

Original Research Article**SCREENING OF CHLOROPHYLL MUTANTS INDUCED BY
INDIVIDUAL AND COMBINATION TREATMENTS OF GAMMA RAYS
AND EMS IN TWO VARIETIES OF *VICIA FABA* L.****ABSTRACT**

Chlorophyll mutations form an important part of study during the mutation breeding experiments. During a mutation breeding experiment, a breeder has to screen chlorophyll mutations grown in a particular mutant population. The present experiment was conducted to develop and screen different chlorophyll mutations by single and combination treatments of gamma rays and ethyl methanesulphonate. The screening of mutants was carried out in M₂ generation of two varieties of *Vicia faba* L. viz., vikrant and PRT-12. The seeds of both varieties were given individual doses/concentrations of gamma rays (100 Gy, 200 Gy, 300 Gy and 400 Gy), EMS (.01%, .02%, .03% and .04%) and their combinations (100 Gy + .01% EMS, 200 Gy + .02% EMS, 300 Gy + .03% EMS and 400 Gy + .04% EMS). The seeds were sown to raise the M₁ generation. The M₁ generation seeds were collected and sown in next season to raise the M₂ generation. M₂ generation was screened for different chlorophyll mutations. Different chlorophyll mutations observed were chlorina, albina, xantha, viridis, aurea, tigrina and maculata. The frequency of chlorophyll mutations increased with increasing concentrations of both individual and combination treatments. Combination treatments produced more chlorophyll mutations followed by individual concentrations/doses of EMS and gamma rays. Both individual and combination treatments induced more chlorophyll mutations in variety vikrant than in variety PRT-12.

Key words: Chlorophyll mutations, ethyl methanesulphonate, gamma rays, *Vicia faba*

INTRODUCTION

Induced mutagenesis has been successfully served as a tool to induce desired genetic variability in different plants. Chlorophyll mutation form a most important dependable index for assessing the effects of mutagens on genetic makeup of plants (Waghmare and Mehra., 2001). The potency of mutagens can be tested by induction of chlorophyll mutations by these mutagens and these chlorophyll mutations have successfully served as genetic markers regarding the response of different mutagens towards different mutagens in mutation breeding programmes (Wani *et al.*, 2011). Some chlorophyll mutations are easily detectable and are most conspicuous and are used to test the sensitivity of crop plants towards mutagens (Gustafsson., 1940). Both nuclear gene mutation and extra chromosomal mutations may result in chlorophyll mutations (Levine, 1972; Walles, 1973 and Wildman, 1973). Many authors have reported chlorophyll mutations in several crops after physical and chemical mutagenesis (Reddy and Gupta, 1989; Kharkwal,1998; Mitra and Bhowmik, 1999 and Solanki, 2005).

Vicia faba L. commonly known as faba bean is a well known pulse crop. It belongs to family fabaceae and has a diploid chromosome number 12 (i.e., $2n=12$). Faba bean is a well known ancient crop that probably served as food for Mediterranean and Near East civilizations including Romans, Greeks and Egyptians (Khursheed and Khan., 2015).

The current experiment was conducted to study the effect of individual and combination treatments of gamma rays and EMS on the frequency of different chlorophyll mutations in two varieties of *Vicia faba* L. viz., vikrant and PRT-12.

MATERIALS AND METHODS

Fresh and healthy seeds of two varieties of *Vicia faba* L. were used for this experiment. 13 sets of each variety was used for this experiment. Out of 13 sets, first four sets were given individual doses of gamma rays (100, 200, 300 and 400 Gy), another four sets were given individual concentrations of EMS (.01, .02, .03 and .04%) and the last four sets were given combination treatments of gamma rays and EMS (100 Gy + .01% EMS, 200 Gy + .02% EMS, 300 Gy + .03% EMS and 400 Gy + .04% EMS). One set was used as control. The seeds were sown in Agricultural Field, Aligarh Muslim University, Aligarh to raise the M₁ generation in the crop season of 2012-2013. The M₁ generation seeds were collected and sown in the next crop season of 2013-2014 to raise the M₂ generation. In M₂ generation, screening of different chlorophyll mutants were carried out.

Some statistical formulas used in this experiment were as:

Chlorophyll mutations were scored when the seedlings were 10-20 days old. They were identified and classified according to Gustafsson (1940). The frequency of chlorophyll mutations was calculated by the following formula:

$$\text{Mutation Frequency (\%)} = \frac{\text{Number of mutant seedlings}}{\text{Total number of M}_2 \text{ seedlings}} \times 100$$

The effect of combined treatments on chlorophyll mutations frequency was analyzed following the method of Sharma (1970):

$$\text{Coefficient of interaction (k)} = \frac{(a + b)}{(a) + (b)}$$

66 Where, $(a + b)$ = The mutation frequency induced by the two mutagens in
67 combination treatments

68 $(a) + (b)$ = The mutation frequencies induced by the two mutagens
69 when applied alone

70 k = Hypothetical interaction coefficient.

71 The value of 'k' should be one, if the interaction is additive. Any deviation from this value
72 would show synergistic or less than additive effects.

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74 **RESULTS AND DISCUSSION**

75 The current experiment was conducted to induce and screen the chlorophyll mutants in M_2
76 generation of *Vicia faba* L. Two varieties were used in this experiment as: Vikrant and PRT-12.
77 Both physical and chemical mutagen was used in this experiment. The physical and chemical
78 mutagen used were gamma rays and ethyl methanesulphonate.

79 Different chlorophyll mutations like albina, chlorina, xantha,
80 viridis, aurea, tigrina and maculate were observed in this experiment. (Plate-I & II). Albina
81 mutants survived only for few days due to complete lack of chlorophyll :Plate-I & Plate-II (Fig:
82 B). Xantha (Bright yellow leaves) and Aurea (golden yellow leaves) also survived for a few days
83 due to block in the synthesis of chlorophyll (Blixt., 1961): Plate-II (Fig: C & G). Chlorina and
84 viridis (Plate-II, Fig: E & H, I, J, K) survived till maturity while as the other chlorophyll
85 mutations like maculata and tigrina (Plate-II, Fig: D & F) survived for almost 50 days.

86 The results of the current experiment are shown in Table 1 & 2.

87 As is clear from Tables, the frequency of chlorophyll mutations increased with increasing
88 doses/concentrations of both individual and combination treatments. Combination treatments in
89 both varieties induced maximum chlorophyll mutations followed by EMS and gamma rays
90 (Gamma rays + EMS > EMS > gamma rays). Variety vikrant showed more frequency of
91 chlorophyll mutations towards mutagens than variety PRT-12.

92 Chlorophyll development are under the control of several genes
93 located adjacent to centromere (Goud., 1967; Swaminathan., 1964). The mutations in these genes
94 thus result in chlorophyll mutations in the plant. Spectrum of different chlorophyll mutations has
95 already been reported by many workers ; Sjodin (1962) in *Vicia faba*, Ambarkar (2005) in
96 chickpea, Lal *et al.*(2009) in Black gram, Khan *et al* (2005) in chickpea. The nature of mutation
97 at the molecular level can be understood by the isolation and characterization of chlorophyll
98 mutant genome (Kolar *et al.*, 2011). Some authors have described chlorophyll mutations
99 occurring due to somatic mutations (Sparrow and Woodwell, 1962). Gupta and Sharma (1990)
100 stated earlier that chemical mutagens produce generally chlorina and xantha types while as
101 ionizing radiations generally produce albino types. Different workers have studied chlorophyll
102 mutations in different plants treated with different physical and chemical mutagens. The
103 combination treatments have been found to induce more chlorophyll mutations than individual
104 doses/concentrations of mutagens (Srinivas and Veerabadhiran., 2010) The synergistic effect of
105 combination treatment has already been reported by Sharma (1970). The main causes of
106 synergistic effect of two mutagens in inducing maximum chlorophyll mutations in combination
107 treatment are: (1) The denaturation of DNA by first mutagen (Scholes and Weiss, 1962; Cox et
108 al., 1955) results in accessibility of sites for the second mutagen to react (Arnason et al.,1963).

(ii) The non-functionality of repair enzymes by the second mutagen facilitates the fixation of already induced pre-mutational lesions (Sobels., 1966). Some authors are of the view that chlorophyll mutations increase with an increase in doses/concentrations of mutagens and is independent of variety used (Thilagavathi and Mullainathan., 2009). Some authors have described chlorophyll mutations to be caused by the change in nucleotide sequences controlling the chlorophyll biosynthesis (Nair et al., 2014).

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Plate-I

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SELECTED ALBINO PLANTS FROM M₂ POPULATION DURING THE EARLY STAGES OF THEIR DEVELOPMENT

Plate-II





Plate-II

A: Control plant

B: Albina mutant (white leaves of seedling)

C: Xantha mutant (bright yellow colour)

D: Maculata mutant (Yellow patches on leaves)

E: Chlorina mutant (light green colour)

F: Tigrina mutant (patches of green dots on leaflets)

G: Aurea mutant (Golden yellow leaves of seedling)

H, I, J, K: Viridis mutant (viridine green colour)

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273 **Chlorophyll mutants**

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Table 1: Frequency and spectrum of chlorophyll mutants induced by gamma rays, EMS and their combinations in *Vicia faba* L. var. vikrant in M₂ generation.

Treatment	N*	A*	X*	T*	C*	V*	YV*	PV*	Au*	M*	CMS*	F*(%)	K [#]
Control	1290	-	-	-	-	-	-	-	-	-	-	-	-
100 Gy	1235	3	-	1	2	-	-	-	1	1	8	0.64	-
200 Gy	1205	2	3	-	1	-	-	-	1	-	7	0.58	-
300 Gy	1181	3	2	1	1	1	-	-	1	-	9	0.76	-
400 Gy	1155	2	1	1	2	2	1	1	2	1	13	1.12	-
.01% EMS	1197	2	2	3	1	1	-	-	2	-	11	0.91	-
.02% EMS	1143	2	3	2	4	2	1	2	3	3	22	1.92	-
.03% EMS	1089	4	5	3	8	3	2	3	5	-	33	3.03	-
.04% EMS	1044	6	3	2	13	4	2	1	3	5	39	3.73	-
100 Gy + .01% EMS	1074	5	2	3	10	2	1	1	5	-	29	2.70	1.74
200 Gy + .02% EMS	990	4	1	2	12	3	2	3	3	6	36	3.63	1.45
300 Gy + .03% EMS	940	5	3	3	14	4	3	5	2	-	39	4.14	1.09
400 Gy + .04% EMS	902	7	2	1	18	5	4	6	4	6	53	5.87	1.21

A*: Albina; X*: Xantha; T*: Tigrina; C*: Chlorina; V*: Viridine; YV*: Yellow viridine; PV*: Patched viridine; Au*: Aurea; M*: Maculata; CMS*: Chlorophyll mutated seedlings; F: Frequency; K[#]: Coefficient of interaction based on %M₂ chlorophyll mutation frequency.

Table 2: Frequency and spectrum of chlorophyll mutants induced by gamma rays, EMS and their

combinations in *Vicia faba* L. Var. PRT-12 in M₂ generation.

Treatment	N*	A*	X*	T*	C*	V*	YV*	PV*	Au*	M*	CMS*	F*	K [#]
Control	1348	-	-	-	-	-	-	-	-	-	-	-	-
100 Gy	1305	-	-	-	3	2	1	1	-	-	7	0.53	-
200 Gy	1292	-	2	-	4	-	2	-	1	-	9	0.69	-
300 Gy	1254	-	3	1	3	1	2	-	1	1	12	0.95	-
400 Gy	1220	2	4	-	4	2	-	-	2	2	16	1.31	-
.01% EMS	1285	2	-	2	3	1	-	-	-	-	8	0.62	-
.02% EMS	1247	3	2	1	5	1	1	-	-	2	15	1.20	-
.03% EMS	1229	2	4	2	5	2	3	2	1	-	21	1.70	-
.04% EMS	1190	3	4	3	6	4	1	2	-	3	26	2.18	-
100 Gy + .01% EMS	1210	4	1	3	5	2	2	3	2	-	22	1.81	1.57
200 Gy + .02% EMS	1165	4	-	3	7	2	3	2	-	3	24	2.06	1.08
300 Gy + .03% EMS	1098	4	5	4	8	4	5	3	2	5	40	3.64	1.37
400 Gy + .04% EMS	1028	5	6	5	10	7	4	3	2	6	48	4.66	1.33

A*: Albina; X*: Xantha; T*: Tigrina; C*: Chlorina; V*: Viridine; YV*: Yellow viridine; PV*: Patched viridine; Au*: Aurea;
M*: Maculata; CMS*: Chlorophyll mutated seedlings; F: Frequency; K[#]: Coefficient of interaction based on %M₂ chlorophyll
mutation frequency.