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Assessment on Seedling Traits of Black Gram (*Vigna mungo* L. Hepper) Mutants Raised during M_2 Generation

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Abstract

The present study was conducted at department of Genetics, Osmania University, Hyderabad, Telangana, India, during September to November, 2021 with the blackgram accession- IC-436524 obtained from NBPGR regional centre, Hyderabad, Telangana, India and the check T9 was collected from ICAR-CRIDA Hyderabad. The present study aimed to estimate the mutagenic sensitivity of black gram accession- IC-436524 with Ethyl methane sulfonate. Accordingly, this study checked whether the mutagenic activity has been inherited to the next generation M2. Data analysis has been performed for the determination of significance in germination percentage, seedling length (cm), seedling vigor index, root length (cm) and seed survival rate (Day 1 to Day 15) that were observed to be highly significant (p<0.01) for above characters except root length (cm) for treatments. It clearly indicated that there was adequate amount of variability among the black gram mutant due to treatments. The collected data for 7th day observed that seed germination percentage was higher at 0.2% and 0.3% EMS treated mutants and lowest germination percentage was observed in 0.5% EMS treated mutants. Seedling length (cm) was recorded highest in 0.2% EMS treated mutants and lowest seedling length (cm) was recorded in control. Maximum seedling vigor index was recorded in 0.2% EMS treated mutants and lowest seedling vigor index was showed for 0.5% EMS treated mutants. Seedling survival rate data was recorded for 15th day showed higher in 0.2% EMS treated mutants. Data showed EMS has induced mutations during M_1 generation and inherited to offspring (M_2 generation).

Keywords: Blackgram, EMS, seed germination percentage, seed survival rate

1. Introduction

Essence of genetic variability present in the germplasm/base population becomes the main source of success of breeding program (Kaur et al., 2022, Laskar et al., 2019). Since the essence lies within the genotypes, deciphering such potential needs remarkable genetic tools (Rather et al., 2022). Hence, in crop improvement program, mutation breeding provides new genetic variability in a shortest possible period of time as compared to hybridization and selection (Goyal et al., 2019a, Chakravarti et al., 2017, Devi et al., 2022). The global demand for the grain legumes especially black gram has been found to increasingly greatly in the recent years because of their tremendous nutritional value (Rather et al., 2022). The researchers seek intense interest to improve the quality of blackgram particularly in the countries with increased population growth. This urges the necessary to induce the genetic variation for constant supply of better yielding and enhanced varieties to the producer (Gurumurthy et al., 2019). Blackgram (*Vigna mungo* L. Hepper) is generally referred as urdbean and is the ancient and significant crop because of its high nutritional quality, sustainability and the ability to fix atmospheric nitrogen (Gummadala et al., 2022, Gurumurthy et al., 2019). It is also termed as poor man's meat due to its protein value (Kaur and Sharma, 2015). India which is the largest producer need attentive research to be updated in its cultivation and export thereby the economic benefits of the crops can be maintained and uplifted (Yadav et al., 2021). The most ideal soil for cultivation of black gram crop is well drained loamy soil with a pH of 6.5–7.8 (Priyanka et al., 2022). In any crop improvement program variability is the prerequisite. As the black gram is a self-pollinated crop which is having lesser variability, hence it is very importance to create variability by inducing mutagenic agent artificially (Yadav et al., 2021). Because of the autogamous value, this crop has only little or no genetic variability. This genetic variability is considered as a prerequisite for any kind of successful breeding and hence the occurrence of mutagenic agents for the induction of new genetic variation is necessary. The mutation breeding is now

intensively popular in recent days and functions as an effective tool in the supplementation of the prevailing germplasm for improved cultivation (Gummadala et al., 2022). The basic steps involved in mutation breeding programme for finding appropriate treatment dose are lethal dose fixation (LD50). To obtain effective and useful mutation at high probability lethal dose (LD_{50}) was used and indicated their importance by many researchers. The lethal dose (LD50) utilized an assumption that lower doses of treatment which effect less influence on the genomic and phenotypic change. Goyal et al. (2019b) investigated the mutagenic effect in the M2 population of U-30 and T-9 varieties of black gram with EMS (Mistry et al., 2022, Savant, 2020). These mutants with increased number of parts and about size were found to be correlated with plant yield and hence subsequent research has to be carried out with several clinical parameters. Tamilzharasi et al. (2019a), Tamilzharasi et al. (2022c) observed various mutation frequencies and span with different concentration of EMS in the M2 generation of black gram. The study Tamilzharasi et al. (2021b) founded a significant correlation between the biological damage in M1 generation with the information provided in the studies the mutagenic sensitivity can be understand on the surviving plants (Goyal et al., 2020, Serrat et al., 2014, Ramya et al., 2014).

2. Materials and Methods

The present study focused on EMS mutation that can modify the cultivar characteristics. To induce EMS mutation to the selected black gram variety. To separately harvest the seeds of M1 plants and to sow in the upcoming plant progeny season. To raise M2 generation and to estimate the frequency of mutation in M2 generation.

2.1. Collection of seeds

Black gram seeds of variety IC-436524 were obtained from NBPGR regional centre, Hyderabad, Telangana, India.

2.2. Processing of seeds

This research selected 90 healthy and uniform seeds and soaked in water for duration of 3–4 h. The soaked seeds were cleaned with a tissue paper and dried.

2.3. Inducing of mutation

The concentration of EMS mutagen has been prepared in the concentration between 0.2%, 0.3%, 0.4% and 0.5% as per mutagenesis protocol. Under each concentration of EMS, fifteen seeds at each concentration were soaked for 6 hours under rotary shaker at 180 rpm in a room temperature of 27±1°C. For uniform and efficient absorption of EMS, it must be noted that the volume of EMS solution must be at the proportion of 10X of seed volume.

2.4. Control

Along with T9, the untreated 15 seeds were kept as control. The untreated seeds-T9 and EMS treated seeds were being sown in the Randomized Block Design field during *kharif* season, mid June, 2021 at experimental farm, Department of Genetics and biotechnology, Osmania University, Hyderabad (Latitude and longitude coordinates are, 17.418974, 78.526596), Telangana, India. The sowing in field has been processed with three kinds of replication with the distance of 10×30 cm^2 between the rows and plants respectively.

2.5. M_1 population

M_1 population was evaluated for agronomic and morphological characters by phenotypical observations which are yield and yield contributing traits like plant height, branches $plant^{-1}$, leaves $plant^{-1}$, clusters $plant^{-1}$, pods $plant^{-1}$, seed yield $plant^{-1}$, 100 seed weight, root length $plant^{-1}$ and root nodules $plant^{-1}$. Plants with high quantitative characters with high yielding in each row of each concentration were separated and seeds from those plants were collected. The data was prepared based on the yield and yield contributing characters.

2.6. M_2 generation

For raising M_2 generation total 180 seeds of 30 healthy seeds from each treatment were collected from high yielding mutant and control along with T9 and were sown with 10 cm×30 cm distance between plants row^{-1} in a field in RBD (Randomized Block Design) with three replications each along with control during *rabi* season, mid September to November, 2021 at experimental farm, Department of Genetics and biotechnology, Osmania University, Hyderabad (Latitude and longitude coordinates are, 17.418974, 78.526596), Telangana, India.

2.7. Parameters

Seedling length (cm), Root length (cm), Seed germination percentage, Seedling vigor index. The following parameters were measured and recorded after sowing from 1st day to 15th day. Seedling survival rate was also determined on 15th day.

2.7.1. Seed germination

The requirements of seed germinations are carefully considered for optimal plant growth. This process is highly crucial that influence the quality and crop yield. Seed germination begins with imbibition 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 (Dhulgande et al., 2015, Rani et al., 2013).

2.7.2. Seed germination rate

The seed germination rate depends on the genetic composition, environmental factors and morphological features. The rate of germination is beneficial for the estimation of the number of seeds for the provided area or the chosen number of plants. The germination capacity is denoted as the number of seeds for complete germination in a population (Zhu et al., 2014).

2.7.3. Seed vigour

The measurement of seed quality with seed variability, germination rate, germination percentage and the seedling strength (Rajjou et al., 2012).

2.7.4. Survival rate

Survival rate is defined as the percentage of living crop seedlings against the total number of planted crop seedlings. The quality of the seed reduces with age and is correlated with the accumulation of genetic modification.

Survival rate = (Number of living seedlings)/(Total number of seedlings)×100……(1)

3. Results and Discussion

This section provides the results and discussion in detail. The statistical analysis of the obtained data is processed and the ANOVA results are depicted below

3.1. Analysis of variance (ANOVA)

Development of desired variants or characters from the genotype is the basic objective of crop improvement program to meet the farmer needs and market needs. The ANOVA data results for seedling length, germination percentage, vigor index, root length and seed survival rate were observed in which highly significant (p<0.01) for above characters except rot length for treatments (Table 1). It clearly indicates that there is adequate amount of variability among the black gram mutant due to treatments.

Table 1: Analysis of variance (ANOVA) of black gram mutant under EMS mutagenesis for germination and seedling characters in M_2 generation

Source of variations	DF	MSSQ				
		Characters				
		Germination percentage	Seedling Length (cm)	Root Length (cm)	Seed Vigor Index percentage	Seed Survival Rate percentage
Replications	2	39.472	0.125	0.002	1872.889	48.958
Treatments	5	109.153**	15.748**	0.017 NS	155483.162**	351.886**
Error		18.755	0.055	0.020	1517.029	47.408
SEd		3.536	0.191	0.115	31.802	5.622
CV (percentage)		5.15	2.48	4.70	4.87	8.35

3.2. *Germination percentage*

The Mean data of germination percentage, Seedling length, Root Length (cm), Germination percentage, Seed Vigor Index percentage, and Seed Survival Rate percentage was depicted in Table 2 and Figure 1. Seed germination percentage was recorded highest in 0.2%, 0.3% EMS treated mutants and control by 88.89% followed by T9 (84.44%), 0.4% EMS treated mutants (77.78%) and lowest seed germination percentage was recorded in 0.5% EMS treated mutants (75.56%). The observation revealed that germination percentage was higher and maximum in 0.2% EMS treated mutants and 0.3% EMS treated mutants and gradually decreased in 0.4% EMS treated mutants, 0.5% EMS treated mutants. The decrease in seed germination rate may be due to factors like high EMS dose treatment. The percentage of seed germination was reduced in EMS treated populations. This is occurred due to the acute and physiological chromosomal damage (Gaur et al., 2003, Nawale et al., 2006). Wang et al. (2014) suggested the reduction in seed germination percentage in high dose in the cucumber seeds. Further these reductions are caused due to that the seed absorbs the mutagen that subsequently reaching the meristem region and impact the germ cell line (Serrat et al., 2014). Same observation has been reported by Ariramana et al. (2014), Kumar and Mishra (2004) and Baghery et al. (2016).

3.3. Seedling length (cm)

Seedling length was recorded highest in 0.2% EMS treated mutants by 12.63 cm, followed by 0.3% EMS treated mutants

Table 2: Mean performance of seedling characters and germination in the M_2 blackgram generation

	Seedling length (cm)	Root length (cm)	Germination percentage	Seed vigor index percentage	Seed Survival Rate percentage
0.2% mutant	12.63	3.07	88.89	1121.78	95.24
0.3% mutant	11.77	3.07	88.89	1046.00	92.49
0.4% mutant	9.63	2.97	77.78	748.89	80.30
0.5% mutant	7.63	2.97	75.56	577.33	65.00
Control	7.17	2.87	88.89	636.67	82.60
T_9	7.97	2.97	84.44	672.67	78.85

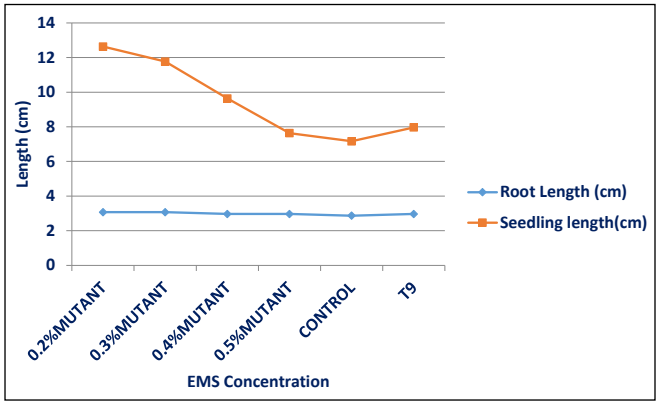

Figure 1: Effect of EMS mutagen on Root length (cm) and Seedling length(cm) in M_2 generation

by 11.77 cm followed by 0.4% EMS treated mutants by 9.63 cm followed by T_9 (7.97 cm), 0.5% EMS treated mutants by 7.63 cm and lowest seedling length was recorded in control by 7.17 cm. Seedling length was highly influenced in lower concentrated EMS treated seeds when compared to higher concentrated EMS treated seeds (Figure 2).

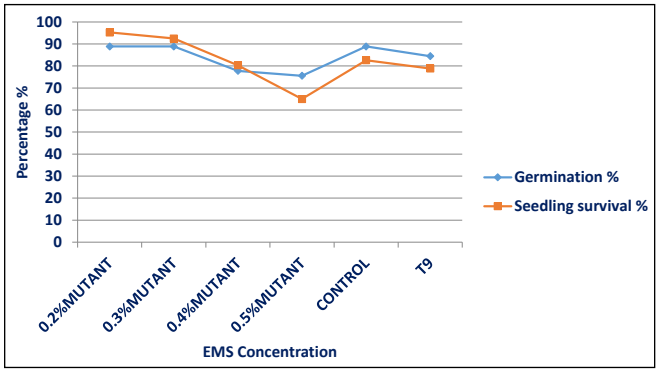

Figure 2: Effect of EMS mutagen on germination % and Seedling survival % in M_2 generation

3.4. Seedling vigor index

Seedling vigor index is calculated by germination percentage multiplying with seedling length. Maximum seedling vigor index was recorded for 0.2% EMS treated mutants by 1121.78 followed by 0.3% EMS treated seeds by 1046.00 followed by 0.4% (748.89), T_9 (672.67), Control (636.67) and lowest seedling vigor index was recorded for 0.5% EMS treated mutants by 577.33. This shows that EMS treated seeds at lower concentration has stimulatory effect on germination rate and growth of seedling length and root length. High doses treated seeds showed reduced stimulatory effect. This shows that the inhibitory effect of EMS with increasing concentration of EMS treated seeds (Zaka et al., 2004) (Figure 3).

3.5. Seedling survival rate

Seedling survival rate data was recorded on 15th day and observation revealed that seed survival of plants was recorded higher at0.2% EMS treated mutants by 95.24% followed

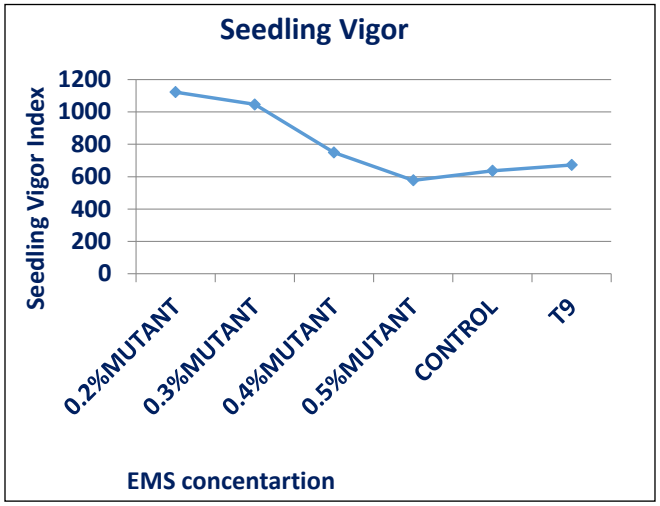

Figure 3: Effect of EMS mutagen on seedling vigor percentage in M_2 generation

by 0.3% EMS treated mutants (92.49%), Control (82.60%), 0.4% EMS treated mutants (80.30%), T_9 (78.85%) and lowest observation at 0.5% EMS treated mutants by 65.00%. Data revealed that seed survival rate decreased with increased EMS concentrations from 0.2% EMS treated seeds sown to 0.5% EMS treated seeds (Din et al., 2003). Decrease in survival percent due to mutagenic treatments was also reported by Dhanavel et al. (2008) in various pulse crops (Figure 4).

3.6. Root length (cm)

Figure 4: Seedling traits in black gram mutant

Root length was recorded highest in 0.2%, 0.3% EMS treated mutants and control by 3.07 cm followed by T9, 0.4% and 0.5% EMS treated mutants by 2.97 cm and lowest Root length was recorded in control by 2.87 cm. The observation revealed that root length character showed no significant change when compared with other treatments. The decrease in root length with increased higher dose of EMS resulted non significant. The decrease in root length due to mutagenic treatment was similar to earlier reports of Veni et al. (2017) in black gram, in cluster bean and Chakravarty et al. (2017) in rice (Figure 5).

Figure 5: Seedling traits in black gram mutant

3.7. *Analysis*

Induced mutation is one of the best methods to evolve new cultivars by producing variability at gene level. Lasker et al. (2019) reported 2832 cultivars, improved through induced mutagenesis in different crops. Currently, large numbers of known chemical compounds are able to induce mutations in both prokaryotic and eukaryotic cells. Despite the large number of mutagenic compounds, only a small number has been tested in plants. Among them, there is only a very restricted group of alkylating agents viz., sodium azide (SA) and ethyl methane sulfonate (EMS) which produce different side effects on the genetic structure of treated populations. Among different chemical mutagens, ethyl methane sulphonate is reported as the most efficient, effective and powerful mutagen for generating variations. In plants, EMS usually causes point mutations but loss of a chromosome segment or deletion can also occur (Mistry et al., 2022). Motivated by the study, the present research focused to induce mutation with EMS.

Similar to the research work done by (Savant, 2020) who studied mutagenic effectiveness and efficiency of gamma rays in three genotypes of Indian mustard (two local cultivars and one improved variety), from the results obtained mutagenic effectiveness was found to be higher at lower doses. It is observed that in our study also, the mutagenic effect was found at low concentration. Tamilzharasi et al. (2022) created variability for significant morphological traits by induced mutagenesis in the black gram variety using the combination of EMS and gamma rays. Goyal et al. (2019a) selected and cultivated mutant traits similar to our study. The study stated that the furthermore developments in research has to be established for significant improvement in molecular markers and for the development of farmer friendly cultivation. Goyal et al. (2020c) analyzed the efficiency effectiveness of various combined and individual treatments of EMS and gamma rays in the M2 generation of black gram.

Goyal et al. (2019a) analyzed the combined and individual effects of EMS and gamma rays on several biological parameters like plant survival, seed germination pollen fertility, seedling height, in the M1 and M2 generations of black gram. Devi et al. (2022) conducted an investigation to provide information on the immediate effect of mutagenesis on the black gram with different dosage of EMS. The study would greatly helpful for understanding the stages of mutation and for cost effective selection of mutagenic agents. EMS has induced mutations during M_1 generation and inherited to offspring (M2 generation). Hence the proposed research effectively inherited the M2 population with the successful implementation of EMS mutation.

4. Conclusion

Data showed EMS has induced mutations during M_1 generation and inherited to offspring (M_2 generation). The results revealed that seed germination percentage, seedling length, seedling vigor index, seed survival rate and root length has showed variation at higher doses compared to lower dose treated EMS seeds in M_2 generation. The results can be concluded that the mutant which is treated with 0.2% EMS was recorded highest germination percentage, seedling length, seedling vigor index, seed survival rate and root length.

5. Acknowledgement

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