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Authors

Ronald, P C

Albano, B

Tabien, R

et al.

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Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*

Pamela C. Ronald[1,*], Beng Albano[2], Rodante Tabien[2], Lleva Abenes[2], Kung-sheng Wu[1], Susan McCouch[1,2], and Steven D. Tanksley[1]

[1] Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca NY 14853, USA
[2] International Rice Research Institute, PO box 1033, Manila, Philippines

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Summary. Nearly isogenic lines (NILs) of rice (*Oryza sativa*) differing at a locus conferring resistance to the pathogen *Xanthomonas oryzae* pv. *oryzae* were surveyed with 123 DNA markers and 985 random primers using restriction fragment length plymorphism (RFLP) and random amplified polymorphic DNA (RAPD) analysis. One chromosome 11 marker (RG103) detected polymorphism between the NILs that cosegregated with *Xa21*. All other chromosome 11 DNA markers tested were monomorphic between the NILs, localizing the *Xa21* introgressed region to an 8.3 cM interval on chromosome 11. Furthermore, we identified two polymerase chain reaction (PCR) products (RAPD2148 and RAPD818) that detected polymorphisms between the NILs. Genomic sequences hybridizing with RAPD818, RAPD248 and RG103 were duplicated specifically in the *Xa21* NIL. All three markers cosegregated with the resistance locus, *Xa21*, in a F_2 population of 386 progeny. Based on the frequency with which we recovered polymorphic *Xa21*-linked markers, we estimated the physical size of the introgressed region to be approximately 800 kb. This estimation was supported by physical mapping (using pulsed field gel electrophoresis) of the sequences hybridizing with the three *Xa21*-linked DNA markers. The results showed that the three *Xa21*-linked markers are physically close to each other, with one copy of the RAPD818 sequences located within 60 kb of RAPD248 and the other copy within 270 kb of RG103. None of the enzymes tested generated a DNA fragment that hybridized with all three of the markers indicating that the introgressed region containing the resistance locus *Xa21* is probably larger than 270 kb.

Key words: *Oryza sativa* – RAPD – RFLP – *Xanthomonas oryzae* pv. *oryzae* – Physical mapping

* *Permanent address:* Department Plant Pathology, University of California Davis, Davis CA, 956165, USA

Correspondence to: P. Ronald at his permanent address

Introduction

Loci conferring disease resistance have been identified in virtually every plant species examined. Genetic analysis of many plant-pathogen interactions has demonstrated that plants often contain single loci that confer resistance against specific races of a pathogen containing a complementary avirulence gene (Flor 1971). While considerable effort has been directed toward cloning plant genes conferring resistance to a variety of bacterial, fungal and viral diseases, there have been few reports of success in this area (Johal and Briggs 1991). The isolation of a disease resistance gene would open the door to analyzing and ultimately understanding the molecular basis of plant defense against pathogen invasion.

Bacterial blight disease of rice (*Oryza sativa*), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), provides an attractive system for studies of disease resistance because both the host and pathogen are amenable to molecular genetic techniques. Races of *Xoo* that induce resistant or susceptible reactions on rice cultivars with distinct resistance (*Xa*) genes to the pathogen have been identified (Mew 1987). Recently, a new source of resistance (*Xa21*) was identified in the wild species *Oryza longistaminata* (Khush et al. 1989). Unlike other *Xa* genes identified, the dominant resistant locus *Xa21* confers resistance to all Indian and Philippine races of *Xoo* tested (Ikeda et al. 1990; Khush et al. 1991). Molecular studies of *Xa21* should provide clues regarding the complexity of the locus and the mechanism by which it confers broad spectrum resistance.

Map-based cloning provides a promising method for isolation of plant disease resistance genes that have been located on a genetic linkage map. This strategy consists of identifying DNA markers that are tightly linked to the gene of interest, isolation of clones containing these markers from a genomic library and complementation of the recessive phenotype by transformation with candidate clones. The introduction and application of new analytical techniques, such as restriction fragment length polymorphisms (RFLPs), the polymerase chain reaction

(PCR), pulsed field gel electrophoresis (PFGE), and yeast artifical chromosome (YAC) cloning methods (Botstein et al. 1980; Burke et al. 1987; Schwartz and Cantor 1984; Williams et al. 1990) have increased the efficiency of map-based cloning approaches (Koenig et al. 1987; Rommens et al. 1989).

Rice, the most widely-consumed crop plant worldwide, has several attributes that make it especially amenable to gene isolation by map-based cloning. Breeding and genetic studies are facilitated by the diploid character ($2n=24$) of rice and the availability of a vast reservoir of germplasm (>200000 accessions) of both domestic and wild rices. Rice researchers have developed a high-density RFLP genetic map of rice containing nearly 500 markers and covering 1700 cM (McCouch et al. 1988; Causse et al. in preparation). Rice has a DNA content and an average kilobasepair per centiMorgan ratio less than those of any other grain species (C= 450 Mb, kb/cM=265) and is among the smallest of any crop plant (Arumuganathan and Earle 1991). The small genome of rice includes a large percentage (ca. 75%) of single or low copy number DNA (Deshpande and Ranjekar 1980; McCouch et al. 1988). This combination of marker density (one marker per 3.4 cM), low kb/cM ratio and the small fraction of repetitive DNA makes chromosome walking feasible using a YAC or cosmid library. Finally, rice has proven to be the most readily transformable of any graminaceous species, which facilitates analysis of cloned genes (Zhang et al. 1988; Shimamoto et al. 1989).

We report here the genetic and physical mapping of the bacterial blight resistance locus *Xa21*. Through RFLP and random amplified polymorphic DNA (RAPD) analysis, we identified three polymorphic DNA markers that are within 1.2 cM of *Xa21* on rice chromosome 11. Based on the frequency with which we discovered polymorphic *Xa21*-linked markers, we estimate the size of the introgressed region containing *Xa21* to be approximately 800 kb. The three markers hybridize with multiple sequences specifically in the *Xa21* resistant line. Finally, we used PFGE to show that the three *Xa21*-linked markers are physically linked to each other. These markers will be used as starting points for a chromosome walk to the *Xa21* locus.

Materials and methods

Genetic stocks. Two populations were used for linkage mapping. One population (P1) consisted of 120 backcross individuals derived from a cross between *O. sativa* (BS125) and *O. longistaminata* (WLO2). This population was not segregating for *Xa21*. The second population (P2) consisted of 386 plants derived from a cross between *Xa21* nearly isogenic lines (NILs). The resistant isoline (1188) was constructed by backcrossing the wild African species *O. longistaminata* containing *Xa21* (*Xa21* donor parent) five times to the recurrent parent *O. sativa* (IR24) followed by five selfings (Khush et al. 1991).

Bacterial inoculations and resistance scoring. The resistant isoline used in this study, 1188, is resistant to all six Philippine *Xoo* races and the resistance segregates as a single gene (Ikeda et al. 1990; Khush et al. 1991). There are no other known *Xa* resistance genes present in 1188. *Xoo* race 6 causes disease on all differential rice cultivars except those containing *Xa21*, therefore resistance to race 6 indicates the presence of the *Xa21* resistance activity in our populations (Ikeda et al. 1990). A total of 386 F2 plants were scored for resistance to *Xoo* race 6 (strain PX099). The strains were grown for 48 h at 30° C in peptone sucrose broth (Tsuchiya et al. 1982). The final inoculum was adjusted with sterile water to 10^9 cfu/ml. Two-month-old, greenhouse-grown plants were cut 2 in from the tip with scissors dipped in the bacterial suspension (Kauffman et al. 1973). Reaction to the pathogen was scored on three independent leaves as either susceptible (lesions range from 5–20 cm) or resistant (lesions less than 1.5 cm). Thirty plants that gave unclear reactions were progeny tested for resistance to *Xoo* race 6.

Molecular techniques. Agarose gels were treated for 15 min with 0.25 M HCl and blotted to Hybond N+ (Amersham) membranes using 0.4 N NaOH. Hybridization conditions have been described (Bernatzky and Tanksley 1986). DNA probes were labeled using the random-hexamer method (Feinberg and Vogelstein 1983). RAPD analysis was performed as described by Williams et al. (1990). PCR products were isolated from agarose using the GENECLEAN kit (Biolabs) and cloned using the TA cloning kit (Stratagene).

Survey of RFLP clones, RAPD primers and linkage analysis. One hundred twenty three markers well distributed on the rice linkage map (McCouch et al. 1988; Causse et al., in preparation) were used as hybridization probes to survey filters containing DNA extracted from the NILs digested with four enzymes, *ScaI*, *Xba*I, *Hind*III and *Eco*RV. A total of 985 10-nucleotide primers (synthesized at DuPont, University of British Columbia and the Cornell Plant Science Center) were surveyed for their ability to amplify polymorphic bands between the NILs according to the RAPD method (Williams et al. 1990; Martin et al. 1991). RFLP and RAPD markers detecting polymorphism with at least one of the enzymes were subjected to linkage analysis.

Leaf samples from 20 F_3 plants per F_2 individual were harvested 6 weeks after sowing. Equal amounts of tissues from all 20 plants per line were combined and mechanically ground using liquid nitrogen and the DNA was extracted as described by Tai and Tanksley (1990). DNA was digested with *Xba*I, separated by gel electrophoresis and blotted to Hybond N+(Amersham). The *Xba*I filters were then hybridized with the *Xa21* linked markers.

Markers were placed on the linkage maps by using the program MAPMAKER (Lander et al. 1987). Standard errors were calculated by the maximum likelihood method (Allard 1956). Distances between markers are presented in centiMorgans derived using the Kosambi function (Kosambi 1944). Confidence intervals for the estimation of introgression were obtained using the exact binomial method (Mendenhall and Scheaffer 1973).

Pulsed field gel electrophoresis: Preparation of rice protoplasts, isolation of high molecular weight DNA, and digestion of DNA in agarose was performed as described (Wu et al. 1992). A contour clamped homogeneous electroc field (CHEF) type gel was used as described by Chu et al. (1986). Gels were made in 0.5 × TBE (1 × TBE = 0.089 M TRIS, 0.089 M boric acid, 0.002M EDTA) at an agarose concentration of 1.1%. Lambda concatamers (InCert) were used as molecular weight markers. For separation up to 300 kb, we used a switch time of 10 s at 150 V for 45 h. For separation of DNA fragments >200 kb we used a switch time of 40 s at 150 V for 55 h. The DNA blotting procedure was performed as described above except that the acid treatment was substituted by treatment with UV light (254 nm for 5 min using a Fotodyne Transilluminator Model 3-4400).

Results and discussion

RFLP analysis

Map-based cloning depends on the tight linkage of a gene of interest to a genetically mapped DNA probe. NILs can be used to find markers that are very close to an introgressed gene from a wild relative owing to the polymorphism of such a segment in the background of the recurrent parent (Young et al. 1988). NILs for *Xa21* were surveyed with 123 RFLP markers that were located, on average, 14 cM apart on the rice RFLP map (McCouch et al. 1988; Causse et al. in preparation). According to estimates of Hanson (1959), the average length of introgressed segments at backcross 5 (BC5) is at least 36 cM. Therefore, we estimated that the clones used in this survey should identify most of the introgressed intervals. Out of 123 clones tested, only 1 chromosome 11 marker (RG103) and 1 chromosome 3 marker (RG944) showed polymorphism between the NILs.

RG103 hybridized with one *Hind*III DNA fragment in the susceptible cultivar, but hybridized with six additional fragments in the *Xa21* donor cultivar and the resistant isoline 1188 (Fig. 1c). The *Xa21* donor, *O. longistaminata*, is a self-incompatible species and therefore most loci are heterozygous. As expected for a heterozygous, outbreeding species, not all of the alleles observed in the donor cultivar were transmitted to the resistant isoline (Fig. 1). The six donor-derived DNA fragments cosegregate with *Xa21* resistance (see below). *O. sativa* BS125 lacks six *Xa21*-linked DNA fragments and *O. longistaminata* WLO2 lacks five of these DNA fragments (Fig. 1c). Both of these cultivars lack the *Xa21* resistance activity (data not shown). These results suggest that a duplication of the RG103 sequence has occurred specifically on the segment of introgressed DNA containing the *Xa21* locus and that between one and five of these DNA fragments are correlated with *Xa21* resistance in the five lines shown here.

RG944 hybridized with two DNA fragments in the susceptible cultivar, one of which showed a polymorphism in the resistant isoline. The polymorphic band was not the same size as the hybridizing band of the *Xa21* donor parent and segregated independently of *Xa21* resistance. These results indicate that the polymorphic band was not inherited from the *O. longistaminata* donor line and is not linked to *Xa21* (data not shown). It is possible that the line IR24 was actually a mixture of pure lines and that the RG944 polymorphism observed between the two NILs is due to polymorphism present in lines of the recurrent parent IR24 (McCouch et al. 1988; Wang et al. 1992).

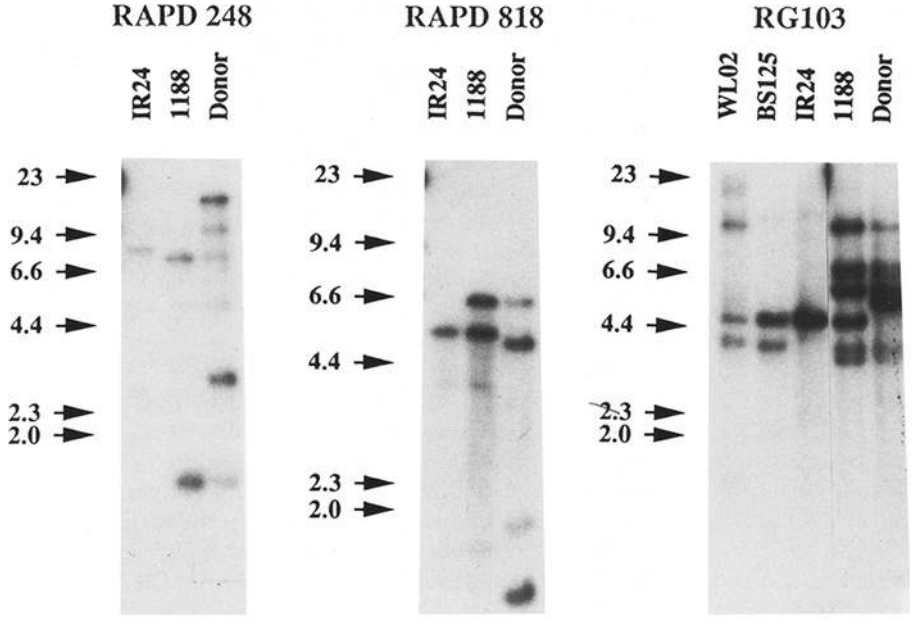

Fig. 1a–c. Surveys of *Xa21* nearly isogenic lines (NILs) showing *Xa21*-linked polymorphic markers. **a** *Xba*I digests of recurrent parent IR24, nearly isogenic resistant cultivar 1188 and the *Oryza longistaminata Xa21* donor parent blotted to Hybond N+ and hybridized with RAPD248. **b** *Xba*I digests of IR24, 1188 and donor DNA hybridized with RAPD818. **c** *Hind*III digests of *O. longistaminata* mapping parent WL02, *Oryza sativa* mapping parent BS125, IR24, 1188 and donor DNA hybridized with RG103. Lambda DNA size markers are shown to the left of each panel in kilobasepairs

RAPD analysis

In an effort to isolate additional markers linked to *Xa21*, we used the RAPD technique (Williams et al. 1990, Martin et al. 1991) to identify PCR products that are polymorphic between the NILs. From a survey of 985 random primers (corresponding to 2357 amplification products observed after separation by gel electrophoresis), we identified two primers (RAPD248 and RAPD818) that amplified polymorphic bands between the NILs. RAPD818 amplified a polymorphic band from the resistant isoline. RAPD248 amplified two polymorphic bands, one from the susceptible and one from the resistant isoline, indicating an insertion/deletion type polymorphism.

Amplified products were cloned and the 1 kb inserts used as hybridization probes to DNA survey filters prepared from the NILs (Fig. 1a, b). RAPD248 hybridizes with one *Xba*I DNA fragment in the susceptible cultivar, but hybridizes with two different DNA fragments in the resistant isoline. The *Xa21 O. longistaminata* donor contains six RAPD248-hybridizing DNA fragments, two of which are the same size as the resistant isoline-specific band (Fig. 1a) RAPD818 hybridizes with the same size *Xba*I DNA fragment in both the susceptible and resistant isolines, and hybridizes with an additional DNA fragment in the resistant isoline. The *Xa21 O. longistaminata* donor contains four RAPD818-hybridizing DNA fragments, one of which comigrates with the resistant isoline specific band (Fig. 1b). These results indicate that the sequences corresponding to the RAPD products 248 and 818 have been duplicated specifically at the *Xa21* locus. The duplicated DNA fragments cosegregate with each other and *Xa21* resistance (see below). The two polymorphic bands of RAPD248 cross-hybridize and are allelic to one another but do not cross-hybridize with RAPD818 (data not shown).

Verification of linkage and chromosomal location of the Xa21 *locus*

The polymorphic DNA markers identified through RFLP and RAPD analysis of NILs were mapped in populations segregating for these markers and for the bacterial blight resistance locus *Xa21*. In our standard mapping population (P1), RAPD248 cosegregated with marker RG103 and mapped to chromosome 11 (Fig. 2). All other chromosome 11 DNA markers tested were monomorphic between the NILs, localizing the *Xa21* introgressed region to an 8.3 cM interval on chromosome 11 between markers CDO534 and RZ537 (Fig. 2). Because RAPD818 was monomorphic and *Xa21* was absent in population P1, an additional F_2 population (P2) was used to determine linkage of RG103, RAPD248, RAPD818 and *Xa21*. The combined data from the two populations led to the consensus RFLP map shown in Fig. 2. No recombinants between RAPD818, RAPD248, RG103 and *Xa21* were found out of 386 F_2 progeny scored. These results indicate that the three markers and the *Xa21* locus are located within 1.2 cm (99% upper confidence limit) from one another on chromosome 11. On average 1 cM corresponds to approximately 265 kb based on a genome size of 450000 kb and a total map size of 1700 cM. Independent experiments using morphological markers conducted at the International Rice Research Institute, The Philippines have confirmed the chromosome 11 location of *Xa21* (R. Ikeda, personal communication).

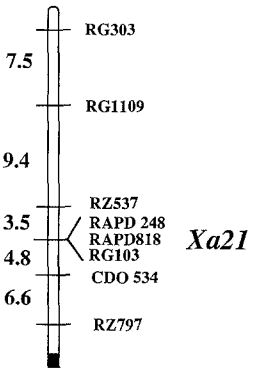

Fig. 2. Restriction fragment length polymorphism (RFLP) map of the *Xa21* genomic region on rice chromosome 11. The data for this map were derived from the two populations described in Materials and methods. The distance between markers is shown in centi Morgans on the left. The *rounded end* and the *dark square* represent the telomere and the orientation toward the centromere, respectively. RAPD248, RAPD818, RG103, *Xa21* and RZ537 are ordered with LOD < 2 using Mapmaker software. All other markers are ordered with LOD > 6

Estimated physical size of the introgressed region of rice chromosome 11

Genetic linkage maps (including those based on RFLPs) reflect the frequency of meiotic crossovers between chromosomal loci. However, map distances provide only a very rough estimate of physical distances along a chromosome, since the kilobase/map unit ratio can vary over at least an order of magnitude depending on what part of the chromosome is being examined (Ganal et al. 1989). Studies have shown that recombination is not constant throughout the genome and that suppression of recombination frequently occurs in regions introgressed from wild species (Ganal et al. 1989; Messegue et al. 1991; Paterson et al. 1990; Young and Tanksley 1989). We therefore used direct physical methods to obtain a more accurate estimate of the region of introgression from the wild species *O. longistaminata* carrying *Xa21*.

Out of a total of 2357 RAPDs and 123 RFLPs analyzed, we identified 3 DNA fragments that are polymorphic between the NILs. Based on the rice genome size (450000 kb), the 65% polymorphism between the recurrent and donor parents (data not shown), and the frequency with which we recovered polymorphic *Xa21*-linked loci (3/2480), we estimated the physical size of the introgressed region to be 837 kb or 3.1 cM ($3/2480 \times 450000 \times 1/0.65$). Using the exact binomial method

Table 1. Size determination of fragments hybridizing to tightly linked DNA markers in the *Xa21* region of rice (all sizes in kilobases)

Enzyme	Marker		
	RAPD248	RAPD818	RG103
*Bsst*I	80, <40	<40	125, 100
*Csp*I	120	120, 115	170
*Mlu*I	125	125, 50	225
*Nar*I	60	60, <40	50, <40
*Not*I	140	190, 140	160, 110, 75
*Nru*I	75	75, <40	60, 50, <40
*Sal*I	60	140, 50	100, 70
*Sfi*I	300	300, 270	270
*Sgr*I	70	70, 50	nd
*Sma*I	75	160, 75	300, 100, 60

Fragments of <40 kb in size are not necessarily identical since they were not in the separation range resolved here. nd, no data

(Mendenhall and Scheaffer 1973), this value lies within a 95% confidence interval of 159–2444 kb. Comparison of this value with the genetic distance covered by the three *Xa21*-linked markers and the resistance locus *Xa21* (<1.2 cM) suggests that recombination may be suppressed in the *Xa21* introgressed region.

Physical linkage of DNA markers in the Xa21 *genomic region*

To utilize the *Xa21*-linked markers to isolate the *Xa21* locus by map-based cloning, it is necessary that the markers be very close to one another and to *Xa21* on the chromosome. PFGE provides a direct method for determining the physical distance between genetically linked markers. A list of rare-cutting enzymes used to map physically the *Xa21* genomic region and the corresponding size of the DNA fragments generated with the three *Xa21* linked probes is shown in Table 1. All hybridizations were performed with at least three gels using different pulse times and yielding an effective separation range from 40 to approximately 300 kb. DNA fragments greater than 200 kb were, in addition, analyzed on PFGs with an effective separation range of 100 to approximately 1000 kb (data not shown).

RAPD248 and one copy of RAPD818 showed clear physical linkage to one another. Both clones were found to be located reproducibly on common DNA fragments generated by eight different enzymes (Table 1). An autoradiogram showing a subset of the enzymes used for this analysis is shown in Fig. 3a, b. The two RAPD products hybridize with the same 140 kb *Not*I, 75 kb *Nru*I, 75 kb *Sma*I, and 125 kb *Mlu*I fragment. RG103 did not hybridize with any of these four DNA fragments.

RG103 and one copy of RAPD818 hybridize reproducibly with the same 270 kb *Sfi*I DNA fragment, using three different PFG conditions (Fig. 3b, c). RAPD248 did not hybridize with the 270 kb *Sfi*I DNA fragment but hybridized with a 300 kb DNA fragment shared with the other RAPD818 copy (Fig. 3a, b). The low molecular weight hybridizing band present in the *Sfi*I lanes was not present in the other three experiments and is probably a DNA degradation product. RG103 and RAPD818 (but not RAPD248) also hybridize to the same 250 kb DNA fragment in partial *Sma*I digests (data not shown).

The results presented in Table 1 show that the smallest DNA fragment shared by RAPD248 and one copy of RAPD818 is 60 kb (*Nar*I). RG103 and the other copy of RAPD818 share a 270 kb *Sfi*I DNA fragment. None of the enzymes tested generated a DNA fragment that hybridized with all three of the markers indicating that the region of introgression containing the resistance locus *Xa21* is probably larger than 270 kb.

RAPD248 and RG103 hybridized with two and six

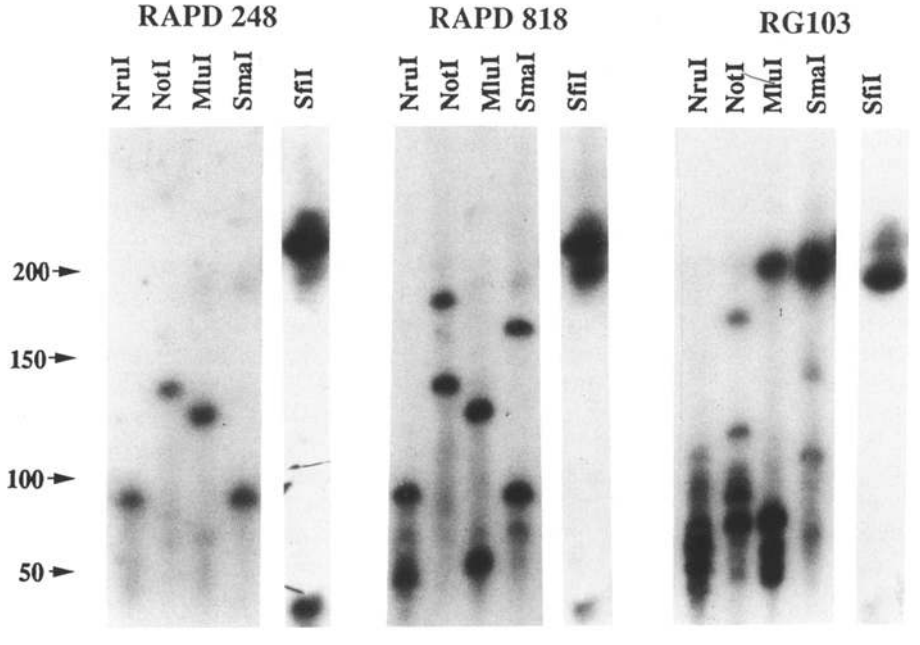

Fig. 3a–c. Autoradiogram showing hybridization of two pulsed field gel electrophoresis (PFGE) gels with the three *Xa21*-linked markers. The same filter was used for each hybrization, stripped after use, and exposed to film to assure that the previous signal had been completely removed. High molecular weight protoplast DNA was digested with rare cutting restriction enzymes *Nru*I, *Not*I, *Mlu*I, *Sma*I, and *Sfi*I and hybridized with the marker RAPD248 (**a**), RAPD818 (**b**) or RG103 (**c**). Lambda DNA concatamer size markers are shown to the left in kilobasepairs

sequences, respectively, in DNA digests electrophoresed on regular agarose gels (Fig. 1a, c) but each of these markers hybridized with single DNA fragments using PFGE techniques (Fig. 3a, c). These results indicate that, in each case, the cosegregating duplicated sequences are physically close in the rice genome. In contrast, no rare-cutting enzyme was found that generated a single DNA fragment containing both RAPD818-hybridizing sequences (Fig. 3b). This raises the question whether both copies of RAPD818 are present in the same region of the genome. Evidence from the PFGE experiments described above indicates that one RAPD818 copy is tightly linked to RAPD248 and the other copy is contained on a separate large DNA fragment shared with RG103. Although only one RAPD818 copy was mapped to the *Xa21* locus (the other copy is nonomorphic between the NILs) each copy hybridizes with PFGE separated DNA fragments that contain RAPD248 or RG103 and both of these markers map to the *Xa21* locus. Thus it appears that the two RAPD818 sequences are not adjacently located on the genome, but rather, are separated in the introgressed region by a DNA segment of unknown length. Because no recombinants between *Xa21* and the three DNA markers were identified, the exact location of *Xa21* in relation to the markers could not be determined.

Map-based cloning of Xa21

The three DNA markers identified in this study can be used as hybridization probes to identify cosmid and YAC clones containing markers linked to the rice disease resistance locus *Xa21*. Identification of plants showing recombination in the *Xa21* genomic region will be essential for precise location of *Xa21* in relation to the three markers cosegregating with *Xa21*. Rare recombinants between the DNA markers and *Xa21* can be rapidly identified using a pooled sample strategy (Tanksley 1991). These recombinants will serve as ordered genomic landmarks so that chromosome walking proceeds in the correct direction.

Molecular basis of disease resistance in rice

Isolation of the *Xa21* locus will provide an opportunity to investigate the molecular basis for the broad spectrum resistance of *Xa21*. The mechanism by which the *Xa21* locus can confer multi-race resistance is unknown. It is possible that the *Xa21* locus consists of a complex locus of several tightly linked genes each recognizing a unique determinant present in different races of the pathogen. Alternatively, the *Xa21* locus may encode a single gene product that can recognize a determinant present in every race of the pathogen.

Studies on *Xoo* have contributed to our understanding of the bacterial genes encoding determinants involved in eliciting a disease resistance response in rice lines carrying different *Xa* genes (Kelemu and Leach 1990; Reimers and Leach 1991). Race-specific interactions in the bacterial blight disease of rice have been shown to follow the gene-for-gene model, which predicts that incompatible interactions are the consequence of positive functions encoded by pathogen avirulence genes and corresponding host resistance genes (Flor 1971; Kelemu and Leach 1990). J. Leach et al. (personal communication), have identified three independent clones from *Xoo* each of which contains an avirulence gene (*avrxa5*, *avrXa7*, and *avrXa10*) what controls bacterial elicitation in rice cultivars containing the *xa5*, *Xa7* and *Xa10* resistance genes. The clones are members of a multigene family and are highly similar to *avrBs3*, a gene from *Xanthomonas campestris* pv. *vesicatoria* that specifies disease resistance on pepper cultivars carrying the resistance gene *Bs3* (Bonas et al. 1979). *avrBS3* encodes a 122 kDa protein, the internal portion of which contains a 34 amino acid motif that is repeated 17.5 times in tandem (Knoop et al. 1991). In the pepper pathogen, deletion derivatives carrying varying number of repeats have altered race specificities (Knoop et al. 1991). In *Xoo*, *avrXa7* and *Xa10* contain 15 and 25 repeats, respectively (J. Leach, personal communication). These results suggest that the number of repeat units determines the avirulence specificity on the resistant host and that novel avirulence specificities can be generated by altering the number of repeats. In addition, the sequence relatedness of the avirulence genes implies similarity in avirulence gene function and in the mechanism of host recognition.

In many instances, genetic analysis has revealed that genes specifying resistance to races of a pathogen are clustered in the plant genome (Shepherd and Mayo 1972; Ellingboe 1978; Saxena and Hooker 1968). For example, genetic studies in lettuce have revealed that resistance to downy mildew is arranged in three linkage groups and that sequence duplication has occurred in some of these regions (Hulbert and Michelmore 1985; R. Michelmore, personal communication). In rice, 17 bacterial blight resistance genes have been identified (Ogawa and Khush 1989). *Xa10* is linked to *Xa21* on chromosome 11 and this cluster is located 27 cM from *Xa3* and *Xa4* (Yoshimura et al. 1983). Two other linkage groups consisting of *Xa1*, *Xa2* and *Xa12* on chromosome 4 and *xa5* and *Xa13* on chromosome 5 have been identified (Ogawa and Khush 1989; McCouch et al. 1991). It has been proposed that a disease resistance locus is made up of many alleles that can recombine to produce a locus with a novel specificity (Pryor 1987; Saxena and Hooker 1968). It is interesting to speculate that, similar to the mechanism for generating new *Xoo* avirulence alleles, duplications and rearrangements in the plant genome may have given rise to the complexity and race specificity observed for many disease resistance loci. Interestingly, our results reveal that sequences of all three *Xa21*-linked DNA markers have been duplicated specifically in the *Xa21* genomic region. Analysis of the molecular structure of the disease resistance locus *Xa21* may shed light on the basis for its broad spectrum resistance and the physical and functional relatedness of the *Xa* loci.

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