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Full Length Research Paper

Identification of RAPD marker for the White Backed Plant Hopper (WBPH) resistant gene in rice

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The experimental material consisted of two parents Gurjari (white backed plant hopper resistant) and Jaya (white backed plant hopper susceptible) and their F2 progeny. The purpose of the study was the identification of RAPD (Random Amplified Polymorphic DNA) marker for white backed plant hopper (WBPH) resistant gene. The RAPD analysis was done group wise as well as combined for the bulk segregant analysis (BSA). For the BSA, of the total 50 random primers surveyed, a single linked primer, OPA 08, was identified. This primer generated 8-bands, one of which, OPA08-7, was putatively linked to resistant gene as was evident by its presence in almost all the resistant bulks and vice-versa. This band had molecular weight equal to 1219.38 bp and was found in resistant parent, Gurjari, and in almost all the resistant bulks (the four susceptible bulks revealed absence of the same band) indicating the band OPA08-7 as a marker for WBPH resistance among the screened rice genotypes.

Key words: Rice, random amplified polymorphic DNA, white backed plant hopper, resistant, susceptible, gene.

INTRODUCTION

The white backed plant hopper (WBPH), *Sogetella furcifera* (Horvath) (Hemiptera: Delphacidae) is an important pest of rice and has gained a status of serious pest for rice in Asia. It is widely distributed throughout South and South-east Asia, India, China, South Pacific Islands and the Northern part of Australia. Under favorable conditions, the insect can completely destroy rice plants, a condition known as hopperburn (Suenaga, 1963). This pest has also attained a place of major pest of paddy in Gujarat. The outbreak during 1991, 1992, 1993 and 1996 ruined the crop completely (Korat et al., 1999). Genetic analysis of resistant varieties has identified five genes for WBPH resistance. Four of these genes are dominant and designated as WBPH 1, WBPH 2, WBPH 3 and WBPH 5 while WBPH 4 is recessive (Angeles et al., 1981; Saini et al., 1982; Wu and Khush, 1985). Because of the high cost of insecticides and the problem of insecticide-induced WBPH resurgence (Nagata, 1979), varietal resistance is considered as the most promising and practical approach in the integrated control of this pest (Khan and Saxena, 1986).

The knowledge of the genetic architecture of rice is essential in genetic and breeding research. The classical genetic map is based on morphological mutant markers. Molecular markers, including DNA markers and isozymes, are discrete, codominant, non-deleterious and unaffected by the environment. Moreover, molecular markers are numerous and thus can cover the entire genome (McCouch et al. 1988; Tanksley et al., 1992; Saito et al., 1991). RAPD analysis is one of the important molecular techniques, which can be used for molecular characterization as well as to identify a linked marker for a resistant gene for various biotic and abiotic stresses.

MATERIALS AND METHODS

One week old rice seedlings were used for DNA extraction. The DNA was extracted by CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1990) with some modifications. The quantitation was carried out by UV-spectrophotometer. For the quantitation, the stock of genomic DNA was diluted to 1:50 in distilled water and optical density (O.D) was measured at 260 nm.

Quality test was done by running DNA on 0.8% agarose con-

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Table 1. Details about parents Jaya and Gurjari regarding origin, pedigree, and agricultural features.

Name of variety	Parentage	Year of notification	Duration (in days)	Ecosystem	Salient features
Jaya (IET-723)	T(N)1 x T-141	1969-78	130	Irrigated	Dwarf (82 cm), grains: long bold, AWP, white, susceptible to WBPH, BLB, SB, RTV, GM and resistant to blast, Yield: 50-60 Q/ha.
Gurjari (IET-10750)	Asha x Kranti	1999	150-160	Irrigated Medium	Grains: long bold and resistant to WBPH, blast.

Source: Directorate of Rice Development (Hyderabad).

taining 10 µl of ethydium bromide (1 mg/ml) per 100 ml of gel. 5 µl of genomic DNA from stock was mixed with 1 µl of Bromo-phenol blue (BPB) tracking dye and loaded on to the gel. The gel was run at 80 V for 1 h. The DNA bands were visualized under UV and scanned by gel documentation system. The O.D. was also measured at 280 nm. The DNA samples with the ratio of 1.7 to 1.8 at O.D. $_{260/280}$ were retained for DNA finger printing. The stock solution was diluted to the concentration of 30 ng/µl and used for further analysis. The genomic DNA was amplified using random primers OPA-1 to OPA-50. Polymerase chain reaction (PCR) was carried out in a reaction volume of 25 µl (Lanham et al., 2000). All the PCR products were carried out in 25 µl thin walled PCR tubes. PCR tubes containing reaction mixture were tapped gently and spin briefly at 10,000 rpm. The PCR amplification was carried out in eppendorf master cycler and subjected to PCR protocol. All the PCR products were run on 1.5% agarose containing 10 µl of ethydium bromide (1 mg/ml). Ten µl of PCR product was mixed with 2 µl of 6 x tracking dye and loaded on to the well. The gel was run at 80 V (constant) to separate the amplified bands. The standard DNA marker was also run along with the samples. The separated bands were seen under ultraviolet light, photographed by gel documentation system and analyzed by gene tool.

RAPD through bulk segregant analysis (BSA)

The two parents viz., Gurjari (Asha x Kranti), source of resistance for WBPH and Jaya (TN-1 x T-141) as susceptible one (Korat et al., 1999), were crossed to obtain F_2 generation. The information regarding parents, their pedigree, and agricultural features are given in Table 1. The parents and their F_2 generation were screened against WBPH at field conditions through natural infestation at Main Rice Research Station, Nawagam, which is considered to be a hot spot for WBPH incidence. Damage was recorded for parents and F_2's on the basis of standard evaluation system for rice at International Rice Research Station (Khan and Saxena, 1986).

The DNA was isolated from various resistant and susceptible bulks and pooled separately and afterwards subjected to RAPD analysis with 50 random oligomer primers.

RESULT

The F_2 population of a cross between Gurjari x Jaya, was subjected to RAPD analysis using BSA. F_2 generation produced from highly resistant parent, Gurjari, and highly susceptible parent, Jaya, comprised of the resistant as well as susceptible plants. Aliquots of DNA of each homozygous individual for one or other allele of the target gene were bulked together. Eleven homozygotes, resistant to WBPH and eight susceptible homozygotes along with parents were used for the bulk segregant analysis with RAPD and the screening was done under field condition against WBPH through natural infestation and the results are as shown in Table 2. Out of the total 50 random primers surveyed, a single linked primer, OPA 08 was identified. This primer generated 8-bands, one of which was putatively linked to resistant gene as was evident by its presence in almost all the resistant bulks and vice-versa.

DISCUSSION

RAPD through bulk segregant analysis employed for the F_2 population of a cross between WBPH resistant parent, Gurjari, and highly susceptible parent, Jaya, comprised of the resistant as well as susceptible plants. Out of the total 50 random primers surveyed, a single linked primer, OPA 08, was identified. This primer generated 8-bands, one of which was putatively linked to resistant gene, as was evident by its presence in almost all the resistant bulks and vice-versa. The marker to resistance against WBPH was designated as OPA08-7. This band had molecular weight equal to 1219.38 bp and was found in resistant parent Gurjari and in all the resistant bulks, that is, 10 out of 11 resistant bulks, except only one bulk, designated as R_4. On the other hand, the four susceptible bulks revealed absence of the same band indicating the band OPA08-7 as a marker for WBPH resistance among the screened rice genotypes.

Out of 7 susceptible bulks, three recorded the presence of the OPA08-7 band. The absence of OPA08-7 band in one resistant bulk and its presence in three susceptible bulks may be attributed to the incomplete linkage of the linked marker with the gene of resistance for WBPH. As the recombination takes place due to crossing over in the subsequent generations, the marker OPA08-7 might have separated from the resistant gene resulting in the presence of OPA08-7 band in three susceptible bulks while absent in one resistant bulk as shown in Figure 1. Selvi et al. (2002) also reported similar results while conducting an experiment for identification of molecular markers for yellow stem borer (*Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae)). They subjected an F_2 population developed using parents contrasting in their reaction to yellow stem borer resistance, to RAPD analysis,

Table 2. Distinct bulks (resistant and susceptible) discriminated with primer OPA08.

S/N	F_2 Generation	Presence / Absence (OPA08-7)	WBPH score under field study
1	Gurjari	+	0
2	Jaya	-	9
3	R1	+	0
4	R2	+	0
5	R3	+	0
6	R4	-	3
7	R5	+	0
8	R6	+	0
9	R7	+	0
10	R8	+	0
11	R9	+	0
12	R10	+	0
13	R11	+	0
14	S1	-	9
15	S2	-	7
16	S3	-	9
17	S4	+	5
18	S5	+	5
19	S6	-	9
20	S7	+	5
21	S8	-	9

0 = Highly resistant; 1 = resistant; 3 = moderately resistant; 5 = moderately susceptible; 7 = susceptible; 9 = highly susceptible.

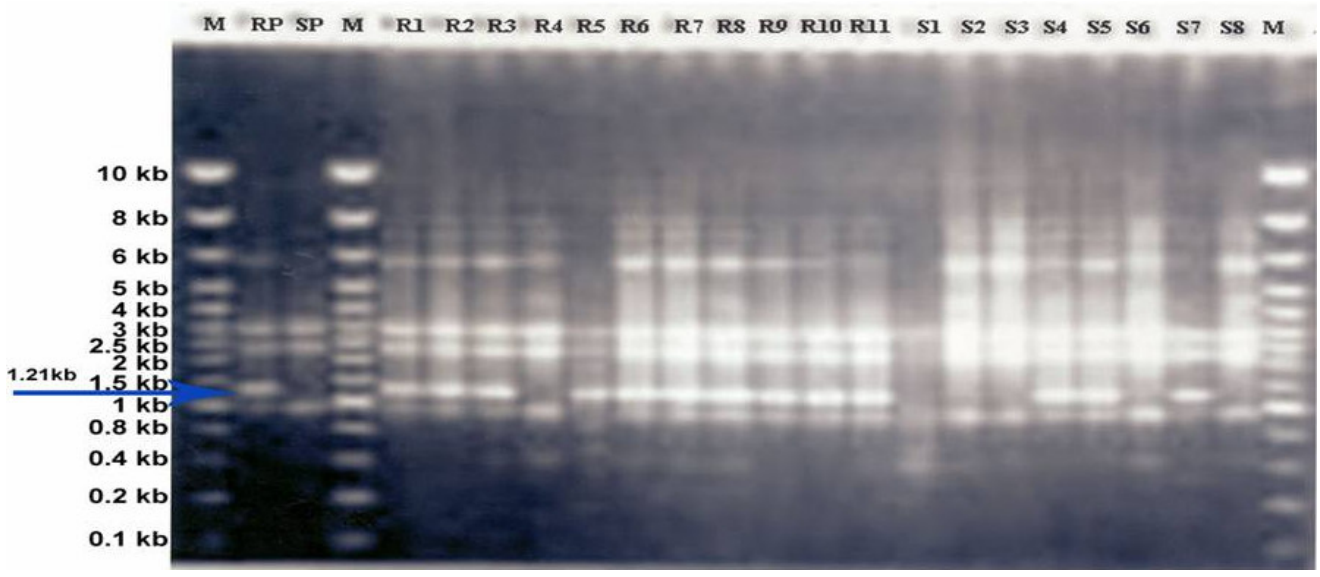

Figure 1. RAPD based bulk segregant analysis in rice against WBPH using F_2 generation of Gurjari X Jaya. M, Marker; RP, resistant parent; SP, susceptible parent; R1 – R11, resistant bulks; S1 – S8, susceptible bulks.

in conjunction with bulked segregant analysis. They identified four phenotype-specific RAPD markers. The markers C1320 and K6695 were linked with resistance and AH5660 and C41300 with susceptibility against yellow stem borer.

Jeon et al. (1999) employed RAPD and restriction frag-

ment length polymorphism (RFLP) analysis using BSA for DNA markers linked to resistance genes. An F_2/F_3 population from a Gayabyeo x Nagdongbyeo cross was developed and evaluated for brown planthopper (BPH) resistance. Three bulked DNAs from two groups of homozygous BPH resistant (each for BPH-1 and the other unknown gene) and homozygous susceptible F_2 plants were analyzed. One primer, OPD-7 yielded a 700-bp fragment that was present in Gayabyeo and resistant F_2 plants but absent in Nagdongbyeo and susceptible F_2 plants. The presence of 700-bp fragment in Gayabyeo and F_2 resistant bulks and its absence in Nagdongbyeo and F_2 susceptible bulks confirms the co- segregation of this marker with BPH-1.

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