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EVALUATION OF EMS-INDUCED MUTANTS FOR GROWTH AND YIELD TRAITS IN M_2 GENERATION OF GREENGRAM (*VIGNA RADIATA* (L.) WILCZEK)

Biotechnology

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ABSTRACT

An experiment was conducted to evaluate the effect of ethyl methane sulphonate (EMS) on growth, phenological, and yield traits in the M_2 generation of greengram (Vigna radiata (L.) Wilczek). Seeds were treated with different concentrations of EMS, and the resulting M_2 population was assessed for variability in quantitative traits. Significant differences were observed among treatments, indicating the effectiveness of EMS in inducing genetic variability. Among the concentrations, 0.1% EMS showed superior performance by recording maximum plant height, number of branches, leaves, clusters, pods per plant, seeds per plant, and seed yield per plant. It also promoted early flowering and maturity compared to higher doses. In contrast, higher concentrations (≥0.3%) exhibited detrimental effects on most traits, resulting in reduced growth, delayed phenology, and lower yield. Seed traits such as seeds per pod and 100-seed weight showed relatively minor variation across treatments. The results indicate a dose-dependent response of EMS, with lower concentrations inducing beneficial mutations and higher concentrations causing physiological and genetic damage. It was concluded that the 0.1% EMS was identified as the most effective dose for generating useful variability without significant adverse effects, suggesting its potential application in mutation breeding programs for greengram improvement.

KEYWORDS

Greengram, Ems, M_2 Generation, Yield Traits, Variability.

INTRODUCTION

Greengram (*Vigna radiata* (L.) Wilczek) is an important pulse crop widely cultivated in tropical and subtropical regions, particularly in India. It serves as a rich source of easily digestible protein, vitamins, and minerals, and plays a significant role in improving soil fertility through biological nitrogen fixation (Singh et al.,2018; Nair et al.,2019). Despite its nutritional importance and adaptability, the productivity of greengram remains relatively low due to various biotic and abiotic constraints, along with a narrow genetic base in cultivated varieties (Kumar and Prasad, 2020).

Enhancing genetic variability is a key prerequisite for crop improvement. However, the limited natural variability available in greengram restricts the scope of conventional breeding approaches. In this context, induced mutagenesis has emerged as an effective tool for creating novel genetic variation (Ahloowalia et al., 2004). Among chemical mutagens, ethyl methane sulphonate (EMS) is widely used due to its high mutagenic efficiency and ability to induce point mutations, which can lead to desirable alterations in plant traits without causing extensive chromosomal damage (Greene et al., 2003; Till et al., 2007).

The success of mutation breeding largely depends on the optimization of mutagen dose, as lower concentrations may induce beneficial mutations, while higher concentrations often result in deleterious effects, including reduced growth, sterility, and lethality (Konzak et al., 1965; IAEA, 2018). The M_2 generation is particularly important in mutation studies, as recessive mutations are expressed and stabilized, allowing effective selection of desirable mutants (Gaul, 1964).

Therefore, the present study was undertaken to evaluate the effect of different concentrations of EMS on growth, phenological, and yield traits in the M_2 generation of greengram. The objective was to identify an optimal EMS dose capable of inducing useful variability and improving agronomic performance, thereby contributing to the development of superior greengram genotypes.

Materials and Methods

Experimental Material and Mutagen Treatment

The experimental material comprised mutants developed from greengram (*Vigna radiata* (L.) Wilczek) genotype WGG-42 through chemical mutagenesis using ethyl methane sulphonate (EMS). Healthy and uniform seeds of the genotype were subjected to different concentrations of EMS to induce mutations. The treated seeds, along with untreated control, were advanced to raise the M_2 generation.

Raising of M_2 Population

The M_2 population was grown during the Rabi season from November 2024 to February 2025 under field conditions. Standard agronomic practices were followed to raise a healthy crop. Each EMS treatment constituted a separate population, and proper isolation was maintained to avoid mechanical mixture.

Selection of Plants and Data Recording

For the assessment of induced variability, 100 normal-looking and randomly selected plants from each EMS treatment, including the control, were evaluated in the M_2 generation. Observations were recorded on important quantitative traits, namely plant height (cm), number of branches per plant (primary and secondary), number of clusters per plant, number of pods per cluster, number of pods per plant, pod length (cm), number of seeds per pod, and single plant yield (g).

Identification and Advancement of Mutants

In addition to quantitative traits, plants exhibiting distinct and desirable morphological variations were carefully identified, tagged, and harvested separately. These selected mutants were advanced to subsequent generations for further evaluation and stabilization.

Statistical Analysis

The data recorded on various quantitative traits in the M_2 generation were subjected to statistical analysis to assess the significance of variation among different EMS treatments. The analysis of variance (ANOVA) was performed following a completely randomized design (CRD) to test the differences among treatment means. The significance of differences among the mean values was tested using the F-test at 5% probability level. When the F-values were found to be significant, critical difference (CD) values were calculated to compare the treatment means. Standard error of mean (SEm ±) was also computed to estimate the variability within treatments. In addition, descriptive statistics such as mean, range, and percentage increase or decrease over control were calculated for all the traits under study. The relative performance of each treatment was evaluated based on these parameters. All statistical analyses were carried out using standard statistical procedures as described by Panse and Sukhatme (1985).

Experimental Results

The significant variation observed among the EMS-induced mutants in the M_2 generation demonstrates that EMS mutagenesis successfully generated genetic diversity for several yield and yield-associated traits in greengram. Traits such as plant height, branching pattern, earliness (days to flowering and maturity), number of pods, seed yield, and seed weight showed pronounced variability and could be effectively utilized in selection programs to identify promising mutant lines with improved yield potential and agronomic performance (Table 1).

Table 1. Analysis of variance (ANOVA) for yield and yield attributes in the mutants of greengram induced by EMS –M2 Generation

Source of variations	DF	MSSQ												
		Characters												
		Pl. Ht(cm)	No.of P.Br./Pl	No.of S.Br./Pl	Days to F.Ini.	Days to P.M	No. L/Pl	No. Cl/Pl	No.P/Cl	No. P/Pl	No. S/PD	No S/Pl	S.Y (g)/Pl	100 S.Wt(g)
Replications	29	26947.286	25319.843	32558.314	53153.896	26716.220	23,735.305	15,749.957	11,091.443	13,530.881	14,435.458	11657.646	14,945.786	22,088.039
Treatments	6	163.512**	929.168**	528.336	1,300.569	598.216**	344.060**	334.249**	188.027**	195.378**	121.793	297.314**	1,543.991**	439.386**
Error	174	-	-	-	-	-	-	-	-	-	-	-	-	-
SE(m)		9.620	10.227	11.272	11.674	8.122	7.701	5.840	5.926	5.877	5.422	5.518	8.630	9.197
SE(d)		13.604	14.463	15.941	16.509	11.486	10.891	8.259	8.381	8.312	7.668	7.804	12.204	13.006

*Significant at 0.05% and ** at 0.01 % level, respectively*

Pl.Ht(cm)- Plant Height (cm); No.of P.Br./Pl- Number of Primary Branches per Plant; No.of S.Br./Pl- Number of Secondary Branches per Plant; Days to F.Ini.- Days to Flower Initiation; Days to P.M- Days to Pod Maturity; No.L/Pl- Number of Leaves per plant; No.Cl/Pl- Number of Clusters per plant; No.P/Cl- Number of Pods per Cluster; No.P/Pl- Number of Pods per Plant; No.S/PD- Number of Seeds per Pod; No S/Pl- Number of Seeds per Plant; S.Y(g)/Pl- Seed Yield (g/pl); 100 S.Wt(g)- 100 Seed Weight (g).

The evaluation of EMS-induced mutants in the M_2 generation of greengram revealed considerable variability in growth, phenological, and yield-related traits, indicating the effectiveness of mutagenesis in generating useful genetic variation. Plant height exhibited significant variation among the treatments. The maximum plant height (62.43 cm) was recorded at 0.1% EMS, suggesting that lower mutagenic doses stimulated vegetative growth in the M_2 population. The untreated control also showed relatively high plant height (55.47 cm), whereas a drastic reduction (17.20 cm) was observed at 0.5% EMS, indicating the persistence of deleterious mutations at higher doses even in the M_2 generation.

Branching pattern showed a similar response. The number of primary branches per plant was highest (9.60) at 0.1% EMS, followed by the control (8.23), while the lowest (2.17) was recorded at 0.5% EMS. Secondary branches were also maximum (4.00) at 0.1% EMS and minimum (1.47) at 0.5% EMS. The improved branching in lower EMS treatments suggests the expression and stabilization of beneficial mutations in the M_2 generation, whereas higher doses likely retained harmful mutations affecting plant architecture. Phenological traits such as days to first flowering and pod maturity were notably influenced. Early flowering (47.27 days) was recorded at 0.1% EMS, followed by 0.2% EMS (48.17 days), while delayed flowering (61.73 days) was observed at 0.5% EMS. Similarly, pod maturity was earliest (86.73 days) at 0.1% EMS and delayed (94.67 days) at 0.5% EMS. These results indicate that desirable early-maturing mutants were effectively expressed in the M_2 generation at lower EMS concentrations, whereas higher doses resulted in delayed phenological development.Leaf production was also enhanced at lower EMS levels, with the highest number of leaves per plant (22.00) recorded at 0.1% EMS, followed by 0.05% EMS (20.40). In contrast, the lowest leaf number (6.53) at 0.5% EMS reflects reduced photosynthetic capacity due to mutagen-induced stress. The improved leaf production in M_2 suggests stabilization of favorable mutations influencing vegetative growth.

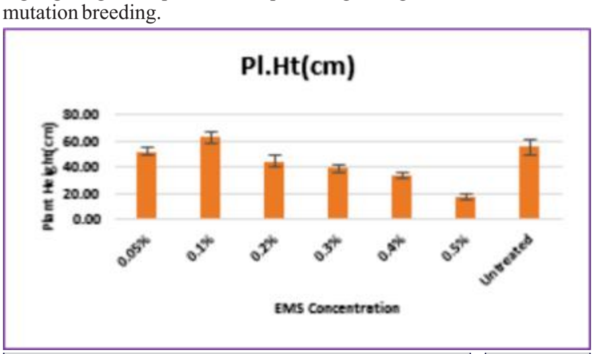
Table 2. Effect of induced EMS mutagenesis in yield and yield contributing traits in greengram of M_2 generation.

EMS Conc.	Pl.Ht(cm)	No.of P.Br./Pl	No.of S.Br./Pl	Days to F.Ini.	Days to P.M	No. L/Pl	No.Cl/Pl	No. P/Cl	No. P/Pl	No.S/PD	No S/Pl	S.Y (g)/Pl	100 S.Wt(g)
0.05 %	51.60	8.20	3.03	50.13	89.90	20.40	4.70	3.77	17.43	9.93	172.97	5.65	3.26
0.1%	62.43	9.60	4.00	47.27	86.73	22.00	5.93	3.83	22.57	10.03	225.30	7.59	3.37
0.2%	44.07	8.17	2.93	48.17	89.47	18.27	4.17	3.50	14.63	9.30	137.13	4.42	3.22
0.3%	39.03	5.87	2.47	54.07	91.47	14.20	3.20	2.60	8.13	9.30	76.20	2.46	3.23
0.4%	32.80	4.17	1.80	56.03	94.27	11.47	2.87	2.30	6.77	8.87	60.60	1.96	3.21
0.5%	17.20	2.17	1.47	61.73	94.67	6.53	2.00	2.27	4.50	8.70	38.80	1.23	3.17
Untreated	55.47	8.23	3.13	52.83	90.77	20.20	4.57	3.43	15.57	10.43	161.67	5.45	3.37
Mean	***43.23***	***6.63***	***2.69***	***52.89***	***91.04***	***16.15***	***3.92***	***3.10***	***12.80***	***9.51***	***124.67***	***4.11***	***3.26***
Min	***17.20***	***2.17***	***1.47***	***47.27***	***86.73***	***6.53***	***2.00***	***2.27***	***4.50***	***8.70***	***38.80***	***1.23***	***3.17***
Max	***62.43***	***9.60***	***4.00***	***61.73***	***94.67***	***22.00***	***5.93***	***3.83***	***22.57***	***10.43***	***225.30***	***7.59***	***3.37***
Sd	***15.24***	***2.67***	***0.86***	***5.02***	***2.77***	***5.65***	***1.32***	***0.69***	***6.52***	***0.64***	***68.08***	***2.31***	***0.08***

Pl.Ht(cm)- Plant Height (cm); No.of P.Br./Pl- Number of Primary Branches per Plant; No.of S.Br./Pl- Number of Secondary Branches per Plant; Days to F.Ini.- Days to Flower Initiation; Days to P.M- Days to Pod Maturity; No.L/Pl- Number of Leaves per plant; No.Cl/Pl- Number of Clusters per plant; No.P/Cl- Number of Pods per Cluster; No.P/Pl- Number of Pods per Plant; No.S/PD- Number of Seeds per Pod; No S/Pl- Number of Seeds per Plant; S.Y(g)/Pl- Seed Yield (g/pl); 100 S.Wt(g)- 100 Seed Weight (g).

Yield-contributing traits such as clusters per plant, pods per cluster, and pods per plant were significantly improved at lower EMS doses. The number of clusters per plant was highest (5.93) at 0.1% EMS and lowest (2.00) at 0.5% EMS. Pods per cluster were maximum (3.83) at 0.1% EMS and minimum (2.27) at 0.5% EMS. Similarly, pods per plant were highest (22.57) at 0.1% EMS and drastically reduced (4.50) at 0.5% EMS. These results indicate that beneficial mutations affecting reproductive efficiency were successfully expressed and fixed in the M_2 generation at lower EMS concentrations. The number of seeds per pod showed relatively minor variation, with the highest value (10.43) recorded in the control and slightly lower values at 0.1% EMS (10.03). The lowest value (8.70) at 0.5% EMS suggests a slight negative effect at higher doses, although this trait remained comparatively stable across treatments.

In contrast, the number of seeds per plant varied widely, with the highest value (225.30) at 0.1% EMS, followed by 0.05% EMS (172.97), and the lowest (38.80) at 0.5% EMS. This improvement at lower concentrations reflects the cumulative effect of enhanced branching and pod formation in the M_2 mutants. Seed yield per plant was significantly influenced by EMS treatments. The maximum yield (7.59 g) was recorded at 0.1% EMS, followed by 0.05% EMS (5.65 g), whereas the lowest yield (1.23 g) was observed at 0.5% EMS. The superior performance at lower EMS concentrations indicates the successful selection and expression of high-yielding mutants in the M_2 generation, while higher doses resulted in reduced plant vigor and yield. The 100-seed weight showed minimal variation across treatments, with a slight increase at 0.1% EMS (3.37 g) and a marginal decrease at 0.5% EMS (3.17 g), suggesting that seed size is a relatively stable trait and less influenced by mutagenesis. The results from the M_2 generation clearly demonstrate that lower concentrations of EMS, particularly 0.1%, were effective in inducing beneficial mutations that enhanced growth and yield traits in greengram. In contrast, higher concentrations (0.5%) resulted in the persistence of deleterious effects, highlighting the importance of optimizing mutagen dose for successful mutation breeding.



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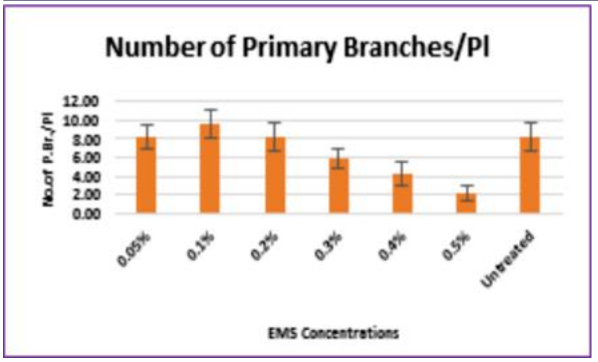

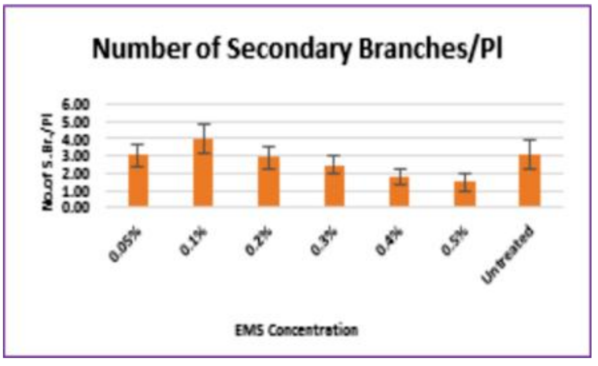

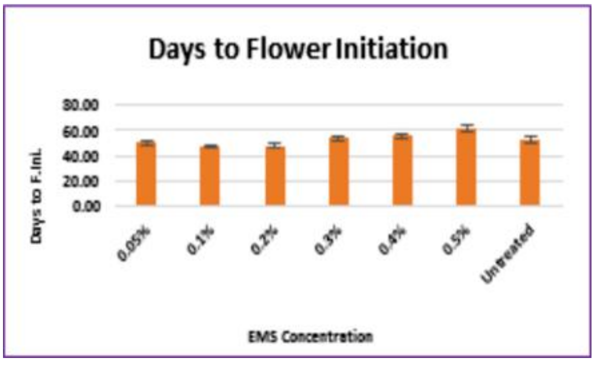

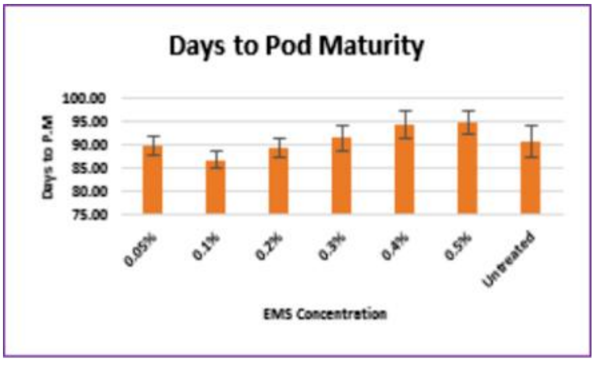

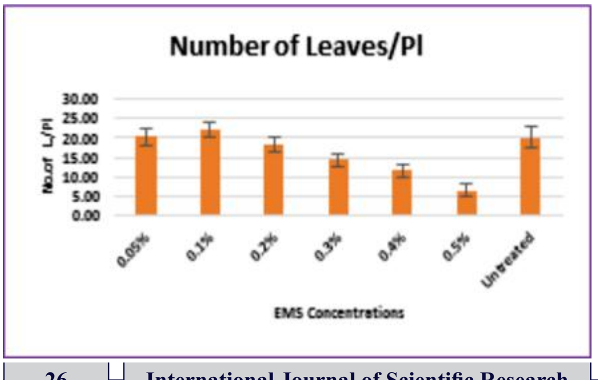

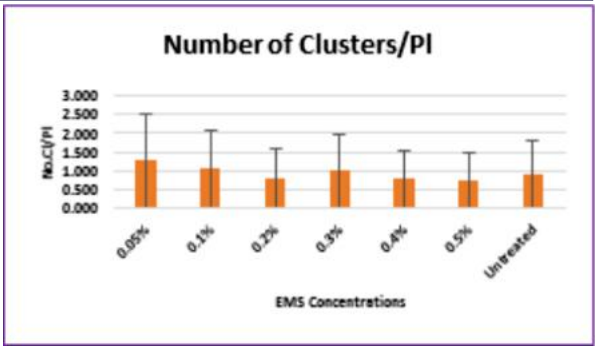

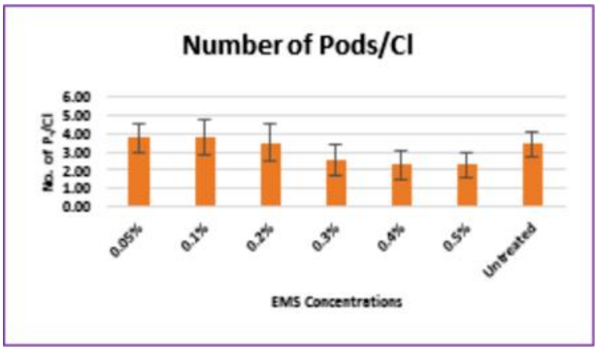

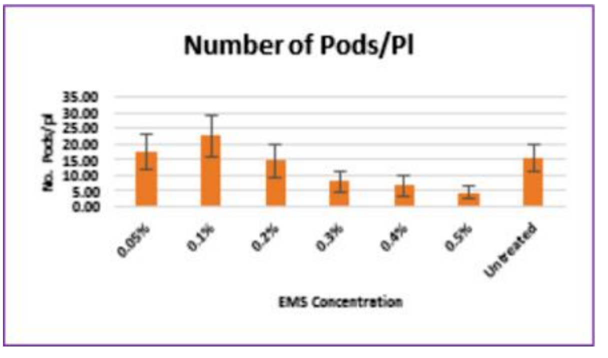

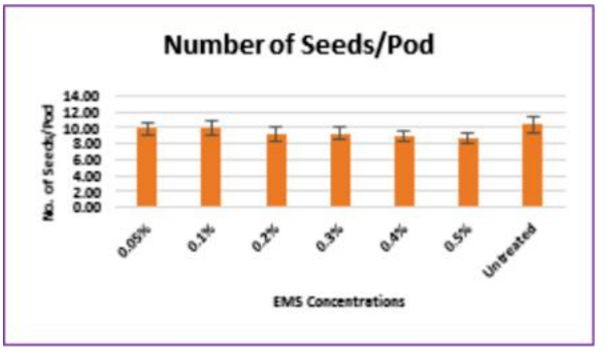

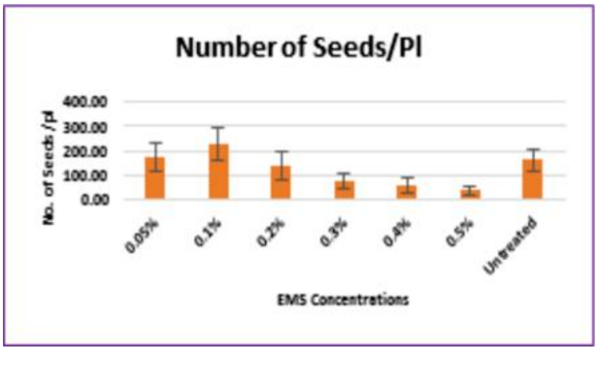

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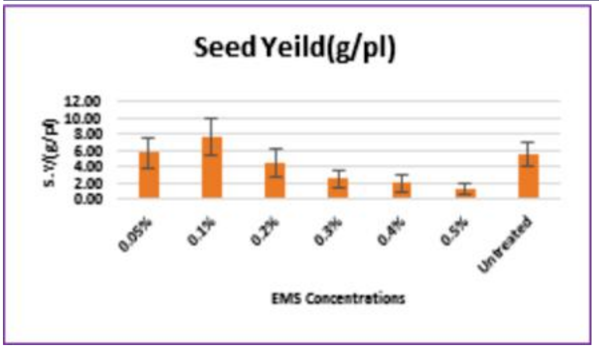

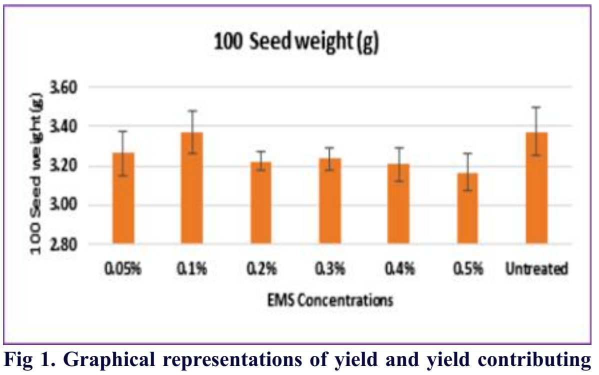

Fig 1. Graphical representations of yield and yield contributing traits in mutants of greengram in M2 generations

DISCUSSION

The present M_2 generation study demonstrated that EMS (ethyl methane sulphonate) induced significant variability in growth, phenology, and yield traits of greengram, with responses largely dependent on mutagen concentration. Lower doses, particularly 0.1% EMS, exhibited stimulatory effects, whereas higher concentrations (≥0.3%) were inhibitory, confirming the dose-dependent nature of mutagenesis (Kumar et al., 2022; Hassan et al., 2023).

Vegetative traits such as plant height, number of branches, and leaf production were enhanced at 0.1% EMS, indicating the expression of beneficial mutations that improved growth and metabolic activity. In contrast, higher concentrations caused a sharp decline in these traits, likely due to chromosomal aberrations, disrupted cell division, and hormonal imbalance. This reflects a hormetic response, where low mutagen doses stimulate growth while higher doses induce physiological damage (Babu et al., 2023; Devi et al., 2023).

Phenological traits showed that lower EMS doses (0.05-0.2%) promoted early flowering and maturity, whereas higher doses delayed these stages. The earliness at lower concentrations may be attributed to accelerated transition to the reproductive phase, while delays at higher doses suggest genetic and physiological disturbances affecting floral initiation (Singh & Sharma, 2022; Patel et al., 2021; Rahman et al., 2023).

Yield and its components, including clusters per plant, pods per cluster, pods per plant, seeds per plant, and seed yield, were significantly improved at 0.1% EMS, indicating enhanced reproductive efficiency and favorable gene modifications under mild mutagenic conditions. However, concentrations above 0.2% led to a drastic reduction in all yield traits, likely due to mutagen-induced sterility, meiotic abnormalities, and reduced assimilate partitioning (El-Nahas et al., 2022; Rani et al., 2024; Maheshwari et al., 2024).

Table 3. Increased/decreased% of yield and yield related traits over its control (untreated) under EMS induced mutagenesis in greengram-M2 generation

EMS Conc.	Pl.Ht(cm)	No.of P.Br./Pl	No. of S.Br./Pl	Days to F.Ini.	Days to P.M	No. L/Pl	No.Cl/Pl	No.P/Cl	No.P/Pl	No.S/PD	No S/Pl	S.Y(g)/Pl	100 S.Wt(g)
0.05%	-7.49	-0.41	-3.30	-5.39	-0.96	0.98	2.84	8.85	10.71	-5.03	6.53	3.60	-3.36
0.1%	11.16	14.24	21.67	-11.78	-4.65	8.18	23.03	10.43	31.02	-3.99	28.24	28.21	0.01
0.2%	-25.87	-0.82	-6.82	-9.69	-1.45	-10.58	-9.60	1.90	-6.38	-12.19	-17.89	-23.35	-4.62
0.3%	-42.10	-40.34	-27.03	2.28	0.77	-42.25	-42.71	-32.05	-91.39	-12.19	-112.16	-121.38	-4.28
0.4%	-69.11	-97.60	-74.07	5.71	3.71	-76.16	-59.30	-49.28	-130.05	-17.67	-166.78	-178.64	-5.10
0.5%	-222.48	-280.00	-113.64	14.42	4.12	-209.18	-128.33	-51.47	-245.93	-19.92	-316.67	-344.13	-6.51

Pl.Ht(cm)- Plant Height (cm); No.of P.Br./Pl- Number of Primary Branches per Plant; No.of S.Br./Pl- Number of Secondary Branches per Plant; Days to F.Ini.- Days to Flower Initiation; Days to P.M- Days to Pod Maturity; No.L/Pl- Number of Leaves per plant; No.Cl/Pl- Number of Clusters per plant; No.P/Cl- Number of Pods per Cluster; No.P/Pl- Number of Pods per Plant; No.S/PD- Number of Seeds per Pod; No S/Pl- Number of Seeds per Plant; S.Y(g)/Pl- Seed Yield (g/pl); 100 S.Wt(g)- 100 Seed Weight (g).

Seed traits such as seeds per pod and 100-seed weight showed comparatively less variation, suggesting their relative stability against mutagenic treatments, as also reported in legumes (Mansour et al., 2022; Nguyen et al.,2024). The results confirm a dose-dependent biphasic effect of EMS in the M_2 generation, where 0.1% EMS proved to be the most effective concentration for inducing beneficial variability without significant detrimental effects. Higher concentrations were associated with severe growth inhibition and yield reduction, emphasizing the importance of optimizing mutagen dose in mutation breeding programs (Kumar et al 2023.; Hassan et al.2023).

CONCLUSION

The present study on EMS-induced mutagenesis in the M_2 generation of greengram (Vigna radiata (L.) Wilczek) genotype WGG-42 revealed significant variability in growth, phenological, and yield traits. Among the different treatments, 0.1% EMS was found to be the most effective concentration, as it enhanced vegetative growth, promoted early flowering and maturity, and significantly improved yield and its components. In contrast, higher EMS concentrations (≥0.3%) exhibited detrimental effects, resulting in reduced growth, delayed phenology, and poor yield performance due to mutagen-induced physiological and genetic damage. The study clearly demonstrated a dose-dependent response of EMS, where lower concentrations induced beneficial mutations while higher concentrations were inhibitory. The identification of superior mutants with desirable traits in the M_2 generation highlights the potential of EMS as an effective tool for generating useful genetic variability in greengram. These promising mutants can be further advanced and evaluated in subsequent generations for stabilization and utilization in breeding programs aimed at yield improvement.

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