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Mutagenic Effectiveness and Efficiency of Gamma Rays, EMS and their Synergistic Effects in Blackgram (*Vigna mungo* (L.) Hepper)

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ABSTRACT: *A comparative study on effect of different dose/concentration of gamma rays, ethyl methane sulfonate (EMS) and combination treatments of gamma rays and EMS on various biological parameters (plant survival, plant height and seed fertility) in M_1 generation and frequency of viable mutations induced in the M_2 generation was carried out in blackgram var. VBN 4 in the present investigation. Mutagenic effectiveness of viable mutants was high in gamma rays treatment than EMS. The efficiency was found to be highest at lower and intermediate concentration of mutagenic treatments. Based on lethality, injury and sterility, gamma rays were more efficient than EMS and combination of both in producing viable mutants. Combination treatments in general proved to be most effective followed by physical mutagens and EMS in inducing maximum frequency of mutations.*

Key words: *Blackgram, Biological abnormalities, Viable mutations, Gamma rays, EMS.*

INTRODUCTION

The presence of genetic variability is necessary for the crop improvement. The variability available to the breeders comes from spontaneous or artificially induced mutations. The artificial induction of mutation in a crop species is achieved through the use of physical and/or chemical mutagens that enlarge the mutation frequency, when compared to the spontaneous occurrence. Almost all mutagens have the property of reacting with DNA and thereby bringing about changes in nucleotide sequences. However, the mode of action of each mutagen is distinct. Besides, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary to recover high frequency of desirable mutations (Badere and Chaudhary, 2007). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded in the M_2 generation in relation to the biological damage caused in M_1 (Khan *et al.*, 2009; Wani, 2009). According to Kumar *et al.*, (2003), M_1 test data along with the frequency and spectrum of morphological mutations in M_2 provide good guidelines for determination of efficient mutagenic treatments.

Blackgram *(Vigna mungo* (L.) Hepper) is an important pulse crop of our country and is commonly known as Urdbean. It belongs to the family leguminosae and subfamily papilionaceae. The chromosome number of this crop is 2n = 2x = 22 (Bhatnagar *et al.*, 1974). It is a highly self pollinated crop with cleistogamous nature. Creation of variability through pollination and artificial hybridization is very difficult as the flowers are cleistogamous and very delicate to handle. As genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central base to crop breeding. Experimentally, induced mutations provide an important source for variability. The present investigation was undertaken with the aim to study the effects of gamma rays, ethyl methane sulfonate (EMS), and their combination on the frequency of macro-mutants and to evaluate the relative effectiveness and efficiency of these mutagens with the main aim of identifying suitable mutagenic treatments that can induce maximum frequency of mutations in this crop.

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MATERIALS AND METHODS

Seeds of VBN 4 urdbean were subjected to three different doses of gamma rays *viz*., 40 kR, 50 kR and 60 kR, EMS 50 mM, 60 mM and 70 mM and and combination treatments *viz*., 40 kR + 50 mM, 40 kR + 60 mM, 40 kR + 70 mM, 50 kR + 50 mM, 50 kR + 60 mM, 50 kR +70 mM, 60 kR + 50 mM, 60 kR + 60 mM and 60 kR + 70 mM. To raise M_1 generation, a total of fifteen treatments along with the control was sown in the field at the rate of 150 seeds for each treatment at a spacing of 30 x 15cm at Agricultural College and Research Institute, Madurai during August, 2010 in Randomized block design (RBD) with three replications. All the surviving individual plants were harvested in each treatment in M_1 generation. The M_2 generation was raised from individual M_1 plant (*i.e.*) M_1 plant basis following plant to progeny method during January, 2011. Thirty plants per treatment were forwarded from the M_1 to the M_2 generation. The seeds were sown with adequate spacing. All the recommended agronomic practices were carried out during the growth period of the crop. Frequency of viable mutations was calculated on M_2 plant basis. Data on biological abnormalities such as injury, lethality and sterility in M_1 generation and macro mutation frequencies in M_2 generation were used to determine the mutagenic efficiency and effectiveness according to the formula suggested by Konzak *et al*., (1965).

$$\text{Mutagenic effectiveness} = \frac{M \times 100}{\text{kR or c} \times \text{t}}$$

$$\text{Mutagenic efficiency} = \frac{M \times 100}{L};\ \frac{M \times 100}{I};\ \frac{M \times 100}{S}$$

Where,

M - Chlorophyll mutation frequency
kR - Dose of gamma radiation
c - Concentration of the chemical mutagen (mM)
L - Percentage of lethality
t - Duration of treatment with chemical mutagen (hrs)
I - Percentage of injury
S - Percentage of sterility

To evaluate the effect of combined treatments on mutation frequency the data was analyzed using the formula suggested by Sharma and Swaminathan (1969).

$$K = \frac{(a+b)}{(a)+(b)}$$

a & b – two mutagens
K – hypothetical interaction coefficient

Any deviation from the unit value of K would reveal either a synergistic effect (K > 1) or antagonistic effect (K < 1). The interaction coefficient was calculated, by dividing mutation frequency of combination treatment (a + b) by a value which was addition of mutation frequencies of two mutagens (a) + (b) while used singly.

RESULTS AND DISCUSSION

Biological abnormalities in M_1 generation

The date of plant survival (%), plant height and seed fertility (%) in M_1 generation for various mutagenic treatments in VBN 4 is given in the Table 1. The data of survival percentage was taken on 30th day in M_1 generation. The survival percentage was more in control than all mutagenic treatments. In both gamma ray and EMS treatments, the percentage of seedlings survival progressively decreased with the increasing dose or concentration of mutagens. Similar results were reported by Kouser *et al*., (2007), Jain and Khandelwal (2008), Lal *et al*., (2009), Sagade and Apparao (2011). The survival percentage ranged from 62.38 (60 kR) to 88.17 (40 kR) per cent over control for gamma rays, for EMS the range is from 76.55 (70 mM) to 91.63 (50 mM) per cent over control. In case of combination treatment the survival percentage ranged from 65.56 (40 kR + 70 mM) to 95.11 (60 kR + 50 mM) per cent over control and there was an irregular trend observed in mean survival of plants.

In gamma ray treatment, the mean plant height ranged from 13.20 (60 kR) to 15 (40 kR) and it showed irregular trend. The plant height reduction was high at 60 kR and low at 50 kR. In case of EMS treatment, the mean plant height was found to be decreased from 50 mM (15.40) to 70 mM (13.60) and it showed declining trend with increasing doses of EMS. In case of combination treatment, there is no dose dependent increase or decrease in plant height. The mean plant height ranged from 11.02 (60 kR + 70 mM) to 17.50 (60 kR + 50 mM) and the control being 18.92. Reduction in plant height on 30th day and maturity was reported by Kundu and Singh (1982) and Chaturvedi *et al*., (1983) in blackgram, in greengram the similar results have been reported by Yogesh kumar and Mishra (1999).

The seed fertility for gamma irradiated population ranged from 82.59 (60 kR) to 92.37 (40 kR) per cent on control, in EMS treated population the seed fertility ranged from 80.91 (70 mM) to 95.12 (50 mM) per cent over control. In case of combination treatments the seed fertility ranged from 66.79 (60 kR + 70 mM) to 91.84 (40 kR + 50 mM). The reduction in the seed

Table 1
Estimation of plant survival (%), plant height and seed fertility (%) in M_1 generation

	Plant survival (%)			*Plant height*			*Seed fertility (%)*		
Treatment	*Mean*	*% over control*	*% reduction*	*Mean*	*% over control*	*% reduction*	*Mean*	*% over control*	*% reduction*
Gamma rays (kR)									
Control	86.56	100.00	0.00	18.35	100.00	0.00	92.02	100.00	0.00
40 kR	76.32	88.17	11.83	15.00	81.74	18.26	85.00	92.37	7.63
50 kR	65.43	75.59	24.41	16.20	88.28	11.72	82.00	89.11	10.89
60 kR	54.00	62.38	37.62	13.20	71.93	28.07	76.00	82.59	17.41
Mean	70.58	81.54	18.46	15.69	85.49	14.51	83.76	91.02	8.98
CD (0.05)	2.20			0.68			3.02		
EMS (mM)									
Control	86.22	100.00	0.00	18.92	100.00	0.00	91.46	100.00	0.00
50 mM	79.00	91.63	8.37	15.40	81.40	18.60	87.00	95.12	4.88
60 mM	74.67	86.60	13.40	14.20	75.05	24.95	81.00	88.56	11.44
70 mM	66.00	76.55	23.45	13.60	71.88	28.12	74.00	80.91	19.09
Mean	76.47	88.69	11.31	15.53	82.08	17.92	83.37	91.15	8.86
CD(0.05)	2.34			0.67			2.98		
Gamma rays + EMS									
Control	86.22	100.00	0.00	18.92	100.00	0.00	91.46	100.00	0.00
40 kR + 50 mM	74.62	86.55	13.45	13.20	69.76	30.24	84.00	91.84	8.16
40 kR + 60 mM	76.00	88.15	11.85	15.67	82.82	17.18	81.12	88.69	11.31
40 kR + 70 mM	56.53	65.56	34.44	16.00	84.57	15.43	80.05	87.52	12.48
50 kR + 50 mM	72.00	83.51	16.49	15.00	79.28	20.72	78.50	85.83	14.17
50 kR + 60 mM	65.83	76.35	23.65	14.67	77.54	22.46	78.34	85.65	14.35
50 kR + 70 mM	63.00	73.07	26.93	17.00	89.85	10.15	75.76	82.83	17.17
60 kR + 50 mM	82.00	95.11	4.89	17.50	92.49	7.51	65.40	71.51	28.49
60 kR + 60 mM	65.02	75.41	24.59	13.00	68.71	31.29	64.00	69.98	30.02
60 kR + 70 mM	76.43	88.65	11.35	11.02	58.25	41.75	61.09	66.79	33.21
Mean	71.77	83.24	16.76	15.19	80.33	19.67	75.97	83.06	16.94
CD(0.05)	2.43			0.77			2.87		

fertility was gradual and the maximum was observed at highest dose or concentration of the mutagens (Sagade and Apparao, 2011). Singh and Mohapatra (2004) estimated the effect of M_1 plant traits *viz*., injury, lethality and pollen sterility and their possible relationship with M_2 population frequency. This was also reported by Sassikumar *et al*., (2003) and Verma *et al*., (2004) in lima bean, Yogesh Kumar and Mishra (1999) in green gram.

Frequency of viable mutants in M_2 generation

An accurate method of estimating induced mutation frequencies must compensate for the bias introduced by factors such as diplontic selection, small progeny size and increased size of mutated sector at higher doses (Nilan *et al*., 1964). Gaul (1960) concluded that the mutants per 100 M_2 plants will be the best estimate of initial mutation frequency, since it may not distort the functional relationship between dose and mutational response. Later this was confirmed by Blixt (1966) in peas, Mohan Rao (1972) in barley. In the present study, observations on viable mutations were recorded from early seedling stage to maturity. The frequency of viable mutants ranged from 4.14 per cent in 60 kR to 5.22 per cent in 40 kR for gamma rays treatment. The viable mutation frequency decreases with increasing doses of gamma rays. In EMS treatment the frequency ranged from 2.40 per cent in 50 mM to 5.91 per cent in 60 mM. A gradual increase in mutation frequency was associated with obtained with the increased in combination treatments, being highest at 60 kR + 60 mM of combination treatment

Table 2
Frequency of viable mutants in M_2 generation

Treatment	*Number of M_2 seedlings*		Mutation frequency
	Examined	*Showing viable mutants*	
Gamma rays (kR)			
Control	342	–	–
40kR	287	15	5.22
50kR	249	11	4.41
60kR	193	8	4.14
EMS (mM)			
Control	328	–	–
50mM	249	6	2.40
60mM	203	12	5.91
70mM	182	8	4.39
Gamma rays + EMS			
Control	328	–	–
40kR+50mM	302	12	3.97
40kR+60mM	254	4	1.57
40kR+70mM	223	11	4.93
50kR+50mM	312	14	4.48
50kR+60mM	276	7	2.53
50kR+70mM	243	4	1.64
60kR+50mM	300	10	3.33
60kR+60mM	272	16	5.88
60kR+70mM	241	8	3.31

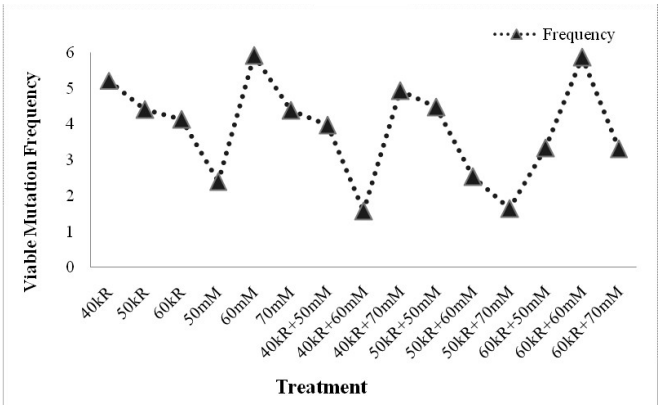

Figure 1: Frequency of viable mutants

(Table 2 and Fig. 1). Similar results were reported by Makeen and Babu (2010) in urdbean. The viable mutants were grouped into plant height, leaf modifications, variation in branching habit, floral mutants, pod and seed mutants and others. In the present investigation viable macro mutations with changes in attributes like stature, duration, leaf, pod and seed mutants were recorded in all the treatments. The highest number of viable mutants was recorded in combination treatments. Similar results were obtained by Wani (2011) in chickpea.

Mutagenic effectiveness

The effectiveness of gamma rays and EMS in inducing viable mutations was calculated on the percentage basis of M_2 seedlings. The effectiveness of gamma rays in inducing viable mutations ranged from 6.9 to 13.05 per cent and the highest effectiveness was observed at 40 kR and the effectiveness decreased with increasing concentration of the mutagen. The effectiveness of EMS in inducing viable mutations ranged from 0.80 to 1.64 per cent and the highest effectiveness was observed at 60 mM. The effectiveness for combination treatments cannot be calculated because of the difference in units of the two mutagens (Table 3). The high effectiveness of gamma rays was reported by Deepalakshmi and Ananda Kumar (2003) in blackgram and Mehraj *et al.*, (1999) in greengram. Gunasekaran *et al.*, (1998) also reported that gamma rays were more effective than chemical mutagen in inducing viable mutants in cowpea. There was no consistent trend between mutagen dose and effectiveness in viable mutants in this study.

Table 3
Mutagenic effectiveness and efficiency based on viable mutants - VBN 4

Treatments	% Survival reduction at 30th day (lethality)	%Height reduction at 30th day (injury)	% Seed fertility reduction (Sterility)	Mutation (M) per 100 M_2 seedlings	Effectiveness MX100 Cxt (or) kR	Mutagenic Efficiency MX100 L	MX100 I	MX100 S	Interaction coefficient (K)
Gamma rays (kR)									
40 kR	11.83	18.26	7.63	5.22	13.05	44.12	28.58	68.41	–
50 kR	24.41	11.72	10.89	4.41	8.82	18.06	37.62	40.49	–
60 kR	37.62	28.07	17.41	4.14	6.90	11.00	14.74	23.77	–
EMS (mM)									
50 mM	8.37	18.60	4.88	2.40	0.80	28.67	12.90	49.18	–
60 mM	13.40	24.95	11.44	5.91	1.64	44.10	23.68	51.66	–
70 mM	23.45	28.12	19.09	4.39	1.04	18.72	15.61	22.99	–
Gamma rays + EMS									
40 kR + 50 mM	13.45	30.24	8.16	3.97	–	29.51	13.12	48.65	0.52
40 kR + 60 mM	11.85	17.18	11.31	1.57	–	13.24	9.13	13.88	0.14
40 kR + 70 mM	34.44	15.43	12.48	4.93	–	14.31	31.95	39.50	0.51
50 kR + 50 mM	16.49	20.72	14.17	4.48	–	27.16	21.62	31.61	0.65
50 kR + 60 mM	23.65	22.46	14.35	2.53	–	10.69	11.26	17.63	0.24
50 kR + 70 mM	26.93	10.15	17.17	1.64	–	6.08	16.15	9.55	0.18
60 kR + 50 mM	4.89	7.51	28.49	3.33	–	68.09	44.34	11.68	0.51
60 kR + 60 mM	24.59	31.29	30.02	5.88	–	23.91	18.79	19.58	0.58
60 kR + 70 mM	11.35	41.75	33.21	3.31	–	29.16	7.92	9.96	0.38

Mutagenic efficiency

The efficiency of both the mutagens were used both in single and in combination, in inducing viable mutations and the data is presented in Table 3. Gamma ray treatments were more efficient than EMS in producing viable mutations (Malarkodi, 2008). Since maximum efficiency of 44.12 and 68.41 per cent was obtained at 40kR based on lethality and sterility basis. Based on injury, the highest level of efficiency (37.62 per cent) was observed at 50 kR. Based on lethality, injury and sterility the 60 mM of EMS treatment was noticed for the highest level of efficiency *viz*., 44.10 per cent, 23.68 per cent and 51.66 per cent respectively. Solanki and Sharma (1994) considered that the higher efficiency of a mutagen indicates relatively less biological damage (*i.e*., lethality, seedling injury, sterility etc.) in relation to mutations induced. Based on lethality, efficiency of combination treatments in inducing viable mutations ranged from 6.08 to 68.09 per cent and the highest efficiency was observed at 60 kR + 50 mM. Based on injury, efficiency ranged from 7.92 to 44.34 per cent and the highest efficiency was observed at 60 kR + 50 mM. Based on sterility, efficiency ranged from 9.55 to 48.65 per cent and the highest efficiency was observed at 40 kR + 50 mM. Based on interaction coefficient (K < 1), all the combination treatments show less than additive effects in inducing viable mutations. Similar results were reported by Srinivas and Veerabadhiran (2010) in lablab.

In the present study, the mutagenic efficiency was higher, mostly at lower and intermediate doses of viable mutants than at higher doses. This is in confirmation with the findings of Khan (1999) in blackgram. The reason for the greater efficiency at lower concentrations of mutagens is relating to the fact that lethality, injury and sterility increased with the mutagen level at a much faster rate. So the lower concentration causes relatively less damage enabling the organisms to manifest the induced mutations more frequently (Shadakshari *et al*., 2001).

CONCLUSION

The result of present study indicates that mutagenic effectiveness of viable mutants was high in gamma rays treatment than EMS. Combination treatments in general proved to be most effective followed by physical mutagens and EMS in inducing maximum frequency of mutations.

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