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Ascertaining Lethal Dose 50 (LD_{50}) and Simultaneous Effect of Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) On Seedling Characters in Mungbean Genotypes 'Pusa 1031' and 'Pusa 1431'

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ABSTRACT

Background: Finding effective dose is the most crucial step before commencing any mutagen treatment. The present study determined the lethal median dose in two mungbean genotypes, 'Pusa 1031' and 'Pusa 1431', using two different chemical mutagens namely Ethyl Methane Sulphonate and Sodium Azide.

Methods: A pair of mungbean genotypes "Pusa 1031" and "Pusa 1431" were subjected to varying concentrations of two chemical mutagens *i.e.*, Ethyl Methane Sulphonate (EMS) (10-100 mm with a ten mm different) and Sodium Azide (SA) (0.01 mm - 0.05 mm) followed by germinating the seeds in trays containing soil rite.

Result: EMS treatment in two genotypes displayed LD_{50} values as 58.81 mM and 45.04 mM for the genotypes 'Pusa 1031' and 'Pusa 1431', respectively. Lethal dose 50 was determined as 0.047 mM for both genotypes when treated with sodium azide. Seedling characters exhibited a linear response with dose augmentation for both the chemical mutagens, despite displaying similar LD_{50} values both genotypes exhibited remarkable differences in seedling parameters when treated with sodium azide.

Key words: Ethyl Methane Sulphonate, LD_{50}, Mungbean, Sodium azide.

INTRODUCTION

Mungbean, one of India's 13 dietary legumes, is the country's third-most significant pulse after chickpea and pigeonpea (Singh *et al*., 2015). Mungbean and other pulses have historically been farmed on the minimal-fertility ground with low productivity with limited input (Khan and Goyal, 2009). Genetic reconstitution is required for these crops to evolve distinct plant kinds due to genetic improvement for enhanced production (Siddique *et al*., 1999). Mutation breeding has emerged as one of the essential methods in mungbean for developing and disseminating novel genotypes and high-yielding cultivars (Pathirana, 2011). The fundamental advantage of utilizing induced mutations is the capacity to ameliorate a single or a small number of desirable traits in a crop without materially changing the remainder of its genetic makeup (Awan, 2005). Mutagenesis using Ethyl Methane Sulphonate (EMS) and sodium azide (SA) has shown to produce a diverse range of mutations in mungbean *viz*., Chlorophyll mutants (Khan and Siddiqui, 1993), Pod mutants and seed mutants (Wani *et al*., 2017), High and lower yielding mutants (Wani *et al*., 2011) and Pollen Fertility (Kulthe, 2019). Chemical mutagens typically exert their effects through single base pair and single nucleotide polymorphisms (Sikora *et al*., 2011). By adding an ethyl group to the guanine bases at the O-6 position, EMS encourages DNA polymerase to insert thymine mismatches with O-6-ethyl guanines, resulting in point mutations at arbitrary loci (Suprasanna *et al*., 2014). Sodium azide is a potent mutagen that can cause various gene or point mutations by uncharged HN_3 molecule found in acidic state enters the cells and increase their ability to cause mutations (Singh and Olejniczak, 1983).

The efficacy of the strategies employed will significantly influence the outcomes of mutation breeding programme. The fact that mutagens effects depend on their dose is well apparent. Establishing the appropriate dose is the most crucial step before beginning any mutagen treatment. In the lack of comprehensive data, it is occasionally required to calculate the LD_{50} through a small-scale test to determine the ideal dose in the M_1 population. However, the

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standardization of the lethal dose 50 estimates for the mungbean genotypes, *viz*., 'Pusa 1031' and Pusa 1431', has not been attempted in any prior studies. Because of this, the current study was conducted to determine the LD_{50} dosage of sodium azide and ethyl methane sulphonate for two mungbean genotypes and to evaluate how differently seedlings respond to various mutagen concentrations.

MATERIALS AND METHODS

Plant material

The genetically pure seeds of two mungbean genotypes, 'Pusa-1031' and 'Pusa-1431', used in this study were obtained from the ICAR-Indian Agricultural Research Institute (IARI), Pusa, New Delhi. Since these cultivars already have valuable genetic backgrounds, we chose them as the subject matter for this study and median lethal dose for two chemical mutagens namely ethyl methane sulphonate and sodium azide, which was not estimated prior to this.

Experimental site

The experiment was carried out during January 2023, in Plant Breeding Laboratory, College of Post Graduate Studies in Agricultural Sciences, CAU (I), Umiam, Meghalaya.

Ethyl methane sulphonate (EMS) treatment

One of the chemical mutagens employed in this experiment was Ethyl Methane Sulphonate (EMS). The investigation was carried out in a lab environment with three replications. To activate the seeds metabolism and boost the mutagen's effectiveness, 50 uniform dry seeds of each genotype per replication were pre-soaked in distilled water for 3 hours. The seeds were then exposed to ten concentrations of EMS (10 mM to 100 mM with a ten mM difference) in 0.1 M phosphate buffer at pH 7.0 for six hours while being gently shaken occasionally. The seeds were then rinsed under running water thrice for 30 minutes to eliminate any remaining mutagens. Seeds for the control were submerged in distilled water for the duration of the treatment.

Sodium azide (SA) treatment

This experiment used sodium azide (SA) as another chemical mutagen studied in a controlled laboratory environment with three replications. Fifty uniform dry seeds of each genotype per replication were pre-soaked in distilled water for 3 hours to make the seeds metabolically active and increase the efficacy of mutagens. The seeds were then treated with different concentrations of SA (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM and 0.05 mM) prepared in 0.1 M phosphate buffer at pH 3.0 with occasional gentle shaking for six hours. Afterward, seeds were washed under running water thrice for 30 minutes to remove residual mutagen. Seeds for control were soaked in distilled water for the total treatment time without SA treatment.

Treated seeds were germinated in trays and kept at room temperature. Following the International Seed Testing Association (ISTA) rules, observations on numerous seedling parameters were recorded. The methodology of the work is represented in (Fig 1). For the evaluation of LD_{50}, data were subjected to probit analysis and various parameters were determined using a mean of three replicates using the OPSTAT Statistical Software Package for Agricultural Research Workers (Sheoran *et al.,* 1998).

RESULTS AND DISCUSSION

What is the significance of finding the LD_{50} values for different mutagens in different genotypes of plants? Lethal Dose 50 (LD_{50}) is the dose that causes a fifty per cent reduction in viable plants or seeds (Gad, 2014). The ideal mutagen dose is one of the critical factors in the success of mungbean mutation breeding programme. Maximum mutation and minimal fatality are produced by a mutagen at its optimal dose (Kodym *et al*., 2012). Many scientists believe the ideal dose should resemble the Lethal Dose 50 (LD_{50}). Based on seed germination rates of treated seeds at various mutagen concentrations compared to untreated controls, LD_{50} values were determined. Median lethal dose for the mutagen Ethyl Methane Sulphonate (EMS), of two mungbean genotypes namely 'Pusa 1031' and 'Pusa 1431' was determined as 58.81 mM and 45.04 mM, respectively (Fig 2). The finding mentioned above is consistent with past research in different pulse crops conducted by various authors, *viz*., chickpea (Khan and Kozgar, 2011), cowpea (Nair and Gayatri, 2022), greengram (Jeevi, 2020; Vairam, 2014; Singh and Kole, 2005) and blackgram (Jain and Khandelwal, 2009). For the mutagen sodium azide, the LD_{50} was determined to be 0.047 mM for both genotypes (Fig 3). The above observation was confirmed by a previous study in chickpea by Khan and Kozgar, (2011) and linseed by Jahan *et al.* (2021). For EMS, "Pusa 1031" displayed a higher LD_{50} value; however, both genotypes displayed comparable LD_{50} values for the mutagen sodium azide. For the mutagen ethyl methane sulphonate, the differences in LD_{50} values between cultivars show that median lethal dose values vary from genotype to genotype, which variations in the genetic background and pedigree of the cultivars may bring about. Comparable LD_{50} values were obtained with sodium azide treatment for both genotypes, indicating that this may be the optimal LD_{50} dose for both genotypes.

Effect of ethyl methane sulphonate and sodium azide on germination of two mungbean genotypes

The proportion of seeds that germinated following treatment compared to the control for two mungbean genotypes was used to calculate seed germination. Ethyl Methane Sulphonate substantially reduced the germination percentage for both genotypes. The germination percentage at the control and 100 mM, when treated with EMS, was observed as 89.66% and 23.07% in 'Pusa 1031' and 89.83% and 15.78% in 'Pusa 1431' respectively (Table 1). The per cent reduction of germination over control at 100 mM EMS was noted as 74.26% and 82.42% for the genotypes' Pusa 1031' and 'Pusa 1431' respectively (Table 1). With a corresponding rise in EMS concentration,

both the genotypes displayed linear trends for germination percentage. Observing the percent reduction in germination of treated seed over control for the mutagen EMS, which has noticeably distinct values for both genotypes at various mutagen doses. Different genotype susceptibility to mutagen EMS or various innate seed germination abilities could cause this difference. For EMS, the maximum germination was recorded at a concentration of 10 mM, with "Pusa 1031" (83%) leading the way, followed by "Pusa 1431" (78.04%) (Table 1).

When the genotypes were treated with Sodium Azide, germination percentage values at the control and 0.05 mM SA were recorded as 89.66% and 34.61% in 'Pusa 1031' and 89.83% and 40% in 'Pusa 1431' respectively (Table 1). The percentage reduction of germination over control at 0.05 mM SA was observed as 61.39 % and 54.61% for the genotypes 'Pusa 1031' and 'Pusa 1431' respectively (Table 1), both the genotypes revealed linear trend with a matching increase in SA concentration. The percentage drop of treated versus control for the mutagen Sodium azide has shown significantly different results for the two mungbean genotypes. At concentrations of 0.01 mM and 0.02 mM, a significant germination percentage drop over control, *i.e.*, 6.39 to 25.28%, in the genotype Pusa 1431 (Table 1), demonstrating that mutagen activity at these concentrations is inhibiting germination. For the mutagen sodium azide, the highest germination was observed at a concentration of 0.01 mM, with 'Pusa 1031' (85%) leading the way followed by 'Pusa 1431' (82%) (Table 1).

Declining trends in germination percentage with a commensurate increase in mutagen dosage were reported by Lavanya *et al.* (2023), Wani *et al.* (2021), Jyothsna *et al.* (2022), Omosun *et al.* (2022), Chaudhary *et al.* (2021), Khan and Kozgar (2011), Shahwar *et al.* (2019), Nair and Gayathri (2022), Singh and Kole (2005). In seeds treated with EMS and SA, a five and four-days delayed germination was noticed in both genotypes. A similar pattern of post-chemical mutagen delayed germination was observed by Taziun *et al.* (2017) and Nair and Gayathri (2022). According to Kurobane *et al.* (1979), mutations impair the enzyme's ability to function, which affects germination. The production of germination-related enzymes may be impacted by mutation. At specific dosages, different genotypes responded to seed germination differently from one another. The physiological effects of EMS and SA, which impede the metabolic activities required for germination and have a more noticeable effect at larger dosages, may explain why germination decreases with increasing mutagen dose. 'Pusa 1431', which illustrated the most considerable per cent reduction in germination over the control, is more affected than Pusa 1031, on the whole.

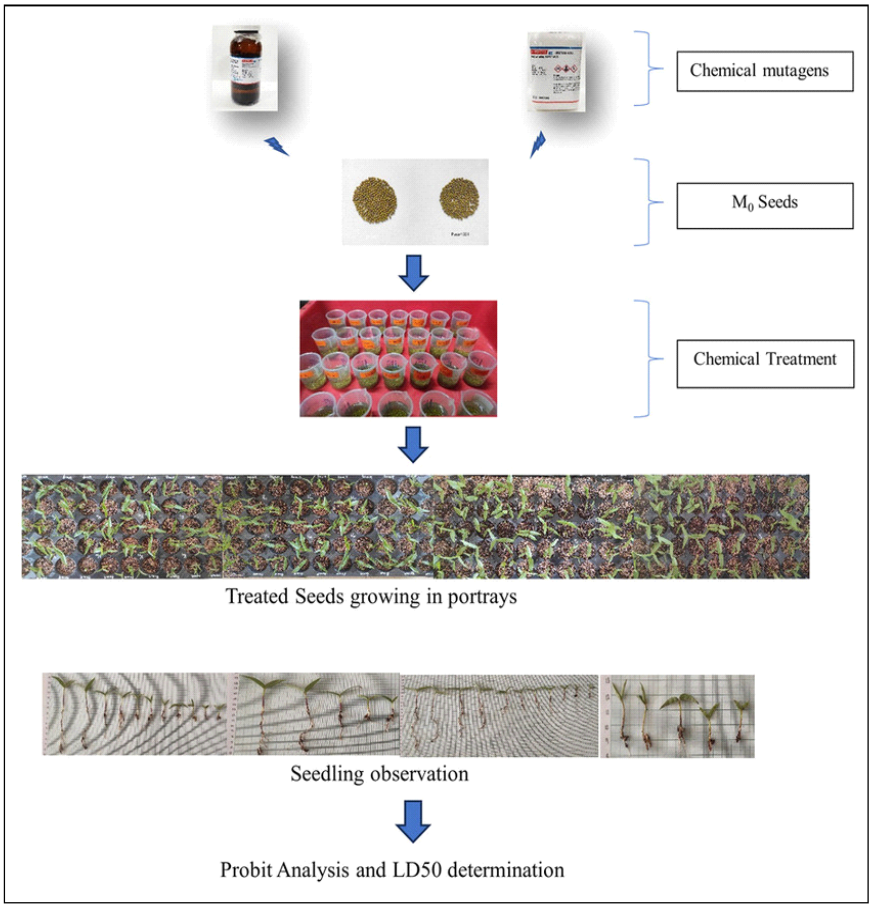

Fig 1: Flow chart demonstrating chemical mutagenesis procedure.

The above finding has been confirmed by Pusa 1431 possessing a lower LD_{50} for the EMS than 'Pusa 1031'. Maximum germination reduction was seen at mutagen doses above LD_{50} values for each genotype, indicating that LD_{50} is the most effective dose for inducing viable mutations and any value above LD_{50} results in plants with the most detrimental effects, which makes them challenging to advance for further generations.

Effect of ethyl methane sulphonate and sodium azide on seedling shoot length, root length, total seedling length and seedling vigour index of two mungbean genotypes

In the current study, the severity of these defects increased as the concentration of chemical mutagens increased. For both genotypes, 0.01 mM and 0.05 mM of Sodium Azide concentration and 10 mM and 100 mM of Ethyl Methane

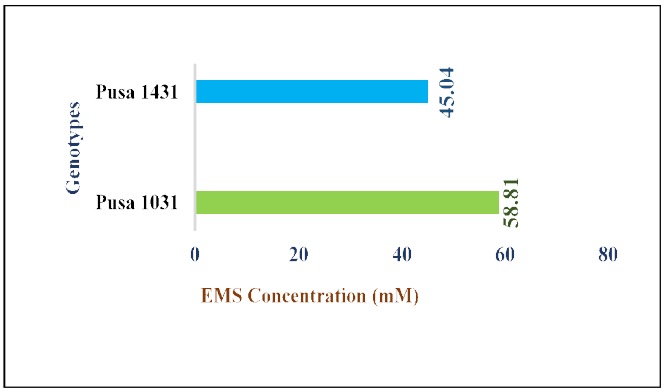

Fig 2: LD_{50} variation among mungbean genotypes for ethyl methane sulphonate.

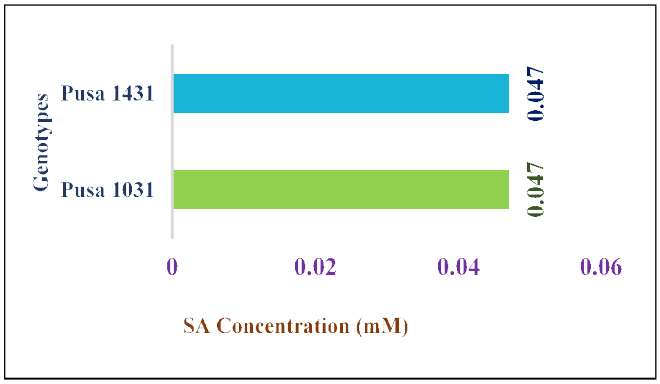

Fig 3: LD_{50} variation among mungbean genotypes for Sodium Azide.

Table 1: The effect of varying concentrations of ethyl methane sulphonate (EMS) and sodium azide (SA) on the germination percentage of two mungbean genotypes.

	Genotype			Genotype	
EMS conc. (mM)	Pusa 1031	Pusa 1431	SA conc. (mM)	Pusa 1031	Pusa 1431
Control	89.66	89.83	Control	89.66	89.83
10 mM	83.33 (7.05)	78.04 (11.44)			
20 mM	76 (15.23)	69.23 (21.44)	0.01 mM	85.71 (4.39)	82.5 (6.39)
30 mM	70.83 (20.99)	55.55 (36.96)			
40 mM	60 (33.07)	45.45 (48.42)	0.02 mM	80.76 (9.91)	65.85 (25.28)
50 mM	57.14 (36.26)	40 (54.61)			
60 mM	45.83 (48.87)	36.17 (58.96)	0.03 mM	73.07 (18.49)	60 (31.92)
70 mM	37.5 (58.17)	31.81 (63.89)			
80 mM	33.33 (62.82)	25.58 (70.97)	0.04 mM	52.17 (41.80)	54.05 (38.66)
90 mM	28 (68.76)	25 (71.63)			
100 mM	23.07 (74.26)	15.78 (82.08)	0.05 mM	34.61 (61.39)	40 (54.61)

Values for the percentage of germination reduction over control are shown in parenthesis.
EMS: Ethyl methane sulphonate, SA: Sodium azide.

Sulphonate concentration have been determined to be the highest and lowest levels for seedling shoot length, seedling root length and total seedling length, respectively (Table 2 and Table 3). Higher mutagenic concentrations of both chemical mutagens induced a drastic reduction in total seedling length, which ranged from 14.87 cm to 2.77 cm in 'Pusa 1031' and 8.17 cm to 3.11 cm in 'Pusa 1431' for EMS (Table 2) and for SA-treated seedlings, it was recorded as 11.20 cm to 5.10 in 'Pusa 1031' and 8.17 cm to 4.50 in "Pusa 1431", respectively (Table 3). These results are consistent with those of Nilahayati *et al.* (2023), Omosun *et al*., (2022), Jyothsna *et al*. (2022) and Chaudhary *et al*. (2021). Seedling root length and shoot length was observed to be decreasing trend as the concentration of both the chemical mutagens increased (Table 2), similar trend was observed by Ravi *et al*. (2023). Genotype 'Pusa 1431' has shown similar root length at 0.01mM and 0.02 mM sodium azide concentration (Table 3).

The seed vigor index was calculated based on the formula proposed by Abdul Baki and Anderson (1973), *viz*.

Seed vigor index =

Seed germination (%) × Seedling total length

With higher mutagen doses, the seedling vigor index showed a sharp decline for both chemical mutagens and it ranged from 1238.89 to 63.85 in 'Pusa 1031' and 637.40 to 48.95 in 'Pusa 1431' for EMS treatment (Table 4). However, for SA treatment, the seed vigor index ranged from 960.00 to 176.54 in "Pusa 1031" and 673.75 to 180.00 in 'Pusa 1431' (Table 4). While the higher concentrations of SA, *i.e*., 0.04 mM to 0.05 mM, genotype 'Pusa 1431' displayed a greater seed vigor index compared to 'Pusa 1031' (Table 4).

Sodium azide and ethyl methane sulphonate treatments had a detrimental effect on various seedling characteristics because they hindered physiological and enzymatic processes. In the current study, the severity of these defects increased as the concentration of chemical mutagens increased. Similar declining trends for various seedling characters were observed by Ravi *et al*. (2023), Omosun *et al*. (2022), Jyothsna *et al*. (2022) and Chaudhary *et al*. (2021).

Table 2: Effect of varying concentrations of ethyl methane sulphonate (EMS) on shoot length, root length and total seedling length of two mungbean genotypes.

EMS conc. (mM)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)	
	Genotype					
	Pusa 1031	Pusa 1431	Pusa 1031	Pusa 1431	Pusa 1031	Pusa 1431
Control	6.76±0.12	5.33±0.17	15.06±0.58	6.3±0.35	21.83	11.63
10 mM	5.2±0.2	4.43±0.12	9.66±0.33	3.73±0.12	14.87	8.17
20 mM	4.5±0.15	3.96±0.08	8.73±0.12	3.2±0.05	13.23	7.17
30 mM	3.93±0.21	3.63±0.08	8.3±0.36	3.06±0.06	12.23	6.70
40 mM	3.43±0.12	3.13±0.08	4.63±0.03	3.03±0.03	8.07	6.17
50 mM	3.46±0.13	2.96±0.03	3.36±0.40	2.9±0.05	6.83	5.87
60 mM	3.13±0.06	2.6±0.05	3.56±0.39	2±0.05	6.70	4.60
70 mM	3.03±0.08	2.43±0.08	3.1±0.2	1.9±0.1	6.13	4.33
80 mM	2.76±0.08	2.2±0.05	2.53±0.13	1.76±0.03	5.30	3.97
90 mM	2.33±0.03	1.96±0.03	1.66±0.03	1.56±0.17	4.00	3.53
100 mM	1.9±0.05	1.9 ±0.05	0.86±0.08	1.2±0.11	2.77	3.10

EMS: Ethyl methane sulphonate, conc.: Concentration.

Table 3: Effect of varying concentrations of sodium azide (SA) on shoot length, root length and total seedling length of two mungbean genotypes.

SA conc. (mM)	Shoot length (cm)		Root length (cm)		Total seedling length (cm)	
	Genotype					
	Pusa 1031	Pusa 1431	Pusa 1031	Pusa 1431	Pusa 1031	Pusa 1431
Control	6.76±0.12	5.33±0.17	15.06±0.58	6.3±0.35	21.83	11.63
0.01	4.03±0.03	3.9±0.15	7.16±0.60	4.26±0.31	11.20	8.17
0.02	3.33±0.08	3.5±0.32	4.33±0.41	4.26±0.29	7.67	7.77
0.03	3.13±0.06	3.2±0.09	3.1±0.37	2.83±0.14	6.23	6.03
0.04	2.83±0.08	2.93±0.13	2.53±0.16	2.96±0.16	5.37	5.90
0.05	2.6±0.05	2.46±0.14	2.5±0.05	2.03±0.47	5.10	4.50

SA: Sodium Azide, conc.: Concentration.

Table 4: Effect of different concentrations of ethyl methane sulphonate (EMS) and Sodium Azide (SA) on seed vigor index of two mungbean genotypes.

EMS conc. (mM)	Seedling vigor index				
	Genotype			Genotype	
	Pusa 1031	Pusa 1431	SA conc. (mM)	Pusa 1031	Pusa 1431
Control	1957.17	1025.31	Control	1957.47	1025.31
10 mM	1238.89	637.40			
20 mM	1005.73	496.15	0.01 mM	960.00	673.75
30 mM	866.53	372.22			
40 mM	484.00	280.30	0.02 mM	619.23	511.46
50 mM	390.48	234.67			
60 mM	307.08	166.38	0.03 mM	455.51	362.00
70 mM	230.00	137.88			
80 mM	176.67	101.47	0.04 mM	280.00	318.92
90 mM	112.00	88.33			
100 mM	63.85	48.95	0.05 mM	176.54	180.00

EMS: Ethyl methane sulphonate, SA: Sodium azide.

CONCLUSION

The degree of reaction of a particular genotype to a certain mutagen differs from that of other genotype because each genotype has a distinct genetic makeup and relevant enzymatic and physiological responses. The statement is supported by the results of the present investigation, in which two different mungbean genotypes showed different LD_{50} values for the chemical mutagen-Ethyl Methane Sulphonate. Despite having obtained similar LD_{50} values for both genotypes when treated with sodium azide, both genotypes showed remarkable differences in seedling parameters like seedling vigor, which depicted genotype “Pusa 1431” having shown higher seedling vigor compared to “Pusa 1031” at higher concentrations when treated with sodium azide, which is indicative of having more significant potential for better seedling growth and survival at higher doses. In order to establish the best mutagenic doses, the study assessed the LD_{50} for sodium azide and ethyl methane sulphonate on two mungbean genotypes. For “Pusa 1031” and “Pusa 1431,” the LD_{50} for ethyl methane sulphonate was determined to be 58.81 mM and 45.04 mM, respectively. When treated with sodium azide, the LD_{50} values for both genotypes was 0.047 mM. With varying dosages of SA and EMS, several seedling parameters responded differently in both genotypes. The results showed that sodium azide at lower concentrations is more effective than EMS for obtaining the median lethal dose in both genotypes. Conducting the required field testing is also encouraged to confirm the results and retrieve beneficial mutants with higher crop value in subsequent generations.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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