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SHORT RESEARCH ARTICLE

Assessment of genetic diversity and population structure in mungbean [*Vigna radiata* (L.) Wilczek] local collections and prominent varieties using SSR markers

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Abstract

Mungbean (*Vigna radiata* L.) is an important legume crop in South and Southeast Asia. Assessment of genetic diversity is vital for species diversity conservation, and crop improvement programs. The present study assessed the genetic diversity of 87 germplasm collections, including 12 released varieties using SSR markers. Of the 35 markers screened, 19 were informative, generating 35 alleles with an average of 4.11 alleles per locus. PIC values ranged from 0.24 to 0.75 and expected heterozygosity averaged 0.47 for local collections and 0.41 for released varieties. Genetic variation was primarily within populations (91.3%), with low population divergence (Fst = 0.12). Local collections were more genetically diverse, offering valuable resources for future breeding programs.

Keywords: Greengram, genetic structure, genetic diversity, narrow genetic base.

Mungbean (*Vigna radiata* L. Wilczek) which is popularly known as greengram, is a grain legume crop of the Fabaceae family. India contributes 60% of global production from 3.8 mha (Somta et al. 2022, Nair and Schreinemachers 2020). Its short life cycle and adaptability to diverse agroecosystems make it valuable for sustainable agriculture. Mungbeans is also crucial for nutritional security, providing high-quality protein, vitamins, and essential minerals (Saini et al. 2010). However, its stagnant productivity due to limited genetic diversity utilization poses challenges, making the crop vulnerable to biotic and abiotic stresses (Gayacharan et al. 2023; Bhardwaj et al. 2023). Despite the availability of over 43,000 conserved germplasm collections globally, many remain underutilized (Gayacharan et al. 2020a, 2020b). Identifying genetic divergence through molecular analysis is essential for advancing breeding programs and understanding genetic connections. Therefore, this study aims to assess the genetic diversity and population structure of local mungbean collections compared to popular released varieties using SSR markers. The findings are expected to find effective conservation strategies and breeding programs, contributing to the crop's improvement and sustainable cultivation in diverse agroecosystems.

A total of 87 diverse mungbean genotypes, including local collections and released varieties obtained from the National Genebank NBPGR, New Delhi were selected for this study. Genotypes represented the major mungbean-growing regions of India. The experiment was conducted at the NBPGR experimental farm. DNA was extracted using the Qiagen DNeasy kit, and quantified withNanoPhotometer®. Out of 35 SSR primer pairs, 19 were polymorphic and selected for diversity analysis. PCR amplification was performed, and DNA fragments were resolved in 4% agarose gel. The polymorphism information content (PIC) for each primer was calculated to assess marker usefulness (Anderson et al. 1993). The dendrogram and principal coordinate analysis (PCoA) was performed using MEGA (Zargar et al. 2016) to visualize genotype diversity. Population structure analysis

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Table 1. Summary of allelic information and genetic diversity parameters for 19 SSR primer pairs used on 87 genotypes of mungbean

Marker	PIC	Na	Ne	Maf	Ho	He	I
MB56315	0.71	5.00	3.22	0.34	0.00	0.75	1.26
MB15469	0.24	4.00	1.08	0.86	0.00	0.25	0.15
MB33094	0.48	4.00	2.07	0.49	0.00	0.57	0.76
MB26622	0.58	4.00	1.74	0.54	0.00	0.63	0.75
MB29460	0.62	4.00	1.79	0.46	0.00	0.67	0.66
MB9543	0.60	5.00	2.53	0.52	0.00	0.65	1.05
MB26637	0.44	5.00	1.94	0.70	0.00	0.47	0.82
MB51985	0.67	5.00	2.57	0.40	0.00	0.72	1.04
MB13673	0.27	4.00	1.48	0.84	0.08	0.29	0.42
MB34120	0.42	3.00	1.66	0.67	0.00	0.49	0.59
MB15445	0.36	3.00	1.65	0.75	0.00	0.41	0.56
MB29754	0.42	3.00	1.61	0.61	0.05	0.51	0.53
MB22568	0.58	4.00	1.97	0.41	0.00	0.65	0.72
MB15212	0.69	4.00	2.56	0.33	0.00	0.74	1.00
MB11659	0.42	4.00	1.24	0.69	0.00	0.47	0.36
MB17985	0.42	3.00	1.69	0.68	0.00	0.48	0.61
MB22067	0.72	6.00	2.69	0.37	0.00	0.75	1.16
MB16610	0.53	4.00	1.89	0.61	0.00	0.57	0.75
MB19286	0.55	4.00	1.98	0.45	0.00	0.62	0.72
Mean	0.51	4.11	1.96	0.56	0.01	0.56	0.73
Pop 1		3.21	2.11		0.02	0.47	0.80
Pop 2		2.42	1.81		0.03	0.41	0.66

PIC = Polymorphism information content, Na = Average number of alleles per locus, Ne = Number of effective alleles per locus, Maf = Major Allele Frequency, Ho = Observed heterozygosity, He = Expected heterozygosity and I = Shannon's Information Index

was done using STRUCTURE 2.3.4 software (Pritchard et al. 2000) with Bayesian clustering and simulations across K values (1 to 10) to determine subpopulations (Evanno et al. 2005). Genetic variation was analyzed using F-statistics and AMOVA through GenAlEx 6.5 software (Peakall and Smouse, 2006) to determine population differentiation and source of variance. Population genetics indices, including allele number, major allele frequency, number of private alleles, effective alleles, expected heterozygosity (He), and Shannon's information index (I), were also computed.

Application of molecular markers for diversity analysis

Findings provide insights into the genetic relationships, diversity patterns, and population structure among mungbean genotypes. The robust use of SSR markers, coupled with advanced data analysis techniques, offers critical information for mungbean conservation and breeding strategies, guiding the selection of superior genotypes for crop improvement and enhancing genetic resource utilization. The SSR markers analysis generated 35 alleles with an average of 4.11 alleles per locus (Table 1). Key parameters such as gene diversity, major allele frequency (Maf), and polymorphism information content (PIC) highlighted the genetic variability present within the mungbean accessions. Gene diversity ranged from 0.25 (MB15469) to 0.75 (MB56315 and MB22067), while PIC values ranged from 0.24 to 0.71, with a mean of 0.51. High Fis values indicated strong inbreeding within populations, further corroborating mungbean self-pollinating behavior. The Fst values averaged 0.061, indicating moderate genetic differentiation between populations, with gene flow (Nm) values ranging from 1.10 to 182.13 across loci (Table 1).

Cluster and structure analysis

Cluster analysis using SSR data revealed two major clusters: Cluster I, comprising 19 accessions, and Cluster II containing 68 accessions. Sub-clustering further divided Cluster II into two groups (IIA and IIB) (Fig. 1). PCoA results were also similar to the dendrogram analysis (Fig. 2). The dendrogram as well as PCoA analysis showed genetic proximity among accessions and highlighted the genetic distinctiveness of certain mungbean genotypes(Figs 1 & 2). Notably, breeding lines and released varieties clustered together, while local collections formed distinct groups.

STRUCTURE analysis confirmed the existence of two major sub-populations (K = 2), irrespective of geographical origin. The unrestricted exchange of seeds between regions likely contributed to this pattern. Genotypes from both populations exhibited admixtures, as evidenced by mixed genomic regions in certain accessions.

Analysis of molecular variance (AMOVA) indicated that most

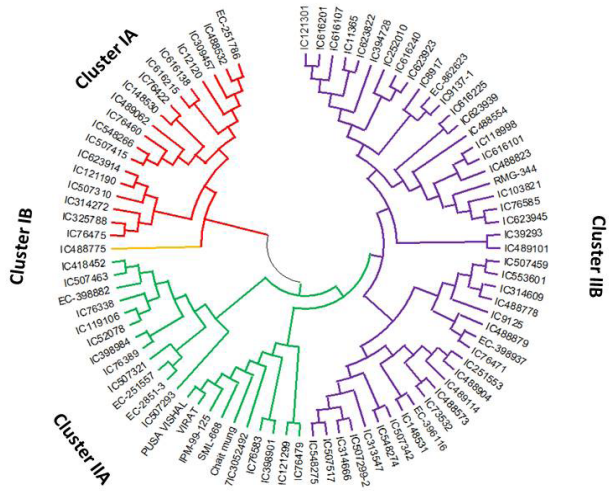

Fig. 1. Depiction of the relationship among 87 mungbean genotypes using the dendrogram generated by the neighbor-joining method of the MEGA software with the maximum composite likelihood substitution model using 19 polymorphic SSR markers

Table 2. AMOVA for two mungbean populations

Source of variation	df	Variance	%Variation	p-value
Among populations	1	0.27	4.83	0.001
Within populations	85	5.18	91.30	0.001
Within individual	87	0.22	3.85	0.001
Total	173	5.67		
F-Statistics	Fst	F'st	Nm	
	0.05	0.11	4.92	

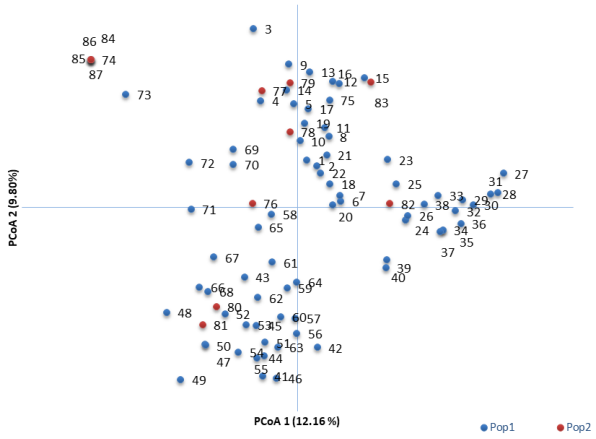

Fig. 2. PCoA of the mungbean accessions using SSR primer

genetic variation (91.3%) was within populations, with only 4.83% attributed to differences between populations and 3.85% within individuals. The low Fst value (0.048) further supported the finding of minimal genetic differentiation between populations (Table 2).

The study underscores the need to broaden the genetic base of the mungbean crop and the usefulness of the SSR markers in assessing genetic diversity. The identification of genetically diverse accessions may find their direct use in the mungbean improvement program. Local collections demonstrated higher genetic diversity, as reflected by higher allele counts, Shannon's information index (I), and private alleles compared to release varieties.

The findings suggest that conservation and breeding strategies should focus on maintaining genetic variability and enhancing gene flow within mungbean populations. The observed genetic diversity, despite the self-pollinating nature of mungbean, indicates that targeted breeding efforts can leverage this variability for improved crop varieties.

Authors' contribution

Conceptualization of research (G); Designing of the experiments (G, RB, SK); Contribution of experimental materials (G, AKS, RB); Execution of field/lab experiments and data collection (G, RB); Analysis of data and interpretation (G, RB); Preparation of the manuscript (G, RB).

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