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Impact of EMS Induced Mutagenesis in Seedling Characters of Black Gram (*Vigna mungo* L. Hepper) During M_3 Generation

Bhattu Rajesh Nayak[1], K. Srinivas Naik[2], A. Yugandhar[1] and G. Vijay Kumar*[1]

[1] Osmania University, Hyderabad - 500 007, Telangana, India
[2] Centre for Plant Molecular Biology, Osmania University, Hyderabad - 500 007, Telangana, India

Abstract

The major objective of the present research is to examine the characters viz., seedling length, germination percentage, seed survival rate, seed vigor index, root length of M_2 generation black gram mutants when raised as M_3 generation as a part of research work carried for studying the yield and yield contributing characters induced with EMS. Data was recorded for studying the above characters of mutants from 1st day to 15th day. The ANOVA analysis revealed that significantly high was recorded for the studied characters except root length (cm). The results revealed that the highest seed germination % was observed in 0.2% mutant followed by the mutants 0.3% mutant, 0.5% mutant, T_9 (check), 0.4% mutant while, lowest germination % was observed in IC-436524-Control (untreated). The results also revealed that highest seedling length (cm) was observed in 0.2% mutant followed by 0.3% mutant, 0.4% mutant and 0.5% mutant. Highest seed vigor index (%) was observed in 0.2% mutant followed by the mutants 0.3% mutant, 0.4% mutant, 0.5% mutant, T_9 (check). Highest seed survival rate (%) was observed in 0.2% mutant followed by 0.4% mutant, control (untreated), 0.3% mutant, 0.5% mutant. Highest root length (cm) was observed in 0.2% mutant followed by 0.3% mutant, IC-436524 (control (untreated), 0.5% mutantand 0.4% mutant. The overal results revealed that all the traits under study, observed significant variability at 0.2% and 0.3% mutants. Investigation studies revealed that there is a huge genetic variability among the mutants studied in which EMS treated at lower concentration (i.e., 0.2% and 0.3% mutants) showed high germination percentage and survival rate when compared with EMS treated at higher concentration mutants and control. This shows the efficiency of EMS to induce mutations and generate variability in black gram and can be useful tool in the future for crop improvement programmes.

***Key words*:** Seed germination percentage, Seedling length, Seed survival, Seed vigor index

In our country like India, mostly to vegetarian population, pulses are an essential source of protein, and they play a significant role in our diet. Legumes are a great way to get protein in your diet. Protein, macro and micronutrients (Ca, P, K, Fe, and Zn), B vitamins (niacin, vitamin A, ascorbic acid, inositol), dietary fibre, and carbohydrates are all abundant in these plants. They contain high concentrations of lysine, an important amino acid that is uncommon in wheat proteins. Urdbean, or blackgram (*Vigna mungo*), is a nutrient-dense and highly adaptable legume that thrives under high levels of stress. For developing nations in Asia and Africa, it is a low-cost vegetable protein, amino acid, etc. source. The crop is vital to restoring soil health (via nitrogen fixation from atmosphere). It's also adaptable to many other types of farming (i.e., dry farming and intercropping). Average daytime temperatures between 25ºC and 35ºC and annual precipitation between 600 and 1000 mm are ideal for growth. Although it cannot tolerate humid tropical climates well, in regions with high rainfall, it can still be cultivated throughout the dry season using stored moisture. Rich black vertisols or loamy soils with a pH of 6.5-7.8 are best for growing it [1]. P (40 kg/ha) and K (30 kg/ha) are responsive to *Vigna mungo*, and it just requires rough tillage and two weedings [1]. Saline and alkaline soils are vulnerable to the *Vigna mungo* [2]. Blackgram is a fantastic addition to a rice dinner from a nutritional perspective due to its high lysine level. Blackgram was first developed in India and is now mostly cultivated in those nations as well as others in Asia, including Myanmar, Pakistan, Bangladesh, and Thailand. India is the world's largest producer of blackgram, accounting for around 70% of total output. When compared to cereals, black grams protein content is almost three times higher at 26%. When compared to the other main pulses grown in India, it comes in at number four. It uses 12.7% of the total area planted to pulses and accounts for 8.4% of the overall output. In 2017–2018, it

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Correspondence to: G. Vijay Kumar, Osmania University, Hyderabad - 500 007, Telangana, India, Tel: +91 9032294499; E-mail: vijay.genetics@gmail.com

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was grown on around 5.44 million hectares and yielded 3.56 million metric tons. A poor average seed output of 650–800 kg/ha for blackgram has been documented in both India and Thailand. This means that increasing yield and its related characteristics is the fundamental objective of any blackgram programme. Breeding for traits relevant to yield is dependent on genetic diversity. The amount of natural genetic variability contained in the germplasm pool directly correlates with the efficacy of conventional crop breeding programs. Genetic variability is a key component of every successful crop improvement programme since it offers a range of variants that can be obtained by mutation, hybridization, recombination, and selection processes, allowing for more efficient and better selection [3-6].

However, it is challenging to achieve a genetic gain in blackgram by recombination because of the autogamous blooming pattern and reduced genetic variability in the top gene pool. Recent efforts to improve blackgram breeding have been hampered by the species' short genome size (574 Mb) and restricted gene pools, both of which have reduced the quality of parent materials available for selection. Genetic enhancement of the crop for sustainable food production and other features may benefit from a greater range of genetic variety. As a result, there is room for exploring new variations for yield-related variables in blackgram via mutation breeding. The selection of promising mutants based on phenotypic characteristics is crucial to the success of mutation breeding programmes [7]. Induce mutagenesis is a crucial addition to conventional breeding for crop development projects since physical and chemical mutagens have caused a significant amount of genetic variability [8]. Since new food crop types embedded with various induced mutations have contributed to the large increase in agricultural production, induced mutations have played a vital role in enhancing both crop productivity and global food security [9]. Induced mutagenesis (IM) is widely used to produce mutant plants with desired plant characteristics [10-12]. The breeding of improved mutant versions of several crop species has become commonplace [13-14]. Since after following the use of inducing mutagens, plant breeding programs has revolutionized a lot in crop improvement programs. About 3,500 varieties have been released around the globe through mutation breeding of which majority are food crops among which very few are pulses [15]. Chemical mutagens increase genetic variety in plants for effective breeding programmes [16]. When compared to normal plants, Adamu and Aliyu's [17] earlier research found that chemical mutagens caused a wide variety of morphological features to vary [18-19]. In mutant breeding operations, choosing an effective and efficient mutagen concentration and growth setting is crucial to generating a high frequency of desirable mutations [20-21]. Chemical mutagens used for inducing mutagenesis provide a new allelic permutation in the characteristics of interest for genetic improvement without altering the plant's fundamental chromosomal structure. The efficiency of any mutagen depends on the rate at which the mutagen induces mutations for changes or aberrations or alterations in the genetic material by creating a range of desirable and undesirable effects. As opposed to natural mutation and artificial recombination, it significantly reduces the time needed to produce offspring. In the case of legume crops including mungbean, blackgram, cowpea, and lentil, mutagenesis was employed to efficiently customize several plant properties. Selection of progeny via breeding cycles may be facilitated by employing multivariate analysis to assess the genetic diversity contained in germplasm through morphological features, and by identifying the connections between seed yield and yield-related factors. Chemical mutagens are not only mutagenic themselves but also affect mutation in specific ways [22]. It produces random point mutations in genetic material. Among the chemical mutagens used for inducing mutational studies, Ethyl methane sulfonate (EMS) has been found highly reactive in inducing mutations and efficient in creating diversity in agronomic traits of food crops including black gram. EMS, as a chemical mutagen, can be used as a supplementary approach to improve desired identifiable characters such as yield related characters [23-24]. EMS offers chances to increase the genetic diversity of quantitatively inherited traits, which has been suggested as a feasible solution to difficulties in plant cultivation [25]. EMS treatment for seeds can have a variety of negative side effects on germination and survival [26]. Seed germination reduced as EMS concentration increased. The effect of EMS on seed meristematic tissues may have caused a decrease in seed germination [27-30]. The EMS chemical mutagen, which causes a high frequency and broad range of mutations, is currently generally recognized as the most effective and impactful mutagen among chemical mutagens. Strong mutagens become more hazardous than greater doses of relatively weaker mutagens when doses are increased past a certain point, but they do not increase the frequency of mutations [31]. It might be caused by EMS, a chemical mutagen that creates new mutation sites.

MATERIALS AND METHODS

Collection of seeds

Black gram accession- IC-436524 was obtained from NBPGR (National Bureau of Plant Genetics Resources) regional centre, Hyderabad and T-9 variety was collected from ICAR-CRIDA (Central Research Institute for Dry land Agriculture), Hyderabad.

Preparation of EMS concentrations

Ninety uniform and healthy seeds were selected and presoaked with water for 3-4 hours [32] and cleaned with tissue paper and dried. EMS mutagen concentration was prepared ranging from 0.2% to 0.5% as per mutagenesis protocol [33]. Seeds under each concentration of EMS, fifteen seeds at each concentration were soaked for 6 hrs under rotary shaker at 180 rpm in a room temperature of 27±1°C. Volume of EMS solution should be ten times to the proportion of seed volume for effective and uniform absorption of EMS. Untreated fifteen seeds were used as control along with T_9.

Sowing of seeds (Design)

EMS treated and untreated seeds along with T_9 were sown in field in a RBD (Randomized Block Design) with three replications each with 10cm × 30 cm distance between plants and rows respectively.

Evaluation of M_1 seeds

M_1 population was evaluated for agronomic and morphological characters by phenotypical observations which are yield and yield contributing traits like plant height, seed yield, pods per plant, and cluster per plant. Plants which have high quantitative characters with high yielding in each row of each concentration were separated and seeds from those plants were collected and data was prepared based on the yield and yield contributing characters.

M_2 generation

The ability to distinguish between mutant and wild species based on morphological traits is thought to be highly effective. Because morphological mutants are essential for altering cultivar features and creating new types of plants, their morphological characteristics have been used to track changes in a variety of plants.

During M_1 generation, probably identification of recessive character is difficult only mutations of dominant characters can be identified. In the M_2 generation, the mutation will segregate to create homozygotes for recessive or dominant alleles [34]. For raising M_2 generation total 120 seeds of 20 healthy seeds from each treatment were collected from high yielding mutant and control along with T_9 and were sown in the pots in Departmental of Genetics, OU. The same agricultural procedures that were used to grow the M_1 generation were continued. In each row of each concentration, plants with high quantitative characteristics and high yields were identified, and the seeds from these plants were gathered and placed in paper bags. Based on the yield and yield contributing characters, the data was prepared.

M_3 generation

For raising M_3 generation total 300 seeds of 50 healthy seeds from each treatment were collected from high yielding mutant and control along with T_9 and were sown in the field at Kodad, Suryapet District Telangana. Same agronomical practices were maintained as in growth of M_1 and M_2 generation.

The following parameters were studied in M_3 generation

Seed germination percentage, seedling length (cm), root length (cm), and seedling vigor index was measured and recorded after sowing from 1st day to 15th day. Seedling survival rate was determined on 15th day.

Seed germination

Understanding seed germination requirements is important for growing plants successfully from seed. Seed germination is a crucial process that influences crop yield and quality. Germination is essential for plants to exist. Seed germination begins with imbibitions of water. As the seed takes in water, it gets bigger and produces an enzyme that enhances the metabolic activity in the seed for breaking the endosperm to provide energy [35-36].

Seed vigor rate is a measure of the quality of seed, and involves the viability of the seed, the germination percentage and the strength of the seedlings produced [37].

Survival rate is the percent of living crop seedlings against total crop seedlings planted. Seed quality deteriorates with age, and this is associated with accumulation of genome damage.

$$\text{Survival rate} = \frac{\text{Number of Living seedlings}}{\text{Number of total seedlings}} \times 100$$

RESULTS AND DISCUSSION

The ANOVA results studied for seedling length, germination percentage, vigor index, root length (cm) and seed survival rate recorded higher significant for the studied characters except root length.

Table 1 ANOVA of seedling characters in M_3 generation of blackgram mutants

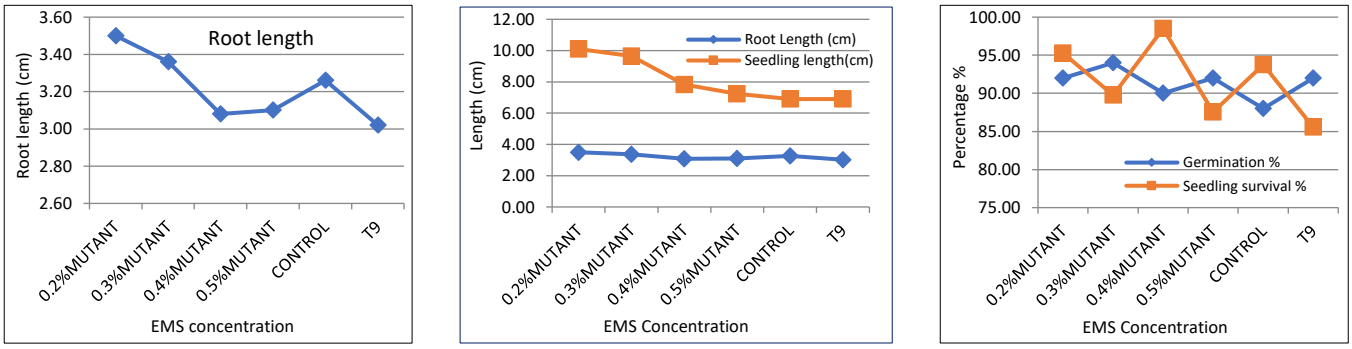
Source of variations	DF	MSSQ				
		Characters				
		Germination %	Seedling length (cm)	Root length (cm)	Seedling vigor index	Seedling survival %
Replications	2	55.000	0.086	0.177	1222.717	53.961
Treatments	5	269.333*	10.132**	0.014NS	119261.713**	67.843**
Error		91.000	0.178	0.036	5439.997	44.813
Mean		87.333	8.103	3.220	735.567	95.361
S.Ed		6.033	0.267	0.119	46.648	4.234
CV (%)		10.92	5.21	5.86	10.03	7.02

Table 2 Mean values of seedling characters in the M_3 blackgram generation

	Root length (cm)	Seedling length (cm)	Seed germination %	Seedling vigor %	Seedling survival %
0.2% mutant	3.50	10.10	92.00	930.40	95.28
0.3% mutant	3.36	9.66	94.00	907.40	89.78
0.4% mutant	3.08	7.82	90.00	704.80	98.50
0.5% mutant	3.10	7.24	92.00	665.60	87.56
Control (untreated)	3.26	6.90	88.00	607.00	93.78
T_9	3.02	6.90	92.00	634.40	85.56

The mean values of the character seed germination % ranged from 88.00% to 92.00%. Highest seed germination % was observed in 0.2% mutant by 92.00% followed by the mutants 0.3% mutant by 94.00%, 0.5% mutant by 92.00%, T_9 (check) by 92.00%, 0.4% mutant by 90.00%. Lowest germination % was observed in IC-436524 (Control (untreated)) by 88.00%. The mean values of the character seedling length ranged (cm) from 6.90 cm to 10.10 cm. Highest seedling length (cm) was observed in 0.2% mutant by 10.10 cm followed by the mutants 0.3% mutant by 9.66cm, 0.4% mutant by7.82cm and 0.5% mutant by 7.24cm. Lowest seedling length was observed in mutant IC-436524 and T_9 by 6.90. Similar outcomes were also observed in cluster beans [38], cow peas [39], and tomatoes [40]. The character seed vigour index's mean values (%) varied from 607.00% to 930.39%. According to Ignacimuthu and Babu [41], a rise in mutagenesis dosages causes an increase in physiological harm to seeds and seedlings. It also causes differences in stimulation owing to cell division rates and an activation of growth hormones like auxin. Lowest seed vigor index (%) was observed in mutant IC-436524

(control (untreated)) by 607.00%. The character seed survival rate (%) ranged from 85.56% to 95.28%. Highest seed survival rate (%) was observed in 0.2% mutant by 95.28% followed by 0.4% mutant with 98.50%, IC-436524(control (untreated)) by93.78%, 0.3% mutant by 89.78%, 0.5% mutant by 87.56%. Lowest seed survival rate (%) was observed in T_9 (check)by 85.56%. The results showed that character root length (cm) ranged from 3.02cm to 3.5cm. Highest root length (cm) was observed in 0.2% mutant by 3.5cm followed by 0.3% mutant by 3.35cm, IC-436524 (control (untreated)) by3.25cm, 0.5% mutant by 3.10cm and 0.4% mutant by 3.08cm. Lowest root length (cm) was observed in T_9 (check)by 3.02cm.

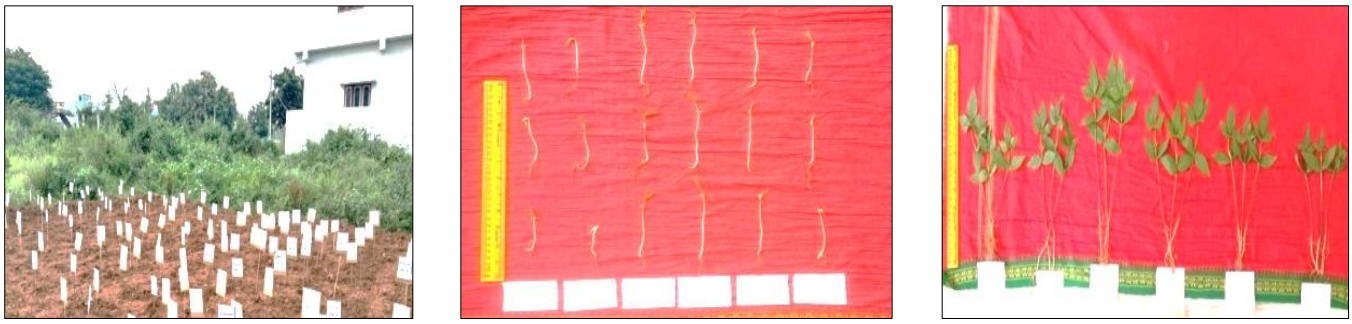


(a) Effect of EMS on root length

(b) Effect of EMS on root length and seedling length

(c) Effect of EMS on germination % and seedling survival %

Fig 1 Graphical representation of seeding traits in M_3 generation of blackgram mutants



a). Field view of blackgram mutants' seedling

b). Seedling traits observation

c). Seedling observation on 15th day

Fig 2 Effect of EMS on seedling length characters and root length in M_3 germination (field Study conducted at Kodad, Suryapet, Telangana)

As the concentration of mutants treated by EMS increased, seed germination decreased. The action of mutagens on the meristematic tissues of the seed may have reduced or stimulated seed germination. The lower seed germination at higher mutagen doses/concentrations may be attributed to cellular disturbances (caused either at physiological or physical level), such as chromosomal damages or altered enzyme activity. The lower seed germination in populations exposed to mutagens may also be caused by the delay or inhibition of physiological and biological processes required for seed germination [42]. The results showed that 0.2% mutant had the highest germination percentage, which was followed by 0.3% mutant, 0.5% mutant, T_9 (check), and 0.4% mutant, while IC-436524-Control had the lowest germination percentage (untreated). The findings showed that seed germination percentage dropped as mutagen concentration or dose increased (EMS). This demonstrates unequivocally that the mutagens inhibited seed germination. Mutagens have been linked to similar inhibitory effects on seed germination in the past, according to studies by [43-45] in pea, in chickpea [46-48], as well as others in cowpea, [51-53] in mungbean, [54] in soybean and [55] in lentil. The results also revealed that highest seedling length (cm) was observed in 0.2% mutant followed by 0.3% mutant, 0.4% mutant and 0.5% mutant. Lowest seedling length was observed in mutant IC-436524 and T_9. Highest seed vigor index (%) was observed in 0.2% mutant followed by the mutants 0.3%mutant, 0.4% mutant, 0.5% mutant, T_9 (check). Lowest seed vigor index (%) was observed in control (untreated). Highest seed survival rate (%) was observed in 0.2% mutant followed by 0.4% mutant, control (untreated), 0.3%mutant, 0.5% mutant. Lowest seed survival rate (%) was observed in T_9 (check). Highest root length (cm) was observed in 0.2% mutant followed by 0.3% mutant, IC-436524 (control(untreated), 0.5% mutant and 0.4% mutant. Lowest root length was observed in T_9 (check). The overall results revealed that all the traits under study, observed at 0.2% and 0.3% mutants.

CONCLUSION

Optimum concentration of EMS is economic and productive approach to induce mutations in any crops. Seed germination and seed survival decreased with increased EMS concentrations Investigation studies revealed that there is a huge genetic variability among the mutants studied in which EMS treated at lower concentration (i.e., 0.2% and 0.3% mutants) showed high germination percentage and survival rate when compared with EMS treated at higher concentration mutants and control. Thus, these show the efficiency of EMS to induce mutations and generate variability in blackgram and can be useful tool in the future for crop improvement programmes.

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