

Mutagenic Effectiveness and Efficiency of Individual and Combination Treatments of Gamma Rays and Ethyl Methanesulfonate in Black Gram [*Vigna mungo* (L.) Hepper]

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Abstract Induced mutagenesis creates new allelic combination of genes without disturbing the basic genomic structure of the plant. Effectiveness relates mutagen dose to mutational events, while mutagenic efficiency shows the proportion of mutations in relation to biological damage in M₁ generation. Present investigation was undertaken to assess the effectiveness and efficiency of different individual and combination treatments of gamma rays and EMS in M₂ generation of black gram. Mutagenic effectiveness and efficiency were calculated in relation to biological damage in M₁ and chlorophyll mutations in M₂ generation. The order of mutagenic effectiveness based upon the percent of mutated plant progenies was EMS > gamma rays+EMS > gamma rays. Combination treatments of gamma rays+EMS were found to be the most efficient followed by the individual treatments of EMS and gamma rays in both the varieties. Moderate doses of gamma rays and EMS alone as well as in combination proved more effective and efficient in inducing mutations. Among the varieties, variety Pant U-30 proved more responsive towards mutagenic treatments than the variety T-9. These results not only provide valuable information about mutagenic treatments and germplasm resources of black gram, but also provide guidance for generating black gram mutants through mutation breeding.

Keywords Black Gram, Gamma Rays, EMS, Mutagenic Effectiveness, Mutagenic Efficiency, Mutational Rate

1. Introduction

Black gram [*Vigna mungo* (L.) Hepper] also known as urdbean is one of the important self-pollinating diploid crops of the world belonging to family Fabaceae. It is a short duration grain legume having wider adaptability and low input requirement. The crop possesses unique ability to fix atmospheric nitrogen in symbiotic association with *Rhizobium* bacteria, which not only enables it to meet its own nitrogen requirement but also profits the succeeding crops. Black gram is rich in proteins and has a low concentration of sulphur containing amino acids like methionine and cysteine [1]. Comparatively higher lysine content makes it an excellent complement to cereals vis-à-vis balanced human nutrition [2]. Being rich in quality proteins, amino acids, vitamins and minerals, it is an inseparable ingredient in the diets of vast majority of Indian population. Despite holding prodigious promise, black gram is often pushed to marginal lands with limited input making it prone to number of abiotic stresses causing tremendous loss in productivity. The stumpy productivity is also due to low yield potential and narrow genetic base of the existing cultivars [3]. In these situations, induced mutation may be resorted to develop superior genotypes due to their direct and cumulative effect on genotypic background [4-5].

The response of biological system to physical and chemical mutagens is influenced by numerous biological, environmental and chemical factors. These factors not only modify the effectiveness and efficiency of the mutagens, but also impact the mutational rate [6]. The early information on relative effectiveness and efficiency of

mutagens helps in optimization and selection of working concentrations/doses that could produce desirable mutations at high rate [7-8]. Although, both effectiveness and efficiency estimates reveal entirely different properties of a mutagen, however collectively they indicate the efficacy of any mutagen [9]. Also, an effective mutagen may not necessarily be an efficient one [10]. Biological parameters of M_1 generation are helpful in determining the effectiveness and efficiency of the mutagens, besides identifying the plants with maximum genetic damage that carry high frequency of micro-mutations in subsequent generations [11]. In mutation breeding programs, the selection of effective and efficient mutagens is indispensable to recover high frequency of desirable mutations [12]. Present investigation was undertaken to understand the response of black gram genotypes to both physical and chemical mutagens with a view to identify mutagenic treatments that induce maximum frequency of desirable mutations in M_2 generation. The prior information on mutagenic effectiveness and efficiency is considered necessary to recover significant mutations at high rate with lesser lethality in mutation breeding. The estimates of effectiveness and efficiency of the mutagens, in the present study, will reveal the relative efficacy of the applied individual and combined treatments of gamma rays and EMS in black gram genotypes T-9 and Pant U-30, which could facilitate the selection of desirable mutations in subsequent generations and be utilized in other mutation breeding experiments as well.

2. Materials and Methods

Field experiment was conducted during the summer season of 2008 and 2009 at Agricultural Farm, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. One set

of healthy and dry seeds of varieties T-9 and Pant U-30 of black gram (*Vigna mungo* (L.) Hepper) were irradiated with 100 to 400 Gy doses of gamma rays from cobalt-60 source at National Botanical Research Institute, Lucknow, India. Another set of 9-hour water presoaked seeds was treated with 0.1 to 0.4% EMS (ethyl methanesulfonate) prepared in phosphate buffer of pH-7 for 6 hours with constant intermittent shaking at $25 \pm 1^\circ\text{C}$ temperature. The seeds were thoroughly washed in running tap water to eliminate the residual mutagen from seed coat after completion of treatment period.

Regarding combination treatments, dry seeds of each variety were firstly irradiated with 200 and 300 Gy doses of gamma rays followed by the treatment with 0.2% and 0.3% of EMS (i.e. 200 Gy+0.2% EMS, 300 Gy+0.2% EMS, 200Gy+0.3%EMS, 300Gy+0.3% EMS). Three replications of 100-seeds each for every treatment and controls in both the varieties were sown in randomized complete block design (RCBD) to raise M_1 generation. The spacing was maintained at 0.30 m (seed to seed in a row) and 0.60 m (between the rows) in the field. Recommended agronomic practices were employed for field preparation, seed sowing and subsequent management of the crop. Data on biological parameters such as seedling injury (I), pollen sterility (S) and meiotic aberrations (Me) were recorded in M_1 generation. Twenty-five healthy seeds from each normal looking M_1 plants of different treatments and controls were sown in plant progeny rows to raise M_2 generation. Different treatments and controls comprised of 50 progenies. Chlorophyll mutations were recorded in M_2 generation when seedlings were 7-15 days old. Mutagenic effectiveness and efficiency of gamma rays, EMS and their combination treatments in M_2 generation were calculated as per the formula suggested by Konzak *et al.* [13] given below:

$$\text{Rate of mutation (Mp)} = \frac{\text{No. of plant progenies segregating in } M_2}{\text{No. of } M_1 \text{ plant progenies}} \times 100$$

$$\text{Mutagenic effectiveness (physical mutagen)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose in (Gy)}}$$

$$\text{Mutagenic effectiveness (chemical mutagen)} = \frac{\text{Rate of mutation (Mp)}}{\text{concentration} \times \text{duration of treatment}}$$

$$\text{Mutagenic effectiveness (combination)} =$$

$$\frac{\text{Rate of mutation (Mp)}}{\text{Dose of physical mutagen (Gy)} \times \text{concentration of chemical mutagen} \times \text{duration of treatment}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Rate of mutation (Mp)}}{* \text{Biological damage in } M_1 \text{ generation}}$$

*Biological damage:

For measuring biological damage, three different criteria were used:

- Injury - i.e. percentage reduction in seedling height (Mp/I)
- Sterility - i.e. percentage reduction in pollen fertility (Mp/S)
- Meiotic abnormalities - i.e. percentage of meiotic abnormalities (Mp/Me)

Seedling injury was measured in terms of reduction in seedling height with respect to control. Pollen fertility was estimated by staining the pollen grains with 1% acetocarmine solution. Pollen grains which took stain and had regular shape and outline were considered as fertile, while shrunken, empty and unstained ones were treated as sterile. For meiotic studies, young flower buds were fixed in Carnoy's fluid for 24 hours. Ferric chloride was added as fixative to achieve better staining. After 24 hours of fixation, flower buds were transferred to 70% ethyl alcohol. Anthers were smeared in 1% acetocarmine solution and pollen mother cells were examined at various stages of microsporogenesis under Olympus compound microscope.

3. Results

Perusal of the data revealed that mutagenic effectiveness and response of black gram varieties to gamma rays, EMS and their combination treatments were varying. In case of gamma rays and EMS, the moderate concentrations were found more effective as compared to lowest and the highest ones in both the varieties (Tables 1, 2). In general, EMS proved more effective than combination (gamma rays+EMS) and gamma ray treatments in both the varieties. The estimates of effectiveness ranged from 0.020 to 0.050

in the variety T-9 and 0.040 to 0.060 in the variety Pant U-30 of gamma ray treatments, whereas the effectiveness of EMS treatments ranged from 6.66 to 13.33 and 8.33 to 15.00 in the T-9 and Pant U-30 varieties, respectively. The effectiveness of the combined gamma rays + EMS treatments ranged from 0.041 to 0.058 in the var. T-9 and 0.051 to 0.062 in the var. Pant U-30 (Tables 1 and 2). The decreasing order of mutagenic effectiveness was found to be EMS, gamma rays + EMS and gamma rays. Mutagenic efficiency was calculated on the basis of seedling injury (Mp/I), pollen sterility (Mp/S) and meiotic abnormalities (Mp/Me) which resulted in highest efficiency at 0.2% EMS among EMS treatments and 200 Gy γ rays among gamma ray treatments in both the varieties. Among the combination treatments, 200Gy γ rays+ 0.2% EMS in var. T-9 and 300 Gy γ rays+0.2% EMS in var. Pant U-30 were found to be the most efficient mutagen treatments. Overall, on the basis of seedling injury, pollen sterility and meiotic abnormalities, the efficiency of mutagens in descending order was: gamma rays + EMS > EMS > gamma rays in both the black gram varieties T-9 and Pant U-30. Mutagenic efficiency varied as per the criteria selected for its estimation. The efficiency calculated on the basis of meiotic aberrations was significantly higher as compared to the efficiency calculated on the basis of seedling injury and pollen sterility in both the varieties. Further, mutational rate (MR) based upon injury (I), sterility (S) and meiotic aberrations (Me) was the highest in combination treatments followed by the individual treatments of EMS and gamma rays (Table 3). Assessments on the estimated effectiveness and efficiency in the present study revealed that the genotypic sensitivity of var. Pant U-30 towards the mutagen treatments was higher compared to var. T-9 and thus, var. Pant U-30 responded favorably to the applied mutagen treatments, especially the highest effective EMS treatments.

Table 1. Effectiveness and efficiency of gamma rays, EMS and their combination treatments in M₂ generation of black gram variety T-9

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
Gamma rays								
100 Gy	10.41	6.72	4.53	2.00	0.020	0.19	0.30	0.44
200 Gy	13.42	13.08	5.76	10.00	0.050	0.74	0.76	1.74
300 Gy	30.34	18.35	7.85	12.00	0.040	0.39	0.65	1.53
400 Gy	36.96	24.56	9.66	14.00	0.035	0.38	0.57	1.45
EMS								
0.1%	8.69	6.36	2.28	4.00	6.66	0.46	0.63	1.75
0.2%	13.07	13.03	5.45	16.00	13.33	1.22	1.23	2.93
0.3%	21.47	17.91	6.84	16.00	8.89	0.74	0.89	2.34
0.4%	34.06	24.32	8.73	20.00	8.33	0.59	0.82	2.29
Gamma rays+EMS								
200 Gy+0.2%	11.59	13.49	4.72	14.00	0.058	1.21	1.04	2.97
300 Gy+0.2%	16.91	19.54	6.80	20.00	0.055	1.18	1.02	2.94
200 Gy+0.3%	33.47	22.78	8.59	20.00	0.055	0.60	0.88	2.33
300 Gy+0.3%	41.75	28.60	10.45	22.00	0.041	0.53	0.77	2.10

Table 2. Effectiveness and efficiency of gamma rays, EMS and their combination treatments in M₂ generation of black gram variety Pant U-30

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
Gamma rays								
100 Gy	9.85	7.41	4.63	4.00	0.040	0.41	0.54	0.86
200 Gy	13.04	14.48	6.29	12.00	0.060	0.92	0.83	1.91
300 Gy	25.91	21.61	8.51	16.00	0.053	0.62	0.74	1.88
400 Gy	40.58	27.78	10.82	20.00	0.050	0.49	0.72	1.85
EMS								
0.1%	7.25	8.20	2.73	6.00	10.00	0.83	0.73	2.20
0.2%	11.88	13.58	6.08	18.00	15.00	1.51	1.32	2.96
0.3%	16.81	20.77	7.20	20.00	11.11	1.19	0.96	2.78
0.4%	27.54	26.90	9.38	20.00	8.33	0.73	0.74	2.13
Gamma rays+EMS								
200 Gy+0.2%	10.20	13.37	5.00	14.00	0.058	1.37	1.05	2.80
300 Gy+0.2%	13.91	19.81	7.45	22.00	0.062	1.58	1.11	2.95
200 Gy+0.3%	32.46	26.78	9.02	22.00	0.061	0.68	0.82	2.44
300 Gv+0.3%	41.16	28.75	12.54	28.00	0.051	0.68	0.97	2.23

Table 3. Mutational rate of the mutagens in relation to biological damage of M₁ generation in varieties T-9 and Pant U-30 of black gram

Treatment	Var. T-9			Var. Pant U-30		
	MRI	MRS	MRMe	MRI	MRS	MRMe
Gamma rays	0.42	0.57	1.29	0.61	0.71	1.62
EMS	0.75	0.89	2.33	1.06	0.94	2.52
Gamma rays + EMS	0.88	0.93	2.58	1.08	0.99	2.60

MRI: Mutation rate based on seedling injury

MRS: Mutation rate based on pollen sterility

MRMe: Mutation rate based on meiotic aberrations

4. Discussion

In mutation breeding programs, the knowledge about effectiveness and efficiency of mutagens are imperative for inducing desirable mutations in crop plants. Different workers have reported different mutagenic effectiveness and efficiency in various pulse crops like grass pea [14], lentil [15], cluster bean [16], cowpea [17-18], soybean [19], faba bean [20] and rice bean [21]. The gamma rays and EMS are widely used mutagens for inducing viable mutations in crop plants. Gamma rays generally induce cytological and morphological alterations along with physio-chemical effects that directly affect the normal growth and development of the exposed plant [22-23]. Mutagenic effectiveness and efficiency of gamma rays, EMS and their combination treatments were estimated on the basis of frequency of progenies segregating for chlorophyll mutations in M₂ generation. In both the varieties, the mutagenic effectiveness and efficiency varied not only between the mutagenic treatments, but also between the mutagens. EMS, individually proved more

effective than combination treatments of gamma rays + EMS and gamma rays. EMS was reported as superior mutagen to gamma rays in inducing useful mutations in lentil [24], mungbean [25], chickpea [26], cowpea [27] and urdbean [28]. In this study, the gamma rays and EMS had proved more effective at moderate doses. The decline in mutagenic effectiveness at higher doses indicates that the increase in mutational rate was not proportional with increasing mutagenic doses.

Like effectiveness, the efficiency was generally found higher at moderate or lower mutagenic doses. The greater efficiency of moderate or lower mutagen doses is due to the fact that biological damage generally increases with increasing mutagen doses at a faster rate than the mutations yielded at the same dose [9]. Higher efficiency at lower and intermediate mutagenic doses has been earlier reported in *Lens culinaris* [29], *Vigna mungo* [30-31], *Vigna radiata* [32] and *Glycine max* [19]. The optimal lower and intermediate treatments of mutagens comparatively induce a tolerable genetic change that leads to successful expression of useful mutations. Mutagenic efficiency

based upon Mp/Me was generally higher as compared to Mp/S and Mp/I. This may be due to the fact that induced meiotic aberrations were comparatively less as compared to the amount of pollen sterility and seedling injury in mutagenized population.

While physical mutagens have been exploited to a greater extent for inducing useful mutations in crop plants and majority of the mutant varieties have been released through them, nevertheless some crop genotypes respond better to chemical mutagens. In such genotypes, appropriate dose and efficient handling of mutated population could yield desirable results vis-à-vis agro-economic traits [11].

5. Conclusions

Results show that moderate doses of gamma rays and EMS alone as well as in combination proved more effective and efficient vis-à-vis mutations induced which could be exploited effectively for the improvement of black gram crop.

Significant Statement

Overview of the results reveal that varieties T-9 and Pant U-30 of black gram proved highly responsive towards the mutagens applied. Intermediate doses of gamma rays and EMS proved more effective and efficient in inducing different mutations. Such mutagenic treatments could be utilized for isolating promising mutant lines with desirable agro-economical traits in various food crops.

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Competing Interest

The authors have declared that no competing interest exists.

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