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Evaluation of Seedling Trait Responses to EMS-Induced Mutagenesis in Greengram (*Vignaradiata* (L.) Wilczek)

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***Abstract*— *The present study was conducted at the Department of Genetics and Plant Breeding, Acharya Nagarjuna University, Lab, Guntur, Andhra Pradesh, and at the Centre for Plant Molecular Biology, Osmania University, Hyderabad, during the period from February 2024 to April 2024. The greengram (Vignaradiata (L.) Wilczek) genotype WGG-42 was procured from ICAR–CRIDA, Santosh nagar, Hyderabad. Ethyl Methane sulfonate (EMS), obtained from HiMedia Laboratories Limited, Mumbai, India, was employed as the chemical mutagen for inducing mutations in greengram seeds.The analysis of variance (ANOVA) results revealed highly significant differences ($p \leq 0.01$) among the treatments for all the studied seed germination and seedling traits. This indicates that EMS-induced mutagenesis caused considerable variation in the early growth and seedling performance of greengram.The seedling traits results revealed that the lower EMS concentrations (0.05-0.1%) have not exerted any harmful effects and, in some cases, slightly enhanced seedling growth and vigour. In contrast, moderate to higher EMS doses (≥0.2%) resulted in a gradual decline across all measured traits. An EMS concentration of 0.1% was identified as optimal for inducing genetic variability while minimizing injury. The observed traits showed a clear dose-dependent inhibitory response at higher EMS levels, highlighting the toxic effects of EMS when applied at higher concentrations. The findings indicate that EMS at lower concentrations is effective in generating genetic variability without causing substantial adverse effects on seed germination and seedling growth. In contrast, higher concentrations of EMS exert lethal and sublethal effects, resulting in reduced seedling establishment and diminished vigour.*

***Keywords*— Greengram, Ethyl Methane Sulfonate, Induced mutagenesis, Seedling trait**

I. INTRODUCTION

Greengram or mungbean (*Vignaradiata* (L.) Wilczek) is a major pulse crop widely grown in the tropical and subtropical regions of Asia, Africa, and parts of South America. It belongs to the family Fabaceae, it is highly valued for its rich nutritional profile, short growth duration, and its capacity to improve soil fertility through symbiotic biological nitrogen fixation. Greengram seeds contain about 24-26% protein, 1-1.5% fat, and 60-65% carbohydrates, and are particularly rich in essential amino acids such as lysine and leucine. Owing to these attributes, mung bean serves as an important dietary protein source, especially in vegetarian diets (Kaur et al., 2023).Globally, greengram is cultivated on nearly 7 million hectares, with an annual production of around 4.5 million tonnes (FAO, 2023). Owing to its short duration of 60-75 days, minimal water requirement, and adaptability to marginal and rainfed environments, the crop has emerged as a climate-resilient legume of global importance (Rani et al., 2022). Furthermore, greengram contributes significantly to sustainable agricultural systems by enhancing soil fertility through effective biological nitrogen fixation, adding approximately 30-50 kg of nitrogen per hectare to the soil (Kumar et al., 2023).In India, greengram is grown across nearly all agro-climatic regions, occupying about 4.5 million hectares with an annual production of approximately 2.5 million tonnes and an average productivity of 550-600 kg ha^{-1} (ICAR-DARE, 2024). Rajasthan, Maharashtra, Madhya Pradesh, Karnataka, Tamil Nadu, and Andhra Pradesh are among the leading greengram-producing states. Owing to its short growth duration and low input demands, the crop integrates efficiently into diverse cropping systems, including rice-mungbean, maize-mungbean, and cotton-mungbean rotations (Singh et al., 2021). This wide adaptability has established greengram as a key component of sustainable and integrated farming systems.Despite its considerable nutritional and agronomic significance, greengram (*Vignaradiata* (L.) Wilczek) has yet to realize its full yield potential, largely due to the narrow genetic base of existing cultivars and the crop's predominantly autogamous nature. Being a self-pollinated species with a limited effective population size, the scope for natural recombination is minimal (Singh et al., 2021). Consequently, the genetic variability accessible through mutational breeding approaches such as EMS chemical mutagenesis remains highly constrained, resulting in stagnation of productivity improvements in recent decades.

Ethyl methanesulfonate (EMS) is a potent alkylating agent ($C_3H_8SO_3$) widely used as a chemical mutagen due to its high mutagenic efficiency and comparatively low physiological toxicity. EMS predominantly induces point mutations, particularly G:C → A:T transitions, by ethylating the O^6 position of guanine residues in DNA (Greene et al., 2019). This alkylated guanine mispairs with thymine during DNA replication rather than with cytosine, resulting in stable base substitution mutations that are inherited in subsequent generations.Although EMS mutagenesis has demonstrated considerable potential for creating useful genetic variability, integrated studies encompassing morphological, physiological, biochemical, and molecular assessments across multiple generations in greengram remain scarce. Most earlier investigations have been largely confined to M_2 populations, resulting in limited insight into the heritability and stability of induced traits in later generations. In this context, the present study is designed to systematically assess the effects of EMS-induced mutagenesis on seed germination, seedling parameters of greengram.

II. MATERIALS AND METHODS

The present investigation was carried out at the Department of Genetics and Plant Breeding, Acharya N.G. Ranga Agricultural University, Lam, Guntur, Andhra Pradesh and Centre for Plant Molecular Biology, Osmania University, Hyderabad during Feb, 2024 to April, 2024.The greengram (Vignaradiata (L.) Wilczek) genotype-WGG-42, obtained from ICAR-CRIDA, Santoshnagar, Hyderabad and thee ethyl methane sulphonate (EMS) was obtained from HIMEDIA Laboratory limited, Mumbai, India, was used as chemical mutagen to induce the blackgram seeds. To determine the optimal mutagen concentration, seeds soaked for 5 hours and were subjected to various EMS concentrations (0.05%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% and control (untreated). Keep the above seven sets in incubator shaker at 180 rpm at room temperature of (27 ± 1 °C) for 3hrs. After that, seeds were thoroughly washed under running tap water for 30 minutes to remove residual EMS. The treated seeds were sown in germination trays, and germination percentage and seedling survival were recorded after 7 days and 13th day. The LD_{50} value (concentration causing 50% reduction in germination) was calculated following the method of Konzak et al. (1965).

Seedling parameters recorded

Seed germination

Germination counts were recorded, after regulating the germination percentage of the control, beginning on the third day after sowing and continuing until the seventh day. The results were expressed as the proportion of germinated seedlings to the total number of seeds sown.

Seedling survival

Observation on survival was made on 30th day of sowing. Plants with at least two primary leaves above the cotyledonary leaves were considered as surviving. It was expressed as percentage of surviving plants to the total number of seedlings emerged.

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 (Rani et al., 2013).

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 (Baskin and Baskin, 2014).

Seed vigour

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

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 = $\frac{\text{Number of living seedlings}}{Totalnumberofseedlings} \times 100 \ldots\ldots\ldots(1)$

Results and Discussions

The analysis of variance (ANOVA) revealed highly significant differences (p ≤ 0.01) among the treatments for all the studied seed germination and seedling traits (Table 1). This indicates that EMS-induced mutagenesis caused considerable variation in the early growth and seedling performance of greengram.

Table 1. Analysis variation (ANOVA) for seed germination and Seedling traits in greengram under induced EMS mutagenesis

Source of Variation	DF	MSSQ				
		Characters				
		Seed germination%	Shoot Length (cm)	Root length cm	Seedling Vigor Index	Seedling Survival rate %
Treatment	7	873.81**	34.57**	3.42**	523039.44**	1047.61**
Error	16	116.667	0.379	0.00	16,459.41	236.83
C.D.		18.857	1.074	0.09	223.976	26.866
SE(m)		6.236	0.355	0.03	74.071	8.885
SE(d)		8.819	0.502	0.042	104.752	12.565
C.V.		14.243	6.132	1.511	15.865	19.839

Significant at: (p=0.05) and **: (p=0.01) level

For seed germination percentage, a highly significant difference (MSS = 873.81**) was observed among the treatments, suggesting that EMS treatments influenced the germination potential of seeds. Similarly, shoot length (MSS = 34.57**) and root length (MSS = 3.42**) also exhibited significant variation, implying differential response of seedlings to mutagenic treatment.The seedling vigor index (SVI) showed a highly significant variation (MSS = 523039.44**) among treatments, indicating that EMS-induced mutations affected overall seedling vigor through their influence on germination and seedling growth. Moreover, seedling survival rate (%) differed significantly (MSS = 1047.61**) across treatments, highlighting that mutagenic stress altered the ability of seedlings to establish successfully.The coefficient of variation (CV) ranged from 1.51% for root length to 19.83% for seedling survival rate, indicating high experimental precision and reliability of the data. The critical difference (C.D.) values further confirmed the presence of substantial genetic variability among treatments for the measured parameters.

Effect of seed germination and seedling traits in greengram under EMS induced mutagenesis

Seed Germination (%)

The seed germination percentage showed a decreasing trend with increasing EMS concentration. The highest germination (93.33%) was observed at 0.05% and 0.1% EMS, which was equal to the untreated control (86.67%), indicating that lower concentrations had little or no adverse effect on germination. A gradual decline was recorded beyond 0.2% EMS, where germination dropped to 86.67%, followed by 73.33% at 0.3%, 66.67% at 0.4%, 60.00% at 0.5%, and 46.67% at 0.6% EMS (Table 2). The reduction in germination at higher concentrations suggests that EMS exerts a dose-dependent inhibitory effect on seed viability and embryo metabolism.

Shoot Length (cm)

Shoot length exhibited slight stimulation at lower EMS concentrations and a marked reduction at higher doses. The maximum shoot length (14.27 cm) was recorded at 0.1% EMS, which was higher than the control (12.86 cm), indicating a mild stimulatory effect of low EMS doses on seedling elongation. Beyond this level, shoot length declined steadily to 12.06 cm (0.2%), 7.63 cm (0.3%), 7.17 cm (0.4%), 6.97 cm (0.5%), and 5.98 cm (0.6%)(Table 2).

The continuous decline at higher doses reflects growth retardation due to mutagen-induced physiological and cytological damage.

Root Length (cm)

Root length followed a similar pattern as shoot length. The longest roots (5.34 cm) were observed at 0.1% EMS, followed by 4.05 cm at 0.05%, both higher than the control (3.98 cm). However, a progressive reduction was recorded at higher EMS concentrations -3.78 cm (0.2%), 3.00 cm (0.3%), 2.45 cm (0.4%), 2.46 cm (0.5%), and 2.23 cm (0.6%)(Table 2). The reduction in root length at higher doses indicates inhibition of cell division and elongation in the root apical meristem due to toxic and mutagenic effects of EMS.

Table 2. EMS mutagenic effect on seed germination% and seedling traits in greengram

EMS Conc.	Seed germination%	Shoot Length (cm)	Root length cm	Seedling Vigor Index	Seedling Survival rate %
0.05%	93.33	13.33	4.05	1244.13	93.33
0.1%	93.33	14.27	5.34	1331.87	93.33
0.2%	86.67	12.06	3.78	1045.20	85.00
0.3%	73.33	7.63	3.00	559.53	83.33
0.4%	66.67	7.17	2.45	478.00	72.22
0.5%	60.00	6.97	2.46	418.20	55.56
0.6%	46.67	5.98	2.23	279.07	44.44
Untreated	86.67	12.86	3.98	1114.53	93.33
Min	***46.67***	***5.98***	***2.23***	***279.07***	***44.44***
Max	***93.33***	***14.27***	***5.34***	***1331.87***	***93.33***
SD	***17.067***	***3.395***	***1.068***	***416.999***	***18.687***

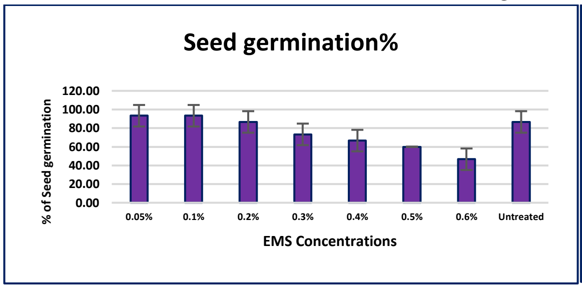
Seedling Vigor Index (SVI)

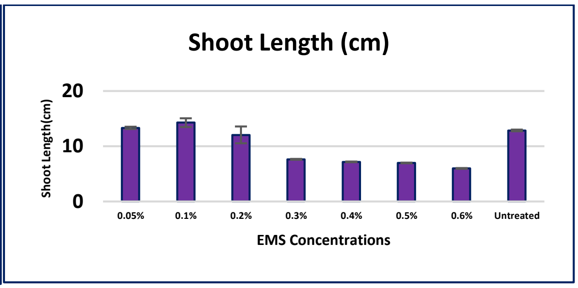
The Seedling Vigor Index (SVI) increased slightly at lower concentrations and decreased drastically at higher doses. The highest vigor index (1331.87) was obtained at 0.1% EMS, followed by 1244.13 at 0.05%, and 1114.53 in the control(Table 2). This suggests that low EMS concentrations may enhance early seedling growth through a hormetic (stimulatory) effect. However, higher EMS levels caused a pronounced reduction in vigor: 1045.20 (0.2%), 559.53 (0.3%), 478.00 (0.4%), 418.20 (0.5%), and 279.07 (0.6%), showing a dose-dependent inhibition of growth vigor

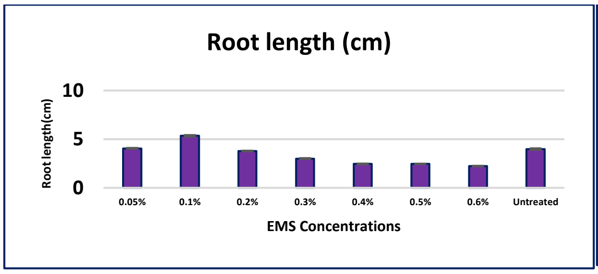
Seedling Survival Rate (%)

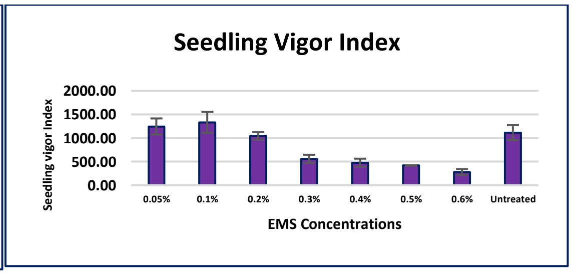
Seedling survival percentage remained high at low EMS doses and declined sharply with increasing concentration. The highest survival (93.33%) was observed in 0.05% and 0.1% EMS, equal to the untreated control. A gradual decline occurred beyond 0.2% EMS with 85.00% (0.2%), 83.33% (0.3%), 72.22% (0.4%), 55.56% (0.5%), and 44.44% (0.6%) survival(Table 2). The decrease in survival at higher EMS levels indicates that mutagenic stress and cellular damage increased proportionally with EMS concentration, reducing the ability of seedlings to establish and survive.

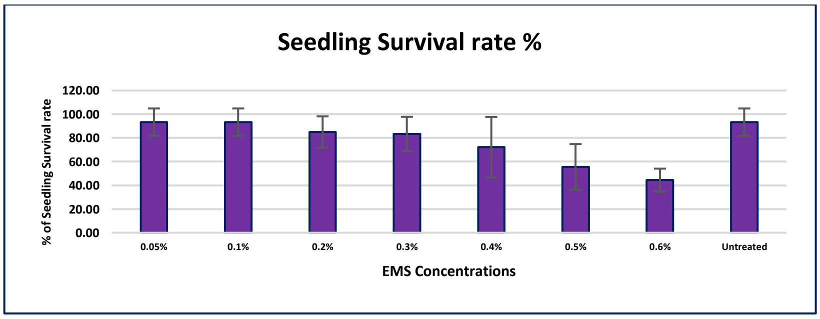
The study revealed that the response of greengram seedlings to different concentrations of Ethyl Methane Sulphonate (EMS) varied significantly across all seedling traits, including germination percentage, shoot and root length, seedling vigor index, and survival rate. The observed variation indicates that EMS exerted both stimulatory and inhibitory effects depending on the dose applied.At lower EMS concentrations (0.05% and 0.1%), seed germination, shoot length, root length, and seedling vigor index were either similar to or slightly higher than the untreated control. This suggests that mild doses of EMS may have stimulated cellular metabolism and enzymatic activity, leading to better germination and seedling growth. Similar low-dose stimulation has been reported in greengram by Sen et al, 2022; Bhattu et al., 2023 in blackgram; Yugandhar et al., 2023 in groundnut. However, as the EMS concentration increased beyond 0.2%, there was a progressive and significant decline in all the measured parameters. The decline in germination percentage and seedling survival rate at higher concentrations (0.3-0.6%) indicates that EMS caused deleterious genetic and physiological damage to the embryo, impairing its normal development. The reduction may be attributed to alterations in nucleic acids, enzyme inactivation, and disruption of metabolic pathways involved in germination and seedling growth. These results align with findings by several researchers who reported dose-dependent inhibition of growth and survival in EMS-treated seeds of crops byKhan and Goyal, 2009, Sen et al, 2022 in green gram; Bhattu et al., 2023 in blackgram; Yugandhar et al., 2023 in groundnut. The reduction in shoot and root length at higher EMS levels indicates that cell division and elongation in meristematic tissues were adversely affected. EMS is an alkylating mutagen that ethylates guanine bases in DNA, leading to point mutations and disturbances in gene expression. Such genetic alterations can inhibit cell proliferation, resulting in stunted shoot and root development. The root growth was more sensitive to EMS than shoot growth, possibly because root apical meristems are directly exposed to the mutagenic solution and are more susceptible to cytotoxic effects.The seedling vigor index (SVI), which integrates both germination and seedling growth, showed a clear dose-dependent reduction. The highest vigor observed at 0.1% EMS suggests that low concentrations may induce mild stress, activating adaptive physiological mechanisms that temporarily enhance growth. However, at higher concentrations, oxidative stress and metabolic imbalance caused a severe reduction in vigor, reflecting mutagenic lethality and growth retardation.The seedling survival rate followed a similar pattern, decreasing sharply at higher EMS doses. The reduced survival percentage is indicative of chromosomal abnormalities, gene mutations, and physiological damage, which limit the ability of seedlings to recover and establish normal growth. The pattern of decline across parameters suggests that EMS toxicity in greengram is concentration-dependent, with 0.1% EMS being the optimum mutagenic dose for inducing variability with minimal lethality.The results demonstrate that EMS, at low concentrations, can be effectively used to induce genetic variation without severely compromising germination and growth. However, higher EMS concentrations lead to lethal and sublethal effects, which manifest as poor seedling establishment and reduced vigor. These findings are consistent with the general mutagenic response observed in pulse crops, where moderate EMS doses are recommended to achieve maximum mutation frequency with minimum biological damage (Khan and Goyal, 2009, Sen et al, 2022 in greengram; Bhattu et al., 2023 in blackgram; Yugandhar et al., 2023 in groundnut).











III. CONCLUSION

The results can concluded that lower EMS concentrations (0.05-0.1%) showed no adverse effects and even stimulated growth and vigor slightly. Moderate and high EMS concentrations (≥0.2%) caused progressive reductions in all traits. The optimum EMS concentration for inducing variability with minimal injury was found to be 0.1%. All traits exhibited a dose-dependent inhibitory response at higher EMS levels, confirming the toxic nature of EMS at elevated concentrations.

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