Studies of Induced Chlorophyll Mutations in *Lathyrus sativus* L.

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The scoring of chlorophyll mutations in M_2 generation has been proved to be the most dependable index for evaluating the genetic effects of mutagenic treatments (Gustaffson 1951). This investigations deal with the studies of frequency and spectrum of mutations induced in six varieties by gamma radiation and one variety by both differential and combined treatment of gamma radiation and 0.2% MES (Methyl Ethane Sulfonate) of *Lathyrus sativus* L ($2n=14$). One previous worker, Nerkar (1976) has also worked on *Lathyrus sativus* but on varieties other than one studied in the present investigations.

Materials and methods

Dry and dormant seeds of *Lathyrus sativus* L. var. P_{10} , P_{24} , P_{288} , P_{293} , P_{585} and LC_{78} (10% moisture) were irradiated with 10, 20, 30, 40 and 50 kR gamma rays at F. C. I., Sindri, India. A detailed description of method of irradiation and post-irradiation treatment and handling has already been described by Prasad and Das—Unpublished.

M_2 generation was raised taking ten seeds from each plant and one plant from each fertility group (0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100). The spectrum of different chlorophyll mutations were recorded per 100 M_2 plants (Gaul 1960) treatmentwise in each variety. Chlorophyll mutants were classified according to Gustaffson (1940).

Experimental results

Chlorophyll mutation frequency: Frequency of chlorophyll mutations per 100 M_2 plants (Gaul 1960) in each variety were scored and presented in histograms (Figs. 1A-G). It appears from the histograms that the chlorophyll mutation frequency increases with increasing dose of ^{60}Co in all varieties, except in P_{585} at 50 kR, where it was almost lower only to its immediate lower dose, i.e. 40 kR.

0.2% MES was found to be more effective than the lowest dose (10 kR) used in the present experiment and it was equal to 20 kR in P_{288} .

Combination treatment of different doses of gamma rays with 0.2% MES showed increase in chlorophyll mutation with increase in gamma rays doses. It means post-irradiation treatment with 0.2% MES produce additive effects.

Although response to gamma rays in different varieties differ greatly in relation to frequency of chlorophyll mutations, LC₇₆ appeared to produce more chlorophyll mutants than others.

Frequency and spectrum of chlorophyll mutants: —Frequency of chlorophyll mutant types are summarized in Tables (1A–F and 2) and different types of mutations observed are described below:—

1. Albino (A): Seedlings were white in colour and relatively smaller than the normal looking seedlings of the same age. These mutants survived for seven to ten days only.

2. Xantha (X): Seedlings were straw yellow in colour. They all showed normal growth in the beginning but started withering away on the 10th day and ultimately died within fifteen days.

3. Albo-Xantha (AX): Base of the stem and leaf of the seedlings were yellow. Stem gradually turned green. Leaves showed yellowish base and green tip in an early stage of development, but the tip gradually turned whitish. This type of mutant lived for 30 days and then died.

4. Xanthalba (XA): Seedlings were albino type in the beginning, but later on they become yellowish. Growth of the mutants were less than normal as evidenced from the seedling height. All died within fifteen days, except in P₁₀ at 10 kR. Here they survived till flowering but did not set any seeds.

5. Albo-Viridis (AV): Seedlings were quite normal in the beginning but thirty days thereafter the tip of some of the leaves become white. These mutants survived till flowering and also set seeds.

6. Virescence (V): All seedlings which became dark-green at later stage of development, were light green in the early stages. These mutants produced normal looking flower and also set seeds.

7. Chlorina (C): Seedlings were pale yellowish-green to yellowish-green. Most of the yellowish-green type died within thirty days. However, light yellowish-green types survived till maturity and set a few seeds.

8. Albescence (AS): Original green colour changed gradually to white after ten days. Although this mutant was recorded only in P₁₀ at 30 kR and in P₂₈₈ at 20 and 50 kR, none of them set any seeds.

9. Tigrina (T): This mutant had characteristic transverse yellowish and whitish bands on their leaves and had survived till maturity. They produced a few seeds.

10. Maculata (M): Seedlings showed yellow or whitish dots on their leaves and sometimes on stem too. These mutants survived till maturity and set seeds.

A perusal of the Tables (1A–F and 2) revealed that gamma rays produced the highest frequency of xantha, chlorina and virescence in all the varieties but MES alone produced maximum number of chlorina followed by xantha, virescence,

Figs. A–G. A–B, chlorophyll mutations induced after ⁶⁰Co irradiation in six varieties of *Lathyrus sativus* L. in M₂ generation. A, var. P₁₀. B, var. P₂₄. C–D, chlorophyll mutations induced after ⁶⁰Co irradiation in six varieties of *Lathyrus sativus* L. in M₂ generation. C, var. P₂₈₈. D, E–G, chlorophyll mutations induced after ⁶⁰Co irradiation in six varieties of *Lathyrus sativus* L. in M₂ generation. E, var. P₂₈₈. F, var. LC 76. G, chlorophyll mutations after differential and combined treatment of ⁶⁰Co and 0.2% MES in M₂ generation (var. P₂₈₈).

tigirina and maculata but no albino. Albino was commonly found only in gamma rays and post-irradiation treatment with 0.2% MES. However, the frequency of albino was noted to be more than the albo-viridis and albo-xantha, and less than xantha and chlorina in gamma rays treated population.

Table 1. Spectrum of chlorophyll mutations in six different varieties of *Lathyrus sativus* L.

Doses (kR)	Total number of plants scored	Mutants types									
		A	X	AX	XA	AV	V	C	AS	T	M*
(A) P ₁₀											
10	100	1	2	—	1	—	2	—	—	—	—
20	130	2	3	—	2	—	1	2	—	—	—
30	120	1	2	1	1	—	—	3	1	1	1
40	100	—	3	1	1	3	1	3	—	2	—
50	97	1	3	—	2	2	2	3	—	2	1
(B) P ₂₄											
10	105	2	1	—	2	—	1	—	—	1	—
20	100	2	2	—	1	1	1	2	—	—	—
30	189	1	3	—	1	2	2	3	—	1	1
40	98	2	1	2	1	—	3	2	—	3	1
50	100	4	3	—	1	—	2	4	—	2	2
(C) P ₂₈₈											
10	160	—	1	—	1	2	1	—	—	—	—
20	180	2	3	—	2	—	3	1	—	—	—
30	130	1	3	1	—	1	3	2	1	1	3
40	150	2	2	1	1	3	2	4	—	2	1
50	110	1	4	2	2	4	2	2	3	1	1
(D) P ₂₉₃											
10	130	1	2	—	1	—	1	1	—	—	—
20	140	—	3	—	2	—	2	1	—	2	—
30	150	2	1	1	—	—	2	3	—	2	4
40	130	2	3	2	2	—	3	4	—	3	1
50	97	1	4	3	2	1	2	2	—	2	4
(E) P ₅₈₅											
10	109	2	1	1	1	—	2	1	—	1	—
20	125	—	2	1	2	—	2	2	—	—	2
30	104	1	3	1	3	—	2	5	—	2	2
40	150	2	5	1	1	—	—	6	—	2	7
50	98	2	4	1	3	2	1	4	—	1	5
(F) LC ₇₆											
10	140	2	3	—	1	—	1	1	—	—	—
20	130	1	4	1	2	—	2	5	—	—	—
30	140	1	2	—	2	—	2	5	—	2	5
40	100	1	5	—	3	3	2	7	—	2	4
50	103	3	6	1	4	—	1	7	—	3	6

Table 2. Spectrum of chlorophyll mutations in var. P₂₈₈ after differential and combined treatment of 0.2% MES + ⁶⁰Co

0.2% MES 160	—	2	—	—	—	2	5	—	1	1
10 kR + MES 150	1	4	1	—	1	1	6	—	3	2
20 kR + MES 130	3	4	—	—	—	1	9	—	3	3
30 kR + MES 140	4	7	—	—	2	1	6	—	4	—
40 kR + MES 155	1	5	1	1	2	2	7	—	2	7
50 kR + MES 160	3	6	—	—	1	4	7	—	3	6

* A—Albino, X—Xantha, AX—Alboxantha, XA—Xanthalba, AV—Albo-viridis, V—Virescens, C—Chlorina, AS—Albescens, T—Tigrina, M—Maculata.

The spectrum of chlorophyll mutation showed different frequency in different genotypes. The mutant (pooled data) on the basis of their occurrence in different genotypes could be arranged in the following sequences in decreasing proportion:

Variety	Mutagen	Sequence in decreasing order
P ₁₀	⁶⁰ Co	X→C→XA→V→ {A→ {M→ AS {T {AX
P ₂₄	„	A→X→V→XA→M→AV C
P ₂₈₈	„	X→V→C→AV {A→ {AX {XA {AS {T M
P ₂₉₃	„	X→C→ {V→M→ {A→AV {T {AX XA
P ₅₈₅	„	C→M→X→XA→A→V→ {AX→AV {T
LC ₇₆	„	C→X→M→XA→ {A→AV→AX {V
P ₂₈₈	0.2% MES	C→ {X→ {T {V {M
P ₂₈₈	⁶⁰ Co + 0.2% MES	C→X→M→T→A→V→AV→AX→XA

Discussion

Dose dependent increase in the mutation frequency has been reported by Blixt (1964, 1966a) and Nerkar (1976). In the present studies, the frequency of chlorophyll mutations recorded in M₂ generation was found to be concomitant with doses. However, this trend was maintained in all the genotypes except in P₅₈₅ where at 50 kR it was noted to be lower than 40 kR but higher than rest of other doses.

Several workers have reported higher mutation frequency in legumes following treatments with chemical mutagens as compared to physical mutagens (Blixt *et al.* 1963, Wellensick 1965, Monti and Donini 1968). In the present investigations 0.2% MES caused more chlorophyll mutations (cf. Ramana and Natrajan,

1965) than 10 kR and equal to 20 kR gamma rays in P_{288} . It seems, therefore, that 0.2% MES is more effective than 10 kR and is equal to 20 kR as regards its mutagenic efficiency.

Two mutagens acting in sequence are known to produce additive effects on mutation frequency. This could be expected, if, for example one of the mutagens exposes the previously protected sites for the action of the second mutagens. In case both mutagens compete for the same site, the effects are completely independent of each other. It could either be additive or less than additive. The combined effect of many chemical mutagens have been demonstrated to be more than additive for chlorophyll mutations (Arnason *et al.* 1963, Favret 1963). Aasveit (1968) found that combination treatment of gamma rays and EMS gave less than additive frequency of chlorophyll mutations as measured on progeny basis and more than additive effects when estimated on M_2 population basis. The present studies indicate that the combination treatment of gamma rays and MES enhances chlorophyll mutation than their separate treatments.

The varieties also differ in their response to mutagens as measured by estimating the mutation frequency on the basis of M_2 population. For example, LC_{76} gave maximum number of chlorophyll mutations following gamma rays treatment than the other varieties indicating that LC_{76} is more radiosensitive than other varieties for this character. The mutation frequency and spectrum of mutations have been reported to be controlled by the nature of mutagens as well as genotypic architecture of the varieties in peas by Sidorova (1966). Despite the fact that different frequencies of similar mutations are induced by different mutagens, the chief limiting factor in the induction and recovery of mutations is the genetic constitution of the experimental material (Gregory 1965).

Summary

Dry and dormant seeds (10% moisture) of *Lathyrus sativus* L. var. P_{10} , P_{24} , P_{288} , P_{293} , P_{585} and LC_{76} were irradiated with 10, 20, 30, 40 and 50 kR gamma rays. Both control and irradiated seeds of variety P_{288} was treated with 0.2% MES for six hours. Albino, Xantha, Albo-Xantha, Xanthalba, Albo-viridis, Virescence, Chlorina, Albescence, Tigrina and Maculata were the general types of chlorophyll mutations observed in all the six varieties at M_2 generations.

The spectrum of chlorophyll mutations was found to be dependent on the nature of genotypes. This was further noted to have linear relationship with doses. Maximum chlorophyll mutations were observed in LC_{76} which was proved to be more sensitive than rest of other varieties.

High frequency of mutations was recorded in P_{288} as a result of differential and combined treatment of 0.2% MES which was seen to be as effective as 20 kR gamma rays.

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