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Full Length Research Paper

Effect of gamma rays and ethyl methane sulphonate (EMS) in M_3 generation of blackgram (*Vigna mungo* L. Hepper)

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Induced mutation in plant improvement has been proven to be one of the ways used to generate new sources of genetic variations in creating new varieties of blackgram. This additional tool is important in plant improvement, which is a valuable approach for plant breeding in crop research. A study of the effects of gamma rays and ethyl methane sulphonate (EMS) on mutagenesis of blackgram was conducted to determine the effects of induced mutation on the species. Seeds of blackgram were treated with various doses/concentrations of gamma rays (40, 60 and 80 kR) and ethyl methanesulfonate (10, 15 and 20 mM). Mean performance of different quantitative traits was significantly better in 15 mM of EMS followed by 60 kR of gamma rays when compared with the control and other doses. Generally, higher doses of gamma rays and EMS (80 kR and 20 mM) that were particularly decreased had a pronounced effect on the plant growth and yield of blackgram. High values of heritability and genetic advance indicate the possibility of inducing desirable mutations for polygenic traits accompanied by effective selection in M_3 and later generations.

Key words: *Vigna mungo*, gamma rays, ethyl methane sulphonate (EMS), quantitative traits, M_3 generation.

INTRODUCTION

The pulse 'blackgram' plays an important role in Indian diet, as it contains vegetable protein and supplement to cereal based diet. It includes about 26% protein, which is almost three times that of cereals and other minerals and vitamins. Blackgram provides a major share of the protein requirement of the vegetarian population of the country. In addition, it is an important source of human and animal feed. The essential amino acid composition of blackgram seed is tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine and isoleucine. The crop is resistant to adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen in the soil (Kanade, 2006). Although legume seeds are important as protein source in human nutrition in many parts of the world (Brohult and Sandegren, 1954), the relative improvement of blackgram is limited by lack of variability for the components of seed yield particularly pod length, pod number and seed mass (Kajjidoni et al., 2009).

Hence, an attempt was made to compare the variability generated through mutation breeding to improve the productivity of blackgram.

Induced mutation using physical and chemical mutagens is one way to create genetic variation resulting in new varieties with better characteristics. The application of radiation and chemical mutation in mungbean breeding found high variation in yield per plant and nutritional quality, especially contents of protein, methionine and total sugars (Ngampongsai et al., 2009). The aim of this study was to generate information on the magnitude of induced genetic variability and magnitude of associations between yield and its components with the application of gamma rays and ethyl methane sulphonate (EMS).

MATERIALS AND METHODS

Blackgram var. VBN_1 was irradiated with 40, 60 and 80 kR amid a ^{60}Co gamma cell at the Sugarcane Breeding Institute, Tamilnadu

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Table 1. The mean values of different characters of blackgram in M_3 generation.

Mutagens	Plant height (cm)	Days to first flowering	Number of cluster per plant	Number of pods per plant	Number of seeds per pod	100 seed weight (g)	Seed yield per plant (g)	Seed protein (%)
Mean±SE								
Control	42.11±1.26	31.39±0.94	11.9±0.36	26.13±0.78	7.0±0.21	4.80±0.14	6.18±0.18	23.5±0.71
Gamma rays (kR)								
40	48.46±1.45	30.56±0.91	14.24±0.43	25.51±0.76	5.80±0.17	4.92±0.14	6.00±0.18	23.3±0.70
60	52.23±1.57	29.46±0.88	15.26±0.46	28.72±0.86	7.40±0.22	5.14±0.15	6.63±0.19	24.0±0.72
80	46.63±1.39	32.61±0.98	12.03±0.36	27.85±0.83	4.68±0.14	4.74±0.14	5.60±0.16	22.8±0.68
EMS (mM)								
10	54.20±1.62	31.38±0.94	14.89±0.45	23.7±0.71	4.68±0.14	4.62±0.14	5.88±0.17	23.8±0.71
15	55.91±1.67	30.52±0.92	16.13±0.48	31.52±0.95	8.56±0.26	5.08±0.15	7.33±0.21	24.3±0.72
20	53.59±1.60	33.26±0.10	13.04±0.39	26.57±0.79	5.31±0.16	4.25±0.13	5.62±0.16	23.0±0.69

Agricultural University, Coimbatore, India. Another quantity of 100 seeds for each treatment was presoaked for 4 h in distilled water, blotted dry, and treated with 10, 15 and 20 mM of freshly prepared solutions of ethyl methane sulfonate for 6 h with intermittent shaking. After treatment, seeds were thoroughly washed in running water for 4 h to leach out the residual of chemicals and sown in the field plots along with untreated control. The seeds were passed to the present status (M_3 generation) through progeny production under natural condition. The M_2 generation seeds were sown in a randomized block design in three replications with a spacing of 30×15 cm between rows and plants to increase the M_3 generation. The recommended package of practice for the crop was followed and was also observed routinely for any sort of change in them. The study was carried out with various observations, such as plant height (cm), days to first flowering, number of cluster per plant, number of pods per plant, number of seeds per pod, 100 seed weight (g), seed yield/plant (g) and seed protein content (%) in M_3 generation. Nonetheless, analysis of variance was computed by NPRC software package.

RESULTS

The data recorded for various quantitative traits in black gram are presented in Table 1. It is clear from the table that the mean values increased significantly for all the yield contributing traits under study. Higher values of mean for days to first flowering (minimum number of days was taken to flowering) and 100 seed weight were recorded at 60 kR of gamma ray treatment. The plant height, number of cluster per plant, number of pods per plant, number of seeds per pod, seed yield per plant and seed protein content was found in 15 mM of EMS.

In M_3 generation, mean values were shown to increase with the increase of mutagenic doses up to a certain level (that is, 60 kR of gamma rays and 15 mM of EMS). Thereafter, higher doses (80 kR and 20 mM) specified a decline of quantitative mean performance. Due to the stimulating effect of mutagens in M_3 generation, quantitative and qualitative traits of mean values were increased in dose/concentration of 15 mM of EMS followed by 60 kR of gamma rays. Higher mean values were observed on plant height (55.91), number of clusters per plant (16.13), number of pods per plant (31.52), number of seeds per pod (8.56), seed yield per plant (7.33) and protein content (24.3) in 15 mM of EMS. The gamma rays' dose, particularly 60 kR, was taken as the minimum number of days to flowering (29.46), whereas its maximum at 20 mM of EMS (33.26) had negative effect on the flowering derived from treated seeds. Similarly, gamma rays also enhanced the mean values of 100 seed weight (5.14).

Variability was found to be larger in EMS treatments than in the gamma rays and control (untreated) population for all characters studied in

Table 2. Genetic parameters for various quantitative traits in M_3 generation of black gram.

	Plant height (cm)				Days to first flowering				Number of cluster per plant				Number of pods per plant			
Mutagen	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	G A	GA (% of mean)
Gamma rays (kR)																
40	10.73	29.40	10.42	29.19	15.71	32.44	19.18	25.30	11.21	33.31	23.43	37.45	12.31	23.40	16.01	26.29
60	19.19	65.63	25.55	50.56	15.87	42.28	26.26	70.83	21.23	40.22	36.37	73.88	23.05	31.33	28.54	59.52
80	18.09	14.29	14.29	21.24	22.64	27.91	22.05	38.91	13.65	25.35	17.85	41.63	9.74	24.03	11.93	45.24
EMS (mM)																
10	20.45	69.45	17.75	37.86	20.97	12.11	22.11	31.91	14.02	26.75	22.01	49.44	8.99	48.25	17.55	46.04
15	23.78	70.43	19.73	50.59	27.63	40.81	24.57	53.51	28.59	51.79	41.80	87.71	32.46	87.93	72.09	57.98
20	17.89	77.05	8.40	22.54	13.58	27.70	13.36	38.60	14.31	29.48	33.80	61.47	8.14	51.35	18.29	38.01

	Number of seeds per pod				100 seed weight				Seed yield per plant (g)				Seed protein (%)			
Mutagen	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	GA	GA (% of mean)	GCV (%)	H_0	G A	GA (% of mean)
Gamma rays (kR)																
40	10.12	33.55	22.14	57.87	9.19	43.50	9.77	44.71	7.40	23.85	41.94	98.57	7.89	26.89	12.37	50.26
60	12.25	65.89	35.24	61.70	15.49	62.69	21.29	69.07	11.65	31.07	51.96	65.97	8.83	43.94	15.67	69.05
80	12.73	67.39	17.71	33.83	8.84	26.42	10.63	42.13	6.47	25.50	44.68	74.50	7.57	17.69	9.47	56.76
EMS (mM)																
10	7.16	63.54	10.31	54.92	10.58	30.04	8.09	59.47	8.61	22.58	48.79	74.22	15.50	63.45	17.55	65.87
15	13.54	72.81	22.40	63.08	11.36	58.71	14.44	58.41	16.43	34.49	67.10	71.43	17.92	80.90	27.39	70.09
20	5.15	74.42	9.54	25.21	11.12	31.48	10.20	21.84	7.57	20.86	43.95	68.03	16.85	70.90	7.84	45.59

the investigation. The genetic parameters were also found to be at their maximum in mutagenic treated plants over the control. Heritability for number of pods per plant (87.93) and seed protein content (80.90) was very high at 15 mM EMS as compared to all other treatments and characters. Genetic coefficient variation (GCV) and genetic advance (GA) showed similar trends and higher values at 60 kR of gamma rays and 15 mM of EMS in black gram (Table 2).

DISCUSSION

As yield increment was the primary objective in most of the plant breeding programme, mutation breeding played a key role in achieving the goal of this study. The stimulating effect of low doses of gamma rays irradiation on plant growth may be due to the stimulation of cell division or elongation, or the alteration of metabolic processes that affect the synthesis of phytohormones or nucleic acids (Pitirmovae, 1979). In addition, high doses of gamma irradiation were reported to be harmful in several studies like that of Ramachandran and Goud (1983), who reported that higher doses of gamma irradiation reduced plant height, number of leaves and branching capacity of safflower.

The minimum number of days taken to flowering was observed at 60 kR of gamma rays when compared to EMS and the control. Similar findings blackgram. The number of pods per plant considerably increased as compared to the control. This is supported by previous reports of Singh and Malhotra (1970) in green gram and Swaminathan (1973) who stated that an increase in the yield of pulses could be achieved by enhancing pod number.

Genetic parameters for various quantitative traits in M_3 generation of black gram are shown in Table 2. There were significant differences among entries for number of pods per plant, 100 seed weight and protein content. High coefficients of phenotypic (PCV) and genotypic variation (GCV) were observed for number of pods per plant followed by number of cluster per plant, number of seeds per pod, and seed yield per plant. Similar results were also reported by Cherian (2000) and Manju and Sreelathakumary (2002). EMS showed high PCV and GCV in number of clusters per plant, number of pods per plant and number of seeds per pod. Khurana and Sandhu (1972) obtained the highest PCV and GCV values in number of pods per plant and seed yield per plant in soybean, while Singh and Mehndiratta (1970) obtained the highest in mung bean. Our results are also supported by previous published studies that were reported by Samad (1991), Deb (1994), Nahar (1997), Deb and Khaleque (2004) and Alam et al. (2004) for different characters in different crops.

The high PCV and GCV observed from their high variability in turn offers good scope for selection. From the results, the heritability values recorded were greater than 25% in all the traits studied. These values were considered to be high and they varied from 26.10 to 87.93%. In addition, they were generally higher in the treated plants than in the control. These results were also demonstrated by Mensah and Eruotor (1993) and Mensah et al. (2005), and were further confirmed by Mensah and Obadoni (2007). Johnson et al. (1955) advocated that heritability estimates along with genetic advance are usually more helpful than the heritability value alone in predicting the resultant effects of selection.

The coefficient of variation was higher in mutagenic treatments as compared to the control, indicating that improvement in seed protein content was possible. Seed protein did not show any association with yield per plant, indicating the independent genetic control of protein content and total plant yield. Sandhu et al. (1979) suggested that the lack of association between grain yield and grain protein can be easily combined in a single genotype. The seed protein content was also high when compared to the control. Moreover, the 100 seed weight in the mutant plants was more than double in the parent of M_3 generation (Wani and Anis, 2001).

Conclusion

Mutation induction has proven to be a workable, sustainable, highly efficient, environmentally acceptable, flexible, unregulated, non-hazardous and a low-cost technology in the breeder's toolbox to enhance crop improvement. In this study, the quantitative traits of the M_3 generation revealed the enhancement of the significant level of yield attributes in blackgram. Among the various dose/concentration treatments, 15 mM of EMS and 60 kR of gamma rays treatment were more desirable, which resulted in low plant damage and higher genetic effects. As such, the maximum variation in quantitative characters may show the stable gene mutations in subsequent generation. The results indicate that blackgram mutant lines are useful for crop improvement and further study is needed for the analysis of the mutants.

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