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IDENTIFICATION OF RAPD MARKERS LINKED TO MYMV RESISTANCE IN MUNGBEAN (*VIGNA RADIATA* (L). WILCZEK)

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ABSTRACT

The present study was undertaken to identify random amplified polymorphic DNA (RAPD) marker associated with mungbean yellow mosaic virus (MYMV) resistance in mungbean (*Vigna radiata* (L). Wilczek) by employing bulk segregant analysis in recombinant inbred lines (RILs). Out of 20 random decamers, only ten primers *viz;* OPA-03, A-06, A-03, OPA-09, A-09, OPB 7, UBC-391, OPC-08, UBC 499 and A-04 showed polymorphism between parents Chinamung and BL 849. Out of these ten primers only one primer *i.e.* UBC 499 amplified a single 700 bp band in the genotype BL 849 (resistant parent) and MYMV resistant bulk which was absent in Chinamung (susceptible parent) and MYMV susceptible bulk. PCR amplified 700 bp marker band was detected in individuals of F_5 RILs constituting the MYMV resistant bulk indicating that primer UBC 499 was linked to MYMV resistance. This marker can be used in screening mungbean genotypes for resistance to MYMV disease.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is a short duration legume crop cultivated primarily for its dry seeds. India alone accounts for 65% of the world acreage and 54% of the world production. It became third largest pulse crop in the country which is grown in an area of 2.53 m ha with 0.86 mt production and productivity of 340 kg/ha (Anonymous, 2010). Mungbean yellow mosaic virus (MYMV) is the most prevalent and important disease to occur on mungbean. This disease has been found to be widely distributed in India causing enormous losses in the production of several leguminous crops (Chenulu and Verma, 1988). MYMV disease can be controlled by chemical, cultural and genetic methods. Nevertheless, if host resistance alone is sufficient to check the disease, then it is to be preferred over other methods because it is the least expensive and has no adverse environmental effects (Selvi *et al.,* 2006). Selection of resistant genotype in conventional methods relies on field or glass house screening and it requires evaluation at 'hot spot' regions. Hence, the screening process is complex and time consuming.

The use of molecular markers for resistance genes is particularly powerful as it removes the delays in breeding programmes associated with the phenotypic analysis (Karthikeyan *et al.,* 2012). Nowadays, this is possible due to availability of many kinds of markers viz. RAPD, AFLP and SSR that are used for genotyping purposes. RAPD is one among the various molecular markers that are being used to screen resistant genes. This RAPD technique developed by Williams *et al.* (1990) is reliable, faster and easier for exploiting genetic polymorphism within and among species and populations. RAPD markers have been already used successfully in many crops for mapping genes, as RAPD markers are more rapidly and easily detectable than RFLP markers (Welsh and McClelland, 1990; Williams *et al.,* 1990). The RAPD marker had also been used effectively for tagging MYMV resistant genes in mungbean (Selvi *et al.,* 2006; Karthikeyan *et al.,* 2012). Lambrides *et al.* (1999) reported RAPD primer OPAJ20 to be distantly linked with the MYMV resistance. Two MYMV-resistance marker loci, YR4 and CYR1, were identified in mungbean and urdbean using R gene homologue sequences (Maiti *et al*., 2011). In molecular marker assisted selection, once a gene of interest is tagged with molecular marker, selection for that gene can be made based on the marker rather than the phenotype of individual which embodies the gene. Moreover selection can be performed in the seedling stage itself by using molecular markers (Tanksley, 1983; Beckmann and Soller, 1986). Thus, the present investigation was carried out with an objective to identify RAPD marker linked to MYMV resistance.

MATERIALS AND METHODS

The experiment was carried out at the University of Agricultural Sciences, Bangalore, Karnataka, India. Chinamung and BL-849 were selected as parents for developing RILs based on field and glass house screening experiment for MYMV resistance (Marappa and Savithramma, 2008). Chinamung

was found to be susceptible to MYMV disease whereas, BL-849 was found to be resistant to MYMV disease.

Ninety three F_5 recombinant inbred lines (RILs) of the cross Chinamung x BL-849 developed through single seed descent method. These RILs and their parents were evaluated in randomized complete block design (RCBD) with three replications. All the recommended cultivation packages were followed except spraying of plant protection chemicals for buildup of whitefly population. Highly susceptible cultivar 'FV-Local' was used as spreader row for MYMV infection and was sown 14 days earlier to test materials. Every six rows of RILs were alternated with cultivar 'FV-Local'. The individuals in each F_5 family were screened for resistance to MYMV under field condition using 1-9 scale rating (Singh *et al.,* 1992). Lines which showed maximum resistance and maximum susceptibility to MYMV in all the 3 replications were used for bulk segregant analysis.

DNA from 10 highly resistant F_5 RILs, 10 highly susceptible F_5 RILs and their parents was extracted as per the Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987) with modification. Twenty RAPD primers given Table 1 were used to detect polymorphism between the MYMV susceptible parent Chinamung and resistant parent BL-849 (Table 1). Each 25 µL reaction mixture consisted of 50 ng of template DNA, 100 ng of RAPD primer, 200 µM of dNTPs, 2 U of *Taq* DNA polymerase and 1X Assay buffer (20 mM Tris-HCL pH 8.8, 10 mM KCl, 2.0 mM $MgCl_2$ 10 mM Ammonium sulphate, 0.1% Triton-X 100). Amplification was carried out on an Eppendorf mastercycler gradient PCR. The reaction was carried out at 94ºC for 2 min as predenaturation step. This was followed by 39 cycles of denaturation at 94ºC for one minute, primer annealing at 40ºC for one minute and primer elongation at 72ºC for 2 min. Additionally a final cycle allowed extension for 2 min at 72ºC. The amplified products were electrophoresed on 1.4% agarose gel at 75 V for 2 hr and photographed using Gel Documentation Unit.

Bulk segregant analysis (Michelmore *et al.,* 1991) was employed to identify RAPD markers linked to MYMV resistant gene of BL 849. Highly resistant bulk (positive bulk or B+) or highly susceptible bulk (negative bulk or B-) were constructed based on phenotypic data on *per cent* MYMV infection. Resistant bulk contained 9 individuals and susceptible bulk contained 10 individuals. An equal concentration of DNA from each individual was added to each pool. The primers which showed polymorphism between the parents were further used to screen resistant and susceptible bulks. The primers which showed polymorphism between positive bulk (B+) and negative bulk (B-) are said to be linked to MYMV resistance.

Table 1: List of RAPD primers used for Bulk segregant analysis in F_5 RILs of Chinamung x BL 849

SL.No.	Primer name	Primer sequence
1	A-01	5'CAGGCCCTTC3'
2	A-03	5'AGTCAGCCAA3'
3	A-04	5'AATCGGGCTG3'
4	A-06	5'GGTCCCTGAC3'
5	A-09	5'GGGATTCGCC3'
6	OP-03	5'CATAGAGCGG3'
7	OP-08	5'CTGGCTCAGA3'
8	OPA-03	5'GTCCATGCCA3'
9	OPA-05	5'GTGGTCCGCA3'
10	OPA-9	5'GGGTAACGCC3'
11	OPB-7	5'GGTGACGCAG3'
12	OPB-17	5'AGGGAACGAG3'
13	OPC-08	5'TGGACCGGTG3'
14	OPM-12	5'GGGACGTTGG3'
15	UBC-284	5'CAGGCGCACA3'
16	UBC-391	5'GCGAACCTCG3'
17	UBC-412	5'TGGCCCGGTG3'
18	UBC-431	5'CTGCGGGTCA3'
19	UBC-499	5'GGCCGATGAT3'
20	UBC-602	5'GCGAAGACTA3'

RESULTS AND DISCUSSION

The advent of DNA marker technology in the 1980s has dramatically enhanced the efficiency of plant breeding. On the other hand, efficiency of marker-aided selection in breeding programs depends on the strength of linkage between molecular markers and the target trait. Some of the extensively used mapping populations are F_2 population, double haploid lines (DHLs), near isogenic lines (NILs), recombinant inbred lines (RILs) and backcross inbred lines (BILs). Recombinant inbred populations derived from single seed descent method have many advantages over other mapping populations. RILs find wide usage in both breeding and molecular work owing to the various purposes they serve. Recombinant inbred lines are the breeding output from which good stabilized segregants can be directly used as breeding lines. They are the means of obtaining recombinants with desirable traits from both the parents involved in the cross. In RILs the early genetic variability realized in the population is maintained. RILs serve as a good mapping population and if the RILs are from wide crosses, the mapping and dissecting of the yield and resistant genes could be carried at any time, as the population revealing segregation in these traits in stabilized condition would be readily available whenever required for testing in various environments.

In the present investigation, an attempt was made to identify RAPD marker associated with MYMV resistance, using bulk segregant analysis in 93 F_5 RILs. Based on MYMV disease scoring (1-9 scale) following observations was made. Thirty nine lines which scored 1 with 0.1-5% infection were resistant, 18 lines scored 3 with 5.1-10% infection were moderately resistant, 27 lines having score of 5 with 15.1-25% infection were moderately susceptible, 8 lines having score of 7 with 25.1-50% infection were susceptible and one line scored 9 with 50.1-100% infection were highly susceptible to MYMV. F_5 RILs which showed maximum resistance and maximum susceptibility to MYMV in all the 3 replications were used for bulk segregant analysis.

One of the most time consuming requirements in marker development is the need to screen entire mapping population with every primer has been reduced by bulked segregant analysis (Michelmore *et al.,* 1991). The screening of contrasting bulks made from individuals of same phenotype of segregating population, suggests that testing the entire population is required only when polymorphisms between the bulks are detected. This result in saving considerable time particularly when used with PCR based technique such as RAPD markers.

A total of 20 RAPD primers were used for this initial survey

(Table 1). Out of these 20 primers only ten primers *viz;* OPA-03, A-06, A-03, OPA-09, A-09, OPB 7, UBC-391, OPC-08, UBC 499 and A-04 showed polymorphism between parents Chinamung and BL 849. These ten primers were used for bulk segregant analysis. Only one primer *i.e.* UBC 499 out of these ten primers exhibited polymorphism between both parents and the bulks. UBC 499 amplified a single 700 bp band in the BL 849 (resistant parent) and resistant bulk (B+) which was absent in Chinamung (susceptible parent) and susceptible bulk (B-).

To confirm that the polymorphism in DNA of bulks was not artifact, both the parents, bulks and individuals used to construct the bulks were examined with UBC 499. PCR amplified 700 bp marker band was detected in resistant parent (BL 849), resistant bulk and also in 9 individuals of F_5 RILs constituting the resistant bulk indicating that primer UBC 499 was linked to MYMV resistance. Similar attempts to find RAPD markers linked MYMV resistance were made by several researchers (Lambrides *et al*., 1999; Selvi *et al*., 2006; Karthikeyan *et al*., 2012). This UBC 499 can be used in screening mungbean genotypes for resistance to MYMV disease. SCAR (Sequence Characterized Amplified Region) represent an alternative to increasing the reproducibility of RAPD markers. UBC 499 can be converted into SCAR for increasing reproducibility and efficiency of marker for selection of MYMV resistant genotypes

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