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Tagging of SSR markers associated to yellow mosaic virus resistance in black gram (*Vigna mungo* (L.) Hepper)

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Abstract In black gram, yellow mosaic virus (YMV) disease is a serious problem causing severe yield reduction. In the present study, F_2 and RIL Population ($F_{4:5}$) of the cross IC436656 (YMV susceptible) x KKB14045 (YMV resistant) were evaluated for YMV resistance through bulked segregant analysis (BSA) using SSR markers. Out of 91 SSR markers studied, thirteen were polymorphic between IC436656 and KKB14045 which showed 14.3% of genome diversity. The F_2 population segregated as 3:1 for susceptibility and resistance in chi-square test which indicated monogenic recessive gene control the YMV resistance. From single marker analysis (SMA), CEDG141 and CEDG008 were strongly associated to YMV resistance. From BSA, CEDG141 differentiated IC436656 (200 bp) and KKB14045 (280 bp) in F_2 population. CEDG141, CEDG 264 and CEDG 008 differentiated the RILs of the same population in BSA. The identified SSR markers would be valuable in black gram breeding programs for YMV resistance studies.

Keywords Black gram · Yellow mosaic virus resistance · SSR markers · Bulked segregant analysis · Single marker analysis

Abbreviations

%	Percent
AC & RI	Agricultural College and Research Institute
bp	Base pair
BSA	Bulked segregant analysis
cM	Centimorgan
CTAB	CetylTrimetyl ammonium bromide
DNA	Deoxyribo nucleic acid
dNTPs	Deoxynucleoside triphosphates
dATP	Deoxyadenosine triphosphate
dGTP	Deoxyguanosine triphosphate
dCTP	Deoxycytidine triphosphate
dTTP	Deoxythymidine triphosphate
EDTA	Ethylene diamine tetra acetic acid
ml	Milliliter
mM	Millimolar
MYMIV	Mungbean yellow mosaic Indian virus
MYMV	Mungbean yellow mosaic virus
NBPGR	National bureau of plant genetic resources
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
SSR	Simple sequence repeats
SMA	Single marker analysis

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SCAR	Sequence characterized amplified regions
TBE	Tris-borate EDTA
µg	Microgram
µl	Microliter
YMV	Yellow mosaic virus

Introduction

Pulses are the main source of protein in a vegetarian diet and they are called as the poor man's meat due to the high protein content available in the seeds. Globally pulses are grown in 21–22 million hectares with an annual production of 12–16 million tonnes (Jeevitha et al. 2018). Pulses are cultivated in an area of about 25.26 million hectares with a production of 19.98 million tonnes and productivity of 652 kg per hectare of yield in India (Devegowda et al. 2019). Black gram or urd bean is a diploid (2n = 2x = 22) short duration and cleistogamous crop, belonging to the family Leguminosae (Naik et al. 2017). Black gram seeds contain 25–26 per cent protein, 60 per cent carbohydrate, 1.5 per cent fat, minerals, amino acids and vitamins (Parveen et al. 2011). Black gram has great source of fodder for milch animals (Fery 2002).

The productivity of black gram is very low and selection becomes complicated under biotic and abiotic stress conditions. Among the biotic stresses, yellow mosaic virus (YMV) disease is a serious problem and yield reduction of up to 100 per cent has been reported (Nene 1972; Rathi 2002). The incidence of mung bean yellow mosaic virus (MYMV) in India was first recorded in Delhi on *Vigna radiata* (Nariani 1960). It is a serious viral disease in major leguminous crops like black gram, mung bean, soybean etc., which leads to a yield loss of $300 million (Varma et al. 1992). The yellow mosaic virus is transmitted by the insect vector *Bemisia tabaci* (Anokhe et al. 2018). Yellow mosaic disease caused by MYMIV (mung bean yellow mosaic India virus) is predominant in Northern and Central India, where as MYMV is predominant in Southern and Western India (Usharani et al. 2004). Yellow mosaic virus belongs to genus *Begomo virus* and infects a number of leguminous crops viz., urd bean, mung bean, cowpea (Nariani 1960), soybean (Suteri 1974), horse gram, lablab bean (Capoor and Varma 1948) and French bean. The inheritance of YMV resistance is controlled by a single recessive gene (Singh and Chaudhary 1979; Thakur et al. 1977; Saleem et al. 1998; Malik et al. 1986; Reddy and Singh 1995; Reddy 2009), dominant gene (Sandhu et al. 1985; Gupta et al. 2005), two recessive genes (Verma and Singh 1988; Ammavasai et al. 2004; Singh and Singh 2006) and complementary recessive genes (Shukla and Pandya 1985). The advanced molecular biology and biotechnological tools such as genetic transformation, marker assisted selection could be used for developing YMV resistance black gram genotypes (Xu et al. 2000).

Molecular markers are used as genetic tool to confirm the presence of a specific gene with high accuracy (Gupta et al. 2015). Various molecular markers were used in tagging of different viral resistant genes in several crops viz., soybean, *Phaseolus* (Urrea et al. 1996) and pea (Gao et al. 2004). ISSR (inter simple sequence repeats) and SCAR (sequence characterized amplified regions) markers were reported to be linked to resistant genes in black gram (Souframanien and Gopalakrishna 2006). Among the different types of markers, simple sequence repeat (SSR) markers are easy to use, highly polymorphic and high reproducibility in nature. These markers are locus specific, tandemly repeated, short sequence repeat of mono, di, tri, tetra nucleotides in the genome (Tóth et al. 2000).

Black gram contains lack of genomic resources and markers have become a major limitation for the number of SSR markers in black gram (Souframanien and Reddy 2015) and this is the major reason for less number of studies on mapping using SSR markers. Identification of markers linked to a trait is important to identify the gene of interest. Bulked segregant analysis (BSA) is a method initially used for generating the genetic map (Michelmore et al. 1991). This method is used to focus on regions of interest or areas of segregation in breeding populations using markers and this method is also useful for a rapid isolation of genes that do not segregate in breeding populations. Single marker analysis is a one of the quantitative trait locus (QTL) technique which has association of molecular markers with various traits. The polymorphic markers differentiate the individuals from the population and the development of polymorphic markers by research group for cultivated crops is still limited (Jayashree et al. 2005). The limitation of marker trait association is overcome by regression technique for future breeding programmes (Pradeep et al. 2007).

In the present scenario, the use of marker assisted selection (MAS) methods would be helpful to identify molecular markers for YMV resistance as they are not influenced by environment. The present investigation was carried out to identify the SSR markers associated to yellow mosaic virus resistance in the cross derivatives of IC436656 (YMV susceptible) x KKB14045 (YMV resistance) using bulked segregant analysis, single marker analysis and validation of linked markers.

Materials and methods

Experimental materials

The field experiments were carried out at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Tamil Nadu, India during 2018–2019. The lines viz., IC436656 and KKB14045 were used as female and male parents respectively in crossing program. The line IC436656 is a land race, obtained from NBPGR, New Delhi and KKB14045 is derived from crossing between PU-0620 and ADT-3. F_2 and RILs ($F_{4:5}$) were developed from this cross for screening against YMV disease. The molecular analysis was carried out at Molecular Biology Laboratory of Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam.

Crossing block and hybridization

The parents were raised in crossing block during *Rabi* 2018.The female and male parents were raised in 3 m rows consisting of 20 plants per row with a spacing of 30 × 10 cm. Flower buds of the female lines were chosen for emasculation. All the emasculated flower buds were tagged for identification. Anthers from freshly opened male flowers were collected and dusted on the stigma of already emasculated flowers. The pollinated flowers were bagged for protection and easy identification.

Development of mapping population

The five F_1 plants were raised in ridges and furrows and hybridity of F_1 was confirmed through the parental polymorphic marker CEDG 141 (Fig. 1). One confirmed F_1 was allowed to selfing to raise F_2 (162 No.) in ridges and furrows with frequent rows of YMV susceptible check ADT3 to attract white flies and to increase infection of YMV under field condition. The F_2 and their RILs were evaluated for YMV resistance along with the parents. All the recommended cultural practices were followed to maintain the field except spraying of insecticides to promote white fly population for spreading of the disease. YMV infection was developed in 6 weeks and the disease was recorded on a 1–9 arbitrary scale (Alice and Nadarajan 2007) mentioned in Table 1.

Chi-square analysis

Chi-square (χ^2) test was applied for testing the deviation of the observed segregation from theoretical segregation. Chi-square test was calculated using the formula $\sum \frac{(O-E)^2}{E}$ where, O = Observed frequency; E = Expected frequency; $\sum$ = Summation of the data.

Extraction of genomic DNA, PCR amplification and electrophoresis

DNA was extracted from the 20 days old leaf samples of F_2 and RILs following CTAB method (Saghai-Maroof et al. 1984) with suitable modifications. The quality and quantity of DNA were checked by using 0.8% of metaphor agarose. The 10 µl volume of PCR reaction was carried out using 91 SSR markers (Supplementary Table 1). Thermal cycler for PCR reaction programmed for an initial denaturation of 95 ºC for 5 min followed by 35 cycles of 94 ºC for 30 s, annealing of 55 ºC for 30 s, extension of 72 ºC for 1 min and final extension of 72 ºC for 7 min**.** After PCR, the products were evaluated in 3% metaphor agarose gel electrophoresis and the gel was visualized in gel documentation system (Bio-Rad).

Molecular analysis

Ninety one SSR markers were selected based on high polymorphism from previous reports of various investigators (Chaitieng et al. 2006; Han et al. 2005; Wang et al. 2004; Naik et al. 2017) which were used to identify the polymorphism between two parents (IC 436,656 and KKB 14,045) based on DNA level variation. BSA is the rapid method of selection of genotypes in mapping populations

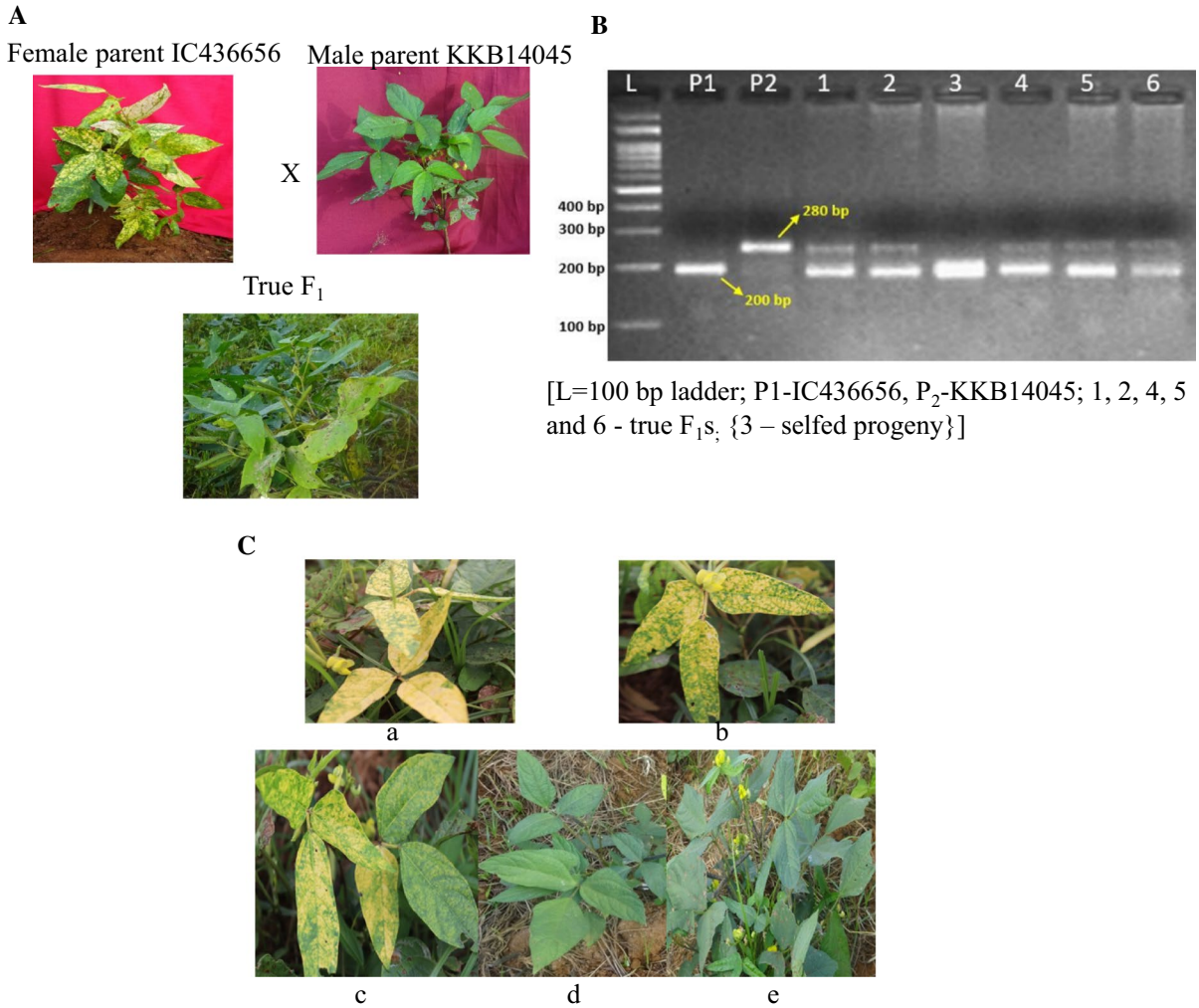


Fig. 1 Identification of true F_1 and their Segregation pattern of YMV incidence in F_2 population in IC436656 x KKB14045 **A** Identification of true F_1 under field condition, **B** Identification of true F_1 under polymorphic marker CEDG141 **C** Segregation pattern of YMV incidence in F_2 population

which are associated to YMV resistance using polymorphic markers. In F_2 and RIL populations, equally pooled DNA samples of YMV resistant and susceptible lines and also DNA samples from the individual plants of resistant and susceptible plants were taken for the analysis. Ten individual plants of resistant and susceptible lines and their bulks (F_2) and five individual plants of resistant and susceptible lines and their bulks (RILs) were taken for the study in BSA. The polymorphic SSR markers were used to test the resistant bulk and susceptible bulk along with parents. The amplified products were scored.

SMA was carried out with the help of student's *t* test using single factor analysis of variance (ANOVA). Each phenotypic trait was taken to determine the simple linear regression using polymorphic SSR markers. The significance of the regression coefficient was calculated based on relationship between polymorphic SSR markers and studied traits. The segregation ratio of 3:1 was analyzed by chi-square test with goodness of fit. Validation of markers helps in

Table 1 Scales and YMV disease reaction ratings

Sl. no.	Symptoms	Scale	Rating	Reaction
1	No visible symptoms on leaves or very minute yellow specks on leaves	1	1.0 to 2.0	Resistant (R)
2	Small yellow specks with restricted spread covering 0.1 to 5% leaf area	2	2.1 to 4.0	Moderately resistant (MR)
3	Yellow mottling of leaves covering 5.1 to 10% leaf area	3		
4	Yellow mottling of leaves covering 10.1 to 15% leaf area	4	4.1 to 5.0	Moderately susceptible (MS)
5	Yellow mottling and discoloration of 15.1 to 30% leaf area	5	5.1 to 7.0	Susceptible (S)
6	Yellow discoloration of 30.1 to 50% leaf area	6		
7	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1 to 75% foliage	7	7.1 to 9.0	Highly susceptible (HS)
8	Severe yellow discoloration of leaves covering 75.1 to 90% of foliage, stunting of plants and reduction in pod size	8		
9	Severe yellow discoloration of entire leaves covering above 90.1% of foliage, stunting of plants and no pod formation	9		

determining the reliability and practical applicability of the markers in predicting the phenotype. Validation is necessary because false associations between markers and the trait of interest can arise if pedigree information is omitted from the discovery population analysis. Markers that cannot be validated have no value as tools for marker-assisted selection. Association of identified markers related to yellow mosaic virus resistance in the present study was verified with the previous related studies and the potentiality of the markers were confirmed.

Results

One hundred and sixty two F_2 plants developed from a cross between IC436656 and KKB14045 in black gram and their RIL populations were raised along with parents and YMV susceptible check (ADT 3). The YMV incidence was scored in the field condition based on the standard disease scoring method (Alice and Nadarajan 2007). From 162 F_2 plants, 121 were YMV susceptible and 41 were YMV resistant which was seventy five per cent (75%) of the population was YMV susceptible and 25 per cent was resistant (Table 2). The Chi-square test validated the goodness of fit to the expected ratio of 3:1 (Susceptible: Resistant) (Table 2). Hence it was concluded that recessive gene controls YMV resistance in the studied population.

Parental polymorphism

Among the studied 91 SSR markers, thirteen markers viz., CEDG141, CEDG008, CEDG020, CEDG269, CEDG011, CEDG077, CEDG015, CEDG264,

Table 2 Segregation pattern and Chi-square analysis for YMV resistance in F_2 cross derivative IC436656 x KKB14045 for YMV reaction

Sl. no.	YMV score	YMV reaction	Number of F_2 plants	YMV incidence				Ratio S:R	χ^2 value
				Observed ratio		Expected ratio			
				Susceptible	Resistance	Susceptible	Resistance		
1	1	Highly resistant	2	121	41	122	40	3:1	0.033
2	2	Resistant	16						
3	2.1 to 4	Moderately resistance	23						
4	4.1 to 5	Moderately susceptible	52						
5	5.1 to 7	Susceptible	41						
6	7.1 to 9	Highly susceptible	28						
Total			162						

CEDG048, CEDG 115, CEDG 271, CEDG 042 and VR 044 were polymorphic (14.3% polymorphism) between parents viz., IC436656 and KKB14045 (Table 3) and other markers were found to be monomorphic.

The parents viz., IC436656 (YMV score 7) and KKB14045 (YMV score 1) were selected based on the results of earlier genetic diversity studies (Priya et al. 2018). The selected parents were crossed during 2018. True F_1s were identified using polymorphic SSR markers. True F_1s were selfed to produce the F_2 segregating population (Fig. 1). The F_2 segregating populations were raised along with parents and YMV susceptible check (ADT 3) for YMV disease screening and molecular characterization studies. The polymorphic SSR markers viz., CEDG 141, CEDG 008, CEDG 020, CEDG 264 and CEDG 048 were used for molecular characterization.

Single marker analysis (SMA)

Among the polymorphic SSR markers, the markers viz., CEDG 141, CEDG 008 and CEDG 264 exhibited the marker-trait association for YMV resistance in F_2 population. The results are given in Table 4**.** The R^2 square value reveals the strong marker relationship with trait which also exhibits the overall percentage of variability of that trait (Anandhan et al. 2010). The R^2 values of polymorphic SSR markers in this study ranged from 15% (CEDG264) to 67% (CEDG141). The markers CEDG008 (63%) and CEDG141 (67%) were highly associated to YMV resistance since the *P* value was very low (0.000) for both the markers. The *P* value ranged from 0.000 to 0.001.

Table 3 List of polymorphic SSR markers identified in parental polymorphism studies

Sl. no.	SSR markers	Sequences	References
1	CEDG141 (F)	CCAGGCATCCATGATGACC	Chaitieng et al. (2006)
	CEDG141 (R)	GAAGTTGTTGGTAATGGTTGCCTC	
2	CEDG008 (F)	AGGCGAGGTTTCGTTTCAAG	
	CEDG008 (R)	GCCCATATTTTTACGCCCAC	
3	CEDG020 (F)	TATCCATACCCAGCTCAAGG	
	CEDG020 (R)	GCCATACCAAGAAAGAGG	
4	CEDG269 (F)	CTGTTACGGCACCTGGAAAG	Han et al. (2005)
	CEDG269 (R)	GCAGAGACACACCTTAACCTTG	
5	CEDG011 (F)	GTCCGACTTTATGTGTGGAG	Chaitieng et al. (2006)
	CEDG011 (R)	TTTCTAGTTCCAGCCCCGAC	
6	CEDG077 (F)	ATCCCGTGACCCTTCTTCCT	Han et al. (2005)
	CEDG077 (R)	GCTCAAGCGAAAACCCAGCA	
7	CEDG015 (F)	CCCGATGAACGCTAATGCTG	
	CEDG015 (R)	CGCCAAAGGAAACGCAGAAC	
8	CEDG264 (F)	GATTCCCTTCCTAGCTATGG	
	CEDG264 (R)	CTGCTGGACATGAAGATTCAG	
9	CEDG048 (F)	TCTCTTCCTCTATGGCTTGG	
	CEDG048 (R)	GCTCCTCTTTTTGCTGCATC	
10	CEDG115 (F)	GGCTCATTGTACCACTGGATAT	Wang et al. (2004)
	CEDG115 (R)	ATGCCTCCTTTCAGGTGATTGT	
11	CEDG271 (F)	GCACTAAAGTTAGACGTGGTTC	
	CEDG271 (R)	CACTCCCACTGCCAAACAAGG	
12	CEDG042 (F)	CACAGTGGTTTGGGCAACAG	
	CEDG042 (R)	TCAGAGGTTCCCATTTCCCG	
13	VR044 (F)	CCCATGAAGGTATGAGACAACA	Naik et al. (2017)
	VR044 (R)	GACTGAGAAAGAGAGAGAAGCATTT	

Table 4 The identified SSR markers linked to YMV resistance in black gram in single marker analysis

Sl. no.	SSR Markers	R^2 square value	*P* value	Population	Allele size (bp)	
					Susceptible	Resistance
1	CEDG141	67	0.000	F_2	200	280
				RILs	200	280
2	CEDG008	63	0.000	RILs	120	110
3	CEDG264	15	0.001	RILs	230	290

Bulked segregant analysis (BSA)

BSA was employed to identify the markers linked to YMV resistant gene in black gram. The parents along with resistant and susceptible bulks were screened using polymorphic markers. Among the polymorphic markers studied, CEDG141 had shown a band at 200 bp for IC436656 and 280 bp for KKB14045 in both F_2 and RILs. CEDG264 and CEDG008 had shown a band at 230 bp for IC436656 and 290 bp for KKB14045 and 120 bp for IC436656 and 110 bp for KKB14045 in RILs respectively. And these markers were able to distinguish the extreme bulks and their corresponding individuals (Fig. 2). From the study, CEDG141, CEDG264 and CEDG008 were linked to YMV resistance in RILs of black gram.

Validation of markers for YMV resistance

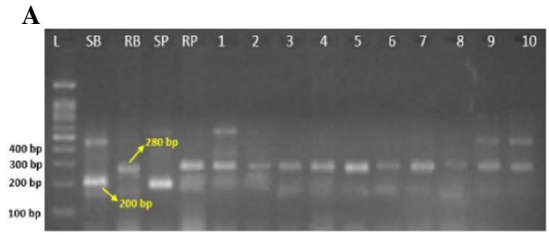
The SSR markers CEDG 141, CEDG 008 and CEDG 264 were validated for their association with YMV resistance over a set of resistant and susceptible genotypes (Table 5). CEDG 141 distinguished the susceptible and resistant genotypes in F_2 and RILs in same allelic size. CEDG 008 also distinguished the susceptible and resistant genotypes and the same result was reported by Gomathi (2020) and Narayanan (2021) in F_2 and RILs of KKM 1 × VBN 6 respectively. CEDG 264 distinguished the susceptible and resistant genotypes and the same result was reported by Narayanan (2021). Hence CEDG 141, CEDG 008 and CEDG 264 were identified as potential markers for marker assisted breeding in YMV studies.

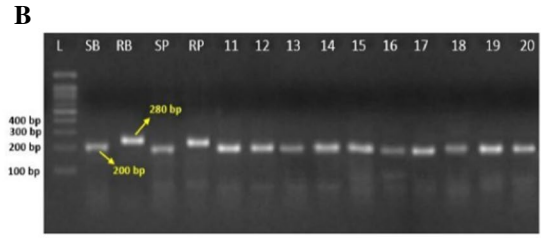
Discussion

The YMV susceptible and resistant parents viz., IC436656 and KKB14045 were selected in this study to generate F_2 segregating population. The susceptibility and resistant ratio suggested that YMV resistance was controlled by monogenic recessive gene and reconfirmed in RIL population. Studies by earlier workers (Anusha 2014; Jain et al. 2013; Reddy 2009; Kundagrami et al. 2009; Basak et al. 2005) suggested similar monogenic recessive pattern for YMV resistance. However, single dominant gene controlling YMV resistance was also reported (Singh and Singh 2006; Gupta et al. 2005, 2013a).

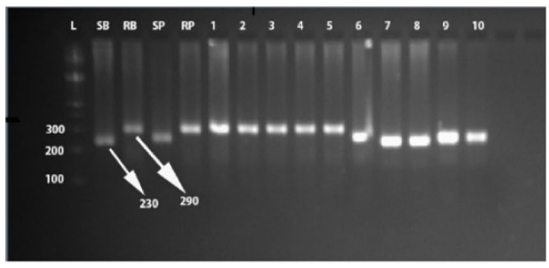
Among the 91 SSR markers, thirteen were polymorphic among the YMV susceptible (IC436656) and the resistant (KKB14045) parents. A total of 469 SSR primers were used for studying the parental polymorphism of TAU-1 × BDU-4 and 24 SSR markers showed the polymorphism (Basamma 2011). The single marker analysis was performed to identify the marker-trait association for YMV resistance. Similar work was reported in cowpea (Manjunatha et al. 2017) and from single marker analysis 4 SSR markers were obtained with phenotypic variances of above 20%. These marker-trait associations were further validated through composite interval mapping. Single marker analysis in mung bean powdery mildew disease (Sarkale 2015) was studied in 37 genotypes using 14 SSR polymorphic markers and five markers viz., DMBSSR199, CEDG259, VrCSSSR1, CEDG290 and VrCSSTS1 were associated to powdery mildew disease resistance and also VrCSSTS1 had high phenotypic variance (R^2) value of 20.18%.

BSA is a rapid method of identification of markers associated with desirable traits. It reduces the handling of the whole mapping population. CEDG141 clearly separated the resistant and susceptible bulks in F_2 and RILs. CEDG264 and CEDG008 separated the resistant and susceptible bulks in RILs. Hence it was concluded that these markers were linked to YMV resistance in this study. Similar results were reported by many workers. The F_2 population of single cross ML267 x CO4 was studied (Selvi et al. 2006) and the YMV

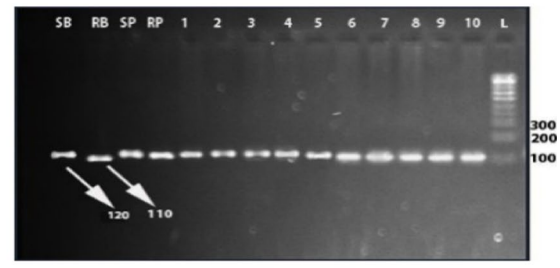




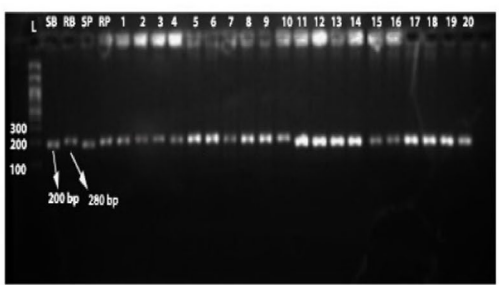
[L-Ladder, SB-Susceptible bulk, RB-Resistant bulk, SP-Susceptible parent, RP-Resistant parent, 1 to 10-Resistant F_2 plants, 11 to 20-Susceptible F_2 plants]



[L-Ladder, SB-Susceptible bulk, RB-Resistant bulk, SP-Susceptible parent, RP-Resistant parent, 1 to 5-Resistant **RIL** plants, 6 to 10-Susceptible **RIL** plants]



[L-Ladder, SB-Susceptible bulk, RB-Resistant bulk, SP-Susceptible parent, RP-Resistant parent, 1 to 5- Susceptible **RIL** plants, 6 to 10 Resistant **RIL** plants]



[L-Ladder, SB-Susceptible bulk, RB-Resistant bulk, SP-Susceptible parent, RP-Resistant parent, 1 to 10-Resistant **RIL** plants, 11 to 20-Susceptible **RIL** plants]

Fig. 2 Bulked segregant analysis for YMV resistance in F_2 and RIL population. **A** & **B** CEDG141 in F_2; **C** CEDG264 in RILs; **D** CEDG008 in RILs; **E** CEDG141 in RILs

resistance gene in mung bean was identified using RAPD markers. The RAPD (Random Amplifed Polymorphic DNA) marker OPBB 05,260 was tightly linked to YMV resistance in F_2 population of mung bean (Karthikeyan et al. 2012) from BSA using 72 random sequence decamer oligonucleotide primers. The SSR marker CEDG 180 was to MYMIV resistance gene in black gram (Gupta and Gupta 2013). This marker was also used to map 168 F2 individuals with a map distance of 12.9 cM and also for evaluating nine resistant and seven susceptible genotypes and the identified marker may be utilized in

Table 5 Marker validation for YMV resistance in black gram

Genotypes	Disease reaction to YMV	YMV score	CEDG 008		CEDG 264		CEDG 141		Pedigree	Reference
			120 bp	**110 bp**	**230 bp**	**290 bp**	**200 bp**	**280 bp**		
IC 436,656	Susceptible	7	Present (RILs)	Absent (RILs)	Present (RILs)	Absent(RILs)	Present (F_2 and RILs)	Absent (F_2 and RILs)	Land race, NBPGR, New Delhi, India	–
KKB 14,045	Resistant	1	Absent (RILs)	Present (RILs)	Absent (RILs)	Present(RILs)	Absent (F_2 and RILs)	Present ($F_2$and RILs)	PU-0620×ADT-3	
KKM 1	Susceptible	4	Absent (F_2 and RILs)	Present (F_2 and RILs)	Present (F_2 and RILs)	Absent(F_2 and RILs)	–	–	COBG 653×VBN 3	Gomathi (2020)#Narayanan (2021)
VBN 6	Resistant	1	Present (F_2 and RILs)	Absent(F_2 and RILs)	Absent (F_2 and RILs)	Present (F_2 and RILs)	–	–	VBN 1×*Vigna mungo var. silvestris,*	

marker assisted breeding (MAB) for developing the MYMIV resistant genotypes in black gram.

Two SCAR markers viz., CM 9 and CM815 in 90 F_2 populations from a cross KMG 189×VBN (Gg) 2 were studied in BSA and CM815 linked at 5.56 cM for YMV resistance gene (Sai et al. 2017). CM 9 had no recombination and closely linked to YMV resistance. The marker CEDG 185 separated the resistant and susceptible bulks by BSA in F_2 population of black gram from the cross T9 x LBG 759 and fourteen markers out of 50 SSR markers showed polymorphism (Rambabu 2016). BSA analysis was done in F_2 population of black gram cross T9 (resistant)×LBG-759 (susceptible) for YMV resistance. The marker VR9 differentiated the resistant and susceptible bulks and this marker was in close association with YMV resistance gene (Naik et al. 2017). The documented SSR markers in the study would be helpful for YMV resistance studies in black gram breeding programs.

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