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Studies on induction of chlorophyll mutations in soybean, *Glycine max* (L.) Merrill

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Abstract The phenotypic response of two soybean cultivars to a chemical mutagen (ethyl methane sulphonate, EMS), physical mutagen (gamma rays) and their combinations were studied in M_1 and M_2 generations and the frequency and spectrum of chlorophyll mutations were worked out. Combined treatment was found to be more effective in inducing chlorophyll mutations compared to individual treatments of gamma rays and EMS in both the cultivars. As far as the spectrum of chlorophyll mutations is concerned, a wider spectrum in both the cultivars was observed in 45 kR + 0.2% EMS combined treatment.

Keywords chlorophyll mutations, gamma rays, ethyl methane sulphonate, soybean

1 Introduction

Mutation breeding is a proven supplement and an effective substitute to conventional breeding so as to confer specific improvement in a variety without significantly affecting its acceptable phenotype. The potential of induced mutation in plant breeding has been well documented by the release of a number of mutant cultivars in the field and other crops. Adoption of new techniques, as a dependable method of crop improvement, depends very much on the identification of more effective and efficient mutagens as well as on the improved methodology adopted to increase the spectrum of useful mutations in the oligogenic and polygenic traits. 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, i.e., gross chromosomal aberrations, lethality and sterility induced by the mutagen. 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 a greater number of *virides* type as attributed to the involvement of polygene in chlorophyll formation. The present study was undertaken to gather information on chlorophyll mutations as a result of induction of physical (gamma rays) and chemical (EMS) mutagens in soybean.

2 Materials and methods

2.1 Experimental material

Seeds of two soybean varieties viz., Pusa-16 and PK-1042 were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

2.2 Methods

2.2.1 Physical and chemical treatments of seeds

The experimental materials were divided into three treatment groups: Group 1 treated with physical mutagen at 15, 30 and 45 kR of gamma rays, by irradiating seeds at the Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi; Group 2 treated with chemical mutagen of EMS at the concentrations of 0.1%, 0.2% and 0.3%, whose seeds were treated for eight hours and washed in running water before sowing and Group 3 treated with both physical and chemical mutagens of 15 kR + 0.2% EMS, 30 kR + 0.2% EMS and 45 kR + 0.2% EMS, by subjecting 100 irradiated seeds of 15, 30 and 45 kR to 0.2% EMS treatment for eight hours, with the treated seeds with chemical mutagen washed in running water before sowing.

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The treated materials along-with two controls (untreated) were immediately sown in a single unreplicated plot with 4 rows at a spacing of 30 cm × 10 cm at the Research Farm of Kisan (P. G.), College, Simbhaoli. The data was recorded from 20 randomly selected plants from each treatment.

2.2.2 Recording of data

Survival of plants was recorded at the time of maturity and expressed in percentage of control. The survival of the plants has been reported to be controlled by the nature of the mutagens as well as genotypic architecture of the varieties. Despite the fact that different frequencies of survival of similar mutations are induced by different mutagens, the main limiting factor in the induction and recovery of mutations is the genetic constitution of the experimental material (Gregory, 1965). Selfed seeds of the individual M₁ plants were harvested separately and were grown as individual M₂ families in separate lines in Modified Single Seed Bulk Design. The treated and the untreated materials were screened for the frequency of chlorophyll mutations in M₂ generation. Mutagenic frequency was estimated as percentage of segregating M₁ plant progenies.

2.2.3 Chlorophyll mutants

2.2.3.1 *Albina*

These seedlings were characterized by their dull white colour and were devoid of chlorophyll, carotenoid and other pigments. Albina seedlings are smaller in height and survive to a maximum of 10 days after germination and then die.

2.2.3.2 *Chlorina*

Normally chlorina mutants do not survive. These mutant seedlings have light yellowish/ yellowish green leaves and culm with yellowish pods. The mutants breed true for the altered characters.

2.2.3.3 *Xantha*

Colours of these mutants vary from deep yellow to yellowish white. Growth of mutants is retarded and most of them die within 7 to 10 days after emergence.

2.2.3.4 *Viridis*

The seedlings are dark green in the early stages of development and turn normal green in the later stages. The mutants produce normal looking flowers and also set seeds.

2.2.3.5 *Tigrina*

The mutants have characteristic transverse yellowish and whitish bands on their leaves and survive till maturity. They produce few seeds.

2.2.3.6 *Xanthalba*

The seedlings are albina type in the beginning, but later on they become yellowish. Growth of mutants is always less than the normal plants. The seedlings die within fifteen days; however, if they survive till flowering they do not set any seed.

3 Experimental results

3.1 Chlorophyll mutations

Gustafsson (1940) classified chlorophyll mutations into various types. However, during the experimentation only 4 types of chlorophyll mutants were identified viz., *albina*, *chlorina*, *xantha* and *virides*. The chlorophyll mutation frequencies were calculated as per cent M₂ plants (Table 1).

3.2 Scoring of chlorophyll mutations

It is evident from the data (Table 1) that in both the cultivars mutation frequency increased with increase in the dose/concentration of mutagen. In general, Pusa-16 exhibited more chlorophyll mutations (1.28%) as compared to the PK-1042 (1.003%). In gamma ray treatment at 15 kR, the mutation frequency of Pusa-16 (2.42%) was double than PK-1042 (1.21%), whereas at 45 kR the frequency of PK-1042 (3.75%) was more than Pusa-16 (3.54%). Similar findings were also noticed in the combined treatments. On the other hand, in the case of EMS treatments, the situation was reverse, i.e., more mutation frequency was exhibited by PK-1042 compared to Pusa-16.

3.3 Spectrum and frequency of chlorophyll mutations

The frequencies and spectra of different chlorophyll mutants in two cultivars of soybean, i.e., Pusa-16 and PK-1042 are presented in Table 2.

3.3.1 *Albina*

In Pusa-16 *albina* mutants (Fig. 1) were observed in all treatments except 0.1% concentration of EMS treatment. The highest frequency (1.03%) was observed in combination treatment (45 kR + 0.2% EMS) and in other treatments the values ranged from 0.26% (0.3% EMS) to 0.68% (30 kR + 0.2% EMS). In the case of PK-1042, the

Table 1 Frequency of chlorophyll mutations recorded as per cent M₂ plants in two cultivars of soybean

serial no.	treatment	total seedling studied		total mutation frequency (% M ₂ plants)	
		PK-1042	Pusa-16	PK-1042	Pusa-16
1	control	580	600	0.00	0.00
2	15 kR	535	740	2.42	1.21
3	30 kR	595	560	2.52	2.32
4	45 kR	480	400	3.54	3.75
5	0.1% EMS	520	600	0.96	1.50
6	0.2% EMS	565	674	1.59	1.78
7	0.3% EMS	685	525	2.33	2.85
8	15 kR + 0.2% EMS	435	485	2.75	2.26
9	30 kR + 0.2% EMS	440	420	3.40	3.09
10	45 kR + 0.2% EMS	385	375	4.93	4.00
total		–	–	(1.280%)*	(1.003%)

Note: * Figures in parenthesis indicate total mutation frequency.

Table 2 Frequency and spectrum of different types of chlorophyll mutants (% M₂ seedlings) in M₂ generation in two cultivars of soybean

serial no.	treatment	total seedling studied	frequency of chlorophyll mutations/%			
			<i>albina</i>	<i>xantha</i>	<i>chlorina</i>	<i>virides</i>
Pusa-16						
1	control	580	0.00	0.00	0.00	0.00
2	15 kR	535	0.37	0.00	0.18	0.18
3	30 kR	595	0.33	0.00	0.50	0.16
4	45 kR	480	0.41	0.62	0.00	0.83
5	0.1% EMS	520	0.00	0.19	0.19	0.00
6	0.2% EMS	565	0.35	0.00	0.35	0.53
7	0.3% EMS	685	0.26	0.00	0.43	0.58
8	15 kR + 0.2% EMS	435	0.45	0.68	0.22	0.00
9	30 kR + 0.2% EMS	440	0.68	0.00	0.68	0.90
10	45 kR + 0.2% EMS	385	1.03	0.51	0.77	0.29
PK-1042						
1	control	600	0.00	0.00	0.00	0.00
2	15 kR	740	0.00	0.00	0.27	0.00
3	30 kR	560	0.17	0.35	0.17	0.35
4	45 kR	400	0.75	0.25	0.50	0.50
5	0.1% EMS	600	0.33	0.00	0.16	0.00
6	0.2% EMS	674	0.44	0.00	0.29	0.14
7	0.3% EMS	525	0.38	0.38	0.76	0.19
8	15 kR + 0.2% EMS	485	0.00	0.41	0.20	0.20
9	30 kR + 0.2% EMS	420	0.71	0.23	0.23	0.47
10	45 kR + 0.2% EMS	375	0.00	1.33	0.80	0.25

albina mutants were observed in six out of nine treatments. The highest frequency (0.75%) was observed at 45 kR of gamma rays, while the lowest value (0.17%) was noticed in 30 kR gamma rays.

3.3.2 *Xantha*

The frequency of *xantha* mutants (Fig. 2) was relatively

high in PK-1042 as compared to Pusa-16. In Pusa-16, the intermediate dose/concentration level of all the three treatments did not show any frequency of *xantha* mutants. The lowest (0.19%) and the highest (0.68%) frequencies of *xantha* mutants were exhibited by 0.1% EMS and 15 kR + 0.2% EMS treatment, respectively. In the case of PK-1042, the highest frequency (1.33%) was exhibited by 45 kR + 0.2% EMS treatment whereas, the lowest value (0.23%)

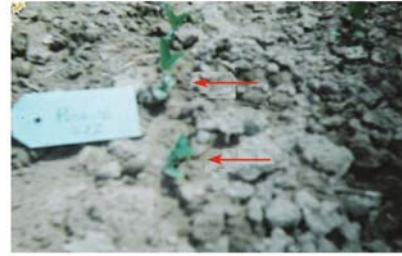
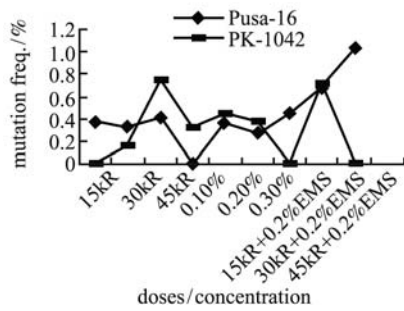


Fig. 1 Frequency and chlorophyll abnormalities in *albina* mutants

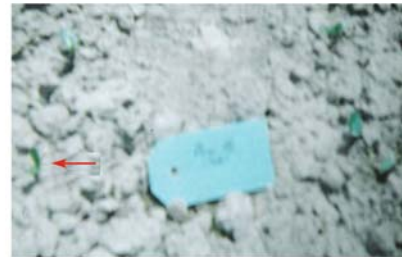
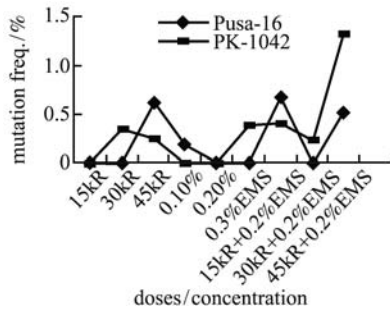


Fig. 2 Frequency and chlorophyll abnormalities in *xantha* mutants

was also exhibited by combined treatment at 30 kR + 0.2% EMS. In gamma ray treatment, an increase in the frequency of *xantha* mutants was noticed at 30 kR which decreased at 45 kR, while in EMS treatments, the frequency was only noticed at 0.3% concentration.

decreasing trend with an increase in the dose up to 30 kR and then increased at 45 kR. The highest frequency of *chlorina* mutants in Pusa-16 (0.77%) and PK-1042 (0.80%) was exhibited by combined treatment of 45 kR + 0.2% EMS.

3.3.3 Chlorina

3.3.4 Virides

In both cultivars total frequencies of *chlorina* mutants were the same (Fig. 3). In EMS and combined treatments, an increase in the frequency of mutants was associated with an increase in the dose/concentration of mutagen in both the cultivars. In Pusa-16, the gamma rays exhibited *chlorina* mutants at 30 and 45 kR which were also correlated with increasing dose. On the other hand, in PK-1042, the frequencies of *chlorina* mutants showed a

In contrast to the *xantha* mutants, the frequencies of *virides* were high in Pusa-16 as compared to PK-1042 (Fig. 4). In cv. Pusa-16, the frequencies of *virides* mutants were higher as compared to the other types of mutants, while a reverse trend was exhibited by PK-1042. In both the cultivars an increase in gamma rays was associated with an increase in the frequency of mutants. The highest frequency of *xantha* mutants (1.29%) in Pusa-16 was exhibited by the

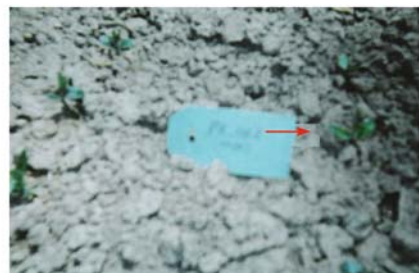
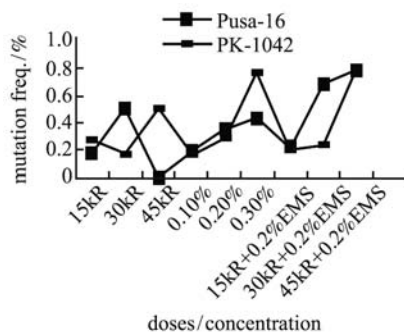


Fig. 3 Frequency and chlorophyll abnormalities in *chlorina* mutants

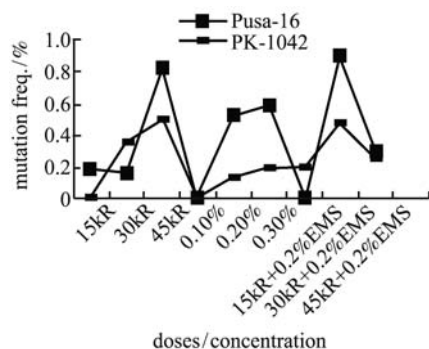


Fig. 4 Frequency and chlorophyll abnormalities in *virides* mutants

combined treatment at 45 kR + 0.2 EMS, whereas in PK-1042, the highest frequency (0.47%) was noticed at 45 kR. In both the cultivars no *virides* mutant were recovered at 0.1% EMS.

The frequencies of different chlorophyll mutant types in cv. Pusa-16 and PK-1042 were found in the following order:

(Pusa-16) *virides* > *albina* > *chlorina* > *xantha*,
 (PK-1042) *chlorina* > *albina* > *xantha* > *virides*.

4 Discussion

Chlorophyll mutations have been used as an index to evaluate the mutagenic potential of various physical and chemical mutagens in a number of crop plants. In the present study the frequency of chlorophyll mutants was high in Pusa-16 as compared to PK-1042. This varietal difference with respect to the frequency of chlorophyll mutations may be attributed to differences in radio sensitivity. Such varietal differences were also reported earlier by (Paul and Singh, 2002), (Das and Kundagrami, 2000), (Geetha and Vaidyanathan, 2000) and (Harb, 1990). However, (Bhatia and Swaminathan, 1963) opined that varietal differences/variations in incidence of chlorophyll mutations are due to the differences in the number of genes controlling the chlorophyll development in different varieties. Among the chlorophyll mutations induced due to the individual treatments of gamma rays and EMS, *virides* was found to be the most frequent type followed by *albina* in Pusa-16 (Table 2), while in PK-1042, *Chlorina* was found to be most frequent followed by *albina*. In several earlier studies, the most frequent type of chlorophyll mutant induced due to gamma rays was also found to be *virides* or *chlorina* (Geetha and Vaidyanathan, 2000; Solanki and Phogat, 2005). It is, therefore, obvious that *virides* in addition to *chlorina* is one of the frequent types of chlorophyll mutation induced by gamma rays and EMS. While comparing mutagens, it has been observed in several studies that chemical mutagens are more effective

than radiation doses and *xantha* instead of *virides* and *chlorina* was the most frequent type with chemical treatment (Prasad and Das, 1980). The present results are in strong contrast with the earlier reports of Prasad and Das. The differences in the effect of gamma rays and EMS on the frequency and spectrum of chlorophyll mutations can be attributed to preferential action of EMS/gamma rays on genes for chlorophyll development located near the centromere. In the case of combined treatment, the frequencies of chlorophyll mutants were high compared to individual doses of gamma rays and EMS. This synergistic effect of combined treatment may be expected due to the fact that the first mutagen makes accessible non-available sites for reaction to the second mutagen or lesions which arise because the first mutagen remains intact due to the inhibitory effect of the second mutagen on repair enzyme or due to the differential mechanism of mutagens in inducing mutation.

In the present study, there was a dose-dependent increase in the chlorophyll mutation frequency. This is supported by Blixt (1968) and Goud (1967) who reported that genes affecting chlorophyll mutation occurred near the centric region of the chromosome where recombinations occur very rarely. In the earlier studies also, the dose-dependent increase in the frequency of chlorophyll mutations has been reported in a variety of crops, i.e., lentil (Rajput and Sarwar, 1996), grasspea (Das and Kundagrami, 2000) and *Brassica campestris* (Zareen and Devi, 1995).

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