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Genetic variability for seed yield and its related traits in M_3 mutants of green gram (*Vigna radiata* (L.) Wilczek)

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Abstract

Mutation breeding is an auxiliary approach to traditional breeding, enhancing genetic diversity and providing essential raw materials for developing high-quality plants through selective breeding techniques. This research was aimed at evaluating the extent of induced genetic variability for various yield-related traits in M_3 mutants of green gram variety PDM-11 after treatments with 0.2% and 0.03% of ethyl methanesulfonate (EMS) and hydrazine hydrate (HZ). Three replications of 100 seeds each were sown for every treatment and control in randomized complete block design (RCBD). Significant increase in mean values for fertile branches per plant, pods per plant, seeds per pod and total seed yield (g) was noticed among the M_3 mutants. EMS treatments were more effective in increasing the mean values for all the yield attributing traits than HZ treatments. The mutant PDM-11A demonstrated the highest increase of 83.7% in seed yield over the control. Compared to the control, analysis of variance revealed increased genetic parameters *viz*., genotypic coefficient of variation (GCV), heritability (H^2) and genetic advance (GA) for seed yield and yield components in the mutants. The rise in mean values, coupled with improved genetic variability for traits associated with seed yield, indicates the potential for identifying more favorable lines with improved yield capacity. Furthermore, a positive phenotypic correlation was noted between the yield components. On the other hand, the protein content of the mutant seeds showed an inverse relationship with total plant yield, indicating that different genetic processes regulate these two traits. These findings highlight the potential of induced mutagenesis, particularly EMS treatments, in enhancing yield-related traits in green gram. The observed genetic variability and improved agronomic performance of the mutants suggest their suitability for further breeding programs aimed at developing high-yielding green gram varieties.

Keywords: Chemical mutagens, Correlations, Green gram, High yield, Protein content.

Introduction

Pulses rank as the second most significant category of crops, after cereals. They are valuable for their role in food and animal feed, for fixing nitrogen biologically, and as materials for industry. Due to their minimal input needs and ability to improve soil health, pulses hold a distinct position within our agricultural framework. Enhancing the productivity of food crops presents a critical challenge in contemporary agriculture, a situation further exacerbated by climate change, especially the increasing temperatures and unpredictable rainfall patterns (Parry et al., 2005; Parry et al., 2009). Green gram- a pulse crop recognized for its high-quality and easily digestible protein, suffers from limited genetic variability, which hinders its improvement. To initiate a green gram breeding program, breeders must understand the inheritance pattern of yield components (Kadam et al., 2015).

The genetic construction of seed yield is more accurately understood through its component traits rather than yield alone because the yield comes from the combined impact of different yield components (Grafius, 1959). Thus, genetic diversity is essential for improving these component traits. Enhancing the quality characteristics of food crops is a vital objective in plant breeding programmes. One of this century's significant challenges is enhancing food production while simultaneously tackling the issue of limited agricultural land. Projections suggest that the increase in crop production using traditional methods will not meet the anticipated future demand for food. To address this challenge, improving the yield and quality of food crops by employing alternative methods such as induced mutagenesis is essential.

Mutation breeding is regarded as a significant advancement of the atomic age, offering a promising shortcut in breeding that can be effectively employed to develop desired varieties of various crop plants (Adamu et al., 2004; Khan and Wani, 2005; Khan and Wani, 2006; Raina et al., 2020; Goyal et al., 2021; Wani, 2021; Wani, 2024; Goyal et al., 2024). Green gram, usually grown in less fertile soils, faces a range of biotic and abiotic stresses such as drought, high temperatures, salinity, cold weather, insect pests, and diseases, all of which

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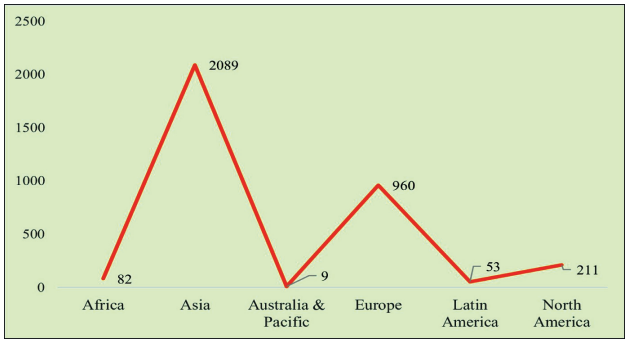

Figure 1. Number of mutant varieties released continent wise (https://mvd.iaea.org)

restrict its productivity. Consequently, the yield of green gram has seen minimal improvement over the last several decades, despite the implementation of various breeding strategies aimed at enhancing its performance. In the agricultural landscape of India, green gram occupies nearly 5.13 million hectare area with 3.08 million tonnes of production (Anonymous, 2021). The average yield of 570 kg per hectare is considered inadequate to satisfy the increasing demand. To overcome the yield stagnation in green gram, it is essential to focus on the development of high-yielding varieties with suitable growth habit. Given that green gram is a self-pollinating crop, the possibility offered by mutagenic agents to induce new genetic variation and to develop high-yielding mutant varieties has become increasingly important (Singh et al., 2000; Khan et al., 2005; Sangsiri et al., 2005).

Induced mutations manifest randomly within the genome, and their specific targets cannot be predetermined. A key benefit of induced mutagenesis lies in its capacity to rectify one or a few undesirable traits of a cultivar without altering most of its genetic framework (Saha- Joshi et al., 2015). Lately, considerable advancements have been achieved in induced mutagenesis, leading to the global release of 3,404 mutant crop varieties (Fig. 1), which encompasses 468 varieties of pulses including 48 varieties of green gram (https://mvd.iaea.org). Binamoong-11 and Binamoong-12 were the most recent mutant varieties of green gram developed in Bangladesh in 2021 and 2022, respectively. The mutant varieties exhibit shorter maturity and higher seed yield, besides being resistant to cercospora leaf spot, yellow mosaic virus, drought, and high temperature. Recent studies have focused on induced mutagenesis in various pulse crops; however, such information is little scanty in green gram. Against this backdrop, the present study was initiated to boost the genetic variability and improve the positive associations between seed yield and its components using ethyl methanesulfonate (EMS) and hydrazine hydrate (HZ) in M_3 generation of green gram to identify beneficial mutations.

Materials and Methods

Seeds of green gram variety PDM-11 were immersed in distilled water for nine hours before undergoing treatment with ethyl methanesulfonate (EMS) and hydrazine hydrate (HZ) at concentrations of 0.2% and 0.03% for six hours. The control group included seeds that were not treated and were merely saturated in distilled water before being planted. The EMS and HZ solutions were formulated using pH 7 phosphate buffer. All seeds underwent a thorough rinse with water to remove left-over mutagen from their surfaces.

The cultivation of green gram requires elevated temperatures, reduced humidity, and moderate levels of rainfall. Loam soil that offers excellent drainage is deemed optimal for growth of green gram. Additionally, well-prepared seedbeds are essential to ensure effective germination and establishment of the crop. Three replications of 100 seeds from each treatment group and the control were planted using randomized complete block design (RCBD) to produce M_1 generation. The seeds were spaced 30 centimeters apart in each row, while the rows were separated by 60 centimeters.

Seeds from different M_1 plants were grown into M_2 families in three replicates. To initiate M_3 generation, ten M_2 progenies were chosen based on their significant improvements in seed yield and yield components (fertile branches per plant, pods per plant and seeds per pod) compared to the control group. Seeds from each selected M_2 progenies were thoroughly blended. A random sample from this mixture was planted to raise M_3 progeny. Statistical analysis was conducted on the data gathered regarding seed yield and yield-associated traits in M_3 generation to assess the extent of induced genetic variation. One way analysis of variance (ANOVA) was done according to Singh and Chaudhary (1985) to find out the variance between the families and within them. To compare the means of various treatments, critical difference (CD) was applied and computed as follows:

Step-1. Construction of data table

The data was compiled in such a way that each treatment occupies a column and their replicates were arranged in rows.

Rows (Replicates)	Column (Treatments) Number			Total of Rows (S)	Squares of (Replicates) total of rows
	T_1	T_2	T_3		
R_1	A_1	B_1	C_1	A_1+..C_1=X_1	$(X_1)^2$
R_2	A_2	B_2	C_2	A_2+..C_2=X_2	$(X_2)^2$
R_3	A_3	B_3	C_3	A_3+..C_3=X_3	$(X_3)^2$
Total of column (Σ)	A_1+..A_3 = Y_1	B_1+..B_3 = Y_2	C_1+..C_3 = Y_3	$(X_1)^2$+..$(X_3)^2$ = Y_1+..Y_3 = X_1+..X_3	= Wr = Wx
Squares of total of columns $(\Sigma)^2$	$(Y_1)^2$	$(Y_2)^2$	$(Y_3)^2$	$(Y_1)^2$+..$(Y_3)^2$	= Wy

Sum of square of total of columns (Σ^2)	$(A_1)^2$+ ..$(A_3)^2$ = Z_1	$(B_1)^2$ +..$(B_3)^2$ = Z_2	$(C_1)^2$ +..$(C_3)^2$ = Z_3	Z_1+ ..Z_3	= Wz

Step-2. Correction Factor (CF)

$$CF = \frac{(\text{Grand total})^2}{\text{t.r.}} \quad \text{or} \quad CF = \frac{(Wx)^2}{\text{t.r.}}$$

where, t = number of treatments
r = number of replicates
Wx = grand total

Step-3. Total sum of squares (SSQT)

$$SSQT = Wz - CF$$

Step-4. Sum of squares of treatments (SSQt)

$$SSQt = \frac{Wy}{r} - CF$$

Step-5. Sum of squares of replicates (SSQr)

$$SSQr = \frac{Wr}{t} - CF$$

Step-6. Sum of squares of error (SSQ_e)

$$SSQ_e = SSQT - (SSQt - SSQr)$$

Step-7. Estimated variance of error (MS_e)

$$MS_e = \frac{SSQ_e}{(t-1)(r-1)}$$

Step-8. Critical difference (CD)

$$\text{CD at 5\% (p=0.05) level} = \sqrt{\frac{2\ MS_e}{r}} \times (t - \text{value at 5\% level})$$

If the difference between any two treatment means exceeds the CD value obtained at 5% level, the difference between the two means is taken to be significant. Protein content in seeds was determined as per the procedure suggested by Lowry et al. (1951). The correlation coefficient, a statistical measure which indicates an association between two or more than two characters (say, the number of pods and plant yield), generally denoted by the symbol 'r,' involved the following steps in computation:

Step-1. Construction of data table

Observations of pair of characters were arranged in the table as follows:

S. No. of pairs of observation	No. of pods (X)	Plant yield (g) (Y)	X^2	Y^2	XY
1	X_1	Y_1	$(X_1)^2$	$(Y_1)^2$	X_1Y_1
2	X_2	Y_2	$(X_2)^2$	$(Y_2)^2$	X_2Y_2
3	X_3	Y_3	$(X_3)^2$	$(Y_3)^2$	X_3Y_3
4	X_4	Y_4	$(X_4)^2$	$(Y_4)^2$	X_4Y_4
5	X_5	Y_5	$(X_5)^2$	$(Y_5)^2$	X_5Y_5
Total	ΣX	ΣY	ΣX^2	ΣY^2	ΣXY

Step-2. Computation of the following

(i) Sum of X-values 'ΣX'
(ii) Sum of Y-values 'Σ Y'
(iii) Square of each X-value and their sum 'Σ X^2'
(iv) Square of each Y-value and their sum 'Σ Y^2'
(v) Product of each pair (X and Y) and their sum 'Σ XY'

Step-3. Computation of 'r' values as follows

$$r = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/N}{\sqrt{[\Sigma X^2 - (\Sigma X)^2/N][(\Sigma Y^2 - (\Sigma Y)^2/N]}}$$

where, N = Number of pairs of observations

Test of significance of 'r'

The 't' test for correlation coefficient was applied to determine whether the relationship between two traits was significant. It was computed as follows:

$$t = r\sqrt{\frac{N-2}{1-(r)^2}}$$

where, r = Correlation coefficient
N = Total number of observations

The observed value of correlation coefficient was compared with the tabulated value for (N-2) degree of freedom. If the observed value was more than the table value of 't', the relationship was said to be significant.

Results and Discussion

Mutation induction within the polygenic system that governs quantitative traits is essential for enhancing crop performance (Wani et al., 2021; Raina et al., 2022; Goyal et al., 2024). In M_2 generation, two mutants, PDM-11A and PDM-11B, were identified for their notable superiority over the control regarding seed yield and other characteristics (Table 1; Figs. 2-3). These mutants were subsequently cultivated in plant progeny rows to raise M_3 generation. The mean seed yield for the mutants ranged from 13.80 to 18.63 g for PDM-11A and from 13.53 to 18.33 g for PDM-11B, in contrast to the control's seed yield range of 8.47 to 9.85 g (Table 2).

Both the mutants showed more fertile branches, pods, seeds per pod, and 100-seed weight than the control group. Notably, mutant PDM-11A was selected to achieve the highest seed yield of 17.49g, surpassing the control's seed yield of 9.52g. Results revealed a substantial increase in number of fertile branches, an important characteristic positively linked to the number of pods and over-all seed yield. This increase in fertile branches may be due to enhanced cell division, more photosynthetic activities, and the synthesis of growth-promoting phytohormones (Raina et al., 2018). The mutagen doses were also successful in increasing the values of genetic parameters viz., GCV, H^2, and GA for all the quantitative traits in both the mutants as compared to the control group. Increase in genetic parameters may be attributed to mutagens or pleiotropic effects of newly mutated genes.

Previous research has shown that mutagenic treatments increased the genetic variability in crops, including grass pea (Waghmare and Mehra, 2000), sesame (Sheeba et al., 2003), chickpea (Wani, 2018), faba bean (Khursheed et al., 2018), urdbean (Goyal et al., 2019) and cowpea (Raina et al., 2020). Enhanced genetic variability for yield and yield

Table 1. Brief description of the mutants isolated in M_3 generation of green gram var. PDM-11

Strain Number	Treatment	Duration of Treatment	Remarks
Var. PDM-11	Control	-	-
PDM-11-A	0.2% EMS	6 hours	High yield
PDM-11-B	0.03% HZ	6 hours	High yield

Figure 2. Control plant (var. PDM-11)

attributes indicates their higher transmittance to subsequent generations (Laskar et al., 2018).

Given that yield is a polygenic trait with intricate inheritance patterns, direct selection for yield is seldom successful. It is essential to utilize indirect selection on traits that have a direct influence on yield. Consequently, understanding the correlation between yield and its associated attributes is vital

Figure 3. High yielding mutant (PDM-11-A) isolated from 0.2% of EMS treatment

for maximizing the selection gains in plant breeding. The correlations elucidate the interrelationships among seed yield and its components. A positive relationship was found between the number of pods and the total seed yield of the plant; both traits also demonstrated a significant positive correlation with the number of fertile branches per plant (Table 3). Additionally, the number of seeds per pod and the weight of 100 seeds unveiled minor positive correlations with the overall seed yield of the plant, suggesting that selecting these traits could also enhance the other traits, ultimately leading to increased seed yield.

Table 2. Estimates of mean values ($\bar{x}$), range and genetic parameters for various quantitative traits of the mutants isolated in M_3 generation of green gram var. PDM-11

Strain No.	Treatment	Mean ± S.E.	Range	Shift in $\bar{x}$	PCV (%)	GCV (%)	H^2(%)	GA (% of $\bar{x}$)
				Fertile branches/plant				
PDM-11	Control	6.41 ± 0.09	5 – 8	-	8.53	4.19	24.15	5.44
PDM-11-A	0.2% EMS	11.06 ± 0.28	8 – 13	+ 4.65	22.39	19.96	79.82	46.99
PDM-11-B	0.03% HZ	10.13 ± 0.23	8 – 12	+ 3.72	19.21	16.31	72.09	36.54
CD (p=0.05)				1.23				
				Pods/plant				
PDM-11	Control	46.33 ± 0.55	42 – 52	-	6.74	3.42	25.64	4.57
PDM-11-A	0.2% EMS	74.25 ± 0.99	66 – 82	+ 27.92	13.16	12.25	86.70	30.07
PDM-11-B	0.03% HZ	68.45 ± 1.07	64 – 87	+ 22.12	14.15	13.10	85.73	31.99
CD (p=0.05)				5.25				
				Seeds per pod				
PDM-11	Control	8.29 ± 0.15	7-9	-	7.06	3.02	15.71	3.79
PDM-11-A	0.2% EMS	9.78 ± 0.32	7-10	+1.49	19.43	16.39	77.61	42.23
PDM-11-B	0.03% HZ	9.53 ± 0.27	6-10	+1.24	16.32	12.58	73.02	37.50
CD (p=0.05)				1.10				
				100- seed weight (g)				
PDM-11	Control	3.69 ± 0.01	3.55-3.95	-	3.76	2.02	20.14	4.12
PDM-11-A	0.2% EMS	4.11 ± 0.07	3.73-4.40	+0.42	16.23	11.96	80.23	27.53
PDM-11-B	0.03% HZ	3.64± 0.09	3.52-3.90	-0.05	13.73	10.68	77.21	25.99
CD (p=0.05)				0.31				
				Seed yield per plant (g)				
PDM-11	Control	9.52 ± 0.14	8.47 – 9.85	-	5.97	2.83	21.91	3.48
PDM-11-A	0.2% EMS	17.49 ± 0.37	13.80 – 18.63	+ 7.97	17.93	15.96	79.25	37.39
PDM-11-B	0.03% HZ	16.14 ± 0.41	13.53 – 18.33	+ 6.62	19.46	17.32	79.50	40.77
CD (p=0.05)				1.80				

SE= Standard error; PCV= Phenotypic coefficient of variation; GCV= Genotypic coefficient of variation; H^2= Heritability; GA= Genetic advance; CD= Critical difference

Table 3. Phenotypic correlation coefficient between different pairs of characters in M_3 mutants of green gram var. PDM-11

Strain No.	Treatment	Fertile branches/plant vs. Pods/plant	Fertile branches/plant vs. Seed yield/plant (g)	Pods/plant vs. Seed yield/plant (g)	Seeds/pod vs. Seed yield/plant (g)	100-seed weight vs. Seed yield/plant (g)
PDM-11	Control	+ 0.24	+ 0.04	+ 0.22	+0.12	+0.03
PDM-11-A	0.2% EMS	+ 0.66*	+ 0.46*	+ 0.63*	+0.36*	+0.18
PDM-11-B	0.03% HZ	+ 0.51*	+ 0.37*	+ 0.49*	+0.29*	+0.09

*Significant at 1% level.

Table 4. Range, mean, coefficient of variation and correlation coefficient for seed protein content in M_3 mutants of green gram var. PDM-11

Strain No.	Treatment	Seed protein content (%)				Seed protein vs.
		Range	Mean ± S.E.	Shift in $\bar{x}$	CV (%)	Seed yield / plant (r)
Var. PDM-11	Control	23.90 – 24.80	24.26 ± 0.09	-	1.23	- 0.112
PDM-11-A	0.2% EMS	24.60 – 26.30	25.50 ± 0.22	+ 1.24	2.74	- 0.382
PDM-11-B	0.03% HZ	24.40 – 25.90	24.93 ± 0.19	+ 0.67	2.52	- 0.219

The relationship between traits that influence seed yield within a population arises from a blend of selection pressures, genetic linkage, and pleiotropic effects (Sehrawat et al., 1996). Studies indicate that in green gram, traits like pods per plant, seeds per pod, and 100-seed weight are strongly linked to seed yield (Moomin and Misra, 2004). In a related study concerning field peas, Patel et al. (2006) identified a positive relationship between seed yield and several other traits, including the number of pods, branches, pod length, and days to maturity. The ability of mutations to alter character associations has been observed in several pulse crops (Kaul and Garg, 1982; Singh and Singh, 2003; Khan and Wani, 2005; Singh et al., 2015). The alterations in correlation among various traits may improve the effectiveness of selection responses in quantitative traits. A trait that exhibits a strong correlation with another preferred trait is deemed more advantageous for indirect selection. Given that the number of fertile branches and pods have demonstrated a significant association with seed yield, these characters may be considered as crucial selection criteria for yield improvement. These findings align with previous research conducted on chickpeas by Raina et al. (2017).

Protein content found in seeds is a complex trait governed by several genes distributed across different chromosomes (Frey, 1977). Protein content of the mutant seeds exhibited negative correlation with seed yield (Table 4). Consequently, improving this trait while simultaneously increasing the seed yield is quite challenging (Andrade et al., 2022). Negative correlation between seed yield and protein content has been earlier reported in various pulse crops (Kaul and Matta, 1976; Gottschalk and Muller, 1982; Karjalainen and Kortet, 1987; Khan and Wani, 2005). Mean protein content in the mutant seeds showed a slight increase in comparison to the control mean. Considering the importance of green gram as a nutritious legume in the human diet, the development of high-yielding mutants with improved protein contents would hold substantial economic and dietary significance.

Conclusion

Present findings indicate that mutagenic treatments have effectively modified the relationships among yield-related traits and generated valuable genetic variability in the mutants. The mutants PDM-11A and PDM-11B of green gram isolated in this study showed improved plant architecture, resulting in increased seed yield and a marginally higher seed protein content compared to the control group. These mutants are promising candidates for assessment in future generations, and after comprehensive testing across various locations, may be introduced as high-yielding varieties. Given that green gram is a highly nutritious legume in the human diet, these high-yielding mutants with elevated protein levels could have significant prospective economic implications. Therefore, the induced genetic variability presents a valuable opportunity for the enhancement of the green gram crop. In essence, these high-yielding green gram mutants exemplify how induced mutagenesis can revolutionize crop breeding—paving the way for a resilient, nutritious, and economically vibrant future in legume production.

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