Academia Journal of Agricultural Research 5(9): 218-223, September 2017
DOI: 10.15413/ajar.2017.1212
ISSN: 2315-7739

Research Paper

Chlorophyll and morphological mutation induction in soybean (*Glycine max* (L.) Merrill.) through modified single seed bulk method

Accepted 11th August, 2017

ABSTRACT

The present investigation was undertaken to study the frequency and spectrum of chlorophyll and morphological mutants induced by gamma rays, EMS and their combination in soybean (*Glycine max* (L.) Merrill.). Chlorophyll mutants were recorded as percent M_2 seedlings and in both the cultivars four types of chlorophyll mutants' viz., *albina*, *xantha*, *chlorina* and *viridis* were observed. The frequency of the chlorophyll mutants increased with the increase in the dose and concentration of the mutagens. Pusa-16 exhibited high chlorophyll mutants as compared to PK-1042 and the frequency of mutants was higher in combined treatment followed by gamma rays and EMS in both the cultivars. In Pusa-16 the frequency of mutants were in the following order: *albina* > *viridis* > *chlorina* > *xantha,* however, in PK-1042 they were present in the following order: a*lbina* > *xantha* > *viridis* > *chlorine.* With regard to the morphological mutants, five types of mutants viz., tall, dwarf, early maturity, gigas and bold seeded were isolated in Pusa-16 and three types of mutants viz., black pod, black spotted seed and early maturing were observed in PK-1042. High frequencies of mutants in Pusa-16 were recorded from combined treatment, while in Pusa-16 highest frequency was observed in EMS treatment.

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Key words: Gamma rays, EMS, chlorophyll mutants, morphological mutants, soybean

INTRODUCTION

The soybean is the world's single greatest source of both vegetable oil and protein. While many crops can produce high levels of oil or protein in their seeds, soybean is uniquely able to produce high amounts of both oil and protein. It contains about 40% proteins, possessing high level of essential amino acids except methionine and cystine,20% oil rich in poly unsaturated fatty acids specially omega-6 and omega-3 fatty acids, 6-7% total minerals, 5-6% crude fiber and 17-19% carbohydrates (Chauhan *et al.,* 1988). Besides, it has good amount of iron, vitamin B-complex and is flavones (Chauhan *et al.*, 2002).

Use of induced mutations in plant breeding is an important means of increasing world food supplies. A number of new varieties, strains and genetically improved variants of various cereals, pluses, oilseed, ornamentals and vegetable crops have been successfully evolved by plant breeders. It 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. One of the advantages of mutation induction is that it allows the development of genotypes with few modifications compared with the original material.

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

Table 1: **Frequency of chlorophyll mutants in M_2 generation in two cultivars of soybean.**

Treatment	Total seedling studies		Total mutations frequency	
	Pusa-16	PK-1042	Pusa-16	PK-1042
Control	642	725	0.00	0.00
15 kR	723	696	0.41	0.71
30 kR	665	584	1.35	1.36
45 kR	421	392	2.37	2.29
0.1% EMS	610	632	0.81	0.63
0.2% EMS	595	545	2.01	1.10
0.3% EMS	336	410	1.95	2.68
15 kR + 0.2% EMS	490	515	0.81	0.97
30 kR + 0.2% EMS	510	492	2.15	2.03
45 kR+ 0.2% EMS	383	346	3.39	2.31
Total			(1.58)	(1.43)

() figure in parenthesis indicates total percentage.

polygenic traits. The potential of induced mutations in widening the genetic diversity is now establishing in soybean. In view of this, the present study was undertaken to gather information in M_2 generation on chlorophyll and morphological mutations as a result of induction of physical (gamma rays) mutagen, chemical (EMS) mutagens and their combinations in soybean.

MATERIALS AND METHODS

Seeds of two soybean cultivars viz., Pusa-16 and PK-1042 were obtained from the Division of Genetics, Indian Agricultural Research Institute, New Delhi, India. 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, 30kR + 0.2% EMS and 45kR + 0.2% EMS, by subjecting 100 irradiated seeds of 15, 30 and 45kR to 0.2% EMS treatment for eight hours, with the treated seeds with chemical mutagen washed in running water before sowing. The treated materials along with two controls (untreated) were immediately sown in Modified single seed bulk method at a spacing of 30 cm × 10 cm between rows and plants at the Research Farm of Genetics and Plant Breeding, Kisan (P.G.), College, Simbhaoli. The data was recorded from 20 randomly selected plants from each treatment for presence of chlorophyll and morphological mutants. The chlorophyll mutations were classified into various types according to Gustafson (1940).

RESULTS

Chlorophyll mutations

The chlorophyll mutations were scored at two leaves seedling stage and the data is presented in Table 1 and 2. In general, it may be noticed from the data that in both the cultivars the chlorophyll mutations increased with the increase in the dose and concentration of the mutagens except in 0.3% EMS in Pusa-16 where a decrease was noticed than the lower concentration of 0.2% EMS. Among the three groups of mutagens, combined treatment exhibited the highest percentage of chlorophyll mutants in both the cultivars followed by gamma rays and EMS. In Pusa-16, the highest percentage of mutants (3.39%) were exhibited by 45 kR + 0.2% EMS combined treatment, while in PK-1042, it was showed by 0.3% EMS concentration (2.68%). The total percentage of mutants was high in Pusa-16 (1.58%) as compared to PK-1042 (1.43%). 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 due to differences in radio sensitivity. However, Bhatia and Swaminathan (1963) opined that varietal differences and variations in incidence of chlorophyll mutations is due to the differences in the number of genes controlling the chlorophyll development in different varieties. Such varietal differences have also been reported earlier by Ahire and Auti (2015), Usharani

Table 2: **Frequency and spectrum of different types of chlorophyll mutants (expressed as % M_2 seedlings) in two cultivars of soybean.**

Doses and concentration	Chlorophyll mutations (%)									
	Pusa-16					PK-1042				
	Total seedling	***Albina***	*Xantha*	***Chlorina***	*Viridis*	**Total seedling**	***Albina***	*Xantha*	***Chlorina***	*Viridis*
Control	642	0.00	0.00	0.00	0.00	725	0.00	0.00	0.00	0.00
15 kR	723	0.00	0.27	0.14	0.00	696	0.43	0.00	0.28	0.00
30 kR	665	0.60	0.30	0.00	0.45	584	0.87	0.17	0.34	0.00
45 KR	421	1.18	0.00	0.47	0.71	392	1.53	0.00	0.00	0.76
0.1% EMS	610	0.00	0.32	0.49	0.00	632	0.00	0.47	0.16	0.00
0.2% EMS	595	0.67	0.00	0.84	0.50	545	0.37	0.00	0.00	0.73
0.3% EMS	336	1.48	0.00	0.00	0.89	410	0.97	0.48	0.73	0.24
15 kR + 0.2%EMS	490	0.00	0.41	0.41	0.00	515	0.00	0.39	0.00	0.58
30 kR + 0.2% EMS	510	0.59	0.00	1.18	0.39	492	1.02	0.42	0.42	0.20
45 kR + 0.2% EMS	383	1.57	0.78	0.00	1.04	346	1.45	0.86	0.00	0.00

and Ananda Kumar (2015), Vairam *et al*. (2014), Das and Kundagrami, (2003) , Paul and Singh (2002).

Albina

In Pusa-16, the *albina* mutants were recorded in 2 higher doses of each of the three treatment groups with high percentage in combined treatment followed by EMS and gamma rays, while in PK-1042, the mutants were recorded from all the treatments except 0.1% EMS and 15kR + 0.2% EMS combined treatment. The gamma rays in PK-1042 exhibited the highest percentage of mutants followed by combined and EMS treatments. In Pusa-16, combined treatment (45kR + 0.2% EMS) exhibited the highest percentage (1.57%) of mutants, while in PK-1042, the highest percentage (1.53%) of mutants were showed by 45kR gamma rays (Table 2).

Xantha

In PK-1042, the frequencies of *xantha* mutants were high as compared to the Pusa-16. In both the cultivars the frequency of *xantha* mutants increased with the increase in the dose and concentration of the combined treatment (Table 2). In Pusa-16, the gamma rays exhibited *xantha* mutants at the two lower doses and these also were correlated with the increasing dose level. EMS treatment exhibited chlorophyll mutants at 0.1% EMS concentration in Pusa-16 and 0.1 EMS and 0.3% EMS concentration in PK-1042. Among the nine treatments, the highest percentage of mutants (0.78 and 0.86%) in both the cultivars that is Pusa-16 and PK-1042 were exhibited by the combined treatment at 45 kR + 0.2% EMS level, respectively.

Chlorina

In both the cultivars the *chlorina* mutants were recorded either from the lower and intermediate dose and concentration of the mutagens except gamma rays in Pusa-16 and EMS treatment in PK-1042, where the mutants were also recorded from the highest dose levels. In Pusa-16, the highest percentage (1.18%) of mutants were noticed in 30kR + 0.2% EMS combined treatment, while in PK-1042, it was noticed in 0.3% EMS concentration (Table 2).

Viridis

The percentage of *viridis* mutants was higher in Pusa-16 as compared to PK-1042 (Table 2). In Pusa-16, the mutants were recorded in two higher doses of each of the three treatment groups and the increase in the dose and concentration of mutagens was associated with the increase in the frequency of mutants. The highest frequencies of mutants were exhibited by combined treatments followed by EMS and gamma rays treatments. On the other hand in case of PK-1042, EMS treatment exhibited mutants at intermediate and higher dose levels, while the combined treatment showed the mutants at lower and intermediate dose levels and in both of these treatment groups, the decrease in the frequency of

Table 3: **Frequency of morphological mutants in two cultivars of soybean.**

Treatment	Total seedling studied		Total mutations frequency	
	Pusa-16	PK-1042	Pusa-16	PK-1042
Control	642	725	0.00	0.00
15 kR	723	696	1.58	0.96
30 kR	665	584	1.88	1.05
45 kR	421	392	1.78	1.43
0.1% EMS	610	632	2.37	1.47
0.2% EMS	595	545	1.47	1.01
0.3% EMS	336	410	2.98	2.68
15 kR + 0.2% EMS	490	515	2.33	1.02
30 kR + 0.2% EMS	510	492	2.03	1.37
45 kR+ 0.2% EMS	383	346	2.60	2.61
Total	-	-	(1.96)	(1.43)

() figure in parenthesis indicates total percentage.

Table 4: Frequency and spectrum of different types of morphological mutants in M_2 generation in two cultivars of soybean.

Treatment	Morphological mutations (%)							
	Pusa-16					PK-1042		
	Dwarf	**Tall**	Bold seed	**Gigas**	Early maturing	**Black pod**	**Black spotted seed**	Early maturing
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15 kR	0.00	0.57	0.71	0.00	0.00	0.96	0.00	0.00
30 kR	0.68	1.02	0.00	0.00	0.00	0.45	0.00	0.60
45 kR	1.02	0.00	0.00	0.76	0.76	0.00	1.43	0.00
0.1% EMS	0.00	0.79	0.47	0.63	0.63	0.49	0.00	0.98
0.2% EMS	0.00	0.37	0.37	0.55	0.55	0.67	0.00	0.34
0.3% EMS	1.70	0.00	0.73	0.00	0.00	1.19	0.89	0.59
15 kR + 0.2% EMS	0.39	0.77	0.58	0.00	0.00	0.40	0.00	0.61
30 kR + 0.2% EMS	0.81	0.61	0.00	0.61	0.61	0.59	0.78	0.00
45 kR+ 0.2% EMS	1.44	0.00	0.00	1.16	1.61	1.57	1.04	0.00

mutants was associated with the increase in the dose and concentration of mutagen.

However, in gamma rays treatments the mutants were recorded only at 45kR. The frequency of different chlorophyll mutants in Pusa-16 were found in the following order: *albina* > *viridis* > *chlorina* > *xantha,* however, in PK-1042, the frequency of mutants showed a different order: a*lbina* > *xantha* > *viridis* > *chlorina*.

Morphological mutants

The data on frequencies (as percent M_2 seedlings) and spectrum of different morphological mutants are presented in Table 3 and 4. In general, the frequencies of mutants were high in Pusa-16 as compared to PK-1042. In Pusa-16, EMS and combined treatments showed decrease in the frequency of mutants' up to the intermediate dose and concentration levels followed by increase at higher dose and concentration levels of the mutagen, however, reverse trend was discernible by gamma rays. On the other hand, in PK-1042, an increase in the frequency of mutants was associated with the increase in the dose and concentration of mutagens in gamma rays and combined treatments, whereas, EMS treatment exhibited decrease up to intermediate level followed by increase at highest concentration level. The highest frequency of mutants in Pusa-16 were exhibited by combined treatment followed by EMS and gamma rays, while in PK-1042 it was exhibited by EMS followed by combined and gamma rays treatments. The maximum frequency of morphological mutants was observed in 0.3% EMS in both the cultivars (Table 3).

Among all the mutants, the maximum frequency in Pusa-16 was exhibited by dwarf mutants followed by tall mutants, while in PK-1042 the maximum frequency was exhibited by black pod mutants. The recovery of other two mutants in PK-1042 was same. The early maturing mutants were recorded in both the cultivars with high frequency in PK-1042. In EMS treatments, the frequency of morphological mutants in both the cultivars was high as compared to gamma rays and combined treatments. In Pusa-16, the frequency of dwarf mutants were recorded from the intermediate, high dose and concentration levels, while the early maturing mutants in both the cultivars were recorded in lower or intermediate dose and concentration levels except one exception in PK-1042, where the mutants were also noticed at highest concentration of EMS. The black pod mutants in PK-1042 were observed in all the mutagenic treatments except 45 kR of gamma rays.

In contrast to the latter mutant, the black spotted seed mutants were recorded only in the higher doses and concentrations of the three treatment groups. The highest frequency of black pod and black spotted seed mutants were scored in combined treatment in PK-1042. The increase in the frequency of these mutants in combined treatment was associated with the increase in the dose and concentration of the mutagen. The bold seed mutant in Pusa-16 were recorded in the lower dose and concentration of gamma rays and combined treatments, while in EMS treatment, all the three concentrations exhibited bold seed mutants. The frequency of gigas mutant was high in combined treatment followed by EMS and gamma rays. The increase in the dose of combined treatment showed an increase in the frequency of gigas mutants and *vica versa* in EMS treatment.

Discussion and Conclusion

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 due to differences in radio sensitivity. However, Bhatia and Swaminathan (1963) opined that varietal differences and variations in incidence of chlorophyll mutations is 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, albina was found to be the most frequent type mutant followed by *viridis* in Pusa-16, while in PK-1042, *albina* was found to be most frequent mutant followed by *xantha*. In several earlier studies, the most frequent type of chlorophyll mutant induced due to gamma rays was also found to be *albina* or *viridis* (Wani, 2011). It is thus obvious from the present study that *albina* in addition to *viridis* or *xantha* is one of the frequent types of chlorophyll mutants induced by gamma rays and EMS. While, comparing mutagens, it has been observed in several studies that chemical mutagens were more effective than radiation doses (Prasad and Das, 1980). The present results are in strong contrast with the report 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 due to preferential action of EMS/gamma rays on genes for chlorophyll development located near the centromere. In case of combined treatment, the frequency of chlorophyll mutants was high as compared to individual doses of gamma rays and EMS. This synergetic 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 due to the lesions which arise due to the first mutagen remains intact due to inhibitory effect of 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 was supported by Goud (1967) and Blixt (1968), who indicated that genes effecting chlorophyll mutation occurred near the centric region of the chromosome where recombination 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 that is soybean (Ahire and Auti, 2015), Urdbean (Usharani and Ananda Kumar, 2015; Gahlot *et al*., 2010), green gram (Vairam *et al*., 2014), chickpea (Bhat *et al*., 2012) and grass pea (Das and Kundagrami, 2003).

In the present investigation, it was observed that all mutants showed several distinct characters. Prominent among them are mutants like tall, dwarf, early maturing and black spotted seed mutants where the morphology of the vegetative parts as well as yield were affected towards the positive side against the control populations. In the earlier, various workers viz., Wani (2011) and Bhat *et al*. (2012) in chickpea, Gahlot *et al*. (2010) in urdbean and Singh *et al.* (2000) in mungbean have also reported induction of viable mutations by the mutagens. The tallness as observed in the present study is due to an initial increase in the internodes length, sometimes accompanied by an increase in internode number (Jana, 1962).

The same author opined that increased length of the cells and their number per unit area can also contribute to tallness. Earlier tall mutants were reported by Wani (2011) and Bhat *et al*. (2012) in chickpea, Gahlot *et al*. (2010) in urdbean and Solanik *et al.* (2004) in lentil. Plants with reduced height (dwarf) were identified in Pusa-16. The mutant plants possessed small leaves, a few flowers, small pods and low grain yield. The dwarfness may be caused due to the distinction of auxins (Smith and Kerstein, 1942), interference with the synthesis of new DNA (Pelc and Howard, 1955) and reduction in the intermodal length (Kumar *et al.,* 1967). Dwarf mutants have earlier been reported by several workers Bhat *et al*. (2012), Wani (2011) and Khan *et al*. (2011) in chickpea, and Solanki and Sharma (2002) also in lentil. Early maturing mutant, obtained in the present study showed normal growth and rapid productivity. The yield and pods per plant and 100 seed weight of these mutants were also high as compared to the parent population. These results are in conformity with the earlier reports of Gopinath and Pavadai (2015), Ha *et al*. (2014), Bhat *et al*. (2012), Wani (2011), Gahlot *et al*. (2010), Solanki and Sharma (2002) and Xue *et al.* (2000). Earlier Jana (1962) explained that early maturity may be due to the physiological changes caused by irradiation and increased production of flowering hormone. Variations in seed coat color (black spotted seed), pod color (black pod) and seed size (bold seed) were also observed in the present investigation. The yield and other yield contributing character of bold seed and black spotted seed mutant were high as compared to the control populations. Variation in the seed coat color may be attributed due to the genetic factors like pigmentation factor, pigment complementary factor and modifying factors. Variation in seed coat color was earlier reported by Gopinath and Pavadai (2015) in soybean, Wani (2011) and Bhat *et al*. (2012) in chickpea, Gahlot *et al*. (2010) in urdbean. One mutant namely gigas were also recorded in the present study which was in conformity with the earlier reports of Khan *et al*. (2011) and Wani (2011). The yield and other contributing characters of this mutant were very low as compared to the control population.

In the present study, some of the above mentioned mutants were differed for more than one character from the parent cultivars. For example, a mutant has tallness as well as high number of pods per plant. Therefore, these characters may be possible due to the pleiotropic gene effects of the mutated gene and a second possibility assumes that they are controlled by several genes (each controlling a separate trait) that was very closely linked but mutated simultaneously during the mutation treatment. Because of their close linkage, the single genes of the group are not separated from each other by crossover events. Therefore, the complex of diverging characters is transferred as a unit from generation to generation, showing a monogenic inheritance in crosses.

ACKNOWLEDGEMENT

Authors are thankful to Department of Genetics and Plant Breeding, Kisan PG College, CCS University, Meerut and Division of Nuclear Research, IARI, Pusa, New Delhi for extended research and financial support.

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