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Spectrum and Frequency of Chlorophyll Mutations Induced by MMS, Gamma Rays and Their Combination in Two Varieties of *Vicia faba* L.

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Abstract: A comparative study of the frequency and spectrum of chlorophyll mutations induced by MMS (0.01, 0.02, 0.03, 0.04 and 0.05%), gamma rays (5, 10, 20, 30 and 40 kR) and their combinations (10 kR+0.01%, 10k R+ 0.02%, 10 kR+0.03%, 10 kR+0.04% and 10 kR+0.05%) in M_2 generation was made in 2 varieties of *Vicia faba* L. viz., minor and major. Six different types of chlorophyll mutants viz., albina, chlorina, maculata, tigrina, virescence and xantha were identified in the treated populations. Chlorophyll mutation frequency was calculated on plant population basis. Frequency of chlorina mutants was highest followed by xantha and other types in both the varieties. Combination treatments in general proved to be most effective followed by MMS and gamma rays in inducing maximum frequency of chlorophyll mutations (Gamma rays + MMS> MMS> Gamma rays). A significant variation in varietal response was observed. The co-efficient of interaction was less than additive, but synergistic effect was also observed.

Key words: *Vicia faba* L., MMS, gamma rays, chlorophyll mutations, varietal response

INTRODUCTION

Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops (Swaminathan *et al*., 1962; Prasad and Das, 1980; Sharma and Sharma, 1981; Reddy and Gupta, 1989; Kharkwal, 1998a; Mitra and Bhowmik, 1999; Solanki, 2005). Combined treatments of physical and chemical mutagens have been reported to alter the mutation frequency and spectrum and also result in additive and synergistic effect (Sharma, 1970; Khalatkar and Bhatia, 1975; Gautam *et al*., 1992; Singh *et al*., 1999; Wani and Anis, 2004; Bhat *et al*., 2005). The chlorophyll mutation frequency in M_2 generation is the most dependable index for evaluating the genetic effects of mutagenic treatments (Kharkwal, 1998a; Waghmare and Mehra, 2001). Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their direct or indirect exploitation in the breeding programme is an important goal of mutation research. The selection of effective and efficient mutagen(s) is very essential to recover a high frequency and spectrum of desirable mutations (Solanki and Sharma, 1994; Kharkwal, 1998b; Mahapatra, 1983). In the present investigation, the effect of gamma rays and Methylmethane Sulphonate (MMS) applied singly as well as in combination on the frequency and spectrum of chlorophyll mutations was studied in 2 varieties of broad bean, one of the most dominating pulse crop of this sub continent.

MATERIALS AND METHODS

Breeder seeds of 2 broad bean varieties viz., minor and major were obtained from the Genetics Division, IARI, New Delhi. Dry (10-12% moisture content) and healthy seeds of both the varieties were irradiated from a ^{60}Co source at Nuclear Research Division at IARI, New Delhi with a dose of 5, 10, 20, 30 and 40 kR. Another set of presoaked seeds in distilled water for 12 h was treated with MMS at different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05%) prepared in sodium phosphate buffer with 7.0 pH for 6 h with constant intermittent shaking. A portion of seeds irradiated at 10 kR gamma ray doses were also treated with MMS independently at 0.01, 0.02, 0.03, 0.04 and 0.05% for 6 h. A total of 16 treatment combinations (including control) were evaluated separately for each variety in CRBD with 3 replications during the rabi season of 2003-2004 and 2004-2005 at the Agriculture Farm,

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Aligarh Muslim University, Aligarh. Each treatment consisted of 300 seeds with 100 seeds in each plot of 3×6 m size. The seed-to-seed and row-to-row distance was maintained at 15 and 40 cm, respectively. Each M_1 plant was harvested separately and M_2 generation was raised from a composite sample of 30 seeds obtained from each M_1 harvested plant of a treatment. M_2 population was also evaluated in CRBD with 3 replications, each replication plot consisting of 500 seeds with seed-to-seed and row-to-row distance being same as in M_1 in both the varieties. The treated as well as control populations were carefully screened for various chlorophyll mutations (Gustaffson, 1940).

The interaction coefficient (K) of each combined treatment was worked out as under (Sharma and Swaminathan, 1969):

$$K = \frac{(a+b)}{(a)+(b)}$$

Where a and b stand for 2 mutagens and K is a hypothetical interaction coefficient. Any deviation from the unit value of K would reveal either a synergistic effect (K>1) or antagonistic (K<1).

RESULTS AND DISCUSSION

Based on the intensity of pigmentation at the seedling stage, 6 types of chlorophyll mutants viz., albina, chlorina, maculata, tigrina, virescence and xantha were recorded in the segregating M_2 plants in both the varieties of broad bean. Most of the chlorophyll mutant types (albina, chlorina, maculata and xantha) were lethal and survived for 25-35 days only. Maculata and tigrina types survived till the maturity, but produced very few seeds only possibly due to very high pollen sterility (75-85%). Significant variation in the spectrum and frequency of different chlorophyll mutations was recorded among the different treatments and also between the 2 varieties (Table 1). Chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segment of chromosome (Swaminathan, 1964; Goud, 1967). Mutations in these chlorophyll genes are reflected in the M_2 and subsequent generations in the form of different types of mutants. In the present study, chlorophyll mutation frequency of all the types has increased linearly with the increase in the dose of the mutagen, with the exception in combination doses where 10 kR+0.04% of MMS showed comparatively more frequency than 10 kR+0.05% of MMS in both the varieties. In case of MMS a slight reduction in chlorophyll mutation frequency was also recorded at 0.03% of MMS. Among the different mutagens used, the maximum frequency was obtained at 0.05% MMS, 40 kR gamma rays and 10 kR+ 0.04% of MMS in both the varieties. However, in case of different types of mutations, the frequency of chlorina, xantha and tigrina was comparatively high than other types in almost all the concentrations of the mutagens. Combination treatment of 10 kR with 0.01, 0.02, 0.03, 0.04 and 0.05% of MMS increased total chlorophyll mutation frequency as well as frequency of different types of chlorophyll mutants as compared to separate treatments of MMS and gamma rays. Among different combination treatments, 10 kR+0.04% of MMS induced maximum frequency of chlorophyll mutations as compared to other combination treatments in both the varieties. It seems that strong mutagens reach their saturation point even at a lower dose in the genotypes having mutable allelic sites and any further increase in the dose does not add to their mutation frequency. It has also been suggested that with the increase in the mutagen dose beyond a certain point, the strong mutagens become more toxic than higher doses of relatively weaker mutagens. Similar observations were made by Kharkwal (1998b) in chickpea and Mitra and Bhowmik (1999) in *Nigella sativa.*

The occurrence of chlorophyll mutations in the present study showed the predominance of chlorina followed by xantha and other types in both the varieties. The frequency of albina and other types was comparatively more in MMS than in gamma rays, although their highest frequency was obtained in the combination treatments. It is generally believed that ionizing radiations produce a high frequency of albina type, whereas, chemical mutagens produce higher frequency of chlorina and xantha types (Gupta and Yashvir, 1975). Kharkwal (1998a) obtained higher frequency of albina followed by chlorina and xantha among EMS treatments in chickpea. Contrary to this, in the present investigation higher frequency of chlorina followed by xantha and other types were obtained in both the varieties.

Among the mutagens, combination treatments in general induced maximum frequency of chlorophyll mutations indicating their greater effectiveness, whereas MMS proved to be more effective than gamma rays. The order of effectiveness was, therefore, gamma rays + MMS > MMS > Gamma rays in both the varieties of *Vicia faba* L. The greater effectiveness of combined treatments in inducing chlorophyll mutation has been reported by many workers (Sharma, 1970; Gautam *et al.*, 1992; Singh *et al.*, 1999). Most of the combination

Table 1: Spectrum and frequency of chlorophyll mutations in M_2 generation in *Vicia faba* L.

Treatments	No. of M_2 plants screened	Chlorophyll mutations (%) Albina	Chlorina	Maculata	Tigrina	Virescence	Xantha	Total mutation frequency (%)	Interaction coefficient (K)
Var. minor	800	-	-	-	-	-	-	-	-
Control									
MMS									-
0.01%	740	0.405	0.540	0.405	0.405	0.270	0.540	2.567	-
0.02%	730	0.410	0.684	0.410	0.410	0.273	0.547	2.739	-
0.03%	745	0.402	0.805	0.536	0.536	0.402	0.671	3.355	-
0.04%	750	0.533	0.666	0.533	0.533	0.400	0.666	3.333	-
0.05%	735	0.544	0.952	0.544	0.680	0.544	0.816	4.081	-
Gamma rays									
5kR	755	0.624	0.397	0.397	0.397	0.132	0.397	1.986	-
10kR	760	0.263	0.526	0.394	0.394	0.263	0.394	2.236	-
20kR	745	0.402	0.536	0.402	0.402	0.268	0.536	2.550	-
30kR	736	0.407	0.679	0.407	0.543	0.407	0.543	2.989	-
40kR	742	0.404	0.808	0.539	0.539	0.404	0.673	3.369	-
Gamma rays + MMS									
10kR+0.01%	746	0.536	0.670	0.402	0.402	0.268	0.536	2.815	0.586
10kR+0.02%	748	0.534	0.668	0.534	0.668	0.401	0.534	3.342	0.673
10kR+0.03%	732	0.683	0.813	0.546	0.683	0.409	0.683	3.688	0.661
10kR+0.04%	736	0.951	1.358	0.815	1.086	0.679	1.222	6.114	1.093
10kR+0.05%	744	0.806	0.940	0.672	0.806	0.537	0.806	4.381	0.686
Var. major	735	-	-	-	-	-	-	-	-
Control									
MMS									
0.01%	733	0.272	0.409	0.272	0.272	0.272	0.409	1.909	-
0.02%	765	0.261	0.522	0.261	0.261	0.261	0.522	2.091	-
0.03%	762	0.393	0.524	0.393	0.393	0.262	0.524	2.493	-
0.04%	754	0.397	0.663	0.397	0.397	0.397	0.663	2.917	-
0.05%	726	0.413	0.826	0.550	0.550	0.413	0.688	3.443	-
Gamma rays									
5kR	728	0.274	0.274	0.274	0.274	0.274	0.412	1.785	-
10kR	737	0.271	0.407	0.271	0.271	0.271	0.407	1.899	-
20kR	747	0.267	0.401	0.401	0.267	0.267	0.401	2.008	-
30kR	749	0.267	0.534	0.400	0.400	0.267	0.400	2.269	-
40kR	753	0.398	0.664	0.398	0.531	0.398	0.531	2.929	-
Gamma rays + MMS									
10kR+0.01%	756	0.398	0.529	0.396	0.396	0.264	0.396	2.381	0.625
10kR+0.02%	751	0.399	0.532	0.399	0.399	0.399	0.399	2.529	0.633
10kR+0.03%	764	0.523	0.654	0.392	0.523	0.392	0.523	3.010	0.685
10kR+0.04%	734	0.810	1.220	0.810	0.950	0.680	0.960	5.440	1.129
10kR+0.05%	758	0.659	0.791	0.659	0.659	0.527	0.659	3.957	0.740

- = Not found

treatments showed less than additive effects. However, additive and synergistic effects were also obtained at 10 kR+0.04% of MMS in both the varieties. Similar findings have been reported by several workers (Khalatkar and Bhatia, 1975; Gautam *et al*., 1992). Prasad and Das (1980) reported that if 2 different mutagens compete for the same site, the effects are completely independent of each other, then the result could be either additive or less than additive. The synergistic effect of combined treatments has been attributed by Sharma (1970) to either (i) the first mutagen treatment makes accessible otherwise non-available sites for reaction to the second mutagen or (ii) premutational lesions induced by the first mutagen became fixed on repair enzymes. Both these pathways should yield a frequency of mutations higher than the total of 2 mutagens. In the present investigation, the induction of high frequency of chlorophyll mutations may be attributed to the method of treatment and efficient scoring and handling of the mutaginized populations. High frequency of different chlorophyll mutations in var. minor as compared to var. major indicated influence of genetic background and response to the different mutagens a finding observed by most of the research workers in several crops. It is therefore, concluded that although the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations.

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REFERENCES

Bhat, T.A., A.H. Khan and P. Sahba, 2005. Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in *Vicia faba* L. Vegetos, 18: 143-145.

Gautam, A.S., K.C. Sood and A.K. Riccarria, 1992. Mutagenic effectiveness and efficacy of gamma rays, ethylmethane sulphonate and their synergistic effects in blackgram (*Vigna mungo* L.). Cytologia, 57: 85-89.

Goud, J.V., 1967. Induced polygenic mutation in hexaploid wheats Rad. Bot., 7: 321-331.

Gupta, P.K. and Yashvir, 1975. Induced mutations in foxtail millet (*Setaria italica* Beauv) 1 Chlorophyll mutations induced by gamma rays, EMS and DES Theor. Applied Genet., 45: 242-249.

Gustaffson, A., 1940. The mutation system of chlorophyll apparatus. Lund Univ. Arskr., 36: 1-40.

Khalatkar, A.S. and C.R. Bhatia, 1975. Synergistic effect of combined treatments of gamma radiation and ethyl methane sulphonate in barley. Rad. Bot., 15: 223-229.

Kharkwal, M.C., 1998a. Induced mutations in chickpea (*Cicer arietinum* L.). Frequency and spectrum of chlorophyll mutations. Ind. J. Genet., 58: 465-474.

Kharkwal, M.C., 1998b. Induced mutations in chickpea (*Cicer arietinum* L.) I. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Ind. J. Genet., 58: 159-167.

Mahapatra, B.K., 1983. Studies on comparative spectrum and frequency of induced genetic variability in green gram (*Vigna radiata* (L.) Wilczek). Unpubl. Ph.D Thesis, IARI, New Delhi.

Mitra, P.K. and G. Bhowmik, 1999. Studies on the frequency and segregation of induced chlorophyll mutations in *Nigella sativa* L. Adv. Plant Sci., 12: 125-129.

Prasad, A.B. and A.K. Das, 1980. Studies on induced chlorophyll mutations in *Lathyrus sativus* L. Cytologia, 45: 335-341.

Reddy, V.R.K. and P.K. Gupta, 1989. Induced mutations in Triticale. Frequency and spectrum of chlorophyll mutations. Indian J. Genet., 49: 183-190.

Sharma, R.P. and M.S. Swaminathan, 1969. Radiation and Radiomimetic substances. In: Mutation Breeding FAD DAE Sud., pp: 70-78.

Sharma, R.P., 1970. Increased mutation frequency and wider mutation spectrum in barley induced by combining gamma rays with ethylmethane sulphonate. Indian J. Genet., 30: 180-186.

Sharma, S.K. and B. Sharma, 1981. Induced chlorophyll mutation in lentil. Indian J. Genet., 41: 328-333.

Singh, V.P., M. Singh and J.P. Pal, 1999. Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macromutations in urdbean (*Vigna mungo* L.) Ind. J. Genet., 59: 203-210.

Solanki, I.S. and B. Sharma, 1994. Mutagenic effectiveness and efficiency of gamma rays, ethyl imine and N- nitroso-N-ethyl urea in macrosperma lentil (*Lens culinaris* Medik.). Ind. J. Genent., 54: 72-76.

Solanki, I.S., 2005. Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.). Ind. J. Genet., 65: 264-268.

Swaminathan, M.S., V.L. Chopra and S. Bhaskaran, 1962. Chromosome aberrations frequency and spectrum of mutations induced by EMS in barley. Ind. J. Genet., 22: 19-207.

Swaminathan, M.S., 1964. A comparison of mutation induction in diploids and polyploids. In: The use of induced mutations in plant breeding. Rad. Mut. Organ. FAO/IAEA Vienna, pp: 619-641.

Waghmare, V.N. and R.B. Mehra, 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Ind. J. Genet., 58: 465-474.

Wani, A.A. and M. Anis, 2004. Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in *Cicer arietinum* L. J. Cytol. Genet., 5: 143-147.