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MUTATION STUDIES IN MUNG BEAN (*PHASEOLUS AUREUS* ROXB.)*

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Abstract

The effects of gamma rays and ethyl methane sulfonate (EMS) in single and combination treatments were studied on seed germination, seedling growth, survival, pollen and seed fertility, recovery index (RI) in M1, and frequency and spectrum of chlorophyll mutations in M2. The percentage of germination, growth of seedlings and survival decreased with an increase in dose of the mutagens used. Combination treatments caused more drastic effects than the single treatments. A linear dependence of pollen and seed fertility on dosage was evident in both the varieties, G-65 and PS-16. The 30kR of gamma rays and 0.3% of EMS treatments were found to be biologically equivalent doses because they induced almost equal amounts of seedling growth depression. In M2 generation, both single and combination treatments induced chlorophyll mutants but the frequencies were different. The frequency of chlorophyll mutation does not appear to be dependent on the dose. However, the mutation rate increased slightly in the combination treatments. In combination treatments, the chlorophyll mutation frequency showed negative synergism. The frequency of chlorophyll mutation was found to depend not only on the type of mutagen used and dose applied but also on the genotype of the material.

Introduction

The present investigation was undertaken to assess the effects of single and combination treatments of gamma rays and ethyl methane sulfonate (EMS) on the different parameters of the two varieties, G-65 and PS-16 of mung bean.

Materials and Methods

Dry seeds of uniform size of two varieties, viz., G-65 (released at Punjab

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Agricultural University, Ludniana) and PS-16 (released at Indian Agricultural Research Institute, New Delhi) were presoaked in distilled water for 9 hr. prior to treatment with four concentrations of EMS ranging from 0.1 to 0.4%. Moisture content of seeds at the time of treatment was 12.60% and 12.79% for the varieties G-65 and PS-16 respectively. The EMS solution was prepared using standard buffer of pH 7. The presoaked seeds were soaked in freshly prepared solution of EMS of each concentration for 6 hr at a constant room temperature of 27±1°C. Control treatments were maintained by treating the seeds with buffer solution.

For combination treatments, dry seed of both varieties were irradiated with 30 kR acute exposure of gamma rays at the dose rate of 40,676 rads per hour. For post-irradiation treatments with EMS, the irradiated seeds were presoaked in distilled water for 9 hr prior to treatment. One lot of 250 presoaked irradiated seeds was treated with different concentrations of EMS. The presoaked seeds of 30 kR gamma rays were treated as checks for the combination treatments. The duration of treatment was 6 hr as in the single treatment.

After completion of the treatments, the treated seeds were washed thoroughly for half an hour in running tap water and sown in the petri-dishes as well as in the field. The M1 generation was studied for the following characteristics to bring out the relative effects of the single and combination treatments: 1. germination and seedling height on 10th day, 2. survival at maturity and 3. pollen and seed fertility. The mean values of 30 plants (10 from each block) selected at random were considered for seedling growth studies. A total of 20 to 30 young flower buds from each treatment was collected for the pollen fertility studies in both the varieties. The seed fertility was calculated on the total population basis for each treatment. Chlorophyll mutations were scored in the M2 generation when the seedlings were 8 to 15 days old. Only 40 progenies were screened in all the single and combination treatments. The classification of various chlorophyll types has been given using the terminology of Gustafsson (1940) and Blixt (1961).

The interaction effect of combination treatments on mutation frequency was calculated by the formula: $K = (a+b)/(a)+(b)$, where (a) and (b) are the mutation frequencies induced by the two mutagens when applied singly, whereas $(a+b)$ is the mutation frequency produced by the two mutagens in combination treatments and "K" is the hypothetical interaction coefficient (Sharma, 1970). If the value of "K" is one, the interaction is additive. Synergistic interaction may alter the value in positive or negative direction. The frequency of chlorophyll mutations was calculated according to the method suggested by Gaul (1957).

"Number of mutants/100 M2 plants"

Recovery index (RI) of single and also of combination treatments in M1 generation was calculated using the formula: $\bar{X} \times S$, where $\bar{X}$ is the mean seedling height on the 10th day and "S" is the corresponding percentage of survival in the same treatment.

Results and Discussion

The germination and survival in both of the varieties decreased with the increased dose of the mutagen used in single and combination treatments (Table 1, 2). And the seed germination and final survival in combination treatment were found to be more adverse than those of single treatments. This may be due to a negative synergistic effect by enhanced toxicity. A decrease in percentage of seed germination and final survival has also been reported by Chang and Hsieh (1957), Bhaskaran and Swaminathan (1961), Khan and Saheal (1981), Goud *et al.* (1970), Ramulu (1970), Chakrabarti (1975). The height of seedlings decreased with the increased dose of the mutagen used either singly or in combination (Table 2). However, in combination treatments, the seedling injury is positively correlated with dose, thus indicating that the combination treatments showed an enhanced effect as compared with single treatments (Table 2). The changes induced by mutagens in the two varieties as regards to seedling height reduction may be expressed as follows:

1. Root was more sensitive than shoot with chemical mutagen.
2. Shoot was more sensitive than root with gamma rays.

The extent of decrease in seedling height with the increased dose of

Table 1. *Effect of single and combination treatments on germination percentage*

Treatments	Germination-actual %	(Var. G-65) % control	Germination-actual %	(Var. PS-16) % control
Buffer control	89.33	100.00	88.00	100.00
0.1% EMS	87.33	97.76	88.00	100.00
0.2% EMS	84.00	94.03	86.00	97.73
0.3% EMS	84.00	94.03	81.33	92.42
0.4% EMS	79.33	88.81	79.33	90.15
30 kR	85.33	95.52	84.67	96.22
30 kR + 0.1% EMS	80.00	88.56	80.67	91.67
30 kR + 0.2% EMS	74.00	82.84	77.33	87.88
30 kR + 0.3% EMS	70.76	79.11	70.67	80.31
30 kR + 0.4% EMS	66.67	74.63	65.33	74.24

Table 2. *Effect of single and combination treatment on different parameters in M1 generation*

Treatments	Root length Mean±S. E.	Injury (%)	Shoot length Mean±S. E.	Injury (%)	Seedling height		Survival % control	Recovery index	Index (RI) % control
					Mean	% control			
(Var. G-65)									
Buffer control	8.1±0.35	—	23.1±0.66	—	31.2	100.0	100.0	3,124.3	100.0
0.1% EMS	7.9±0.35	2.5	21.2±0.58	8.2	29.1	93.3	87.5	2,553.2	81.7
0.2% EMS	7.5±0.31	7.4	21.0±0.39	9.1	28.5	91.7	75.0	2,146.5	68.7
0.3% EMS	6.6±0.27	18.5	19.3±0.48	16.4	25.9	83.0	62.5	1,624.1	52.0
0.4% EMS	7.3±0.27	9.9	17.1±0.55	26.0	24.4	78.5	21.8	1,761.5	56.4
30 kR	7.9±0.16	2.5	19.9±0.37	13.9	27.8	89.1	92.5	2,578.5	82.5
30 kR + 0.1% EMS	6.1±0.23	24.7	17.5±0.37	24.2	23.6	76.0	77.5	1,839.5	58.9
30 kR + 0.2% EMS	4.9±0.14	39.5	16.6±0.37	28.1	21.5	69.2	82.5	1,783.0	57.1
30 kR + 0.3% EMS	4.7±0.16	42.0	16.8±0.35	27.3	21.5	69.2	67.5	1,462.0	46.8
30 kR + 0.4% EMS	4.7±0.13	42.0	15.7±0.40	32.0	20.4	65.4	50.0	1,023.2	32.7
(Var. PS-16)									
Buffer control	8.9±0.19	—	22.2±0.62	—	31.1	100.0	100.0	3,120.6	100.0
0.1% EMS	6.5±0.42	27.0	19.5±0.64	12.2	26.0	83.3	100.0	2,609.6	83.6
0.2% EMS	7.2±0.34	19.1	18.9±0.66	14.9	26.1	83.6	96.6	2,530.5	81.1
0.3% EMS	7.1±0.46	20.2	19.7±0.64	11.3	26.8	85.9	63.3	1,703.6	54.6
0.4% EMS	6.0±0.32	32.6	17.0±0.63	23.4	23.0	73.7	76.6	1,766.5	56.6
30 kR	7.0±0.33	21.3	20.2±0.40	9.0	27.2	87.5	88.0	2,402.4	77.0
30 kR + 0.1% EMS	6.8±0.23	23.6	19.4±0.42	12.6	26.2	84.3	77.3	2,039.4	65.3
30 kR + 0.2% EMS	6.0±0.30	32.6	17.9±0.51	19.4	23.9	76.6	90.6	2,175.5	70.0
30 kR + 0.3% EMS	5.5±0.36	38.2	15.7±0.41	29.3	21.2	65.1	58.6	1,250.8	40.1
30 kR + 0.4% EMS	4.9±0.58	45.0	15.2±0.53	31.5	20.1	67.6	50.6	1,020.1	32.7

mutagen was not the same in the two varieties. This kind of differential response has also been reported by Bhatia (1960), Nirula (1961) and Kawai and Sato (1975). It was also noticed in the present study that 0.3% EMS and 30 kR of gamma rays induce almost equal amounts of seedling growth depression in M1 generation and, hence, they may be considered as biologically equivalent doses (Table 2).

The regression and correlation were calculated to ascertain the relationship between the root and shoot lengths for the single and combination treatments (Table 3). The root and shoot were found to be highly correlated in the combination treatments for the two varieties, G-65 and PS-16. These two variables are positively correlated.

Pollen and seed fertility

Pollen and seed fertility decreased with the increased doses of single and combined treatments of gamma rays and EMS, revealing a linear dependence of fertility on dose. The combination treatments caused more drastic effects on fertility than the single treatments (Table 4). The dependence of fertility on dose was also reported by Chang and Hsieh (1957), Yamaguchi (1963), Sharma (1970), Chakrabarti (1975) and Khan and Hashim (1978). Gamma rays are known to produce chromosomal aberrations in contrast to EMS which produces more of genic changes. Sato and Gaul (1967), while studying the effect of EMS induced sterility in barley, reported that the seed sterility occurred with a low frequency of translocation or due to cryptic chromosome deletions and ruled out that genetic change is a cause of seed sterility. They argued that the seed sterility induced by EMS is too large to be caused by gene mutations alone. In the present study, the differential effect of chemical mutagen on fertility may be due to the low frequency of gross structural changes and other meiotic disturbances. According to Katayamma (1963), the pollen and seed fertility correspond approximately to the frequency of translocations showing adjacent segregation.

Table 3. *Relationship between root and shoot length* (*r-values*)

Variety	Mutagens	Regression equation	Correlation coefficient(r)
G-65	EMS	Y = + 1.1302 + 2.5671 X	+0.6793
G-65	Gamma rays + EMS	Y = + 11.1167 + 1.0890 X	+0.9657**
PS-16	EMS	Y = + 8.5118 + 1.5315 X	+0.9174*
PS-16	Gamma rays + EMS	Y = + 2.6372 + 2.4801 X	+0.9809**

* Significant at P=0.01 level.
** Significant at P=0.001 level.

Table 4. *Effect of single and combination treatments on pollen and seed fertility*

Treatments	Fertility (Var. G-65)		Fertility (Var. PS-16)	
	Pollen	Seed	Pollen	Seed
Buffer control	100.00	100.00	100.00	100.00
0.1% EMS	86.33	81.90	87.81	80.59
0.2% EMS	80.30	79.12	82.40	77.06
0.3% EMS	71.65	76.29	75.95	75.40
0.4% EMS	70.67	73.72	73.59	73.36
30 kR	83.23	83.31	83.52	84.68
30 kR + 0.1% EMS	77.53	76.49	78.28	78.11
30 kR + 0.2% EMS	71.18	75.57	72.14	73.83
30 kR + 0.3% EMS	63.60	33.76	61.15	73.34
30 kR + 0.4% EMS	62.17	69.72	52.95	72.69

Chlorophyll mutations in M2

A comparison of the chlorophyll mutation frequency indicates that the mutation rate, in general, increased in the combination treatments with the increase of seedling injury (Table 5). The two varieties differed considerably in their mutagenic response as evidenced from the germination, seedling growth, survival at maturity and fertility. The spectrum of different types of chlorophyll mutants included: *albina*, *xantha*, *chlorina*, *viridis*, *chlorotica*, and *virescent* was recorded in the single and combination treatments of the two mutagens.

A synergistic effect may cause if the sites of action protected during treatment with first mutagen are exposed to the action of the second (Arnason *et al.* 1963). An additive effect or even lowering of the effect may result, in case their actions are independent or the two mutagens compete for the same site (Aastveit, 1968). In the present study, less than additive effect was observed in the combination treatments, because of the fact that both the mutagens compete for the same site. Our results confirm the findings of Reddy *et al.* (1973) in rice. Thus, it is obvious that combination of mutagenic agent is another useful method for explaining the mechanism of their action.

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Table 5. *Frequency and spectrum of chlorophyll mutations in M2 generation*

Treatments	Relative percentage of different types of M2 Chlorophyll mutants						Total frequency (%)	Coefficient of interaction (K)
	Albina	Chlorina	Xantha	Viridis	Chlorotica	Virescent		
Var. (G-65)								
Buffer control	—	—	—	—	—	—	—	—
0.1% EMS	38.46	23.08	30.77	7.69	—	—	9.52	—
0.2% EMS	14.63	29.27	34.15	14.63	4.88	2.44	16.21	—
0.3% EMS	24.24	39.39	21.21	6.06	9.09	—	14.16	—
0.4% EMS	16.28	39.53	20.93	11.63	6.98	4.65	17.99	—
30 kR	13.64	27.27	31.82	18.18	9.09	—	12.15	—
30 kR + 0.1% EMS	25.00	30.56	27.78	—	16.67	—	17.22	0.79
30 kR + 0.2% EMS	26.32	31.58	18.42	13.16	—	8.53	18.54	0.65
30 kR + 0.3% EMS	23.53	31.37	23.53	11.76	9.80	—	23.53	0.91
30 kR + 0.4% EMS	20.51	30.27	12.82	17.95	10.26	7.69	19.60	0.65
Var. (PS-16)								
Buffer control	—	—	—	—	—	—	—	—
0.1% EMS	18.52	37.04	22.22	14.81	7.41	—	10.15	—
0.2% EMS	31.25	37.05	25.00	—	3.13	3.13	12.65	—
0.3% EMS	20.69	34.38	31.03	13.79	—	—	13.55	—
0.4% EMS	34.48	37.93	20.69	—	6.90	—	16.02	—
30 kR	17.39	56.52	17.39	8.70	—	—	10.04	—
30 kR + 0.1% EMS	17.65	29.41	32.35	8.82	8.82	2.94	18.38	0.91
30 kR + 0.2% EMS	9.76	31.71	29.27	14.63	14.63	—	19.60	0.84
30 kR + 0.3% EMS	19.85	38.71	35.48	—	6.45	—	16.49	0.70
30 kR + 0.4% EMS	15.15	36.36	30.30	18.18	—	—	16.84	0.65

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綠豆突變之研究

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以伽瑪射線或以 Ethyl Methane Sulfonate (EMS) 等誘變劑單獨或聯合處理綠豆，再研究其對種子發芽、幼苗生長、存活、花粉或種子授粉有效性，突變第一代 (M1) 回收指數 (Recovery Index, RI)，和突變第二代 (M2) 的葉綠素突變之頻度及其範圍之影響。種子之萌芽率、幼苗之生長及存活率隨着突變劑處理劑量之增加而相對降低。聯合處理比單一劑量處理所產生之作用更爲劇烈。在綠豆二品種 G-65 和 PS-16，花粉或種子授粉有效性和誘變劑量間有直線性之關係。以劑量 30 kR 的伽瑪射線或以 0.3% 的 EMS 處理對綠豆幼苗產生相同的抑制作用，故由所謂"生物劑量"而言，兩者作用大略相同。在突變二代 (M2) 單一或聯合二種突變劑處理皆可產生葉綠素變異，但是突變頻率有異。葉綠素突變之頻率似乎和劑量無關。然而聯合處理則比單一處理其突變率略有增加。但是，聯合處理對葉綠素之誘變率則却有相尅之作用。葉綠素之突變不僅和所應用之突變劑之種類、劑量、並且和實驗材料之遺傳型有關。