

# Comparative mutagenic analysis in $M_1$ generation of mungbean (*Vigna radiata* (L.) Wilczek)

Mohammad Rafiq Wani\*

Department of Botany, Government Degree College Tral- 192123, Pulwama, Jammu and Kashmir, India

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## Abstract

Mutation breeding is a robust technique used to enhance the genetic diversity of crops. It plays a crucial role in creating desirable varieties by boosting the crop productivity and improving the crop resilience against diseases. Under this backdrop, three chemical mutagens, namely EMS, HZ, and SA were employed in the present study to examine the biological damage and genetic diversity for quantitative traits incurred by the mutagens in  $M_1$  generation of mungbean (*Vigna radiata* (L.) Wilczek) varieties PDM-11 and NM-1. The biological damage induced in  $M_1$  generation was assessed by examining the immediate effects of mutagens on seed germination and pollen fertility. Both varieties experienced a dose-proportional decrease in seed germination and pollen fertility as the concentration of the mutagens increased. Variety NM-1 exhibited a higher susceptibility as compared to var. PDM-11 in inducing biological damage (inhibition in seed germination and pollen fertility). Various quantitative traits were also scrutinized to measure the impact of induced variability in  $M_1$  generation. The treated population saw mostly unchanged means for all the quantitative traits; however, the variability coefficients were different for each trait, and the fertile branches per plant had the highest variability over the controls. Treatments that result in significant variation in quantitative traits holds potential for improving the mungbean crop.

**Keywords:** Biological damage, Chemical mutagens, Mungbean, Quantitative traits.

## Introduction

India holds the label of being the top producer of pulses globally growing a vast types of pulse crops under diverse agro-climatic settings. Pulses are an essential part of sustainable agriculture, especially in dryland areas, because of their unique qualities, which include high protein content, the ability to fix nitrogen (Stevenson and Van Kessel, 1996), soil-improving qualities, and the capacity to flourish under harsh climatic conditions. Although cultivated primarily in Asia, Africa, and South America, mungbeans are relished by people worldwide. Like many legumes, mungbeans contain a substantial number of plant-based proteins, complex carbohydrates, fibres, and other nutrients. Mungbeans, despite their mild flavour, are a great

base for various flavourful recipes, such as soups, stews, salads, and curries. Several vegans utilize mungbeans to prepare egg-free scrambles and omelettes. During germination, the sprouted mungbean seeds produce ascorbic acid (vitamin-C) and exhibit higher amounts of riboflavin and thiamine.

The majority of pulses, including mungbean, have experienced a significant reduction in genetic diversity due to the influence of natural selection at low management levels. Genetic diversity is a fundamental necessity for enhancing crop plants. However, the scope for selecting better genotypes in crop plants is restricted by the lack of required genetic variability. Mungbean- being an autogamous crop, demands a boost in the existing genetic variability to

\*Author for Correspondences: Phone: +91 9906517207, Email: botanyrafiq@gmail.com

achieve the desired enhancement in the crop. Due to insufficient natural variability, traditional techniques of plant breeding hold limited scope to improve the crop. Inducing mutations artificially is the most efficient method to enhance genetic diversity within a short time frame (Patil et al., 2003; Wani et al., 2021; Goyal et al., 2021a; Raina et al., 2022a). It has been well-documented in numerous plant species that the effects caused by mutagens vary depending upon the specific mutagen and the dosage administered. Therefore, choosing a mutagen and determining the ideal dosage based on the genotype of individual plant species is a crucial aspect of mutation breeding initiatives. An effort has been made to assess the level of biological damage caused by the mutagens and to analyse their effects on different quantitative traits subsequent to mutagenesis with EMS, HZ, and SA in M<sub>1</sub> generation, considering the importance of mungbean in both economic and nutritional fronts.

## Materials and Methods

Mungbean (*Vigna radiata* (L.) Wilczek) seeds of varieties PDM-11 and NM-1 were immersed in distilled water for 9 hours prior to being exposed to 0.1-0.4% of EMS and 0.01-0.04% of HZ and SA for 6 hours. The untreated seeds, which had been immersed solely in distilled water for a period of 15 hours were employed as the experimental controls. The solutions of EMS and HZ were formulated using a pH-7 phosphate buffer, whereas the SA solution was formulated in a phosphate buffer with pH set to 3. All mutagenic treatments were conducted using exclusively freshly prepared solutions. The solution and seeds in the flasks were regularly shaken to maintain proper aeration during the treatment process. To eradicate the residual mutagen from seed coat, all seeds that underwent mutagen treatment were thoroughly rinsed using a continuous flow of tap water.

The M<sub>1</sub> generation was raised by sowing 300 seeds in RCBD for both treatment and control groups in the field. The distance separating each seed within a row was maintained at 30 centimetres, while the

distance between the rows was set at 60 centimetres. The mungbean population was cultivated in the field after implementing the recommended agronomic practices, which were diligently adhered to throughout the field preparation, sowing, and subsequent maintenance. The seed germination percentage was determined using the remaining batch of 50 seeds. The seeds from both varieties were evenly distributed on moist cotton in petri-plates for each treatment and control group. Subsequently, the petri-plates were positioned in BOD incubator adjusted to a temperature of 27±1°C. The seed germination percentage was determined by counting the number of seeds sown in the petri-dishes and the number of seeds that sprouted successfully.

Pollen fertility was assessed by examining 30 plants chosen at random from each treatment and the controls during the flowering period. In order to ascertain fertility, pollen grains were subjected to 1% aceto-carmin solution. Pollen grains that displayed staining and maintained a uniform outline were designated as fertile, whereas withered, empty, and colourless pollen grains were identified as sterile. For all the nine quantitative traits, data was collected from twenty-five to thirty healthy plants from every treatment, besides controls. Mean ( $\bar{x}$ ), standard error ( $\pm$  SE), and coefficient of variation (CV%) were calculated using the standard statistical formulas.

## Results and Discussion

Table 1 displays the data on seed germination in M<sub>1</sub> generation. In both mungbean varieties, an incremental decline in seed germination was noted as the concentrations of the mutagens increased. The reduction in seed germination varied to different degrees among the two varieties. Var. NM-1 suffered a greater impact on seed germination than the var. PDM-11. The greatest decrease in seed germination was observed with sodium azide treatments. In var. NM-1, the seed germination inhibition percentage was recorded as 13.26 and 23.46 when exposed to SA concentrations of 0.01%

*Table 1.* Effects of mutagens on seed germination and pollen fertility in M<sub>1</sub> generation of mungbean varieties PDM-11 and NM-1

Treatment	Var. PDM-11				Var. NM-1			
	Seed germination (%)	(%) inhibition in seed germination	Pollen fertility (%)	(%) reduction in pollen fertility	Seed germination (%)	(%) inhibition in seed germination	Pollen fertility (%)	(%) reduction in pollen fertility
Control	98.00	-	96.00	-	98.00	-	96.00	-
0.1% EMS	94.00	4.08	75.00	21.87	92.00	6.12	73.91	23.01
0.2% EMS	91.00	7.14	66.66	30.56	90.00	8.16	65.21	32.07
0.3% EMS	89.00	9.18	62.50	34.89	87.00	11.22	60.86	36.60
0.4% EMS	87.00	11.22	58.33	39.23	85.00	13.26	56.52	41.12
0.01% HZ	91.00	7.14	83.33	13.19	89.00	9.18	82.60	13.95
0.02% HZ	88.00	10.20	75.00	21.87	87.00	11.22	73.91	23.01
0.03% HZ	86.00	12.24	70.83	26.21	84.00	14.28	69.56	27.54
0.04% HZ	82.00	16.32	66.66	30.56	81.00	17.34	65.21	32.07
0.01% SA	88.00	10.20	91.66	4.52	85.00	13.26	91.30	4.89
0.02% SA	85.00	13.26	87.50	8.85	83.00	15.30	86.95	9.42
0.03% SA	81.00	17.34	83.33	13.19	79.00	19.38	82.60	13.95
0.04% SA	77.00	21.42	79.16	17.54	75.00	23.46	78.26	18.47

and 0.04%, respectively. Similarly, in the var. PDM-11, the inhibition percentages were observed as 10.20 and 21.42 with the same mutagenic concentrations (Table 1).

The mutagenic sensitivity of the biological system is commonly assessed by measuring the percentages of inhibition in seed germination and pollen fertility in M<sub>1</sub> generation. In both mungbean varieties, treatments with EMS, HZ, and SA were found to cause a dose-dependent decrease in seed germination. Various factors such as enzyme activity, hormonal imbalances, damage to cell constituents, disruption of seed layers, and inhibition of mitotic processes have been identified as potential causes for decline in seed germination after exposure to mutagenic treatments (Chrispeels and Varner, 1967; Ananthaswamy et al., 1971; Kurobane et al., 1979; Talebi and Shahrokhifar, 2012; Kumar et al., 2013).

The rise in EMS, HZ, and SA concentrations resulted in a decline in pollen fertility, which was observed in both mungbean varieties (Table 1). The var. PDM-11 exhibited the highest decrease in pollen fertility with percentages of 39.23 at 0.4% EMS and 30.56 at 0.04% of HZ. On the other hand, the var. NM-1 exhibited the highest decrease in pollen fertility percentages of 41.12 and 32.07 when

exposed to the highest concentration of EMS and HZ. SA treatments resulted in reduced levels of pollen sterility in both varieties in comparison to EMS and HZ treatments.

Despite a 4% occurrence of pollen sterility in the control plants, the sterility remained prominent in the mutagenic treatments and exhibited a relationship that depended on the dosage. Previous studies have also reported a similar dose-dependent relationship between mutagen dosage and pollen fertility in different crop plants (Mitra and Bhowmik, 1999; Muthusamy and Jayabalan, 2002; Kumar and Singh, 2003; Wani et al., 2004; Barshile et al., 2006; Khan and Wani, 2006; Sharma et al., 2006; Wani, 2021). Comparatively, EMS treatments resulted in higher pollen sterility than HZ and SA treatments in both varieties.

Tables 2-5 display the data collected on nine quantitative traits in M<sub>1</sub> generation. The examination of the mutagenic treatments indicated that there were no notable variances in the mean values of the majority of the quantitative traits, as depicted in Tables 3 and 5. Nevertheless, in contrast to the controls, there was a noticeable rise in the coefficient of variation values for all the characters. The greatest increase in coefficient of variation over the controls

**Table 2.** Estimates of mean values ( $\bar{x}$ ) and coefficient of variation (CV%) for various quantitative traits in M<sub>1</sub> generation of mungbean var. PDM-11

Treatment	Days to flowering	Plant height (cm)	Days to maturity	Fertile branches/plant	Pods/plant	Pod length (cm)	Seeds/pod	100-seed weight (g)	Total plant yield (g)
Control	39.10±0.25 (2.51)	43.05±0.19 (1.79)	70.07±0.22 (1.25)	6.45±0.12 (6.97)	47.13±0.22 (2.63)	6.15±0.04 (2.60)	8.85±0.12 (5.42)	3.67±0.03 (3.13)	9.30±0.10 (4.18)
0.1% EMS	39.34±0.65 (6.35)	43.13±0.83 (7.45)	70.37±0.85 (4.76)	7.05±0.45 (25.10)	48.20±1.20 (9.67)	6.72±0.08 (4.61)	9.45±0.28 (11.53)	3.96±0.04 (3.91)	10.17±0.38 (14.45)
0.2% EMS	38.76±0.67 (6.65)	42.88±0.76 (6.86)	69.80±0.88 (4.94)	7.18±0.39 (21.45)	48.60±1.16 (9.24)	6.57±0.08 (4.72)	9.39±0.25 (10.54)	3.91±0.06 (5.94)	10.30±0.36 (13.51)
0.3% EMS	38.10±0.57 (5.78)	42.10±0.71 (6.53)	68.90±0.84 (4.70)	6.72±0.40 (23.21)	48.10±0.91 (7.36)	5.90±0.07 (4.57)	9.32±0.24 (10.08)	3.88±0.06 (5.98)	9.90±0.31 (12.14)
0.4% EMS	37.90±0.67 (6.83)	41.76±0.82 (7.60)	68.84±0.91 (5.13)	6.37±0.42 (25.43)	46.90±0.93 (7.72)	6.31±0.08 (4.91)	8.26±0.23 (10.65)	3.52±0.05 (5.48)	9.15±0.23 (9.70)
0.01% HZ	39.70±0.98 (9.57)	42.98±0.86 (7.75)	70.73±0.96 (5.24)	6.98±0.37 (20.91)	48.46±0.99 (7.94)	6.48±0.07 (4.17)	9.39±0.23 (9.69)	3.95±0.04 (3.92)	10.14±0.29 (11.14)
0.02% HZ	38.37±0.87 (8.83)	42.68±0.82 (7.44)	70.14±0.97 (5.39)	6.92±0.33 (18.78)	48.24±1.12 (8.99)	6.32±0.06 (3.48)	9.25±0.20 (8.64)	3.87±0.05 (5.01)	10.19±0.25 (9.46)
0.03% HZ	38.17±0.62 (6.27)	41.86±0.84 (7.77)	69.13±0.88 (4.94)	6.66±0.33 (18.92)	47.90±0.79 (6.43)	6.28±0.07 (4.29)	8.53±0.21 (9.26)	3.55±0.04 (4.37)	10.07±0.24 (9.23)
0.04% HZ	38.03±0.65 (6.63)	41.60±0.80 (7.45)	68.94±0.91 (5.13)	5.72±0.27 (18.35)	45.90±0.86 (7.25)	5.97±0.06 (3.69)	8.39±0.19 (8.82)	3.62±0.05 (5.33)	8.78±0.19 (8.46)
0.01% SA	39.37±0.72 (7.21)	42.58±0.88 (8.01)	70.40±0.82 (4.54)	5.80±0.35 (23.10)	46.10±0.88 (7.37)	6.36±0.05 (3.01)	9.19±0.21 (8.92)	3.90±0.04 (3.97)	9.05±0.20 (8.46)
0.02% SA	38.63±0.54 (5.38)	42.38±0.92 (8.42)	69.53±0.80 (4.47)	6.79±0.31 (17.96)	48.06±0.86 (6.97)	6.22±0.05 (3.09)	9.12±0.16 (7.02)	3.82±0.04 (4.02)	9.97±0.18 (7.02)
0.03% SA	38.50±0.52 (5.29)	41.83±0.81 (7.50)	70.46±0.58 (3.18)	6.58±0.25 (14.58)	47.50±0.89 (7.28)	6.06±0.04 (2.69)	9.05±0.17 (7.18)	3.58±0.02 (2.26)	9.20±0.16 (6.68)
0.04% SA	38.30±0.54 (5.46)	41.77±0.85 (7.88)	69.34±0.62 (3.47)	5.86±0.19 (12.28)	46.01±0.70 (5.91)	6.03±0.05 (3.15)	8.59±0.15 (6.86)	3.78±0.03 (3.12)	8.85±0.14 (6.21)

±Standard error; Values in parenthesis represent CV (%).

**Table 3:** Comparative effects of mutagens on mean and coefficient of variation for various quantitative traits in M<sub>1</sub> generation of mungbean var. PDM-11

Trait	Control		EMS		HZ		SA	
	Mean	CV (%)	Mean*	CV (%)**	Mean	CV (%)	Mean	CV (%)
Days to flowering	39.10	2.51	38.52	6.40	38.57	7.82	38.70	5.83
Plant height (cm)	43.05	1.79	42.47	7.11	42.28	7.60	42.14	7.95
Days to maturity	70.07	1.25	69.48	4.88	69.73	5.17	69.93	3.91
Fertile branches/plant	6.45	6.97	6.83	23.79	6.57	19.24	6.26	16.98
Pods/plant	47.13	2.63	47.95	8.49	47.62	7.65	46.92	6.88
Pod length (cm)	6.15	2.60	6.37	4.70	6.26	3.91	6.17	2.98
Seeds/pod	8.85	5.42	9.10	10.70	8.89	9.10	8.99	7.49
100-seed weight (g)	3.67	3.13	3.82	5.32	3.75	4.66	3.77	3.34
Total plant yield (g)	9.30	4.18	9.88	12.45	9.79	9.57	9.27	7.09

\* Each value is an average of means of four treatments. \*\*Each value is an average of coefficient of variation of four treatments.

was observed for fertile branches per plant, while the smallest increase was observed for pod length in both varieties.

Genetic variability within a crop species is crucial for selecting economically important traits. In particular, the introduction of minor mutations in

the polygenic system that governs quantitative traits greatly contributes to the improvement of crops. The results of this study undeniably prove that considerable level of genetic diversity for quantitative characters has been triggered within the population treated with mutagens. A change in variability in various quantitative traits after

**Table 4.** Estimates of mean values ( $\bar{x}$ ) and coefficient of variation (CV%) for various quantitative traits in M<sub>1</sub> generation of mungbean var. NM-1

Treatment	Days to flowering	Plant height (cm)	Days to maturity	Fertile branches/plant	Pods /plant	Pod length (cm)	Seeds /pod	100-seed weight (g)	Total plant yield (g)
Control	38.06±0.15 (1.55)	42.95±0.23 (2.07)	68.60±0.49 (2.75)	6.10±0.12 (7.86)	47.35±0.29 (2.36)	6.20±0.04 (2.26)	8.20±0.10 (5.00)	3.60±0.02 (2.78)	9.45±0.12 (4.87)
0.1% EMS	38.94±0.99 (9.86)	42.52±0.82 (7.48)	69.33±0.95 (5.35)	6.97±0.52 (29.27)	48.80±1.20 (9.57)	6.60±0.09 (5.23)	9.06±0.31 (13.46)	3.90±0.06 (6.41)	10.31±0.39 (14.84)
0.2% EMS	37.47±0.84 (8.67)	42.16±0.73 (6.71)	67.45±0.95 (5.45)	7.10±0.49 (26.90)	49.01±1.26 (9.93)	6.75±0.12 (6.96)	8.94±0.28 (12.30)	3.95±0.07 (6.58)	10.44±0.40 (14.98)
0.3% EMS	37.40±0.66 (6.82)	41.89±0.78 (7.26)	67.67±0.80 (4.61)	6.70±0.45 (25.97)	48.45±1.28 (10.22)	6.40±0.09 (5.31)	8.80±0.24 (10.70)	3.66±0.05 (5.27)	10.28±0.42 (15.76)
0.4% EMS	37.53±0.62 (6.37)	41.53±0.86 (7.99)	67.60±0.87 (5.00)	6.67±0.39 (22.79)	48.10±0.90 (8.53)	6.04±0.04 (2.48)	8.73±0.27 (11.80)	3.73±0.05 (5.89)	9.40±0.35 (14.36)
0.01% HZ	39.13±0.98 (9.71)	42.34±0.76 (7.01)	68.73±1.07 (6.08)	6.90±0.36 (20.43)	48.90±1.06 (8.42)	6.64±0.08 (4.52)	8.94±0.25 (10.74)	3.92±0.05 (5.61)	10.26±0.35 (13.16)
0.02% HZ	37.33±0.98 (10.20)	41.82±0.83 (7.65)	68.80±1.11 (6.20)	6.91±0.39 (21.71)	48.60±1.13 (9.03)	6.40±0.07 (4.22)	8.86±0.23 (10.27)	3.78±0.07 (6.88)	10.37±0.32 (11.96)
0.03% HZ	37.27±0.95 (9.84)	41.62±0.81 (7.54)	67.73±0.95 (5.42)	6.80±0.31 (17.76)	48.70±0.93 (7.43)	6.09±0.07 (4.43)	7.80±0.14 (11.02)	3.75±0.05 (5.16)	10.05±0.29 (11.24)
0.04% HZ	38.46±0.84 (8.50)	41.33±0.90 (8.47)	67.53±0.97 (5.59)	5.90±0.35 (23.39)	46.50±0.97 (8.08)	6.28±0.06 (3.82)	7.86±0.16 (8.14)	3.46±0.05 (5.49)	9.09±0.28 (11.88)
0.01% SA	39.00±0.71 (7.10)	42.21±0.93 (8.57)	69.13±0.92 (5.13)	6.64±0.40 (23.34)	48.33±0.96 (7.76)	6.55±0.07 (4.12)	8.73±0.20 (9.05)	3.89±0.04 (4.11)	10.22±0.25 (9.59)
0.02% SA	37.80±0.64 (6.56)	41.73±0.90 (8.36)	69.20±0.78 (4.39)	6.83±0.33 (18.74)	48.53±0.83 (6.67)	6.35±0.05 (3.15)	8.67±0.18 (8.30)	3.81±0.04 (4.19)	10.14±0.24 (9.57)
0.03% SA	37.60±0.65 (6.65)	41.36±0.79 (7.42)	67.86±0.64 (3.64)	5.88±0.27 (18.19)	46.70±0.82 (6.79)	6.32±0.05 (3.32)	7.93±0.15 (7.44)	3.52±0.04 (4.26)	9.66±0.25 (10.18)
0.04% SA	37.53±0.62 (6.39)	41.30±0.96 (8.98)	67.67±0.73 (4.17)	5.84±0.25 (16.61)	46.40±0.76 (6.33)	6.09±0.06 (3.44)	8.60±0.19 (8.49)	3.50±0.03 (3.42)	9.06±0.22 (9.27)

±Standard error; Values in parenthesis represent CV (%)

**Table 5.** Comparative effects of mutagens on mean and coefficient of variation for various quantitative traits in M<sub>1</sub> generation of mungbean var. NM-1

Trait	Control		EMS		HZ		SA	
	Mean	CV (%)	Mean*	CV (%)**	Mean	CV (%)	Mean	CV (%)
Days to flowering	38.06	1.55	37.83	7.93	38.05	9.56	37.98	6.67
Plant height (cm)	42.95	2.07	42.02	7.36	41.78	7.67	41.65	8.33
Days to maturity	68.60	2.75	68.01	5.10	68.20	5.82	68.46	4.33
Fertile branches/plant	6.10	7.86	6.86	26.23	6.63	20.82	6.30	19.22
Pods/plant	47.35	2.36	48.59	9.56	48.17	8.24	47.49	6.89
Pod length (cm)	6.20	2.26	6.45	4.99	6.35	4.25	6.33	3.51
Seeds/pod	8.20	5.00	8.88	12.06	8.36	10.04	8.48	8.32
100-seed weight (g)	3.60	2.78	3.81	6.04	3.73	5.78	3.68	3.99
Total plant yield (g)	9.45	4.87	10.11	14.98	9.94	12.06	9.77	9.65

\* Each value is an average of the means of four treatments. \*\*Each value is an average of the coefficient of variation of four treatments.

mutagenic treatments in M<sub>1</sub> generation has also been previously reported in *Vigna mungo* (Thilagavathi and Mullainathan, 2011; Ramya et al., 2014) and *Vigna unguiculata* (Mensah and Akomeah, 1992; Rizwana et al., 2005).

To enhance the quality of crop plants, it is imperative

to introduce micro-mutations into the polygenic system in order to impact quantitative characters. Previous studies conducted by various authors on different crops (Ignacimuthu and Babu, 1993; Khan et al., 1998; Joshi and Verma, 2004; Khan and Wani, 2005; Singh et al., 2006; Goyal et al., 2021b; Raina et al., 2022b; Wani et al., 2023; Goyal et al., 2024)

have clearly demonstrated that micro-mutations lead to a significant amount of genetic variability within the population subjected to mutagens. Initial research on utilizing mutations to enhance quantitative traits revealed that different attributes responded differently to mutagenic treatments. While one trait may exhibit reduced responsiveness in terms of variance levels, another trait may display significant responsiveness. Additionally, the genetic makeup of the biological material also influences the outcome of polygenic mutations (Loesch, 1964). Therefore, the extent of genetic factors and the breeding approach employed are crucial in determining how these traits can be genetically improved.

The current investigation reveals that the average values for all the quantitative characters undergo shifts in both positive and negative directions. Different viewpoints emerge concerning the direction of the mutations. Gaul (1965) propose that induced polygenic mutations occur in a random manner rather than following a specific sequence. On the other hand, Brock (1965) and Goud (1967) present a contrasting viewpoint, suggesting that polygenic mutations consistently exhibit a direction that is contrary to the previous selection history. Given the complex genetic basis of most of the quantitative traits, which involves the interaction of multiple genes, it is expected that shifts will occur in both directions.

## Conclusion

Seed germination and pollen fertility in M<sub>1</sub> generation are typically evaluated to assess the mutagenic sensitivity of biological systems. Seed germination and pollen fertility in the present study exhibited a proportional decrease with increasing mutagenic concentrations. Nevertheless, lower doses of the mutagens induced comparatively less damage than the higher ones, presenting an opportunity to enhance the genetic variability for quantitative traits in future generations. Additionally, the immediate data derived from M<sub>1</sub> biological parameters can assist in the preliminary elimination of less significant mutagen

concentrations and mutagenized populations, thereby saving time and effort for plant breeders in advancing mutation breeding research with better precision and greater likelihood of achieving desired outcomes. Further, mutagenic treatments that exhibited the most significant variation in quantitative traits have the potential to result in stable gene mutations in subsequent generations, which could be exploited to improve the mungbean crop.

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