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# Genetic Diversity and Population Structure Analyses in Mungbean (*Vigna radiata* L. Wilczek)

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## Abstract

Mungbean (*Vigna radiata* L. Wilczek) is an important protein rich pulse crop produced globally, with high nutritional and economic value. In the present study, a set of 35 simple sequence repeat (SSR) markers was used to analyze 48 mungbean genotypes including released varieties and advanced breeding lines. The major allele frequency ranged from 0.13 to 0.60 with an average of 0.36. Similarly, the gene diversity varied between 0.32 and 0.90, whereas polymorphism information content (PIC) ranged from 0.30 to 0.90 with the average of 0.75. Further, model-based clustering generated five distinct subpopulations, with a maximum of 18 genotypes forming subpopulation-IV, while the subpopulation-V contained the least number of genotypes (3). Five genotypes were considered as admixtures. Four principal components (PCs1 to 4) were extracted from the original data and had latent roots greater than one, accounting for nearly 75.00% of the total variation. The maximum Eigen root value (3.53) was recorded for the 1$^{st}$ PC, which explained 32.12% variation. The rest three PCs (2$^{nd}$, 3$^{rd}$ and 4$^{th}$) explained 26.18%, 9.67% and 7.91 individual variation. Of these seven clusters, cluster VII comprised eighteen genotypes, forming the largest group followed by cluster I comprised seventeen genotypes. Clusters IV, V and VI each contained two genotypes, whereas cluster III comprised of six genotypes. Cluster II harbored a single genotype. The clustering patterns resulting from hierarchical clustering based on phenotypic scores remained in close agreement with STRUCTURE and UPGMA clustering. The informative SSR markers identified in this study will be great supplementary tools for hybridization programs in mungbean breeding.

## Keywords

Mungbean, Simple sequence repeat markers, Population structure, Polymorphic information content, Germplasm

## Introduction

Mungbean is a self-pollinated diploid species having chromosome number of 2n=22 with an estimated genome size of about 543 Mb [1, 2]. Like other legumes, it also improves the soil health by fixing the atmospheric nitrogen into the soil and increase the yield of subsequent crop [3]. Despite its importance, the productivity of mungbean is still poor, which may be due to lack of synchronous maturity and high-yielding varieties. Increasing yield gains in mungbean warrants accelerated development of new high-yielding verities with synchronous maturity. Deployment of such mungbean cultivars in rice-wheat cropping system would help expand the area of mungbean without reducing the area under other crops. Trait association analysis and genetic variation studies based on morphological features has been performed by several researchers in mungbean for yield improvement [4] gave more emphasis on selection indices followed by correlation studies, which will be more powerful approach to discriminate the genotypes for yield improvement.

In recent years, genomics approaches have emerged as great support to phenotypic evaluation for targeted trait improvement. The small genome size makes it an attractive and valuable model for advancing the understanding of diversity and evolution of legumes such as mungbean. Reducing crop duration, enhancing nutritional quality, introgressing disease resistance genes and increasing climate resilience remain the prime objectives of mungbean breeding. Prior to the availability of draft genome of mungbean, several workers demonstrated transferability of markers from other legume crops to mungbean, and more than thousand DNA markers now available for genotyping applications in mungbean. Many reports are available on the use of molecular markers for estimation of genetic variability of mungbean cultivars such as RAPD, SSR, ISSR, RGA, etc. Several workers successfully utilized the SSR markers in developing mungbean maps [5]. Due to co-dominant nature, multiple allelism, high polymorphism and reproducibility of SSRs have been extensively used in genetic studies across a broad range of crops [6]. The molecular breeding approach is now being increasingly adopted for accelerated improvement of important traits in plants. Keeping the above in view, this study was undertaken to assess the genetic variation and population structure of the 48 mungbean elite lines using thirty-five SSRs.

# Materials and Methods

## Plant materials and field experimentation

The plant materials comprised of 48 mungbean genotypes including released varieties and elite lines (Table 1). The experiment was conducted at natural field conditions at Rajaula Research Farm of Nana Ji Deshmukh New Agricultural Campus, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidhyalaya, Chitrakoot, Satna, Madhya Pradesh, India. The recommended package of cultural practices was followed to raise a healthy crop. Phenotypic observations were made on eleven traits including yield and component traits like plant height (cm) (PH), number of branches per plant (NBP), number of clusters per plant (NCP), number of pods per plant (NPP), number of seeds per pod (NSP), 100-seed weight (g) (SI), seed yield per plant (g) (SYP), biological yield per plant (g) (BY) and harvest index (%) (HI) based on five randomly selected plants in each genotype; whereas two traits viz., days to 50% flowering (DFF), days to maturity (DM) were recorded on plot basis [7].

**Table 1:** List of the mungbean genotypes used for analysis in the present study.

| S. No. | Genotypes | Origin/Source |
|---|---|---|
| 1 | Samrat | IIPR, Kanpur |
| 2 | PDM 04-123 | IIPR, Kanpur |
| 3 | PDM 281 | IIPR, Kanpur |
| 4 | PDM 54 | IIPR, Kanpur |
| 5 | PDM 262 | IIPR, Kanpur |
| 6 | PDM 288 | IIPR, Kanpur |
| 7 | PDM 178 | IIPR, Kanpur |
| 8 | PDM 191 | IIPR, Kanpur |
| 9 | IPM 02-14 | IIPR, Kanpur |
| 10 | IPM 06-5 | IIPR, Kanpur |
| 11 | IPM 409-4 | IIPR, Kanpur |
| 12 | IPM 312-43K | IIPR, Kanpur |
| 13 | Meha | IIPR, Kanpur |
| 14 | IPM 2K–14-9 | IIPR, Kanpur |
| 15 | IPM 205-7 | IIPR, Kanpur |
| 16 | IPM 2-23 | IIPR, Kanpur |
| 17 | IPM 03-1 | IIPR, Kanpur |
| 18 | IPM 03-3 | IIPR, Kanpur |
| 19 | IPM 02-17 | IIPR, Kanpur |
| 20 | IPM 02–3-1 | IIPR, Kanpur |
| 21 | IPM 02-16 | IIPR, Kanpur |
| 22 | IPM 02-3 | IIPR, Kanpur |
| 23 | IPM 03-2 | IIPR, Kanpur |
| 24 | IPM 02-19 | IIPR, Kanpur |
| 25 | ML 818 | PAU, Ludhiana |
| 26 | ML 5 | PAU, Ludhiana |
| 27 | ML 512 | PAU, Ludhiana |
| 28 | ML 515 | PAU, Ludhiana |
| 29 | ML 682 | PAU, Ludhiana |
| 30 | ML 729 | PAU, Ludhiana |
| 31 | ML 935 | PAU, Ludhiana |
| 32 | ML 1059 | PAU, Ludhiana |
| 33 | ML 1256 | PAU, Ludhiana |
| 34 | ML 1257 | PAU, Ludhiana |
| 35 | AKM 99-4 | PDKV, Akola |
| 36 | AKM 96-1 | PDKV, Akola |
| 37 | AKM 96-2 | PDKV, Akola |
| 38 | EC 393410 | Exotic collection |
| 39 | Prateeksha Nepal | AVRDC, Taiwan |
| 40 | EC 398894 | Exotic collection |
| 41 | Pusa Vishal | IARI, New Delhi |
| 42 | IPM 312-394 | IIPR, Kanpur |
| 43 | Taram 1 | BARC, Trombay |
| 44 | Taram 18 | BARC, Trombay |
| 45 | TMB 37 | BARC, Trombay |
| 46 | TMB 96-2 | BARC, Trombay |
| 47 | RMG 991 | RAU, Pusa |
| 48 | MG 331 | Gurdaspur, Panjab |

## Molecular characterization

We selected a set of 35 SSRs from different *Vigna* species for conducting the diversity analysis. Genomic DNA was extracted from the young leaves following the Cetyl Tri-methyl Ammonium Bromide method [8]. The quality of the extracted DNA was equated on 0.8% agarose gel and the quantity was determined using Nanodrop spectrophotometer ND 1000 (Nanodrop Technologies, DE, USA).The PCR amplification was carried out in 10 µl reaction mixture containing 10X Taq buffer with 20 mM $MgCl_2$, 2 mM dNTPs and 1U of Taq DNA polymerase (Fermentas, Mumbai), 50 ng template DNA and 5 pmol each of forward and reverse primers (ILS, India) in a thermocycler. PCR conditions were programmed at initial denaturation for 3 min at 94 ˚C, followed by 30 cycles of denaturation for 30 sec at 94 ˚C, annealing for 30 sec at 45 - 55 ˚C (primer specific), extension at 72 ˚C for 1 min and final extension of 72 ˚C for 7 min. The PCR products thus obtained were resolved on 3% agarose gel in 1X TAE buffer and stained with ethidium bromide.

## Statistical analysis

To establish the patterns of morphological variation, prin-

cipal component analyses (PCA) were conducted with SAS ver.9.2 (SAS Institute, 1996, Cary, NC) [9]. To assess the genetic variation among genetic groups, histograms were built with standard error (SE) estimated for 11 quantitative traits using agricolae package of R software [10].

Amplified fragments were scored and subjected to analysis using Power Marker ver. 3.25 [11]. Estimation of the hidden population was done by multi-locus Bayesian analysis using STRUCTURE ver. 2.3.4 [12]. The optimum number of populations (ΔK) was calculated using a web-based program Structure Harvester [13]. The cluster analysis was done by DARwin ver. 6.0.014 for evaluating the phylogenetic relationship among characterized genotypes. The product size of SSRs was further used to formulate the genetic groups using unweighted pair group method of arithmetic average (UPGMA) through DARwin ver.6.0.14 [14].

Duncan's Multiple Range Test was used to clarify significance among six groups (Five genetic groups and an admixed group) by population structure analysis using structure software [12] for the 11 phenotypic traits data, such as DF, DM, PH, NBP, NCP, NPP, NSP, SI, SYP, BY and HI.

# Results and Discussion

## Genetic variation and trait association

Maximum SYP was recorded for IPM 06 - 5 (11.21) and IPM 2K 14 - 9 (11.10), while less SYP. Yield is a very complex trait, and it is cumulative effects of independent associated traits. High genetic variation offers more chance to select the traits and genotypes for its manipulation for yield improvement. The analysis of variance revealed significant variation for all traits considered in the present study [15]. Knowledge of trait associations would help to identify the important yield component traits to be selected for yield improvement during selection. Correlation coefficients were worked out between eleven yield component traits with seed yield and among themselves. Seed yield showed positive significant association with branches per plant, pods per plant, clusters per plant, SI, HI, whereas PH, DFF and DM were negatively associated with seed yield.

## PCA

Four PCs (PCs1 to 4) were extracted from the original data and having latent roots greater than one, accounting nearly 75.00% of the total variation (Table 2 and figure 1) as similar reported by SAS Institute [8]. The maximum Eigen root value (3.53) was recorded for the 1st PC, which explained 32.12% variation. The rest three PCs (2nd, 3rd, and 4th) explained 26.18%, 9.67% and 7.91 individual variation. The first PC was predominantly related to yield and yield contributing traits like NPP, NCP, SYP, NBP and BY, which indicated that these components were proven more important towards the genetic diversity. Whereas, the second PC contrast variables were related solely to DFF, DM and PH which are associated with reproductive development and accounted for 26.18% of total variation. The third principal component was named seed weight component since positively correlated with SI. This factor accounted for 9.67%. The fourth PC accounted for 7.91% of the variation. In this component, correlation of seed yield and its components were highly positive. Because of that, this component entitled as factor seed yield [16] of PCA indicated that these traits are important for trait manipulation, and the genetic diversity in this population could be credited to these traits.

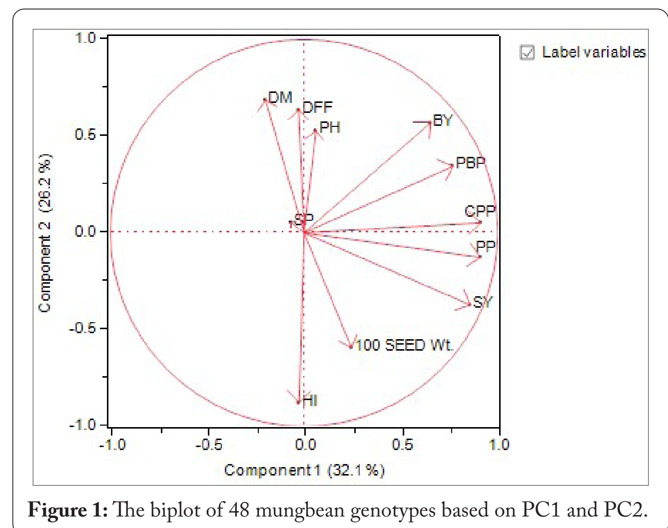


**Figure 1:** The biplot of 48 mungbean genotypes based on PC1 and PC2.

**Table 2:** Eigenvalues, variability, and correlation coefficient of each trait with respect to its PCs.

| PCs | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| Eigene value (root) | 3.53 | 2.88 | 1.06 | 0.87 |
| %Var. exp. | 32.12 | 26.18 | 9.67 | 7.91 |
| Cum. var. exp. | 32.12 | 58.29 | 67.96 | 75.87 |
| DFF | -0.03 | 0.64 | -0.15 | 0.12 |
| DM | -0.20 | 0.69 | -0.14 | -0.22 |
| PH | 0.06 | 0.53 | 0.18 | 0.71 |
| NBP | 0.77 | 0.34 | -0.03 | -0.11 |
| NPP | 0.91 | -0.13 | -0.02 | 0.10 |
| NSP | -0.07 | 0.05 | 0.95 | -0.22 |
| SI | 0.24 | -0.59 | -0.25 | -0.23 |
| NCP | 0.91 | 0.05 | -0.01 | 0.19 |
| BY | 0.65 | 0.57 | 0.05 | -0.29 |
| HI | -0.02 | -0.88 | 0.09 | 0.26 |
| SYP | 0.86 | -0.37 | 0.11 | -0.02 |

## Cluster analysis

After reducing the dimension, eight PCs were transformed into a single index and data were subjected to non-hierarchical euclidean clustering. The clustering categorized the 48 mungbean genotypes into seven distinct clusters (Table 3). Of these, cluster VII comprised eighteen genotypes, forming the largest group followed by cluster I comprised seventeen genotypes. Clusters IV, V and VI each contained two genotypes, whereas cluster III comprised of six genotypes. Cluster II harbored a single genotype.

## Genetic variation studies

Genotyping variation was estimated using SSR loci generating valuable information about the diversity parameters such as major allelic frequency (MAF), number of allele (NA), gene diversity (GD) and polymorphic information content (PIC) (Table 4). Based on 35 polymorphic SSRs, a total of 336 alleles were obtained across 48 mungbean genotypes. The number of alleles ranged from 4 - 16 with a mean of 9.60 alleles/ locus. GD and PIC values ranged between 0.32 - 0.92 and 0.30 - 0.90 with the mean value of 0.77 and 0.75, respectively.

## Population structure analysis

Population genetic structure of 48 mungbean genotypes was carried out and K = 5 was found to converge well than K = 2 - 10. Forty-eight mungbean genotypes were categorized into the five groups (Figure 2), representing 25%, 12.5%, 18.75%, 37.75% and 6.25%, respectively. Subpopulation-I included 12 genotypes, whereas subpopulation-II was represented by 6 genotypes. The subpopulations III, IV and V included 9, 18 and 3 genotypes, respectively. Membership fractions were used to classify these populations as pure or an admixture. Subpopulation-I showed nine pure (75.00%) and three admixed (25.00%) individuals, subpopulation-II showed six pure (100.00%) individuals, subpopulation-III showed five pure (55.55%) and four (44.44%) admixed individuals, subpopulation-IV showed eighteen pure (100.00%) individuals, and subpopulation-V contained one pure (33.33%) and two (66.67%) admixed individuals. To explore the genetic variation among subpopulations for yield and yield contributing traits, mean performance was calculated over two years (Figure 3). DFF ranged from 60 to 78 days represented by subpopulation-I and III, respectively. PH ranged from 34.02 to 39.47 cm assigned for subpopulation-II and III, respectively. Likewise, SY and SI ranged from 5.14 g to 11.71 g and 2.10 g to 4.62 g, respectively. This also noted significant variation in SI (2.10 - 4.62 g) and NSP (8.54 - 11.88) (Table 5).

## Neighbor-joining UPGMA analyses

The SSR data were further used to study genetic diversity based on NJ-UPGMA analysis (Figure 4). Interestingly, this analysis exhibited a similar pattern as structure analysis revealing five different clusters. Considerable differences were observed among the genotypes belonging to different geographical regions.

Genetic variation and association studies are very important for yield improvement. The direction and magnitude of association of traits with seed yield helps prioritize traits for plant selections. Several research groups have performed genetic diversity studies based on their morphological traits and DNA markers. Pattern of positive relationship of seed yield with its yield component traits have been reported earlier by Siddique et al. [17] for SI, NSP and PL; Biradar et al. [18] for NCP; Rahim et al. [19] for NSP and SI; Tabasum et al. [20] for NCP, PH, NSP and SI; Khajudparn and Tantasawat [21] for PL and NSP and Singh et al. [22] for PL, SI and HI; Singh et al. [23] for NSP and PL; for NBP, NCP, NSP, SI and HI [24-26]. The positive association between number of branches and other agro-morphological traits suggested that the genotypes with more NBP had more NCP, PH, high SP, SI and HI. Such trait correlations may help accelerate yield gains via assisting indirect selections. Thus, these yield component traits may be taken into consideration during the selection for yield improvement of mungbean [27-29].

Results of PCA suggested a substantial contribution of different traits such as NPP, NCP, SYP, NBP, and BY based

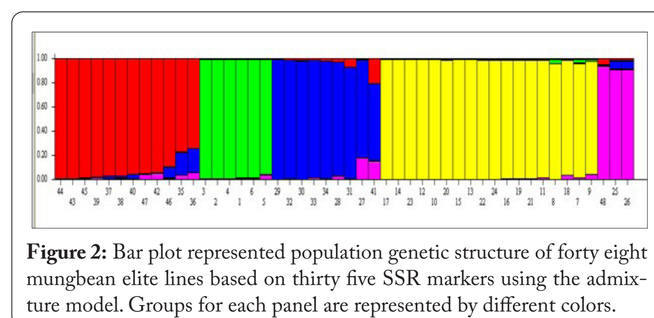

**Figure 2:** Bar plot represented population genetic structure of forty eight mungbean elite lines based on thirty five SSR markers using the admixture model. Groups for each panel are represented by different colors.

**Table 3:** Distribution of 48 mungbean genotypes based on quantitative parameters.

| Cluster no. | Frequency | Name of the genotypes |
|---|---|---|
| I | 17 | Samrat, IPM 312-43K, IPM 03-2, AKM 96-1, AKM 96-2, EC 393410, PDM 04-123, PDM 288, Pusa Vishal, IPM 03-3, ML 515, IPM 02-3, ML 935, IPM 06-5, TMB 96-2, PRATEEKSHA NEPAL, TMB 37 |
| II | 1 | IPM 2K-14-9 |
| III | 6 | PDM 281, IPM 312-394, IPM 02-14, IPM 02-19, Meha, AKM 99-4 |
| IV | 2 | ML 682, EC 398894 |
| V | 2 | IPM 409-4, IPM 205-7 |
| VI | 2 | Taram 1, Taram 18 |
| VII | 18 | PDM 54, PDM 262, IPM 02-16, ML 818, ML 512, PDM 178, IPM 02-17, MG 331, PDM 191, ML 1059, ML 1256, ML 1257, IPM 2-23, IPM 02-3-1, ML 5, IPM 03-1, ML 729, RMG 991 |

**Table 4:** Details of diversity statistics for each microsatellite marker.

| Marker name | MAF | NA | GD | PIC |
|---|---|---|---|---|
| MM1 | 0.14 | 7.00 | 0.86 | 0.84 |
| MM2 | 0.73 | 7.00 | 0.46 | 0.44 |
| MM3 | 0.22 | 9.00 | 0.84 | 0.82 |
| MM4 | 0.60 | 9.00 | 0.61 | 0.59 |
| MM5 | 0.53 | 11.00 | 0.69 | 0.68 |
| MM6 | 0.30 | 11.00 | 0.84 | 0.83 |
| MM7 | 0.26 | 8.00 | 0.83 | 0.81 |
| MM8 | 0.33 | 7.00 | 0.78 | 0.75 |
| MM9 | 0.47 | 7.00 | 0.71 | 0.68 |
| MM10 | 0.82 | 4.00 | 0.32 | 0.30 |
| MM11 | 0.49 | 9.00 | 0.72 | 0.69 |
| MM12 | 0.33 | 7.00 | 0.81 | 0.78 |
| MM13 | 0.21 | 13.00 | 0.89 | 0.89 |
| MM14 | 0.29 | 10.00 | 0.85 | 0.83 |
| MM15 | 0.42 | 9.00 | 0.77 | 0.75 |
| MM16 | 0.13 | 16.00 | 0.92 | 0.92 |
| MM17 | 0.25 | 11.00 | 0.85 | 0.84 |
| MM18 | 0.23 | 8.00 | 0.85 | 0.83 |
| MM19 | 0.35 | 7.00 | 0.79 | 0.76 |
| MM20 | 0.48 | 6.00 | 0.69 | 0.65 |
| MM21 | 0.25 | 9.00 | 0.84 | 0.82 |
| MM22 | 0.46 | 11.00 | 0.74 | 0.72 |
| MM23 | 0.23 | 14.00 | 0.88 | 0.87 |
| MM24 | 0.47 | 7.00 | 0.72 | 0.69 |
| MM25 | 0.24 | 13.00 | 0.88 | 0.87 |
| MM26 | 0.45 | 7.00 | 0.67 | 0.62 |
| MM27 | 0.21 | 9.00 | 0.86 | 0.84 |
| MM28 | 0.39 | 10.00 | 0.79 | 0.78 |
| MM29 | 0.29 | 11.00 | 0.84 | 0.83 |
| MM30 | 0.45 | 8.00 | 0.73 | 0.70 |
| MM31 | 0.30 | 11.00 | 0.84 | 0.83 |
| MM32 | 0.13 | 13.00 | 0.90 | 0.90 |
| MM33 | 0.18 | 16.00 | 0.90 | 0.90 |
| MM34 | 0.67 | 9.00 | 0.53 | 0.50 |
| MM35 | 0.23 | 12.00 | 0.88 | 0.87 |
| Mean | 0.36 | 9.60 | 0.77 | 0.75 |

**Figure 3:** Subpopulations mean of yield and its component traits.

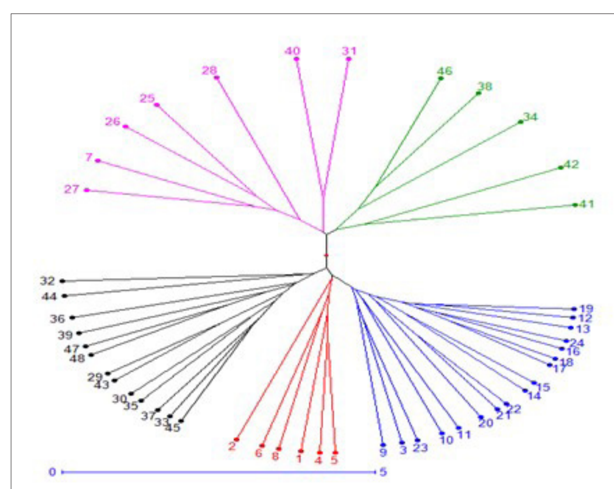

**Figure 4:** UPGMA clustering of forty-eight mungbean elite lines obtained from thirty five SSR markers.

on respective variation [30-33]. Researchers also found a positive high weight for DFF and DM [34], whereas others found high positive weight for DFF, PH, NCP, and SI [35]. Similarly high weight for NSP has earlier been reported by Mehandi et al. [36]. This deviation may be noted due to differences in germplasm lines of mungbean and growing environment and characters studied as well.

A total of 336 alleles were obtained across 48 mungbean genotypes using SSR markers, which is higher than earlier published reports in mungbean [31, 32]. Adequate amount of genetic variation was noticed among the lines. In general, high levels of DNA polymorphism is enabled by SSRs, which is believed to have contributed to higher genetic dissimilarity as also observed by Mehandi et al. [33].

The clustering arrangements arising from non-hierarchical clustering showed concordance with the patterns inferred

**Table 5:** Duncan's multiple range test and analysis of variance of population structure analysis.

| Traits | DFF | DM | PH | NBP | NCP | PC | NSP | SI | SYP | BY | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 35.91[a] | 70.15[a] | 39.68[a] | 1.99[a] | 6.69[a] | 26.69[a] | 9.60[a] | 3.59[a] | 8.04[c] | 26.62[a] | 31.09[a] |
| Group 2 | 37.20[b] | 70.28[a] | 39.64[a] | 2.41[a] | 6.39[a] | 25.99[a] | 9.87[a] | 3.88[a] | 8.25[c] | 26.47[a] | 31.63[a] |
| Group 3 | 41.38[c] | 75.98[a] | 43.65[a] | 2.31[a] | 6.30[a] | 24.53[a] | 10.03[a] | 3.72[a] | 7.42[b] | 28.26[a] | 26.77[a] |
| Group 4 | 37.94[a] | 69.76[a] | 41.37[a] | 2.57[a] | 6.58[a] | 27.01[a] | 10.13[a] | 3.78[a] | 7.96[b] | 27.78[a] | 29.35[a] |
| Group 5 | 36.33[a] | 70.44[b] | 43.14[a] | 2.14[a] | 5.41[a] | 20.17[a] | 10.32[a] | 3.69[a] | 6.53[a] | 29.16[a] | 23.14[a] |
| Admixture | 34.10[d] | 68.80[a] | 41.99[a] | 2.31[a] | 6.43[b] | 27.96[b] | 9.56[a] | 4.02[a] | 8.18[c] | 26.13[a] | 33.13[a] |
| C.D. @ 5% | 1.60 | 1.99 | 2.35 | 1.81 | 0.75 | 2.78 | 1.32 | 0.96 | 0.53 | 2.98 | 5.48 |
| SE(d) | 0.71 | 0.88 | 1.04 | 0.21 | 0.33 | 1.23 | 0.49 | 0.13 | 0.24 | 1.63 | 2.43 |
| C.V. | 2.34 | 1.52 | 3.07 | 10.97 | 6.48 | 5.94 | 6.05 | 4.12 | 3.73 | 7.26 | 10.19 |
| F value | 23.81 | 16.88 | 5.29 | 1.94 | 3.76 | 10.37 | 0.75 | 2.80 | 15.45 | 1.07 | 4.58 |

from STRUCTURE and NJ-UPGMA clustering. Placement of genotypes within the same cluster points to a possibility of their shared ancestry [34]. It is interesting to note that we corroborated inference obtained from phenotyping analysis with the marker genotyping data. We anticipate that 35 microsatellite markers used in the study were successful in detecting genetic variation of the mungbean population. In this study, microsatellite markers were proved successful to detect genetic variation in mungbean genotypes for reasonable management and crossbreeding purposes [35, 36].

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## Conflict of Interest

The authors have declared no conflict of interest.

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