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RAPD and RFLP mapping of the bacterial blight resistance gene *xa-13* in rice

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Abstract Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the most serious diseases of rice. The recessive gene *xa-13* confers resistance to Philippine race 6 of *Xoo*. To tag *xa-13* with molecular markers, RAPD analysis was conducted with the combined use of near-isogenic lines and bulked segregant analysis. From the survey of 260 arbitrary 10-nucleotide primers, one primer (OPAC05) was detected to amplify specifically a 0.9-kb band from the DNA of susceptible plants. The distance between the RAPD marker OPAC05-900 and *xa-13* was estimated to be 5.3 cM. The RAPD marker was then mapped on chromosome 8 using a mapping population of doubled haploid lines derived from the cross of IR64/Azucena. The linkage between RFLP markers and the RAPD marker was analyzed using an F_2 population of 135 plants derived from a cross between a near-isogenic line for *xa-13*, IR66699-5-5-4-2, and IR24. No recombinants were found between RZ28 and CDO116 and their distance from *xa-13* was estimated to be 4.8 cM. RG136 was located at 3.7 cM on the other side of *xa-13*. The mapping of *xa-13* with closely linked DNA markers provides the basis for marker-aided selection for rice improvement.

Key words Molecular mapping · *Xanthomonas oryzae* · Resistance gene · Marker-aided selection · *Oryza sativa*

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Introduction

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the most serious diseases of rice. The pathogen varies in its virulence to different rice varieties. Six races of the bacterium have been identified in the Philippines. Each race has specific virulence to varieties with different resistance genes, showing a gene-for-gene relationship in the host-pathogen interaction (Mew 1987; Vera Cruz and Mew 1989). To-date, at least 18 genes conferring resistance to specific races, or clusters of races of *Xoo* have been identified through classical genetic analysis (Ogawa 1987; Kinoshita 1991). The recessive gene *xa-13* confers resistance to Philippine races of *Xoo* (Ogawa et al. 1987).

DNA markers provide a unique opportunity to map genes of agronomic importance. Several RFLP linkage maps have been developed in rice (McCouch et al. 1988; Saito et al. 1991; Causse et al. 1994; Kurata et al. 1994). One of the main uses of the RFLP maps is to locate markers linked to genes of interest. Random amplified polymorphic DNA (RAPD) has been used to quickly identify markers within a genomic region of interest using near-isogenic lines (Williams et al. 1990; Klein-Lankhorst et al. 1991; Martin et al. 1991; Paran et al. 1991; Penner et al. 1993). Bulked segregant analysis has been used for focusing on regions of interest or areas sparsely populated with markers (Michelmore et al. 1991). The RFLP map developed from a doubled haploid population in rice is a useful tool in locating RAPD markers on the map (Huang et al. 1994).

Recently, several bacterial blight resistance genes in rice have been mapped with RFLP markers and RAPD markers (McCouch et al. 1991; Ronald et al. 1992; Yoshimura et al. 1992). This information is useful in facilitating breeding efforts aimed at developing more durable forms of resistance to the bacterial blight. In the present report, mapping of the *xa-13* gene will be described. RAPD analysis was used as an initial step to

identify potential markers linked to the gene. Then the RAPD marker was located on a chromosome using a mapping population of doubled haploid lines. The linkage between *xa-13* and RFLP markers was investigated using an F_2 population.

Materials and methods

Plant materials

The near-isogenic line for *xa-13* (IR66699-5-5-4-2), the donor cultivar for the resistance gene (Long Grain, IRRI Acc. 35023), and the susceptible recurrent parent (IR24) were used in this study. The near-isogenic line was developed from the cross between IR24 and an F_3 plant derived from the cross TN1/Long Grain, backcrossed six times to the recurrent parent IR24 followed by selfing. The F_2 population of 135 plants was produced by crossing IR66699-5-5-4-2 and IR24. Another isogenic line, IRBB5, homozygous for *xa-5*, a recessive gene for resistance to bacterial blight, was also used.

Reaction to bacterial blight

The parents and the F_2 population were grown in the screenhouse. At maximum tillering stage, the plants were inoculated with race 6 (PXO 99) of *X. oryzae* using the leaf clipping method (Kauffman et al. 1973). Plant reaction to the disease was recorded 14 days after inoculation. Plants were classified either resistant (R) or as susceptible (S).

DNA extraction, restriction digests, electrophoresis, and Southern analysis

Rice DNA was prepared from fresh-frozen leaf tissues using the Dellaporta et al. (1983) method. Total genomic DNAs of IR24, IR66699-5-5-4-2, Long Grain, resistant bulk and susceptible bulk were digested with 12 restriction enzymes (*Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, *Sac*I, *Sca*, *Xba*I and *Xho*I). The resistant bulk and the susceptible bulk were prepared by mixing DNA from ten resistant plants and ten susceptible plants of the F_2 population. For the F_2 progeny filters, only those enzymes giving positive results were used. Electrophoresis and Southern analysis were conducted according to standard protocols (Sambrook et al. 1989).

RAPD analysis

A total of 260 arbitrary 10-nucleotide primers (Operon 10-mer Kits, Operon Technologies, Inc.) were surveyed for their ability to amplify the polymorphic band among parents and the resistant and susceptible bulks according to the RAPD method (Williams et al. 1990; Martin et al. 1991). The positive polymorphic band was isolated and purified from gels and was radioactively labeled using the random primer method (Feinberg and Vogelstein 1983). The linkage between the RAPD marker and the *xa-13* locus was analyzed using the specific band as a probe to survey filters of 135 F_2 plants. The RAPD marker was located on a chromosome using the mapping population of 135 doubled haploid lines generated from the cross of IR64/Azucena (Huang et al. 1994).

Linkage between *xa-13* and RFLP markers

Ten RFLP markers on the RAPD marker region were selected to survey IR66699-5-5-4-2, Long Grain, IR24, and the resistant and the susceptible bulks. The RFLP markers showing polymorphism between resistant and susceptible lines were used to analyze their linkage to *xa-13* using the F_2 population of 135 plants.

Computer analysis

Linkage values were calculated using the MAPMAKER program (Lander et al. 1987). Distances between markers are presented in centiMorgans derived using the Kosambi function (Kosambi 1944). The distances shown on the genetic map were calculated from multipoint analysis.

Results and discussion

Segregation of resistant and susceptible plants in the F_2 population

F_2 plants derived from the cross IR66699-5-5-4-2/IR24 were classified either as resistant (R) or as susceptible (S) according to the reaction to race 6 of *Xoo*. Of the 135 F_2 plants, 108 plants were susceptible and 27 plants were resistant. The segregation of susceptible and resistant plants in the F_2 population agreed with a 3:1 ratio (Table 1). The results confirm that the *xa-13* gene conferring resistance to race 6 of *Xoo* is recessive.

Identification of the RAPD marker linked to the *xa-13* locus

RAPD analysis was used as an initial step for mapping the *xa-13* gene. From the survey of 260 random primers, we identified one primer (OPAC05) that amplified a polymorphic band between the resistant plants (resistant parent, donor, and resistant bulk) and susceptible plants (susceptible parent and susceptible bulk). A 0.9-kb band was amplified from DNA of susceptible parent IR24 and the susceptible bulk. A trace of the band was also detected in the resistant bulk, but it could not be detected in the resistant parent and the donor (Fig. 1). When DNAs from each of the ten resistant and ten susceptible plants in the F_2 bulks were amplified with primer OPAC05, the 0.9-kb band was found in all susceptible plants but only in two of the resistant ones.

To confirm the linkage between *xa-13* and the RAPD marker (OPAC05-900), the specific band was isolated from gels and used as a hybridization probe to blots of

Table 1 The chi-square test for the ratios of the scores for the phenotype of *xa-13* and genotypes at the marker loci in the F_2 population of IR66699-5-5-4-2/IR24

Locus	Score[a]			Total	χ^2 (ratio)
	1	2	3		
xa-13	108 (S)		27 (R)	135	1.68 (3:1)
OPAC05-900	34	75	23	132	4.29 (1:2:1)
RG136	33	76	24	133	3.93 (1:2:1)
RZ28	36	67	23	126	3.19 (1:2:1)
CDO116	36	67	23	126	3.19 (1:2:1)

[a] 1 = homozygote for the IR24 allele, 2 = heterozygote, 3 = homozygote for the IR66699-5-5-4-2 allele, S = susceptible for race 6 of *Xoo*, R = resistant for race 6 of *Xoo*

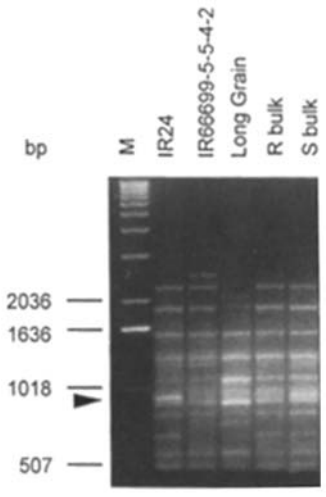


Fig. 1 Electrophoresis of random primer OPAC05 amplication products showing a PCR polymorphism. The ethidium bromide-stained bands represent genomic sequences amplified on DNA isolated from IR24, IR66699-5-5-4-2, Long Grain, resistant (*R*) bulk, and susceptible (*S*) bulk. A 0.9-kb product indicated by the *arrow head* was amplified from the DNA of IR24 and the susceptible bulk

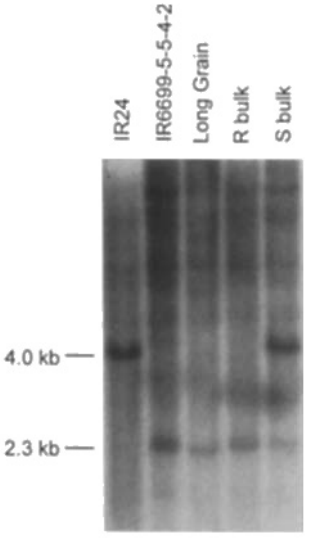


Fig. 2 Blot analyses of the specific band. The specific PCR product, OPAC05-900, was excised from the gel and used as a hybridization probe to blots of genomic DNA digested with *Xba*I. A 4.0-kb band is present in the DNA of IR24 and the susceptible (*S*) bulk, and a 2.3-kb band is present in the DNA of IR66699-5-5-4-2, Long Grain, resistant (*R*) bulk and susceptible (*S*) bulk

genomic DNA digested with 12 restriction enzymes. Polymorphic DNA fragments were detected between the susceptible parent (IR24) and the resistant plants (IR66699-5-5-4-2, Long Grain, and resistant bulk). The susceptible bulk showed both bands because it contained some heterozygotes (Fig. 2). From the survey of 132 plants in the F_2 population of the cross IR66699-5-5-4-2/IR24 using the OPAC05-900 probe, it was found that the segregation of homozygotes for the IR24 allele, heterozygotes, and homozygotes for the IR66699-5-5-4-2 allele at the OPAC05-900 locus in the F_2 population was in the ratio 1:2:1 (Table 1). The distance between OPAC05-900 and the *xa-13* locus was estimated to be 5.3 cM.

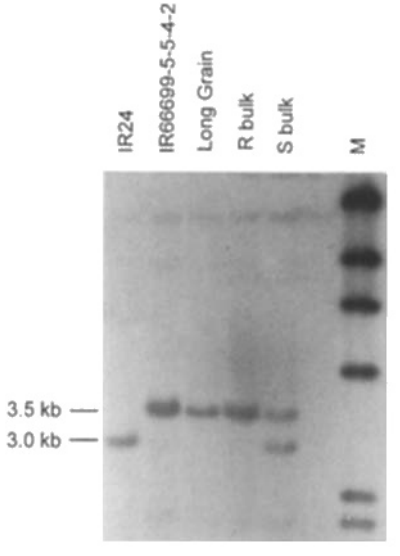
Location of the RAPD marker

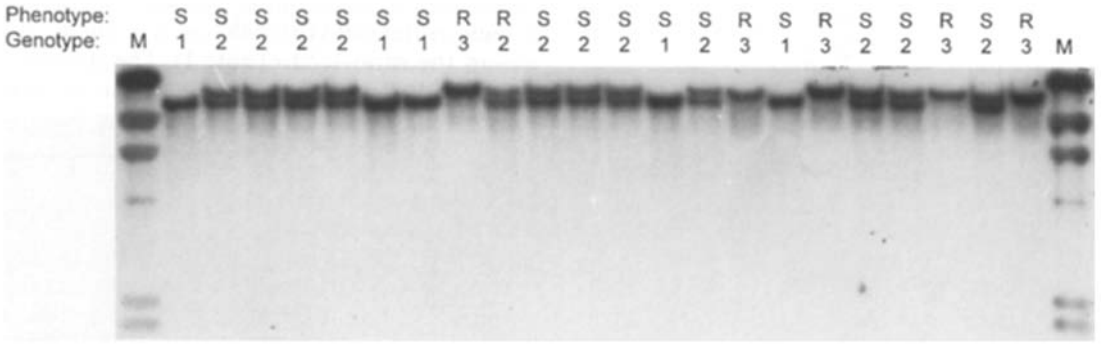
The OPAC05-900 probe was then used to survey filters containing *Sca*I-digested DNA from the mapping population of 135 doubled haploid lines derived from the cross of IR64/Azucena after the parental survey of DNA polymorphism. The genotypes for the restriction fragment from the 135 doubled haploid lines were scored. Scores were incorporated to the data pool on the RFLP map (Huang et al. 1994). The RAPD marker was then located on the RFLP map using the MAPMAKER program (Lander et al. 1987). The results indicated that the RAPD marker was located between RFLP markers RZ66 and CDO99 on chromosome 8 (see Fig. 5 A).

Mapping of *xa-13*

Ten RFLP markers on the OPAC05-900 region were selected to hybridize the DNA blots of IR66699-5-5-4-2, Long Grain, IR24, and the resistant and susceptible bulks. With the use of 12 restriction enzymes, DNA polymorphisms between the resistant lines and the susceptible lines were detected for RZ28 and CDO116 when the DNA was digested with *Xho*I. RG136 showed polymorphisms when DNA was digested with *Dra*I, *Bgl*II, *Eco*RI, *Sac*I, *Xba*I and *Xho*I (Fig. 3). The other seven markers (RZ926, CDO595, RZ66, RZ649, RZ572, CDO99 and RZ997) showed monomorphism. The three

Fig. 3 Autoradiograph showing hybridization pattern of RG136 in the DNA digested with *Dra*I





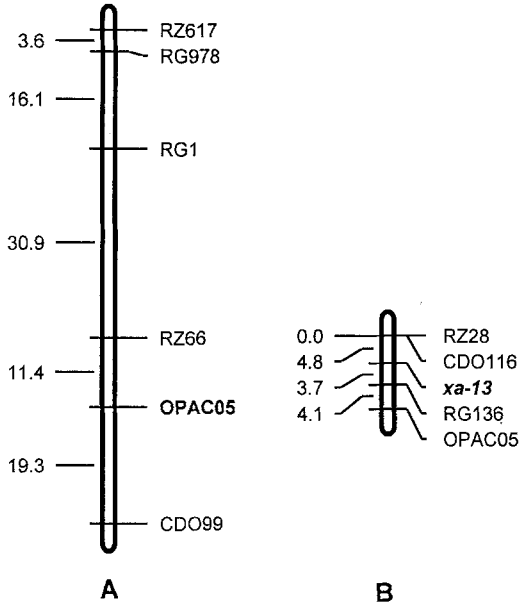


Fig. 5A, B RFLP maps of chromosome 8 of rice. The distance between markers is shown in centiMorgans on the left. **A** A map of chromosome 8 developed using the mapping population of doubled haploid lines derived from the cross of IR64/Azucena (Huang et al. 1994). The OPAC05-900 marker is located between RFLP markers RZ66 and CDO99 on the map. **B** A map of chromosome 8 developed using the F_2 population of 135 plants derived from the cross IR66699-5-5-4-2/IR24, showing the location of the *xa-13* and closely linked markers.

polymorphic RFLP markers were then used to survey the filters of 135 F_2 plants (Fig. 4). At all three loci, the segregation of homozygotes for the IR24 allele, heterozygotes, and homozygotes for the IR66699-5-5-4-2 allele in the F_2 population showed no significant difference from the ratio of 1:2:1 (Table 1). No recombinants were found between RZ28 and CDO116. The distance between the RFLP markers (RZ28 and CDO116) and *xa-13* was estimated to be 4.8 cM. RG136 is on the other side of *xa-13* at a distance of 3.7 cM. (Fig. 5 B).

Independence of *xa-13* and *xa-5*

The *xa-13* gene was previously reported to be linked with another recessive resistant gene, *xa-5* (Ogawa et al. 1987); *xa-5* has been located on chromosome 5 through trisomic analysis (Yoshimura et al. 1984) and RFLP analysis (McCouch et al. 1991). Following the assumption of linkage between these two genes, the 38 available RFLP markers on chromosome 5 were surveyed, but we failed to identify any linkage between these markers and *xa-13* (data not shown). We then carried out a linkage analysis between *xa-13* and *xa-5* by observing the segregation in the F_2 population of the cross IRBB5/ IR66-699-5-5-4-2. The results indicate that *xa-13* and *xa-5* segregate independently (Table 2).

We also crossed IR66699-9-1-1-4-1-3-2 with BJ1, the variety in which *xa-13* was originally identified. The F_1 plants were resistant and the F_2 population of 957 plants did not segregate for susceptibility. These results confirmed that BJ1 and Long Grain both carry the *xa-13* gene.

Fig. 4 Southern analysis using probe RG136 of *Xho*I-digested DNA from the F_2 population of the cross IR66699-5-5-4-2/IR24. *Phenotype*: *R* = resistance to race 6 of *Xoo*, *S* = susceptible to race 6 of *Xoo*. *Genotype* at the RG136 locus: *1* = homozygote for the IR24 allele, *2* = heterozygote, *3* = homozygote for the IR66699-5-5-4-2 allele

Table 2 Reaction of the F_2 population segregating for *xa-5* and *xa-13* to races 1 and 6 from the cross IRBB5/IR66699-5-5-4-2

Reaction of Race 6 (*xa-13*)	Reaction to Race 1 (*xa-5*)		
	Resistant	Susceptible	Total
Resistant	7	13	20
Susceptible	13	33	46
Total	20	46	66

χ^2 for 1:3:3:9 = 2.525, non-significant at the 5% level

Marker-aided selection

Identifying molecular markers which are closely linked to genes of agronomic significance represents an important step towards increasing the breeding efficiency through marker-aided selection (MAS). MAS has been used in recurrent selection and backcross breeding (Edwards and Johnson 1994; Openshaw et al. 1994), and in

Table 3 Identification of plants carrying *xa-13* in three F_4 families via the RFLP marker RG136

Cross	Genotype[a]			Total
	R	H	S	
IR68312-10-4-6/IR68311-13-3-13	7	4	3	14
IR68312-15-2-21/IR68311-13-6-19	15	2	5	22
IR68312-15-2-21/IR68311-13-3-4	12	1	4	17
Total	34	7	12	53

[a] R = homozygote for the allele from IR68311 lines carrying *xa-13*, S = homozygote for the allele from IR68312 lines carrying *Xa-21* without *xa-13*, H = heterozygote

gene pyramiding (Abenes et al. 1993; Hittalmani et al. 1994). The mapping of *xa-13* provides a basis for marker-aided selection in rice breeding.

In a breeding program to combine genes for bacterial blight resistance, brown plant-hopper resistance and intermediate amylose, the two parents in each cross carry either *xa-13* or *Xa-21*. The dominant resistant gene *Xa-21* confers resistance to all the known Philippines races of *Xoo* (Ikeda et al. 1990; Khush et al. 1991). In the progenies derived from the crosses, the segregants with *Xa-21* could be identified by conventional approaches but the presence of *xa-13* could not be determined because the effect of *xa-13* was masked by *Xa-21*. To identify the plants carrying both *xa-13* and *Xa-21*, F_4 plants carrying *Xa-21* were tested for the presence of the marker RG136 which is closely linked to *xa-13* (Fig. 5 B). A total of 34 plants (64.2%) were homozygotes for the RG136 allele linked to *xa-13* (Table 3). The F_4 plants with *xa-13* as well as *Xa-21* were thus identified.

The results indicate that marker-aided selection is useful to increase breeding efficiency. MAS is especially useful in selecting for recessive genes, such as *xa-13*, where the presence of the gene in the heterozygous condition cannot be detected through traditional approaches without progeny testing, and/or for cases where different genes have the same effect on a character and their genotypes cannot be identified through conventional approaches.

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