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MAPPING MOLECULAR VARIATION IN MUNGBEAN TO ACCELERATE GENETIC GAINS

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ABSTRACT

Mungbean (*Vigna radiata*) plays a critical role in sustainable agriculture due to its high protein content and nitrogen-fixing ability, which naturally improves soil fertility. Its genetic diversity is valuable for breeding programs aimed at enhancing yield, disease resistance, and resilience. A molecular diversity analysis using 30 SSR markers revealed that only seven were polymorphic, indicating limited genetic variation among the tested genotypes. The observed heterozygosity (He) and polymorphic information content (PIC) were 0.48 and 0.37, respectively. Genetic dissimilarity ranged from 0.07 to 0.30, confirming low diversity. Adzuki bean-derived SSR markers proved effective in assessing mungbean diversity. These results emphasize the necessity of using a wider range of genotypes and more primers in subsequent research. Breeding robust, high-yielding mungbean variants requires the identification of polymorphic markers. While combining genomic and phenotypic data improves breeding strategies for sustainable agriculture and food security, SSR markers aid in parental selection and genetic conservation.
Keywords **:** *Vigna radiata*; Molecular Diversity; Adzuki bean; PIC; SSR.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is a highly prized legume crop valued for its short growth duration and vital role in enhancing cropping intensity, particularly when used as an intercrop (Singh *et al*., 2020). It is an excellent source of protein, vitamins, and essential minerals, making it an important component of human diets across Asia and beyond (Nair *et al*., 2019). In addition to its nutritional importance, mungbean plays a crucial role in sustainable agriculture by fixing atmospheric nitrogen, thereby improving soil fertility and reducing dependence on synthetic fertilizers (Ali & Gupta, 2012). This ecological benefit is particularly significant for Indian agricultural systems, where input efficiency and soil health are major concerns.Globally cultivated in tropical and subtropical regions, mungbean contributes significantly to food security and cropping system diversification (Somta & Srinives, 2007). In India, the crop covers more than 5.5 million hectares with an average productivity of 570 kg/ha, producing nearly 3.17 million tons annually. Bihar alone accounts for 0.22 million hectares with a higher productivity of 727 kg/ha (Government of Bihar, 2024). Despite its potential, mungbean productivity is constrained by the absence of stable high-yielding cultivars, asynchronous pod maturity, and susceptibility to biotic stresses such as mungbean yellow mosaic virus (MYMV) and pod borers (Pandey *et al*., 2018). The crop's adaptability allows cultivation in all three seasons Kharif, Rabi, and Zaid with increasing adoption in summer and spring due to its tolerance to high temperatures (Chauhan *et al*., 2021). However, the narrowing genetic base under modern agricultural systems threatens crop resilience and long-term food security. Therefore, harnessing genetic diversity in mungbean germplasm is crucial for breeding resilient and high-yielding cultivars. DNA-based molecular markers, particularly Simple Sequence Repeats (SSRs), have proven effective in assessing genetic variation and identifying diverse parental lines for crop improvement (Somta *et al*., 2008). The present study was undertaken to evaluate

the genetic diversity in mungbean germplasm using SSR markers, with the aim of identifying diverse and promising lines that can be utilized in breeding programs for developing high-yielding, stress-tolerant cultivars.

Materials and Methods

Plant Materials

The experiment was conducted at the Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour (Bihar). A total of 36 mungbean genotypes, including released varieties, advanced breeding lines, and local collections (LC), were used (Table 1). These genotypes were sourced from IIPR-Kanpur, NBPGR-New Delhi, GBPUAT-Pantnagar, BHU-Varanasi, CSKHPKV-Palampur, IARI-New Delhi, and local regions of Bihar and Uttarakhand. Check varieties are denoted as (C).

Table 1: List of mungbean genotypes used in the study.

S.No.	Entry name	Source
1.	GM-99-25	IIPR, Kanpur
2.	KL-4	LC, Kashipur, Uttarakhand
3.	Banka Local Mung-5	LC, Etahari, Banka, Bihar
4.	IPM-99-125	IIPR, Kanpur
5.	IC-683	NBPGR, New Delhi
6.	GP-276	IIPR, Kanpur
7.	IPM-2-3	IIPR, Kanpur
8.	Samrat (C)	IIPR, Kanpur
9.	BRM-8-1	LC, Pantnagar, Uttarakhand
10.	Meha	IIPR, Kanpur
11.	Banka Local Mung-2	LC, Etahari, Banka, Bihar
12.	Banka Local Mung-7	LC, Simariya, Banka, Bihar
13.	Banka Local Mung-1	LC, Simariya, Banka, Bihar
14.	IC-314326	NBPGR, New Delhi
15.	IPM-409-4	IIPR, Kanpur
16.	PM-5 (C)	GBPUAT, Pantnagar, Uttarakhand
17.	DMG-1103	IIPR, Kanpur
18.	IPM-2-14	IIPR, Kanpur
19.	DMG-1105-1-2	IIPR, Kanpur
20.	IC-39403	NBPGR, New Delhi
21.	HUM-12	BHU, Varanasi
22.	IC-369233	NBPGR, New Delhi
23.	LM-249	IIPR, Kanpur
24.	HUM-16 (C)	BHU, Varanasi
25.	IC-324012	NBPGR, New Delhi
26.	BRM-1	LC, Kashipur, Uttarakhand
27.	SML-668	CSKHPKV, Palampur
28.	GG-1980	IIPR, Kanpur
29.	KL-1	LC, Kashipur, Uttarakhand
30.	LM-126	IIPR, Kanpur
31.	DMG-1105-2-2	IIPR, Kanpur
32.	Pusa Vishal (C)	IARI, New Delhi
33.	LM-3	IIPR, Kanpur
34.	IC-16033	NBPGR, New Delhi
35.	Banka Local Mung-4	LC, Simariya, Bihar
36.	IPM-205-7	IIPR, Kanpur

Where, C – Check, LC- Local Collection

DNA Extraction

Genomic DNA was isolated from 11–12-day-old seedlings using the CTAB method described by Doyle and Doyle (1990) with minor modifications. DNA quality and concentration were checked on 0.8% agarose gel and quantified using a NanoDrop spectrophotometer.

SSR Markers and PCR Amplification

A total of 30 SSR primers were used, including 20 mungbean-specific SSRs developed by Somta *et al.* (2008) and 10 adzuki bean-derived SSRs reported by Wang *et al.* (2004). These primers have been widely utilized for genetic diversity studies in Vigna species due to their high polymorphism and transferability.

PCR amplification was carried out in a 25 µL reaction mixture containing:

a. 50 ng template DNA,
b. 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$),
c. 200 µM of each dNTP,
d. 0.5 µM of each forward and reverse primer, and
e. 1 U Taq DNA polymerase (Thermo Scientific, USA).

Amplification was performed in a thermal cycler (Eppendorf Mastercycler) using the following conditions:

a) Initial denaturation at 94 °C for 4 min,
b) 35 cycles of:
c) Denaturation at 94 °C for 30 s,
d) Annealing at 50–60 °C (depending on primer Tm) for 45 s,
e) Extension at 72 °C for 1 min,
f) Final extension at 72 °C for 7 min.

PCR products were resolved on 3% agarose gel stained with ethidium bromide and visualized under UV light.

Data Scoring and Analysis

SSR polymorphisms were scored manually based on the presence (1) or absence (0) of alleles. The binary data matrix was used to estimate genetic diversity among the genotypes using DARwin 6.0 software (Perrier & Jacquemoud-Collet, 2006). A dendrogram was constructed using the unweighted pair group method with arithmetic averages (UPGMA).

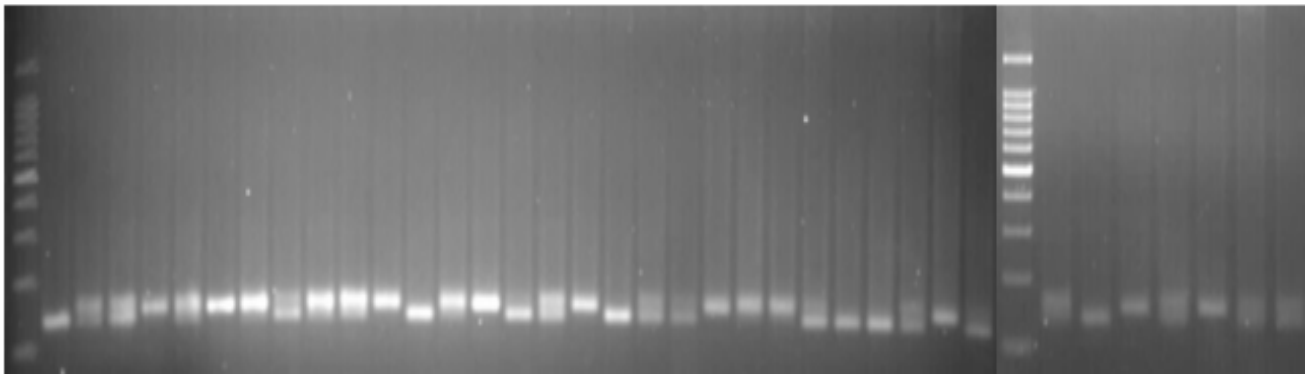
Results and Discussion

A total of 30 SSR markers were used to evaluate the genetic diversity among 36 mungbean genotypes. Of these, 7 primers were polymorphic, 16 were monomorphic, and 7 failed to amplify. The seven polymorphic primers (Table 2) revealed a total of 14 alleles, with an average of 2 alleles per primer, suggesting a relatively low level of genetic diversity within the tested germplasm. The number of alleles per marker (2) was lower than that reported in earlier studies by Lestari *et al.* (2014) and Khajudparn *et al.* (2023), who observed higher allelic numbers using larger germplasm sets. The Polymorphic Information Content (PIC) values ranged from 0.28 (CEDG006) to 0.37 (CEDAAG002), with an average of 0.33, indicating moderate informativeness of the SSR markers used (Table 2). Among these, CEDAAG002 emerged as the most informative marker for differentiating genotypes, consistent with reports of cross-transferability of adzuki bean SSRs to mungbean (Wang *et al.*, 2004). The expected heterozygosity (He) ranged from 0.34 to 0.48, averaging 0.41, indicating that the studied mungbean germplasm harbors narrow but exploitable variability. The genetic dissimilarity indices among the mungbean genotypes ranged from 0.07 to 0.30 (Fig. 1). Some genotype pairs (e.g., GM-99-25 and GP-276; Banka Local Mung-1 and Banka Local Mung-2) exhibited complete similarity (0.00), suggesting close relatedness. In contrast, the highest dissimilarity (0.30) was recorded between KL-1 and Samrat, indicating their potential utility as diverse parental combinations in breeding

Table 2 : Details of polymorphic SSR markers studied in the experiment

Sl. No.	Primer	Repeat motif	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing Temp (°C)	No. of alleles	He	PIC	Reference
1	MAB128079	$(GA)_{18}$	AGCTTGTGCT TCTGTCTGTG	GACACCAACAT GGGAGAAGT	55	2	0.38	0.31	Somta *et al.*, 2008
2	MBM-00201	$(CT)_{16}$	CCTTCCTTGTT GTTCTTCTC	ATGTGCTTGTG GTGTGATGT	56	2	0.48	0.36	Somta *et al.*, 2008
3	MBM-03131	$(AT)_{13}$	GCTGGATGAG TGAACTTGAG	TGTGGGTAGTT GGGAGTAGG	55	2	0.38	0.31	Somta *et al.*, 2008

4	CEDGAG001	$(GA)_{15}$	TGGCTCTTCTT CTCCACTTT	CGGGTAGTCAA GTTGGAGAT	54	2	0.40	0.32	Wang *et al*., 2004
5	CEDAAG002	$(AAG)_{12}$	CCTTCCATGTC TTCTGCTCT	GAGTGGAAGGT GGTAGTGGA	55	2	0.48	0.37	Wang *et al*., 2004
6	CEDG006	$(GA)_{10}$	CCAAGTCCCT TGTCTCTTCT	GAGTGAGCCTG TTTGACTGA	53	2	0.34	0.28	Wang *et al*., 2004
7	CEDG008	$(GA)_{12}$	GTTTCCAGTG TTGGTTTGTG	AGATTTGTGGG TGTTGTTGG	54	2	0.42	0.33	Wang *et al*., 2004



Note: **L**- Ladder, **1**- GM-99-25, **2**- KL-4, **3-** Banka Local Mung-5, **4-** IPM-99-125, **5-** IC-683, **6-** GP-276, **7-** IPM-2-3, **8-** Samrat (C), **9-** BRM-8-1, **10-** Meha, **11-** Banka Local Mung-2, **12-** Banka Local Mung-7, **13-** Banka Local Mung-1, **14-** IC-314326, **15-** IPM-409-4, **16-** PM-5 (C), **17-** DMG-1103, **18-** IPM-2-14, **19-** DMG-1105-1-2, **20-** IC-39403, **21-** HUM-12, **22-** IC-369233, **23-** LM-249, **24-** HUM-16 (C), **25-** IC-324012, **26-** BRM-1, **27-** SML-668, **28-** GG-1980, **29-** KL-1, **30-** LM-126, **31-** DMG-1105-2-2, **32-** Pusa Vishal (C), **33-** LM-3, **34-** IC-16033, **35-** Banka Local Mung-4, **36-** IPM-205-7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
2	0.22																																		
3	0.29	0.24																																	
4	0.21	0.09	0.24																																
5	0.21	0.13	0.23	0.12																															
6	0.27	0.22	0.16	0.22	0.21																														
7	0.23	0.09	0.25	0.10	0.14	0.23																													
8	0.30	0.25	0.13	0.24	0.23	0.16	0.26																												
9	0.30	0.25	0.10	0.25	0.24	0.17	0.26	0.14																											
10	0.29	0.24	0.00	0.24	0.23	0.16	0.25	0.13	0.10																										
11	0.29	0.24	0.17	0.23	0.22	0.10	0.24	0.17	0.18	0.17																									
12	0.30	0.25	0.10	0.24	0.24	0.16	0.26	0.14	0.07	0.10	0.18																								
13	0.14	0.15	0.22	0.14	0.13	0.20	0.16	0.22	0.23	0.22	0.21	0.23																							
14	0.23	0.09	0.25	0.10	0.14	0.23	0.00	0.26	0.26	0.25	0.24	0.26	0.16																						
15	0.28	0.23	0.17	0.23	0.22	0.10	0.24	0.17	0.18	0.17	0.07	0.18	0.21	0.24																					
16	0.26	0.21	0.17	0.20	0.20	0.15	0.22	0.17	0.18	0.17	0.16	0.17	0.19	0.22	0.16																				
17	0.24	0.19	0.19	0.18	0.18	0.17	0.20	0.19	0.20	0.19	0.18	0.20	0.17	0.20	0.18	0.16																			
18	0.25	0.20	0.20	0.19	0.18	0.18	0.21	0.20	0.21	0.20	0.19	0.21	0.18	0.21	0.19	0.17	0.11																		
19	0.30	0.25	0.10	0.25	0.24	0.17	0.26	0.14	0.00	0.10	0.18	0.07	0.23	0.26	0.18	0.18	0.20	0.21																	
20	0.30	0.25	0.10	0.25	0.24	0.17	0.26	0.14	0.00	0.10	0.18	0.07	0.23	0.26	0.18	0.18	0.20	0.21	0.00																
21	0.26	0.21	0.17	0.20	0.20	0.15	0.22	0.17	0.18	0.17	0.16	0.17	0.19	0.22	0.16	0.07	0.16	0.17	0.18	0.18															
22	0.24	0.19	0.19	0.18	0.17	0.17	0.20	0.19	0.20	0.19	0.18	0.20	0.17	0.20	0.18	0.15	0.10	0.07	0.20	0.20	0.15														
23	0.23	0.09	0.25	0.10	0.14	0.23	0.00	0.26	0.26	0.25	0.24	0.26	0.16	0.00	0.24	0.22	0.20	0.21	0.26	0.26	0.22	0.20													
24	0.29	0.24	0.10	0.24	0.23	0.15	0.25	0.13	0.11	0.10	0.17	0.11	0.22	0.25	0.17	0.16	0.19	0.20	0.11	0.11	0.16	0.19	0.25												
25	0.30	0.25	0.10	0.24	0.24	0.16	0.26	0.14	0.07	0.10	0.18	0.00	0.23	0.26	0.18	0.17	0.20	0.21	0.07	0.07	0.17	0.20	0.26	0.11											
26	0.23	0.09	0.25	0.10	0.14	0.23	0.00	0.26	0.26	0.25	0.24	0.26	0.16	0.00	0.24	0.22	0.20	0.21	0.26	0.26	0.22	0.20	0.00	0.25	0.26										
27	0.21	0.16	0.23	0.15	0.15	0.21	0.17	0.24	0.24	0.23	0.23	0.24	0.14	0.17	0.22	0.20	0.18	0.19	0.24	0.24	0.20	0.18	0.17	0.23	0.24	0.17									
28	0.22	0.08	0.25	0.09	0.13	0.22	0.07	0.25	0.26	0.25	0.24	0.25	0.15	0.07	0.24	0.21	0.19	0.20	0.26	0.26	0.21	0.19	0.07	0.24	0.25	0.07	0.16								
29	0.19	0.15	0.22	0.14	0.13	0.20	0.15	0.22	0.23	0.22	0.21	0.23	0.12	0.15	0.21	0.19	0.16	0.17	0.23	0.23	0.19	0.16	0.15	0.22	0.23	0.15	0.07	0.15							
30	0.28	0.23	0.17	0.23	0.22	0.10	0.24	0.17	0.18	0.17	0.07	0.18	0.21	0.24	0.00	0.16	0.18	0.19	0.18	0.18	0.16	0.18	0.24	0.17	0.18	0.24	0.22	0.24	0.21						
31	0.29	0.24	0.17	0.23	0.22	0.10	0.24	0.17	0.18	0.17	0.00	0.18	0.21	0.24	0.07	0.16	0.18	0.19	0.18	0.18	0.16	0.18	0.24	0.17	0.18	0.24	0.23	0.24	0.21	0.07					
32	0.29	0.24	0.17	0.23	0.22	0.10	0.24	0.17	0.18	0.17	0.00	0.18	0.21	0.24	0.07	0.16	0.18	0.19	0.18	0.18	0.16	0.18	0.24	0.17	0.18	0.24	0.23	0.24	0.21	0.07	0.00				
33	0.30	0.25	0.13	0.24	0.23	0.16	0.26	0.07	0.14	0.13	0.17	0.14	0.22	0.26	0.17	0.17	0.19	0.20	0.14	0.14	0.17	0.19	0.26	0.13	0.14	0.26	0.24	0.25	0.22	0.17	0.17	0.17			
34	0.30	0.25	0.10	0.24	0.24	0.16	0.26	0.14	0.07	0.10	0.18	0.00	0.23	0.26	0.18	0.17	0.20	0.21	0.07	0.07	0.17	0.20	0.26	0.11	0.00	0.26	0.24	0.25	0.23	0.18	0.18	0.18	0.14		
35	0.30	0.25	0.10	0.25	0.24	0.17	0.26	0.14	0.00	0.10	0.18	0.07	0.23	0.26	0.18	0.18	0.20	0.21	0.00	0.00	0.18	0.20	0.26	0.11	0.07	0.26	0.24	0.26	0.23	0.18	0.18	0.18	0.14	0.07	
36	0.21	0.16	0.23	0.15	0.15	0.21	0.17	0.24	0.24	0.23	0.23	0.24	0.14	0.17	0.22	0.20	0.18	0.19	0.24	0.24	0.20	0.18	0.17	0.23	0.24	0.17	0.00	0.16	0.07	0.22	0.23	0.23	0.24	0.24	0.24

1- GM-99-25, **2**- KL-4, **3-** Banka Local Mung-5, **4-** IPM-99-125, **5-** IC-683, **6-** GP-276, **7-** IPM-2-3, **8-** Samrat (C), **9-** BRM-8-1, **10-** Meha, **11-** Banka Local Mung-2, **12-** Banka Local Mung-7, **13-** Banka Local Mung-1, **14-** IC-314326, **15-** IPM-409-4, **16-** PM-5 (C), **17-** DMG-1103, **18-** IPM-2-14, **19-** DMG-1105-1-2, **20-** IC-39403, **21-** HUM-12, **22-** IC-369233, **23-** LM-249, **24-** HUM-16 (C), **25-** IC-324012, **26-** BRM-1, **27-** SML-668, **28-** GG-1980, **29-** KL-1, **30-** LM-126, **31-** DMG-1105-2-2, **32-** Pusa Vishal (C), **33-** LM-3, **34-** IC-16033, **35-** Banka Local Mung-4, **36-** IPM-205-7

Fig. 1: Dissimilarity matrix between 36 genotypes of mungbean (DARwin 6.0 program)

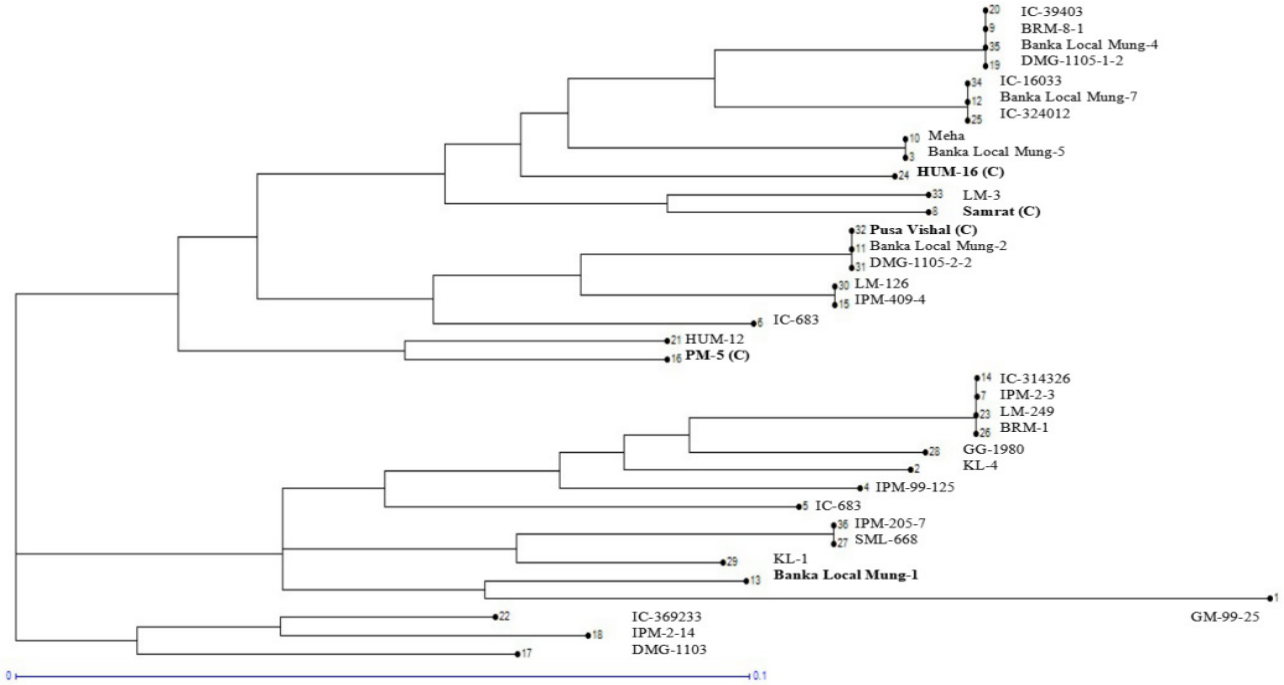


Fig. 2: Clustering of mungbean genotypes by UPGMA using molecular data

Interestingly, the molecular clustering pattern did not strongly correspond to geographical origin, as also observed in mungbean by Molla *et al.* (2016) and Rohilla *et al.* (2022). This implies that mungbean breeding and seed exchange have facilitated genetic mixing across regions. The SSR primers used in this study were sourced from both mungbean-specific SSRs (Somta *et al.*, 2008) and adzuki bean-derived SSRs (Wang *et al.*, 2004). The choice of adzuki bean primers was based on their proven cross-transferability to mungbean due to the close phylogenetic relationship within the Vigna genus (Somta & Srinives, 2007). In this study, 4 of the 10 adzuki bean-derived markers were polymorphic, confirming their utility for diversity studies in mungbean.These results collectively demonstrate that while the studied mungbean germplasm exhibits narrow molecular diversity, the identified polymorphic SSRs can serve as valuable tools for parent selection in breeding programs. Expanding germplasm coverage and integrating additional cross-transferable markers could provide a more comprehensive view of mungbean genetic diversity, aiding in the development of improved cultivars with higher yield potential and stress resilience.

Conclusion and Future Research

The present study highlights the genetic diversity available among mungbean genotypes, underscoring its potential for varietal improvement. However, the genetic base of mungbean remains relatively narrow compared to other pulses, necessitating the incorporation of wild relatives and novel germplasm. Future research should emphasize integrating conventional breeding with molecular and genomic tools for trait-specific improvement. The use of genomic selection, coupled with high-throughput phenotyping and advanced bioinformatics, will further accelerate breeding efficiency. Moreover, research should also focus on enhancing traits linked with sustainability such as resource-use efficiency, resilience to climate variability, and nutritional fortification to ensure mungbean remains a key crop for food, nutrition, and livelihood security in the future.

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Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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