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Validation of DNA Markers Linked to MYMV Resistance in Mungbean (*Vigna radiata* (L). R. Wilczek)

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ABSTRACT

Mungbean yellow mosaic virus (MYMV) is the most important disease of mungbean causing significant yield loss. Present investigation was carried out to identify molecular marker linked with MYMV resistance gene by investigating F_5 recombinant inbred lines (RILs) developed from the cross between Meha (resistant) and GM-4 (susceptible) at hot spot with GM-4 as an infector. The RILs segregated in 1R:1S ratio in the field screening indicating that the MYMV resistance gene inherited as a monogenic trait and MYMV resistance is under monogenic control. Out of 124 primers studied including RAPD, SSR, SCAR and RGA primers, only one primer RGA pair 1F-CG/RGA 1R amplified a single 445 bp band in the genotype Meha (resistant parent) and MYMV resistant bulk which was absent in GM-4 (susceptible parent) and MYMV susceptible bulk. Same amplicon was detected in individual samples of F_5 RILs constituting the MYMV resistant bulk indicating that this RGA was linked to MYMV resistance. This marker can be used in screening mungbean genotypes for resistance to MYMV disease.

Key words: *Recombinant Inbred Lines, Mungbean Yellow Mosaic Virus, Resistant Gene Analogue.*

INTRODUCTION

Mungbean (*Vigna radiate* (L.) R. Wilczek, 2n=22) is an important self-fertilizing pulse crop, ranks third in production after chickpea and pigeon pea[3]. The estimated genome size of mungbean is 579 Mb[5]. White fly transmit mungbean yellow mosaic virus (MYMV), a member of family Geminiviridae, has emerged as a great threat in a wide range of legume crops including mungbean. Common approach to control spread of MYMV is by limiting the vector population using insecticides, which is mostly ineffective under severe whitefly infestations.

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The most efficient, eco-friendly and economic approach to reduce damage due to mosaic disease in mungbean is the use of host resistance. Inheritance pattern of MYMV resistance varies from a single recessive gene[12,9,2], two recessive genes[1], complementary recessive genes[11] and a dominant gene[10] depending upon the source of resistance and genetic background. Identification of MYMV resistance source as well as screening of advanced generations segregating for resistance is difficult task as development of epiphytotic condition depends upon vector population and environmental conditions. Generally, screening under hot spot is considered as an efficient method for evaluation of mungbean genotypes for MYMV resistance. However, there are chances of disease escape under hot spot. Marker assisted selection is an efficient method to screen genotypes for MYMV resistance as it eliminates the need of epiphytotic condition.

Different DNA markers linked with MYMV resistance gene have been reported in black gram and mungbean[2,14,6]. The markers identified using one source of MYMV resistance gene may not work for another source, as different sources may have different MYMV resistance gene as revealed by inheritance studies. Recombinant Inbred Lines (RILs) are mapping population of choice, as they give better mapping resolution due to higher number of recombination events during their development. Ignoring rare residual heterozygote plant, F_5 RILs are almost stabilized population following statistical and independent segregation in accordance with 1:1 ratio. Therefore, the present study was aimed at validation of reported molecular markers linked with MYMV resistance using F_5 RILs.

MATERIAL AND METHODS

Mapping population for identification of marker linked to MYMV resistance was developed from the cross between the susceptible variety GM-4 as female parent and resistant variety Meha as a pollen parent. F_5 RILs were developed from F_2 population of this cross by single seed descent method. Total 165 RILs (F_5) and parents were screened in summer, 2017 under hot spot condition at College Farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat. After every two rows, one row of highly susceptible genotype GM-4 was grown as an infector. All recommended cultural practices were followed except insecticide spraying to build up white fly population. For screening of RILs against MYMV infection, the disease-rating scales (0–9) were used to score the disease infection according to Singh *et al.*[13] Disease reaction was scored when 90% of the infector rows showed MYMV incidence under field conditions. The chi-square test was performed to determine the goodness of fit of observed segregation for MYMV reaction in RILs.

Genomic DNA was isolated from leaves of parents and RILs according to the method described by Doyle and Doyle[4] with some modifications. DNA was quantified by taking the reading at 260nm and 280nm absorbance by Nano-drop (Thermo, USA). Total 124 primers including 100 RAPD primers, 12 cowpea SSR markers, 6 soybean SSR markers, 1 black gram ISSR-SCAR, 3 RGA markers and 2 RAPD-SCAR (mungbean and black gram) were used to detect polymorphism between parents Meha and GM-4. Bulk segregant analysis according to Michelmore *et al.*[7] was used to identify linked marker. Equal amount

of DNA was pooled from each of the 10 resistant and 10 susceptible RILs to create resistant and susceptible bulks, respectively. These pooled bulks along with the parental DNA samples were used for marker analysis. PCRs were performed in 25 µl volume containing 100 ng of template DNA, 3 units of Taq DNA polymerase (Bangalore Genei Ltd., Bangaluru, India), 2.5 mM of dNTPs, 0.2 µM primers in a 1× PCR Taq buffer containing $MgCl_2$. The amplification was carried out by Bio-Rad thermal-cycler. PCR conditions include 94°C of 5 min. for initial denaturation followed by 35 cycles consisting each of a denaturation step for 1 min at 94°C, an annealing step for 45 sec at 40°C, an extension step for 1 min at 72°C and the final extension for 10 min at 72 °C. Amplified products were separated by 2.0 % agarose gel electrophoresis at 80 V. The gels were stained with ethidium bromide and visualized on a digital gel-documentation and image analysis system (Bio-Rad).

RESULTS AND DISCUSSION

In order to validate the marker associated with MYMV resistance, RILs obtained from a cross Meha x GM-4 were phenotyped as resistant and susceptible based on the field evaluation by using rating scale. Parents Meha and GM-4 showed uniform expression of resistance and susceptibility, respectively. Infector rows GM-4 were heavily (90–100%) infected, while resistant parent Meha remained uninfected till maturity. RIL population showed various levels of reactions *viz*., resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on disease score[13]. (Table 1)

The screening of RILs at MYMV hot spot in summer season resulted in precise phenotyping for MYMV reaction. Heavy incidence and the uniform MYMV reaction of these RILs could be attributed to the high humidity and favourable temperature in summer season in Navsari Agricultural University, Navsari for conductive growth and development of MYMV vector whiteflies and also due to availability of the inoculums throughout the year.

Table 1: Response of RILs to MYMV infection

Disease reactions	Rating scale	Percentage of plants foliage affected	Reaction of RILs	Final classification of F_5 RILs on the basis of MYMV infection
Resistant	1	No visible symptoms (or) minute yellow specks covering 0.1–5.0% of the leaf area	78	85 (RILs resistant)
Moderately resistant	3	Mottling of leaves covering 5.1–10.0% of the leaf area	7	
Moderately susceptible	5	Mottling and yellow dis-colouration of 10.1-25.0% of the leaf area	6	80 (RILs susceptible)
Susceptible	7	Mottling and yellow discoloration of leaves on 25.1–50% of the leaf area	37	
Highly susceptible	9	Yellow mottling on more than 50% and up to 100% of the leaf area	37	

Fig. 1: Field screening of individuals F_5 RILs by using infector row (GM-4)

The RILs with resistant and moderately resistant reactions showed consistent reaction, either R or MR, while the RILs with MS, S and HS reactions were either S or HS. Hence, RIL population was grouped into two major categories *viz*., resistant (R) and susceptible (S). Out of 165 RILs, 85 lines manifested resistant reaction, while 80 showed susceptible reaction. The RIL population showed a good fit with 1:1 ratio for disease resistance and susceptible reaction (Table 2) revealing monogenic biallelic inheritance of MYMV resistance. Good fit of RILs in 1:1 ratio for resistance and susceptibility also showed the suitability of the F_5 RIL population for molecular tagging. Monogenic inheritance for MYMV resistance has also been reported in mungbean[12,9,2] and black gram[6].

Table 2: Chi-square test for segregation of disease resistant reaction in F_5 RIL population

RILs (F_5)	Total plants	Disease Resistant Reaction				Ratio R:S	χ^2
		Observed		Expected			
		Resistant	Susceptible	Resistant	Susceptible		
GM-4 × MEHA	165	85	80	82.5	82.5	1:1	0.151 (ns)

Out of 124 primers used to survey parental polymorphism between parents GM-4 (susceptible) and Meha (resistant), only one RGA primer pair 1F-CG/RGA 1R was able to deduce polymorphism with an allele size 445 bp (Fig.2,3). The RGA marker was present in the resistant parent, bulk and all 10 individual samples of resistant RILs, while absent in susceptible parent, bulk and all 10 individual samples of susceptible RILs (Fig. 2, 3). Basak *et al*.[2] also found linkage of this RGA marker with MYMV resistance in blackgram. Meha is a very popular variety of mungbean and has been developed from the inter-specific cross of *Vigna radiata* (Pant moong-2) and *Vigna mungo* (AMP-46)[8]. The results are in

accordance with the lineage of mosaic resistance in Meha from blackgram.

Lack of polymorphism between two parents observed in present study may be attributed to inherent low genetic and morphological variability present in mungbean crop. This happens when released varieties/elite genotypes are screened for polymorphism. Due to common breeding objectives, most of the loci governing seed yield and its attributes are almost identical leaving very few loci with sequence differences which are very difficult to detect. Detection of few loci in given genotypes demands genome wide survey using NGS (Next Generation Sequencing) based marker detection, validation and utilization for fine mapping and molecular dissection of any morphological trait.

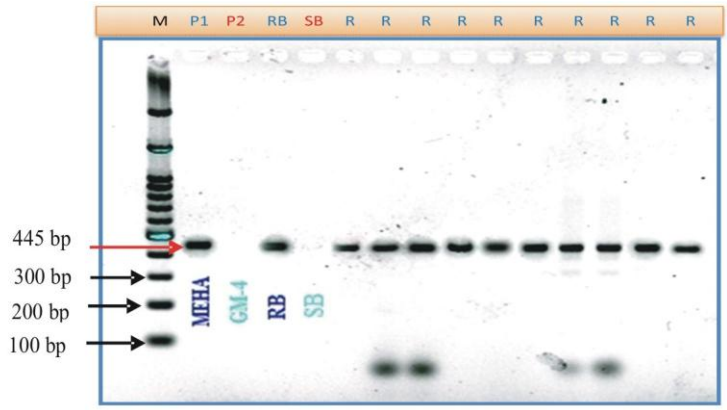

Fig. 2 PCR amplification of RGA marker in two parents, resistant bulk, susceptible bulk and ten individuals resistant F_5 RILs of mungbean.

Lane M= 100 bp ladder, P_1= MEHA (Resistant), P_2= GM-4 (Susceptible), RB= Resistant Bulk, SB= Susceptible Bulk, R= Individual F_5 Resistant RILs

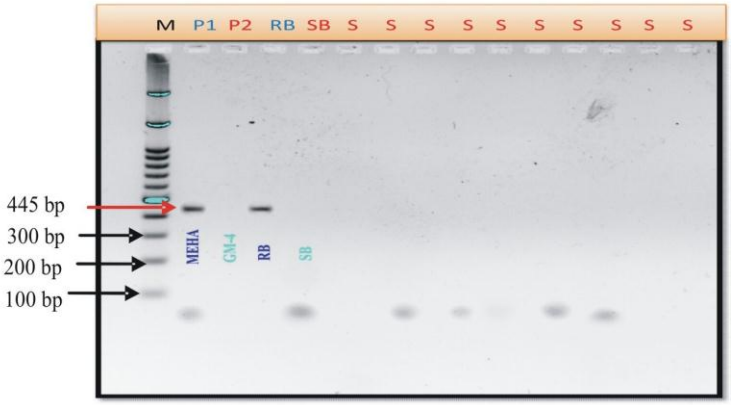

Fig. 3 PCR amplification of RGA marker in two parents, resistant bulk, susceptible bulk and ten individuals susceptible F_5 RILs of mungbean

Lane M= 100 bp ladder, P_1= MEHA (Resistant), P_2= GM-4 (Susceptible), RB= Resistant Bulk, SB= Susceptible Bulk, S= Individual F_5 Susceptible RILs

Mungbean is one of the important pulse crops of India. MYMV is the major production constraint limiting mungbean yield sustainability. Therefore, there is an urgent need to develop varieties with resistance to MYMV with greater yield stability. Using conventional methods, selection of the MYMV-resistant genotypes depend on the field screening for more than one year or season. Further, the inconsistencies in scoring disease reaction may affect the introgression of MYMV resistance into elite mungbean genotypes.

CONCLUSION

The RGA marker validated in this study may hasten breeding programme aimed at introgression of resistance gene in high yielding elite cultivars. However, efficiency of this molecular marker is to be checked using linkage mapping. Amplicon sequencing and homology search with BLAST (Basic Local Alignment Search Tool) may

provide better insight into functional role of this locus in MYMV resistance. Available mungbean genome sequence information may also be utilized to identify and characterize this RGA locus for development of co-dominant marker as well as for molecular dissection of MYMV resistance in a mungbean.

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