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Short Communication

Validation of CYR-1 marker linked with yellow mosaic virus resistance in black gram (*Vigna mungo* L. Hepper)

K. K. Panigrahi, T. R. Das[1]*, B. Baisakh, A. Mohanty and J. Pradhan

Orissa University of Agriculture and Technology, Bhubaneswar, Orissa; [1]IARI RS, Pusa, Samastipur, Bihar 848 125

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Abstract

The inheritance of yellow mosaic virus (YMV) resistance was studied in blackgram using the molecular marker and F_2 population derived from the cross Keonjhar Local(susceptible) × OBG-31(resistant). Assuming Mendelian inheritance, almost perfect fit to a ratio of 3S:1R in segregating population suggested a single recessive gene controlling YMV resistance in *V. mungo*. The presence of recessive gene was further confirmed by the linked DNA marker of 1236 bp named 'CYR1'. Amplification of CYR1 marker from the genomic DNA of phenotypic resistant individuals from segregating F_2 populations and F_3 families revealed a tight linkage between this marker with the YMV resistance.

Key words: *Vigna mungo*, yellow mosaic virus, CYR1, molecular markers

Blackgram (*Vigna mungo* L. Hepper) is one of the important grain legumes in India and an excellent source of good quality protein. Among different diseases that are responsible for low productivity in black gram, yellow mosaic disease (YMD) is one of the major disease in India caused by yellow mosaic virus (YMV), a whitefly transmitted bigemini virus. Yield loss due to this disease varies from 5 to 100 percent depending upon disease severity, susceptibility of cultivars and population of whitefly (Rathi 2002). 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 (Sowmini and jayamani 2014). It is assuming increased importance due to lack of uniform field screening procedure as well as indirect selection due to complex virus vectors, host and environmental interaction (Souframanien and Gopalkrishna 2006). Few molecular markers linked with YMV resistance have identified earlier (Basak et al. 2004; Maiti et al. 2011). Use of RGA-markers is comparatively recent and can be conveniently designed from the diagnostic motifs of known disease resistance genes (Yan et al. 2003). So validation of such markers becomes an essential criterion with regard to the intricate disease nature. Thus the present study was undertaken to validate available RGA markers linked with YMV resistance in blackgram.

A cross between Keonjhar Local (YMV-susceptible) and OBG-31 (YMV-resistant) was made by taking Keonjhar local as the female parent. Out of a total of 600 individual flowers crossed, 230 putative F_1 plants were sown during December 2012 along with the parents. Seeds from selfed individual F_1 plants were collected; half of the seeds from each F_1 plants were stored and the other half sown to raise F_2 populations along with the parental lines and a known resistant check PU-30 for advancing the F_2 population. The parental lines and the F_2 populations were screened for YMV reaction from September to December 2013 in forced inoculation environment as well as under natural field epiphytotic conditions. In the forced inoculation experiment, YMV-reaction was observed on the parental lines, 230 F_2 plants obtained from 12 putative F_1s. Data on YMV-reaction under

*Corresponding author's e-mail: trdas.iari@gmail.com
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natural epiphytotic condition was recorded from 298 F_2 individuals. In March 2014, 100 F_3 families (25 plants/family) from the cross Keonjhar Local × OBG-31 were sown. The goodness of fit to Mendelian segregation of pattern in the segregating populations was tested by Chi-square. YMV reaction of the F_2 and F_3 plants were assessed by using molecular markers to confirm the inheritance pattern of YMV resistance. One pair of resistance gene analogue (RGA) primers RGA22F2/RGA24R2 designed from 'R' (Resistance) genes of soybean (Table 1) was used in PCR for detection of polymorphism as suggested earlier (Basak et al. 2004; Maiti et al. 2011; Sowmini and Jayamani 2014). Amplification was carried out in 50 µl reaction mixture containing 100 ng of genomic DNA, 100ng of each primer, 1X PCR buffer containing 1.5mM $MgCl_2$, 250 µM of each dNTPs and 1unit of Taq DNA polymerase (Bangalore Genei, India), using a AB system thermal cycler. The PCR cycle consisted of a denaturation step at 94^{o}C for 300s, followed by 35 cycles of denaturation at 94^{o}C for 45s, then 45s at the annealing temperature and extension at 72^{o}C for 60s followed by a final extension at 72^{o}C for 600s. The amplified PCR products were separated in 1.5% agarose gels and visualized by ethidium bromide staining.

Table 1. RGA primers and respective annealing temperatures

Primer	Nucleotide sequence	Annealing temperature (^{o}C)
RGA22F2	5'-GGGTGGNTTGGG TAAGACCAC-3'	60
RGA24R2	5'-NTCGCGGTGNGT GAAAAGNCT-3'	58

The lines were evaluated for YMV reaction under field as well as artificial forced inoculated method with surplus population of white fly (*Bemisia tabaci* Genn.). A total of 230 plants of Keonjhar local × OBG-31 were evaluated visually under forced inoculated conditions. The F_2 population segregated into 177 susceptible and 53 resistant plants fitting well to a theoretical ratio of 3:1 suggesting that a single recessive gene is controlling resistance against YMV (Table 2). Phenotypic categories of resistance or susceptibility were assigned based on the visible expression of disease development on plants. The susceptible cultivars Keonjhar local developed mosaic symptoms very clearly in the field condition as well as under forced inoculation and the resistant cultivar OBG-31 did not show any sign of symptoms of YMV under both of the conditions. Since the observation was made at pod formation stage, which has been considered optimum for disease development, the symptoms were clearly expressed on the test material. Three-fourths of the F_2 plants of Keonjhar local × OBG-31 cross showed susceptible reaction with disease symptoms under field conditions (Table 3). The heterogeneity test revealed significant differences and the pooled data also confirmed to the monogenic hypothesis of segregation in F_2. Based on the forced inoculated and natural conditioned of F_2, it can be concludes that YMV is controlled by a single recessive gene and, therefore, simply inherited.

The marker CYR1 produced an allele in resistant bulk and was absent in susceptible bulk behaved as dominant, either with presence or absence of allele (Basak et al. 2004; Kundagrami et al. 2009; Srimathy et al. 2012). Amplified fragments were generated using combinations of both (RGA22F2/RGA24R2) the RGA primers, of which one fragments was identified displaying consistent polymorphism with respect to

Table 2. Segregation of YMV reaction on forced inoculated F_2 individuals

Cross	No. of plants	YMV reaction		Expected ratio	d.f.	χ^2	P value
		Susceptible	Resistant				
Keonjhar Local x OBG-31	230	177	53	3:1	1	0.469	<0.05 (3.84)
				15.1	1	110.7025	>0.05 (3.84)

Table 3. Segregation in F_2 population for YMV reaction under natural conditions

Cross	No. of plants	YMV reaction		Expected ratio	d.f.	χ^2	P value
		Susceptible	Resistant				
Keonjhar Local x OBG-31	298	233	65	3:1	1	1.614	<0.05 (3.84)
				15.1	1	126.8450	>0.05 (3.84)

disease reaction phenotypes i.e., amplified polymorphic fragment of 1236 bp in resistant lines (Fig. 1).

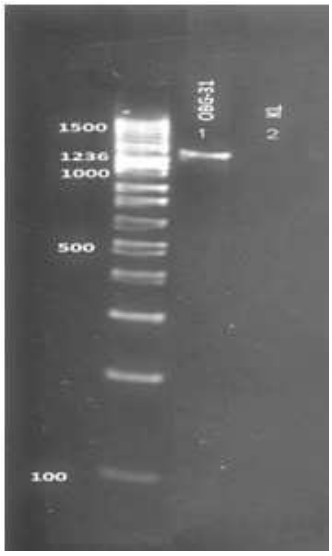

Fig. 1. PCR amplification of a single 1236 BP band in tolerant parent OBG-31. The band is absent in the susceptible parent Keonjhar Local. Molecular weight marker-100bp ladder. KL = Keonjhar Local

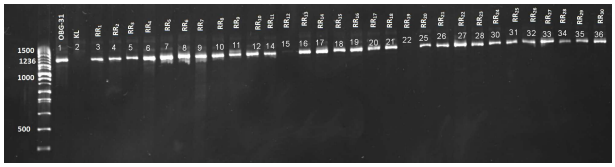

Fig. 2. Detection of PCR amplified CYR1 marker, linked with YMV resistance in selected phenotyped individuals in F_2 segregating population

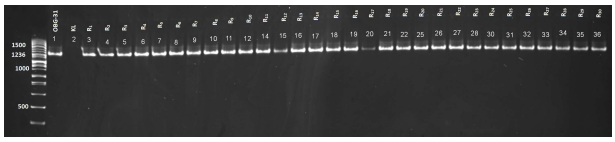

Fig. 3. Detection of PCR amplified CYR1 marker, linked with YMV resistance in phenotyped resistant members of F_3 families. RGA 22 F2/ RGA 24 R2 primer M = 100 bp 1.5 kb molecular weight marker

In this experiment, YMV resistance of *V. mungo* is controlled by a single recessive gene as determined by classical genetic analysis, which was confirmed by the linked DNA marker of 1236bp named 'CYR1'. The 1236 bp DNA marker co-segregated with the YMV resistance individuals of F_2 population (Fig. 2) and F_3 families (Fig. 3) confirming its linkage with YMV resistance. The present study demonstrated that the marker is efficient and ubiquitous for genotyping of YMV reaction. Breeders could even screen high yielding heterozygous susceptible lines for advance breeding or gene pyramiding. The tightly linked marker developed in this study will be of use in marker assisted selection and will hopefully aid in the development of resistant cultivars in relatively shorter time span.

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