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# Induced polygenic variability using combination treatment of gamma rays and ethyl methane sulphonate in blackgram (*Vigna mungo* (L.) Hepper)

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**Induced mutation in plant improvement has been proven to be one of the alternative ways to generate new sources of genetic variation in blackgram. In this study, dry seeds of VBN 4 blackgram were treated with combination treatment of both gamma rays (400, 500 and 600 Gy) and ethyl methane sulphonate (EMS) (50, 60 and 70 mM) to study the polygenic characters in $M_2$ generation. The mean values for plant height, number of primary branches, number of clusters per plant, number of pods per plant, number of seeds per pod and single plant yield decreased below the control in most of the treatments. The mean single plant yield was more than for the control at 400 Gy+60 mM and 600 Gy+50 mM. Moderate and high phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were recorded in yield component characters such as plant height, number of primary branches, number of clusters per plant and number of pods per plant. A high amount of heritability and GA as per cent of mean was noted for plant height, number of clusters per plant, number of primary branches per plant, number of pods per plant, pod length, number of seeds, 100 seed weight and single plant yield. This denoted that these characters are governed largely by additive gene effect, which may be favorably exploited for improvement through simple selection in $M_2$ generation.**

**Key words:** Gamma rays, ethyl methane sulphonate (EMS), blackgram, polygenic variability, $M_2$ generation.

## INTRODUCTION

Pulses constitute an important role in human dietary. They are important source of protein and are essential adjuncts to a predominantly cereal based diet and enhance the biological value of protein consumed. Pulses are often attributed as “poor man’s diet,” which are really important in Indian diet as a source of protein. Though pulses contributed significant role in human consumption but they have not yet reached a comfortable level of production. Blackgram [*Vigna mungo* (L.) Hepper], one of the important proteinaceous pulse crop grown largely in

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**Abbreviations: EMS,** Ethyl methane sulphonate; **RBD,** randomized block design; **GA,** genetic advance; **PCV,** phenotypic coefficients of variation; **GCV,** genotypic coefficients of variation.

India, needs crop improvement to a considerable extent. It contains about 26% protein, which is almost three times more than cereals. 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. In India, it covers an area of about 3.24 million hectares and produces 1.46million tonnes. Its productivity is only 526 kg per ha. In Tamil Nadu, blackgram covers an area of about 3.41 lakh hectares with production of 1.21 lakh tonnes and 355 kg per ha. For any successful breeding programme, the variability is the basic tool on which the selection is exercised for genetic improvement. Creation of variability through pollination and artificial hybridization is very difficult in this crop as the flowers are cleistogamous and delicate to handle. Even if hybridization is carried out the seed set is less than 5%. Also, this crop lacks proper male sterility system commercially to be utilized for hybridization (Anbu Selvam et al., 2010).

Research on blackgram is lagging behind than that of cereals and other legumes (Arulbalachandran and Mullainathan, 2009). In order to improve yield and other polygenic characters, mutation breeding can be effectively utilized (Deepalakshmi and Ananda Kumar, 2004). Induction of mutation forms an important part of breeding programme as it widens the gene pool through creation of genetic variability. According to Raje and Rao (2000), genetic variability is essential in order to realize response to selection pressure as the estimates of genetic parameters of variation are specific for a particular population and the phenotypic expression of the quantitative character may be altered by environmental stress that affect plant growth and development. Therefore, an attempt has been made to study the magnitude of variability through induction of mutation using both gamma rays and ethyl methane sulphonate (EMS) and their combinations.

## MATERIALS AND METHODS

Blackgram variety Vamban-4 (VBN 4) was selected to induce mutation by combination treatment of gamma rays and EMS to analyze polygenic variability in $M_2$ generation. 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 2009 to 2011.

### Induction of mutation: fixing $LD_{50}$ value under laboratory

#### *Physical mutagen (gamma rays)*

For fixing $LD_{50}$ value of physical mutagen, nine sets containing 75 well filled seeds were treated with gamma rays (100 Gy to 900 Gy with an interval of 100 Gy) 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 were also taken as control.

#### *Chemical mutagen (EMS)*

Five different concentrations of EMS ranging from 30 to 70 mM with 10 mM interval were used initially to fix $LD_{50}$ value. Five sets containing 75 well filled seeds were presoaked for 16 h in distilled water initially (Malarkodi, 2008). Then soaked seeds were treated with EMS for 6 h. A sample of 150 seeds was soaked in distilled water for the respective duration to utilize it as control. After soaking the seeds in the mutagen, they were thoroughly washed in running tap water for half an hour. The seeds were then subjected to germination test. Based on the effect of physical and chemical mutagen on germination, $LD_{50}$ value was obtained.

#### *Combination treatment (gamma rays + EMS)*

Based on the $LD_{50}$ value of both the mutagens, initially the seeds were exposed to gamma irradiation and then same seeds were treated with EMS following the same procedure as mentioned above. Non-irradiated wet seeds are also taken to utilize it as control for combination treatment.

#### *Raising $M_1$ generation*

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 500 Gy for gamma rays and at 60 mM for EMS treatments. Based on this, the mutagenic doses studied under field condition for combination treatments *viz.*, 400 Gy + 50 mM, 400 Gy + 60 mM, 400 Gy + 70 mM, 500 Gy + 50 mM, 500 Gy + 60 mM, 500 Gy + 70 mM, 600 Gy + 50 mM, 600 Gy + 60 mM and 600 Gy + 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 three 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).

#### *Raising $M_2$ generation*

The $M_2$ generation was raised on $M_1$ plant basis following plant to progeny method in a RBD with three replications. Thirty plants per treatment were forwarded from the $M_1$ to the $M_2$ generation. Data on various quantitative traits *viz.,* seed yield (g), plant height (cm), days to 50% flowering (days), primary branches number, cluster number, pod number, number of seeds per pod, pod length (cm) and 100 seed weight (g) were taken and analyzed statistically to estimate the PCV, GCV (Burton, 1952), broad sense heritability ($h^2$) and genetic advance (GA) as per cent of mean (Johnson et al., 1955).

## RESULTS AND DISCUSSION

### Effect of population mean

Aastveit (1968) and 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 quantitative traits in the $M_2$ families of different

**Table 1.** Mean performance of blackgram in relation to different concentration of gamma rays + EMS in $M_2$ generation.

| Gamma rays (Gy) + EMS (mM) | Plant height (cm) | Days to 50% flowering | 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) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 25.40 | 39.13 | 2.00 | 7.36 | 15.80 | 5.82 | 5.20 | 4.57 | 4.75 |
| 400 Gy + 50 mM | 26.71 | 38.95 | 2.20 | 7.98 | 12.15 | 5.60 | 4.73 | 3.54 | 4.15 |
| 400 Gy + 60 mM | 22.41 | 40.00 | 2.15 | 6.80 | 9.92 | 6.35 | 4.99 | 4.25 | 4.88 |
| 400 Gy + 70 mM | 20.84 | 41.99 | 1.40 | 9.89 | 15.35 | 6.40 | 4.49 | 3.23 | 4.18 |
| 500 Gy + 50 mM | 27.14 | 38.80 | 2.40 | 7.65 | 8.98 | 5.50 | 3.93 | 4.14 | 3.39 |
| 500 Gy + 60 mM | 22.80 | 41.35 | 1.75 | 7.58 | 19.85 | 5.35 | 4.94 | 4.65 | 4.68 |
| 500 Gy + 70 mM | 24.59 | 39.90 | 1.33 | 6.90 | 12.64 | 5.20 | 5.04 | 3.99 | 3.04 |
| 600 Gy + 50 mM | 26.70 | 42.69 | 2.00 | 8.25 | 18.65 | 4.60 | 4.58 | 2.77 | 5.01 |
| 600 Gy + 60 mM | 23.30 | 38.87 | 1.95 | 6.88 | 11.00 | 5.95 | 3.95 | 4.51 | 4.75 |
| 600 Gy + 70 mM | 26.03 | 39.00 | 1.51 | 7.50 | 9.72 | 5.77 | 4.92 | 3.77 | 4.82 |

mutagenic treatments. The mean values of the treatments are presented in Table 1. The study reveals that, the mean values for plant height, number of primary branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod and single plant yield decreased below the control in most of the treatments. Similar results were reported in blackgram (Vanniarajan, 1989), cowpea (Palanisamy, 1975), greengram (Krishnaswami, 1977), redgram (Natarajan et al., 1982) and lentil (Sinha and Lal, 2007).

According to Prema et al. (1998), the mean values for plant height, number of pods per plant and single plant yield decreased than the control in all the mutagenic treatments in blackgram. In the present study, the mean single plant yield was more than for the control at 400 Gy + 60 mM and 600 Gy + 50 mM doses. This was in confirmation with the results of Singh et al. (2000) in blackgram. Samiullah et al. (2000) in greengram reported increased mean values for number of branches per plant, Khan et al. (1994) for number of pods per plant and plant height and Tickoo and Chandra (1999) for number of seeds per pod and yield per plant in greengram. Murugan and Subramanian (1993) reported that mean of most of the polygenic traits was shifted in both the direction in cowpea. The changes in the mean values after mutagenic treatments has been reported earlier in many pulse crops including mungbean (Wani et al., 2005, Arulbalachandran and Mullainathan, 2009), lentil (Singh et al., 2006) and urdbean (Deepalakshmi and Ananda Kumar, 2003).

## Variability

Knowledge on genetic variability of the available population is very essential for any crop improvement programme, as it positively enhance the efficiency of selection. Gaul (1964) stated that radiation induced variability could be determined in M2 generation whereas Borojevic and Borojevic (1969) opined that variability increased in wheat up to four times in M2 and M3 which decreased in M4 and stabilized around M5 generation. Increase in variability following mutagenic treatments was reported by Kharkwal (2000), Khan et al. (2004) and Sengupta and Datta (2004). In order to know the breeding utility of this variability and selection value of various quantitative traits, it is essential to determine various components and heritable proportion of variability.

In general, PCV values were more than GCV values (Figure 1). This indicates that the apparent variation was not only due to genotypes, but also due to the influence of environment on the expression of the characters. The characters plant height at maturity, number of primary branches, number of clusters per plant and number of pods per plant recorded moderate and high PCV and GCV. The low, moderate and high PCV and GCV were observed for number of seeds per pod, pod length, 100 seed weight and seed yield per plant in the mutated population. Low PCV and GCV were recorded in days to 50% flowering (Table 2). Similar results were reported in blackgram

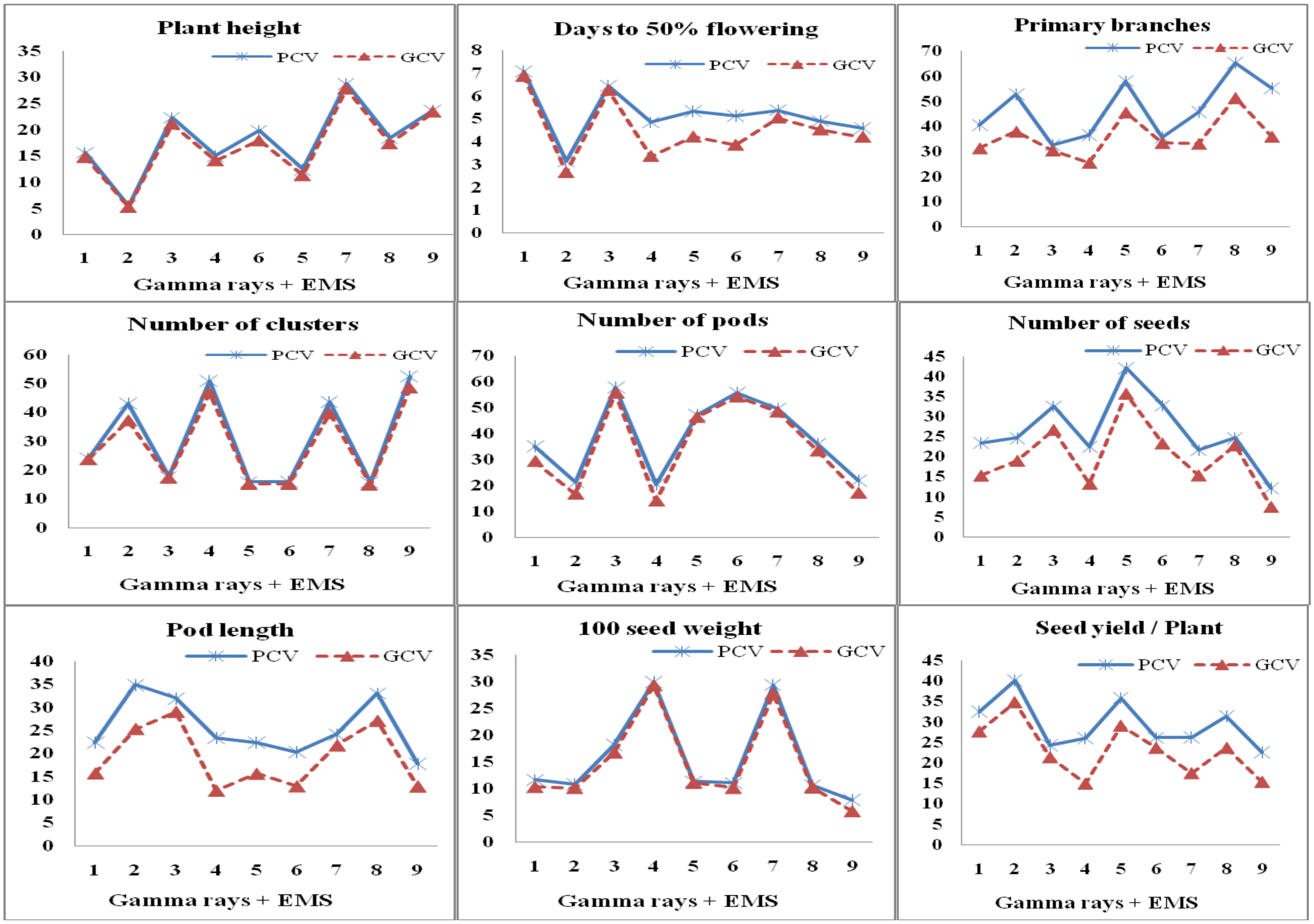


**Figure 1.** Comparative estimation of genetic parameters for yields and its components in $M_2$ generation of blackgram. Gamma rays + EMS (x axis) : 1) 400 Gy + 50 mM; 2) 400 Gy + 60 mM; 3) 400 Gy + 70 mM; 4) 500 Gy + 50 mM; 5) 500 Gy + 60 mM; 6) 500 Gy + 70 mM; 7) 600 Gy + 50 mM; 8) 600 Gy + 60 mM and 9) 600Gy + 70 mM.

**Table 2.** Phenotypic (PCV) and genotypic coefficient of variation (GCV) induced by 400 Gy + EMS in $M_2$ generation.

| Gamma rays + EMS | 400 Gy + 50 mM | | 400 Gy + 60 mM | | 400 Gy + 70 mM | |
|---|---|---|---|---|---|---|
| | PCV | GCV | PCV | GCV | PCV | GCV |
| Plant height (cm) | 15.67 | 14.78 | 5.39 | 5.25 | 22.33 | 21.14 |
| Days to 50% flowering | 7.09 | 6.88 | 3.14 | 2.66 | 6.45 | 6.26 |
| Number of primary branches | 40.66 | 31.42 | 52.80 | 38.07 | 32.58 | 30.56 |
| Number of clusters per plant | 24.09 | 23.74 | 43.10 | 37.24 | 17.70 | 17.39 |
| Number of pods per plant | 35.05 | 29.33 | 21.40 | 16.70 | 57.70 | 55.64 |
| Number of seeds per pod | 23.46 | 15.33 | 24.65 | 19.04 | 32.62 | 26.72 |
| Pod length (cm) | 22.38 | 15.74 | 34.91 | 25.34 | 31.95 | 29.03 |
| 100 seed weight (g) | 11.63 | 10.28 | 10.82 | 10.10 | 18.10 | 16.75 |
| Yield / plant (g) | 32.50 | 27.63 | 40.13 | 34.79 | 24.28 | 21.36 |
| **PCV and GCV induced by 500 Gy + EMS in $M_2$ generation** | | | | | | |
| **Gamma rays + EMS** | **500 Gy + 50 mM** | | **500 Gy + 60 mM** | | **500 Gy + 70 mM** | |
| | **PCV** | **GCV** | **PCV** | **GCV** | **PCV** | **GCV** |
| Plant height (cm) | 15.01 | 14.16 | 19.86 | 17.98 | 12.41 | 11.31 |
| Days to 50% flowering | 4.85 | 3.37 | 5.34 | 4.22 | 5.14 | 3.86 |

**Table 2.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| Number of primary branches | 36.70 | 25.65 | 58.20 | 45.70 | 35.63 | 33.59 |
| Number of clusters per plant | 50.94 | 46.89 | 15.83 | 15.23 | 15.95 | 15.23 |
| Number of pods per plant | 20.38 | 14.05 | 47.12 | 46.27 | 55.68 | 54.13 |
| Number of seeds per pod | 22.46 | 13.33 | 42.14 | 35.70 | 32.78 | 23.33 |
| Pod length (cm) | 23.39 | 11.97 | 22.38 | 15.65 | 20.33 | 12.93 |
| 100 seed weight (g) | 29.90 | 29.39 | 11.33 | 11.06 | 11.02 | 10.11 |
| Yield / plant (g) | 25.98 | 14.92 | 35.77 | 29.06 | 26.09 | 23.65 |
| **PCV and GCV induced by 600 Gy + EMS in $M_2$ generation** | | | | | | |
| **Gamma rays + EMS** | **600 Gy + 50 mM** | | **600 Gy + 60 mM** | | **600 Gy + 70 mM** | |
| | **PCV** | **GCV** | **PCV** | **PCV** | **GCV** | **PCV** |
| Plant height (cm) | 28.80 | 27.89 | 18.39 | 17.41 | 23.72 | 23.46 |
| Days to 50% flowering | 5.38 | 5.05 | 4.91 | 4.53 | 4.60 | 4.21 |
| Number of primary branches | 45.85 | 33.22 | 65.45 | 51.45 | 55.28 | 36.08 |
| Number of clusters per plant | 43.59 | 39.75 | 15.66 | 14.92 | 52.54 | 48.61 |
| Number of pods per plant | 49.55 | 48.35 | 36.00 | 33.34 | 21.87 | 17.09 |
| Number of seeds per pod | 21.62 | 15.38 | 24.64 | 22.88 | 12.10 | 7.56 |
| Pod length (cm) | 24.12 | 21.78 | 33.09 | 27.07 | 17.74 | 12.90 |
| 100 seed weight (g) | 29.37 | 27.81 | 10.59 | 10.18 | 7.84 | 5.74 |
| Yield / plant (g) | 26.16 | 17.49 | 31.30 | 23.66 | 22.49 | 15.39 |

(Arulbalachandran and Mullainathan, 2009). Similarly, Tabasum et al. (2010) recorded high PCV and GCV for number of cluster per plant, number of pods per plant, seeds per pod, 100 seed weight and seed protein content in blackgram and soybean by Patil and Wakode (2011). Highest magnitude of variability for number of pods per plant in blackgram due to induced mutagenesis was reported by Singh et al. (2000), Anbu Selvam et al. (2010) and Arulbalachandran et al. (2010) in blackgram, Samiullah et al. (2000) in greengram, Waghmare and Mehra (2000), Talukdar and Biswas (2008) in grass pea, Rai et al. (2008) and Lekharam (2010) in French bean, Renuka and Singh (2006) in rice bean and Ali et al. (2011) in chickpea.

## Heritability and GA as per cent of mean

Johnson et al. (1955) suggested heritability in combination with genetic advance was more effective and reliable in prediction of resultant effect of selection than heritability alone. This is because, the heritability estimates are subjected to certain estimation errors, that is, overestimation of the parameters would affect the selection efficiency more than the underestimation (Lin et al., 1979) and genotype-environment interaction (Kaul and Garg, 1979). The estimate of heritability acts as a predictive mechanism in expressing the reliability of phenotypic values. Hence, it helps plant breeders to make a selection for a particular character when heritability is high (Arulbalachandran et al., 2010). Base on the fact that heritability is also influenced by the environment, the information on heritability alone may not suffice in pinpointing characters enforcing selection. Therefore, an estimation of heritability, coupled with genetic advance, is also needed to assess the heritable portion of total variation and the genetic gain expected for effective selection. Heritability estimates provide information on the extent to which a particular genetic character can be transmitted to successive generations, whereas genetic advance helps in formulating suitable selection indices. Such estimates facilitate the evaluation of genetic and environmental effects, thereby aiding in selection. Estimates of heritability can be used to predict genetic advance under selection, so that breeders can anticipate improvement from different types and intensities of selection. 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.

Among the characters investigated in the present study, high heritability accompanied with high genetic advance as per cent of mean was observed for the trait like plant height, number of clusters per plant, number of primary branches per plant, number of pods per plant, pod length, number of seeds, 100 seed weight and single plant yield (Table 3). This indicates that these traits are under the control of additive gene action and directional selection for these traits could be effective for

**Table 3.** Heritability ($h^2$) and genetic mean (%) induced by 400Gy + EMS in $M_2$ generation.

| Gamma rays + EMS | 40 0Gy + 50 mM | | 400 Gy + 60 mM | | 400 Gy + 70 mM | |
|---|---|---|---|---|---|---|
| | $h^2$ | GA% | $h^2$ | GA% | $h^2$ | GA% |
| Plant height (cm) | 88.97 | 8.09 | 94.89 | 10.54 | 89.61 | 9.59 |
| Days to 50% flowering | 94.17 | 4.07 | 71.84 | 3.02 | 93.94 | 3.76 |
| Number of primary branches | 59.72 | 42.13 | 51.84 | 44.12 | 87.98 | 51.48 |
| Number of clusters per plant | 97.10 | 14.34 | 74.65 | 27.47 | 96.51 | 11.50 |
| Number of pods per plant | 70.04 | 18.13 | 60.89 | 14.57 | 92.98 | 19.12 |
| Number of seeds per pod | 42.72 | 15.67 | 59.64 | 19.29 | 67.11 | 23.64 |
| Pod length (cm) | 49.49 | 18.70 | 52.68 | 23.82 | 82.59 | 29.32 |
| 100 seed weight (g) | 78.12 | 18.71 | 87.19 | 29.43 | 85.70 | 31.95 |
| Yield / plant (g) | 72.31 | 30.26 | 75.18 | 31.35 | 77.40 | 26.48 |

**Heritability ($h^2$) and genetic mean (%) induced by 500 Gy + EMS in $M_2$ generation**

| Gamma rays + EMS | 500 Gy + 50 mM | | 500 Gy + 60 mM | | 500 Gy + 70 mM | |
|---|---|---|---|---|---|---|
| | $h^2$ | GA% | $h^2$ | GA% | $h^2$ | GA% |
| Plant height (cm) | 88.94 | 7.86 | 81.91 | 10.27 | 83.22 | 7.64 |
| Days to 50% flowering | 48.48 | 2.99 | 62.59 | 3.62 | 56.61 | 3.40 |
| Number of primary branches | 48.65 | 33.21 | 61.52 | 57.59 | 88.87 | 54.73 |
| Number of clusters per plant | 84.71 | 28.16 | 92.58 | 14.38 | 91.17 | 15.57 |
| Number of pods per plant | 47.51 | 12.54 | 96.39 | 13.33 | 94.51 | 19.77 |
| Number of seeds per pod | 35.21 | 13.15 | 71.79 | 30.25 | 50.66 | 21.96 |
| Pod length (cm) | 26.18 | 12.19 | 48.93 | 18.13 | 40.45 | 14.71 |
| 100 seed weight (g) | 96.57 | 59.49 | 95.29 | 22.24 | 84.17 | 19.11 |
| Yield / plant (g) | 32.98 | 17.02 | 66.02 | 28.71 | 82.19 | 32.20 |

**Heritability ($h^2$) and genetic mean (%) induced by 600Gy + EMS in $M_2$ generation**

| Gamma rays + EMS | 600 Gy + 50 mM | | 600 Gy + 60 mM | | 600 Gy + 70 mM | |
|---|---|---|---|---|---|---|
| | $h^2$ | GA% | $h^2$ | GA% | $h^2$ | GA% |
| Plant height (cm) | 93.77 | 10.02 | 89.71 | 9.30 | 97.79 | 7.42 |
| Days to 50% flowering | 88.19 | 3.78 | 85.08 | 3.87 | 83.57 | 3.74 |
| Number of primary branches | 52.50 | 42.97 | 61.70 | 57.98 | 42.59 | 46.21 |
| Number of clusters per plant | 83.16 | 25.23 | 90.79 | 15.54 | 85.62 | 28.75 |
| Number of pods per plant | 95.20 | 14.96 | 85.76 | 19.63 | 61.08 | 14.90 |
| Number of seeds per pod | 50.59 | 18.94 | 86.20 | 22.02 | 39.03 | 10.29 |
| Pod length (cm) | 81.52 | 25.28 | 66.94 | 30.26 | 52.87 | 17.14 |
| 100 seed weight (g) | 89.60 | 54.22 | 92.37 | 20.16 | 53.70 | 8.67 |
| Yield / plant (g) | 44.70 | 18.13 | 57.13 | 24.42 | 46.81 | 17.79 |

desired genetic improvement. This is in conformity with Sinha and Bharati (1990), Deepalakshmi and Ananda Kumar (2004) in blackgram, Khan (1988) in greengram, Gunasekaran et al. (1998) in cowpea and Ali et al. (2011)

in chickpea.

## Conclusions

From the results of present study, it may be inferred that the mean values for plant height, number of primary branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod and single plant yield decreased below the control in most of the treatments. Induced mutations delivered fairly good amount of genotypic coefficient of variation with respect to the number of branches per plant, the number of clusters per plant, pod and seed yield. A high amount of heritability and GA as per cent of mean was noted for plant height, number of clusters per plant, number of primary branches per plant, number of pods per plant, pod length, number of seeds, 100 seed weight and single plant yield. This denoted that these characters are governed largely by additive gene effect, which may be favourably exploited for improving these characters by selection.

## Conflict of interests

The authors did not declare any conflict of interest.

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