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Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean [*Glycine max* (L.) Merrill.]

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Mutagenic effectiveness and efficiency of gamma rays, EMS and combined treatments was studied in terms of M_2 (progenies) lethality and chlorophyll mutations in two cultivars of soybean (Pusa-16 and PK-1042). In general the frequencies of chlorophyll mutations were high in gamma rays and combined treatments. Four types of mutants viz., albina, xantha, chlorine and viridis were observed in the study. Gamma rays were found to be more effective to induce chlorophyll mutations in both cultivars. PK-1042 cultivar exhibited higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment.

Key words: Effectiveness, efficiency, gamma rays, EMS, soybean.

INTRODUCTION

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen, while mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility induced by the mutagen (Wagner and Foster, 1965). The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. Chlorophyll mutations are used to evaluate the genetic effects of various mutagens. Gaul (1964) reported the appearance of greater number of viridis type which is attributed to the involvement of polygene in chlorophyll formation. The present study was undertaken to gather information on efficiency, effectiveness and chlorophyll mutations on the consequences of induction of physical (Gamma rays) and Chemical (EMS) mutagens in Soybean.

MATERIALS AND METHODS

Dry seeds (9 - 12% moisture) of two cultivars Pusa-16 and PK- 1042 of soybean were treated with ethyl methane sulphonate (EMS) (0.1, 0.2 and 0.3% concentration) and Gamma rays (15, 30 and 45 Kr) at

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NRL, IARI, New Delhi. 100 irradiated seeds (Gamma ray) were subjected to 0.2% EMS for eight hours. The treated material along side with two controls (untreated) were immediately sown in a single unreplicated plots with 4 rows at a distance of 30 and 10 cm between rows and plants, respectively at Research Farm of Kisan (P.G.) College, Simbhaoli, UP, India. The seeds treated with chemical mutagen were washed in running water before sowing. The data was recorded from 20 randomly selected plants from each treatment.

Survival of plants was recorded at the time of maturity in the field and was expressed as percentage of control. Selfed seeds of the individual M_1 plants were harvested separately and were grown as individual M_2 families in separate lines in Modified Single Seed Bulk Design. The treated and the control material were screened for the frequency of chlorophyll mutations in the M_2 generation. Mutagenic frequency was estimated as percentage of segregating M_1 plant progenies (Gaul, 1964). The mutagenic efficiency and effectiveness were determined by the methods suggested by Konzak et al. (1965).

RESULTS

Chlorophyll mutation

The chlorophyll mutation frequencies were calculated as per cent M_1 plants (M_2 progenies) and the data is presented in Table 1. It may be noticed from the data that in cultivar PK-1042, the mutation frequency increased with increase in the dose of gamma rays, while in case of

Table 1. Frequency of chlorophyll mutations in EMS and Gamma rays treated M₂ generations of soybean cultivars.

Cultivars	Dose	Number of M ₂ seedlings		Mutation frequency/100 M ₂ seedlings	Spectrum (relative %) of Chlorophyll mutants			
		Tested	Chlorophyll mutants		Albina	Xantha	Chlorina	Viridis
	Control							
PK-1042	0	600	-	-	-	-	-	-
Pusa-16	0	580	-	-	-	-	-	-
	Gamma rays (kR)							
PK-1042	15	500	9	1.80	0.00	0.00	55.56	44.44
	30	420	13	3.09	7.69	23.07	30.76	38.46
	45	400	15	3.75	13.33	33.34	20.00	33.33
Pusa-16	15	535	15	2.80	6.66	20.00	33.34	40.00
	30	495	17	3.43	0.00	29.41	41.17	29.41
	45	480	13	2.70	23.07	0.00	15.38	61.53
	EMS (%)							
PK-1042	0.1	600	9	1.50	0.00	33.33	22.22	44.45
	0.2	574	15	2.61	6.66	33.33	26.67	33.33
	0.3	525	12	2.28	16.67	16.67	41.67	25.00
Pusa-16	0.1	520	5	0.96	0.00	25.00	50.00	25.00
	0.2	515	9	1.74	11.11	22.22	33.34	33.33
	0.3	485	16	3.29	0.00	31.25	25.00	43.75
	Combination [EMS + Gamma rays (%)]							
PK-1042	15 kR + 0.2	485	11	2.26	0.00	36.36	27.27	36.36
	30 kR + 0.2	320	15	4.68	20.00	0.00	33.34	46.46
	45 kR + 0.2	315	13	4.12	7.69	38.46	23.07	46.15
Pusa-16	15 kR + 0.2	435	12	2.75	16.66	41.66	25.00	16.67
	30 kR + 0.2	410	15	3.65	20.00	6.67	33.33	40.00
	45 kR + 0.2	385	19	4.93	10.52	26.31	36.84	26.31

EMS and combined treatments, the mutation frequencies exhibited addition up to the intermediate dose levels and then showed a decline with further increase in the dose of mutagen. In Pusa-16, the situation was somewhat different. An increase in the dose of gamma rays showed increase in the mutation frequencies at intermediate level and a gradual decrease at higher doses, whereas EMS and combined treatment showed slight increase in the mutation frequency with the increase in the dose/conc. of mutagen.

Spectrum and frequency of chlorophyll

The data of frequencies and spectra of different chlorophyll mutants in the two soybean cultivars that is PK-1042 and Pusa-16 are presented in Table 1. The details for individual mutation types

are as follows: -

a) Albina: In PK-1042, out of nine mutagenic treatments the *albina* mutants were observed in six treatments. Among these treatments, the highest frequency (20.00%) of mutants was observed at 30 + 0.2% combination treatment. In other treatments, the values ranged from 0.00 to 16.67%. In Pusa-16, the *albina* mutants were observed in six treatments. The highest frequency (23.07%) was observed at 45 Kr gamma rays and 30 + 0.2% (20.00) combination treatment. Other treatments showed a range of 0.00 to 16.66%.

b) Xantha: The frequency of *xantha* chlorophyll mutants showed a slight increase in PK-1042 as compared to Pusa-16 in EMS and combined treatments. The frequencies of *xantha* mutants showed a decrease at intermediate dose levels and then increase with the increase in dose of mutagen, whereas in Pusa-16 it showed a decreasing trend. With gamma rays treatments in PK-1042, an increase in the frequency of xantha mutants was noticed with the increase in the doses of mutagen, while in Pusa-16 an increase upto intermediate level and thereafter a gradual decrease (0.00%) at higher dose (45 Kr) was noticed.

c) Chlorina: Dissimilar to the xantha mutants, the frequency of the chlorina mutants were high in Pusa-16 then PK-1042. In Pusa-16, the frequen-cies of chlorina mutants showed an increase with the increase in dose of mutagens. In PK-1042, on the other hand, the increase in the dose of gamma rays was associated with the decrease in the frequency of chlorina mutants, whereas EMS and combined treatments showed increase at the intermediate levels. In Pusa- 16 as compared to other treatments, the frequencies of *chlorina* mutants were high following combined treatments. The increase in the dose was associated with the increase in the frequencies of mutants. 45 Kr gamma rays and combination treatment (45Kr + 0.2%) showed the highest frequency of chlorina mutants in Pusa-16 among all the treatments in both cultivars.

d) Viridis: The frequencies of the *viridis* mutants were high as compared to the other types of chlorophyll mutants. In both the cultivars, the frequencies of the *viridis* showed an increase at the intermediate level of combination treatment. Contrary to this, an increase in the dose of gamma rays was associated with the decrease in the frequency of mutants, whereas it showed a reverse trend in case of EMS treatment.

The frequencies of different chlorophyll mutant types in both the cultivars were found in the following order: - *Viridis > Chlorina > Xantha > Albina*.

Mutagenic effectiveness and efficiency

The effectiveness of different mutagens and that of different treatments of mutagens were calculated and the data are presented in Table 2. The mutagenic effective-

ness was as high as 1.6310 in gamma rays treatments. Whereas the highest effectiveness of EMS treatments was 1.3291 in the cultivar Pusa-16. EMS was 19 to 60 times more effective than gamma rays, while it was less effective that is 0.40 to 5 times in case of PK-1042. In both cultivars mutagenic effectiveness decreased with the increase in the dose of the mutagens except in EMS treatment of Pusa- 16 where the increase (1.3291) was observed at higher concentration (0.3%). Thus, the order of mutagens based on effectiveness was: - EMS > Gamma rays.

The mutagenic efficiency was calculated on the basis of lethality and the data are presented in Table 2. The highest (0.0297) and lowest (0.092) mutagenic efficiency was exhibited by Pusa-16 (15 Kr + 0.2%) and Pk-1042 and Pusa-16 (0.3%), respectively. In general, PK-1042 exhibited higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment. However, Pusa- 16 showed higher frequency in combined treatment. The mutagenic efficiency decreased with the increase in the dose of the mutagen in both cultivars the differences in number of genes controlling chlorophyll development in the two cultivars. It may also be noted that the frequencies of chlorophyll mutants were higher in gamma rays and combined treatments. This is in conformity with the previous reports of Prasad and Das (1980). In 50% of the treatments (Gamma rays, PK-1042; EMS, Pusa-16 and Combined treatment, Pusa-16) an increasing trend in the frequency of mutants with increase in the dose of mutagen was observed but the increase was not linear. The non-linearity in the fre-quency of mutants and dose of mutagens may to some extent, be attributed to the irregular segregation ratios of chlorophyll mutants leading to deficits of recessives in the progenies.

Albina, xantha, chlorina and viridis were the different types of chlorophyll mutants found in the present study. Xantha and Chlorina types of chlorophyll mutants in soybean were earlier reported by Geetha and Vaidyanathan (2000) . In general, viridis occurred in maximum proportion than other types of chlorophyll mutants in gamma rays and combined treatments.

Since chlorophyll mutations are most conspicuous and easily detectable as they have been extensively used to find out sensitivity of crop plants to mutagens and to elucidate effectiveness and efficiency of mutagens (Gaul, 1960). It may be noted from the Table 2 that relative to the EMS, gamma rays were more effective in inducing chlorophyll mutation in both the cultivars. Similar results have also been reported in Lathyrus by Nerkar (1977). In the present study, the efficiency of EMS, Gamma rays and combined treatments decreased considerably except Pusa-16 (combined treatment) with the increase in the dose of mutagens in both cultivars. The decrease in efficiency may be attributed to the failure in proportionate increase in dose of mutagens (Table 2).

Similar findings have also been reported by Dixit and Dubey (1986). It is obvious that the higher efficiency

Dose	Mutation frequency/100 M₂ seedlings PK-1042 Pusa-16		Chlorophyll mutar N	Mutagenic effectiveness		Mutagenic efficiency		
-			PK-1042	Pusa-16	PK-1042	Pusa-16	PK-1042	Pusa-16
Control			0.00	0.00	0.00	0.00	0.00	0.00
Gamma rays (kR)								
15	1.80	2.80	1.80	2.80	0.1200	0.1866	0.181	0.203
30	3.09	3.43	3.09	3.43	0.1030	0.1143	0.253	0.137
45	3.75	2.70	3.75	2.70	0.0833	0.0600	0.098	0.108
EMS (%)								
0.1	1.50	0.96	1.50	0.96	1.8750	1.2000	0.166	0.168
0.2	2.61	1.74	2.61	1.74	1.6310	1.0875	0.169	0.133
0.3	2.28	3.29	2.28	3.29	0.9500	1.3291	0.092	0.092
Combination								
15 kR + 0.2(%)	2.26	2.75	2.26	2.75			0.208	0.297
30 kR + 0.2(%)	4.68	3.65	4.48	3.65			0.172	0.243
45 kR + 0.2(%)	4.12	4.93	4.12	4.93			0.106	0.232

Table 2. Effectiveness and efficiency of various EMS and Gamma rays treatments in M₂ generation in two cultivars of soybean.

at lower and intermediate doses of mutagens may be due to the fact that the biological damage (lethality and sterility)increased with the dose at a r ate greater than the frequency of mutations (Konzak et al., 1965).

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