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RESEARCH ARTICLE

Tagging of Yellow Mosaic Virus resistance gene using SSR markers in Black gram (*Vigna mungo* L. Hepper)

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Abstract

Black gram is one of the most important pulse crops grown in India. The productivity of black gram is very low as it is cultivated in marginal lands with less care. Yellow mosaic virus disease in black gram is a serious concern as the yield reduction varies from 30-100%. Breeding and cultivation of resistant varieties is the potential option to minimize yield reduction. However, the breakdown of resistance due to the evolution of new pathotypes in resistant varieties evolved through conventional breeding leads to the adoption of biotechnological tools such as marker-assisted breeding. Identifying molecular markers linked with YMV resistance paved way for changing black gram varieties with durable resistance. In the present study, 72 RILs developed by crossing the black gram lines ADT 3 and KKM 15052 as susceptible and resistant parents were used as mapping populations to tag YMV resistance with SSR markers. Among the 72 RILs screened for YMV resistance, 7 RILs (1,2,14,39,55,66 and 67) were resistant to YMV. Of 69 SSR markers, 14 were polymorphic between ADT 3 and KKM 15052. Single-marker analysis showed significant association of VR 086, VR 148 and CEDG 186 markers with YMV resistance. Bulked Segregant Analysis (BSA) confirmed that the SSR markers VR 086 and VR 148 are linked with the genes conferring resistance to YMV in KKB 15052.

Keywords

Black gram; YMV; tagging; SSR markers; BSA

Introduction

Pulses are essential food crops rich in protein and are cultivated all over the world. Black gram is one of India's significant pulses and india is the world's largest producer and consumer. In India, 24.19 lakh tonnes of black gram was produced during 2020-21 (1). The low yield in black gram is attributed to biotic factors such as high susceptibility to yellow mosaic disease. In Blackgram, Yellow Mosaic Disease (YMD) was reported in 1966 (2). In black gram, YMD was predominantly observed in Asian nations such as India, Thailand, Pakistan, Philippines, Sri Lanka and Nepal (3). YMD is caused by a virus belonging to the genus Begomovirus. Studies reported that two virus species causing YMD are prevalent in the Indian subcontinent. One of these species, the Mung bean Yellow Mosaic India Virus (MYMIV), commonly occurs in the northern Indian subcontinent.

In contrast, the Mung bean Yellow Mosaic Virus (MYMV) is mainly confined to the peninsular region of India (4, 5). These two virus species can easily be distinguished based on nucleotide sequence identity (6). The virus causes yellowing and mosaic symptoms in leaves, leading to poor photosynthesis, poor seed set and ill-filled seeds. Significant yield loss due to YMV ranges from 10% to 100% depending on crop growth stages and genotypes (7-10). Since the vector whitefly transmits the virus (*Bemissia tabaci*), controlling YMV using an insecticide is ineffective during severe whitefly infestations. As the use of insecticide is not environment friendly, the only alternative to prevent the occurrence of YMD is to develop black gram varieties resistant to YMV.

Knowledge of the mode of inheritance of MYMV resistance in black gram is essential to develop resistant genotypes. However, numerous studies have reported contrasting results regarding the resistance to YMD in black gram, including single or double-recessive, monogenic, digenic dominant and two-gene models with epistasis (11-14). Conventional breeding involves selection based on phenotypic screening, which is susceptible to environmental influence and may lead to frequent resistance breakdown. Molecular breeding techniques, including MAS (Marker Assisted Selection), MABC (Marker Assisted Backcross), marker-assisted gene pyramiding, MARS (Marker Assisted Recurrent Selection), QTL mapping (Quantitative Trait Loci) and genomic selection, are cost-efficient, enable early selection and facilitate efficient screening. Molecular markers are now commonly employed as genetic tools to establish the existence of a target gene of interest in a given locus with high precision (15). Molecular markers are used for gene tagging and QTL mapping, enabling researchers to identify and locate specific genes and genetic regions associated with desired traits. The YMV resistance gene in black gram has been tagged and mapped using SSR (CEDG 180) and ISSR markers (ISSR8111357) (16, 17).

The present study aimed to identify SSR markers associated with the YMV resistance gene in the black gram genotype KKB 15052.

Materials and Methods

Plant Materials

ADT 3 and KKB 15052 were used as susceptible and resistant parents and hybridization was carried out to develop the mapping population at VOC Agricultural College and Research Institute, Killikulam. The genotype KKB 15052 was used as the resistant parent, an advanced breeding line selected from the cross PU-0620 x 2-107 of the same institute. In contrast, ADT 3 was the susceptible parent, which was evolved from Tamil Nadu Rice Research Institute, Aduthurai. The F_1 was raised and the true F_1 were identified through molecular marker analysis. The F_2 population were raised from selected F_1. Subsequently, the Recombinant Inbred Line (RIL) population was developed using the single seed descent method. The mapping population used for tagging YMV resistance consists of 72 RILs.

Screening of RILs for YMV reaction

All 72 RILs of the mapping population, developed from the cross ADT3 x KKB 15052 and the parents were raised in a plot measuring 4m x 3m with a spacing of 30cm x 10cm. The susceptible check CO 5 was raised for every two plots of RIL as an infector row. The black gram variety VBN8 was raised as a YMV-resistant check. Based on the prevalence of YMV during the summer season in the last ten years, Killikulam was identified as a hotspot for YMV. No pesticides were used to increase the natural whitefly population in the field, enabling the spread of YMV to all the RILs and resistant check VBN8 from the infector row CO 5. YMV disease reaction was scored in all the RIL populations, resistant parents and susceptible parents, after developing 80 percent of the disease in the infector genotype CO 5.

The disease infestation was calculated using the following formula:

$$\textbf{Percent disease incidence (PI)} = \frac{\text{No. of infected plants in the plot}}{\text{Total number of plants in the plot}} \times 100$$

The severity of the disease was categorized based on the percent disease incidence (18) (Table 1). Then, the YMV resistance scoring was done based on the modified (0-9) scale of the All India Coordinated Research Project on MULLaRP (19) (Table 2).

Table 1. YMV disease incidence and scoring for resistance

Disease severity percent	Rating	Reaction
0.1-5	1.0-2.0	Resistant (R)
5.1-15	2.1-4.0	Moderately Resistant (MR)
15.1-30	4.1-5.0	Moderately susceptible (MS)
30.1-75	5.1-7.0	Susceptible (S)
75.1-100	7.1-9.0	Highly susceptible (HS)

Table 2. Modified MULLaRP scale for YMV resistance

Scale	Description
0	No visible symptoms on leaves
1	Very minute yellow specks on leaves
2	Tiny yellow specks with restricted spread covering 0.1 - 5.0% of the leaf area of the plant
3	Yellow mottling of leaves covering 5.1 - 10.0% of the leaf area of the plant
4	Yellow mottling of leaves covering 10.1 - 15.0% of the leaf area of the plant
5	Yellow mottling and discolouration of 15.1 - 30.0% of the leaf area of the plant
6	Yellow discolouration of 30.1 - 50.0% leaf area of the plant
7	Pronounced yellow mottling and discolouration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1 - 75.0% of foliage of the plant
8	Severe yellow discolouration of leaves covering 75.1 - 90.0% foliage, stunting of plants and reduction of pod size
9	Severe yellow discolouration of leaves covering above 90.1% foliage, stunting of plants and no pod formation

SSR markers used for the study

69 SSR markers were used to study parental polymorphism and identify the SSR markers linked with YMV resistance using BSA in the RIL population of black gram.

DNA extraction

Total genomic DNA was isolated using the CTAB method (19) from young leaf samples of all 72 RILs, the resistant and susceptible parents and the resistant and susceptible checks. The quality of extracted DNA was checked by electrophoresis using lambda DNA as a standard and the quantity was determined using a spectrophotometer. The working DNA sample was prepared by diluting the DNA to a standard concentration of 20 ng/µl.

Simple Sequence Repeat analysis

A total of 69 SSR markers, specific to YMV resistance as reported previously by several researchers (21-24), were used in this study. Primers used for genotyping the parental lines ADT 3 and KKB 15052 in this study were synthesized by Integrated DNA Technologies (IDT), USA. Polymerase Chain Reaction (PCR) was carried out in a 10 µl PCR reaction mixture consisting of 5 µl 2X PCR master mixture, 0.5 µl SSR markers (both forward and reverse primers), 0.5 µl template DNA and 4 µl sterile distilled water, using a thermal cycler. The PCR profile was programmed with initial denaturation at 94°C for 5 min, followed by 35 cycles of amplification, consisting of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The amplified products were resolved by electrophoresis in 3% agarose gel with ethidium bromide stain. Polymorphism between parents were detected based on the presence or absence of marker alleles or differences in the size of marker alleles visualized in the gel.

Preparation of DNA bulks for Bulked Segregant Analysis (BSA)

Two bulks were prepared for BSA, one each from highly resistant RILs and susceptible RILs (25). An equal quantity of DNA from five RILs was taken and pooled for each bulk. The polymorphic markers identified between the parents were used for genotyping the parents and the corresponding resistant and susceptible bulks. The association between the marker and the gene conferring resistance was established based on the amplification pattern of the polymorphic marker in the resistant parent and bulk and susceptible parent and bulk. The marker associated with the resistance gene was used for genotyping the individual RILs for cosegregation analysis.

Single-marker analysis was carried out using student's t-test and one-way ANOVA. Simple linear regression was performed for each phenotypic trait using the polymorphic SSR markers. The significance of the regression coefficient was calculated to determine the relationship between the polymorphic SSR markers and resistance to YMV.

Results

YMV resistance reaction of RILs

Among the 72 RILs screened for YMV resistance, 7 RILs (1, 2, 14, 39, 55, 66 and 67) were resistant to YMV with a disease severity score of 1-2 and corresponding percent disease incidence. Twenty-two RILs showed moderate resistance with a disease severity score of 2.1-4, 19 RILs showed moderate susceptibility with a disease severity score of 4.1-5, and 24 RILs were susceptible with a disease severity score of 5.1-7. The disease severity scores and percent disease incidence for RILs, along with resistant and susceptible parents and checks, are presented in Table 3 and Fig. 1.

Tagging of YMV resistance in KKB 15052

Parental Polymorphism: The polymorphism between the resistant line KKB 15052 and the susceptible line ADT 3 was analyzed with 69 SSR primers. Among these, 14 markers *viz.*, VR086, VR148, DMBSSR160, CEDG247, CEDG139, CEDG186, CEDG191, CEDG166, CEDG149, CEDG056, CEDG030, CEDG215, CEDG121 and CEDG214, were found to be polymorphic between the parental genotypes. (Table 4).

Table 3. YMV resistance score for RILs, parents, resistant and susceptible check

S.No.	YMV Score	YMV Response	Number of RILs
1	1	Highly resistant	3(14, 66, 67)
2	2	Resistant	4 (1, 2, 39, 55)
3	2.1-4	Moderately resistant	22(4, 6, 10, 11, 13, 15, 16, 19, 22, 24, 31, 32, 35, 37, 42, 43, 46, 49, 51, 58, 64, 65, 69)
4	4.1-5	Moderately susceptible	19(3, 5, 7, 17, 18, 33, 36, 38, 40, 41, 44, 47, 48, 50, 52, 59, 63, 68, 72)
5	5.1-7	Susceptible	24(8, 9, 12, 19, 20, 21, 23, 25, 26, 27, 28, 29, 30, 34, 45, 53, 54, 56, 57, 60, 61, 62, 70, 71)
6	7.1-9	Highly susceptible	-
		Total	72
	Parents/ Check Varieties		
1.	7	Susceptible	ADT3
2.	1	Highly resistant	KKB15052
3.	7	Susceptible	CO 5
4.	2	Highly resistant	VBN8

Fig. 1. Disease reaction of RILs to YMV

Table 4. List of SSR markers showing parental polymorphism

S. No	SSR primer	Sequences
1.	CEDG139 F	CAAACTTCCGATCGAAAGCGCTTG
	CEDG139 R	GTTTCTCCTCAATCTCAAGCTCCG
2.	CEDG149 F	GGCTGAAGGTGATGACAGAAG
	CEDG149 R	GGCACTGGTTTTCTAAGGTTGTTG
3.	CEDG215 F	CGTACTGAGATTGAGGTC
	CEDG215 R	CACCATGTGTTCCTCAAG
4.	CEDG121 F	CTTTCAAAATAATGTTGAGGCATA
	CEDG121 R	CAATACATAAATAACCTTTTCTGC
5.	CEDG214 F	CACTCACTGCAAAGAGCAAC
	CEDG214 R	CTACCTATCTGAGGGACAC
6.	CEDG247 F	GTAGACACTGATCATCACC
	CEDG247 R	GACCATCATCGATACGATTC
7.	CEDG186 F	GGATGGGAGAGTAAGAAG
	CEDG186 R	GCATGGCATGATGACTTG
8.	CEDG191 F	CAATAAGCAATCTGTGGAGAG
	CEDG191 R	CTGCAGGAAACTTGGAATTGC
9.	CEDG056 F	TTCCATCTATAGGGGAAGGGAG
	CEDG056 R	GCTATGATGGAAGAGGGCATGG
10.	CEDG166 F	GGTACAACATTCTTCTATTTG
	CEDG166 R	GGCTTATGAGTTTATCTTATC
11.	VR086 F	GAGATCCTCCTACGGATTTGC
	VR086 R	TTTCCTTCTCCAATTCTTGCTC
12.	VR148 F	CCGTTGTTGTTGCTGTTGTG
	VR148 R	GAGCTTGCTAACCCTCTCCAAT
13.	DMB-SSR160 F	GGTGGATCAAATCCATTTTAGG
	DMB-SSR160 R	ACAGATCACATAGCAACCAAACA
14.	CEDG030 F	TGAGGGAATGGGAGAGAGGC
	CEDG030 R	TGAGGGAATGGGAGAGAGGC

Single-marker Analysis: The association of markers with YMV resistance was assessed by single-marker analysis. Among the polymorphic SSR markers, VR 086, VR 148 and CEDG 186 exhibited significant marker-trait association, with R^2 values of 46.81%, 43.29% and 48.65%, respectively. The results are presented in Table 5. The R^2 value indicates the strength of marker association with the trait and the percentage of trait variability explained. This study's R^2 value of polymorphic SSR markers ranged from 20.87 (CEDG 139) to 48.65 (CEDG 186). The markers VR 086, VR 148 and CEDG 186 are strongly associated with YMV resistance, with a highly significant p-value (<0.001). The results are presented in Table 5.

Bulked Segregant Analysis (BSA): The YMV resistance genes in black gram were tagged using bulked segregant analysis. By combining equal quantities of DNA from five

Table 5. Single-marker analysis

S.NO	Polymorphic SSR marker	R^2 value	P value
1	VR086	46.81	5.87E-21
2	VR148	43.29	4.06E-13
3	DMBSSR160	35.98	3.08E-17
4	CEDG247	32.98	4.27E-11
5	CEDG139	38.87	3.25E-09
6	CEDG186	48.65	8.18E-20
7	CEDG191	23.87	5.12E-16
8	CEDG166	29.57	6.12E-24
9	CEDG149	20.87	8.08E-13
10	CEDG056	23.67	2.09E-56
11	CEDG030	24.66	7.13E-17
12	CEDG215	32.87	3.09E-16
13	CEDG121	33.67	4.23E-18
14	CEDG214	38.65	5.21E-16

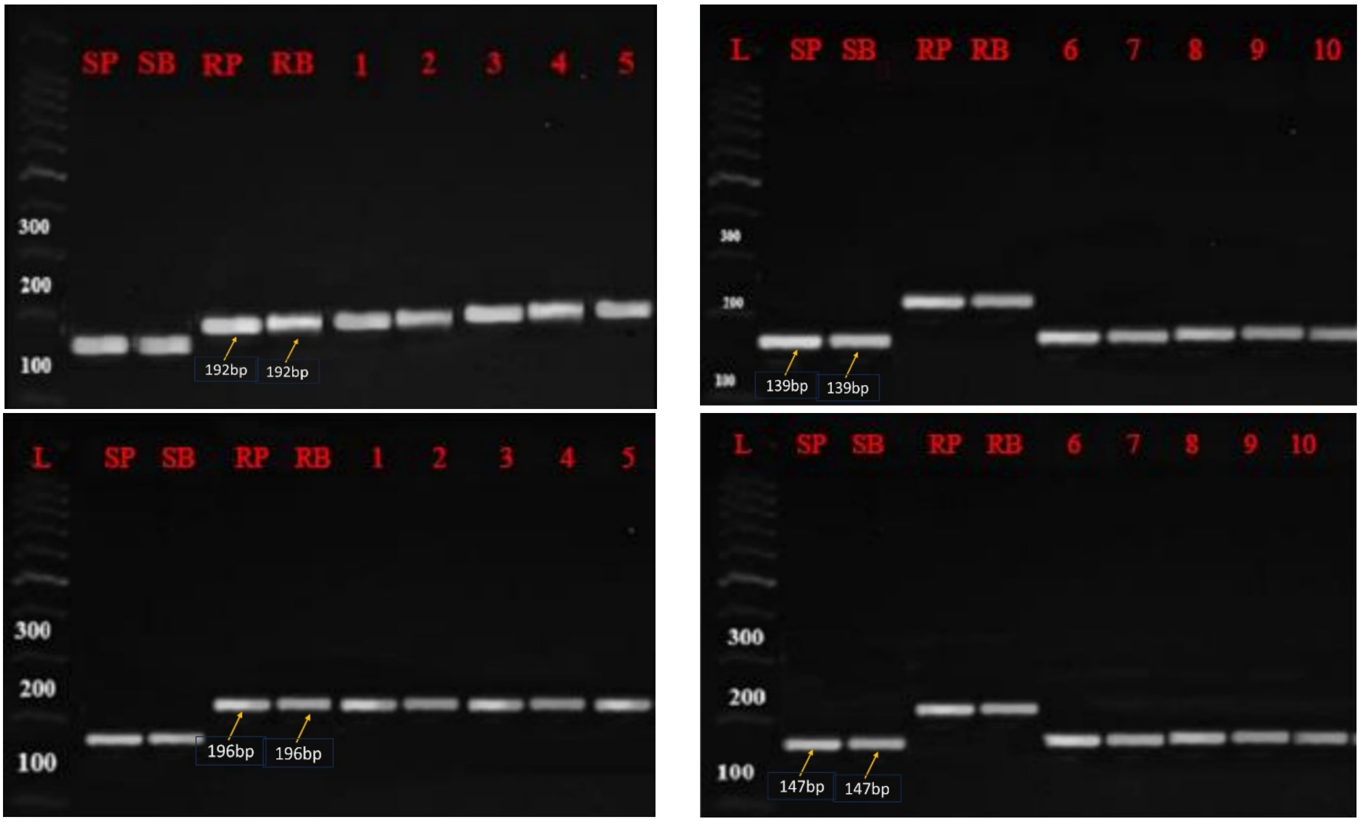
resistant and five susceptible plants from the same RIL population, resistant and susceptible bulks were created. The parents and the pooled susceptible and resistant bulks were screened using the identified polymorphic markers. Further, these 14 primers were used to analyze polymorphism between the susceptible bulk (SB) and resistant bulk (RB). Among these, the marker VR 086 amplified an allele of 139 bp in the susceptible parent and susceptible bulk, while the allele size was 192 bp in the resistant parent and resistant bulk. Similarly, the marker VR 148 amplified an allele of 147 bp in the susceptible parent and susceptible bulk, while the allele size was 196 bp in the resistant parent and resistant bulk. The SSR marker alleles differentiated the resistant parent and resistant bulk from the susceptible parent, and the susceptible bulk is depicted in Fig. 2.

Cosegregation analysis: Cosegregation of marker alleles with resistant and susceptible individuals was studied with the SSR markers VR 086 and VR 148, which differentiated the resistant and susceptible bulks. Fig. 3 showed that the allele 139 bp of VR 086 was present in the susceptible parent and the susceptible bulk was present in all five susceptible RILs. Similarly, the allele 192 bp of VR 086 was present in the resistant parent, and the resistant bulk was present in all five resistant RILs. The allele 147 bp of VR148 was present in the susceptible parent and the susceptible bulk was present in all five susceptible RILs. The allele 196 bp of VR 148 present in resistant parent and resistant bulk was also present in all the five resistant RILs (Fig. 4). The amplification of resistant parental alleles in resistant individuals and susceptible parental alleles in susceptible individuals indicated that these markers are linked to the genes conferring resistance to YMV in KKB 15052.

Discussion

Marker-assisted breeding plays a major role in breeding varieties with traits of interest. Developing YMV-resistant varieties through conventional breeding in black gram breeding is inefficient, as the selection is based on phenotypic scoring, which is easily influenced by environmental factors. Hence, identifying molecular markers linked with YMV resistance aids the transfer of genes to popular black gram varieties. In the present study, 72 RILs developed by crossing ADT 3 (susceptible parent) and KKB 15052 (resistant parent) were used as the mapping population to identify molecular markers linked with YMV resistance. All 72 RILs were screened for resistance to YMV. A total of 69 SSR markers were used to study parental polymorphism and 14 SSR markers were found to be polymorphic between the YMV-susceptible and -resistant parents, ADT 3 and KKB 15052, respectively. These markers included VR086, VR148, DMBSSR160, CEDG247, CEDG139, CEDG186, CEDG191, CEDG166, CEDG149, CEDG056, CEDG030, CEDG215, CEDG121 and CEDG214. Several SSR markers have been reported for polymorphism between resistant and susceptible parents for YMV. Similar studies were conducted in an F_2 population, where 32 polymorphic markers for YMV out of 469 SSR markers were identified (26).

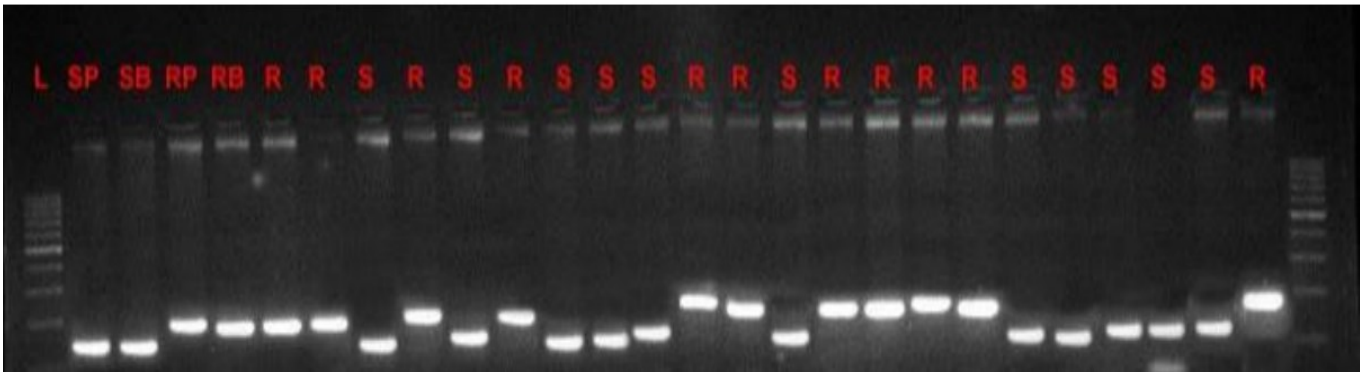
A single-marker analysis was performed to identify the marker-trait association with YMV resistance**.** The association of 14 polymorphic SSR markers with YMV resistance was



(L - Ladder, SP - Susceptible parent, SB- Susceptible bulk, RP- Resistant parent, RB - Resistant bulk, 1-5: resistant individuals, 6-10: susceptible individuals)

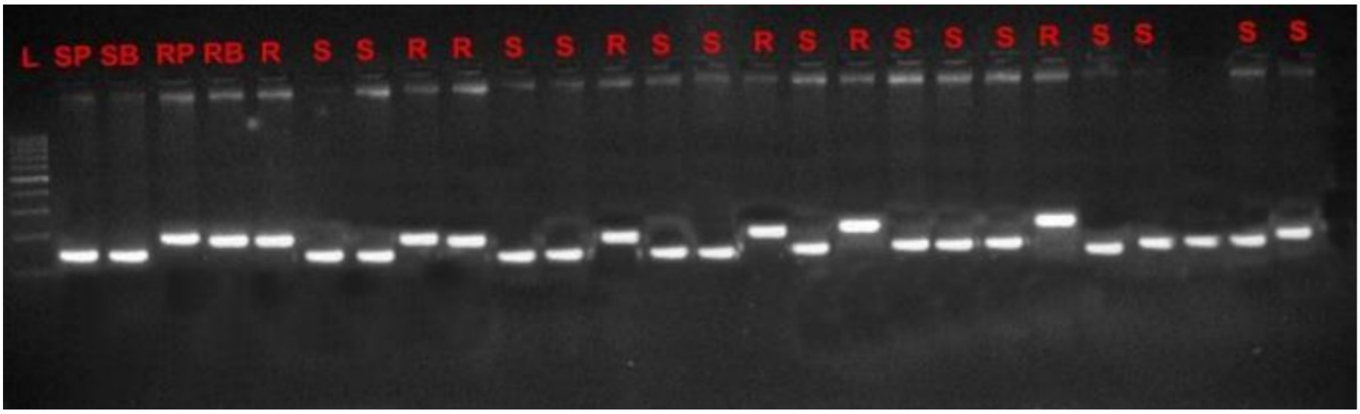
A & B: VR086, C & D: VR148

Fig. 2. Bulk segregant analysis for YMV resistance in RIL population



L - Ladder, SP - Susceptible parent, SB - Susceptible bulk, RP - Resistant parent, RB - Resistant bulk, R - Resistant RIL, S - Susceptible RIL

Fig. 3. Co-Segregation of SSR marker, VR 086 with YMV resistance in the RILs



L - Ladder, SP - Susceptible parent, SB - Susceptible bulk, RP - Resistant parent, RB - Resistant bulk, R - Resistant RIL, S - Susceptible RIL

Fig. 4. Co-Segregation of SSR marker, VR 148 with YMV resistance in the RILs

examined. The markers VR086 (46.81%), VR148 (43.29%) and CEDG186 (48.65%) showed significant association with YMV resistance.

Similarly, four markers associated with more than 20% phenotypic variance were reported in cowpeas (27). Two markers, CEDG141 and CEDG008, were reportedly strongly associated with YMV resistance in black gram (28). The marker-trait association was further confirmed through composite interval mapping. Single-marker analysis for mung bean powdery mildew disease was conducted using 14 SSR polymorphic markers in 37 genotypes. Among them, five markers (DMBSSR199, CEDG259, VrCSSR1, CEDG290 and VrCSSTS1) showed high phenotypic variance (R^2) of 20.18% (29). Similar results were reported in F_2 populations of mung bean and groundnut for MYMV resistance and early leaf spot resistance, respectively (30,31).

Bulked segregant analysis showed that the SSR markers VR086, VR148 and CEDG186 distinguished the extreme bulks and their respective parents. The marker VR086 amplified at 139 bp for the susceptible parent, susceptible bulks and corresponding individuals. The marker VR148 amplified at 142 bp for the susceptible bulk and 197 bp for the resistant bulk, respectively. 59 SSR markers associated with resistance to yellow mosaic virus were utilized for polymorphic study in the F_2 segregating populations arising from the cross between T986 (R) and LBG-759 (S). Among the 59 SSR primers, only 12 showed parental polymorphism (32).

Studies on tagging MYMV resistance using 67 SSR primers in an F_2 mapping population (SML668 x Mash114) through BSA showed that 46 markers were polymorphic between the parental lines. One SSR marker, MBM0378, was able to distinguish between resistant bulk (135 bp) and susceptible bulk (150 bp) by BSA (33). The F_5 RIL population of the cross Maha x GM-4 exhibited MYMV resistance linked to 124 genetic markers (100 RAPD, 12 cowpea SSR, six soybean SCAR and 1 black gram RGA) (34).

The susceptible parent and susceptible bulk amplified at 139 bp and the resistant parent and resistant bulk amplified at 192 bp, respectively, for the marker VR086. The susceptible parent and susceptible bulk amplified at 147 bp and the resistant parent and resistant bulk amplified at 196 bp, respectively, for the marker VR148. Several findings revealed an association of SSR markers with YMV resistance in mung beans, which are helpful for breeding and selecting YMV-resistant varieties faster and more accurately than conventional breeding programmes (35, 36).

Conclusion

From the present study's findings, it is concluded that the SSR markers VR086 and VR148 were linked to YMV resistance in black gram. Several earlier studies also reported that the SSR markers viz., CEDG 180, CEDG 141, CEDG 264 and CEDG 008 were linked with YMV resistance in black gram through BSA. The association of these markers with resistance was validated by molecular marker analysis of numerous YMV-resistant and susceptible genotypes. The marker-trait association can be confirmed through composite interval mapping. The findings agree with previous studies on various legume crops, including mung bean, groundnut and cowpea. The identified markers can transfer YMV resistance genes to popular black gram varieties through marker-assisted breeding, viz., marker-assisted back cross-breeding, gene pyramiding, etc. This marker-assisted breeding enhances breeding efficiency and accuracy.

Further fine mapping of genes conferring YMV resistance can be done, which aids in developing YMV-resistant black gram varieties, eliminating the undesirable genes linked with the YMV-resistant gene. Furthermore, integrating molecular marker data with genomics, proteomics and phenomics data allows researchers to link sequenced genome data with observed traits, bridging the genome to the phenome divide. These markers can then be used routinely in crop breeding programs.

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Authors' contributions

RL carried out field experiments, analyzed data and prepared this manuscript. LL carried out molecular analysis. DS carried out a statistical analysis. MA mentored research programme. SK carried out data interpretation. KE mentored screening for YMV resistance. KK scored YMV resistance.

Compliance with ethical standards

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

Ethical issues: None.

References

1. ANGRAU. Blackgram Outlook Report-January to May 2021, Agricultural Market Intelligence Centre, ANGRAU, Lam. 2021
2. Williams F, Grewal J, Amin K. Serious and new diseases of pulse crops in India in 1966. Plant Disease Reporter. 1968; 52(4):300-4.
3. Karthikeyan A, Sudha M, Nagrajan P, Pandiyan M, et al. Using SSR marker to identify the MYMV resistance gene in mungbean [*Vigna radiata* (L.) Wilczek]. Rom J Biol-Plant Biol. 2012; 57:105-13.
4. Varma A, Malathi VG. Emerging geminivirus problems: A serious threat to crop production. Ann Appl Biol [Internet]. 2003;142 (2):145-64 https://doi.org/10.1111/j.1744-7348.2003.tb00240.x
5. Malathi V, John P. Mungbean Yellow Mosaic Viruses", Desk Encyclopaedia of Plant and Fungal Virology in Encyclopaedia of Virology. Amsterdam: Elsevier Ltd; 2008. https://doi.org/10.1016/B978-012374410-4.00708-1
6. Fauquet CM, Bisaro DM, Briddon RW, Brown JK, Harrison BD, Rybicki EP, et al. Revision of taxonomic criteria for species demarcation in the family Geminiviridae and an updated list of begomovirus species. Arch Virol. 2003; 148(2):405-21 https://doi.org/10.1007/s00705-002-0957-5
7. Nene YL. A survey of viral diseases of pulse crops in Uttar Pradesh. A survey of viral diseases of pulse crops. Uttar Pradesh Book; 1972.

8. Marimuthu T, Subramanian C, Mohan R. Assessment of yield losses due to yellow mosaic infection in mungbean. In: Pulse Crop Newsletter1:104. 1981.

9. Gurha S, Misra D, Kamthan K. Studies on some aspects of yellow mosaic disease of black gram". Madras Agricultural Journal. 1982;69(7):435-8. https://doi.org/10.29321/MAJ.10.A02739

10. Bashir M, Ahmad Z, Ghafoor A. Sources of genetic resistance in mungbean and blackgram against Urdbean leaf crinkle virus (ULCV). Pakistan Journal of Agricultural Sciences. 2005; 37(1):47 -51.

11. Singh DP. Inheritance of resistance to yellow mosaic virus in blackgram (*Vigna mungo* (L.) Hepper). Züchter Genet Breed Res. 1980;57(5):233-5 https://doi.org/10.1007/BF00264676

12. Verma RPS, Singh DP. The allelic relationship of genes giving resistance to mungbean yellow mosaic virus in blackgram. Züchter Genet Breed Res. 1986;72(6):737-8. https://doi.org/10.1007/BF00266537

13. Sai CB, Nagarajan P, Raveendran M, Rabindran R, Senthil N. Understanding the inheritance of mungbean yellow mosaic virus (MYMV) resistance in mungbean (*Vigna radiata* L. Wilczek). Mol Breed. 2017;37. https://doi.org/10.1007/s11032-017-0650-8

14. Thamodhran G, Geetha S, Ramalingam A. Genetic study in urd bean (*Vigna mungo* (L.) Hepper) for inheritance of Mungbean Yellow Mosaic Virus resistance. International Journal of Agriculture, Environment and Biotechnology. 2016; 9(1):33. https://doi.org/10.5958/2230-732X.2016.00005.X

15. Gupta SK, Souframanien J, Reddy KS. Validation of molecular markers linked to yellow mosaic virus disease resistance in diverse genetic background of blackgram. Electron J Plant Breed. 2015; 6:755-63.

16. Souframanien J, Gopalakrishna T. ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [*Vigna mungo* (L.) Hepper]. Plant Breed. 2006;125(6):619-22 https://doi.org/10.1111/j.1439-0523.2006.01260.x

17. Gupta S, Gupta DS, Anjum TK, Pratap A, Kumar J. Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* L. Hepper). Euphytica. 2013; 193(1):27-37. https://doi.org/10.1007/s10681-013-0884-4

18. Gantait S, Das PK. Genetic divergence, adaptability and genotypic response to YMV in blackgram. Legume Research. 2009;32(2):79-85.

19. Alice D, Nadarajan N. Screening techniques and assessment methods for disease resistance, Department of Pulses. 2007.

20. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci . 1984; 81(24):8014-8. https://doi.org/10.1073/pnas.81.24.8014

21. Wang XW, Kaga A, Tomooka N, Vaughan DA. The development of SSR markers by a new method in plants and their application to gene flow studies in azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). Züchter Genet Breed Res. 2004;109(2):352-60 https://doi.org/10.1007/s00122-004-1634-8

22. Han OK, Kaga A, Isemura T, Wang XW, Tomooka N, Vaughan DA. A genetic linkage map for azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). Züchter Genet Breed Res. 2005; 111(7):1278-87. https://doi.org/10.1007/s00122-005-0046-8

23. Chaitieng B, Kaga A, Tomooka N, Isemura T, Kuroda Y, Vaughan DA. Development of a black gram [*Vigna mungo* (L.) Hepper] linkage map and its comparison with an azuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi) linkage map. Züchter Genet Breed Res. 2006; 113(7):1261-9. https://doi.org/10.1007/s00122-006-0380-5

24. Somta P, Seehalak W, Srinives P. Development, characterization and cross-species amplification of mung bean (*Vigna radiata*) genic microsatellite markers. Conserv Genet. 2009; 10:1939-43. https://doi.org/10.1007/s10592-009-9860-x

25. Michelmore RW, Paran I, Kesseli RV. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci. 1991; 88:9828-32. https://doi.org/10.1073/pnas.88.21.9828

26. Basamma K, Salimath P, Malagouda P, Suma B. Genetics, molecular dissection and identification of high yielding disease resistant lines to MYMV in blackgram (*Vigna mungo*). Legume Res. 2015;38(6):851-4. https://doi.org/10.18805/lr.v38i6.6735

27. Manjunatha N, Rangaswamy KT, Sah RP, Nagaraju N, Rudras-Wamy P. Characterization and identification of SSR markers for screening of cowpea genotypes against Beanommon mosaic virus (BCMV) disease resistance. Legum Res. 2017; 40(878). https://doi.org/10.18805/LR-3673

28. Sathees N, Shoba D, Mani N, Saravanan S, Kumari MP, Pillai MA. Tagging of SSR markers associated to yellow mosaic virus resistance in black gram (*Vigna mungo* (L.) Hepper). Euphytica. 2022; 218(3):23. https://doi.org/10.1007/s10681-022-02976-3

29. Sarkale AP. Validation of molecular markers associated with powdery mildew resistance in mungbean. M. Sc, Thesis. University of Dharward. 2015.

30. Mogali SC, Abhilash BN. Development of Mapping Population and Validation of Molecular Markers Associated with MYMV Resistance in Mungbean. Legume Research - An International Journal. 2021;1. https://doi.org/10.18805/LR-4272

31. Zongo A, Khera P, Sawadogo M, Shasidhar Y, Sriswathi M, Vishwakarma MK, et al. SSR markers associated to early leaf spot disease resistance through selective genotyping and single marker analysis in groundnut (*Arachis hypogaea* L). Biotechnology Reports. 2017; 15:132-7. https://doi.org/10.1016/j.btre.2017.07.005

32. Naik BJ, Anuradha CH, Kumar PA, Sreedhar V, Chary S. Identification of simple sequence repeats (SSR) markers linked to yellow mosaic virus (YMV) resistance in blackgram [*Vigna mungo* (L). Hepper]. Agric Update. 2017; 12(SP-3):812-9. https://doi.org/10.15740/Has/au/12.techsear(3)2017/812-819

33. Lekhi P, Gill RK, Kaur S, Bains TS. Generation of interspecific hybrids for introgression of mungbean yellow mosaic virus resistance in *Vigna radiata* (L.) Wilczek. Legume Res [Internet]. 2018;(00). https://doi.org/10.18805/LR-3808

34. Patel P, Modha K, Kapadia C, Vadodariya G, Patel R. Validation of DNA markers linked to MYMV resistance in mungbean (*Vigna radiata* (L.) R. Wilczek). International Journal of Pure & Applied Bioscience. 2018;6(4):340-6. https://doi.org/10.18782/2320-7051.6820

35. Panigrahi K, Baisakh B. Reaction of local and mutant lines of black gram (*Vigna mungo* (L) Hepper) to yellow mosaic virus and powdery mildew. Journal of Plant Protection and Environment. 2013; 10(2):57-63.

36. Somta P, Srinives P. Genome research in mungbean [*Vigna radiata* (L.) Wilczek] and blackgram. Science Asia. 2007; 3(1):69-74. https://doi.org/10.2306/scienceasia1513-1874.2007.33(s1).069