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Assessment of Genetic Diversity for MYMV Resistance in Mungbean [*Vigna radiata* (L.) Wilczek] using SSR Markers

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ABSTRACT

Background: Mung bean (*Vigna radiata* L.) is among the most significant edible legume crops and consumed in various parts of the world. Among different biotic factors, mungbean yellow mosaic virus (MYMV) affects this crop. The objective of the present investigation was to assess genetic variability among mungbean genotypes for MYMV resistance using simple sequence repeat markers.

Methods: The present research was conducted to assess the genetic diversity among selected mungbean genotypes from field screening of 303 genotypes with an emphasis on resistance against MYMV. The field screening was done at Seed Breeding Farm, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh during summer 2022, *kharif* 2022 and summer 2023. Thirty SSR markers have been utilized to measure genetic variation among the 32 studied genotypes.

Result: Marker MB-SSR033 stands out as the most informative locus with the greatest PIC and gene diversity value. The cluster analysis, classified the 32 genotypes into two major groups and maximum distance was noted between PDM 139 and IC 314523. The SSR markers CEDG293 (10.14%) and DMBSSR008 (9.11%) were found considerably linked to MYMV resistance. Harnessing multiple sources of resistance gives a good possibility to boost the resistance and production of mungbean cultivars.

Key words: Cluster analysis, Marker-trait association, SSR markers, Yellow mosaic resistance.

INTRODUCTION

Mung bean (*Vigna radiata* L.) or green gram, is one the most significant legume crops mostly grown for consumption as food. This important crop is cultivated on over six million hectares globally and is consumed by a large proportion of Asian households (Hou *et al*., 2019). High amounts of proteins and amino acids are found in mungbeans, which greatly enhances their nutritional value (Hadidi *et al*., 2021). According to Mehandi *et al*. (2019), these legumes are strategically positioned in Southeast Asian nations to sustain crop production and ensure nutritional security. Mungbean Yellow Mosaic Virus (MYMV) is considered the deadliest virus-related disease in the regions where it is grown (Mishra *et al*., 2020). Yearly, the disease manifests in a severe form, with yield penalties reaching 85-100% during epidemic years (Singh *et al*., 2018).

MYMV is a chronic circulative member of the genus Begomovirus, which is spread by whiteflies (*Bemisia tabaci*) (Mishra *et al*., 2020). The lack of a standard screening process and the fact that recessive genes often control resistance, make it difficult for YMV resistance to introgress in mungbean necessitates the application of modern biotechnological tools for precisely identifying genotypes resistant to MYMV (Jeevitha *et al*., 2022). It has long been believed that using mungbean cultivars resilient to MYMV is an efficient and cost-effective method of controlling the virus (Karthikeyan *et al*., 2011). Numerous investigations assessed the germplasm of mungbean for MYMV resistance and only few exhibited resistance (Basavaraj *et al*., 2019). Field level studies have some limitations due to their dependencies on environmental conditions. However, studies under laboratory conditions always add values to the field data and help researchers to draw a conclusion. Therefore, it is important to combine field level screening of mungbean genotypes with DNA based screening against MYMV to identify MYMV resistant mungbean genotypes.

DNA based markers especially microsatellites have proven their potential in the field of molecular breeding (Sao *et al*., 2024 and Pratap *et al*., 2015) due to their excellent repeatability and ease of application. The accessibility of credible markers for marker-assisted selection (MAS) and the detection of QTLs for traits of interest were demonstrated by the effective implementation of SSR markers in the construction of linkage maps for mungbean (Isemura *et al*., 2012). In light of the aforementioned, this

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study was undertaken to assess the genetic variation and analyses the population structure of the 32 mungbean lines using thirty microsatellite markers.

MATERIALS AND METHODS

Experimental details

The field experiment was conducted in the breeder seed farm at the College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh, to evaluate the MYMV disease reaction of 303 genotypes including three checks (Virat, Shikha and MH 421) under natural field conditions with augmented block design in summer 2022, *kharif* 2022 and summer 2023. All standard procedures and package of practices were followed, with the exception of systemic pesticides used to control whiteflies. To increase MYMV infection, two rows of infectors were placed all around the experiment. The crop was often observed to check for the emergence of MYMV symptoms. Infection on the leaves of each plant at 50% flowering was recorded and Percentage Disease Incidence (number of plants infected) was worked out using the formula:

Percentage disease incidence =

$$\frac{\text{Total number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

The genotypes were then scored based on PDI using a scale of 0 to 9 (Mohan *et al*. 2014) where, 0: immune (I); 1: resistant (R); 3: moderately resistant (MR); 5: moderately susceptible (MS); 7: susceptible (S) to 9: highly susceptible (HS).

Based on the performance of three seasons, total thirty-two mungbean genotypes (Table 1), including resistant check Virat and a suitable susceptible check, *i.e*., China mung was selected for the further molecular analysis.

Genomic DNA extraction

For genomic DNA isolation, juvenile leaf specimens from a seedling that was one month old were collected. Following the guidelines in the handbook, the gathered leaves were crushed and homogenised in liquid nitrogen and then genomic DNA was extracted utilising the NucleoSpin® Plant II kit. Isolated DNA was qualitatively assessed on a 0.8% agarose gel, by electrophoresis in 1X TAE buffer. During electrophoresis, an uncut λHind III DNA ladder was used as a reference.

Microsatellite based diversity analysis

In order to identify polymorphism in markers for further investigation, the SSR markers were first examined using template DNA from four mungbean genotypes. Only thirty SSR markers (Table 2) out of all examined were able to generate polymorphic alleles and were further processed with all of the mungbean genotypes. The amplification was performed using the Mastercycler Nexus PCR apparatus. The PCR master mix was prepared using 2 µl of DNA sample, 7 µl of master mix, 4 µl of nuclease-free water and 0.5 µl of each of the forward and reverse primers. A total of 14 µl was used. The PCR was programmed to run for five minutes at 94°C for initial denaturation, for one cycle, further 35 cycles of 30 s for annealing at 52-55°C (depending on the primer) and 60 s for elongation at 72°C. The last extension was scheduled for 5 minutes at 72°C. The products of amplification were resolved on 3% agarose gel through electrophoresis and visualized under the Syngene gel documentation system. Identification of polymorphic SSR markers was based on amplicon sizes as determined by comparing them with a 100-bp DNA ladder.

Data analyses

Powermarker version 3.25 software (Liu and Muse, 2005) was used to calculate the major allele frequency, average allele number, gene diversity, heterozygosity and polymorphic information content (PIC) values from molecular data. The UPGMA (Unweighted Pair Group Approach with Arithmetic Mean) technique was used to construct a dendrogram using Molecular Evolutionary Genetics Analysis (MEGA) software. Based on statistical results, TASSEL 5.0, software version 2.1 (https://www.maizegenetics.net), was used to determine the marker-trait association among specific markers and MYMV resistance.

RESULTS AND DISCUSSION

Field screening for MYMV resistance

Along with other desired features, it is important for plant breeders to develop mungbean cultivars that are resistant to MYMV. The discovery of seasonally suited, disease-resistant genotypes is essential for the success of the genetic improvement programs (Buttar *et al*., 2023). Based on the PDI values all 303 genotypes provided different disease reactions against MYMV in three different seasons *viz*., Summer 2022, *Kharif 2022* and Summer 2023. However, according to the pooled analysis after considering average performance of genotypes based on the per cent infection, 135 lines were recorded as resistant, 92 lines showed moderately resistant reaction, 39 were moderately susceptible and 24 were susceptible, while 13 were highly susceptible (Data not shown). The infector line used was completely infected by MYMV in all the three seasons. The genotypes were selected on the basis of their agronomic performance and MYMV resistance reaction in the field throughout the screening period of study. The identified consistently resistant genotypes were further used for microsatellite markers-based analysis.

Molecular analysis

Understanding the genetic structure of mungbean diversity is crucial as it aids in the selection of necessary parents for breeding programmes by breeders. It has been revealed that SSR markers-based method is an effective method for identifying the genetic diversity of mungbean populations as they are independent of the environment, molecular markers are useful for estimating the genetic

variation found in the germplasm (Mwangi *et al*., 2021 and Rohilla *et al*., 2022). The PIC of these markers is their capacity to identify polymorphisms and is crucial in choosing markers for genetic research (Serrote *et al*., 2020). The PIC values varied from 0.850 (MB-SSR033) to 0.110 (CEDG225), with an overall average of 0.506, indicating that the majority of the markers could be used for germplasm genotyping. However, 18 markers were more informative since their PIC values were greater than 0.5. The most relevant locus, according to the PIC values, was MB-SSR033, with the greatest PIC value. The current study showed that the PIC values of the markers ranged from 0 to 1, which was consistent with earlier research findings in mungbean (Sahoo *et al*., 2022; Kaur *et al*., 2016). The major allele frequencies of 30 SSR markers varied from 0.938 (CEDG225) to 0.219 (MB-SSR033) throughout the co-dominant loci in the current study. Among the primers, the major allele frequency had an average value of 0.586 (Table 2). The average value for gene diversity was noted as 0.1561. MB-SSR033 (0.864) had the largest gene diversity, followed by primers CEDG211 (0.825), CEDG136 (0.777), CEDG121 (0.753), MB-SSR86 (0.729), DMBSSR043 (0.726) and Satt226 (0.705). As revealed in Table 2, the markers with the lowest diversity were CEDG225 (0.117) and DMBSSR024 (0.174).

Cluster analysis

During the selection process, a prominent technique called cluster analysis is most often employed to identify genotypes from the germplasm harboring desired traits. Initially, the 32 genotypes of mungbean formed two groups (group I and group II) in the dendrogram created based on the UPGMA (Unweighted Pair Group Method with Arithmetic Mean), as shown in Fig 1. Group I was further divided into two sub-groups IA and IB. Sub-group IA was differentiated into IA-1 and IA-2. Sub-group IA-1 consisted of total 10 genotypes *viz*., CHINA MUNG, IC 314523, IC 103821, IC

Table 1: Mungbean genotypes identified for molecular analysis based on pooled values of MYMV resistance.

Genotypes	PDI (%)				Reaction group
	Summer 2022	*Kharif* 2022	Summer 2023	Pooled	
IPM 2K-14-9	0.86	0.55	0.41	0.61	R
IC 314523	0.71	0.47	0.50	0.56	R
MH-3-18	0.39	0.18	0.38	0.32	R
IC 348964	0.36	0.67	0.78	0.60	R
IC 103821	0.59	0.72	1.00	0.77	R
IC 121301	0.27	0.30	0.94	0.50	R
IC 52078	1.00	0.63	0.94	0.86	R
IC 73395	0.57	0.45	0.70	0.57	R
OMG-1045 (PMR)	0.77	0.11	0.24	0.37	R
IC 373199	0.40	0.92	0.55	0.62	R
IC 417873	0.66	0.65	0.42	0.58	R
IC 15567	0.82	0.12	0.76	0.57	R
EC 520041	0.29	0.93	0.76	0.66	R
IC 119033	0.66	0.34	0.76	0.59	R
IC 314697	0.52	0.68	0.56	0.59	R
SML 2122	0.76	0.11	0.54	0.47	R
EC 398885	0.26	0.56	0.83	0.55	R
EC 398891	0.10	0.96	0.77	0.61	R
EC 520026	0.35	0.77	0.56	0.56	R
EC 520029	0.40	0.23	0.94	0.52	R
EC 520038	0.52	0.27	0.33	0.37	R
VGG-15-030	0.31	0.37	0.47	0.38	R
VGG 17-019	0.16	0.21	0.51	0.29	R
PDM 139	0.54	0.26	0.75	0.52	R
BHUTAN LM-95	0.97	0.95	0.70	0.88	R
PRAKASH NEPAL	0.63	1.00	0.91	0.85	R
EC 520024	0.42	0.66	0.80	0.63	R
MUNG LOCAL NASURULLAGANJ	0.38	0.70	0.76	0.61	R
IPM 312-86-1	0.40	0.68	0.43	0.50	R
MH 421	0.18	0.64	0.29	0.37	R
Virat (Resistant check)	0.89	0.89	0.09	0.62	R
CHINA MUNG (Susceptible check)	66.69	66.21	41.16	58.02	HS

Table 2: List of SSR primer with sequences.

SSR primer	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	MAF	NA	GD	H	PIC	MTA	
								R^2	P value
CEDG293	GGATGGTAATGGTAGTTGCTG	CTTCTAGAAACCCGTCACTG	0.688	6	0.496	0.625	0.466	10.14	0.05
CEDG211	GAGTGTGCATATGTGAGAG	CAAGTCTAATCTCTGACTCC	0.234	9	0.825	0.156	0.802	2.70	0.06
CEDG121	CTTTCAAAATAATGTTGAGGCATA	CAATACATAAATAACCTTTTCTGC	0.313	6	0.753	0.031	0.712	2.10	0.06
DMBSSR059	TGCCAGATTTGAGAAGAAAGGT	CATGCATGTGGATAAGAATTCAG	0.844	5	0.281	0.031	0.270	1.50	0.05
DMBSSR008	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC	0.484	6	0.687	0.031	0.647	9.11	0.05
CEDG275	CACACTTCAAGGAACCTCAAG	GTAGGCAACCTCCATTGAAC	0.547	8	0.634	0.688	0.593	1.15	0.05
CEDG006	AATTGCTCTCGAACCAGCTC	GGTGTACAAGTGTGTGCAAG	0.594	4	0.580	0.000	0.531	5.64	0.07
CEDG041	GCTGCATCTCTATTCTCTGG	GCCAACTAGCCTAATCAG	0.500	3	0.594	0.000	0.511	5.13	0.07
VES0503	CGCTTTTGTAGGATTGGAACA	TGAAGGATGAGGGGAAGATG	0.797	2	0.324	0.406	0.271	3.14	0.05
MB-SSR033	CTATTCCTGAGTGCAGGTTC	GTGTGTGTGTTCTCGTGTGT	0.219	12	0.864	0.031	0.850	2.17	0.07
CEDG042	CACAGTGGTTTGGGCAACAG	TCAGAGGTTCCCATTTCCCG	0.469	6	0.632	0.906	0.565	1.90	0.07
CEDG100	CCCATCAAGTAACTACATAACA	ATGTGGGACTGGACAAATAAAA	0.563	6	0.621	0.063	0.580	1.84	0.07
CEDG225	GAGGAAGTGTTGCAGCACC	GTAGACTCTGCAGAGGGATG	0.938	2	0.117	0.000	0.110	2.35	0.06
DMBSSR024	TTTCTGCGAAGAATCTGAAGG	TTTTGAGTACCATGCTCTTCCT	0.906	3	0.174	0.000	0.166	1.11	0.06
CEDG118	AACCCAACCAACCCTTGTGGTAAG	GCTGGAATCATAATACCGCCTTGT	0.469	4	0.623	0.000	0.549	3.46	0.07
CEDG146	GGTGATCGGATTTCAGAG	GGAGAAGAGAATAGAGACG	0.813	3	0.314	0.000	0.281	2.10	0.06
CEDG097	GTAAGCCGCATCCATAATTCCA	TGCGAAAGAGCCGTTAGTAGAA	0.594	5	0.594	0.000	0.554	2.46	0.23
CEDG136	GTTCCAAGTCTCCAATCCGTAC	CACTTCACTAGAACTGGTTCAG	0.313	6	0.777	0.000	0.744	1.24	0.19
CEDG174	GAGGGATCTCCAAAGTTCAACGG	GAAGGCTCCGAAGTTGAAGGTTG	0.656	4	0.512	0.000	0.461	1.10	0.11
DMBSSR043	GATATGGTTTTAGAGGCGATCACA	CAATACTTCGCCAACAAATCA	0.359	4	0.726	1.000	0.676	1.56	0.13
MB-SSR8	ACCATTACCTCCACAATCTC	AATGGAGTTCTACGTGATGG	0.859	4	0.253	0.156	0.241	1.11	0.27
DMBSSR16	GTGCGAAAATTCGAAATGGT	TCAACTCAAGCAATGCTAAGTCA	0.641	6	0.554	0.094	0.525	1.86	0.06
Satt226	GCGAAACAACTCACTTAAGCAATACAT	GCGTCCTCCTACCTTTCTTATC	0.438	6	0.705	0.000	0.661	2.41	0.07
MB-SSR21	ACATCCGGGAACAAACAAAACG	AACTGAGGCTTGAGAAGATGAC	0.688	3	0.471	0.000	0.416	2.01	0.19
DMBSSR13	ACACAGATCATCATCACCAATC	ATCACACACTTTGGGTCTCAAT	0.516	5	0.670	0.844	0.633	0.33	0.07
Satt244	GCGCCCCATATGTTTAAATTATATGGAG	GCGATGGGGATATTTTCTTTATTATCAG	0.547	5	0.640	0.344	0.601	1.90	0.07
MB-SSR86	AGCGGTGGTCCGACGAGG	AAGGACCGACCACCACGAG	0.422	6	0.729	0.469	0.690	1.37	0.06
Satt500	GCGAACGACCATGATAATCACA	GCGCTCATTTGAAAGCATTGTTATA	0.766	4	0.385	0.281	0.352	1.50	0.57
MB-SSR136	ATGATGAGGTCGCAAGAGGG	TCTACCGTGCAGTCGTCGC	0.719	2	0.404	0.000	0.323	3.37	0.18
Satt044	AAAAAATATTTATAGGTTACATGTG	TTACCACTAAGAATTAGGTCTAA	0.688	3	0.461	0.000	0.398	1.79	0.13
Mean			0.586	4.93	0.547	0.205	0.506		

MAF- Major allele frequency, NA- Number of alleles, GD- Genetic diversity, H- Heterozygosity, PIC- Polymorphism information content, MTA- Marker trait association.

119033, IC 121301, EC 520029, EC 520038, IC 520041, EC 520026 and MH 421. The sub-group IA-2 was monogenotypic consisting solely of Virat. Sub-group IB was further sub-divided into two parts *i.e*. IB-1 and IB-2. IB-1 is monogenotypic and consisting EC 398885 only while, IB-2 having four genotypes EC 398891, EC 520024, IC 15567 and IC 314697 respectively. This indicated presence of considerable diversity among the genotypes studied. Out of thirty-two genotypes, 16 belong to the cluster I. Cluster II was further divided into two sub-clusters *i.e*. II A and II B. Sub-cluster II A was further named II A-1 consisted of total 4 genotypes *viz*., BHUTAN LM-95, IPM 312-86-1, MH-3-18 and IPM 2K-14-9. The Cluster II B was further sub-divided into three subgroups *i.e*., II B-1 consisting of OMG-1045 PMR and PDM-139, II B-2 having three genotypes SML2122, VGG 17-019 and VGG-15-030. The cluster II B-3 was occupied by seven genotypes *viz*., IC 373199, IC 73395, NASURULLAGANJ LOCAL, IC 348964, IC 52078, IC 417873 and PRAKASH NEPAL. The genotypes PDM-139 and IC 314523 showed the greatest degree of genetic dissimilarity in the context of the dendrogram analysis. Following that, there was a noteworthy genetic divergence between PDM-139 and CHINA MUNG, VGG-15-030 and IC 103821, PDM-139 and EC 520024 and OMG-1045 (PMR) and IC 314523. Earlier researchers also confirmed the existence of genetic variation for MYMV resistance in mungbean (Goyal *et al*., 2022; Prathyusha *et al*., 2023; Sugandh *et al*., 2018). Overall, the investigation provides a significant opportunity for the use of hybridization techniques. Harnessing different sources of resistance offers a wonderful chance to combine beneficial traits in unique ways that may increase the adaptability and productivity of mungbean. The results demonstrate the potential advantages of using genetic diversity to further agricultural biotechnology and sustainable crop development endeavors.

Marker trait association

A maximum likelihood model was used to analyze the association between the SSR markers and MYMV resistance. The model tested the correlation between the presence of specific alleles and the phenotypic expression

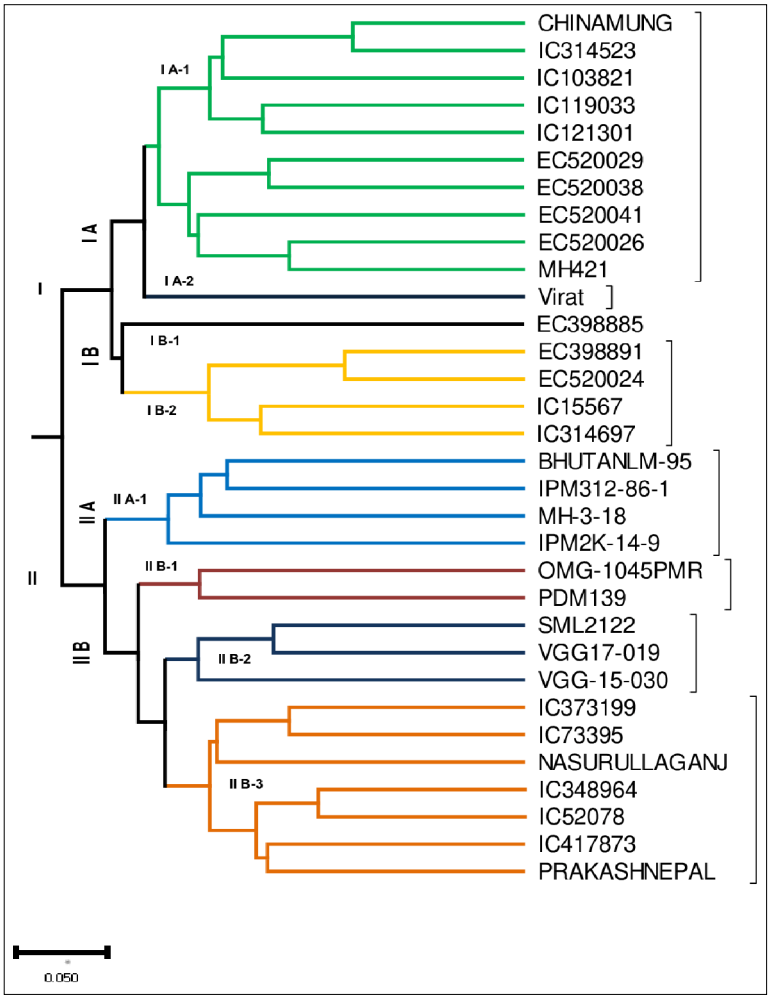

Fig 1: Clustering analysis dendrogram of 32 mungbean genotypes based on SSR data.

of resistance. Significant associations were determined based on a threshold P-value of <0.05. Specifically, association mapping was performed using the genotypic data from SSR markers and the phenotypic data for MYMV resistance. Markers trait association (MTA) was analyzed using the maximum likelihood model, 5 MTAs for MYMV were discovered in a pooled study. This correlation accounted for 0.33-10.14% of overall phenotypic variation. The findings revealed a substantial association of five microsatellite markers with MYMV at P value (P<0.05), out of five microsatellite loci, CEDG293 (10.14%) and DMBSSR008 (9.11%), being substantially linked with MYMV resistance, accounting for more than 5.00% of phenotypic variance (Table 2). The identified SSR markers emerge as strong candidates for use in future breeding programs aimed at enhancing MYMV resistance. These results were in agreement with the results of Singh *et al*. (2020) and Rohilla *et al*. (2022).

CONCLUSION

This research provides valuable insights into the genetic diversity and associations focusing on markers related to resistance, particularly against Mungbean Yellow Mosaic Virus (MYMV). Notably, MB-SSR033 emerged as the most relevant locus with the highest PIC value. The study identified 5 Marker Trait Associations (MTAs) for MYMV resistance and among these, CEDG293 and DMBSSR008 showed substantial association with MYMV resistance, providing potential markers for future breeding programs. Importantly, the study emphasized the potential for hybridization techniques to harness genetic diversity for improving traits, particularly MYMV resistance. The study provides a foundation for further exploration and utilization of genetic resources in breeding programs aimed at enhancing disease resilience and productivity.

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Conflict of interest

The authors declare that they have no conflicts of interest relevant to the content of this article.

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