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Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in mungbean

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Abstract : Mutation frequency, effectiveness and efficiency of gamma rays, ethyl methane sulphonate individually and in their combination treatments were assessed in mungbean cultivars PDM 11 and PDM 54. The study revealed that ethyl methane sulphonate (EMS) was found to be more effective in inducing mutations in both the cultivars than gamma rays. However EMS was also found more efficient than gamma ray treatment. The efficiency of combination treatments was also higher than the gamma rays and EMS alone. There was a progressive increase in mutation frequency of chlorophyll and viable mutations like injury, pollen sterility and reduction in ovule fertility with the increase in doses of the mutagen. Both mutagenic effectiveness and efficiency were found to be higher at higher doses of gamma rays and ethyl methane sulphonate. Varietal preference to mutagens was also noticed in the present study.

Key words : Mutagens, gamma rays, ethyl methane sulphonate, pollen sterility, ovule sterility, mutation frequency.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is an important pulse crop of India having a good nutritional quality and rich source of vegetarian protein. Mutation breeding is relatively a quicker method for improvement of crops. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens. The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. Mutagenic effectiveness denotes the frequency of mutations induced by a unit dose of a mutagen, while mutagenic efficiency is a measure of the proportion of mutations in relation to undesirable changes like, lethality, sterility, injury, survival *etc*. When the combinations of gamma rays and ethyl methane sulphonate (EMS) were used it resulted in synergistic as well as antagonistic effects. An experiment was undertaken to assess the relative effectiveness and efficiency of gamma rays and ethyl methane sulphonate (EMS) in the grain legume, mungbean and the results presented.

Materials and methods

The two established and promising cultivars of mungbean namely, PDM 11 & PDM 54 with superior agronomic characters were employed as the experimental material and treated with the gamma rays, ethyl methane sulphonate and their combinations. The pure, healthy, uniform sized, dry seeds of both the varieties were used for the treatment.

Table 1. Effect of different doses of mutagens on pollen sterility and ovule fertility.

Mutagenic treatments	Pollen sterility as per cent of control		Ovule fertility as per cent of control	
	cv. PDM11	cv. PDM 54	cv. PDM 11	cv. PDM 54
Gamma rays (kR)				
10	14.64	8.95	87.72	88.57
20	21.07	17.69	81.46	81.27
30	31.16	27.14	73.56	74.34
40	49.09	37.54	70.61	65.29
EMS (M)				
0.01	16.39	6.87	73.56	82.11
0.02	26.26	25.57	68.97	76.95
0.03	40.97	41.40	62.23	73.42
0.04	48.04	50.36	51.11	71.22
Combination treatment (Gamma rays + EMS)				
10kR+0.02M	22.48	22.06	63.19	73.20
20 kR+ 0.02 M	34.39	31.01	57.51	61.33
30kR+0.02M	47.97	50.50	48.34	57.47
40kR + 0.02 M	56.58	56.09	39.67	45.51

Methods of Treatment:

Gamma rays:

The 300 uniform sized, dry seeds with 12 per cent moisture mungbean varieties namely, PDM 11 and PDM 54 were exposed to gamma rays at 10, 20, 30 and 40 kR doses (with irradiation source of ^{60}Co gamma cell with capacity to release 3000 Ci delivery 7200r/ min) at Indian Agricultural Research Institute, New Delhi.

Ethyl methane sulphonate :

Four samples comprising 300 seeds each of the cultivars were presoaked in distilled water for six hours and then treated with freshly prepared ethyl methane sulphonate concentration of 0.01, 0.02, 0.03 and 0.04 M. The treatment was carried out at room temperature (23±1°C) with intermittent shaking of the container. The graded EMS were freshly prepared in phosphate buffer (pH 7.0). The seeds were completely submerged in the solution for six hours, then washed thoroughly for one hour in running tap water to terminate the reaction of the chemicals.

Combination treatments of Gamma rays and EMS:

The seeds of each cultivar were irradiated with gamma rays at 10, 20, 30 and 40 kR doses as described above. Then these gamma ray irradiated seeds were presoaked in distilled water for 6 hrs and then treated with 0.02 M EMS solution. The procedure for EMS treatment was applied same as described above.

After the completion of the treatment with gamma rays, EMS, and their combinations alongwith untreated seeds of both cultivars treated as controls were immediately sown

Table 2. Frequency, effectiveness, efficiency of physical and chemical mutagens and their combination in M_2 generation of Mungbean cv. PDM 11.

Mutagenic treatment	Number of M_1 plant progenies	Number of plants in M_2	Number of M_1 Plant progenies mutated	Number of M_2 plants mutated	Mutation frequency		Effectiveness		Efficiency		Synergism on % M_2 plants mutated
					Number of M_1 plant progenies mutated (Mp)	M_2 plants mutated (Ms)	Mp/dose	Ms/dose	Ms/Ps	Ms/Of	
Gamma rays (kR)											
10	94	1776	6	11	06.383	0.619	0.638	0.062	0.064	0.007	-
20	86	1679	11	29	12.791	1.727	0.640	0.086	0.065	0.021	-
30	77	1480	19	39	24.675	2.635	0.823	0.088	0.068	0.036	-
40	61	1232	23	46	37.705	3.734	0.943	0.093	0.071	0.053	-
EMS (M)											
0.01	97	1537	7	10	07.216	0.651	120.275	10.884	0.085	0.009	-
0.02	88	1389	13	34	14.773	2.448	123.106	20.398	0.088	0.035	-
0.03	73	1462	17	55	23.288	3.762	129.376	20.900	0.091	0.060	-
0.04	64	1167	23	61	35.938	5.227	149.740	21.779	0.102	0.102	-
Combination treatment (Gamma rays + EMS)											
10 kR + 0.02M	89	1480	14	36	15.730	2.432	13.109	2.027	0.097	0.038	1.915
20 kR+ 0.02 M	78	1255	21	56	26.923	4.462	11.218	1.859	0.119	0.078	1.069
30kR + 0.02 M	71	1233	27	79	38.028	6.407	10.563	1.780	0.117	0.132	1.002
40kR + 0.02 M	62	1082	29	76	46.774	7.024	9.745	1.463	0.120	0.177	0.784

in the field to raise the M_1 generation in a randomized block design (RBD) with three replications. Survival of plant was recorded on 30th day and was expressed as percentage of control. Pollen sterility in fifteen buds and ovule fertility from the pods at the time of maturity were recorded. Randomly marked 30 plants each in both the varieties were harvested separately in each treatment.

For raising of M_2 generation the seeds of randomly selected plants of M_1 generation were space planted in the field in three-meter long single row in three replications. M_2 generation was screened for chlorophyll and viable mutations. Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.* (1965).

I. Mutagenic Effectiveness =
(Physical mutagens)

$$\frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Dose in kilo Roentgen (kR)}}$$

Mutagenic Effectiveness =
(Chemical mutagens)

$$\frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Concentration of mutagen (mM) x Time of Treatment}}$$

Mutagenic Effectiveness =
(Combination Treatment)

$$\frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{Dose in kilo Roentgen (kR) x Concentration of mutagen (mM) x Time of Treatment}}$$

II. Mutagenic Efficiency =

$$\frac{\text{Mutation frequency on the basis of } M_1 \text{ plant progenies (Mp) or } M_2 \text{ population (Ms)}}{\text{\% Pollen sterility (Ps) or \% Ovule fertility (O}f\text{)}}$$

When two kinds of mutagens were combined, their synergistic or additive effects were tested with reduction in biological parameters, *viz.* pollen sterility and reduction in ovule fertility. To evaluate the effect of combination treatments on mutation frequency, the data were analyzed using the formula adopted by Doll and Sandfaer (1969)

$$(a) + (b) = 1/K \ (a + b),$$

Where, a and b stands for two treatment and k is a 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.

Results and Discussion

The relative effectiveness and efficiency of both physical and chemical mutagens in inducing pollen sterility and ovule fertility were computed on 100 M_1 plant basis presented in Table 1. In general, the effect of different doses of gamma rays, EMS and their combinations on pollen sterility (%) and ovule fertility (%) in both the cultivars, revealed that high doses/concentrations of treatments gradually increased the pollen sterility and were more effective in reducing ovule fertility. There was a dose dependent increase in both the cultivars for pollen sterility and reductions in ovule fertility. Gamma rays and EMS treatments of the mutagens suggesting the direct relationship with the dose dependent increase in PDM 11 and PDM 54, the similar results

Table 3. Frequency, effectiveness, efficiency of physical and chemical mutagens and their combination in M_2 generation of Mungbean cv. PDM 54.

Mutagenic treatment	Number of M_1 plant progenies	Number of plants in M_2	Number of M_1 Plant progenies mutated	Number of M_2 plants mutated	Mutation frequency		Effectiveness		Efficiency		Synergism on % M_2 plants mutated
					Number of M_1 plant progenies mutated (Mp)	M_2 plants mutated (Ms)	Mp/dose	Ms/dose	Ms/Ps	Ms/Of	
Gamma rays (kR)											
10	1 12	1806	5	11	4.464	0.609	0.446	0.061	0.06805	0.007	-
20	98	1662	13	27	13.265	1.625	0.663	0.081	0.092	0.020	-
30	93	1342	19	36	20.430	2.683	0.681	0.089	0.099	0.036	-
40	82	1123	23	46	28.049	4.096	0.701	0.102	0.109	0.063	-
EMS (M)											
0.01	103	1492	5	7	4.854	0.469	[r] 80.906	7.819	0.068	0.006	-
0.02	86	1362	15	24	17.442	1.762	145.349	14.684	0.069	0.023	-
0.03	77	1298	21	47	27.273	3.621	151.515	20.116	0.087	0.049	-
0.04	69	1167	26	56	37.681	4.799	157.005	19.994	0.095	0.067	-
Combination treatment (Gamma rays + EMS)											
10 kR+ 0.02 M	89	1411	11	25	12.360	1.772	10.300	1.476	0.080	0.024	1.643
20 kR+ 0.02 M	82	1395	20	49	24.390	3.513	10.163	1.464	0.113	0.057	1.037
30kR + 0.02 M	74	1249	24	69	32.432	5.524	9.009	1.535	0.109	0.096	0.876
40kR + 0.02 M	66	1041	28	79	42.424	7.589	8.838	1.581	0.135	0.167	0.853

were obtained with their combination treatments also. The maximum pollen sterility and reduction in ovule fertility occurring at higher doses of gamma rays and EMS. However, the combination treatments of gamma rays with EMS increased the pollen sterility and reduced ovule fertility than their individual treatments. This, post irradiation treatments with EMS produced synergistic effects in both cultivars. Similar synergism had been reported by Sharma (1972) in barley and Gautam *et al.* (1998) in rajmash.

The data revealed that, effectiveness of gamma rays increased with the increase in dose from 10 kR to 40 kR in both the cultivars *viz.* PDM l1 (Table 2) and PDM 54 (Table 3). Most effective gamma irradiation dose was found to be 40 kR on the basis of maturity at M_1 plant progeny basis as well as on the basis of M_2 plants mutated. The effectiveness of chemical mutagens (EMS) was also increased with the increase in concentration from 0.01 to 0.04M concentrations and 0.04 M concentration found to be most effective. Whereas, 10 kR + 0.02 M (EMS) was found to be most effective in combination treatments. In general, ethyl methane sulphonate were found to be most effective mutagens as compared to gamma rays and/or their combination treatments.

Mutagenic efficiency, calculated on the basis of both percentage pollen sterility and percentage ovule fertility, 40 kR gamma rays treatments was most efficient among gamma rays treatments and in EMS, 0.04 M concentration was found to be most efficient on the basis of both pollen sterility and reduction in ovule fertility. Among combination treatments, 40 kR+0.02 M EMS was the most efficient on the basis of pollen sterility and in causing reduction in ovule fertility. In general, EMS was more efficient than gamma rays. The above findings were in accordance to the observations of several other workers (Konzak *et al.,* 1965) in barley, (Nerker, 1977) in lathyrus, (Gautam *et al.,* 1992) in black gram and (Gautam *et al.,* 1984) in rajmash.

Mutation frequency calculated on the basis of M_1 plant progenies and M_2 plant basis showed dose dependent increase. A gradual increase in mutation frequency was obtained with the increase in combination treatments, being highest at 40 kR+0.02 M combination treatments of gamma rays and EMS. Similarly, in other combination treatments, the rate of mutation frequency is higher than the individual mutagenic treatments of gamma rays and EMS. In combination treatment, all the doses of gamma rays with 0.02 M EMS produced synergistic effect on mutation frequency on the basis of per cent M_2 plants mutated (Table 2 & 3). Synergistic effect of physical and chemical mutagen had also been reported by several workers (Khalatkar and Bhatia, 1975 in barley, Jana and Appa Rao, 1975 in black gram, Ignacimuthu and Babu, 1989 in urd and mungbean, Gautam *et al.,* 1992 in blackgram, Solanki and Sharma, 1994 in lentil and Gautam *et al.,* 1998 in rajmash).

The synergism between two mutagens may be because of first mutagen treatment making accessible otherwise non-available sites for induction to the second mutagen, and pre - mutational lesions induced by the first mutagen becomes fixed due to an inhibitory effect of the second mutagen on repair enzyme (Sharma, 1972). Both these pathways should yield a frequency of mutations applied individually.

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