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Principal component analysis of mutagen-induced variability and trait interrelationships in greengram (*Vigna radiata* L.)

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ABSTRACT

The present investigation was undertaken to evaluate the pattern of variability and trait interrelationships among nine quantitative characters of greengram crop in a set of fifteen mutagenic treatments along with the parent variety Sujata as the control using principal component analysis (PCA), which indicated that the first principal component (PC_1) explained 56.18% of the total variation, followed by PC_2 (16.38%) and PC_3 (10.65%), cumulatively accounting for 83.21% of the total variability. Eigenvector analysis revealed that PC_1 was largely influenced by positive contributions from traits such as plant height, pods per plant, seeds per pod, and 100-seed weight whereas days to 50% flowering and days to maturity contributed negatively, reflecting a major contrast between trait groups. The second principal component was dominated by yield per plant, indicating its independent role, while PC_3 was mainly associated with clusters per plant, contributing to secondary variability. The PCA biplot further confirmed the clustering of traits namely plant height, clusters per plant, pods per plant, pod length, seeds per pod and 100-seed weight and the distinct separation of days to 50% flowering and days to maturity, with yield per plant forming an independent axis. Mutagenic treatments, i.e. gamma rays (20 kR, 40 kR) and EMS (0.2%, 0.4%) are closely associated with major contributing traits, appear to be promising candidates for yield improvement whereas MH (0.02%, 0.03%) and 40 kR gamma rays + 0.02% MH were positioned in the opposite direction, showing a negative association with major yield-related traits. Treatments NG (0.010% and 0.015%) and MH (0.01%) were aligned with yield per plant, indicating their specific influence on this trait. The results demonstrate that induced mutagenesis effectively enhanced genetic diversity in greengram and that PCA serves as a reliable tool for identifying key traits and promising genotypes, thereby facilitating selection strategies in greengram breeding programs.

Key words: Genetic variability, greengram, induced mutation, mutagens, principal component analysis, trait association, yield components

INTRODUCTION

Greengram (*Vigna radiata* L.) is an important pulse crop widely cultivated in Asia and other parts of the world due to its high nutritional value and adaptability to diverse environments. It serves as a major source of plant protein and plays a vital role in ensuring food and nutritional security, particularly in developing countries (Nair et al., 2013). Additionally, its ability to fix atmospheric nitrogen enhances soil fertility, making it an integral component of sustainable agriculture systems. Despite its importance, the productivity of greengram remains relatively low due to limited genetic variability and susceptibility to environmental stresses. Yield in greengram is

a complex trait governed by multiple interacting components, which often exhibit varying degrees of association among themselves. This complexity poses challenges for breeders in identifying key traits for selection (Das et al., 2020). The bottlenecks in its improvement have been the lack of variability in different traits and improvement of one trait on its own will affect the performance of other traits because of genotypic correlations between traits (Das and Baisakh, 2019).

Induced mutagenesis has been widely used to create genetic variability in pulse crops. It provides a powerful means of creating new and useful variability in crop plants both in qualitative and quantitative traits (Das and Misra, 2005; Das and Baisakh, 2020). Physical and chemical mutagens induce genes to mutate at rates above spontaneous baselines, thus producing a range of novel traits and broadening the genetic diversity of plants (Das and Baisakh, 2013). Physical or chemical mutagen-induced quantitative variation not only serves as an alternative source of germplasms for natural variation but is also useful in generating appropriately linked gene complexes that are responsible for the improvement in yield and other characteristics of economic interest (Das and Prusti, 2020). Selection of efficient mutagens and their treatment doses is a prerequisite for successful mutagenesis in crop plants as mutagens are potential tools for direct improvement of qualitative and quantitative characters. Several studies have demonstrated the effectiveness of mutagenesis in improving yield and related traits in pulses (Kharkwal and Shu, 2009; Das et al., 2021). However, the evaluation of such variability requires proper statistical tools capable of handling multiple traits simultaneously.

Multivariate statistical techniques, particularly Principal Component Analysis (PCA), have been extensively used in plant breeding to analyze complex datasets. PCA is a particularly useful method for identifying traits that differentiate genotypes. It reduces the dimensionality of data by transforming correlated variables into a set of uncorrelated principal components, each explaining a portion of total variance (Jolliffe & Cadima, 2016). This approach helps in yield associated traits contributing to variability and simplifies the selection process. Correlation analysis complements PCA by revealing the strength and direction of relationships among traits. Positive correlations indicate that traits can be improved simultaneously, whereas negative correlations suggest trade-offs. The interpretation of PCA results involves examining eigenvalues, eigenvectors, and loadings. Eigenvalues indicate the importance of each component, while eigenvectors represent the contribution of individual traits (Abdi and Williams, 2010). Given the importance of multivariate analysis in crop improvement, the present study aims to assess variability among nine traits, evaluate divergence among mutagenic treatments, and identify key traits influencing productivity.

MATERIALS AND METHODS

The experimental material consisted of M_3 genotypes developed from Sujata variety of greengram by fifteen mutagenic treatments i.e three doses each of gamma rays (20, 40 and 60 kR), ethyl methane sulphonate (EMS; 0.2, 0.4 and 0.6%), nitroso guanidine (NG; 0.005, 0.010 and 0.015%) and maleic hydrazide (MH; 0.01, 0.02 and 0.03%) singly, and combined mutagenic treatments of 40 kR gamma rays with 0.4% EMS or 0.01% NG or 0.02% MH. The twelve single mutagenic treatments of gamma rays, EMS, NG, and MH (Sr No. 1 to 12) were coded as G1, G2, G3, E1, E2, E3, N1, N2, N3, M1, M2 and M3, respectively. Three combined treatments of 40 kR gamma rays + 0.4% EMS, 40 kR gamma rays + 0.01% NG and 40 kR gamma rays + 0.02% MH (Sr No.13 to 15) were coded as GE2, GN2 and GM2, respectively. The parent variety (Sujata) used as the control and coded as C (Sr No.16). Observations were recorded on nine quantitative traits i.e. days to 50% flowering (X1), days to maturity (X2), plant height (X3), clusters per plant (X4), pods per plant (X5), pod length (X6), seeds per pod (X7), 100-seed weight (X8) and yield per plant (X9). The data were used to analyze the eigenvalues, percentage variance, cumulative variance and other PCA parameters. PCA was conducted using a correlation matrix. Eigenvectors were extracted to determine trait contributions to principal components. The "R" software (version 3.3.2.) was used for this analysis.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA) was performed to understand the multivariate

relationship among nine plant traits (X1–X9) and to assess the divergence among mutagenic treatments (1–15) in comparison to the control (16). The analysis revealed substantial variability, confirming the effectiveness of mutagenic treatments in generating phenotypic diversity.

Eigenvalues and variance

The PCA results indicated that the first principal component (PC_1) had an eigenvalue of 5.056, explaining 56.18% of the total variation, which clearly dominates the variability structure and the second principal component (PC_2) contributed 16.38% (eigenvalue = 1.474). Together, PC_1 and PC_2 accounted for 72.56% of the total variation, which is sufficiently high to reliably interpret the biplot. Subsequent components contributed progressively less variance: PC_3 (10.65%), PC_4 (7.23%), and remaining components each contributed less than 5% (Table 1). The cumulative variance reached 83.21% up to PC_3 and 90.45% up to PC_4, indicating that the first two to three components capture the major structure of variation. The scree plot showed a sharp decline from PC_1 to PC_2 and gradual flattening thereafter (Fig. 1). The clear "elbow" observed at PC_2 in the scree plot suggests that most of the meaningful variation is captured within the first two principal components, supporting their use for interpretation and graphical representation.

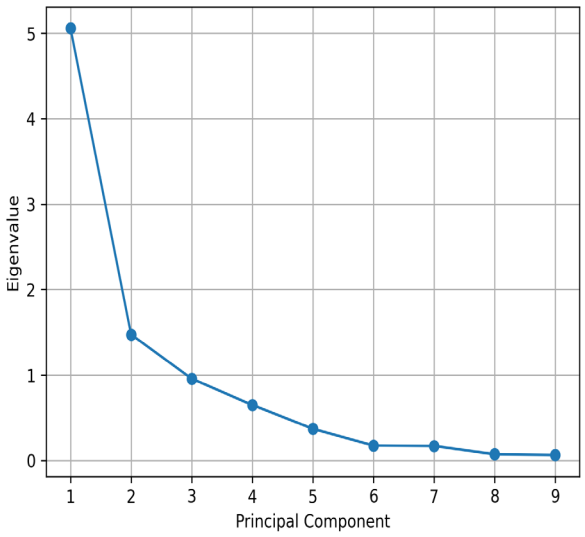

Fig. 1. Scree plot showing eigenvalues of principal components

Trait association and principal component structure

The PCA biplot (Fig. 2) clearly demonstrates that the first principal component (PC_1) is primarily associated with traits X3, X4, X5, X6, X7, and X8, all oriented in the positive direction. The strong clustering and small angular separation among these vectors indicate a high degree of positive correlation. This suggests that these traits are functionally related and may collectively influence yield or productivity. On the contrary, traits X1 and X2 are positioned on the negative side of PC_1, indicating a strong inverse relationship with the above group. This opposition reflects a biological trade-off, where enhancement in X3–X8 may lead to reduction in X1 and X2, or vice versa. Trait X9 is distinctly aligned along PC_2. This indicates that X9 contributes independently to variability and may represent a unique physiological or morphological characteristic. Its vertical alignment indicates that it captures variability not explained by PC_1, highlighting its unique contribution to phenotypic divergence. The predominance of PC_1 in explaining more than half of the total variation highlights the importance of traits X3–X8 in determining overall phenotypic diversity in greengram.

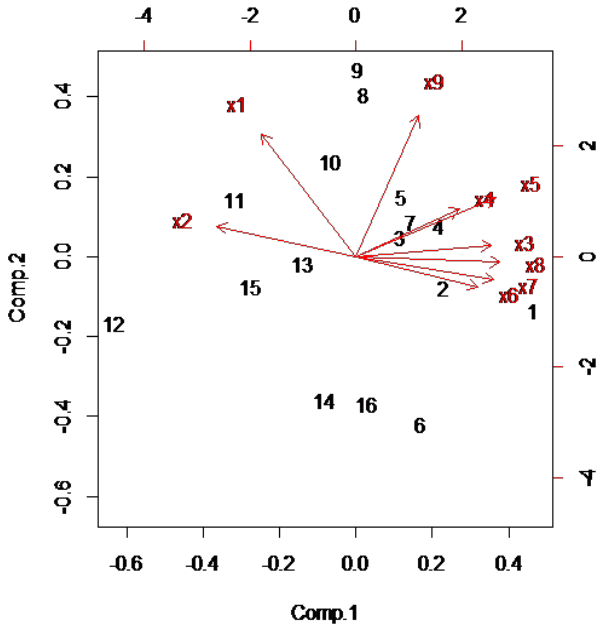

Fig. 2. PCA biplot

Days to 50% flowering (X1), days to maturity (X2), plant height (X3), clusters per plant (X4), pods per plant (X5), pod length (X6), seeds per pod (X7), 100-seed weight (X8) and yield per plant (X9). 1-15 Mutagenic treatments (details in Table 4), 16- control (Sujata)

The PCA biplot (Fig. 2.) clearly demonstrates three major trait groups, i.e., Group I (Positive PC_1 cluster) consists of X3, X4, X5, X6, X7 and X8 traits which are strongly and positively correlated. The alignment of these traits suggests that selection for one trait in this group may simultaneously improve others, Group II (Negative PC_1 cluster) consists X1 and X2 traits are closely associated with each other but negatively correlated with Group I traits, indicating contrasting biological functions or growth patterns and Group III (PC_2 dominant trait) consists X9 trait is relatively independent and contributes distinctly to overall variability, suggesting its importance as a unique selection parameter. Such grouping is typical in mutagenesis studies, where induced variability leads to the emergence of new trait combinations and altered correlations.

Table 1. Eigenvalues and variance

Parameters	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9
Eigenvalue	5.056	1.474	0.959	0.651	0.372	0.177	0.171	0.074	0.065
% of variance	56.18	16.38	10.65	7.23	4.14	1.97	1.90	0.82	0.73
Cumulative (%)	56.18	72.56	83.21	90.44	94.58	96.55	98.45	99.27	100

The component loading matrix revealed that PC_1 was predominantly influenced by traits X3 (0.810), X4 (0.780), X5 (0.740), X6 (0.690), X7 (0.720), and X8 (0.760), all of which exhibited high positive loadings, indicating their major contribution to total variability (Table 2). In contrast, traits X1 (−0.620) and X2 (−0.680) showed negative loadings on PC_1, suggesting an inverse association with the primary trait group. PC_2 was mainly defined by trait X9, which recorded a high loading (0.850), indicating its independent contribution. The third principal component (PC_3), explaining 10.651% of variance, showed moderate loadings for X1, X2, X6, and X7, representing residual variability.

Table 2. PCA loadings values

Trait	PC_1	PC_2	PC_3
X1	-0.620	0.180	0.310
X2	-0.680	0.120	0.275
X3	0.810	0.050	-0.210
X4	0.780	0.220	-0.145
X5	0.740	0.300	-0.095
X6	0.690	-0.150	0.260
X7	0.720	-0.200	0.185
X8	0.760	-0.080	0.140
X9	0.150	0.850	0.065

The eigenvector matrix (Table 3) revealed that PC_1 was dominated by positive coefficients of X3 (0.368), X5 (0.380), X7 (0.375), and X8 (0.391), while X1 (−0.258) and X2 (−0.377) contributed negatively. This confirms that PC_1 represents a major axis of variation contrasting yield-related traits with early growth traits. PC_2 was primarily influenced by X9 (0.678) and X1 (0.592), indicating a separate dimension of variability. PC_3 showed high contribution from X4 (0.669), suggesting its role in secondary variation.

Table 3. Eigenvector matrix for traits

Trait	PC_1	PC_2	PC_3	PC_4
X1	-0.258	0.592	0.301	0.168
X2	-0.377	0.145	-0.032	0.457
X3	0.368	0.055	0.289	-0.020
X4	0.280	0.228	0.669	-0.250
X5	0.380	0.284	-0.238	-0.101
X6	0.332	-0.144	0.047	0.735
X7	0.375	-0.109	0.163	0.347
X8	0.391	-0.026	-0.382	-0.149
X9	0.170	0.678	-0.383	0.090

Traits with lower absolute value closer to zero influence the clustering less than those with

largest absolute value closer to unity within the first principal component. The strong positive association among these traits suggests that they may be governed by similar genetic factors or physiological pathways, making them suitable targets for simultaneous improvement. The negative association of X1 and X2 with these traits indicates the presence of trade-offs, which is a common phenomenon in crop improvement programs where enhancement of certain traits may adversely affect others. Madhukumar et al. (2023) reported similar negative association for X1 and X2 in greengram. The distinct behavior of trait X9, primarily influencing PC_2, suggests that it represents an independent dimension of variability. This trait may be controlled by different genetic mechanisms and should be considered separately during selection. Similar findings have been reported in other crop studies where PCA effectively identified key traits contributing to variability (Oladosu et al., 2016). The contribution of PC_3, particularly through X4, suggests the presence of additional variability that may help in differentiating genotypes more precisely. The eigenvector analysis further supports these findings by showing that PC_1 is largely defined by traits associated with yield and productivity. The negative contribution of X1 and X2 to PC_1 indicates their contrasting role, possibly related to early growth or structural traits. The strong loading of X9 on PC_2 highlights its independence, suggesting that it is governed by different genetic factors and should be considered separately during selection. Positive and negative loadings indicate correlation patterns between the traits and components, as observed in previous studies (Jakhar and Kumar, 2018).

Distribution of mutagenic treatments

The distribution of mutagenic treatments across the PCA space revealed considerable divergence (Table 4). Treatments G1, G2 , E1, E2, E3, N1, and G3 exhibited high positive PC_1 scores (ranging from 4.031 - 1.012), indicating their strong association with traits X3–X8. Among these, G1 recorded the highest PC_1 score (4.031), suggesting superior performance for these traits. Treatment G3 recorded moderate scores on PC_1 (1.012) and PC_2 (0.229) with a higher score on PC_4 (1.167), indicating a balanced contribution across traits without strong association with any particular trait group. Similarly, N1 showed moderate PC_1 (1.269) and slightly positive PC_2 (0.409) values, suggesting a stable but non-extreme expression of traits. E3 exhibited a relatively high PC_1 score (1.467) but a strongly negative PC_2 value (−1.978), indicating its association with the major trait group (X3–X8) while being negatively related to trait X9. Conversely, treatments M3, M2, GM2, GE2, GN2, and M1 showed negative PC_1 scores (−5.502 to −0.590), reflecting their association with traits X1 and X2. Treatment M3 exhibited the most extreme negative value (−5.502), indicating maximum divergence in this direction. Regarding PC_2, treatments N3 and N2 showed the highest positive scores (2.211 and 1.902, respectively), clearly indicating their strong alignment with trait X9 and its role in trait-specific variation. The treatment GE2 displayed a negative PC_1 score (−1.208) along with a high positive PC_4 value (1.852), suggesting its divergence from the primary trait clusters and contribution to secondary variability. These intermediate and divergent positions indicate that while some treatments strongly influence specific trait groups, others contribute to overall variability and balance among traits. E3, GN2 and the parent (C) recorded negative PC_2 values (−1.978, −1.687, and −1.735, respectively), indicating lower expression of this trait. The control (16) was positioned away from most mutagenic treatments, reflecting limited variability compared to induced genotypes.

In addition to clearly associated treatments, several treatments exhibited intermediate positioning in the PCA biplot. G3 and N1 were located near the center of the plot, indicating moderate and balanced contribution across traits without strong association with any specific trait group. E3, although positioned on the positive side of PC_1, was separated along PC_2, suggesting its association with major traits (X3–X8) but a negative relationship with X9. N3 showed a strong alignment along PC_2, indicating its specific association with trait X9. GE2 was positioned away from the primary trait clusters, reflecting its divergent nature and contribution to overall variability.

Table 4. PCA scores

Sl. No.	Codes	PC_1	PC_2	PC_3	PC_4
1	G1	4.031	-0.639	-0.569	-0.012
2	G2	1.999	-0.369	-1.717	-0.443
3	G3	1.012	0.229	0.571	1.167
4	E1	1.890	0.368	0.768	0.047
5	E2	1.050	0.711	0.679	0.676
6	E3	1.467	-1.978	1.651	-0.850
7	N1	1.269	0.409	0.226	0.175
8	N2	0.199	1.902	-0.203	-1.068
9	N3	0.048	2.211	0.192	-0.331
10	M1	-0.590	1.119	0.462	-0.060
11	M2	-2.764	0.670	-0.188	-0.073
12	M3	-5.502	-0.776	1.080	-0.056
13	GE2	-1.208	-0.083	-0.588	1.852
14	GN2	-0.736	-1.687	-1.286	0.776
15	GM2	-2.405	-0.355	-1.739	-1.098
16	C	0.239	-1.735	0.662	-0.702

The clear separation of treatments in the PCA space demonstrates the effectiveness of mutagenesis in generating genetic diversity. Treatments such as G1, G2, E1 and E3, which are closely associated with major contributing traits, appear to be promising candidates for yield improvement. The intermediate positioning of treatments such as G3 and N1, as indicated by their moderate PC_1 and PC_2 scores, suggests that these genotypes maintain a balanced expression of traits and may serve as stable genetic material in breeding programs. On the other hand, treatments like M2, M3 and GM2, which are positioned in the opposite direction, show negative association with major yield-related traits and may be useful for improving specific traits represented by X1 and X2. Treatments N2, N3, and M1 were aligned with X9 along PC_2, indicating their specific influence on this trait. The distinct separation of the parent (control) from most treatments further confirms that induced mutagenesis has successfully altered the genetic architecture and enhanced variability. The strong negative PC_2 value of E3 indicates its limited association with trait X9 despite its positive contribution to the major trait group, highlighting the complexity of trait interactions. The high PC_2 value observed in N3 confirms its specific contribution to trait X9, indicating that certain treatments may influence individual traits independently rather than contributing to overall performance. The divergence of GE2, reflected by its negative PC_1 and high PC_4 score, suggests its potential role in broadening the genetic base, which is essential for long-term breeding strategies. The alignment of treatments with specific trait vectors in the PCA biplot indicates that mutagenesis has successfully altered trait expression patterns. Treatments positioned along the direction of X3–X8 are associated with improved yield-related traits and can be considered promising for selection. Conversely, treatments located opposite to these vectors may represent undesirable combinations or alternative trait expressions. The independent positioning of the trait yield per plant and its associated treatments suggests that this trait is controlled by different genetic factors and should be considered separately in breeding programs. Overall, the PCA results provide a comprehensive understanding of trait relationships and genotype performance, facilitating more informed selection decisions in greengram breeding programs. The overall distribution of treatments in the PCA space indicates that mutagenesis has successfully generated significant variability, enabling the identification of promising genotypes.

CONCLUSION

The present study demonstrated that Principal Component Analysis is an effective approach for dissecting complex trait relationships and assessing genetic divergence in greengram. The first two principal components accounted for a substantial proportion of total variability, enabling clear interpretation of trait associations and treatment performance. Traits X3–X8 emerged as major contributors to variability and can be targeted for simultaneous improvement. The trait yield per plant should be considered independently, while days to 50% flowering and days to maturity require careful handling due to their negative association with major traits. Mutagenic treatments, particularly

gamma rays (20 and 40kR) and EMS (0.2%, 0.4%), showed promising potential for enhancing desirable traits, whereas the control exhibited limited variability. Treatments NG (0.010% and 0.015%) and MH (0.01%) were aligned with yield per plant, indicating their specific influence on this trait. The findings highlight the usefulness of induced mutagenesis combined with multivariate analysis in broadening the genetic base and accelerating breeding progress for improving productivity.

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