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ESTIMATION OF VARIABILITY, HERITABILITY AND GENETIC ADVANCE IN MUTANT POPULATIONS OF BLACK GRAM (*Vigna mungo* L. Hepper)

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SUMMARY

Genetic variability and heritability estimates with genetic advance of traits are essential for crop improvement. In this investigation, variability induced by gamma rays and ethyl methane sulphonate (EMS) for different quantitative traits including plant height (cm), flowering time (days), number of primary branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod, pod length (cm), 100 seed weight (g) and seed yield per plant (g) was studied in M_2 generation of black gram (*Vigna mungo* L. Hepper). Seeds of VBN 4 black gram were treated with various doses/concentrations of gamma rays (40, 50 and 60 kR) and EMS (50, 60 and 70 mM). Mean performance of different quantitative traits was significantly better in 50 mM of EMS followed by 50 kR of gamma rays when compared with the control and other doses. Generally, higher doses of gamma rays and EMS (60 kR and 70 mM) that were particularly decreased had a pronounced effect on the plant growth and yield of black gram. High values of heritability and genetic advance indicate the possibility of inducing desirable mutations for polygenic traits accompanied by effective selection in M_2 and later generations.

Key words: Variability, gamma rays, ethyl methane sulphonate (EMS), induced mutation, genetic advance, quantitative traits, M_2 generation

Key findings: 50 kR of gamma rays revealed highest values of h^2 with GA% for number of primary branches per plant, number of pods per plant, 100 seed weight and seed yield per plant. H^2 coupled with GA% were comparatively high at 50 mM of EMS for number of primary branches per plant, pod length and seed yield per plant. This indicates that these traits are under the control of additive gene action and directional selection for these traits could be effective for desired genetic improvement.

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INTRODUCTION

Grain legumes are the chief source of protein and dietary amino acids for men and farm animals. Black gram (*Vigna mungo* L. Hepper), popularly known as urdbean, urid or mash is an economically important grain legume crop in Asia, widely cultivated on marginal lands with low inputs during *kharif*, *rabi* and summer seasons. Similar to other pulse crops, urdbean also enriches soil nitrogen content for soil fertility restoration with a considerable importance of its adaptation to short growth duration and low water requirement. It is utilized in various ways where seeds are consumed as source of protein, vitamins and minerals, while plant parts are used as fodder. It is an important source of inexpensive vegetable protein, iron, calcium, vitamins and essential amino acids in daily diets of vegetarian and non-vegetarian diets in most of the Asian population. It is an important pulse crop and narrow genetic diversity, which basic cause for its failure in achieving raised yield levels. Because, black gram is being a self-pollinated crop and flower drop (Arulbalachandran *et al.,* 2010). Selection of genotypes based on yield as such is difficult to the integrated structure of plant in which most of the characters are inherited and being governed by the large number of cumulative, duplicate and dominant genes. Urdbean breeding strategy involves generating genetic material, selection of superior genotypes from the variable genetic material to develop superior varieties.

Genetic variability is the basic requirement for making progress in crop breeding (Appalaswamy and Reddy, 2004). Creation of variability through pollination and artificial hybridization is very difficult in this crop as the flowers are cleistogamous and delicate to handle. But, artificial induction of variability by mutation breeding can be effectively utilized can generate new variability and it has been recognized as a valuable supplement to conventional breeding in crop improvement (Singh *et al.,* 2000; Deepalakshmi and Anandakumar, 2004; Khan and Wani, 2006; Anbu Selvam *et al.,* 2010; Ali *et al.,* 2011).

Hence, an attempt was made to compare the variability generated through mutation breeding to improve the productivity of black gram. 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 urd bean breeding found high variation in yield per plant and nutritional quality, especially contents of protein, methionine and total sugars (Arulbalachandran and Mullainathan, 2009). The aim of this study was to generate information on the magnitude of induced genetic variability with the application of gamma rays and EMS.

MATERIALS AND METHODS

Black gram variety Vamban 4 (VBN 4) was chosen to induce mutation by physical and chemical mutagen *viz.,* gamma rays and EMS respectively to estimate the genetic variation on quantitative characters in M_2 generation. The seeds were collected from Vamban, Tamilnadu, India. VBN 4 is resistant to recent developments on yellow mosaic virus (YMV), late senescence suitable for all seasons (June to July, September to October and February to March). This study was carried out in Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai during the year 2010 to 2011.

For fixing LD_{50} value of physical mutagen, 9 sets containing 75 well filled seeds were treated with gamma rays (10 kR to 90 kR with an interval of 10 kR) in the gamma chamber installed at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore where cobalt-60 serves as source of gamma rays. Non-irradiated dry seeds are also taken to utilize it as control. Another quantity of 75 well filled seeds for each treatment was presoaked for 16 hours in distilled water (Malarkodi, 2008), blotted dry, and treated with freshly prepared solutions of EMS (30 mM to 70 mM with an interval of 10 mM) for 6 hours with intermittent shaking. A sample of 150 seeds was soaked in distilled water for the respective duration to utilize it as control. After treatment, seeds were thoroughly washed in running tap water for half an hour to leach out the residual of chemicals. The seeds were then subjected to germination test. Based on the effect of physical and chemical mutagen on germination, LD_{50} value was obtained.

The LD_{50} values for the mutagens were worked out based on observations recorded on seed germination under laboratory conditions. 50% reduction in germination was obtained at 50 kR for gamma rays and at 60mM for EMS treatments. Based on this, the mutagenic doses studied under field condition for gamma rays *viz.,* 40 kR, 50 kR and 60 kR, EMS 50 mM, 60 mM and 70 mM. For M_1 generation, in each treatment a total of 150 seeds were sown in the field along with control in a randomized block design (RBD) with 3 replications by adopting a spacing of 30 cm between rows and 15 cm between plants. Recommended agronomic practices and plant protection measures were followed as per crop production manual (TNAU and Department of Agriculture).

The M_2 generation was raised on M_1 plant basis following plant to progeny method in a RBD with 3 replications. Thirty plants per treatment were forwarded from the M_1 to the M_2 generation. The study was carried out with various observations, such as plant height (cm), flowering time (days), primary branches number, cluster number, pod number, number of seeds per pod, pod length (cm), 100 seed weight (g) and seed yield (g) in M_2 generation. Phenotypic and genotypic coefficient of variation (PCV and GCV) was computed using the formula adopted by Burton (1952) and categorized of the range of variation was done as proposed by Sivasubramaniam and Madhava Menon (1973). Heritability (h^2) in broad sense was computed using the formula according to Lush (1940) and it was classified according to Robinson (1966). Genetic advance (GA) as per cent of mean was estimated, adopted the method suggested by Johnson *et al.* (1955). Analysis of variance of all the parameters was computed using the software TNAUSTAT statistical package (Retrived from https://sites.google.com/site/ tnaustat).

RESULTS AND DISCUSSION

Effect of mutagens on quantitative mean performance

Scossiroli (1977) suggested that an estimation of the extent of induced genetic variability in quantitative traits in M_2 itself would provide valuable information for designing selection programme. It is therefore, considered worthwhile to study the shift of mean values and gather information on induced genotypic variance, heritability and GA as per cent of mean for different polygenic traits in the M_2 families of different mutagenic treatments. The mean values of the treatments are presented in Table 1. Among the different dose of gamma rays and EMS, a gradual increase of mean values was observed up to optimal dose when compared to control in M_2 generation. Beyond the optimal dose of mutagen showed decreasing of mean values of quantitative traits. Variability analysis showed an increase all the traits. The wide range of variation was observed in all the traits of 6 treatments. EMS treatments were more effective and increasing the mean values than gamma rays. Higher values of mean for plant height (26.1), flowering time (minimum number of days was taken to flowering - 38), number of primary branches per plant (2.3) and number of seeds per pod (6.3) were recorded at 50 kR of gamma ray treatment. The plant height (28.8), flowering time (38), number of primary branches per plant (2.4), number of clusters per plant (8.4), number of pods per plant (18.3) and seed yield per plant (5.8) was found in 50 mM of EMS.

Table 1. The mean values of different characters of black gram in M_2 generation.

Treatment	Plant height (cm)	Flowering time (days)	Number of primary branches	Number of clusters per plant	Number of pods per plant	Number of seeds per pod	Pod length (cm)	100 seed weight (g)	Yield / plant (g)
Gamma control	25.6	39.0	2.2	7.6	15.1	5.6	5.4	4.7	4.7
40 kR	24.5	39.0	2.3	8.6	17.3	5.2	5.1	3.4	4.7
50 kR	26.1	38.0	2.3	7.4	12.9	6.3	4.3	4.2	3.9
60 kR	17.3	43.0	1.9	6.5	14.2	4.6	4.2	4.8	4.3
EMS control	25.4	39.0	2.0	7.4	15.8	5.8	5.2	4.6	4.8
50 mM	28.8	38.0	2.4	8.4	18.3	5.7	4.7	2.1	5.8
60 mM	25.0	41.0	1.9	7.7	10.1	5.4	4.8	4.6	4.7
70 mM	23.7	38.0	1.7	6.9	10.4	5.6	4.2	3.8	4.6

Plant height has been shown to be an important trait for predicting the competitive ability of wheat cultivars (Wicks *et al.*, 2004). In this study showed plant height and other growth traits increased than control plant because effect of mutagen on genome may induce genetic variability. These results are akin to the findings of Wani and Anis (2001), Kampli *et al.* (2002) and Arulbalachandran *et al.* (2010). 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. In addition, high doses of gamma irradiation were reported to be harmful in several studies like that of Devi and Mullainathan (2012), who reported that higher doses of gamma irradiation reduced plant height, number of clusters, number of pods and number of seeds of black gram.

Effect of mutagens on Phenotypic and genotypic coefficient variation (PCV and GCV)

Knowledge on genetic variability of the available population is very essential for any crop improvement programme. The success of breeder in selecting genotypes possessing higher yield and growth traits depends largely on the existence and exploitation of genetic variability of the fullest extent. Gaul (1964) stated that radiation induced variability could be determined in M_2 generation. Phenotypic and genotypic coefficient of variability for various quantitative traits in M_2 generation of black gram is shown in Table 2 and Table 3. Variability was found to be larger in gamma ray treatments than in the EMS and control (untreated) population for all characters studied except pod length in the investigation. The genetic parameters were also found to be at their maximum in mutagenic treated plants over the control.

Table 2. Phenotypic and genotypic coefficient of variation induced by gamma rays in M_2 generation.

Gamma rays	40 kR		50 kR		60 kR	
	PCV	GCV	PCV	GCV	PCV	GCV
Plant height (cm)	20.1	18.5	19.4	17.1	15.6	14.3
Flowering time (days)	6.9	6.6	3.9	3.3	5.4	5.1
Number of primary branches	64.8	54.2	49.6	33.1	53.4	30.1
Number of clusters per plant	28.0	22.1	12.9	12.2	43.7	37.3
Number of pods per plant	15.6	14.3	60.6	58.6	42.2	40.3
Number of seeds per pod	30.4	27.2	13.6	10.4	21.6	15.4
Pod length (cm)	19.5	16.0	16.0	9.3	15.8	10.5
100 seed weight (g)	24.4	24.1	19.4	18.8	29.3	27.8
Seed yield per plant (g)	35.6	28.1	23.5	19.9	27.9	26.9

Table 3. Phenotypic and genotypic coefficient of variation induced by EMS in M_2 generation.

EMS	50 mM		60 mM		70 mM	
	PCV	GCV	PCV	GCV	PCV	GCV
Plant height (cm)	13.3	12.4	13.1	11.7	23.2	22.5
Flowering time (days)	4.0	3.6	4.7	4.3	4.8	4.4
Number of primary branches	31.4	26.0	26.6	25.2	27.3	25.6
Number of clusters per plant	34.2	34.0	15.1	14.5	15.1	15.2
Number of pods per plant	36.8	35.5	22.9	18.8	37.5	34.6
Number of seeds per pod	19.8	14.2	25.2	23.4	17.1	11.0
Pod length (cm)	23.2	20.6	22.5	15.4	28.3	20.9
100 seed weight (g)	11.6	9.8	26.7	24.6	14.9	14.1
Seed yield per plant (g)	28.5	25.7	32.6	24.1	33.2	25.6

In general, the estimates of PCV were higher than the estimates of GCV for all the traits under study indicating the apparent variation was not only due to genotypes, but also due to the influence of environment on the expression of the characters (Figure 1). Similar results were reported by Tabasum *et al.* (2010) in greengram, Konda *et al.* (2009) and Reddy *et al.* (2011) in black gram. High coefficients of phenotypic and genotypic variation were observed for number of pods per plant followed by number of primary branches per plant and seed yield per plant. Similar results were also reported by Raje and Rao (2000) and Rai *et al.* (2008). EMS showed high PCV and GCV in number of pods per plant, number of clusters per plant, number of primary branches per plant, pod length and seed yield per plant. Nehru *et al.* (2009) obtained the highest PCV and GCV values in number of branches per plant, number of clusters per plant, number of pods per plant and seed yield per plant in cowpea, while Rahim *et al.* (2010) obtained the highest in mungbean. Our results are also supported by previous published studies that were reported by Khan *et al.* (2004), Renuka and Singh (2006), Sinha and Lal (2007), Lekharam (2010), Patil and Wakode (2011) for different characters in different crops. The high PCV and GCV observed from their high variability in turn offers good scope for selection. Moderate PCV and GCV were observed in plant height, number of seeds per pod and 100 seed weight. Selection will be effective based on the heritable nature of these traits. The wide range of variability was exhibited by heritability and genetic advance as percent of mean.

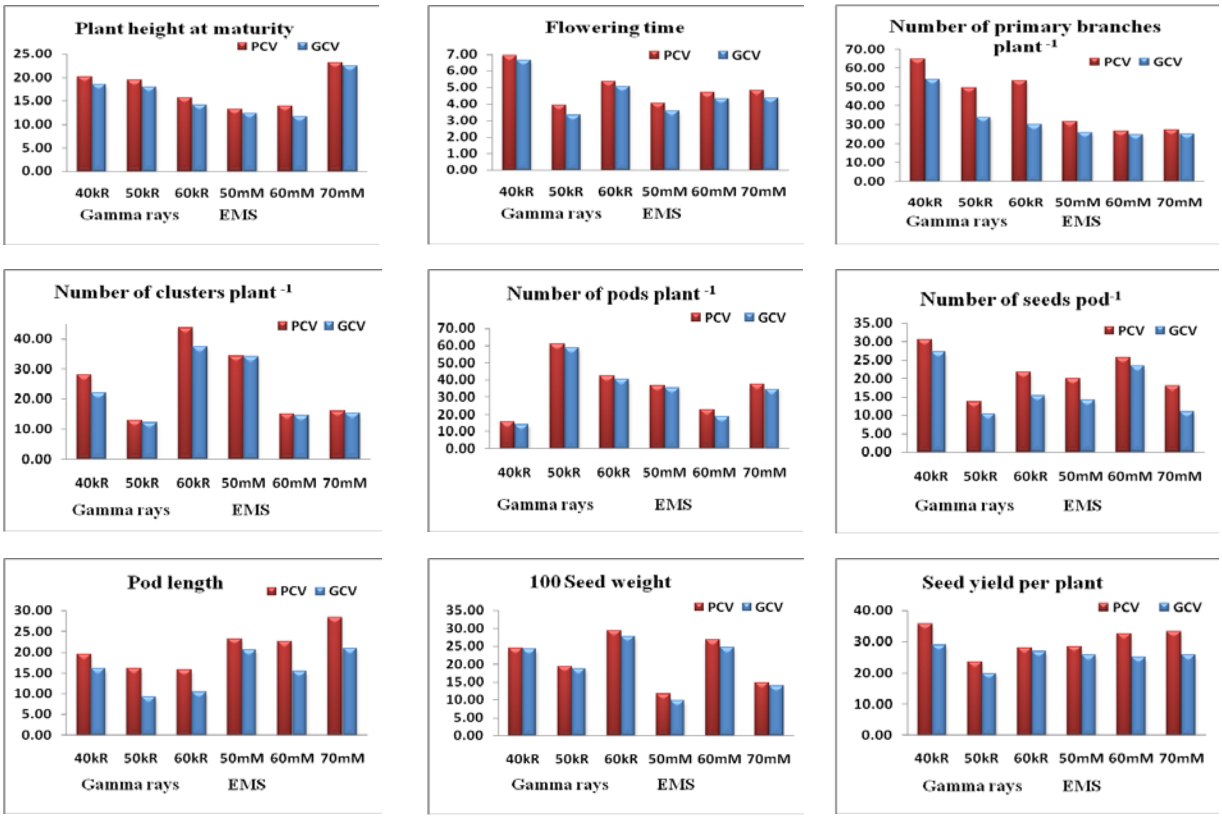

Figure 1. Estimates of PCV and GCV for yields and its components in M_2 generation of black gram.

Effect of mutagens on heritability and genetic advance as percent of mean (h^2 and GA%)

The estimate of heritability acts as a predictive instrument in expressing the reliability of phenotypic values. Therefore, it helps the plant breeders to make selection for a particular character when heritability is high in magnitude (Unche *et al.*, 2008). High value of heritability together with high genetic advance for any character indicates additive gene action and selection will be rewarding for improvement of such traits, whereas high heritability associated with low genetic advance might attribute to the presence of non-additive gene action which indicates epistasis, dominance and genotypic and environmental interaction (Tikka *et al.*, 1977), hence their response to selection would be poor.

Kumar (2008) recorded high heritability for number of seeds per pod, days to flowering, yield per plant, 100 grain weight, number of pods per plant and harvest index in pea. The heritability was high almost all the doses of gamma ray and EMS treatments (Table 4 and Table 5). However, 50 kR of gamma rays revealed highest values of heritability with genetic advance as per cent of mean for number of primary branches per plant (46.7; 38.6), number of pods per plant (93.3; 21.2), 100 seed weight (93.8; 37.5) and seed yield per plant (72.2; 26.5). Heritability coupled with genetic advance as per cent of mean were comparatively high at 50 mM of EMS for number of primary branches per plant (68.7; 38.3), pod length (78.9; 24.5) and seed yield per plant (81.7; 24.4). This indicates that these traits are under the control of additive gene action and directional selection for these traits could be effective for desired genetic improvement. It is in accordance with the findings of Malik *et al.* (2008) and Bhareti *et al.* (2011). High heritability with low genetic advance as percent of mean was recorded for plant height and flowering time indicates that these characters are under the control of non-additive gene action and hence selection would be ineffective.

Table 4. Heritability (h^2) and genetic mean (%) induced by gamma rays in M_2 generation.

Gamma rays	40 kR		50 kR		60 kR	
	h^2	GA%	h^2	GA%	h^2	GA%
Plant height (cm)	84.8	9.9	85.5	9.4	83.5	7.9
Flowering time (days)	91.3	4.3	72.4	3.5	88.2	3.8
Number of primary branches	69.1	56.6	46.7	38.6	31.7	31.9
Number of clusters per plant	62.0	18.2	88.3	14.1	72.1	28.1
Number of pods per plant	83.5	7.9	93.3	21.2	91.2	17.6
Number of seeds per pod	80.1	26.8	58.8	14.3	50.6	18.9
Pod length (cm)	67.6	20.5	33.3	11.9	43.7	15.2
100 seed weight (g)	97.8	49.1	93.8	37.5	89.9	54.3
Seed yield per plant (g)	66.3	28.6	72.2	26.5	92.4	25.4

Table 5. Heritability (h^2) and genetic mean (%) induced by EMS in M_2 generation.

EMS	50 mM		60 mM		70 mM	
	h^2	GA%	h^2	GA%	h^2	GA%
Plant height (cm)	87.0	7.3	69.9	7.1	93.9	9.5
Flowering time (days)	77.9	3.5	85.1	3.8	83.6	3.9
Number of primary branches	68.7	38.3	90.1	39.1	87.9	43.2
Number of clusters per plant	98.7	13.8	92.0	14.1	91.1	15.6
Number of pods per plant	93.0	13.1	67.3	15.8	85.3	20.7
Number of seeds per pod	51.2	16.4	84.1	23.9	37.6	12.4
Pod length (cm)	78.9	24.5	46.8	17.1	54.55	24.1
100 seed weight (g)	71.7	17.1	85.2	46.8	89.9	27.5
Seed yield per plant (g)	81.7	24.4	58.8	25.6	59.5	26.2

CONCLUSION

In this study, the quantitative traits of the M_2 generation revealed the enhancement of the significant level of yield attributes in black gram. Among the various dose/concentration treatments, 50 kR of gamma rays and 50 mM of EMS treatment were more desirable, which resulted in low plant damage and higher genetic effects. The wide range of variability for different traits coupled with high heritability and high genetic advance for important yield traits hence selection is effective for these traits. The maximum variation in polygenic traits may show the stable gene mutations in subsequent generation. The results indicate that urdbean mutant lines are useful for crop improvement and further study is needed for the analysis of the mutants.

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