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Research Article

Molecular diversiry studies among blackgram genotypes for yellow mosaic virus resistance

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Abstract
Yellow mosaic disease (YMD), incited by a geminivirus. *Mungbean Yellow Mosaic Virus* (MYMV), is transmitted by whiteflies, poses a significant threat to various leguminous crops in India. This study focuses on identifying resistant sources and validating molecular markers for their efficacy in detecting YMV resistance in blackgram. In the present study, 150 blackgram genotypes were evaluated to identify resistant sources for YMD under natural field conditions and through molecular markers. Among the 150 genotypes, 22 (both resistant and susceptible) were selected based on field screening. These genotypes were validated using 15 molecular markers, including 8 simple sequence repeats (SSRs) and 7 gene-specific markers linked to MYMV resistance. The markers amplified a total of 48 alleles, with polymorphic information content (PIC) values ranging from 0.82 (CEDG67) to 0.21 (HSP), with an average value of 0.55. The highest PIC values were exhibited by markers CEDG67 (0.82), CEDG115 (0.81), and CEDG20 (0.74). Cluster analysis using DARWIN revealed that most phenotypically resistant genotypes grouped together, while susceptible genotypes formed separate clusters. The genotypes LBG 904, LBG 932, LBG 884, PU 1815, PU 1808, PU 1803, MASH 1008, MASH 114, VBN 10, and PU 31 exhibited resistance to YMV phenotypically. Among these, LBG 904, LBG 932, PU 1815, PU 31, and MASH 1008 produced a 162 bp resistant allele using the marker CEDG180. Similarly, the gene-specific marker DEF produced a 190 bp resistant allele in all the resistant genotypes, whereas the susceptible genotypes produced both 190 and 250 bp alleles. These identified genotypes can serve as valuable sources for MYMV resistance in blackgram breeding programs.

Keywords: SSR markers, Blackgram, Diversity, MYMV

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper], also known as urdbean, is an important short-duration pulse crop cultivated throughout India, belonging to the family Leguminaceae. Yellow mosaic disease (YMD), caused by geminivirus and transmitted by whitefly, is a major cause of significant yield loss in pulse crops, potentially causing up to 100 percent yield loss (Naimuddin, 2001). In India, YMD is primarily caused by two virus species: *Mungbean Yellow Mosaic India Virus* (MYMIV) in northern India and *Mungbean Yellow Mosaic Virus* (MYMV) in the peninsular region (Malathi and John, 2008). Chemical management of the vector is very costly, making the development of MYMV-resistant varieties the most economical and effective method for controlling the disease and achieving higher yields. However, conventional breeding methods for MYMV resistance are time-consuming due to the rapid emergence of new isolates and the complexity of virus-vector-host interactions (Souframanien and Gopalakrishna, 2006).

Molecular marker technology offers a diagnostic tool to accurately predict the presence of specific genes,

facilitating efficient gene transfer across different genetic backgrounds. Marker-assisted selection (MAS) for resistant genotypes using linked markers is an effective approach for developing YMD-resistant blackgram cultivars. Before applying these markers in MAS programs, they must be validated across known resistant and susceptible genotypes to ensure their effectiveness. Inheritance of MYMV resistance studies revealed that the resistance is controlled by a single recessive gene (Reddy and Singh, 1995; Basak *et al.,* 2004 and Rashmi *et al.,* 2013), dominant gene (Sandhu *et al.,*1985), two recessive genes (Dhole and Reddy 2012 and Gajaraj *et al.,* 2013) and complementary recessive genes (Shukla and Pandya, 1985). Previous studies have reported the use of molecular markers linked to YMV resistance in mungbean and urdbean (Selvi *et al.,* 2006; Chen *et al.,* 2013; Dhole and Reddy, 2013; Gupta *et al.,* 2015; Mogali *et al.,* 2021; Vijay *et al.,* 2022). However, these markers were often identified using specific mapping populations, necessitating validation in diverse blackgram genotypes before MAS application. With this background, this study was conducted to screen 150 blackgram genotypes under natural field condition to identify MYMV-resistant and susceptible genotypes, followed by validation using molecular markers linked to MYMV resistance.

MATERIALS AND METHODS

Screening of blackgram genotypes for MYMV resistance: A total of 150 blackgram genotypes comprising advanced breeding lines, released and pre released varieties were screened for YMD resistance under natural field conditions at RARS, Lam during *summer* season 2022. Details of the genotypes are provided in **Table 1**. Each entry was sown in two rows of four-meter length with a spacing of 30 × 10 cm and followed all recommended agronomic practices. No insecticidal spray was used to allow the multiplication of whitefly population, the vector for MYMV, to spread the disease naturally. LBG 623, a highly susceptible cultivar of YMD, was used as an infector refuge, planted after every two rows of a single genotype. The test entries were evaluated for YMD once 80% of the plants in the infector rows showed YMD incidence. The disease severity was scored based on 1-9 scale and the blackgram genotypes were categorized as resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible according to the disease rating scale suggested by Singh *et al.* (1992).

DNA extraction and quantification: The genomic DNA was isolated from young leaves using CTAB method of Dellaporta *et al.* (1983). DNA concentration was determined using Nanodrop ND 1000 spectrophotometer (Thermo Scientific, USA). The DNA samples were diluted to 50 ng μl^{-1} for PCR amplification.

PCR amplification**:** A total of 15 molecular markers reported to be linked to MYMV resistance in blackgram and greengram were used for the validation study (**Table 2**). PCR was performed in a total volume of 10 µl containing 2 µl (50 µg/µl) genomic DNA. The master mix included 0.5 µl of 10 pmol forward and reverse primers, 0.5 µl of 2.5 mM deoxyribonucleotides (dNTPs), 1 µl of Genei 10X assay buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2), 0.1 µl of 5 U/µl Taq DNA polymerase (Bangalore Genei Private Limited, Bangalore), and 5.4 µl of sterile distilled water.

The PCR amplification was conducted in a thermocycler (Biorad thermal cycler) with the following cycle profile: initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes, with a hold at 4°C. The obtained PCR products were analyzed by electrophoresis on a 3% agarose gel (3g of agarose in 100 ml of 1X TAE buffer) using a 100 base pair DNA ladder (Takara) to determine the molecular weight of the PCR products. The gels were viewed under a gel documentation system (iBright 1500-AB system).

Estimation of PIC values and diversity analysis: The polymorphism information content (PIC) for each SSR marker was calculated following the formula given by Anderson *et al.* (1993). Cluster analysis was performed using allelic data to estimate genetic distances among the genotypes using simple matching coefficients,

Grade	Description	Reaction
1	No visible symptoms on leaves or very minute yellow specks on leaves	Resistant (R)
2	Small yellow specks with restrict spread covering 0.1-5 % leaf area	Resistant (R)
3	Yellow mottling of leaves covering 5.1-10 % leaf area	Moderately resistant (MR)
4	Yellow mottling of leaves covering 10.1-15 % leaf area	Moderately resistant (MR)
5	Yellow mottling of leaves covering 15.1-30 % leaf area	Moderately susceptible (MS)
6	Yellow mottling of leaves covering 30.1-50 % leaf area	Moderately susceptible (MS)
7	Pronounced yellow mottling and discoloration of leaves and pod, reduction in leaf size and stunting of plants covering 50.1 to 75 % foliage.	Susceptible (S)
8	Severe yellow discoloration of leaves covering above 75.1 to 90 % of foliage, stunting of plants and reduction in pod size.	Susceptible (S)
9	Severe yellow discoloration of entries covering above 90.1 % of foliage, stunting of plants and no pod formation.	Highly susceptible (HS)

Table 1. Screening of blackgram genotypes for YMD resistance

S. No.	Genotype	Disease Score	Reaction	S. No.	Genotype	Disease Score	Reaction	S. No.	Genotype	Disease Score	Reaction
1	ADT 5	7	S	51	IPU 18-7	3	MR	101	LBG 985	3	MR
2	AZAD URD 12		R	52	IPU 19-27	2	R	102	LBG 989	1	R
3	BCU 20-62	2	R	53	IPU 19-51	3	MR	103	LBG 998	3	MR
4	BCU 20-73	3	MR	54	IPU 19-53	2	R	104	LBG 999	5	MS
5	CO 5	7	S	55	KOTA URD 4	1	R	105	MASH 1008	2	R
6	GB 67	9	HS	56	KPU 175-2	4	MR	106	MASH 114	1	R
7	GBG 1	3	MR	57	KPU 18-1	6	MS	107	MBG 1080	8	HS
8	GBG 11	9	HS	58	KPU 20-01	2	R	108	OBG 45	2	R
9	GBG 12	9	HS	59	KPU 20-05	3	MR	109	PANT U 1305	3	MR
10	GBG 15	9	HS	60	KPU 21-11	3	MR	110	PBG 272	9	HS
11	GBG 18	7	S	61	KPU 405	3	MR	111	PBG 276	6	MS
12	GBG 2	8	S	62	KUG 479	1	R	112	PU 1537	1	R
13	GBG 20	9	HS	63	LBG 1001	1	R	113	PU 1801	1	R
14	GBG 22	4	MR	64	LBG 1002	1	R	114	PU 1803	1	R
15	GBG 23	9	HS	65	LBG 1003	1	R	115	PU 1805	1	R
16	GBG 24	9	HS	66	LBG 1006	1	R	116	PU 1808	1	R
17	GBG 26	9	HS	67	LBG 1008	1	R	117	PU 1810	1	R
18	GBG 3	5	MS	68	LBG 1009	1	R	118	PU 1812	1	R
19	GBG 30	9	HS	69	LBG 1010	1	R	119	PU 1815	1	R
20	GBG 45	9	HS	70	LBG 1012	1	R	120	PU 19	1	R
21	GBG 5	9	HS	71	LBG 1013	1	R	121	PU 31	1	R
22	GBG 65	4	MR	72	LBG 1016	4	MR	122	PU 35	1	R
23	GBG 66	2	R	73	LBG 1024	5	MS	123	PUSA B 28	4	MR
24	GBG 70	6	MS	74	LBG 1026	3	MR	124	PUSA B 55	5	MS
25	GBG 71	9	HS	75	LBG 1044	1	R	125	PUSA B 64	2	R
26	GBG 72	6	MS	76	LBG 1046	2	R	126	SHEKHAR 2	1	R
27	GBG 73	3	MR	77	LBG 1047	2	R	127	SUG 1279	1	R
28	GBG 77	8	S	78	LBG 1049	1	R	128	SUG 1282	3	MR
29	GBG 78	9	HS	79	LBG 1050	1	R	129	SVU 6	9	HS
30	GBG 80	2	R	80	LBG 1051	1	R	130	TBG 104	1	R
31	GBG 81	2	R	81	LBG 1053	2	R	131	TBG 129	1	R
32	GBG 82	5	MS	82	LBG 1058	3	MR	132	TBU 236-6	9	HS
33	GBG 83	2	R	83	LBG 1059	3	MR	133	TJU 130	4	MR
34	GBG 84	5	MS	84	LBG 1059-1	4	MS	134	TJU 339	5	MS
35	GBG 85	9	HS	85	LBG 1064	1	R	135	TU 1-11	2	R
36	GBG 86	9	HS	86	LBG 1068	8	HS	136	TU 1-30	3	MR
37	GBG 87	9	HS	87	LBG 1069	1	R	137	TU 40	1	R
38	GBG 88	9	HS	88	LBG 1071	1	R	138	TU 62	1	R
39	GBG 89	7	S	89	LBG 1072	1	R	139	VBN 10	1	R
40	GBG 9	8	S	90	LBG 685	9	HS	140	VBN 11	4	MR
41	GBG 90	6	MS	91	LBG 752	4	MS	141	VBN 17-021	3	MR
42	GBG 91	6	MS	92	LBG 787	9	HS	142	VBN 19-033	1	R
43	GBG 92	9	HS	93	LBG 806	1	R	143	VBN 6	1	R
44	GBG 93	9	HS	94	LBG 808	1	R	144	VBN 8	1	R
45	GBG 94	9	HS	95	LBG 884	1	R	145	LBG623	9	HS
46	GBG 95	9	HS	96	LBG 904	1	R	146	LBG645	9	HS
47	GBG 96	9	HS	97	LBG 932	1	R	147	ADTBG14003	9	HS
48	GBG 97	9	HS	98	LBG 933	3	MR	148	VBN 9	9	HS
49	IPU 11-02	1	R	99	LBG 941	1	R	149	GBG 234	9	HS
50	IPU 13-6	1	R	100	LBG 944	1	R	150	GBG 210	9	HS

R: Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; S: Susceptible; HS: Highly Susceptible

Table 2. Details of the markers used in the present study

S. No.	Name of the marker	Forward sequence	Reverse sequence	Reference
1	CEDG20	TATCCATACCCAGCTCAAGG	GCCATACCAAGAAAGAGG	Ragul *et al.* 2021
2	CEDG67	AGACTAAGTTACTTGGGCAACCAG	TGACGGCCCGGCTCTCC	Ragul *et al.* 2021
3	CEDG44	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	Singh *et al.* 2018
4	CEDG97	GTAAGCCGCATCCATAATTCCA	TGCGAAAGAGCCGTTAGTAGAA	Vijay *et al.* 2022, Vadivel *et al.* 2021
5	CEDG180	GGTATGGAGCAAAACAATC	GTGCGTGAAGTTGTCTTATC	Gupta *et al.* 2008, Ragul *et al.* 2022
6	CEDG305	GCAGCTTCACATGCATAGTAC	GAACTTAACTTGGGTTGTCTGC	Mogali *et al.* 2021
7	CEDG268	CATCTCCCTGAAACTTGTG	GCTATCAATCGAGTGCAG	-
8	CEDG115	GGCTCATTGTACCACTGGATAT	ATGCCTCCTTTCAGGTGATTGT	Mogali *et al.* 2021
9	CAM	CGAAGAATGCCACAACATGA	CTACTCAGGGCGATTGAAC	Kundu *et al.* 2019
10	HSP	TTCAAACCCTCCTTGGGACAC	GAATGAAAGCTGGCCAGAAG	Kundu *et al.* 2019
11	AGO	GACGTTGTCTCTGCTGGCAG	ACACCTCCTCCTACATCAGC	Kundu *et al.* 2019
12	ANK	TACCACCCGTTGCACATAGC	GCAGGCAAGTACAACCCATC	Kundu *et al.* 2019
13	DEF	GTGGCTCTGAGACTCACATG	CCGGTAAGCCTTCTCCACGC	Kundu *et al.* 2019
14	NAC	GTGGAGGGTGTGAAGGTTATC	CTCCGTCTCAGGTTCCCATGG	Kundu *et al.* 2019
15	PRP	CTGGCAAAGCCAAGAGTGAT	AGCTCTCACAATTATGCAGC	Kundu *et al.* 2019

bootstrapping 1000 times. Genotypes were clustered using the neighbor-joining method based on the dissimilarity matrix, utilizing Darwin 6.0 software (Perrier and Jacquemond, 2006). Principal coordinate analysis was performed using the dissimilarity matrix constructed with Darwin 6.0.

RESULTS AND DISCUSSION

Screening of blackgram genotypes for YMD resistance: In this study, 150 genotypes were screened against yellow mosaic disease under natural field conditions during the *summer* of, 2022. Based on the yellow mosaic disease score, the genotypes were classified as 67 resistant, 25 as moderately resistant, 15 as moderately susceptible, 7 as susceptible, and 36 as highly susceptible to YMD. The classification of genotypes according to YMD resistance is detailed in **Table 3 and Fig. 1.** Similar studies on screening blackgram genotypes for YMD resistance have been conducted by Singh *et al.* (2008), Sundaram *et al.* (2017), Kumar *et al.* (2018), Nair *et al.* (2020), and Prakash *et al.* (2021).

Molecular diversity analysis among the selected blackgram genotypes: In this direction, a total of 15 molecular markers, including 8 SSRs and 7 gene-specific markers, were reported to be associated with MYMV resistance QTLs/genes in different studies by Gupta *et al.* (2012) and Gupta *et al.* (2015), Vijay *et al.* (2022), Kundu *et al.* (2019), Ragul *et al.* (2021) and Mogali *et al.* (2021) were used to screen 22 genotypes (12 susceptible and 10 resistant) identified from the field screening studies based on the disease scores. The 15 SSR markers used in the study amplified a total of 48 alleles (**Table 4**). The number of alleles produced by each marker ranged from 1-8, with an average of 3.2 alleles per marker. The polymorphic information content (PIC) of these markers ranged from 0.82 (CEDG20) to 0.21 (HSP) with an average PIC value of 0.55, indicating the potential of these markers for assessing molecular diversity. The highest PIC values were exhibited by the markers CEDG67 (0.82), followed by CEDG115 (0.81) and CEDG20 (0.74). Markers with high PIC values can efficiently distinguish between genotypes and are considered more informative. Therefore, these markers can be used for diversity and gene mapping studies. The lowest PIC value was exhibited by HSP (0.21), indicating its lower discriminatory power in distinguishing genotypes. PIC provides a more accurate assessment of diversity and also signifies the discriminatory power of a locus, as it considers the number of expressed alleles and the relative frequencies of each allele. In the present study, out of the 15 markers screened, seven markers were found to be highly informative with PIC values greater than 0.5. Thus, based on the PIC values, the markers used in the present study showed an appreciable level of polymorphism among the genotypes studied.

The 22 blackgram genotypes were grouped into three major clusters based on their molecular marker profiles (**Table 5 and Fig. 2**). Cluster I comprise of two genotypes, further divided into two sub-clusters (IA and IB) with one genotype each. Cluster II comprises six genotypes, further divided into two sub-clusters IIA (5 genotypes) and IIB (1 genotype). Cluster III comprises 14 genotypes, further divided into two sub-clusters, IIIA (2 genotypes) and IIIB (12 genotypes). This classification revealed a distinct

Table 3. Classification of genotypes for YMD resistance

S.No.	Category	Number of Genotypes	Name of Genotypes
1.	Resistant (R)	67	AZAD URD 1, BCU 20-62, GBG 66, GBG 80, GBG 81, GBG 83, IPU 11-02, IPU 13-6, IPU 19-27, IPU 19-53, KOTA URD 4, KPU 20-01, KUG 479, LBG 1001, LBG 1002, LBG 1003, LBG 1006, LBG 1008, LBG 1009, LBG 1010, LBG 1012, LBG 1013, LBG 1044, LBG 1046, LBG 1047, LBG 1049, LBG 1050, LBG 1051, LBG 1053, LBG 1064, LBG 1069, LBG 1071, LBG 1072, LBG 806, LBG 808, LBG 884, LBG 904, LBG 932, LBG 941, LBG 944, LBG 989, MASH 1008, MASH 114, OBG 45, PU 1801, PU 1537, PU 1803, PU 1805, PU 1808, PU 1810, PU 1812, PU 1815, PU 19, PU 31, PU 35, PUSA B 64, SHEKHAR 2, SUG 1279, TBG 104, TBG 129, TU 1-11, TU 40, TU 62, VBN 10, VBN 19-033, VBN 6, VBN 8
2.	Moderately resistant (MR)	25	BCU 20-73, GBG 1, GBG 22, GBG 65, GBG 73, IPU 18-7, IPU 19-51, KPU 175-2, KPU 20-05, KPU 21-11, KPU 405, LBG 1016, LBG 1026, LBG 1058, LBG 1059, LBG 933, LBG 985, LBG 998, PANT U 1305, PUSA B 28, SUG 1282, TJU 130, TU 1-30, VBN 11, VBN 17-021
3.	Moderately susceptible (MS)	15	GBG 3, GBG 70, GBG 72, GBG 82, GBG 84, GBG 90, GBG 91, KPU 18-1, LBG 1024, LBG 1059-1, LBG 752, LBG 999, PBG 276, PUSA B 55, TJU 339
4.	Susceptible (S)	7	ADT 5, CO 5, GBG 18, GBG 2, GBG 77, GBG 89, GBG 9
5.	Highly susceptible (HS)	36	GB 67, GBG 11, GBG 12, GBG 15, GBG 20, GBG 23, GBG 24, GBG 26, GBG 30, GBG 45, GBG 5, GBG 71, GBG 78, GBG 85, GBG 86, GBG 87, GBG 88, GBG 92, GBG 93, GBG 94, GBG 95, GBG 96, GBG 97, LBG 1068, LBG 685, LBG 787, MBG 1080, PBG 272, SVU 6, TBU 236-6, LBG623, LBG645, ADTBG14003, VBN 9, GBG 234, GBG 210

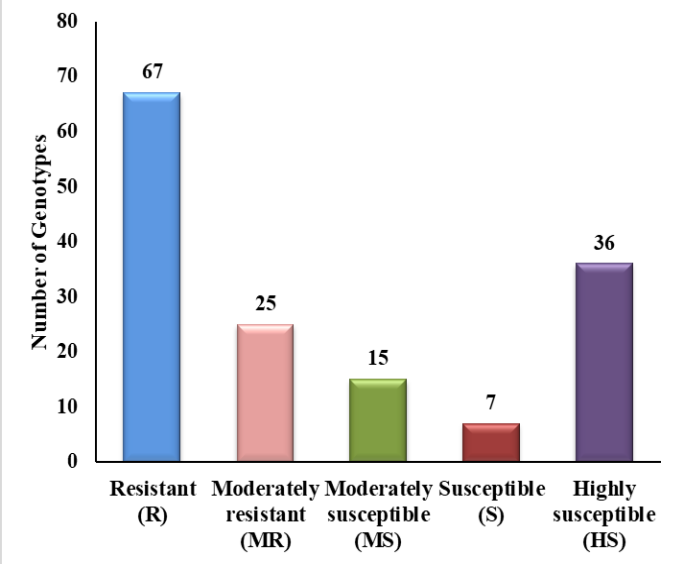

Fig. 1. Classification of blackgram genotypes for YMD resistance

separation between resistant and susceptible genotypes. All the resistant genotypes, namely LBG 884, LBG 932, LBG 904, MASH 114, PU 1808, PU 1815, PU 31, VBN 10, PU1803 and MASH 1008 were grouped together, whereas all the susceptible genotypes were distributed into separate clusters.

This clustering indicated a clear genetic differentiation between resistant and susceptible genotypes, providing valuable insights for breeding programs aimed at developing YMD- resistant blackgram varieties.

Principal Coordinate Analysis : The PCoA demonstrated

Table 4. Polymorphic information content and number of alleles generated by the markers

S.No.	Marker	PIC	No. of Alleles
1	CEDG20	0.74	5
2	CEDG67	0.82	8
3	CEDG44	0.63	5
4	CEDG97	0.65	4
5	CEDG180	0.50	2
6	CEDG305	0.46	2
7	CEDG268	0.43	2
8	CEDG115	0.81	5
9	CAM	0.45	2
10	HSP	0.21	2
11	AGO	0.44	2
12	ANK	0.62	5
13	DEF	0.47	2
14	NAC	-	1
15	PRP	-	1
16	Total		48
17	Maximum	0.82	8
18	Minimum	0.21	1
19	Mean	0.55	3.2

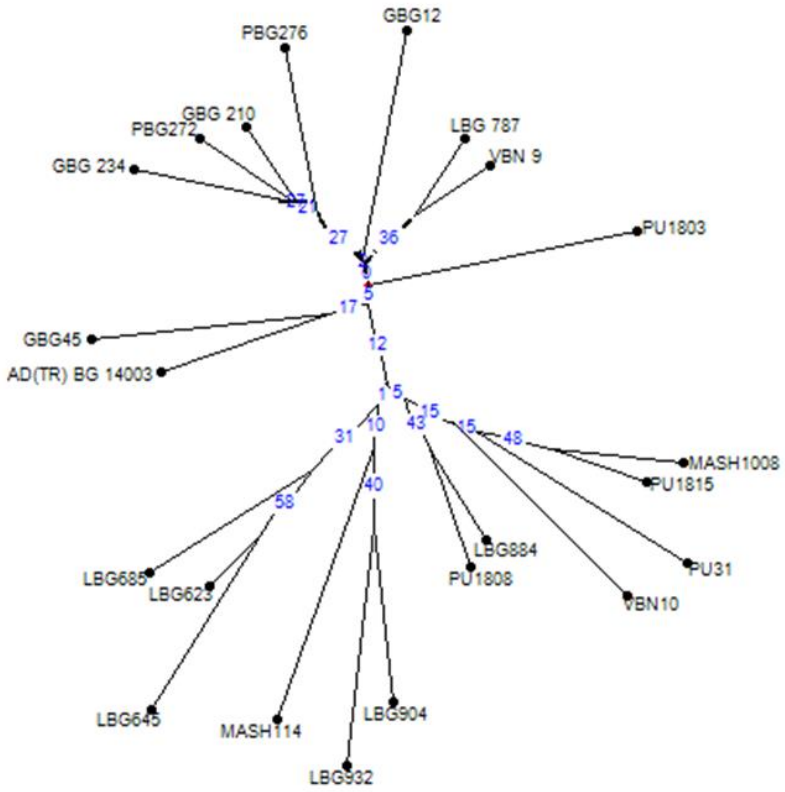

Fig. 2. Grouping of blackgram genotypes based on molecular diversity

Table 5. Grouping of genotypes into clusters based on molecular diversity

S. No.	Cluster	Number of genotypes	Genotypes
1.	IA	01	LBG 787
	IB	01	VBN 9
2.	IIA	05	GBG 234, PBG 272, GBG 210, PBG276 and GBG12
	IIB	01	PU 1803
3.	IIIA	02	AD(TR)BG 14003, GBG45
	IIIB	12	MASH1008, PU1815, PU31, VBN10, LBG884, PU1808, LBG904, LBG932, MASH114, LBG645, LBG623, LBG685

clear differentiation among the 22 blackgram genotypes. Resistant genotypes clustered together, confirming the findings of the dendrogram analysis. The scatter plot of the genotypes illustrated their distribution based on the first two principal coordinates. The first three principal coordinates (22.33%, 16.37% and 12.34%) accounted for a significant portion of the total genetic variation (64.43%) among the genotypes (**Fig. 3**).

Validation of YMD Resistance Using Molecular Markers: Among the 150 blackgram genotypes screened, 22 genotypes comprising 10 resistant (LBG 884, LBG 932, LBG 904, MASH 114, PU 1803, PU 1808, PU 1815, PU 31, VBN 10, MASH 1008) and 12 susceptible genotypes (GBG 12, GBG 45, LBG 623, LBG 645, PBG 272, LBG 685, PBG 276, AD (TR)BG 14003, VBN 9, LBG 787, GBG 234, GBG 210) were identified from phenotypic studies. These genotypes were validated using molecular markers for the presence or absence of MYMV resistance genes. Among the markers tested, CEDG180 could discriminate between resistant and susceptible genotypes. It produced a 162 bp allele in the majority of the resistant genotypes (PU 1815, PU 31, LBG 932, LBG 904, GBG 1, PU 1808, LBG 884, PBG 276) except for VBN 10, MASH 114, and PU 1803, while a 148 bp allele was produced in most of the susceptible genotypes except for LBG 623, LBG 645, LBG 685, and AD (TR)BG 14003 (**Fig. 4**). The marker CEDG180 was previously reported to be associated with YMD resistance QTL by Vijay *et al.* (2022), Vadivel *et al.* (2021) and Ragul *et al.* (2021). Similar validation studies were also conducted by Gupta *et al.* (2015), Tamilzharasi *et al.* (2018) and Madhumitha *et al.* (2019), confirming the association of CEDG180 with YMD resistance.

Similar to the present study, CEDG180 amplified resistance-linked alleles in susceptible genotypes and susceptibility-linked alleles in resistant genotypes. This may be attributed due to the loose linkage of this marker to the YMV resistance gene (12.9 cM). Gupta *et al.* (2015) Another gene-specific marker, DEF, produced a 190 bp allele in the majority of the resistant genotypes, whereas the majority of the susceptible genotypes produced both 190 and 250 bp alleles. Kundu *et al.* (2019) identified that the DEF (Defensin) gene was upregulated in response

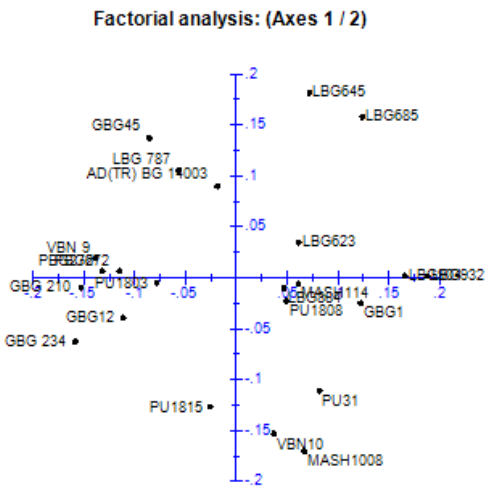

Fig. 3. Principal coordinate analysis of blackgram genotypes

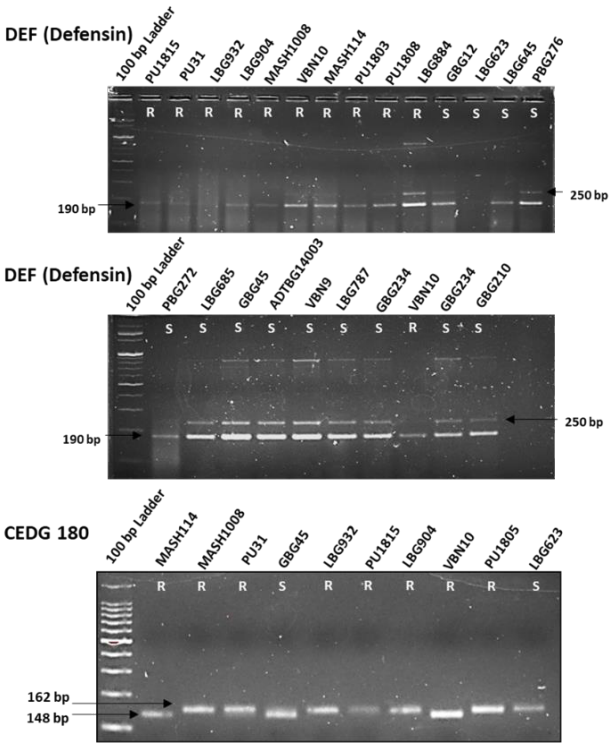

Fig. 4. Amplification of DEF (Defensin) and CEDG180 in known resistant and susceptible genotypes

to 3, 7, and 10 days post-inoculation of MYMV in the resistant *V. mungo* genotypes, indicating a possible association of the DEF marker with YMV resistance.

The genotypes LBG 904, LBG 932, PU 1815, PU 31, and MASH 1008 not only exhibited resistant-reactions phenotypically but also amplified resistant alleles of 162 bp of CEDG180 and 190 bp of PU 31 with DEF gene-specific marker. Hence, these genotypes may be used in the breeding program for the improvement of YMD resistance. Among the markers validated in the present study, these two markers, facilitate the breeding of YMD-resistant varieties by allowing for the selection of resistant genotypes at the seedling stage, thus speeding up the breeding process and improving the efficiency of developing resistant cultivars. Marker-assisted selection (MAS) using these markers helps in the accurate and early identification of resistant plants, reducing the reliance on phenotypic screening, which can be labor-intensive and less precise.

The present study identified the genotypes LBG 904, LBG 932, PU 1815, PU 31, and MASH 1008 as resistant to MYMV through phenotypic analysis and confirmed this resistance using molecular markers. These identified resistant genotypes can be utilized in blackgram breeding programs to enhance MYMV resistance. Amon the markers tested, the marker, CEDG180 could discriminate between resistant and susceptible genotypes.

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