006	1.191
	Germination			
Gamma rays				
Pusa-16	5.066	-0.194	0.003	2.679
PK-1042	5.491	-0.186	0.004	4.271
EMS				
Pusa-16	4.587	-0.305	0.011	0.467
PK-1042	5.068	-0.028	0.005	2.642
Combined				
Pusa-16	4.948	-0.290	0.006	6.663
PK-1042	5.049	-0.329	0.006	1.413

a = Intercept, b = Slope, SE_b = Standard error of b

Table 3. Effect of mutagens on pollen sterility in two cultivars (Pusa-16 and PK-1042) of soybean

S. No.	Doses	Cultivars/Treatments			
		Pollen sterility		Pollen sterility over control (%)	
		Pusa-16	PK-1042	Pusa-16	PK-1042
1.	Control	15.76	14.42	0.00	0.00
2.	15 kR	16.81	19.42	1.05	5.30
3.	30 kR	27.63	47.86	11.87	33.74
4.	45 kR	32.43	32.38	16.67	17.96
5.	0.1% EMS	31.93	35.16	16.17	20.74
6.	0.2% EMS	35.31	42.05	19.55	27.63
7.	0.3% EMS	58.23	45.92	42.47	31.50
8.	15kR + 0.2% EMS	41.20	43.52	25.44	29.10
9.	30kR+ 0.2% EMS	50.85	36.41	35.09	21.99
10.	45kR+ 0.2% EMS	47.83	52.20	32.87	37.78