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Elucidating mutagenic sensitivity and Lethal Dose 50 (LD₅₀) of Black gram variety Vamban 11 under laboratory condition

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Abstract

The investigation examined the impact of different doses of Ethyl methane sulfonate (EMS) (0.2%, 0.4%, 0.6%, 0.8%, and 1.0%) under laboratory condition on black gram variety Vamban 11 to broaden the genetic base and establish optimal mutation breeding parameters. Four seedling parameters were assessed: shoot length, root length, germination percentage and seed survival rate. Finney's probit analysis was used to evaluate EMS toxicity and to calculate the lethal dose causing 50% mortality (LD₅₀). The findings demonstrated clear dose-dependent responses across all traits. Shoot length decreased progressively from 27.20 cm (control) to 13.23 cm (1.0% EMS), a 51.3% reduction. Root length exhibited greater sensitivity, declining 66.2% from 12.50 cm to 4.23 cm. Germination percentage dropped from 95.0% to 41.33% (56.5% reduction), while seed survival rate decreased most dramatically from 95.0% to 33.33% (64.9% reduction). The LD₅₀ value was determined to be 0.68% EMS concentration, confirming its suitability for effective mutation induction while maintaining acceptable survival rates.

Keywords: Black gram, VBN 11, mutation breeding, ethyl methane sulfonate, LD₅₀

Introduction

Vigna mungo L. Hepper; 2n=22, known as black gram or urd bean, represents a critically important pulse crops throughout India and Asian countries. It belongs to the family Leguminosae and is highly valued for its rich protein content (24-26%), making it a crucial component of vegetarian diets (Kaewwongwal *et al.*, 2015) [3]. The species contributes significantly to sustainable farming systems through its atmospheric nitrogen fixation capacity, enriching soil nutrient status while diminishing synthetic fertilizer requirements (Yadav *et al.*, 2006) [24]. India leads global black gram production with 70% of total output, while Myanmar and Pakistan rank second and third. India yields roughly 2.7 million tonnes across 4.4 million hectares, averaging 598 kg per hectare (Nair *et al.*, 2024) [9].

Despite its nutritional and agricultural importance, black gram production faces numerous challenges including modest yield capacity, diseases vulnerability, and constrained genetic diversity in existing cultivars (Patidar *et al.*, 2018) [11]. While hybridization is a conventional method to create variability, it poses significant challenges in black gram due to its cleistogamous nature, which makes emasculation difficult and leads to poor seed set. Additionally, the absence of established male sterility systems in this crop further complicates hybridization efforts (Selvam *et al.*, 2010) [14]. To overcome these constraints and develop superior varieties with enhanced traits, plant breeders have increasingly turned to induced mutagenesis as an effective strategy for generating genetic variability, especially valuable in self-pollinating species like black gram where natural genetic variation is limited (Kharkwal and Shu, 2009) [4]. In order to induce variable mutations in crop plants, both chemical and physical mutagens are most commonly used, with chemical mutagens gaining particular prominence due to their ease of application, cost-effectiveness, and ability to induce point mutations rather than large chromosomal aberrations.

EMS is one of the most widely used and efficient chemical mutagens in plant breeding programs and has proven successful in generating variation for multiple traits including pod seed number, primary branch count, plant height (Usharani and Kumar, 2015; Kuralarasan *et al.*, 2018) ^[18, 7], methionine levels (Arulbalachandran *et al.*, 2009) ^[2], protein content, nitrate reductase activity (Thilagavathi and Mullainathan, 2011) ^[17], and overall seed productivity (Verma *et al.*, 2018) ^[21]. The determination of LD₅₀ (Lethal Dose 50) is a crucial preliminary step in any mutation breeding program. This metric identifies the mutagen concentration or dose producing 50% mortality within the treated population (Tamilzharasi *et al.*, 2021) ^[16]. This parameter serves as a standardized measure to optimize mutagenic treatments, ensuring maximum genetic variation while maintaining adequate survival rates for further selection and breeding work. The concentration of EMS and duration of treatment are critical factors that determine both the mutation frequency and the survival rate of treated seeds (Usharani and Kumar, 2015) ^[18]. Therefore, the present study was conducted as a laboratory-based dose-optimization experiment to investigate the effects of varying EMS concentrations on the seedling traits of black gram variety Vamban 11. The primary goal was to establish the LD₅₀ value based on germination and survival capacity, serving as a prerequisite for future M1 generation field trials.

Materials and Methods

The present investigation was carried out at Plant Breeding and Genetics laboratory, Adhiparasakthi Agricultural College (affiliated to Tamil Nadu Agricultural University, Coimbatore) Ranipet, Tamil Nadu during the month of July, 2025. The black gram variety VBN 11 seeds procured from TNAU, Coimbatore served as experimental material. Uniform, healthy VBN 11

seeds underwent comprehensive washing followed by 5-hour hydration in distilled water, then treated with EMS for 2 hours at five different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0%). Post-treatment, the seeds were rinsed under flowing tap water for 1 hour to eliminate residual mutagen from seed surfaces. For this experiment, 75 seeds from each EMS concentration and 75 control seeds (untreated) were used, with each treatment including the control replicated three times with 25 seeds per replicate using the roll towel method on germination paper as described by Usharani *et al.* (2017) ^[19]. As this was an *in vitro* sensitivity assay, observations were limited to early seedling growth to assess physiological damage. The following parameters were observed on the 8th day after treatment viz., shoot length (cm), root length (cm), germination percentage and seed survival rate (%). Statistical analysis employed a completely randomized design (CRD). Analysis of variance for different parameters was conducted and means were compared using Tukey’s HSD (honestly significant difference) test *via* STAR (Statistical Tool for Agricultural Research) software version 2.0. LD₅₀ calculation utilized Finney’s probit analysis (Mekapogu, 2021) ^[8].

Results and Discussion

The result had shown a significant variation observed in all treatments among all the characters under study (Table 1). The morphological observations indicated that escalating mutagen concentration progressively diminished overall seedling development. Shoot length showed progressive reduction ranged from 27.20 cm in the control to 13.23 cm at the highest concentration (1.0% EMS), representing a 51.3% decrease (Table 2). This substantial reduction indicates significant impact on aerial plant development with increasing mutagen concentration.

Table 1: Analysis of variance for seedling traits of Black gram variety VBN 11

Source	Degree of freedom	Shoot length		Root length		Germination%		Seed survival rate	
		MSS	F Value	MSS	F Value	MSS	F Value	MSS	F Value
Treatment	5	93.31	17.55**	33.38	29.57**	1447.16	33.10**	1898.63	38.01**
Error	12	5.31		1.12		43.72		49.94	

*Significant at *P* < 0.05 level
**Significant at *P* < 0.01 level
(MSS- Mean sum of squares, F value- Fisher’s statistic value)

Table 2: Effect of EMS on shoot length and root length of black gram variety VBN 11

Treatments (EMS Conc.)	Shoot length (cm)	Percent reduction over control	Root length (cm)	Percent reduction over control
Control	27.20 ^a	-	12.50 ^a	-
0.2%	25.53 ^{ab}	6.13	11.76 ^{ab}	5.90
0.4%	21.47 ^{ab}	21.06	9.07 ^{bc}	27.47
0.6%	19.23 ^{bc}	29.29	7.36 ^{cd}	41.08
0.8%	15.01 ^c	44.83	5.47 ^{de}	56.25
1.0%	13.23 ^c	51.36	4.23 ^e	66.17

(Means having same letter(s) are not significantly different at 5% level of probability)

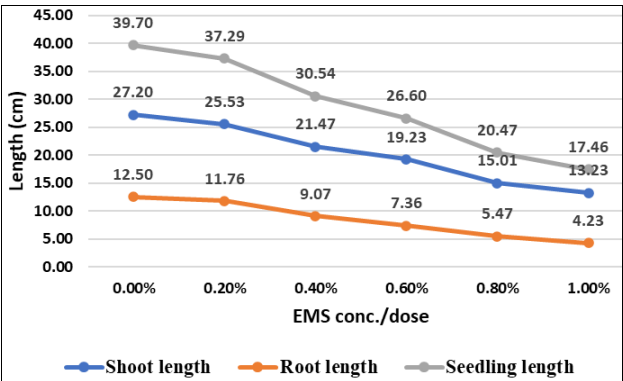


Fig 1: EMS Effects on Seedling length

Root length exhibited even more pronounced sensitivity to EMS treatment, declining from 12.50 cm in control to 4.23 cm at 1.0% concentration - a dramatic 66.2% reduction over control (Table 2). The root system's higher sensitivity suggests that underground growth is more susceptible to mutagenic stress than shoot development (Figure 1), likely due to direct exposure and absorption of the mutagen through the root system as the primary uptake site, and the absence of protective barriers such as cuticles that are present in shoots. These findings correspond with Usharani *et al.* (2017) ^[19], revealed that the progressive reduction was observed as the EMS concentration increases, where the shoot length ranged from 25.72 cm (control) to 7.30 cm (highest EMS dose) and root length ranged from 6.75 cm (control) to 1.20 cm (highest EMS dose) on the black gram

variety VBN 4. Similar declining trends were also reported by Tamilzharasi *et al.* (2021) ^[16] in the crop black gram, Kumar *et al.* (2022) ^[6] in linseed cultivar (Baner, Him Alsi-2 and Surbhi); Ali *et al.* (2024) ^[1] in Mungbean genotypes (Pusa 1031 and Pusa 1431) and Sofia *et al.* (2019) ^[15] in Mungbean variety (WGG-42 and LGG-460). However, as EMS concentration increased, the reductions became more severe: at 0.4% EMS, germination showed 17% reduction and survival 23% reduction over control; at 0.6% EMS, germination had 33% reduction and survival 38% reduction over control; at 0.8% EMS, germination declined by 48% and survival by 56% over control; and at the highest concentration of 1.0% EMS, germination showed 56% reduction while survival had 65% reduction over control (Table 3). The

data reveals that seed survival rates exhibited greater sensitivity to EMS treatment than germination percentages, with survival showing consistently higher percentage reductions compared to control at each concentration level (Figure 3). The greater sensitivity of seed survival rates compared to germination percentages occurs because germination only measures the initial ability of seeds to sprout, while survival rate assesses the seedlings continued viability over time. Seeds that initially germinate may still succumb to cumulative mutagenic damage during subsequent growth stages due to impaired metabolic functions, compromised cellular repair mechanisms, and delayed expression of lethal mutations that only manifest after germination, making survival a more stringent indicator of EMS toxicity.

Table 3: Effect of EMS on germination percentage and seed survival rate of black gram variety VBN 11

Treatments (EMS Conc.)	Percent germination	Percent reduction over control	Seed survival rate (%)	Percent reduction over control
Control	95.00 ^a	-	95.00 ^a	-
0.2%	90.67 ^a	4.56	89.33 ^a	5.96
0.4%	78.67 ^{ab}	17.19	73.33 ^{ab}	22.81
0.6%	64.00 ^{bc}	32.63	58.67 ^{bc}	38.25
0.8%	49.33 ^{cd}	48.07	41.33 ^{cd}	56.49
1.0%	41.33 ^d	56.49	33.33 ^d	64.91

(Means having same letter(s) are not significantly different at 5% level of probability)

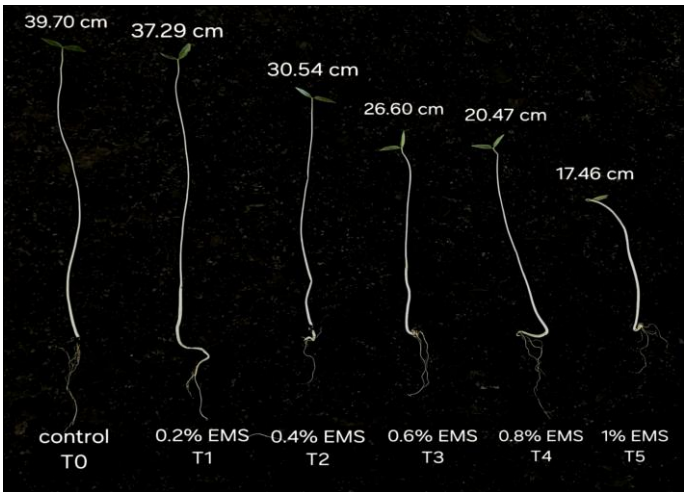


Fig 2: Mean seedling length of different treatments of EMS on VBN 11

and the data also confirmed that the plant survival exhibited greater sensitivity to EMS treatment than germination percentage. Similar results were also reported by Kumar *et al.* (2022) ^[6] in the black gram accession IC436585, where the speed of germination decreased with increase in the EMS concentration and opined that the impact on germination could be attributed to damage in the seeds at the time of cell division in the meristematic tissues. Similar declining trends were also obtained by Vijayakanth *et al.* (2021) ^[22] in black gram variety VBN-3 even though there was different mutagen employed viz., DES (Diethyl Sulphate) and SA (Sodium Azide). These results harmonize with earlier reports including Ramya and Nallathambi (2014) ^[13] in blackgram variety TNAUCo(Bg)6, Veni *et al.* (2017) ^[20] in black gram variety MDU 1 and VBN 4, Vikhe and Nehul (2020) ^[23] in Mungbean cultivar Naval. These findings indicate that while EMS is effective as a chemical mutagen, concentrations exceeding 0.6% may be too severe for practical mutation breeding applications due to excessive seed mortality and poor germination performance, with the optimal range appearing to be between 0.4-0.6% EMS for balancing mutagenic potential with acceptable seed viability, though precise determination of the optimal concentration should be based on LD₅₀ values for more accurate dosage selection in mutation breeding programs.

The probit analysis revealed an LD₅₀ value of 6,844.91 ppm (0.68% EMS) with a 95% confidence interval ranging from 4,803.96 to 9,752.94 ppm (Table 5). This indicates that approximately 50% of the black gram seeds would be expected to die when exposed to 6,844.91 ppm of EMS under the experimental conditions tested. The probit log graph (Figure 3) illustrates the linear relationship between log₁₀ dose and empirical probit values for EMS toxicity. The plotted points (Table 4) show excellent linearity with log₁₀ dose on the x-axis (3.30 to 4.00) and empirical probits on the y-axis (3.76 to 5.43). This strong linear relationship ($R^2 > 0.95$) confirms that the data perfectly fit the probit model assumptions, validating the statistical approach used. The intersection points where the regression line crosses the 50% mortality level (probit value $\approx$

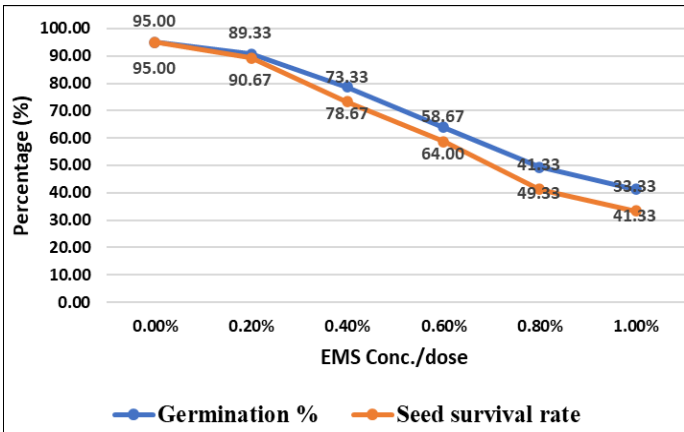


Fig 3: EMS Effects on Seed Germination and Survival Rates

These outcomes align with Kumar *et al.* (2020) ^[5] where the percent reduction over control for both germination percentage and plant survival were observed higher in the highest concentration of EMS (0.8%) used in all the linseed cultivars

5.0) precisely corresponds to the calculated LD₅₀ value of 6,844.91 ppm (log₁₀ dose ≈ 3.835). This graphical validation supports the mathematical derivation of the LD₅₀.

Table 4: Probit analysis for calculating LD₅₀ in VBN-11

EMS Dose/Conc.	Total	Dead	Group	Log10 Dose	Empirical Probits
0.2%	75	8	2	3.30	3.76
0.4%	75	20	3	3.60	4.38
0.6%	75	31	4	3.78	4.78
0.8%	75	44	5	3.90	5.22
1.0%	75	50	6	4.00	5.43

Table 5: Determination of Lethal Dose 50 based on Finney’s probit analysis

LD/LC (%)	LD/LC ppm	95%Fiducial CI	
		Lower	Upper
LD ₁₀	2022.30077	1419.31144	2881.46795
LD ₃₀	4156.15318	2916.91316	5921.87984
LD ₅₀	6844.90763	4803.96181	9752.94192
LD ₇₀	11273.10725	7911.80533	16062.44614
LD ₉₉	62601.80112	43935.82471	89197.95019

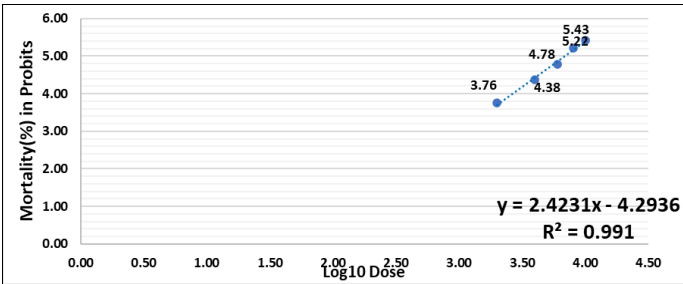


Fig 4: Probit log graphs dose effect of EMS

From the observations, LD₅₀ value for EMS treated black gram variety VBN 11 has been found at 6844.91ppm (0.68%). Usharani *et al.* (2017) ^[19] obtained LD₅₀ value in 60mM of EMS treatments *i.e.*, 0.74% for the black gram variety VBN 4. LD₅₀ value for black gram variety TNAUCo(Bg)6 was observed at 20mM (0.24%) of EMS (Ramya *et al.*, 2014) ^[12]. Kumar *et al.* (2020) ^[5] suggested that 0.3% of EMS concentration can create variability in the black gram genotype IC 436585. Tamilzharasi *et al.* (2020) ^[16] recorded the LD₅₀ value at 23.11mM (0.28%) of EMS concentration in the black gram variety CO 6. Ali *et al.* (2024) ^[1] exhibited LD₅₀ values at 58.81mM (0.73%) and 45.04mM (0.56%) of EMS for the Mungbean genotypes ‘Pusa 1031’ and ‘Pusa 1431’, respectively. LD₅₀ value of soybean seeds (cv. Gepak Kuning) treated with EMS mutagen was at a concentration of 0.63% (Nilahayati *et al.*, 2024) ^[10]. Variations in LD₅₀ among different varieties of identical species represents a common phenomenon in mutation studies because the resistance exhibited by biological material primarily depends on seed size, maturity level and moisture content during treatment (Sofia *et al.*, 2019) ^[15].

Conclusion

This study establishes that EMS-induced mutagenesis in black gram variety VBN 11 follows predictable dose-response kinetics, with root systems showing greater sensitivity (66.2% reduction) than shoots (51.3% reduction) at maximum concentration. The determined LD₅₀ value of 0.68% EMS provides a critical benchmark for mutation breeding protocols and balances mutagenic potential with acceptable plant viability. These findings offer evidence-based guidelines for black gram

improvement programs, confirming variety-specific responses that align with broader legume mutagenesis patterns. Future research can directly utilize the established LD₅₀ value of 0.68% EMS concentration to achieve balanced mutation induction while maintaining optimum population size in M1 generation under field conditions. This standardized approach will ensure adequate genetic variation for selection while preserving sufficient plant survival for effective breeding programs, enabling systematic mutation breeding initiatives with predictable outcomes.

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Conflict of Interest

All authors declared that there is no conflict of interest.

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