Euphytica (2013) 193:27–37
DOI 10.1007/s10681-013-0884-4

Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* L. Hepper)

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Received: 23 July 2012 / Accepted: 6 February 2013 / Published online: 21 February 2013

Abstract Yellow Mosaic disease (YMD) is one of the most destructive diseases of blackgram (*Vigna mungo*) causing heavy yield losses every year. Mungbean Yellow Mosaic India Virus (MYMIV) is one of the YMD causing begomoviruses prevalent in the major blackgram growing area (northern and central part) of India. Inheritance of MYMIV resistance gene was studied in blackgram using F_1, F_2 and $F_{2:3}$ derived from cross DPU 88-31(resistant)× AKU 9904 (susceptible). The results of genetic analysis showed that a single dominant gene controls the MYMIV resistance in blackgram genotype DPU 88-31. The F_2 population from the same cross was also used to tag and map the MYMIV resistance gene using SSR markers. Out of 361 markers, 31 were found polymorphic between the parents. However, marker CEDG 180 was found to be linked with resistance gene following the bulked segregant analysis. This marker was mapped in the F_2 mapping population of 168 individuals at a map distance of 12.9 cm. The validation of this marker in nine resistant and seven susceptible genotypes has suggested its use in marker assisted breeding for developing MYMIV resistant genotypes in blackgram.

Keywords *Vigna mungo* · MYMIV · Bulked segregant analysis · Simple sequence repeats

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Introduction

Yellow Mosaic Disease (YMD) inflicts heavy yield losses in five economically important food legumes including blackgram (*Vigna mungo*), soybean (*Glycine max*), mungbean (*Vigna radiata*), frenchbean (*Phaseolus vulgaris*) and mothbean (*Vigna aconitifolia*). Blackgram, which is mainly cultivated in India, Myanmar, Thialand, Phillipines and Pakistan, is highly prone to YMD. The disease is caused by representative species of the genus *Begomovirus*. Based on several studies, it has been confirmed that at least two virus species causing YMD are prevalent in Indian sub-continent. One of these species, mungbean yellow mosaic India virus (MYMIV) is commonly occurring in northern part of Indian sub-continent while Mungbean Yellow Mosaic Virus (MYMV) is mostly confined to peninsular region of India (Varma and Malathi 2003; Malathi and John 2008). These two virus species can easily be distinguished on the basis of nucleotide sequence identity (Fauquet et al. 2003).

The losses due to MYMIV strain have been observed from 60 to 100 % in Northern India. Since the virus transmission is attributed by the vector-whitefly (*Bemisia tabaci*), control of MYMIV based upon limiting the vector population by using insecticides is ineffective under severe whitefly infestations. Further, this is also not an eco-friendly approach. The most effective way to prevent the occurrence of this disease is to develop genetically resistant cultivars of blackgram.

There are conflicting reports about the genetics of resistance to YMD, claiming both resistance and susceptibility to be dominant. In blackgram, monogenic dominant nature of resistance was reported by Dahiya et al. (1977), Kaushal and Singh (1988) and Gupta et al. (2005) while it was reported to be digenic recessive by Singh (1980), Dwivedi and Singh (1985), Verma and Singh (1986). Monogenic recessive control of yellow mosaic resistance has also been reported (Pal et al. 1991; Reddy and Singh 1995). Nevertheless, these studies have been conducted by taking into account the yellow mosaic disease in general. However, the prevalence of two different species of virus causing YMD and sources of resistance to each of these species might have caused differing results about the genetics of resistance to the disease.

Marker assisted indirect selection of resistant genotypes using linked markers has been reported as an effective breeding approach for developing YMD resistant cultivars in blackgram. It is assuming increased importance due to lack of uniform field screening procedure as well as difficulty in direct selection due to complex interaction of virus, vector and host (Souframanien and Gopalakrishna 2006). Basak et al. (2004) reported use of resistance gene analog (RGA) associated with yellow mosaic tolerance. However, in their study, linkage distance between marker and gene was not estimated and virus species was not specified. Similarly, a SCAR marker linked to MYMV resistance gene with a distance of 6.8 cm was also reported (Souframanien and Gopalakrishna 2006), although in this study also the virus species has not been clearly identified. Therefore, identification of a tightly linked molecular marker with MYMIV resistance is urgently required that could be used easily by plant breeders in blackgram breeding program. In recent years, SSR markers have proved to be most powerful tool for marker assisted breeding in many crops as they are highly polymorphic, reproducible and easy to use for screening large segregating populations. Previously, development of SSR markers has been reported in several *Vigna* species and other pulse crops (for review see Kumar et al. 2011a, b). The studies on disease resistance genes have shown a high level of polymorphism due to the presence of SSR at certain loci (Yu et al. 1996). Therefore, aim of the present investigation was to study inheritance of MYMIV resistance gene, tagging and mapping of the resistance loci and validation of linked marker among diverse genotypes of blackgram.

Materials and methods

Plant materials

The hybridization between DPU 88-31 and AKU 9904 genotypes of blackgram (*V. mungo*) was carried out at Main Research Farm of Indian Institute of Pulses Research, Kanpur and the hybrid seeds of the cross DPU 88-31 × AKU 9904 were harvested during 2009. Genotype DPU 88-31 was used as a resistant parent to MYMIV in this investigation, which is an advanced breeding line developed from the cross PLU 131 × T9 at IIPR, Kanpur while genotype AKU 9904, also a breeding line, was used as susceptible parent. 19 F_1s of this cross were sown in the year 2010 in the field with the two parents sown alongwith as checks for distinguishing the true hybrids from false ones. Hybridity of F_1s was confirmed through molecular marker analysis. Six true F_1 plants of above cross were then harvested individually to produce their respective F_2 populations. The F_2 populations were sown in the field, and the F_3 plants were harvested individually in 2011. These, $F_{2:3}$ families were raised in the field to confirm the segregation ratio observed in F_2.

One F_2 population from the DPU 88-31 × AKU 9904 cross was chosen randomly to tag the MYMIV resistant gene(s) of DPU 88-31. This population had 168 individuals. At the seedling stage, when the second trifoliate leaf unrolled, the young leaves were collected from individual plants of DPU 88-31, AKU 9904, F_1 and F_2 population, and preserved in the refrigerator at –70 °C.

Evaluation for MYMIV reaction

For evaluation of the test material against MYMIV, the infector row method of sowing two test rows alternating with spreader rows of highly susceptible

variety 'Barabanki Local' was adopted at test location which is the hot spot for the disease (Singh and Gurha, 1994). The F_1, F_2 and $F_{2:3}$ were scored for disease incidence after 80 % of the plants in spreader rows showed MYMIV incidence. As evidenced by the disease data over the years, Kanpur is a hot spot for MYMIV and during the test years as well more than 80 % MYMIV incidence was recorded in most susceptible check 'Barabanki Local'. For evaluation of parental genotypes, the MYMIV reaction was observed on the basis of agro-inoculation test as suggested by Mandal et al. (1997) with a few modifications (Jacob et al. 2003). Accordingly, non-viruliferous adult white flies were given acquisition feeding for 24 h on infected leaves and transferred to the healthy test plants for inoculation feeding. For confirmation of virus species on the basis of sequence identity, MYMIV and MYMV specific primers (Naimuddin et al. 2011) were synthesized by ILS, India and used for viral DNA amplification.

MYMIV reaction data were recorded on parents, F_1, each individual plant of F_2 population and $F_{2:3}$ families and accordingly, those were classified as: healthy (no symptoms), necrotic (necrotic mottle, N)] and susceptible (typical yellow mosaic symptoms, S). Both healthy and necrotic plants were grouped together as resistant (R) plants as suggested by Biswas and Varma (2001).

DNA extraction

Total genomic DNA was isolated from parental lines, F_1 and individual F_2 plants following Doyle and Doyle (1987) with slight modifications. The quality of extracted DNA was checked by comparing with λ DNA while quantity was determined using spectrophotometer. The working DNA sample was diluted to a standard concentration of 20 ng/μl. For isolation of viral total DNA from infected leaf samples, a Qiagen DNeasy Plant Mini Kit was used.

Simple sequence repeats analysis

Total 361 SSR markers were used in the present study which were derived from previous reports in other legume species viz., adzuki bean (Wang et al. 2004), mungbean (Somta et al. 2009; Kumar et al. 2002a; Kumar et al. 2002b), cowpea (Li et al. 2001), common bean (Gaitan-Solis et al. 2002; Blair et al. 2003), chickpea (Buhariwalla et al. 2005) and pigeonpea (Datta et al. 2010). These primer sequences were synthesized by IDT, USA and used to genotype the parental lines. Polymerase Chain Reaction was carried out in 20 μl reaction mixture containing 1× PCR buffer (Fermantas, USA), 50 ng genomic DNA, 0.6 U of Taq DNA polymerase (Fermantas, USA), 0.2 mM dNTP mix (Fermantas, USA) and 0.2 μM each of forward and reverse primers (IDT, USA and ILS, India) in a thermocycler (G-Storm, UK). The PCR profile was programmed for an initial denaturation of 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, annealing for 1 min, 72 °C for 2 min and final extension at 72 °C for 7 min. The amplification products were resolved on 10 % PAGE following silver staining. Polymorphic markers were distinguished on the basis of differences in allele size or presence/absence of marker allele visible on the gel. The sequence of reverse and forward primers of polymorphic SSR markers is given in Table 1.

Preparation of DNA bulks for bulked segregant analysis (BSA)

For BSA, two bulks, one each from highly resistant and susceptible plants identified in F_2 population were prepared as described by Michelmore et al. (1991). For each bulk, equal quantity of DNA (25 ng/μl) was taken from 12 individual plants and pooled together. The polymorphic markers identified between the parents were used to genotype the parental genotypes along with resistant and susceptible bulks. On the basis of amplification of an allele, either in resistant bulk and resistant parent or susceptible bulk and susceptible parent, the association of marker with genes controlling the target trait was established. The marker showing association with target gene through BSA was used to genotype individual F_2 plants and genotypic data were recorded on each individual plant as homozygous for parental alleles and heterozygous at marker locus.

Segregation analysis

The phenotypic data of disease reaction and genotypic data of concerned marker, which were recorded on individual F_2 plants and $F_{2:3}$ families were used to classify the segregating population into distinct classes and analyzed with the help of Chi square (χ^2) test

Table 1 Details of 31 polymorphic markers between resistant (DPU88-31) and susceptible (AKU9904) parents for MYMIV

Marker	Forward primer (5′–3′) Reverse primer (5′–3′)	Repeat motif	T_m	Crop	Reference
CEDG204	CCTTGGTTGGAGCAGCAGC CACAGACACCCTCGCGATG	(AG)15	59	Azukibean	Chatieng et al. 2006
CEDG139	CAAACTTCCGATCGAAAGCGCTTG GTTTCTCCTCAATCTCAAGCTCCG	(AG)19	58	Azukibean	Chatieng et al. 2006
CEDG008	AGGCGAGGTTTCGTTTCAAG GCCCATATTTTTACGCCCAC	(AG)26	55	Azukibean	Chatieng et al. 2006
CEDG268	CATCTCCCTGAAACTTGTG GCTATCAATCGAGTGCAG	(AG)16	50	Azukibean	Chatieng et al. 2006
CEDG030	TGAGGGAATGGGAGAGAGGC TCCGCAGATAGAGGCTCACG	(AG)45	59	Azukibean	Han et al. 2005
CEDG092	TCTTTTGGTTGTAGCAGGATGAAC TACAAGTGATATGCAACGGTTAGG	(AG)17	55	Azukibean	Chatieng et al. 2006
CEDG022	AGGAATGTGAGATTTG AATCGCTTCAAGGTCAAGCC	(AG)27	49	Azukibean	Han et al. 2005
CEDG024	CATCTTCCTCACCTGCATTC TTTGGTGAAGATGACAGCCC	(AG)18	54	Azukibean	Han et al. 2005
CEDG198	CAAGGAAGATGGAGAGAATC CCTTCTAAGAACAGTGACATG	(AG)30	50	Azukibean	Han et al. 2005
CEDG013	CGTTCGAGTTTCTTCGATCG ACCATCCATCCATTCGCATC	(AG)22	54	Azukibean	Chatieng et al. 2006
DMB-SSR182	TAGAGCCTTCTGGTTTTTCACA AGGAGGAGGATTTTGATGATGA	(TGA)3..(CT)3…(TCC)4	54	Azukibean	Somta et al. 2009
DMB-SSR186	GAGAGAGAAGGAGAGGGAGA ATTCTTTCTCCACCACAATG	(GGT)4…(GGT)3…(GGT)4	52	Mungbean	Somta et al.2009
CEDG133	GCATACATAATGTGGTGAGATG GTCTCGTGCCTTTCACAC	(AT)3(AG)11	57	Azukibean	Chatieng et al. 2006
CEDG141	CCAGGCATCCATGATGACC GAAGTTGTTGGTAATGGTTGCCTC	(AT)6(AG)13	60	Azukibean	Chatieng et al. 2006
CEDG225	GAGGAAGTGTTGCAGCACC GTAGACTCTGCAGAGGGATG	(AG)8 TG(AG)3(TG)2(AG)4	60	Azukibean	Han et al. 2005
CEDG284	GGTGCTAACGTTGGAAACTGAG CACTCCATTCTGAGGATCAATCC	(AT)19(AG)21 A(AG)5	60	Azukibean	Chatieng et al. 2006
CEDG077	ATCCCGTGACCCTTCTTCCT GCTCAAGCGAAAACCCAGCA	(AG)8	60	Azukibean	Han et al. 2005
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC CTCCTCACTTGGTCTGAAACTC	(TG)3(AG)9	61	Azukibean	Chatieng et al. 2006
CEDG014	GCTTGCATCACCCATGATTC AAGTGATACGGTCTGGTTCC	(AT)12(AG)14	58	Azukibean	Chatieng et al. 2006
CEDG020	TATCCATACCCAGCTCAAGG GCCATACCAAGAAAGAGG	(AT)18(AG)20	56	Azukibean	Chatieng et al. 2006
CEDG067	AGACTAAGTTACTTGGGCAACCAG TGACGGCCCGGCTCTCC	(AG)14	62	Azukibean	Han et al. 2005
CEDG245	GATAGAGCTTAAACCCTC CTTTTGATGACAAATGCCC	(AC)10(AT)9(AG)14	53	Azukibean	Han et al. 2005

Table 1 continued

Marker	Forward primer (5′–3′) Reverse primer (5′–3′)	Repeat motif	T_m	Crop	Reference
CEDG059	AGAAAAGGGTGGCCTCGTTG GCAGGCATTTCCATCGCAG	(AG)8…(AG)18	60	Azukibean	Han et al. 2005
CEDG112	GCAATATTCGCATTATTCATTCA GTGTTTCAAAGCACTATACTTAA	(AT)18(AG)20	53	Azukibean	Han et al. 2005
CEDG269	CTGTTACGGCACCTGGAAAG GCAGAGACACACCTTAACCTTG	(AG)14	60	Azukibean	Han et al. 2005
CEDC011	GTCCGACTTTATGTGTGGAG TTTCTAGTTCCAGCCCCGAC	(AC)9(AT)8…(AT)7(AC)4	59	Azukibean	Chatieng et al. 2006
CEDG056	TTCCATCTATAGGGGAAGGGAG GCTATGATGGAAGAGGGCATGG	(AG)14	61	Azukibean	Chatieng et al. 2006
CEDG180	GGTATGGAGCAAAACAATC GTGCGTGAAGTTGTCTTATC	(AG)11	55	Azukibean	Chatieng et al. 2006
CEDG044	TCAGCAACCTTGCATTGCAG TTTCCCGTCACTCTTCTAGG	(GT)10 AT(AG)18	58	Azukibean	Chatieng et al. 2006
CEDG104	TATGGCCCGAGCAAACCTTG CCGTTCGGTCTTCGGTTGAA	(AG)13	60	Azukibean	Han et al. 2005
VrCS SSR3	GCAGACACAACCATAAATCC GGTCTTTGACGGCAATCTC	(AT)37	57	Mungbean BAC library	Zhang et al.2008

for a fixed ratio hypothesis (Gomez and Gomez 1984). The hypothesis was tested at 5 % level of significance. Homogeneity among F_2 populations and among $F_{2:3}$ families was evaluated using contingency test.

Linkage analysis and validation of linked marker

Linkage between the marker and the target gene was declared when χ^2 value was significantly higher than the table value. The product ratio (z) method was used to determine the per cent recombination between resistance gene and the linked marker locus. The 'z' value was calculated using the formula taking into account that marker and gene are associated in coupling phase (Stevens 1939). The per cent recombination between two loci was determined from the table of recombination fraction given by Stevens (1939) against calculated 'z' value. This recombination frequency was then converted into linkage distance as 1 % recombination equal to 1 cm. The marker linked with MYMIV resistance was also used for its validation into survey the other nine resistant and seven susceptible genotypes of blackgram. The genotypes used for validation are listed in Table 4.

Results

Confirmation of MYMIV species

In order to confirm the species of the virus causing yellow mosaic disease under local conditions, MYMIV-specific primer pairs were used. These primers amplified the desired size of amplicons (i.e. approximately 1,000 bp for DNA-A and 900 bp for DNA-B) in the disease infected leaves of the susceptible parent, which was used as infector in present investigation. Based on these results, it was confirmed that the present study was carried out for Mungbean Yellow Mosaic India Virus (MYMIV) and not for MYMV.

Inheritance of MYMIV resistance gene

Inheritance of MYMIV resistance gene was studied in six individual F_2 populations derived from the cross DPU88-31 (resistant) × AKU9904 (susceptible). Each of the six F_2 populations exhibited segregation for resistance gene, and all segregation ratios of resistant to susceptible fitted a 3:1 ratio under Chi square test. Table 2 shows that homogeneity Chi square test among the six F_2 populations further demonstrated

Table 2 Segregation for MYMIV resistance gene in the F_2 populations of DPU 88-31× AKU 9904

Population no.	Segregants in F_2 population for MYMIV		χ^2 (3:1)*	df	*P* value
	Resistant	Susceptible			
1	114	39	0.019	1	0.90–0.95
2	89	32	0.134	1	0.70–0.80
3	109	34	0.114	1	0.70–0.80
4[a]	128	40	0.126	1	0.70–0.80
5	114	36	0.08	1	0.70–0.80
6	81	23	0.461	1	0.50–0.70
Pooled	635	204	0.210	1	0.50–0.70
Homogeneity			1.328	5	0.90–0.95

*Value for significance of $P = 0.05$

[a] Population used for gene tagging

the segregation ratio as being homogeneously 3:1. Further the pooled population of the six F_2 populations with a total of 640 resistant and 199 susceptible plants also fitted a 3:1 segregation ratio. The frequency of resistant and susceptible plants did not deviate significantly from 3:1 ratio suggesting that single dominant gene is controlling resistance to MYMIV in blackgram.

One F_2 population from the DPU 88-31× AKU 9904 cross (population no. 4) was chosen randomly to develop $F_{2:3}$ families to confirm the observed F_2 segregation ratio for MYMIV resistant gene(s) of DPU 88-31. This population had 168 individuals. The seeds of all individual plants were sown in separate rows. Each row was observed for segregation for resistant and susceptible types for MYMIV. The segregation ratio of the number of non-segregating resistant lines: number of 3:1 segregation of $F_{2:3}$ lines: number of susceptible lines fitted a 1:2:1 ratio ($\chi^2 = 0.540$, $P = 0.706$) as expected.

Tagging of MYMIV resistant gene for DPU 88-31

The polymorphism between resistant line DPU 88-31 and susceptible line AKU 9904 was analyzed with 361 pairs of SSR primers. Among them, 31 SSR markers were found to be polymorphic between the above-mentioned parental genotypes. These 31 pairs of SSR primers were further used to test the polymorphism between the susceptible bulk (S bulk) and resistant bulk (R bulk). Among these, only the marker CEDG180 amplified an allele of 136 bp in the resistant parent and R bulk while in susceptible parent and S bulk, the allele size was 163 bp. The amplification of resistant parental allele in R bulk and susceptible parental allele in S bulk indicated that this marker is associated with the gene controlling MYMIV resistance in blackgram.

Mapping of linked marker and its validation

The SSR marker associated with MYMIV resistance gene was used to genotype each individual of the F_2 population (Fig. 1). Genotyping data were employed to classify this population as homozygote for alleles of resistant parents, heterozygote and homozygote for alleles of susceptible parents at the marker locus. In F_2, though the marker and the target trait segregated separately in 1:2:1 and 3:1 ratio, as expected the two loci did not segregate independently ($P < 0.05$), and

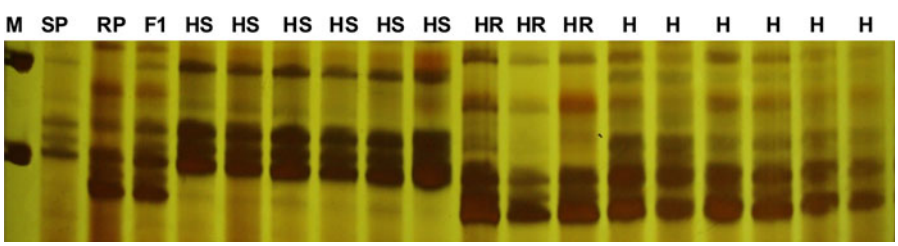

Fig. 1 Profile of CEDG180 marker in F2 mapping population of MYMIV *M* ladder, *SP* susceptible parent (AKU 9904), *RP* resistant parent (DPU 88-31), *F1* hybrid, *HS* homozygous susceptible, *HR* homozygous resistant, *H* heterozygote

Table 3 Segregation of SSR marker CEDG180 in F_2 population

Parents with allele of marker CEDG180	Segregation in F_2 population				χ^2 (9:3:3:1)	'Z' value	Map distance against the 'z' value
	Resistant		Susceptible				
	Homozygote for resistant alleles/ heterozygote at marker locus (a)	Homozygote for susceptible allele at marker locus (b)	Homozygote for resistant alleles/ heterozygote at marker locus (c)	Homozygote for susceptible allele at marker locus (d)			
DPU88-31 (Resistant with 136 bp marker allele) and AKU 9904 (Susceptible with 163 bp marker allele)	112	6	14	36	12.01*	0.028	12.9 cm

* Significant at $P < 0.05$

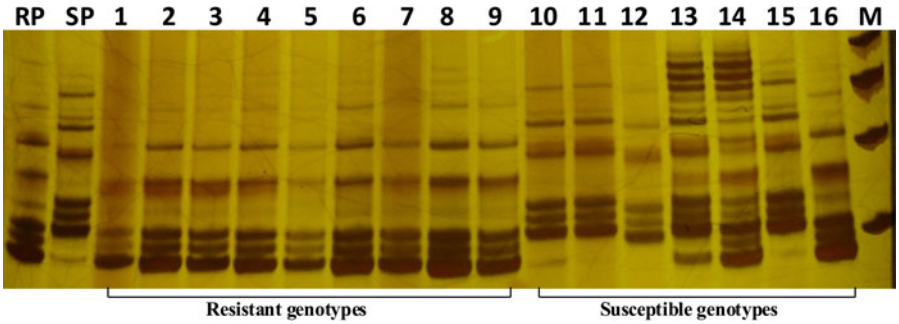

Fig. 2 Validation of the linked marker CEDG180 in MYMIV resistant and susceptible genotypes *RP* resistant parent (DPU 88-31), *SP* susceptible parent (AKU 9904), *Lane 1* PLU-1, *2* IPU 99-79, *3* UH 80-26, *4* NG 2119, *5* V 3108, *6* PLU 312, *7* IPU 91-7, *8* Uttara, *9* IPU 2-43, *10* PGRU95018, *11* IPU 99-147, *12* HPU 180, *13* PDU 1, *14* LBG 20, *15* Shekhar 2, *16* T9

frequency of parental types (148 out of 168) was observed to be higher than the recombinants. These results further indicated association of CEDG180 marker with the gene controlling MYMIV resistance in blackgram. Based on product ratio method, the calculated 'z' value (0.028) corresponded with recombination fraction table given by Stevens (1939) that indicated a map distance of 12.9 cm between the marker CEDG180 and MYMIV resistance gene in coupling phase of linkage (Table 3).

This marker (CEDG180) was also used to survey 9 resistant and 7 susceptible genotypes screened earlier for MYMIV disease reaction. It was able to distinguish the resistant genotypes with 136 bp allele and the susceptible genotypes with 163 bp allele (Fig. 2; Table 4). However two genotypes of susceptible category did not show the susceptible allele. The susceptible genotype LGB 20 was observed to be heterozygote, while another genotype T9, was homozygote for the alleles of resistant parent at marker locus. The heterozygosity at marker locus was further confirmed in one of the genotypes, LBG20 by analysing the individual seeds using the marker CEDG 180.

Discussion

Marker assisted selection for those traits which are difficult to screen phenotypically has become an important tool for breeding programs. Molecular markers are now widely used to track loci and genome regions in many important crops including legume crops and several improved varieties have been developed using molecular markers in recent times (Kumar et al. 2011a, b). Screening for MYMIV resistance in blackgram is also difficult owing to practical difficulties in creation of artificial

Table 4 MYMIV resistance status of different genotypes in this study and presence of MYMIV resistant allele

Genotype	Disease reaction to MYMIV	CEDG 180 marker SSR allele		Pedigree
		136 bp	163 bp	
DPU 88-31	Resistant	Present	Absent	Advanced breeding line (PLU 131× T9)
AKU 9904	Susceptible	Absent	Present	Susceptible genotype
PLU-1	Resistant	Present	Absent	Germplasm collection from North Western Plains of India
IPU 99-79	Resistant	Present	Absent	Germplasm collection from Western Ghats of India
UH 80-26	Resistant	Present	Absent	Advanced breeding line (T9× US 131)
NG 2119	Resistant	Present	Absent	Germplasm collection from Central India
V3108	Resistant	Present	Absent	Germplasm collection from Northern Hills of India
PLU 312	Resistant	Present	Absent	Germplasm collection from North Western Plains of India
IPU 91-7	Resistant	Present	Absent	Germplasm collection from Western Ghats of India
Uttara	Resistant	Present	Absent	Cultivar recommended for commercial cultivation and developed from cross NP19× T9
IPU 2-43	Resistant	Present	Absent	Commercial cultivar developed from cross DPU88-31× DUR-1
PGRU 95018	Susceptible	Absent	Present	Germplasm collection from north India
IPU 99-147	Susceptible	Absent	Present	Germplasm collection from Western Ghats of India
HPU180	Susceptible	Absent	Present	Germplsam collection from Northern Hills of India
PDU 1	Susceptible	Absent	Present	Selection from indigeneous collection accessioned as IC8219
LBG 20	Susceptible	Present	Present	Commercial cultivar developed from cross T-9× Netiminumu
Shekhar 2	Susceptible	Absent	Present	Commercial cultivar developed from cross 7378/2× T9
T9	Susceptible	Present	Absent	Local selection from Bareilly, India

epiphytotic conditions and dependency of MYMIV occurrence on several factors such as vector population, climatic conditions, etc. The aim of present investigation was to tag and map genes controlling MYMIV resistance in blackgram. For this purpose, we used a resistant donor DPU88-31, which was earlier identified to be a resistance source against the MYMV species (Gupta et al. 2005). However, subsequent studies based on sequence similarity analysis confirmed that this resistance was against MYMIV species. This virus species is prevalent in northern, central and eastern regions of India, while MYMV species is common in southern states of India (Fauquet et al. 2003; Haq et al. 2011). Since the confirmatory studies as well the present investigation were conducted under local conditions which is a hotspot for MYMIV, it was established that DPU 88-31 is a resistant donor for MYMIV and nor for MYMV species. Using the disease reaction data of individual F_2 plants, the inheritance of MYMIV resistance gene was also studied. Our results showed that a single dominant gene controls the inheritance of MYMIV resistance in blackgram which confirms the results of Gupta et al. (2005). However, this is contradictory to other study which reported that resistance to MYMIV is controlled by monogenic recessive gene (Basak et al. 2004).

For tagging and mapping of resistance gene in the present study, a F_2 mapping population was developed by crossing between the resistant and susceptible parents and subsequently screening the population with a number of SSR markers. Several studies have suggested that SSR markers are among the most useful markers for marker assisted breeding because these are highly polymorphic, reproducible and their easy handling makes it possible to screen large segregating populations over a limited period of time. The DNA of parental genotypes was surveyed and 8.6 % polymorphism was observed, which was low as compared to overall polymorphism reported in diverse genotypes of blackgram in an earlier study (Gupta et al. 2013). This low level of polymorphism is common in pulse crops due to narrow genetic base (Kumar et al. 2011a, b).

Bulked segregant analysis is an important method to tag a gene without the need of preparing a linkage map and hence it reduces the cost, time and labour required for genotyping of all the plants in a mapping population with large number of polymorphic markers. Therefore, its use becomes more important in those conditions where insufficient polymorphic markers exist for mapping (Shoba et al. 2012). The difficulty of genotyping all the plants in mapping population can be reduced through selective genotyping through BSA. BSA involves selection of extremely resistant and susceptible lines and pooling their DNA into two bulks viz., resistant and susceptible bulks (Michelmore et al. 1991). In this study we used BSA technique to identify SSR marker(s) associated with MYMIV resistance in cultivated blackgram. Among the 31 polymorphic SSR markers, only one marker, CEDG180, was able to distinguish the resistant and susceptible bulks. Genotyping of all 168 F_2 individuals of the cross DPU88-31× AKU9904 resulted in association of this marker with MYMIV resistance gene at a distance of 12.9 cm. In several such studies conducted earlier, molecular markers have been used to tag virus resistance in many legumes like soybean (Jeong and SaghaiMaroof 2004), common bean (Urrea 1996), pea (Gao et al. 2004) and peanut (Shoba et al. 2012). In blackgram, Basak et al. (2004) reported linkage of a RGA marker of 445 bp with YMV resistance. However in our study this marker did not show polymorphism in the parental lines. Nevertheless, this study had not specified the virus species and estimation of linkage was not performed. Similarly, without specifying the virus species, resistance gene for yellow mosaic disease was identified to be linked with a SCAR marker at a map distance of 6.8 cm (Souframanien and Gopalakrishna 2006). The resistant parent TU94-2 used in this study was bred in southern part of India and was screened under south Indian conditions only where MYMV virus is prevalent (Fauquet et al. 2003, Haq et al. 2011). Hence based on these observations, probably Souframanien and Gopalakrishna (2006) had mapped the resistance gene to MYMV rather than MYMIV (prevalent virus species in northern India). In another study, a RGA marker namely CYR1 was shown to be completely linked to the MYMIV resistance gene when validated in susceptible (T9) and resistant (AKU9904) genotypes (Maiti et al. 2011). However, the genotype AKU9904 showed consistently highly susceptible reaction to MYMIV at Kanpur over the years (Gupta et al. 2008; Anjum Tuba et al. 2010) and therefore was used as a highly susceptible parent in our mapping population. Therefore, the marker CYR1 was not used for mapping the MYMIV resistant gene in present study.

Molecular marker identified in the present study has been validated in nine resistant and seven susceptible genotypes (Table 3). Among the two susceptible genotypes showing heterozygosity at marker locus, the DNA profile study of individual seeds of LBG 20 suggested that the seed lot was genetically impure and could be an admixture with seeds of another genotype having the resistant allele. The identified marker with resistant gene is not very close to the resistance locus and hence showed discrepancy in T 9 cultivar. Since the marker CEDG 180 linked to the gene controlling MYMIV resistance validated in majority of test genotypes, this can be effectively utilized for developing the MYMIV resistant genotypes through marker assisted selection. Such cultivars will be of immense use for cultivation in the northern and central part of India, which is the major blackgram growing area of the country.

Acknowledgments This study was supported by research grant from Department of Biotechnology, Government of India. The authors thank Director, IIPR and Head, Crop Improvement Division, IIPR for providing necessary facilities required to carry out this study.

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