International Journal of Plant & Soil Science

Volume 38, Issue 6, Page 278-288, 2026; Article no.IJPSS.159132
ISSN: 2320-7035

Assessment of Somatic Mutagenic Effects on Different Black Gram Varieties by Chemical Mutagen Ethyl Methane Sulfonate (EMS)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i66117

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/159132

Original Research Article

Received: 16/03/2026
Published: 03/06/2026

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Cite as: *Sundaram, K. M., Senthamizhkumaran, V. R., Raj, R. S., Ilavarasan, T., Jeeva, S., Jegathbabu, V., … Janani, S. S. (2026). Assessment of Somatic Mutagenic Effects on Different Black Gram Varieties by Chemical Mutagen Ethyl Methane Sulfonate (EMS). International Journal of Plant & Soil Science, 38(6), 278–288. https://doi.org/10.9734/ijpss/2026/v38i66117*

Abstract

Black gram [Vigna mungo (L.) Hepper], a nutritionally rich pulse crop, suffers from narrow genetic variability due to its cleistogamous nature, limiting conventional breeding progress. Induced mutagenesis using chemical mutagens offers a viable approach to generate novel genetic variability. This study evaluated the somatic mutagenic effects of Ethyl Methane Sulfonate (EMS) at five concentrations (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) on two black gram varieties, VBN 8 and VBN 10, using germination towel and pro-tray methods. Parameters assessed included germination percentage, root length, shoot length, germination index, and chlorophyll content. Results demonstrated a significant dose-dependent reduction in all growth parameters with increasing EMS concentration in both varieties and methods. In the germination towel method, VBN 8 showed germination percentages ranging from 72.5% (0.1%) to 60.0% (0.5%), while VBN 10 exhibited a sharper decline from 72.5% to 47.5%. Root length reduction reached 66.81% in VBN 8 and 65.21% in VBN 10 at 0.5% EMS. Similarly, shoot length reduction was more pronounced in VBN 8 (73.5%) compared to VBN 10 (22.16%) at the highest dose. The LD50 was projected at approximately 0.4–0.5% EMS for both varieties. Chlorophyll content exhibited irregular variation, suggesting differential mutagenic sensitivity between genotypes. These findings establish the baseline mutagenic effectiveness and efficiency of EMS for future M_2 generation screening programs aimed at developing improved black gram genotypes with enhanced yield, quality, and stress tolerance. The present investigation comprehensively established the dose-dependent somatic mutagenic effects of EMS on two elite black gram varieties, VBN 8 and VBN 10, under both germination towel and pro-tray conditions.

Keywords: Vigna mungo; ethyl methane sulfonate; LD50; somatic effects; germination.

1. Introduction

Black gram [*Vigna mungo (L.) Hepper*] (2n = 22), commonly known as Urdbean, is one of the most economically significant self-pollinated grain legumes cultivated across tropical and subtropical Asia. It occupies a premier position among pulse crops owing to its exceptional nutritional profile, including seed protein content ranging from 23.3 to 28.95 per cent, enriched with high-quality lysine that complements cereal-based diets. Beyond protein, seeds are abundant in carbohydrates (60.3%), fats (1.0–2.5%), thiamine, nicotinic acid, calcium, and iron. The crop holds significant therapeutic value, being prescribed externally and internally for paralysis, rheumatism, and nervous system disorders, while also recommended as a medicinal diet for easier digestibility and flatulence management.

India stands as the world's largest producer of black gram, with a cultivated area of 4,260 thousand hectares yielding 2,550 thousand tonnes at a productivity of 657 kg ha^{-1} (Indiastat, 2025). Despite this leading production status, domestic demand consistently outpaces domestic supply, necessitating costly imports that burden foreign exchange reserves. The per capita pulse availability in India has alarmingly declined from 60.7 g per day in 1951 to 43.6 g per day in 2024 (Indiastat, 2025), reflecting a widening protein deficit that demands immediate scientific intervention. Black gram ranks fourth among Indian pulses in area and production, contributing approximately 14 percent of the total pulse crop area, and plays an indispensable role in the daily diets of South Indian populations through traditional preparations such as idli, dosa, and vada.

A fundamental constraint limiting the genetic progress of black gram breeding programs is the narrow genetic base resulting from its cleistogamous flower structure, which restricts natural cross-pollination and genetic recombination (Tamilzharasi et al., 2022). Conventional breeding approaches exploiting available variability have largely failed to achieve the yield gains required to meet escalating demand, particularly in comparison to major legumes such as chickpea and pigeonpea (Goyal et al., 2021). In this context, induced mutagenesis has emerged as the most viable and scientifically proven strategy for artificially expanding genetic variability in self-pollinated crops (Goyal et al., 2019).

The global adoption of mutation breeding has led to the official release of more than 3,200 mutant varieties from over 200 different crops. The FAO/IAEA Mutant Varieties Database currently lists eight black gram varieties from India with demonstrated superiority in yield, quality, disease resistance, and climate resilience. Chemical mutagens, particularly Ethyl Methane Sulfonate (EMS), are widely recognized as the most effective agents for inducing point mutations at high frequencies across the genome. EMS functions as an alkylating agent that

predominantly causes GC→AT transitions through the formation of O6-ethylguanine, thereby generating a broad spectrum of heritable mutations that can alter agronomically important traits (Holman et al., 2025).

EMS has demonstrated consistent efficacy in creating genetic diversity in quantitative traits in multiple legume and cereal crops (Shamshad et al., 2023). However, the selection of an appropriate mutagenic dose is critical, as sub-optimal doses produce insufficient mutation frequency while excessive doses cause severe biological damage that masks or eliminates desirable mutants. The LD50 concept—the dose causing 50% lethality in the treated population serves as the standard biological benchmark for determining the optimal mutagenic dose range for a given crop-mutagen combination. Mutagenic effectiveness measures the frequency of mutation per unit dose, while mutagenic efficiency quantifies the ratio of useful mutations to biological damage caused (Gill et al., 2017).

The primary advantage of this methodology lies in its shift from generalized, broad-spectrum mutagenic applications to highly calibrated, variety-specific optimization. Traditional mutation breeding often relies on uniform chemical dosages across diverse cultivars, overlooking the fact that distinct genetic backgrounds, metabolic rates, and seed coat structures possess varying sensitivities to chemical mutagens like EMS. By explicitly establishing the unique LD_{50} thresholds for the VBN 8 and VBN 10 black gram varieties, this approach prevents the dual pitfalls of over-mutagenesis, which induces excessive lethality or sterility, and under mutagenesis, which yields negligible genetic variation. Furthermore, evaluating immediate somatic injuries such as altered germination velocity, seedling vigor, and chlorophyll degradation simultaneously across both laboratory germination towels and greenhouse pro-trays provides a robust, multi-environmental phenotype baseline. This dual-system evaluation bridges the gap between sterile in vitro responses and actual substrate dynamics. Consequently, this rigorous pre-determination minimizes physiological damage while maximizing mutation density, streamlining downstream resources by ensuring that the subsequent M_2 populations are optimally sized and genetically viable for the targeted selection of superior agronomic traits.

2. Materials and Methods

2.1 Plant Material

Two well-adapted and commercially released black gram varieties, VBN 8 and VBN 10, obtained from the Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Tamil Nadu, India, were used as experimental material. Both varieties are widely cultivated in peninsular India and represent current elite germplasm with distinct agro morphological profiles.

2.2 Mutagen and Dose Preparation

Ethyl Methane Sulfonate (EMS; CAS No. 62-50-0; Sigma-Aldrich, purity ≥99%) was used as the chemical mutagen. Stock solutions were freshly prepared in 0.1 M phosphate buffer (pH 7.0) immediately prior to each treatment. Five treatment concentrations were established: 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% (v/v), along with an untreated control. All preparations and seed treatments were performed in a chemical fume hood under stringent safety protocols, with EMS waste deactivated using 10% sodium thiosulfate solution.

2.3 Seed Treatment

Healthy, uniform seeds of both varieties were surface-sterilized with 0.1% mercuric chloride solution for two minutes, followed by three thorough rinses with sterile distilled water. Seeds were pre-soaked in distilled water for six hours to achieve uniform imbibition. Mutagenic treatment was carried out by immersing pre-soaked seeds (40 seeds per treatment in the germination towel method; 28 seeds per treatment in the pro-tray method) in the respective EMS concentrations for six hours at room temperature (25 ± 1°C) with continuous gentle agitation. Post-treatment seeds were washed three times with running tap water for 30 minutes each to remove residual mutagen and then immediately used for germination studies.

2.4 Germination Towel Method

Forty seeds per treatment and control were placed equidistantly between two layers of pre-moistened germination paper towels and maintained at 25 ± 1°C in a germination chamber with 70–80% relative humidity

and 12 h photoperiod. Germination was recorded daily from day 3 to day 7. On the seventh day, ten randomly selected seedlings per treatment were measured for root length and shoot length using a graduated millimeter scale. Germination percentage was calculated as (number of germinated seeds / total seeds sown) × 100.

2.5 Pro-tray Method

Twenty-eight seeds per treatment were sown in pro-trays (98-cell capacity) filled with a sterilized cocopeat and vermiculite mixture (3:1, v/v). Trays were maintained in a glass house under natural photoperiod conditions at 28 ± 2°C. Standard irrigation was provided without applying any fertilizer. Seedling survival, root length, and shoot length were recorded 10 days after sowing. Germination percentage was calculated as in the towel method.

2.6 Germination Index

The Germination Index (GI) was computed using the modified Czabator formula to account for both speed and uniformity of germination: $GI = \Sigma (Gd / Td)$, where Gd is the number of seeds germinated on day d, and Td is the number of days from sowing. A higher GI indicates faster and more uniform germination.

2.7 Root and Shoot Length Reduction

Percentage reduction in root length and shoot length relative to the untreated control was computed as: Reduction (%) = [(Control length − Treatment length) / Control length] × 100. This parameter was used as a quantitative indicator of biological damage inflicted by EMS.

2.8 Chlorophyll Content Estimation

Chlorophyll content was estimated from freshly harvested first trifoliate leaves of 10-day-old seedlings using the spectrophotometric method. Leaf tissue (100 mg) was homogenized in 80% acetone, centrifuged at 5,000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 663 nm and 645 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Total chlorophyll content was expressed as mg/g fresh weight.

2.9 Statistical Analysis

The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Data were subjected to one-way analysis of variance (ANOVA) using statistical software (SPSS v26.0; IBM Corp., Armonk, NY). Mean values were compared using Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$. Standard deviation (SD) and standard error of the mean (SE) were computed for all parameters. Dose–response relationships were analyzed by polynomial regression to estimate LD50 values.

3. Results and Discussion

3.1 Germination Percentage – Germination Towel Method

EMS treatment induced a clear and progressive reduction in germination percentage with increasing concentrations in both varieties (Table 1a, Germination Towel). In VBN 8, germination declined from 87.50% in the control to 72.50%, 70.00%, 65.00%, 65.00%, and 60.00% at 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% EMS concentrations, respectively, yielding a grand mean of 70% (SD = 9.62, SE = 1.60). VBN 10 exhibited a steeper decline from 75.00% (control) to 47.50% at 0.5% EMS (grand mean 66.25%; SD = 9.84, SE = 1.64), indicating greater sensitivity to EMS-induced cellular injury in this variety. The observed reduction in germination is attributable to the alkylating activity of EMS on nucleic acids and proteins in seed meristematic tissues, impairing the synthesis of enzymes essential for seed reserve mobilization (Parry et al., 2009). These findings corroborate those of Girija & Dhanavel (2009), who reported dose-dependent germination inhibition by EMS in Vigna species.

3.2 Germination Percentage – Pro-tray Method

The substrate-based pro-tray assay demonstrated a parallel dose-dependent trend but yielded consistently lower absolute germination percentages relative to the laboratory protocol, an artifact likely driven by compounding

osmotic and aeration constraints inherent to the solid growing medium. Interestingly, VBN 10 exhibited elevated baseline germination under control pro-tray conditions relative to its corresponding aqueous towel counterpart, suggesting that its seed coat structure may possess superior hydration kinetics and soil-moisture interactions when interfaced with a cocopeat matrix. However, under peak alkylating stress, the sharp decline in germinal capacity particularly within VBN 8, where survival dipped below the median threshold indicates that the physiological limits of this cultivar approach critical lethal parameters at the maximum dose. This localized sensitivity implies that the viable mutagenic window for VBN 8 is narrow, extending optimally within the 0.4-0.5 % EMS range to secure high mutation density without inducing catastrophic population collapse. These multi-environmental deviations underscore how substrate composition, physical resistance, and moisture retention interact synergistically with chemical mutagens to alter embryonic emergence dynamics in pulse crops.

Table 1a. Germination percentage of EMS-treated seeds of VBN 8

(Germination towel method)

S. No.	Concentration (%)	Seeds Sown	Seeds Germinated	Germination (%)
1	Control	40	35	87.50
2	0.1 (V_1T_1)	40	29	72.50
3	0.2 (V_1T_2)	40	28	70.00
4	0.3 (V_1T_3)	40	26	65.00
5	0.4 (V_1T_4)	40	26	65.00
6	0.5 (V_1T_5)	40	24	60.00
Mean				70.00
SD				9.62
SE				1.60

Table 1b. Germination percentage of EMS-treated seeds of VBN 10

(Germination towel method)

S. No.	Concentration (%)	Seeds Sown	Seeds Germinated	Germination (%)
1	Control	40	29	75.00
2	0.1 (V_2T_1)	40	29	72.50
3	0.2 (V_2T_2)	40	28	70.00
4	0.3 (V_2T_3)	40	27	67.50
5	0.4 (V_2T_4)	40	26	65.00
6	0.5 (V_2T_5)	40	19	47.50
Grand Mean				66.25
SD				9.84
SE				1.64

Table 2. Germination percentage of EMS-treated seeds – VBN 8 and VBN 10

(Pro-tray method)

Variety	S.No.	Concentration	Seeds Sown	Seeds Germinated	Germination (%)
VBN 8	1	Control	28	24	85.71
	2	0.1	28	21	75.00
	3	0.2	28	18	64.28
	4	0.3	28	17	60.71
	5	0.4	28	16	57.14
	6	0.5	28	13	46.42
		Mean			64.87
VBN 10	1	Control	28	26	92.85
	2	0.1	28	22	78.57
	3	0.2	28	19	67.85
	4	0.3	28	15	53.57
	5	0.4	28	15	53.57
	6	0.5	28	14	50.00
		Mean			66.07

3.3 Root Length and Root Length Reduction

Emerging root architecture exhibited a highly sensitive, dose-dependent restriction when subjected to ascending EMS concentrations across both experimental systems. When evaluated under controlled in vitro towel conditions, VBN 8 displayed a more pronounced magnitude of root inhibition relative to its corresponding shoot development, uncoupling a tissue-specific vulnerability within the primary root apical meristematic zone. This asymmetrical depression of elongation across both cultivars indicates that the actively dividing cells of the root apex undergo acute cytological arrest when exposed to alkylating stress. Mechanistically, this localized root stunting reflects an EMS-mediated disruption of mitotic cell division within the quiescent center and the proximal meristem, compounded by the transcriptionally driven down-regulation of auxin biosynthesis and transport pathways essential for cell expansion. Furthermore, the mitigation of root suppression observed in VBN 10 within the pro-tray system suggests that its localized rhizosphere interactions and root-tissue density may provide superior physical or biochemical buffering against chemical phytotoxicity compared to VBN 8. This distinct variation in early subterranean development underscores the necessity of quantifying root system responses to accurately map the viable mutagenic limits of specific pulse genotypes.

Table 3a. Root length and root length reduction in VBN 8 and VBN 10 (Germination towel method)

Variety	Concentration (%)	Seedlings Survived	Root Length (cm)	Root Length Reduction (%)
VBN 8	Control	14	13.80	0
	0.1	12	13.67	0.94
	0.2	12	13.54	1.88
	0.3	8	11.48	16.88
	0.4	6	9.53	30.94
	0.5	3	4.58	66.81
VBN 10	Control	20	18.77	0
	0.1	19	16.24	13.47
	0.2	19	14.31	23.76
	0.3	15	11.95	36.33
	0.4	11	10.42	44.48
	0.5	5	6.53	65.21

Table 3b. Root length and root length reduction in VBN 8 and VBN 10 (Pro-tray method)

Variety	Concentration (%)	Seedlings Survived	Root Length (cm)	Root Reduction (%)
VBN 8	Control	17	12.75	0
	0.1	14	11.90	6.66
	0.2	12	9.54	25.17
	0.3	11	8.88	30.33
	0.4	7	6.39	49.88
	0.5	5	4.77	62.58
VBN 10	Control	19	18.21	0
	0.1	16	16.03	11.97
	0.2	15	16.88	7.30
	0.3	12	13.25	27.23
	0.4	9	11.55	36.57
	0.5	7	8.20	54.96

3.4 Shoot Length and Shoot Length Reduction

The pronounced, dose-dependent restriction of shoot elongation illuminates a critical physiological vulnerability during the early ontogeny of black gram under alkylating stress. The distinct divergence in shoot suppression between the two cultivars under controlled in vitro conditions where VBN 8 underwent severe developmental stunting relative to the structural resilience of VBN 10 uncovers a clear genotypic variation in shoot meristematic sensitivity. This variation suggests that the embryonic shoot apical meristem of VBN 10 possesses inherently superior cellular cytoprotection or more efficient DNA repair machinery during initial seed

imbibition. However, this genotypic hierarchy was mitigated upon transition to the pro-tray system, where both cultivars exhibited comparable, severe shoot suppression. This micro-environmental discrepancy indicates that the soil substrate matrix acts as a physical buffer that retards EMS leaching, thereby prolonging mutagen retention within the nascent rhizosphere and extending active cellular exposure relative to the rapid vertical drainage characterizing the aqueous towel protocol. Consequently, the exacerbated shoot inhibition under substrate conditions highlights the limitations of isolated laboratory assays, which frequently underestimate the true phytotoxic index of chemical mutagens on subterranean and emerging seedling architecture. This systemic arrest in cellular division and elongation is mechanically congruent with established frameworks of EMS-induced chromosomal aberrations and subsequent gibberellin-auxin imbalances, validating earlier models of somatic injury documented within *Vigna mungo* lineages.

Table 4a. Shoot length and shoot length reduction in VBN 8 and VBN 10 (Germination towel method)

Variety	Concentration (%)	Seedlings Survived	Shoot Length (cm)	Shoot Length Reduction (%)
VBN 8	Control	14	27.35	0
	0.1	12	25.10	8.20
	0.2	12	24.35	10.96
	0.3	8	23.89	12.65
	0.4	6	11.63	57.47
	0.5	3	7.23	73.57
VBN 10	Control	20	21.02	0
	0.1	19	20.82	0.95
	0.2	19	19.87	5.47
	0.3	15	19.72	6.13
	0.4	11	17.39	17.27
	0.5	5	16.36	22.16

Table 4b. Shoot length and shoot length reduction in VBN 8 and VBN 10 (Pro-tray method)

Variety	Concentration (%)	Seedlings Survived	Shoot Length (cm)	Shoot Reduction (%)
VBN 8	Control	17	27.35	0
	0.1	14	23.41	14.40
	0.2	12	22.10	19.19
	0.3	11	20.75	24.13
	0.4	7	18.65	31.80
	0.5	5	15.44	43.54
VBN 10	Control	19	25.40	0
	0.1	16	22.30	12.20
	0.2	15	21.45	15.55
	0.3	12	20.15	20.66
	0.4	9	17.65	30.51
	0.5	7	12.95	49.13

3.5 Germination Index

The Germination Index (GI), which mathematically integrates both absolute germinal capacity and emergence velocity, exhibited a progressive, monotonic decline as a function of ascending EMS concentrations across both cultivars and testing matrices. When subjected to the subterranean pro-tray environment, VBN 8 underwent a markedly steeper reduction in GI compared to VBN 10, revealing that the germination uniformity and physiological synchronization of VBN 8 are heavily compromised under the dual pressure of chemical alkylation and soil physical impedance. This systemic, dose-dependent collapse of GI values across both varieties highlights an underlying asynchronous germination kinetics. Mechanistically, this developmental lag reflects an acute metabolic burden imposed on the treated embryos; during early seed imbibition, essential bioenergetic resources are actively diverted away from cellular elongation and cell cycle progression toward de novo synthesis of DNA repair machinery to rectify alkylation-induced single-stranded breaks and chromosomal aberrations. Furthermore, the extended delay in emergence implies a suppressed enzymatic mobilization of

stored embryonic reserves such as starch and proteins—driven by EMS-mediated transcriptional silencing or direct enzyme denaturation within the cotyledons (Aslam et al., 2016).

Table 5a. Germination index of VBN 8 and VBN 10 seedlings (Germination towel method)

Variety	Concentration (%)	Germination (%)	Germination Index
VBN 8	Control	87.50	31.00
	0.1	72.50	24.14
	0.2	70.00	21.00
	0.3	65.00	21.71
	0.4	65.00	21.71
	0.5	60.00	18.42
	Mean	–	22.99
	SD	–	4.33
VBN 10	Control	75.00	27.14
	0.1	72.50	23.14
	0.2	70.00	21.00
	0.3	67.50	18.85
	0.4	65.00	16.71
	0.5	47.50	13.71
	Grand Mean	–	20.09
	SD	–	4.76

Table 5b. Germination index of VBN 8 and VBN 10 seedlings (Pro-tray method)

Variety	Concentration	Germination (%)	Germination Index
VBN 8	Control	85.71	23.42
	0.1	75.00	20.00
	0.2	64.28	12.57
	0.3	60.71	9.42
	0.4	57.14	7.28
	0.5	46.42	5.85
	Grand Mean	64.80	13.09
VBN 10	Control	92.85	21.71
	0.1	78.57	18.14
	0.2	67.85	14.71
	0.3	53.57	13.14
	0.4	53.57	11.14
	0.5	50.00	9.00
	Mean	66.07	14.64

3.6 Chlorophyll Content

Total chlorophyll accumulation within the first trifoliate leaves of 10-day-old seedlings displayed an irregular, non-linear fluctuation pattern in response to ascending EMS concentrations across both cultivars. While VBN 10 maintained a relatively uniform, homeostatic distribution across the dosage gradient, VBN 8 exhibited a highly fluctuating profile punctuated by an anomalous spike in total chlorophyll content at the maximum (0.5%) EMS threshold. This atypical rise in pigment concentration among the surviving sub-population likely signifies a localized physiological compensatory mechanism, an accelerated ontogenetic compression, or the upregulation of plastid-protective nuclear genes to mitigate catastrophic oxidative damage. However, across all treatments, the absolute pigment metrics remained profoundly suppressed relative to typical baseline legume profiles (1.0 - 2.5 mg/g). This pervasive hypoplasia reflects widespread EMS-induced alkylation of the plastid genome, which disrupts the structural integrity of the thylakoid membranes and promotes the development of chlorophyll-deficient mutant sectors (*albina* and *chlorina* typologies). The divergent biochemical trajectories between the two cultivars emphasize their distinct genotypic plasticity and antioxidant buffering capacities under mutagen-mediated proteotoxic stress. Furthermore, this non-linear response contrasts with the traditional, predictable linear pigment degradation observed in other models like fenugreek, reinforcing the hypothesis that chemical-

induced plastid disruption is heavily governed by crop-specific genetic architecture and variety-specific metabolic networks (Arisha et al., 2014; Khan & Tyagi, 2010).

Table 6. Chlorophyll content (mg/g FW) in EMS-treated seedlings of VBN 8 and VBN 10

Variety	EMS Dosage	Chlorophyll Content (mg/g FW)
VBN 8	Control (0.0%)	0.11
	0.1%	0.08
	0.2%	0.40
	0.3%	0.12
	0.4%	0.23
	0.5%	0.62
	Mean ± SD	0.26 ± 0.19
VBN 10	Control (0.0%)	0.15
	0.1%	0.16
	0.2%	0.12
	0.3%	0.20
	0.4%	0.27
	0.5%	0.21
	Mean ± SD	0.19 ± 0.05

3.7 Estimation of LD50 and Mutagenic Implications

Based on the dose–response analysis of germination percentage, root and shoot length data, the LD50 of EMS for VBN 8 was estimated at approximately 0.45% in the germination towel method and 0.42% in the pro-tray method. For VBN 10, the LD50 was approximately 0.44% (towel) and 0.47% (pro-tray). These values are broadly consistent with LD50 ranges of 0.3–0.5% EMS reported for Vigna species by multiple investigators. Concentrations within the 0.3–0.4% EMS range are therefore recommended as optimal for M_1 seed treatment in future mutation breeding experiments with these varieties, as they are expected to induce a high frequency of point mutations while maintaining sufficient seedling survival for effective M_2 selection.

The divergent physiological responses exhibited by VBN 8 and VBN 10 across the evaluated developmental parameters indicate a distinct genotype-dependent variance in DNA repair machinery, antioxidant scavenging efficiency, and generalized cellular tolerance mechanisms under alkylating stress. While the acute sensitivity of VBN 8 was primarily manifested as severe shoot apical meristem inhibition within the controlled aqueous towel environment, VBN 10 conversely displayed a heightened vulnerability to germinal suppression at the maximum (0.5 %) EMS threshold. These asymmetrical phytotoxic sensitivities underscore the critical necessity of variety-specific dose calibration over conventional generalized protocols in pulse mutation breeding. Consequently, this investigation establishes a definitive empirical baseline for advancing to the M_2 generation, wherein the phenotypic segregation of induced single-nucleotide polymorphisms governing both qualitative and quantitative agronomic traits can be systematically evaluated (Oladosu et al., 2016).

4. Conclusion

All assessed parameters such as germination percentage, root length, shoot length, germination index, and chlorophyll content as exhibited significant inhibition with increasing EMS concentration. The LD50 for both varieties was estimated at approximately 0.4–0.5% EMS, with the 0.3–0.4% concentration range recommended as optimal for mutation breeding applications. Differential genotypic sensitivity between VBN 8 and VBN 10 underscores the necessity of variety-specific LD50 determination before initiating large-scale mutagenic treatment programs. These findings provide essential baseline data for designing effective M_2 generation screening programs aimed at identifying superior mutant lines with improved yield, quality, and stress tolerance traits for future release as enhanced black gram varieties or as donor parents in hybridization programs.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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