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RESEARCH ARTICLE

Genetic characterization of mung bean (*Vigna radiata*) genotypes based on morphological traits and SSR markers

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Abstract

The genetic diversity among 117 mung bean (*Vigna radiata*) accessions was assessed using eight morphological traits and 70 Simple Short Repeat (SSR) markers. These accessions were grown in an augmented design during the Summer and *Kharif* seasons of 2017. The mean data from the two seasons were subjected to correlation, principal component and cluster analyses. Plant height (r = 0.527), the number of pods in a plant (r = 0.717) and the number of seeds in a pod (r = 0.241) showed a highly significant positive relationship with individual plant yield. Principal Component Analysis (PCA) revealed that the first three components explained 74.32 % of the total variation, with eigenvalues greater than one. Principal Component 1 (PC1) accounted for the maximum variation in traits, including days to first flowering (loading = 0.773), days to 50 % flowering (0.740), number of pods in a plant (0.708) and plant height (0.653). Morphological trait-based clustering grouped the genotypes into two major clusters. Further, the 117 mung bean accessions were analyzed using 70 SSR markers. Of the 70 SSRs, thirteen were polymorphic and generated 55 alleles, averaging 4.23 alleles per locus. The polymorphic information content (PIC) values ranged between 0.52 and 0.79, averaging 0.71. Analysis of genotypic data led to the classification of the accessions into three clearly defined clusters. The findings of the present study were expected to contribute to future mung bean breeding programs aimed at developing trait-specific genotypes.

Keywords : correlation; mung bean; principal component analysis

Introduction

Mung bean, was a predominantly self-pollinated pulse grown primarily in Asia, where it constituted a significant part of the human diet. Mung bean seeds were a rich source of carbohydrates and protein. To date, most mung bean breeding programs had focused on improving yield along with biotic and abiotic stress tolerance, as well as nutritional traits. The selection of diverse parental materials with desirable traits was a key component in achieving the objectives of any successful breeding program (1, 2). However, mung bean breeding programs were constrained by the limited diversity of parental materials (3, 4). This limitation arose from the small genome size of 543 Mb and the restricted gene pools of cultivated mung bean species (5). The largest mung bean germplasm collections were maintained in Asian countries, such as India, Taiwan, China and Pakistan. However, many of these germplasms remained uncharacterized. For effective utilization of such germplasm collections in breeding programs, it was essential to characterize and analyze the genetic diversity among genotypes (6, 7). This characterization was critical not only for the conservation of germplasm but also for identifying genes that might have been valuable in breeding programs (8). Additionally, information on genetic diversity aided in gene-bank management and breeding studies, including germplasm identification, pinpointing and/or removing duplicates in gene stocks, constructing core collections and sorting populations for genome mapping studies (9, 10). Methods for assessing genetic diversity ranged from conventional approaches, which studied morphological traits described in crop descriptor lists, to biochemical and molecular approaches, each with its comparative merits and limitations (11, 12).

Traditionally, mung bean germplasm diversity was assessed based on morphological traits (13-16). Nevertheless, these techniques exhibited certain limitations, such as low heritability, low polymorphism, late expression of traits and susceptibility to biotic and abiotic stresses (17, 12). Therefore, evaluating the genetic diversity of germplasm at the morphological level was complemented with molecular-level studies. Subsequent studies on mung bean diversity incorporated both morphological traits and molecular marker data, such as Random Amplified Polymorphic DNA (RAPD) (18, 19), Amplified Fragment Length Polymorphism (AFLP) (20, 21) and SSRs (22, 23, 12). Among these markers, SSRs provided to be particularly efficient for genotyping because they were reliable, cost-effective, co-dominant and exhibited high allelic diversity (24, 25). As such, SSR markers were widely incorporated in genetic diversity studies of mung bean.

With this backdrop, this study was structured to pursue the following objectives: (1) to determine phenotypic variation and identify traits closely associated with yield, (2) to identify traits responsible for the maximum variation in mung bean accessions and (3) to assess both morphological and molecular diversity among mung bean accessions.

Materials and Methods

Plant genotypes and experimental plot

An aggregate of 117 Mung bean accessions, along with five check varieties (VBN 3, VBN 2, CO 8, CO 7, CO 6), was used in this study. The accessions originated from Asia, with a majority sourced from India. The seeds were obtained from multiple institutions, including AVRDC in Taiwan, NBPGR in New Delhi, India and the Department of Plant Genetic Resources TNAU, Coimbatore, India. The genotypes were examined at the Agricultural Research Station (ARS) of TNAU, located in Bhavanisagar, India. The mung bean accessions, along with the five check varieties, were cultivated in an augmented design during the summer and *Kharif* seasons of 2019. Each accession was sown on ridges and furrows, with row measuring four meters in length and plants spaced at 30 cm × 10 cm. The crop was managed using standard agronomic practices throughout the crop duration.

Phenotypic characterization of accessions

Phenotypic observations were recorded according to the mung bean descriptors (26). Data were collected from ten plants at random within each genotype. Traits observed included plant height (cm), days to first flowering, days to 50 % flowering, number of pods in a plant, pod length (cm), number of seeds in a pod, 100-seed weight (g) and single-plant yield (g).

Simple sequence repeat analysis

Genomic DNA was isolated from the young leaves of mung bean accessions using the CTAB method (27). DNA quality was assessed using 0.8 % agarose gel electrophoresis and genomic DNA concentrations were adjusted to 25 ng/µl. A total of 70 adzuki bean SSR primer pairs were employed for diversity analysis (28). PCR analysis and gel electrophoresis were conducted following the protocol described in previous studies (29). The Polymorphic Information Content (PIC) was calculated using the equation $Hj = 1-\Sigma pi^2$, where *pi* indicates the frequency of i^{th} allele (30). The allelic data generated were subjected to hierarchical clustering using Ward's method (31), implemented in version 6.0 of DARwin software (32).

Statistical analysis

The mean data from the two seasons were used for statistical analysis. Correlation analysis was performed using TNAUSTAT software (33). PCA was conducted using SPSS v.16.0 (34). Cluster analysis was carried out using MINITAB version 17.1, applying Ward's method utilizing Euclidean distance to group the accessions (35).

Results and Discussion

Phenotypic variation among mung bean accessions

The genetic diversity analysis of mung bean germplasm provided key insights into the genetic relationships among accessions and aided in gene bank management as well as the design of mung bean breeding programs (15). This research focused on evaluating the genetic diversity among 117 different mung bean accessions. Basic descriptive statistics based on the mean data for eight quantitative traits are displayed in Table 1. Among the traits studied, plant height had a mean value of 37.44 cm, ranging from 18.65 cm (EC 396117) to 62.80 cm (PLS 274). Days to first flowering showed a mean value of 32.84 days, with earliness observed in genotype LM 294 (29 days) and late flowering recorded in Tenkasi 2 (36 days). Days to 50 % flowering ranged between 33 days (LM 294) and 41 days (Sonamoong, ADT 1 and PLS 274), with a mean of 37.11 days. The number of pods per plant, a key yield-associated trait, had a mean 34.38. The lowest pod-bearing genotype was EC 396120 (19.10), while the highest was EC 118889 (71.10). Pod length varied between 4.96 cm (Binamung 7) and 11.32 cm (EC 396115), with an average 7.69 cm. The number of seeds per pod had a mean value of 11.11, ranging from a minimum of 6.90 (Parjula) to a maximum of 14.91 (LM 420B). The 100-seed weight averaged 3.68 g, with values ranging from 2.32 g (Pantmung 4) to 5.32 g (EC 396116). Single-plant yield ranged from 6.12 g (Binamung 7) to 17.49 g (Velampatti), with a mean of 9.83 g.

Table 1. Descriptive statistics for eight morphological traits in 117 mung bean accessions

S.No.	Traits	Mean	SD	Median	Minimum	Maximum	CV	Skewness	Kurtosis
1.	Plant height	37.44	10.78	36.68	18.65	62.80	28.79	0.29	-0.67
2.	Days to first flowering	32.84	1.57	32.90	29.00	36.10	4.78	0.39	-0.64
3.	Days to 50 per cent flowering	37.11	1.67	37.00	33.00	40.90	4.50	0.18	-0.32
4.	No. of pods in a plant	34.38	9.83	33.15	19.10	71.10	22.58	1.32*	2.02
5.	Pod length	7.69	1.35	7.33	4.96	11.32	17.53	0.59*	-0.19
6.	No. of seeds in a pod	11.11	1.44	11.10	6.90	14.91	12.97	-0.64*	0.61
7.	Hundred seed weight	3.68	0.74	3.57	2.32	5.32	20.02	0.36	-0.66
8.	Single plant yield	9.83	2.70	8.93	6.12	17.49	23.45	1.31*	0.75

SD: Standard deviation, CV: Coefficient of variation.

Quantitative traits controlled by polygenes exhibited a continuous range of variation and were highly influenced by environmental factors. Among the accessions studied, LM 294 was identified as the earliest flowering genotype, while PLS 274 also flowered early and exhibited the maximum plant height. Genotypes with higher number of pods per plant included EC 118889, Kangeyam, S 4, Velampatti and Tenkasi 2. Pod length was highest in EC 396115, EC 396123, EC 396111, EC 396103 and EC 396102. A greater number of seeds in a pod was detected in LM 420B, Harsha, K. Pudur 3, K. Pudur 2 and Rajendram. Bold-seeded genotypes, which are desirable in breeding programs, were identified as EC 396116, EC 396113, PDM 54-1, EC 396117 and EC 396097. Genotypes with the maximum single-plant yield included Velampatti, Tenkasi 2, Salem 1, Sonamoong and S 4. Accessions such as Velampatti, Tenkasi 2, EC 118889, S 4 and Harsha demonstrated high values for important yield-attributing traits, including plant height, number of pods in a plant, number of seeds in a pod and the single-plant yield. These accessions were considered potential candidates for inclusion in mung bean improvement programs.

Out of the eight quantitative traits analyzed, except number of seeds per pod showed positive skewness, implying the potential of directional selection of those characters. Kurtosis analysis revealed leptokurtic distributions for the number of pods in a plant (2.02), individual plant yield (0.75) and number of seeds in a pod (0.61), indicating lower variation in the germplasm for these traits. In contrast, traits such as plant height (-0.67), 100-seed weight (-0.66), days initial flowering (-0.64), days to 50 % flowering (-0.32) and pod length (-0.19) displayed platykurtic distributions, suggesting greater variation in the germplasm for these traits. Analysis of variance indicated significant variation across all morphological traits, suggesting substantial diversity within the germplasm. Similar findings on variability in mung bean were reported (36, 37).

Correlation Analysis

Correlation analysis provided insight into the relationships among quantitative traits and their contributions to yield improvement, assisting in the formulation of an effective selection index. The analysis revealed 13 significant positive associations and eight significant negative associations (Table 2). Traits showing highly significant positive relationship with individual plant yield included number of pods in a plant (0.717), plant height (0.527) and number of seeds in a pod (0.241). These findings were in agreement with previous research (38, 39). A positive correlation was also detected among days to initial flowering and single-plant yield (0.214). Earliness associated with yield was found to be particularly advantageous, as it enables the crop to complete its life cycle with available moisture and minimal irrigation while requiring fewer management practices for pest and disease control. This finding suggested that selecting these morphological traits could be highly effective for yield improvement in mung bean.

Principal component analysis

PCA accounted for more than 70 % of the total variability (74.32 %) across the first three components (Table 3 and Fig. 1), which were consistent with previous findings (40, 41). The eigenvalues for PC1, PC2 and PC3 were 2.96, 1.60 and 1.39, respectively. PC1 explained 37.01 % of the total variation and was strongly influenced by days to first flowering (0.773), days to 50 % flowering (0.740), number of pods in a plant (0.708) and plant height (0.653) which were similar with observations of previous studies (42, 43). PC2 contributed 19.95 % of the total variation, with single-plant yield (0.698) being the dominant contributing trait aligning with previous findings (15). PC3 explained 17.36 % of the total variation, primarily influenced by pod length (0.542), number of seeds in a pod (0.190) and 100-seed weight (0.514). The results for 100-seed weight, pod length and number of seeds per pod were consistent with previous reports (40, 44).

Cluster analysis

Agglomerative cluster analysis, organised 117 entries into two main clusters. The clustering was based on genetic diversity rather than geographic origin, as observed in previous studies (45). Cluster I**,** consisting of 22 genotypes, exhibited the minimum mean value for days to first flowering (32 days) and days to 50 % flowering (44 days) but showed the highest mean

Table 2. Genotypic correlations between the dependent trait (yield) and seven independent traits of 117 mung bean accessions

	PH	DFF	DFPF	PPP	PL	SPP	HSW	SPY
PH	1							
DFF	0.298**	1						
DFPF	0.250**	0.791**	1					
PPP	0.515**	0.278**	0.260**	1				
PL	-0.105	-0.240*	-0.239*	-0.221*	1			
SPP	0.124	0.222*	0.197*	-0.204*	0.021	1		
HSW	-0.261**	-0.332**	-0.295**	-0.333**	0.561**	-0.011	1	
SPY	0.527**	0.214*	0.187	0.717**	-0.004	0.241**	0.038	1

*,** Significant at 5 and 1 % probability respectively. PH: Plant height, DFF: Days to first flowering, DFPF: Days to fifty per cent flowering, PPP: Number of pods in a plant, PL: Pod length, SPP: Number of seeds in a pod, HSW: Hundred seed weight, SPY: Single plant yield

Table 3. Component loadings, Eigenvalues and percent contributions of the first three components from PCA

Characters	Eigen vectors		
	PC1	PC2	PC3
Plant height	0.653	0.425	-0.019
Days to first flowering	0.773	-0.413	0.364
Days to fifty per cent flowering	0.740	-0.432	0.371
Number of pods in a plant	0.708	0.499	-0.336
Pod length	-0.443	0.412	0.542
Number of seeds in a pod	0.190	-0.069	0.650
Hundred seed weight	-0.551	0.384	0.514
Single plant yield	0.594	0.698	0.158
Eigen value	2.96	1.60	1.39
Proportion of variation	37.01	19.95	17.36
Cumulative proportion	37.01	56.96	74.32

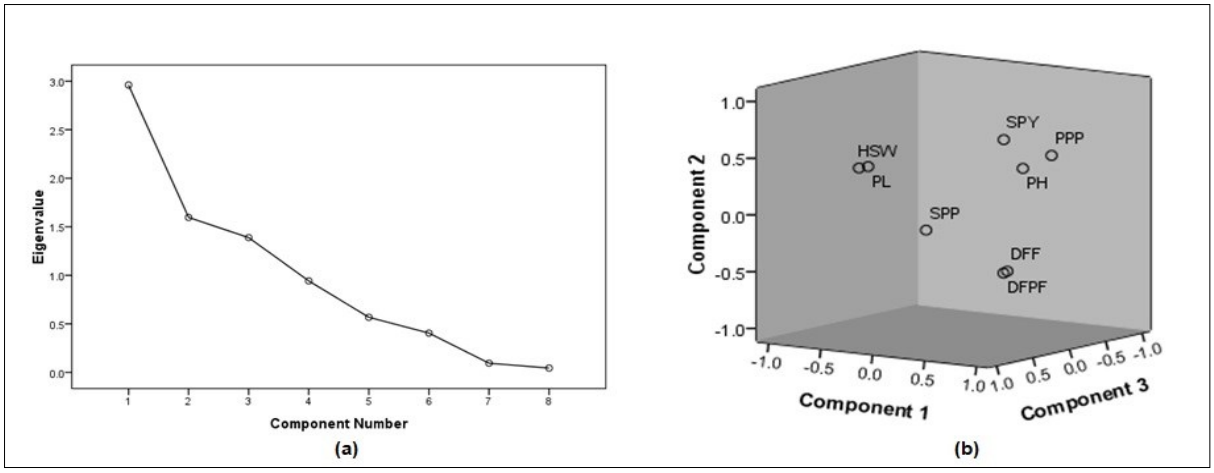

Fig. 1. (a) Scree plot showing the distribution of the principal components and their corresponding eigenvalues (b) Three-dimensional component plot illustrate the grouping pattern revealed by PCA

values for pod length (9.58 cm) and 100-seed weight (4.62 g). Cluster II**,** which contained 95 genotypes, was further subdivided into three sub-clusters: Sub-cluster IIa**:** Contained 20 genotypes with the highest mean from number of seeds in a pod (11.81). Sub-cluster IIb**:** Comprised 53 genotypes with moderate yield potential. Sub-cluster IIc**:** Confined 27 accessions with the maximum mean for plant height (46.69 cm), number of pods in a plant (45.73) and individual plant yield (14.25 g). The expansion of extra-early varieties with increased pod length and bold seeds could be achieved by utilizing genotypes from Cluster I. Selection of genotypes from sub-cluster IIc could facilitate the development of tall plants characterized by a higher number of pods and improved individual plant yield. Hybridization between genotypes from Cluster I and Sub-cluster IIc was expected to produce varieties with a shorter duration and enhanced yield potential. Similar clustering patterns were observed in previous studies, where 41 mung bean accessions into five different clusters. Cluster I exhibited the greater values for seeds per pod and individual plant yield, while Cluster II recorded the lowest values for days to flowering (46). It was also observed that high-yielding genotypes were assembled into separate clusters (15, 47).

Genotypic variation based on SSR markers analysis

The assessment of molecular diversity among accessions was a crucial factor in selecting appropriate materials for breeding programs. SSR markers emerged as markers of choice for various applications, including genetic diversity studies. After evaluating the genetic diversity of germplasm at the morphological level, it was essential to quantify genetic relationships by assessing genetic distance and forming clusters based on relatedness. This approach helped to visualize genetic relatedness among individuals, tracing of geographic origins, dispersion and the selection of parents for hybridization. In this study, hierarchical clustering based on Ward's method was utilized, as it tends to produces balanced clusters without outlier accessions (48).

Seventy SSR primer pairs were utilized to evaluate the genetic diversity among 117 mung bean accessions. Of these, thirteen primers were polymorphic, resulting in detection of 55 alleles, averaging 4.23 alleles per locus. Each locus consisted of three to six alleles. These findings were consistent with previous reports (49, 50). The mean PIC value was 0.71, ranging from 0.52 (CEDG008) to 0.79 (CEDG269) (Fig. 2). The clustering of genotypes did not correlate with geographical distribution. While both morphological and molecular analyses grouped the accessions into clusters- two in morphological and three in molecular. The molecular data provided more refined sub-clustering. These results underscored the limitations of relying solely on morphological traits to assess genetic diversity, as such traits are often influenced by environmental conditions and are governed by non-heritable genes (51). Although phenotypic analysis displayed less differentiation compared to genotypic analysis, it remains valuable for the quick and easy identification of genotypes. This study highlighted the importance of partitioning mung bean genotypes based on both morphological and molecular analyses, as reported in previous studies (41, 52).

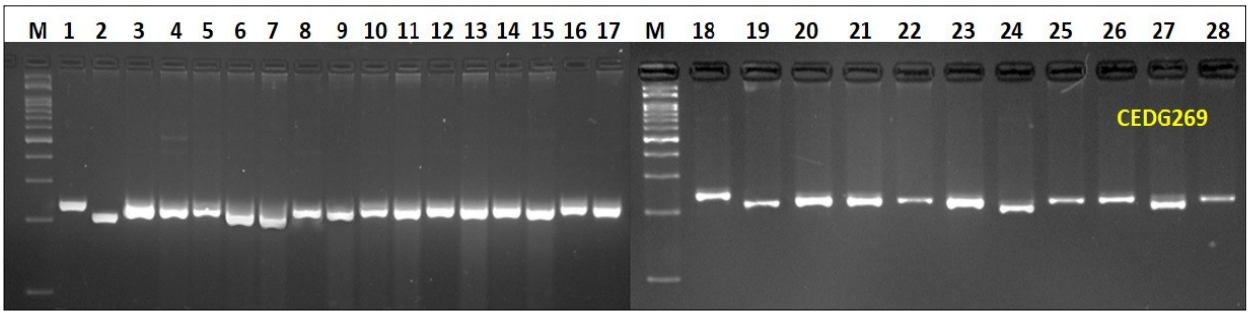

Fig. 2. PCR amplification of the SSR marker CEDG269 in mung bean accessions

Note: M - Ladder (100 bp); 1- K. Pudur 1; 2- K. Pudur 2; 3- K. Pudur 3; 4- Agasthilingapuram; 5- Coimbatore Local Bold; 6- Vilathikulam; 7- Kovilpatti; 8- Kangeyam; 9- Srivilliputhur; 10- Pusavishal; 11- T.V. Malai/1; 12- Maduramoong; 13- S 4; 14- Velampatti; 15- Rajendram; 16- K 1; 17 - T.V. Malai; 18- Salem 1; 19- Sonamoong; 20- LM 294; 21- Pusa 118; 22- AVT/RMI 6/1; 23- HG 19A; 24- SML 1168; 25- PDM 54-1; 26- SML 134; 27- MS 9721; 28- PDM 239.

Conclusion

The present study demonstrated the genetic diversity among 117 mung bean accessions. Plant height and the number of pods in a plant exhibited a highly substantial positive association with single-plant yield and contributed significantly to the variation captured in the first principal component. Thus, selecting for plant height and the number of pods per plant appeared to be a highly effective strategy for enhancing yield in mung bean. Clustering of mung bean accessions based on morphological traits and SSR markers provided valuable insights into the germplasm. The highly diverse accessions identified in this study could serve as potential candidates in future mung bean breeding programs aimed at emerging trait-specific genotypes.

Authors' contributions

MD, JHS and SSM, conceived and designed methods and experiments. SSM and MD managed the fieldwork. SSM and KA conducted phenotype screening. SSM, DM and KA performed the data analysis. MD, JHS and SN provided input and suggestions on experimental design. SSM, KA and JHS drafted the manuscript. All authors contributed to the article and approved the submitted version.

Compliance with ethical standards

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical issues: None

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