

Original Research Article

SCREENING OF CHLOROPHYLL MUTANTS INDUCED BY INDIVIDUAL AND COMBINATION TREATMENTS OF GAMMA RAYS AND ETHYL METHANESULPHONATE 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. This is important for a breeder to check the potency of particular mutagen in inducing mutagens. As chlorophyll is an important pigment in plant for making food during photosynthesis. So, for a breeder it is important to check the optimum dose/concentration of mutagen in inducing chlorophyll mutations in addition to other parameters. 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. Reason for higher frequency of chlorophyll

mutants in combination treatments may be due to higher induction of mutations in genes controlling chlorophyll biosynthesis 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 successfully served as a tool to induce desired genetic variability in different plants. Both physical and chemical mutagen induce mutations in different cell systems by interacting with their DNA. 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).

As chlorophyll is an important pigment used by the plant during photosynthesis. So, the moderate level of mutation tolerable by the plant can be checked by calculating chlorophyll content and chlorophyll mutations of that particular plant. Several

48 authors have reported decrease of chlorophyll content with increasing doses/concentrations of
49 mutagens (Rahimzadeh *et al.*, 2011; Verma *et al.*, 2010).

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

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

59 MATERIALS AND METHODS

60 Fresh and healthy seeds of two varieties of *Vicia faba* L. were used for this experiment. 13 sets
61 of each variety was used for this experiment. Out of 13 sets, first four sets were given individual
62 doses of gamma rays (100, 200, 300 and 400 Gy), another four sets were given individual
63 concentrations of EMS (.01, .02, .03 and .04%) and the last four sets were given combination
64 treatments of gamma rays and EMS (100 Gy + .01% EMS, 200 Gy + .02% EMS, 300 Gy + .03%
65 EMS and 400 Gy + .04% EMS). One set was used as control. The seeds were sown in
66 Agricultural Field, Aligarh Muslim University, Aligarh to raise the M_1 generation in the crop
67 season of 2012-2013. The M_1 generation seeds were collected and sown in the next crop season

68 of 2013-2014 to raise the M₂ generation. In M₂ generation, screening of different chlorophyll
69 mutants was carried out.

70 The statistical formulas used in this experiment were as:

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

74
$$\text{Mutation Frequency (\%)} = \frac{\text{Number of mutant seedlings}}{\text{Total number of M}_2 \text{ seedlings}} \times 100$$

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

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

77

78 Where, (a + b) = The mutation frequency induced by the two mutagens in
79 combination treatments

80 (a) + (b) = The mutation frequencies induced by the two mutagens
81 when applied alone

82 k = Hypothetical interaction coefficient.

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

85

RESULTS AND DISCUSSION

The current experiment was conducted to induce and screen the chlorophyll mutants in M₂ generation of *Vicia faba* L. Two varieties were used in this experiment as: Vikrant and PRT-12. Both physical and chemical mutagen were used in this experiment. The physical and chemical mutagen used were gamma rays and ethyl methanesulphonate.

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

The results of the current experiment are shown in Table 1 and 2. As is clear from Tables, the frequency of chlorophyll mutations increased with increasing doses/concentrations of both individual and combination treatments. Combination treatments in both varieties induced maximum chlorophyll mutations followed by EMS and gamma rays (Gamma rays + EMS > EMS > gamma rays). Variety vikrant showed higher frequency of chlorophyll mutations towards mutagens than variety PRT-12.

Chlorophyll biosynthesis is under the control of several genes located adjacent to centromere (Goud., 1967; Swaminathan., 1964). The mutations in these genes thus result in chlorophyll mutations in the plant. Spectrum of different chlorophyll mutations has already been reported by many workers ; Sjodin (1962) in *Vicia faba*, Ambarkar *et al.* (2005) in

chickpea, Lal *et al.*(2009) in Black gram, Khan *et al* (2005) in chickpea. The nature of mutation at the molecular level can be understood by the isolation and characterization of chlorophyll mutant genome (Kolar *et al.*, 2011). Some authors have described chlorophyll mutations occurring due to somatic mutations (Sparrow and Woodwell, 1962). These usually are the result of plastid mutations and hence they do not breed true in subsequent generations due to irregular distribution of plastid (Goud., 1967). Gupta and Sharma (1990) stated earlier that chemical mutagens produce generally *chlorina* and *xantha* types while as ionizing radiations generally produce albino types. Different workers have studied chlorophyll mutations in different plants treated with different physical and chemical mutagens. The combination treatments have been found to induce more chlorophyll mutations than individual doses/concentrations of mutagens (Srinivas and Veerabhadhiran., 2010) The synergistic effect of combination treatments has already been reported by Sharma (1970). The main causes of synergistic effect of two mutagens in inducing maximum chlorophyll mutations in combination treatment is: The denaturation of DNA by first mutagen (Scholes and Weiss, 1962; Cox et al., 1955) results in accessibility of sites for the second mutagen to react (Arnason et al.,1963). Another reason for higher frequency of chlorophyll mutants in combination treatments may be due to higher induction of mutations in genes controlling chlorophyll biosynthesis. 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 chlorophyll biosynthesis (Nair et al., 2014).

CONCLUSIONS

The present experiment was conducted to induce and screen the frequency and types of chlorophyll mutations in two varieties of *Vicia faba* L. viz., Vikrant and PRT-12. The mutagens used in this experiment were gamma rays and ethyl methanesulphonate. Both individual and combination treatments of these mutagens were used in this experiment. Chlorophyll mutants were screened in M₂ generation. Different chlorophyll mutants like *chlorina*, *albina*, *xantha*, *viridis*, *aurea*, *tigrina* and *maculata* were observed in M₂ generation. Combination treatments of gamma rays and ethyl methanesulphonate induced more frequency of chlorophyll mutants followed by individual treatments of ethyl methanesulphonate and gamma rays. The reason for higher frequency of chlorophyll mutants in combination treatments may be due to higher induction of mutations in genes controlling chlorophyll biosynthesis. Variety Vikrant showed higher frequency of chlorophyll mutants than variety PRT-12 towards treatments.

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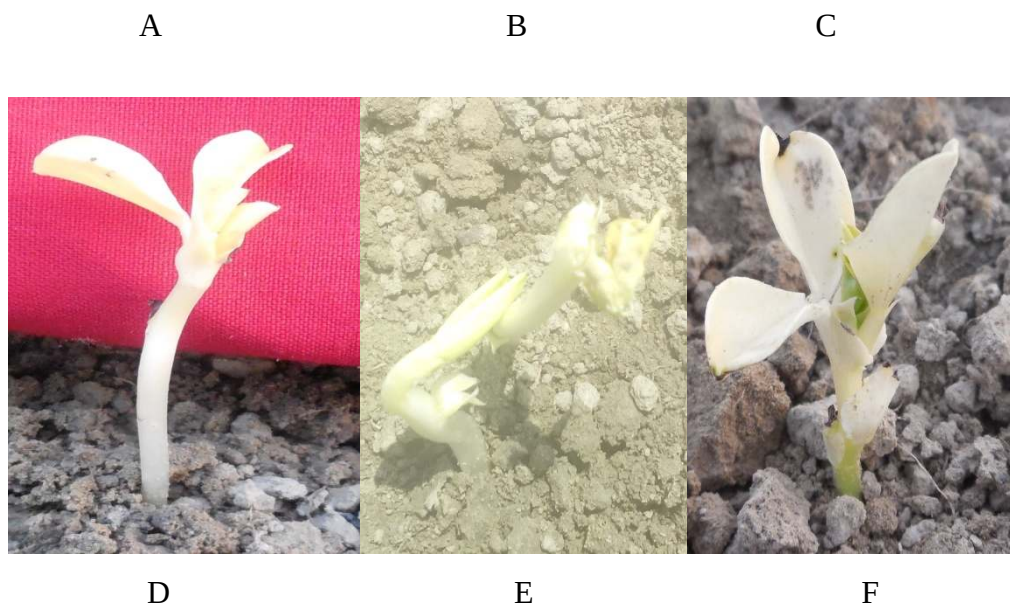
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Plate-I



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

Plate-II



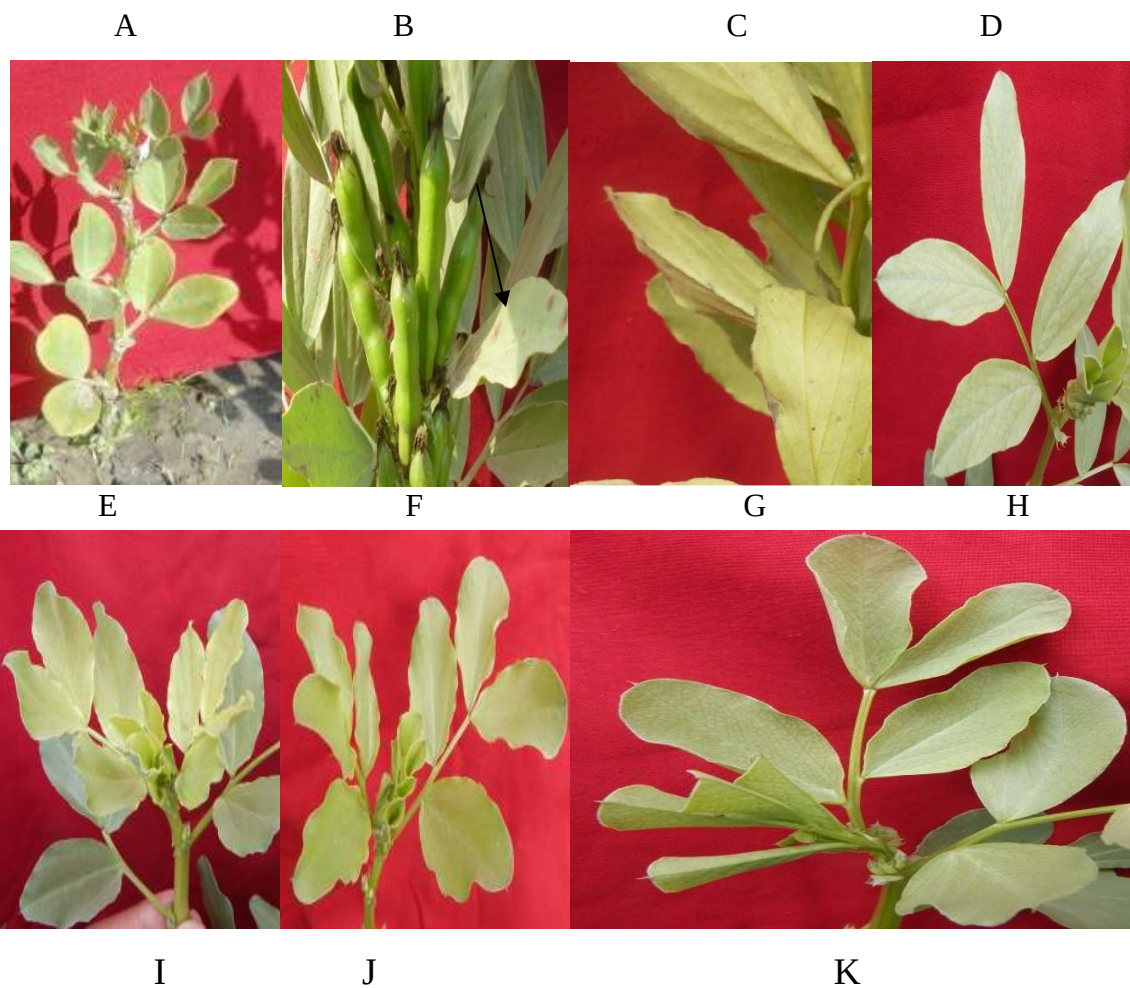


Plate-II

A: Control plant

B: Albina mutant (white leaves of seedling)

C: Xantha mutant (bright yellow colour)

291 D: Maculata mutant (Yellow patches on leaves)
292 E: Chlorina mutant (light green colour)
293 F: Tigrina mutant (patches of green dots on leaflets)
294 G: Aurea mutant (Golden yellow leaves of seedling)
295 H, I, J, K: Viridis mutant (viridine green colour)

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302 **Chlorophyll mutants**

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

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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

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

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

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

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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

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319 A*: Albina; X*: Xantha; T*: Tigrina; C*: Chlorina; V*: Viridine; YV*: Yellow viridine; PV*: Patched viridine; Au*: Aurea;

320 M*: Maculata; CMS*: Chlorophyll mutated seedlings; F: Frequency; K[#]: Coefficient of interaction based on %M₂ chlorophyll

321 mutation frequency.