# RESEARCH ARTICLE





# Unravelling genetic relationships in spring mung bean genotypes using morphological and molecular markers under the foothill condition of Manipur

Mukesh¹, Sonika Yumnam¹⁺, Priyashree Laishram¹, Konsam Sarika², Chongtham Chinglen Meetei² & Shankar Lal Choudhary¹

<sup>1</sup>Department of Genetics and Plant Breeding College of Agriculture, Iroisemba, Central Agricultural University, Imphal 795 004, Manipur, India

<sup>2</sup>Indian Council of Agricultural Research complex for NEH Region, Lamphelphat, Imphal 795 004, Manipur, India

\*Correspondence email - sonikayumnam@gmail.com

Received: 26 April 2025; Accepted: 25 August 2025; Available online: Version 1.0: 26 September 2025; Version 2.0: 16 October 2025

Cite this article: Mukesh, Sonika Y, Priyashree L, Konsam S, Chongtham CM, Shankar LC. Unravelling genetic relationships in spring mungbean genotypes using morphological and molecular markers under the foothill condition of Manipur. Plant Science Today. 2025; 12(4): 1-9. https://doi.org/10.14719/pst.9126

#### **Abstract**

Mung bean (*Vigna radiata*) is a warm-season legume widely grown in tropical and subtropical regions. It is a key source of proteins, carbohydrates and antioxidants in Asian diets. It is well-suited for SSR-based diversity studies. This study sought to evaluate the genetic diversity among twenty-seven mung bean genotypes to support the breeding of improved varieties with enhanced yield and resilience. By integrating morphological and agronomic trait analysis with molecular characterization using highly polymorphic Simple Sequence Repeat (SSR) markers, we aimed to uncover both phenotypic and genetic variation. These insights are expected to guide the selection of diverse parental lines for hybridization programs, fostering the development of robust, high-performing mung bean cultivars. Significant genetic variability was recorded among studied genotypes. The genotypes were grouped into seven clusters; Cluster I included seventeen genotypes. Intra-cluster distances ranged from 0.00 to 9.70 and inter-cluster distances varied from 10.39 to 27.11. Protein percentage (27.5 %) contributed most to divergence. Out of Thirteen primers ten (76.92 %) exhibited polymorphism. Thirteen SSR primers produced amplicons ranging from 100 to 413 bp. An average of 3.2 alleles per locus was observed, highest six alleles were recorded for VrSSR61. PIC ranged from 0.20 (DMBSSR080) to 0.79 (VrSSR61). UPGMA cluster analysis of twenty-seven *Vigna radiata* genotypes clustered into two main clusters (A and B), each with two sub-clusters (A-I, A-II, B-I, B-II). Similarity coefficients ranged from 0.46 to 0.97. Two markers *viz.*, DMBSSR125 and DMBSSR 130 were identified and associated with YVMV and powdery mildew respectively. Future breeding programs should integrate MAS to efficiently screen and select desired resistant genotypes at early developmental stages, significantly reducing the time and resources typically required for traditional phenotypic screening. The identified clusters and inter-cluster distances provide a clear roadmap fo

**Keywords:** genetic diversity; mung bean; PIC; polymorphism; SSR primers

# Introduction

Mung bean (Vigna radiata L.) is a warm-season legume predominantly cultivated in tropical and subtropical regions. Its adaptability to diverse cropping systems prevalent in these areas underscores its agronomic versatility (1). It is a key food legume in Asia, especially in India and surrounding countries, where it serves as a vital source of protein's, carbohydrate's, antioxidants and fibers, suggesting it to be an excellent and balanced source of diet (2, 3). The genome sizes of Vigna species are highly variable, ranging from 416 to 1,394 MB (4-6) and Vigna radiata and have a genomic size of 579 Mb (7) and cytological studies suggest that the fundamental basic chromosome number 2x=2n=22 is consistent across most varieties and species of mung bean. Due to short duration growth cycle of mung bean (typically 55-100 days contingent on cultivar and environmental factors) renders it a suitable crop for integration into various intercropping and double-cropping systems, particularly with

cereal crops, between the rabi and kharif seasons (Break/separate two season) and its ability to enhance soil health (8, 9), it is commonly known as a break crop or catch crop.

Limited genetic diversity, a low harvest index and susceptibility to pests and diseases are major obstacles to achieving high yields (10). A successful breeding program hinges on genetic diversity as a fundamental requirement (9). Genetic analysis plays a crucial role in developing future breeding strategies aimed at enhancing the quality and yield of crops (11). The narrow genetic base of existing cultivars has hindered the development of high-yielding, disease-resistant varieties. To address this, comprehensive characterization of diverse germplasm is crucial for identifying valuable traits and developing superior crop varieties (12). Traditional germplasm cataloging and characterization using morphological, agronomic and physiological markers have been insufficient to abundantly reveal genetic diversity.

Over the past few decades, molecular marker-based germplasm characterization has emerged as a superior alternative to traditional phenotypic markers for genetic diversity analysis (13). DNA markers have become the most powerful and widely used tool for genetic analysis due to their ability to reveal genome sequence composition and their detectability throughout all developmental stages (10). Advancements in molecular technology have introduced easier and more effective tools for genetic analysis and identification. Reliable DNA marker systems for assessing genetic diversity and evolutionary relationships in crop plants include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), microsatellites or SSRs and single nucleotide polymorphisms (SNPs). Among marker systems, SSR markers tend to be chosen because of their numerous advantages, including high polymorphism levels, co-dominant inheritance, locus specificity, high reproducibility and a uniform distribution throughout the genome. These markers have become a good choice for a wide range of applications such as diversity studies, genotype identification, genetic mapping and germplasm conservation.

The current research aimed to investigate the genetic diversity among spring mung bean genotypes utilizing Mahalanobis  $D^2$  and SSR markers. The findings of this study provide crucial insights into the genetic makeup of these genotypes, facilitating the identification of diverse parents for hybridization efforts.

#### **Materials and Methods**

#### Mahalanobis D<sup>2</sup>

Twenty-seven genotypes of mung bean from IIPR, Kanpur, were used for the analysis of genetic diversity in this study (Table 1.). The study was carried out at Central Agricultural University, Research Farm Andro, Imphal East, Manipur during spring 2023. The research farm is located at latitudes 25°4′5.45″ N and

**Table 1.** Details of genotypes and their sources of collection

Table 1. Details C	or genotypes and their sources of collection
S.No.	Germplasm line
1	IPM 1707-1
2	TCA DM-1
3	BCM 20-45
4	JLPM 818-8
5	BCM 20-1
6	TRM 230
7	Pusa M 23-32
8	SML 2108
9	JLPM 707-27
10	TRM 146
11	RMG 1148
12	SVM 66
13	PM 1711
14	MH 18-100
15	Pusa M 2231
16	Pusa M 23-31
17	SVM 88
18	IPM 1604-1
19	PMS 9
20	BCM 20-50
21	MML 2552
22	RMG 1196
23	PM 1803
24	PMS 13
25	GM 6 (Check)
26	Virat (IPM 205-7) (Check)
27	SML 1115 (Check)

longitudes and 94°8'42" E at an elevation of 790 m above the MSL. The soil type is clay with a pH range of 5.1, indicating an acidic environment that may influence mung bean growth and nutrient availability. The mean data was used for statistical analysis. Five plants were randomly selected from each plot and genotype within each replication to record the following quantitative traits: plant height (cm), number of primary branches, days to 50 % flowering, days to maturity, number of clusters per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100-seed weight (g), protein percentage (%) and seed yield per plant (g). Data was collected according to standard procedures and analyzed using mean values. To assess genetic diversity, Mahalanobis D<sup>2</sup> statistical analysis (14) was employed. Subsequently, Tocher's method (15) was utilized to group the genotypes into distinct clusters by using R studio 4.4.2.

## Plant material, DNA isolation and PCR amplification

Total genomic DNA of twenty-seven mung bean (21 days old seedlings) genotypes extracted using CTAB technique (16). Gel electrophoresis 0.8 % (agarose gel) and Nano Drop 1000 Spectrophotometer readings were used to assess the quality and quantity of DNA in the samples. Primer selection was performed based on criteria including annealing temperature, GC content and polymorphic information content (PIC) to optimize amplification efficiency and discriminatory capacity and PCR amplification was performed for primers as mentioned in Table 1 (17). PCR amplification was performed using a 10 µl reaction mixture in an Eppendorf thermal cycler. Two types of PCR, standard PCR (For DMBSSR series markers) and touchdown PCR (For VrSSR series primers), were employed in this study to amplify different types of SSR markers. Standard PCR with initial denaturation at 95 °C for 5 min and then 35 cycles of denaturation at 95 °C for 30 sec followed by primer annealing at 55 °C for 30 sec and primer extension at 72 °C for 30 sec with a finial extension step at 72 °C for 7 min. A touchdown PCR protocol was employed, starting with an initial denaturation at 95 °C for 4 min, followed by 10 cycles of denaturation at 95 °C for 40 seconds, annealing at 65 °C for 50 seconds (During these 10 cycles the annealing temperature was set to decrease by 1°C after each cycle until it reached 55 °C.) and extension at 72 °C for 50 seconds. Subsequently, 25 cycles were performed with denaturation at 95 °C for 40 sec, annealing at 55 °C for 50 sec and extension at 72 °C for 50 sec, concluding with a final extension at 72 °C for 7 min. The amplified products were separated and visualized using gel electrophoresis on a 3 % agarose gel in 1X TAE buffer and the image was captured using the Bio-Rad system with Bio-Vision v1 8.7.0.0 software.

# **Data analysis**

The multi-imaging system was used to observe the PCR amplified alleles and a binary matrix was used to manually assess the alleles' presence or absence labeled as '1' and '0', respectively. To calculate genetic statistics for the 13 primer pairs, including the number of alleles (Na), amplicon size (As), polymorphic information content (PIC) and Jaccard's similarity coefficient matrix was used. The genetic relationships between the 27 mung bean genotypes were examined using the allelic data obtained across all genotypes. Jaccard's similarity coefficient matrix was also determined using the produced data matrix. After scoring the allelic data, a dendrogram illustrating

the genetic relationships among the genotypes was generated using the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) clustering algorithm within the NTSYS-pc version 2.10e software.

#### **Results and Discussion**

The analysis of variance (ANOVA) revealed significant differences among all the studied, traits indicating ample genetic variability within the germplasm. Studied genotypes of mung bean were clustered or grouped into 7 clusters on the basis magnitude of their D<sup>2</sup> levels (Fig.1). Cluster I contained highest number of genotypes holding 17 followed by cluster II, III, IV and V contained two genotypes each cluster and VI (ML 2552) and VIII (USA M 2231) contained one genotype each (Fig 1.). The range of intracluster distances is 0.00 (Cluster VI&VII) to 9.70 (Cluster V) Table 2. The cluster V has the greatest intra-cluster distance (9.70), followed by cluster I (8.45), cluster IV (8.16), cluster III (6.31) and cluster II (5.42). Inter-cluster distance (D) varied from 10.39 to 27.11 (Table 2) it is suggested that crossing between the selected genotypes from very high inter-cluster distance clusters will give high heterotic crosses and wide spectrum of variation among segregates. The highest percentage contribution to divergence was revealed by protein % (27.5 %) followed by 100 seed weight

(17.3 %), number of seed per pod (13.7 %), pod length (14 %), seed yield per plant (5.5 %), number of clusters per plant (5.1 %), plant height (4.1 %), days to 50 % flowering (3.8 %), number of primary branches (3.5 %), days to maturity (3 %) and number of pods per plant (2.5 %) (18, 19). The consistent observation of these traits as major contributors to divergence suggests that they are likely controlled by a complex genetic architecture, possibly involving multiple genes with additive and non-additive effects (20). The mechanism behind this consistent contribution lies in quantitative traits, which are influenced by many genes and environmental factors.

# Polymorphism determination for genetic diversity analysis.

Thirteen SSR primer pairs were used to assess genetic diversity among 27 mung bean genotypes. Out of 13 SSR primers 10 (76.92 %) primers shown polymorphic Table 3. Fig. 1 illustrates a selection of mung bean genotypes amplified with the VrSSR61 and DMBSSR125 primer pair. The amplicon size ranges from 100 bp to 413 bp and amplified alleles ranges from 2 to 6 with an average of 3.2 alleles per locus was obtained from 32 total alleles, a smaller number of alleles recorded in previous study (21), highest 6 alleles were recorded for VrSSR61 marker, 5 alleles DMBSSR105 and 4 alleles DMBSSR125 (Table 3).

**Table 2.** Intra and inter-cluster distances of 27 green gram genotypes

Cluster	i	II	III	IV	V	VI	VII
	71.55	201.17	107.97	188.20	338.42	222.19	157.83
•	(8.45)	(14.18)	(10.39)	(13.71)	(18.39)	(14.90)	(12.56)
II		29.43	195.24	513.34	735.05	656.91	426.59
"		(5.42)	(13.97)	(22.65)	(27.11)	(25.63)	(20.65)
Ш			39.86	246.55	265.94	298.27	123.40
""			(6.31)	(15.70)	(16.30)	(17.27)	(11.10)
IV				66.63	356.75	116.07	232.49
ıv				(8.16)	(18.88)	(10.77)	(15.24)
V					94.26	277.64	164.22
V					(9.70)	(16.66)	(12.81)
VI						0.00	135.67
VI						(0.00)	(11.64)
VII							0.00
VII							(0.00)

Table 3. List of SSR primers used in the study

S.N.	Primer code	Primer sequence	GC %	NA	Tm (°C)	PIC	Amplicon size
2.	DMBSSR125	F: AAAATGAGTGACAGAGGTGGAAA	39.13	4	57°C	0.70	210-300
۷.	DMD33K1Z3	R: ACATGCACATTCTGAACCACAT	40.90	4	37 C	0.70	210-300
3.	DMBSSR130	F: CAACTGCAAATGAGGTGAAGAT	40.90	1	56.5°C		175
٥.	DMD22K120	R: ATCCAAGAGCATTTGAACTTCC	40.90	1	36.3 C	-	175
4.	DMBSSR080	F: CGAGGCAGAGAAACCTTAAGAA	40.90	2	58°C	0.20	100-108
4.	DMD32K000	R: GCTCGATACTCTTGGGTTGAA	47.61	2	36.0	0.20	100-106
1	DMBSSR105	F: TGATTTAAAGACGGACGGAAA	38.10	5	EC E0C	0.50	150 205
1.	DMP22K102	R: AGAAGAAAGCAACCCTTGGAT	40.90	Э	56.5°C	0.59	150-305
_	DMDCCD004	F: CTGAGGGTCCTATGAATTCTGATT	41.66	3	56°C	0.54	205 200
5.	DMBSSR084	R: TGAATGGTAATTGGTGCTTCTC	40.90	3	36°C	0.54	285-300
C	VrSSR61	F: TGGTTTCAAGCCTCCGTATC	50.00	C	CO °C	0.70	205 412
6.	VI22K01	R: GAATGTAATGCCAACCCATGT	42.85	6	60 °C	0.79	365-413
7.	VCCDC2	F: TTCTCAACCAAAAGCCAAAAA	33.30	2	56 °C	0.51	210 240
7.	VrSSR62	R: GGGTTGAGGAATTAAGTGAAGG	42.85	2	36 C	0.51	310-340
8.	VrSSR65	F: CGACATTTCAATTTCCAAAA	30.00	2	52 °C	0.63	235-250
٥.	VISSKOS	R: GAGGAAGCTGACAGGATTGAGT	50.00	2	52 C	0.63	233-230
9.	VrSSR66	F: TGTTCCCTACCAACTTGACCA	47.61	3	62°C	0.51	360-405
9.	VISSKOO	R: GAAAACACTAAGGATGGATCACTAC	40.00	3	62°C	0.51	360-405
10	VCCDC0	F: AGGAGAGAAGGAAGGTAAAGAAAG	41.66	2	CO 9C	0.46	175 105
10.	VrSSR68	R: CTCTCACACGCACGCATATC	55.00	3	68 °C	0.46	175-195
11	VCCD72	F: TGGGAAATTCCGAAAACTGA	40.00	2	56 °C	0.44	200 210
11.	VrSSR73	R: GGAGCGGGAAAAATAAATCA	40.00	2	56 °C	0.44	200-210
12	VCCD74	F: TCAGGGACTGGAAACAACCT	50.00		6006		240
12.	VrSSR74	R: TCTGATTCCTTCGTTTTCCA	40.00	1	60°C	-	240
12	V-CCD00	F: TGTGAGAGTGGAAGAGCAACTT	45.45		6406		200
13.	VrSSR80	R: AATGGTCCCTTTACCCCTTTT	42.85	1	64°C	-	290

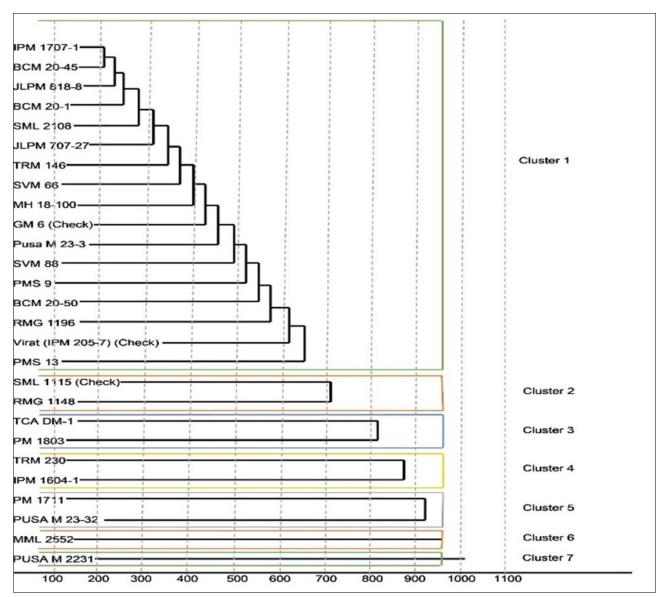


Fig. 1. Cluster dendrogram by Tocher's method.

Previous research has discovered the genetic diversity of extensive mung bean collections using SSR markers, revealing varying numbers of alleles in these studies 2.25 (22) and 2.08 (7). The PIC values for 32 loci ranged from 0.20 (DMBSSR080) to 0.79 (VrSSR61), with an average of 0.55 (Table 3.). This average is higher than what was reported in previous studies on mung bean 0.37 (20), 0.49 (17), 0.20 (10), 0.34 (23) and 0.26 (24), this shows PIC is a strong tool for studying genetic diversity, it determined by allelic richness. The PIC values were lower than those found in other plants like chickpea 0.65 (25), lentil 0.63 (26), rice 0.56 (27), (28) and (29), in wheat 0.790 (30) and (31) maize 0.85 (32). The observed lower PIC values in mung bean likely reflect its relatively narrow genetic base within the studied germplasm, possibly influenced by breeding history and inherent species-specific diversity levels. While the PIC values are lower than some other crops, they still indicate sufficient polymorphism for genetic diversity analysis and provide valuable information for breeding strategies.

## Genetic relatedness of mung bean

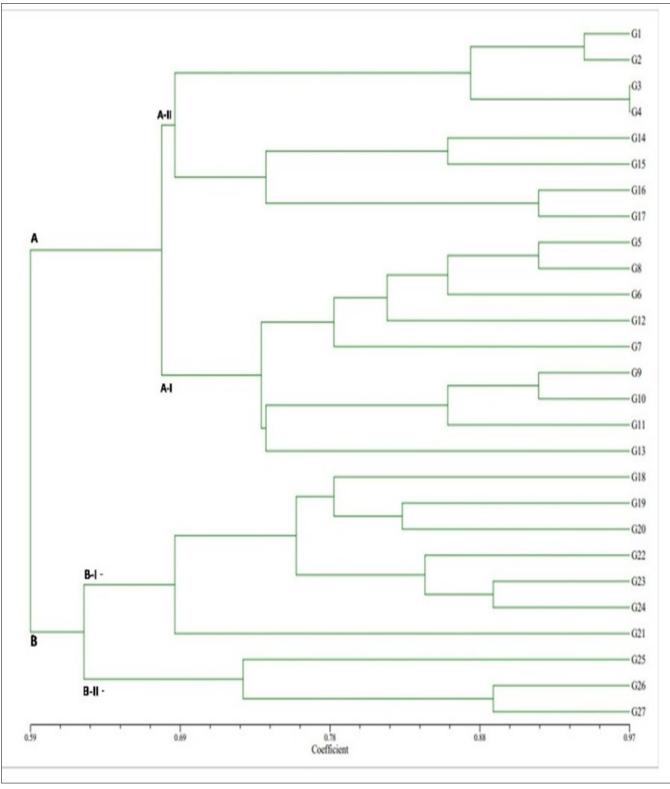
The SSR markers binary data were used for UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis. The UPGMA dendrogram resulting from the cluster analysis of SSR data is presented in the accompanying Fig. 1. The similarity

coefficients observed spanned from 0.46 to 0.97(12, 33) (Table 4). The cluster analysis led using combined SSR markers data produced a dendrogram that distinctly grouped the genotypes into two (A&B) major clusters (Fig. 2) and these major clusters divided into two sub clusters (A-I, II & B-I, II) which were subdivided to form two sub clusters.

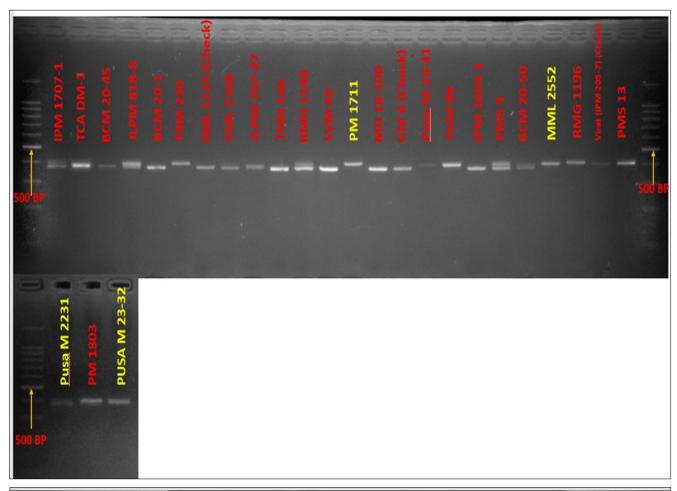
# Quantification of pairwise genetic dissimilarity between 27 mung bean genotypes

The genetic relatedness among the genotypes was elucidated by examining the patterns of shared alleles across their genomes. In the present study, the coefficients varied from 0.46 to 0.97 (Table 4.) underscores the substantial genetic diversity present among the genotypes. Dendrogram clearly distinguish all the genotypes included in the study, BCM 20-45 and JLPM 818-8, which exhibited a very high degree of dissimilarity (0.97) is particularly significant, suggesting that crosses between these two genotypes could lead to a broader range of segregants and potentially unlock novel gene combinations, ultimately fostering greater heterosis and allowing for the selection of superior lines. This phenomenon indicates that factors other than geographical isolation, such as germplasm exchange, selection pressure, genetic drift and shared breeding histories, play a more dominant role in shaping the genetic architecture of cultivated

Genotypes	-	7	ო	4	72	9	7	<b>∞</b>	6	10	11	12	13	14	15	. 91	17	18 1	19 2	20 21	1 22	23	24	25	26	9
₽	1																									
2	0.94	П																								
8	0.86	0.89	1																							
4	0.86	0.89	0.97	П																						
2	0.74	0.71	0.63	0.63	П																					
9	0.74	0.71	0.63	0.63	98.0	П																				
7	0.63	09.0	0.51	0.51	0.80	08.0	П																			
8	0.80	0.77	69.0	69.0	0.91	98.0	08.0	П																		
6	0.74	0.77	0.74	0.74	0.74	0.74	0.63	0.80	н																	
10	69.0	0.71	69.0	69.0	0.74	0.74	0.63	0.80	0.91	н																
11	99.0	0.69	99.0	99.0	0.77	0.71	99.0	0.71	0.89	0.83	⊣															
12	69.0	0.71	69.0	69.0	0.80	98.0	0.74	0.80	0.80	0.86	0.77	1														
13	0.60	0.63	99.0	99.0	0.77	0.77	99.0	0.71	0.71	0.71	08.0	0.77	П													
14	0.69	0.71	69.0	69.0	0.80	0.74	69.0	0.86	0.80	0.80	0.71	08.0	0.71	1												
15	69.0	0.71	0.74	0.74	69.0	0.63	0.57	0.74	69.0	69.0	09.0	69.0	0.71	98.0	П											
16	0.63	99.0	0.74	0.74	0.63	0.74	0.57	0.63	0.63	0.63	09.0	0.74	0.71	0.74	0.80	П										
17	0.57	09.0	69.0	69.0	0.57	69.0	0.63	0.57	0.57	0.57	09.0	69.0	0.71	0.69	0.74 0	0.91	1									
18	0.57	09.0	69.0	69.0	0.57	0.51	0.57	0.63	69.0	69.0	99.0	0.57	09.0	0.69	0.74 0	0 69.0	0.74	н								
19	09.0	0.57	09.0	09.0	0.49	0.49	09.0	0.54	99.0	09.0	0.63	0.49	0.51	0.54	0 09:0	0.54 0	0 09:0	0.77	н							
20	0.57	09.0	0.63	0.63	0.57	0.51	0.51	0.51	69.0	0.63	0.77	0.57	99.0	0.57	0.63 0	0.63 0	0 69.0	0.80 0.	0.83	1						
21	0.49	0.51	0.54	0.54	09.0	0.54	99.0	0.54	99.0	99.0	0.74	09.0	69.0	0.54	0.54 0	0.54 0	0.60 0	0.66 0.	0.74 0.	0.77 1						
22	0.69	99.0	0.63	0.63	0.51	0.51	0.51	0.57	0.57	0.57	0.54	0.46	0.49	0.51	0.57 0	0.51 0	0.57 0	0.74 0.	0.77 0.	0.74 0.66	36 1					
23	09.0	0.57	09.0	09.0	0.49	0.49	0.49	0.54	09.0	09.0	0.57	0.49	0.51	0.54	0.60 0	0.54 0	0.60 0	0.77 0.	0.80 0.	0.77 0.69	99 0.89	6				
24	0.63	09.0	0.63	0.63	0.51	0.57	0.47	0.57	0.63	0.63	09.0	0.51	09.0	0.57	0.63 0	0.63 0	0.63 0	0.74 0.	0.77 0.	0.74 0.60	90 0.80	0.89	9 1			
25	0.63	99.0	0.63	0.63	0.51	0.57	0.51	0.57	0.57	0.57	0.54	0.51	09.0	0.51	0.57 0	0.57 0	0.63 0	0.63 0.	0.60 0.	0.63 0.60	90 0:69	9 0.71	1 0.74	<del>-</del>		
56	0.63	99.0	69.0	69.0	0.57	0.57	0.57	0.63	0.63	0.63	09.0	0.57	09.0	0.57	0.63 0	0.63 0	0 69.0	0.74 0.	0.60 0.	0.63 0.60	50 0.63	3 0.60	0 0.57	7 0.74	1	
77	,	0	i	i		;																				0



**Fig. 2.** Dendrogram showing relationship among 27 genotypes of mung bean generated by UPGMA analysis based on SSR Markers (G1 to G27 IPM 1707- 1, TCA DM-1, BCM 20-45, JLPM 818-8, TRM 230, SML 1115 (Check), SML 2108, JLPM 707-27, TRM 146, RMG 1148, SVM 66, PM 1711, MH 18-100, GM 6 (Check), Pusa M 23-31, SVM 88, IPM 1604-1, PMS 9, BCM 20-50, MML 2552, RMG 1196, Virat (IPM 205-7) (Check), PMS 13, Pusa M 2231, PM 1803, PUSA M 23-32).



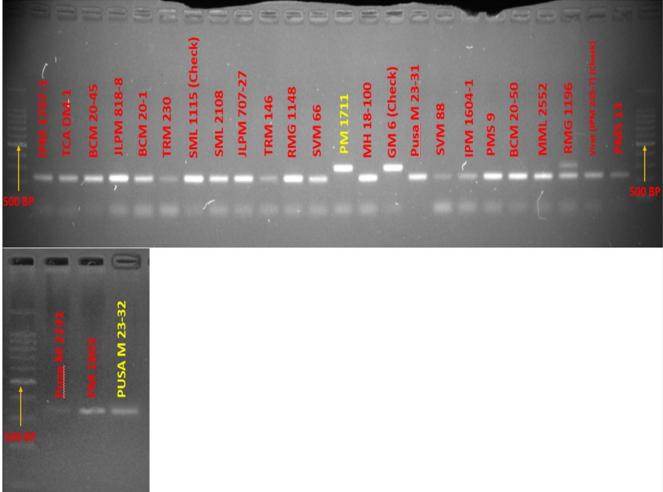


Fig. 3. PCR Amplification profiles generated from 27 *V. radiata* genotypes using primers VrSSR 61 and DMBSSR125 respectively.

mungbean accessions (34, 35). The dendrogram (Fig. 3) analysis showed that two specific genotypes, MML 2552 and Pusa M 2231, were clustered into cluster B-I&II single from the others. The remaining genotypes were clustered sub-clusters (A-I, II and B-I, II) (Fig. 2) in which the genotypes were distributed randomly and no correlation observed with the geographical location from which they were obtained. Maximum number of genotypes was clustered into cluster A-I. The complete distinction of studied genotypes by using 13 SSR markers validates the robustness and utility of this developed marker set for genetic analysis within this species. The clear differentiation achieved highlights their capacity to accurately assess genetic relationships and identify unique genotypes, which is fundamental for effective germplasm management and targeted breeding efforts in mung bean (10). This effective resolution of genetic relationships reinforces the reliability of SSR-based molecular characterization as a cornerstone for future mung bean genetic improvement programs.

#### Comparative study of molecular markers and D<sup>2</sup> statistics

Two distinct clustering approaches were employed in this study; one based on molecular markers and the other using D² analysis. Comparative analysis was done from the resulting dendrograms from both the molecular marker-based and D² analysis methods. The genotypes Pusa M 2231 and MML 2552 fall into the two independent clusters with single genotype in each in both the methods. PMS 9, BCM 20-50, RMG 1196, Virat (IPM 205-7) falls into the same respective clusters. While IPM 1707-1, TCA DM-1, BCM 20-45, JLPM 818-8 and MH 18-100, GM 6 (Check), Pusa M 23-31, SVM 88 were grouped together in different clusters in each of the methods with other genotypes. BCM 20-1, TRM 230 and SML 2108 are also grouped together but in different clusters. This indicates a moderate correlation between the diversity based on morphological traits-based and the SSR marker profile.

# Conclusion

This study conclusively demonstrates the existence of significant genetic diversity within the spring mung bean (Vigna radiata) germplasm, evidenced by both quantitative morphological characters and molecular SSR markers. The observed moderate to high genetic gain across all studied traits underscores a substantial inherent potential for genetic improvement through targeted selection. Specifically, the high informativeness of the VrSSR61 marker, coupled with the moderate yet valuable discriminatory power of the remaining SSR markers, confirms their efficacy in accurately assessing genetic polymorphism and delineating intricate relationships within Vigna radiata germplasm. The congruence between morphological and molecular data further validates the reliability of these findings. Crucially, the identification of markers DMBSSR125 and DMBSSR130 associated with Yellow Vein Mosaic Virus (YVMV) and powdery mildew resistance, respectively, represents a significant revelation. This direct link between specific molecular markers and disease resistance holds immense potential for implementing efficient marker-assisted selection (MAS) in future breeding programs, accelerating the development of resilient mung bean varieties. The substantial genetic diversity confirmed among these twenty-seven green gram genotypes establishes them as invaluable genetic resources, offering a robust foundation for initiating strategic crossing programs aimed at the accelerated genetic improvement of this vital pulse crop.

# **Acknowledgements**

The authors are deeply indebted to the College of Agriculture, Central Agricultural University Imphal, Manipur, for providing the essential resources and infrastructure that facilitated this research. We are immensely grateful for their continuous support throughout the study. Moreover, we express our sincere appreciation to the All India Coordinated Research Project on Chickpea for their generous financial backing, which was crucial for covering the research expenses and ensuring its successful execution.

#### **Authors' contributions**

This work was a collaborative effort, with all authors contributing M, PL, SY and SLC were responsible for the conception, design, editing, data analysis, finalization and submission of the manuscript. CCM and SLC conducted the literature survey, while SY, SLC and CCM contributed to the revision and redrafting of the manuscript. All authors read and approved the final manuscript.

## **Compliance with ethical standards**

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

#### References

- Yimram T, Somta P, Srinives P. Genetic variation in cultivated mung bean germplasm and its implication in breeding for high yield. Field Crops Res. 2009;112(2-3):260-6. https://doi.org/10.1016/ j.fcr.2009.03.006
- Mukesh PL, Dhaked SK, Kumar S, Yumnam S, Choudhary SL. Principal component analysis approach to identify genetic variation in spring mung bean under the foothill condition of Manipur India. Plant Arch. 2025;25(1):171. https://doi.org/10.51470/ PLANTARCHIVES.2025.v25.no.1.171
- 3. Mukesh SY, Laishram P, Kumar H, Senjam P, Singh LNK, Gopimohan N, et al. Genetic basis of yield variation in spring mung bean (Vigna radiata L.): a correlation and path analysis study. Int J Bio-resour Stress Manag. 2024;15:1-7. https://doi.org/10.23910/1.2024.5749
- Parida A, Raina SN, Narayan RK. Quantitative DNA variation between and within chromosome complements of *Vigna* species (Fabaceae). Genetica. 1990;82(2):125-33. https://doi.org/10.1007/ BF00124642
- Lakhanpaul S, Babu C. Symposium on Grain Legumes. New Delhi: India; 1991:47-57. https://doi.org/10.1007/978-981-19-4169-6\_29
- Kang YJ, Kim SK, Kim MY, Lestari P, Kim KH, Ha BK, et al. Genome sequence of mung bean and insights into evolution within *Vigna* species. Nat Commun. 2014;5:5443. https://doi.org/10.1038/ ncomms6443
- Somta P, Musch W, Kongsamai B, Chanprame S, Nakasathien S, Toojinda T, et al. New microsatellite markers isolated from mung bean (*Vigna radiata* (L.) Wilczek). Mol Ecol Resour. 2008;8(5):1155-7. https://doi.org/10.1111/j.1755-0998.2008.02219.x
- 8. Ilyas N, Ambreen F, Batool N, Arshad M, Mazhar R, Bibi F, et al. Contribution of nitrogen fixed by mung bean to the following wheat crop. Commun Soil Sci Plant Anal. 2018;49(2):148-58. https://doi.org/10.1080/00103624.2017.1421215
- Van Haeften S, Dudley C, Kang Y, Smith D, Nair RM, Douglas CA, et al. Building a better mungbean: breeding for reproductive resilience in a changing climate. Food Energy Secur. 2023;12(6):e467. https://

#### doi.org/10.1002/fes3.467

- Tabasum A, Hameed A, Asghar MJ. Exploring the genetic divergence in mungbean (Vigna radiata L.) germplasm using multiple molecular marker systems. Mol Biotechnol. 2020;62(11):547-56. https://doi.org/10.1007/s12033-020-00270-y
- Somta P, Laosatit K, Yuan X, Chen X. Thirty years of mung bean genome research: where do we stand and what have we learned? Front Plant Sci. 2022;13:944721. https://doi.org/10.3389/ fpls.2022.944721
- Lavanya GR, Srivastava J, Ranade SA. Molecular assessment of genetic diversity in mung bean germplasm. J Genet. 2008;87:65-74. https://doi.org/10.1007/s12041-008-0009-3
- Karp A, Edwards KJ, Bruford M, Funk S, Vosman B, Morgante M, et al. Molecular technologies for biodiversity evaluation: opportunities and challenges. Nat Biotechnol. 1997;15(7):625-8. https:// doi.org/10.1038/nbt0797-625
- Mahalanobis PC. The generalized distance in statistics. Proc Indian Natl Inst Sci. 1936;2:49-55. https://www.scirp.org/reference/ referencespapers?referenceid=1001254
- Rao CR. Advanced statistical methods in biometrical research. New York: John Wiley & Sons; 1952:357-63.
- Doyle JJ. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13-
- 17. Shrivastava D, Verma P, Bhatia S. Expanding the repertoire of microsatellite markers for polymorphism studies in Indian accessions of mung bean (*Vigna radiata* L. Wilczek). Mol Biol Rep. 2014;41:5669-80. https://doi.org/10.1007/s11033-014-3436-7
- Nalajala S, Singh NB, Jeberson MS, Sastry EV, Yumnam S, Sinha B, et al. Genetic variability, correlation and path analysis in mung bean genotypes (*Vigna radiata* L. Wilczek): an experimental investigation. Int J Environ Climate Change. 2022;12(11):1846-54. https://doi.org/10.9734/ijecc/2022/v12i1131170
- Gadakh SS, Dethe AM, Kathale MN, Kahate NS. Genetic diversity for yield and its component traits in green gram (*Vigna radiata* (L.) Wilczek). J Crop Weed. 2013;9(1):106-9.
- Singh R, van Heusden AW, Yadav RC. A comparative genetic diversity analysis in mungbean (*Vigna radiata* L.) using inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP). Afr J Biotechnol. 2013;12(47):6574-82.
- Mwangi JW, Okoth OR, Kariuki MP, Piero NM. Genetic and phenotypic diversity of selected Kenyan mung bean (*Vigna radiata* L. Wilczek) genotypes. J Genet Eng Biotechnol. 2021;19(1):142. https://doi.org/10.1186/s43141-021-00207-9
- Seehalak W, Somta P, Sommanas W, Srinives P. Microsatellite markers for mung bean developed from sequence database. Mol Ecol Resour. 2009;9(3):862-4. https://doi.org/10.1111/j.1755-0998.2009.02655.x
- Sanghani JM, Golakiya BA, Dhedhi KK, Patel SV. Molecular characterization of mung bean (*Vigna radiata* L.) genotypes through RAPD, ISSR and SSR markers. Legume Res. 2015;38(4):452-6. https://doi.org/10.5958/0976-0571.2015.00040.9
- Tangphatsornruang S, Somta P, Uthaipaisanwong P, Chanprasert J, Sangsrakru D, Seehalak W, et al. Characterization of microsatellites and gene contents from genome shotgun sequences of mung bean (*Vigna radiata* (L.) Wilczek). BMC Plant Biol. 2009;9:137. https://doi.org/10.1186/1471-2229-9-137
- 25. Mir AH, Bhat MA, Fayaz H, Wani AA, Dar SA, Maqbool S, et al. SSR markers in revealing extent of genetic diversity and phylogenetic relationships among chickpea core collection accessions for Western Himalayas. Mol Biol Rep. 2022;49(12):11469-79. https://doi.org/10.1007/s11033-022-07862-x

- Saidi A, Sarvmeili J, Pouresmael M. Genetic diversity study in lentil (Lens culinaris Medik.) germplasm: a comparison of CAAT box derived polymorphism (CBDP) and simple sequence repeat (SSR) markers. Biologia. 2022;77(10):2793-803. https://doi.org/10.1007/ s11756-022-01089-5
- Tripathi S, Singh SK, Srivashtav V, Khaire AR, Vennela P, Singh DK. Molecular diversity analysis in rice (*Oryza sativa* L.) using SSR markers. Electron J Plant Breed. 2020;11(3):776-82. https://doi.org/10.37992/2020.1103.130
- Nachimuthu W, Muthurajan R, Duraialaguraja S, Sivakami R, Pandian BA, Ponniah G, et al. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa*. Rice. 2015;8:30. https://doi.org/10.1186/s12284-015-0062-5
- Mukta S, Bappy MN, Bhuiyan J, Zohora FT, Afrin D. Assessment of genetic diversity in Bangladeshi rice (*Oryza sativa* L.) varieties utilizing SSR markers. Gene Rep. 2024;37:102051. https:// doi.org/10.1016/j.genrep.2024.102051
- Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet. 2000;100:584-92. https://doi.org/10.1007/s001220050077
- Türkoğlu A, Haliloğlu K, Mohammadi SA, Öztürk A, Bolouri P, Özkan G, et al. Genetic diversity and population structure in Turkiye bread wheat genotypes revealed by simple sequence repeats (SSR) markers. Genes. 2023;14(6):1182. https://doi.org/10.3390/genes14061182
- Islam MA, Alam MS, Maniruzzaman M, Haque MS. Microsatellite marker-based genetic diversity assessment among exotic and native maize inbred lines of Bangladesh. Saudi J Biol Sci. 2023;30 (8):103715. https://doi.org/10.1016/j.sjbs.2023.103715
- Kaur G, Joshi A, Jain D. SSR-marker assisted evaluation of genetic diversity in mung bean (*Vigna radiata* (L.) Wilczek) genotypes. Braz Arch Biol Technol. 2018;61:e16160613. https://doi.org/10.1590/1678 -4324-2016160613
- 34. Gokulakrishnan J, Kumar BS, Prakash M. Studies on genetic diversity in mung bean (*Vigna radiata* L.). Legume Res. 2012;35(1):50 -2. https://doi.org/10.5958/j.0976-0571.35.1.012
- Khan MH, Dar SA, Hussain J, Dar NA, Mehvish S, Qayoom A, et al. Genetic diversity studies in mung bean (*Vigna radiata* (L.) Wilczek).
   Adv Crop Sci Technol. 2023;11(2):1000556. https://doi.org/10.35248/2329-8863.23.11.556

#### **Additional information**

 $\label{per review} \textbf{Peer review:} \ \textbf{Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.}$ 

**Reprints & permissions information** is available at https://horizonepublishing.com/journals/index.php/PST/open\_access\_policy

**Publisher's Note**: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing**: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc

See https://horizonepublishing.com/journals/index.php/PST/indexing\_abstracting

**Copyright:** © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

**Publisher information:** Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.