

Screening of Chlorophyll Mutations in the Mutagenized Population of Two Cultivars of *Vicia faba* L.

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Authors' contributions

This work was carried out in collaboration between two authors. Author SK carried out experiment under the guidance of author S. Khan and drafted the manuscript. Author S Khan finalized the manuscript with necessary corrections.

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 a particular mutagen in inducing mutagenesis. 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 combined 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 the 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 single and combined treatments. Combined 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 single and combined treatments induced more chlorophyll mutations in variety Vikrant than in variety PRT-12.

Keywords: Chlorophyll mutations; ethyl methanesulphonate; gamma rays; *Vicia faba*.

1. INTRODUCTION

Induced mutagenesis has successfully served as a tool to induce desired genetic variability in different plants. Both physical and chemical mutagens induce mutations in different cell

systems by interacting with their DNA. Chlorophyll mutation represents the most important dependable index for assessing the effects of mutagens on genetic makeup of plants [1]. 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 [2]. Some chlorophyll mutations are easily detectable and are most conspicuous and are used to test the sensitivity of crop plants towards mutagens [3]. Both nuclear gene mutation and extra chromosomal mutations may result in chlorophyll mutations [4] [5] [6]. Many authors have reported chlorophyll mutations in several crops after physical and chemical mutagenesis [7] [8] [9] [10].

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 authors have reported a decrease in chlorophyll content with increasing doses/concentrations of mutagens [11] [12].

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 [13].

The current experiment was conducted to study the effect of single and combined 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.

2. 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 M_1 generation in the crop season of 2012-2013. The M_1 generation seeds were collected and sown in the next crop season of 2013-2014 to raise the M_2 generation. In M_2 generation, screening of different chlorophyll mutants was carried out.

The statistical formulae used in this experiment were as follows:

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:

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):

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

Where,

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

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

k = Hypothetical interaction coefficient.

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

3. RESULTS AND DISCUSSION

The current experiment was conducted to induce and screen the chlorophyll mutants in M_2 generation of *Vicia faba* L. Two varieties used in this experiment were the following: Vikrant and PRT-12. Both physical and chemical mutagens 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-1 and 2). *Albina* mutants survived only for few days due to complete lack of chlorophyll: Plate-1 & Plate-2 (Plate 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-2, C & G). *Chlorina* and *viridis* (Plate-2, E & H, I, J, K) survived till maturity while as the other

chlorophyll mutations like *maculata* and *tigrina* (Plate-2, 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 single

and combined treatments. Combined 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.

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



A



B



C



D



E



F

Plate 1. Selected albino plants from M₂ population during the early stages of their growth

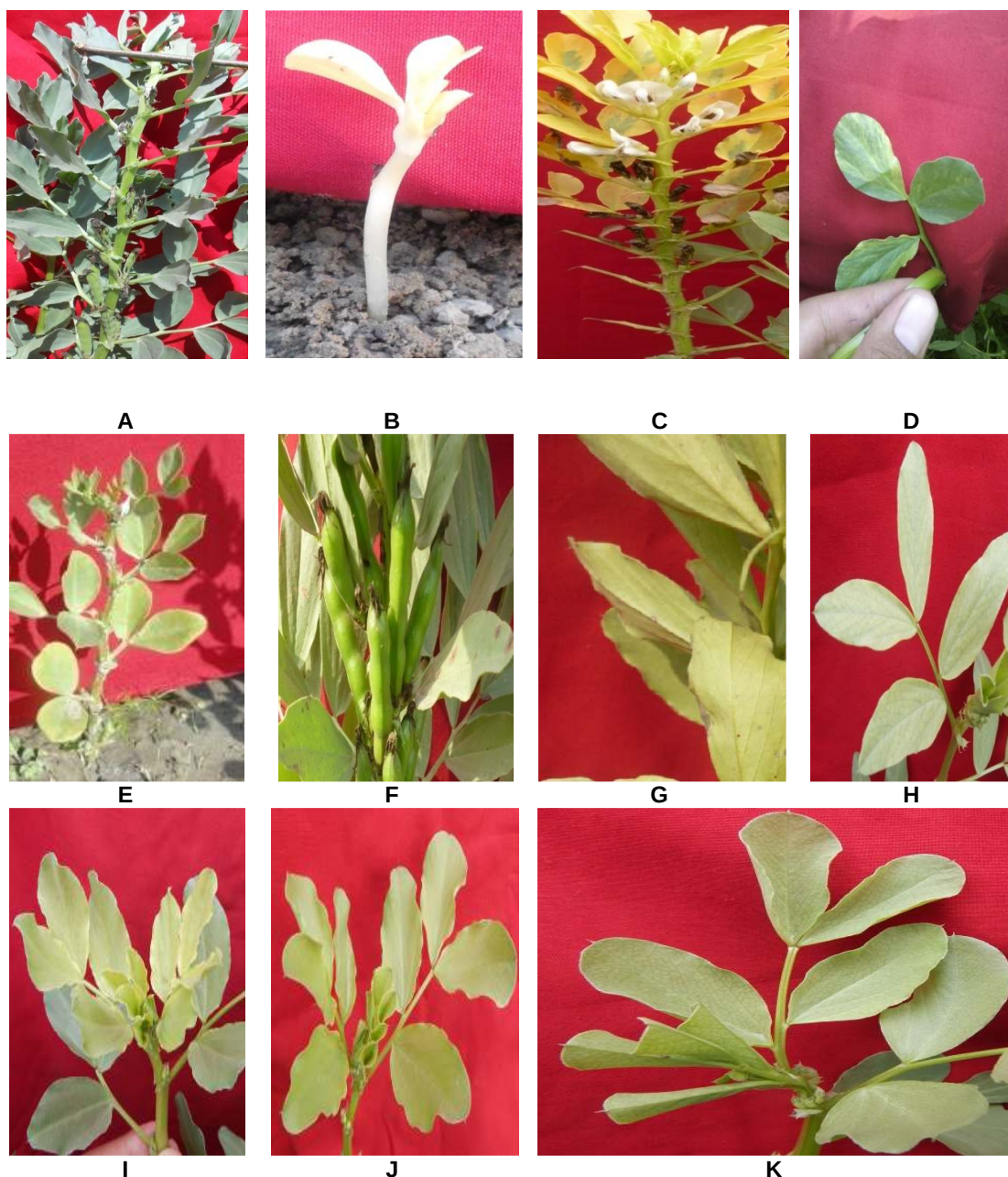


Plate 2. Chlorophyll mutants

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)

Chlorophyll biosynthesis is under the control of several genes located adjacent to centromere [14] [22] [15]. 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 ; [16]

[17] [18] [19]. The nature of mutation at the molecular level can be understood by the isolation and characterization of chlorophyll mutant genome [20]. Some authors have described chlorophyll mutations occurring due to somatic mutations [21]. These usually are the

result of plastid mutations and hence they do not breed true in subsequent generations due to irregular distribution of plastid [22]. Chemical mutagens produce generally *chlorina* and *xantha* types while as ionizing radiations generally produce albino types [23]. 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 [24]. The synergistic effect of combination treatments has already been reported by [25]. The main causes of synergistic effect of two mutagens in inducing maximum chlorophyll mutations in combination treatment are the following: The denaturation of DNA by first mutagen [26] [27] results in accessibility of sites for the second mutagen to react [28]. 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 are independent of variety used [29]. Some authors have described chlorophyll mutations to be caused by the change in nucleotide sequences controlling chlorophyll biosynthesis [30].

4. 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 single and combined 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. Combined treatments of gamma rays and ethyl methanesulphonate induced higher 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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