



RESEARCH ARTICLE

Molecular characterisation and genetic diversity in mungbean (*Vigna radiata* (L.) Wilczek) using SSR markers

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Abstract

Mungbean (*Vigna radiata* (L.) Wilczek) is India's third most important pulse crop, accounting for nearly 16 % of total pulse area. Mungbean is grown in almost all the states. India is the major producer of mungbean in the world. Mungbean crop diversity and mungbean yellow mosaic virus (MYMV) afflicted area has continuously raised since the mid-1990s. Because of its sensitivity to the MYMV, the average yield of mungbean is poor. Mungbean yellow mosaic virus disease (MYMD) is caused by the MYMV, which is transferred by whiteflies (*Bemisia tabaci*). Several types of molecular markers have been used for breeding in mungbean, among which SSR markers have rapidly become a preferred type of molecular marker for germplasm analysis because SSR markers are locus specific, highly polymorphic due to their co-dominant nature. In the current study, a total of 12 genotypes were assessed against the MYMV in Kharif, 2021, together with the susceptible control local mungbean. The detection of genetic variation in mungbean genotypes was effectively done using a total of 14 SSR markers. Disease resistance phenotypes and SSR marker analyses showed a good correlation. Phenotypic screening identified nine resistant, 2 moderately resistant and one susceptible genotype (LGG450). Molecular analysis revealed that 12 out of 14 SSR markers were polymorphic, with polymorphism information content (PI values ranging from 0.15 to 0.63 (average 0.38). These findings demonstrate a high degree of genetic variability across various mungbean genotypes, to improve the resistance of the mungbean genotypes to MYMV, resistance genotypes identified to be useful.

Keywords: begomovirus; disease resistance; genotype screening; polymorphism information content value; UPGMA analysis

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is a legume crop commonly grown in South and Southeast Asia, characterised by rapid growth, good nutrition, antioxidant capacity and a crucial role in ensuring food security (1). India is the global leader in its production, followed by China and Myanmar. This crop is cultivated primarily for its dry seeds, which are rich in easily digestible protein, carbohydrates, essential amino acids (especially leucine, phenylalanine, lysine, valine and isoleucine), vitamin C, folic acid, thiamine and important minerals such as iron, zinc, potassium, magnesium, copper, manganese and phosphorus (2, 3) and are used to prevent heatstroke, support digestive health, promote weight loss and lower LDL cholesterol, among other benefits. The mungbean crop performs best under warm and humid conditions, with optimum temperatures of 25–35 °C and well-distributed rainfall of 400–550 mm during the growing season (1). Its short growing cycle of 75–90 days allows for multiple harvests per year, increasing food availability and farm income. Mungbean has a relatively small genome (579 Mb) and a diploid chromosome number of 22 (2n = 2x = 22) (4–6).

The mungbean crop faces numerous challenges, including a shortage of quality seeds and pest and disease pressure, resulting in low yields, which are exacerbated by the reliance on traditional

farming methods. However, this crop offers several advantages for sustainable agriculture: it has a short shelf life, fixes atmospheric nitrogen, improves soil fertility through biological nitrogen fixation, requires little water and produces good biomass. Furthermore, it helps reduce greenhouse gas emissions by increasing carbon absorption, making it a highly valued legume crop worldwide (7). However, global productivity averages only 0.5 tonnes/ha, far below its potential yield of 2.5–3.0 tonnes/ha of mungbean (8). Both biotic (pesticides and diseases such as mungbean yellow mosaic virus disease (MYMD), powdery mildew and bacterial leaf spot) and abiotic stresses (drought, heat, salinity and waterlogging) limit its expansion, with MYMD being the most serious threat in tropical and subtropical regions (9).

The MYMD is caused by mungbean yellow mosaic virus (MYMV) and it's transmitted by the whitefly (*Bemisia tabaci*). The virus grows and spreads rapidly under favourable environmental condition in the presence of susceptible hosts and virus vectors (*B. tabaci*) (10). Symptoms typically begin as small yellow spots along leaf veins, which in severe cases spread to cover the entire leaf in a single layer. The disease causes stunted growth, reduced flowering and pod formation, delayed crop maturity and significant yield reductions. Losses from early-stage infections are much greater than those from later-stage infections. In heavily affected

plants, chlorosis, reduced branching and suppressed shoot growth lower yield components such as plant height, fresh biomass, seed weight and yield per plant. Pods and seeds often become deformed, though seed germination remains unaffected (11). Yield reduction has been strongly correlated with MYMV severity, with path coefficient analysis indicating that reduced seed weight is the primary indirect effect (12). Economic losses from MYMV can reach up to 85 % in severely affected regions (13). The virus was first reported in India in 1950 at IARI, New Delhi (14).

Both male and female whiteflies can transmit MYMV, although females transmit the virus four times more efficiently than males. Interestingly, neither vector remains infectious throughout its lifespan. Integrated pest management strategies combining cultural, mechanical, biological and chemical control are also used, although reliance on insecticides has raised environmental and health concerns. Resistance to MYMV in host plants is considered the most effective and environmentally friendly strategy for managing MYMD, so breeding for MYMV resistance remains a priority. Early efforts relied on morphological traits, isozymes and biochemical markers to study genetic diversity in *V. radiata* (15). However, advanced breeding programs require more reliable tools such as molecular markers, which can tag multiple genes, aid in variety identification, support genetic mapping and assess diversity within germplasm collections (16).

The use of simple sequence repeat (SSR) markers has significantly improved mungbean breeding efforts. The SSR markers are abundant, co-dominant, highly polymorphic, reproducible and easy to assay using polymerase chain reaction (PCR) (17, 18). Compared to older marker systems such as RAPD and ISSR, SSRs are more informative due to their locus specificity, high polymorphism from repeat variation and wide distribution across the genome (19). Their application has accelerated the identification of MYMV-resistant mungbean genotypes and greatly enhanced the efficiency of marker-assisted breeding programs (10). In this study, we hypothesised that using highly polymorphic SSR markers would reveal diverse genetic variations and markers associated with MYMV resistance in the selected germplasm, thus facilitating differentiation between resistant and susceptible genotypes. For these reasons, we used 14 SSR markers to estimate the genetic diversity and determine the population structure of *V. radiata*. Therefore, determining the genetic diversity of extant species of *V. radiata* and identifying the distribution of genetic differences within and among their populations were the primary goals of this study.

Materials and Methods

A total of 12 different mungbean genotypes (Table 1) collected from the AICRP (All India Coordinated Research Project) on MULLaRP (Mung, Urd, Lentil, Lathyrus, Rajmash and Pea) were used for screening against MYMV. The seeds were germinated under favourable environmental conditions, with adequate water and sunlight, at the College of Basic Science and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa. After 10 to 12 days, the leaves of the plants were collected and used for total genomic DNA extraction.

Total genomic DNA extraction

Young leaves were collected from 10 to 12 days old *V. radiata* plantlets. The modified Cetyl Trimethyl Ammonium Bromide

Table 1. List of *Vigna radiata* genotypes used for SSR marker analysis

Sl. No.	Genotype
1	RMG1132
2	SKNM1911
3	IPM99125
4	IPMD1012
5	SML1082
6	JGPM5042027
7	MHBC203
8	SML1933
9	IPM214
10	IPM23
11	TBMB172
12	LGG450 (S check)

(CTAB) method of total genomic DNA extraction was used (20). Using an autoclaved mortar and pestle, the leaves were ground with preheated 2X CTAB extraction buffer (2 mL) and β -mercaptoethanol (0.4 mL). The 1 mL of the ground leaf mixture was added to a microcentrifuge tube (MCT- 2 mL) and incubated at 65 °C for 5 min with simultaneous inversion. After incubation, 1 mL of chloroform and isoamyl alcohol (24:1) was added to the MCT, then inverted for 5 min and then centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant (upper aqueous layer) was then collected in a new MCT and 5X CTAB buffer (1/5th volume of the supernatant solution) was added. The mixture was inverted and centrifuged at 12000 rpm for 10 min at 4 °C. Again, the supernatant liquid was collected in a new MCT using cut-end tips and added to chilled isopropanol (0.6 volume of the supernatant solution) to precipitate the DNA. We placed the mixture on ice for 10–15 min and then centrifuged it at 12000 rpm for 10 min at 4 °C to precipitate the DNA as a pellet. The supernatant was discarded and the DNA pellet was washed with 70 % ethanol and centrifuged at 8000 rpm for 5 min at 25 °C. The supernatant was removed and the pellet was air-dried. The pellet was dissolved in 0.1X TE buffer (100 μ L) and the total genomic DNA was stored at 4 °C for further use.

SSR marker analysis

Mungbean genotypes were characterised using 14 SSR markers (Table 2).

The SSR marker amplification was performed using 50 ng of template DNA, 0.2 μ M primers (forward and reverse, each), 5 μ L of All-in-one PCR Supermix (amaR OnePCR™ GeneDireX, Inc., Taiwan) and 3 μ L of molecular grade nucleases-free water (MP Biomedicals, USA) to obtain a total of 10 μ L of PCR reaction. The PCR steps were as follows: initial denaturation for 2 min at 94 °C, followed by 30 cycles of denaturation for 1 minute at 94 °C, primer annealing for 30 sec at TA (annealing temperature of primers mentioned in Table 2), primer extension for 1 min at 72 °C and final extension for 3 min at 72 °C. After the reaction was completed, the quality of the PCR product was measured by running 1.2 % agarose gel electrophoresis and an image was captured using a gel documentation system (20). Further, the presence or absence of DNA bands for each SSR marker was given a score of 1 or 0, respectively. By applying the unweighted pair-group approach and the arithmetic mean (UPGMA), the dendrogram was created based on similarity indices produced. The NTSyspc software was used for analysis to generate the dendrogram (21).

Polymorphism information content (PIC) analysis:

The matrix of binary data for each SSR maker was used for data analysis. The PIC was calculated using the following formula:

Table 2. List of SSR marker pairs used to characterise mungbean genotypes

Sl. No.	Primer name	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')	Annealing temperature (°C)
1	MBM00389	GCAAGTGTTCGACGAGGTT	GCTACACGCCATTGTTTTGA	55 °C
2	MBM0003	GGAAGGGAAGGAAAAAGGAA	ATCCAGAAACCGAATCGTTG	53 °C
3	MBM00201	ATTTGCATCTCACGTGTTGG	CCGGGAAGGTTCAAAAAGAT	54 °C
4	MBM01589	ATGGGTGTTCTTGCCCAAC	ACCAATAGCAGCCAGACGTT	56 °C
5	MB112A	TGGTTGGTTGGTTCACAAGA	CACGGGTCTGTCTCCAATA	54 °C
6	MR7322B	TCAGTCAGTGTGATAGCATAGC	GACACAGAGAGAGAGAGAGAG	57 °C
7	MAB128113	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGTTG	56 °C
8	MVR2	CGCCCTCTAGGTTGGTTGG	GGGAAAGACGAAGGTTAGAA	58 °C
9	MSSR182	TAGAGCCTTCTGTTTTTCACA	AGGAGGAGGATTTTGATGATGA	54 °C
10	MSSR217	TCCTTGCCCTTATGATTCTGTGA	TTTGCCCACTTCCAACTTTA	53 °C
11	MSSR177	TGAGAAAGTAAAGGTGGGAGA	TGACAGGAACACCTTCCACA	55 °C
12	MSSR186	GAGAGAGAAGGAGAGGGAGA	GAGAGAGAAGGAGAGGGAGA	55 °C
13	MAB128079	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTACGCCAC	56 °C
14	MAB128093	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	58 °C

$$PIC = 1 - \sum (P_i)^2$$

Eqn. 1

Where, P_i is the proportion of samples carrying the allele of a particular locus. High, medium or low polymorphism is in accordance with $PIC > 0.5$, $PIC > 0.20$ and $PIC < 0.20$, respectively.

Results and Discussion

Genetic diversity analysis among *Vigna radiata* genotypes using SSR markers

In the present investigation, 14 SSR markers were used for diversity analysis among 12 *V. radiata* genotypes (Fig. 1). Out of 14 SSR markers used, 12 SSR markers were found to be polymorphic (Table 3). The PIC value ranged from 0.15 to 0.63 with an average of 0.38. The maximum PIC value (0.63) was observed in MBM0003 marker and minimum PIC value (0.15) was observed in MSSR182 marker.

Data analysis followed the procedures outlined in the materials and methods section, using a matrix of binary data for each SSR marker. All the polymorphic SSR markers employed in this investigation had PIC values ranging from 0.15 to 0.63. (Table 3). Using the Dice similarity coefficient in the NTSYSpc software, the bands produced by all the SSR primers were taken into consideration for analysing the similarity coefficient. The range of the similarity coefficient was 0.2857 to 0.8571. (Table 4). The lowest similarity coefficient (0.0000) was observed between the susceptible check LGG450 and genotypes IPM99125, SML1082 and SML1933, indicating a complete lack of shared polymorphic alleles at the loci examined. Other highly divergent pairs included SKNM1911 and SML1933 (0.2857) and MHBC203 and TBMB172 (0.2857). Conversely, genotypes IPM99125 and IPMD1012 showed the highest genetic

similarity with a coefficient of 0.8571. Subsequently, cluster analysis was performed using the UPGMA to visualise these relationships in a dendrogram.

A dendrogram was prepared using SSR marker data, which divided the 12 mungbean genotypes into 2 main groups I and II (Fig. 2). Group I was further subdivided into 2 subgroups, IA and IB. Two genotypes (RMG1132 and SKNM1911) grouped in group IA showed moderate resistance to MYMV infection. Group IB was further divided to form IB-1 and IB-2. One resistant genotype (MHBC203) was included in group IB-2, although most of the resistant genotypes were grouped under group IB-1, which was further divided into 2 sub-groups. On the other hand, only one genotype (LGG450) in group II was found to be susceptible to MYMV.

Discussion

Evaluation of 12 *V. radiata* genotypes revealed a significant spectrum of viral responses, with infection rates ranging from 6.00 % to 50.00 %. While the absence of completely immune genotypes suggests that complete resistance is rare, the high frequency of resistant responses (75.00 % of the population) indicates strong genetic tolerance within the studied germplasm. The superior performance of nine resistant lines i.e. IPM99125, IPMD1012, SML1082, JGPM5042027, SML1933, IPM214, IPM23, MHBC203 and TBMB172 is particularly significant, as these genotypes maintained high seed yield, effectively bridging the gap between disease resistance and yield stability (22–24). The use of 14 SSR markers revealed a polymorphism rate of 71.43 %, confirming the high-resolution power of these genomic tools for characterization (25–27). However, this efficiency is highly dependent on the genetic background of the specific germplasm,

Table 3. Polymorphism and PIC values in *Vigna radiata* genotypes as revealed by SSR marker

Sl. No.	SSR marker	PIC calculated value	Polymorphism
1	MBM00389	0.28	Medium polymorphism
2	MBM0003	0.63	High polymorphism
3	MBM00201	0.43	Medium polymorphism
4	MBM01589	0.17	Low polymorphism
5	MB112A	0.54	High polymorphism
6	MR7322B	0.23	Medium polymorphism
7	MAB128113	0.61	High polymorphism
8	MVR2	0.24	Medium polymorphism
9	MSSR182	0.15	low polymorphism
10	MSSR217	0.60	High polymorphism
11	MSSR177	0.50	High polymorphism
12	MSSR186	0.37	Medium polymorphism
13	MAB128079	-	Non-polymorphic
14	MAB128093	-	Non-polymorphic

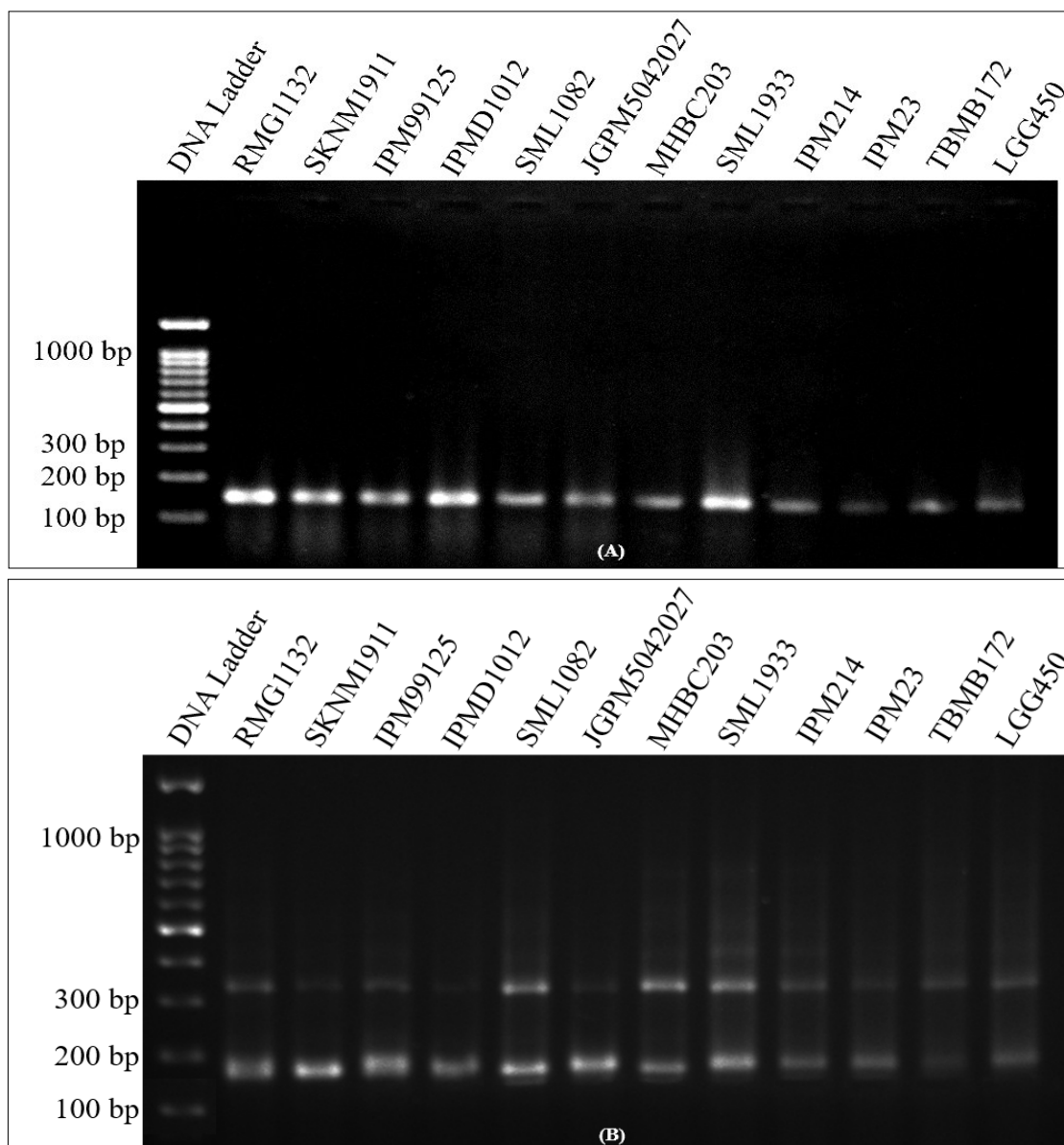


Fig. 1. The Simple Sequence Repeat (SSR) profiles of 11 mungbean genotypes using different SSR markers. The amplified PCR product was separated on 2 % agarose gel. SSR marker analysis using A- MAB128113; B- MR7322B markers in mungbean genotypes.

Table 4. Estimates of 12 polymorphic SSR markers-based similarity coefficient among 12 *Vigna radiata* genotypes

	RMG1132	SKNM1911	IPM99125	IPMD1012	SML1082	JGPM5042027	MHBC203	SML1933	IPM214	IPM23	TBMB172	LGG450
RMG1132	1.0000											
SKNM1911	1.0000	1.0000										
IPM99125	0.5714	0.5714	1.0000									
IPMD1012	0.5714	0.5714	0.8571	1.0000								
SML1082	0.5714	0.5714	0.7143	0.5714	1.0000							
JGPM5042027	0.2857	0.2857	0.7143	0.5714	0.7143	1.0000						
MHBC203	0.4286	0.4286	0.4286	0.4286	0.7143	0.4286	1.0000					
SML1933	0.2857	0.2857	0.7143	0.5714	0.7143	0.7143	0.7143	1.0000				
IPM214	0.2857	0.2857	0.7143	0.5714	0.7143	0.7143	0.5714	0.8571	1.0000			
IPM23	0.2857	0.2857	0.5714	0.4286	0.5714	0.5714	0.4286	0.7143	0.8571	1.0000		
TBMB172	0.4286	0.4286	0.5714	0.4286	0.4286	0.4286	0.2857	0.5714	0.7143	0.7143	1.0000	
LGG450	0.1333	0.1333	0.0000	0.1333	0.0000	0.1333	0.1333	0.0000	0.1333	0.1333	0.4000	1.0000

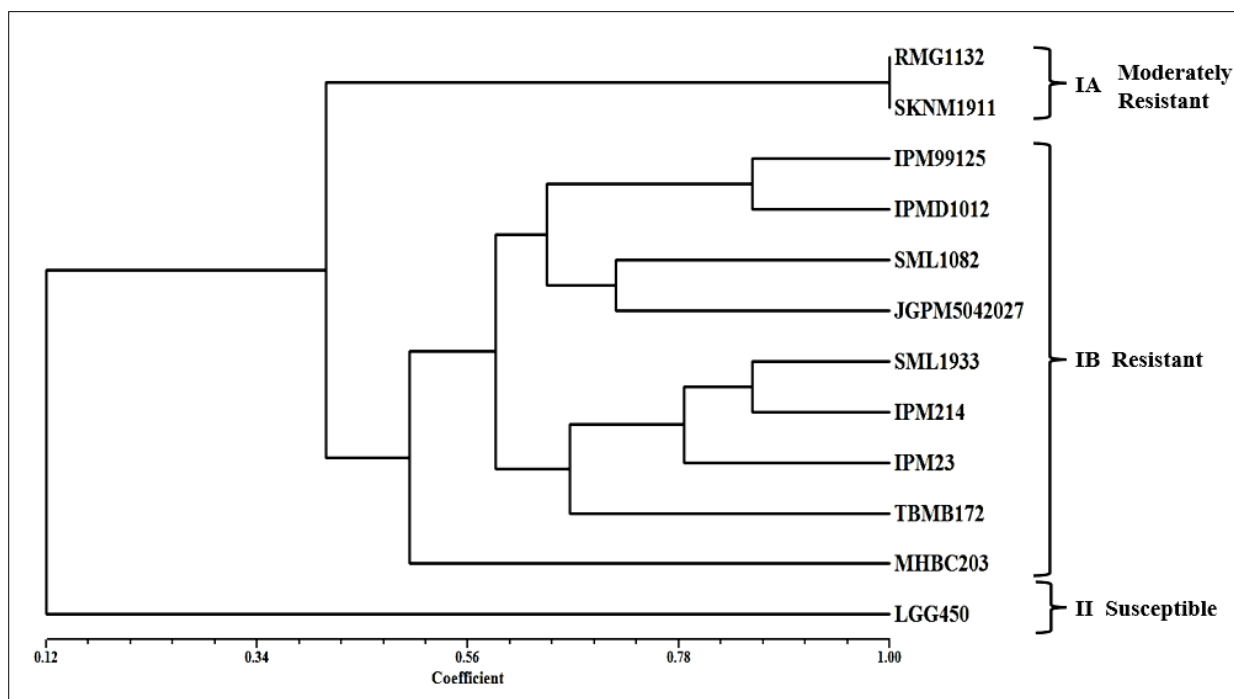


Fig. 2. Dendrogram depicting the classification of mungbean genotypes constructed through UPGMA method and based on SSR marker. The scale at the bottom is Jaccard's coefficient of genetic similarity.

as evidenced by the wide range of polymorphism reported in various studies (20.00 % to 100.00 %) (28–32).

An average PIC of 0.38 indicates that the selected markers possess moderate to high discriminatory power. Specific markers such as MBM0003, MAB128113, MSSR177 and MB112A were found to be highly informative (PIC up to 0.63), providing a valuable toolkit for future marker-assisted selection (MAS). These markers are likely associated with highly variable genomic regions, making them more effective than standard SSRs for diversity mapping and variety identification (25, 33). Although similarity coefficients ranging from 0.00 to 0.86 suggest a relatively narrow genetic base, a common constraint in cultivated mung bean due to intensive selection for specific agronomic traits, this finding should be considered in the context of the focused sample size of 12 genotypes used in this study (33, 34). The UPGMA-based dendrogram successfully translated these molecular distances into a biological hierarchy, clustering the genotypes into 2 main groups. The distinct clustering of all MYMV-resistant genotypes in sub-group IB demonstrates a strong "genotype-to-phenotype" relationship. This suggests that the genomic regions targeted by these SSR markers are either directly linked to or associated with quantitative trait loci (QTLs) controlling MYMV resistance, validating molecular fingerprinting as a reliable alternative for field-level screening.

For practical breeding applications, the combination of high PIC markers and low similarity coefficients provides a strategic roadmap for parental selection. The low similarity values were also observed in several previous studies which support our findings (35, 36). The highest genetic similarity (0.8571) between IPM99125 and IPMD1012 suggests that these lines should not be crossed, as their genomic redundancy offers little potential for genetic gain. Conversely, a cross between MHBC203 and TBMB172 is highly recommended; despite both exhibiting phenotypic resistance, their significant genetic distance (0.2857) indicates that they possess complementary alleles. Using such diverse resistant parents is an ideal strategy for gene pyramiding and achieving transgressive segregation in the progeny, which significantly accelerates the development of elite, MYMV-tolerant mung bean varieties.

Conclusion

The precise relationship established here between SSR marker data and MYMV resistance phenotype provides a crucial roadmap for mungbean improvement. By mapping genetic variation across three distinct clades and identifying MBM0003 as a high-utility marker, this study transforms raw diversity data into a functional breeding toolkit. These findings empower breeders to move beyond existing partially resistant genotypes, facilitating the targeted development of highly resilient *Vigna radiata* varieties. Ultimately, this molecular framework serves as a vital bridge between genetic potential and field-level disease management.

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Authors' contributions

SKK Carried out the research, analysis of data and drafted the manuscript. BDP Supervision, carried out the research, analysis of data and drafted the manuscript. SS Supervision, carried out the research, analysis of data and drafted the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

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

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