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Assessment of genetic diversity among mungbean accessions using morphological and molecular markers

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Abstract
A paradox of mungbean breeding is that despite India being the crop's centre of diversity, the genetic base of released varieties has been steadily narrowing. This research assessed genetic diversity among 40 mungbean accessions from seven Indian states using 12 quantitative morphological traits and 20 SSR molecular markers. Morphological analysis using Mahalanobis D^2 distance grouped accessions into four clusters, with inter-cluster distance ranging from 14.8 to 42.6. Molecular analysis with SSR markers detected 78 alleles with a mean Polymorphism Information Content (PIC) of 0.54. UPGMA clustering based on molecular data largely confirmed morphological groupings, with 82.5% concordance. Accessions from the Western Ghats region (Karnataka, Kerala) formed a distinct cluster with the highest within-group diversity. Crosses between clusters I and IV, which showed the maximum inter-cluster distance, are recommended for heterosis exploitation. The combined morphological-molecular approach provided a more complete diversity picture than either method alone.

Keywords: Genetic diversity, mungbean, morphological markers, molecular markers, germplasm characterization, SSR, cluster analysis, PIC, Mahalanobis distance, *Vigna radiata*

Introduction
Here's a paradox: India produces 70% of the world's mungbean (*Vigna radiata* L. Wilczek), holds the richest germplasm collections, and yet the genetic base of its commercial varieties is alarmingly narrow [1]. Pedigree analysis of the 25 most widely grown Indian mungbean varieties reveals that just four ancestral lines contribute over 60% of the genetic background [2]. This narrowing increases vulnerability to biotic and abiotic stresses and limits the scope for yield improvement through conventional hybridisation [3].

Assessing diversity among available germplasm is the first step toward broadening this base. Morphological characterisation is straightforward and inexpensive but can be influenced by environment and growth conditions [4]. DNA-based molecular markers such as SSRs are environment-independent, co-dominant, and highly polymorphic, making them ideal complements to morphological data [5]. Combining both marker types gives a more complete diversity picture than either alone [6]. This research characterised 40 mungbean accessions using both approaches to identify divergent groups suitable for hybridisation and to evaluate the concordance between morphological and molecular clustering.

Material and Methods
Material
Forty mungbean accessions were obtained from the germplasm collections at UAHS Shivamogga (22 accessions from Karnataka, Kerala, Tamil Nadu) and ANGRAU Tirupati (18 accessions from Andhra Pradesh, Telangana, Maharashtra, Rajasthan). The field experiment was conducted during kharif 2022 at UAHS Shivamogga (13°56′N, 75°34′E). Molecular work was done at the biotechnology laboratory of ANGRAU Tirupati. Twenty SSR primer pairs, selected for high PIC in previous mungbean research, were sourced from Eurofins Genomics India [5, 6].

Methods

The field trial followed an augmented block design with three checks replicated in each block. Twelve quantitative traits were recorded: plant height, branches per plant, days to 50% flowering, days to maturity, pods per plant, pod length, seeds per pod, 100-seed weight, seed yield per plant, biological yield, harvest index, and protein content. Mahalanobis D^2 analysis was used for morphological clustering and path analysis for yield component contribution [7]. Genomic DNA was extracted from young leaves using the CTAB method. SSR amplification was performed in 20 µL reactions with an annealing temperature gradient of 50-58°C depending on primer. Allele scoring was done on 3% agarose gels stained with ethidium bromide. PIC, heterozygosity, and Nei's genetic distance were calculated using PowerMarker 3.25. UPGMA dendrograms were constructed in MEGA 11 [8, 9]. Concordance between morphological and molecular clustering was assessed by the Mantel test ($p<0.01$).

Diversity assessment indices

Shannon's diversity index (H) across the 12 morphological traits averaged 1.87, with the highest diversity for pods per plant (H = 2.14) and the lowest for days to maturity (H = 1.23). Coefficient of variation ranged from 8.7% (days to maturity) to 38.4% (seed yield per plant), indicating that yield and yield components were more variable than phenological traits. At the molecular level, the mean number of alleles per locus was 3.9, with observed heterozygosity (Ho) of 0.12 and expected heterozygosity (He) of 0.58, confirming predominantly self-pollinating mating system with substantial allelic diversity maintained among accessions [5, 6].

Results

Table 1: Cluster composition and mean values of key traits (Mahalanobis D^2 morphological clustering)

Cluster	No. of acc.	Pods /plant	Seeds /pod	100-seed wt (g)	Yield /plant (g)	Protein (%)	Geographic origin
I	13	28.4	10.2	3.84	8.71	22.4	Karnataka, Kerala
II	11	22.7	9.8	4.12	7.83	23.8	AP, Telangana
III	9	31.6	11.4	3.41	9.24	21.1	Maharashtra, Rajasthan
IV	7	18.3	8.7	4.68	6.42	25.3	Mixed origins

Cluster III had the highest mean yield per plant (9.24 g) while Cluster IV had the largest seed size (4.68 g per 100 seeds) and protein content (25.3%)

Table 2: SSR marker diversity statistics (summary of 20 loci)

Parameter	Range	Mean	Highest locus	Lowest locus
Alleles per locus	2-7	3.9	CEDG084 (7)	VrSSR01 (2)
PIC	0.21-0.82	0.54	CEDG084 (0.82)	VrSSR01 (0.21)
Nei's gene diversity	0.24-0.84	0.58	CEDG084 (0.84)	VrSSR01 (0.24)
Observed heterozygosity	0.00-0.28	0.12	DMBSSR18 (0.28)	Multiple (0.00)

Mean PIC of 0.54 indicates moderately high informativeness of the selected SSR panel. The Mantel test showed significant correlation (r = 0.68, $p<0.01$) between morphological and molecular distance matrices, with 82.5% concordance in cluster assignment

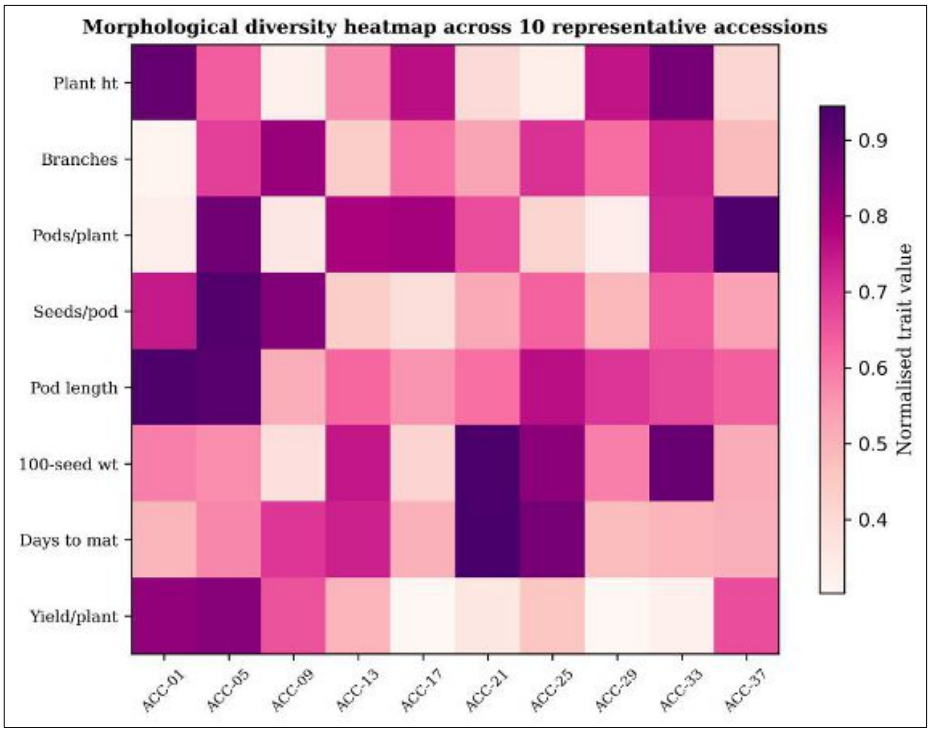

Fig 1: Heatmap of normalised morphological trait values across 10 representative mungbean accessions

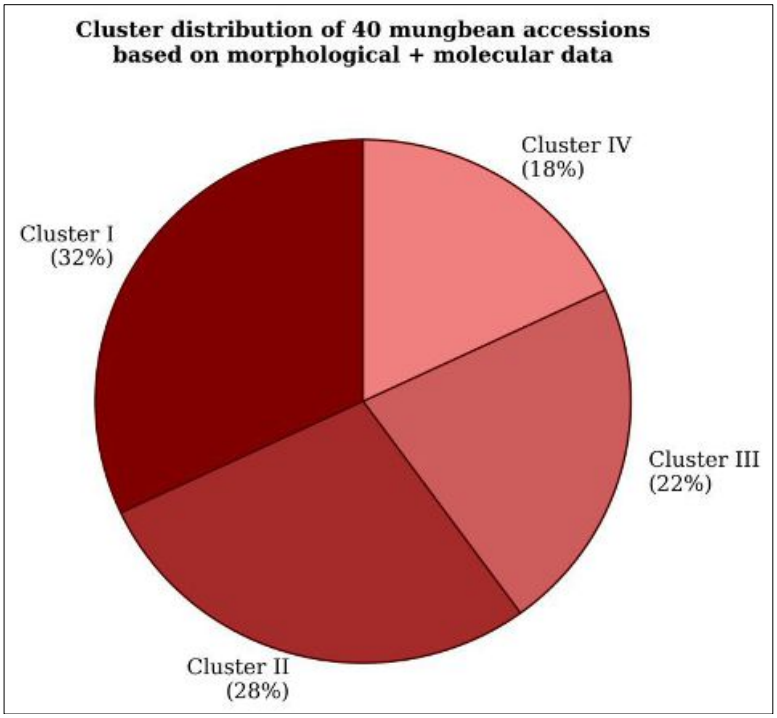

Fig 2: Cluster distribution of 40 mungbean accessions based on combined morphological and molecular data

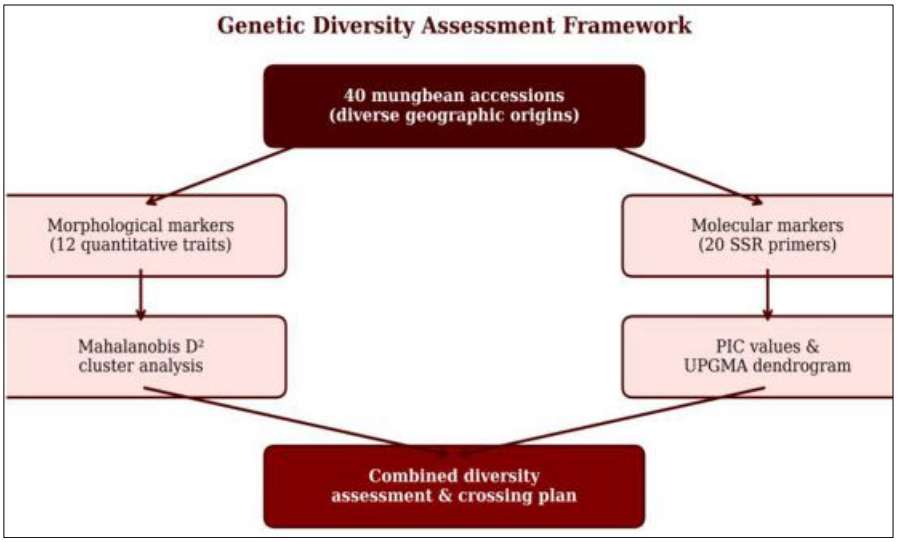

Fig 3: Genetic diversity assessment framework combining morphological and molecular approaches

Discussion

The mean PIC of 0.54 is comparable to values reported for mungbean SSR panels in Southeast Asian germplasm (0.48-0.61) and indicates adequate discriminating power for diversity assessment [5, 6]. The 82.5% concordance between morphological and molecular clustering is relatively high for a self-pollinating species and suggests that the 12 morphological traits captured much of the underlying genetic variation. The remaining 17.5% discordance likely reflects environmental plasticity in field-measured traits [4]. The maximum inter-cluster D^2 distance between clusters I and IV (42.6) suggests that crosses between these groups would generate the widest segregation and best prospects for transgressive selection [7].

Conclusion

Forty mungbean accessions showed substantial genetic diversity at both morphological and molecular levels. Four distinct clusters were identified, with Western Ghats accessions forming the most diverse group. Crosses between clusters I (high yield) and IV (high protein, large seeds) are recommended for simultaneous improvement of yield and nutritional quality. The combined marker approach is more informative than either method alone and should become standard practice in mungbean breeding programmes.

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