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Mapping of Bacterial Blight Resistance Gene *Xa11* on Rice Chromosome 3

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Abstract

The bacterial blight (BB) resistance gene *Xa11* confers resistance in rice to Japanese races IB, II, IIIA, and V of the BB pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Here, we report the mapping of *Xa11* by using randomly amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequence (CAPS), and simple sequence repeat (SSR) markers. To detect DNA markers linked to *Xa11*, we used an *Xa11* near-isogenic line, IR-BB11 (in genetic background of IR24), and the susceptible cultivar IR24. The RAPD fragment $L19_{1200}$, putatively linked to *Xa11*, was identified. Cloning and sequencing of the $L19_{1200}$ product indicated that it was held in the Rice Genome Program database as accession number AC097277, and that it occurs on chromosome 3. On the basis of the sequences of $L19_{1200}$ and the flanking genomic region, we designed CAPS marker KUX11 and selected two SSR markers, RM347 and RM1350, for mapping *Xa11*. To confirm the putative linkage among *Xa11* and the three markers, we conducted linkage analysis using the F_2 population of a cross between IR24 and IR-BB11. Each F_2 plant was inoculated with strain T7156 (race IB) and genotyped with KUX11, RM347, and RM1350. Segregation in the F_2 located *Xa11* between the loci of RM347 (2.0 cM) and KUX11 (1.0 cM) on the long arm of chromosome 3. These results should be useful for the marker-assisted selection of *Xa11* in breeding programs and for cloning *Xa11* by map-based cloning.

Discipline: Plant Breeding
Additional key words: cleaved amplified polymorphic sequence (CAPS), linkage, randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), *Xanthomonas oryzae* pv. *oryzae*.

Introduction

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases in rice (*Oryza sativa* L.) worldwide. Breeding and deployment of resistant cultivars carrying major resistance (*R*) genes have given the most effective approach to controlling BB. More than 30 major genes which confer resistance to various *Xoo* strains have been identified and are used as the main sources for genetic improvement of rice for resistance to *Xoo* in Asia.

Genetic and physical mapping of these *R*-genes not only permits marker-assisted breeding in rice, but also facilitates isolation and characterization of these genes at the molecular level. So far, 17 BB *R*-genes have been mapped on rice chromosomes and used for transferring and pyramiding in marker assisted selection (MAS) [19,26]. In particular, six *R*-genes, *Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27*, were cloned by map-based cloning[3,6,9,20,22,27]. However, *Xa11*, *xa15*, *Xa16*, *Xa17*, *Xa18*, *xa19*, *xa20*, *xa24*, and *xa28* have not been mapped yet.

Ogawa and Yamamoto[14] identified the BB resistance gene *Xa11*, and Ogawa et al.[17] found it in the cultivars or lines IR8, Elwee, RP9-3, Peta, and IR944-102-3-2. *Xa11* confers specific resistance to strains T7156, T7147, T7133, and H75304 (Japanese *Xoo* races IB, II, IIIA, and V, respectively)[13,17]. IR-BB11, a near isogenic line (NIL) carrying *Xa11*, was developed at the International Rice Research Institute (IRRI) and the former Tropical Agriculture Research Center (TARC), now the Japan International Research Center for Agricultural Sciences (JIRCAS)[15,16]. In this study, we report the identification of molecular markers closely linked to *Xa11*, and the mapping of *Xa11* in an IR24 × IR-BB11 cross.

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Materials and methods

1. Plant materials and disease evaluation

We used a near-isogenic line, IR-BB11, carrying *Xa11* in the genetic background of the susceptible cultivar IR24 for RAPD analysis and as a donor of *Xa11.* We used IR24 as a control cultivar for RAPD analysis and as a parent for F_2 analysis. IR-BB11 showed resistance to Japanese races IB, II, IIIA and V (Fig. 1a), as described previously[17]. Two F_2 populations derived from a cross between IR24 and IR-BB11 were used for RAPD analysis of markers putatively linked to *Xa11* and for genetic analysis of *Xa11.* The first population was sown on 1993 May 15, and the second on 2006 May 24. The plants were transplanted 30 days after sowing into concrete beds at Kyushu University, and the parents and F_2 plants showed uniform growth. The soil was fertilized with N-P-K at 9–6–6 g/m^2 before transplanting, and additional nitrogen was applied at 3 g/m^2 before inoculation.

Plants were inoculated with strain T7156 (Japanese race IB). The strain was cultured on potato semi-synthetic agar (PSA) medium at 30°C for 3 days[24]. Bacteria were suspended in sterile distilled water at a concentration of approximately 10^{8-9}cfu/mL as inoculum. At booting stage, five of the uppermost fully expanded leaves of each plant were inoculated by the leaf-clipping method[10]. Plants were inoculated on 2006 August 4, and reactions of individual F_2 plants were evaluated two or three times during 14–21 days after inoculation. The disease reaction was categorized by eye as resistant (R) or susceptible (S) according to the degree of development. For example, a lesion that stopped at the cut margin was categorized as R, but a lesion that expanded along a vein was categorized as S.

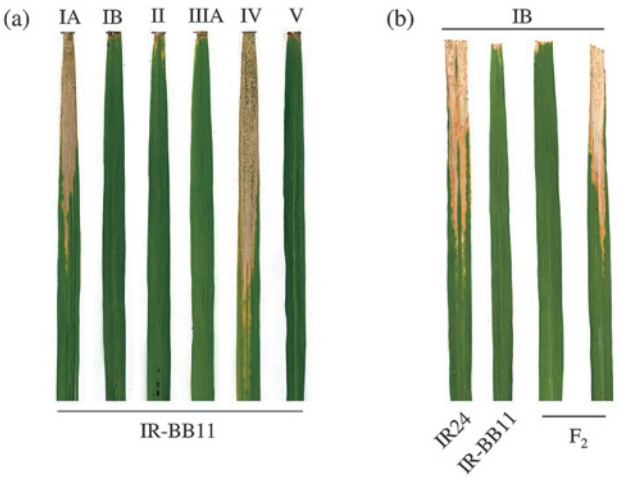

Fig. 1. Pathogenic reaction of *Xanthomonas oryzae* pv. *oryzae* on rice

(a) IR-BB11 inoculated with six *Xoo* strains: T7174 (race IA), T7156 (IB), T7147 (II), T7133 (IIIA), H75373 (IV), H75304 (V). (b) IR24, IR-BB11, and F_2s derived from IR24 × IR-BB11 inoculated with *Xoo* strain T7156.

2. PCR conditions for RAPD, CAPS, and SSR analysis

For RAPD analysis, we used 500 ten-base arbitrary primers (RAPD kits; Operon Biotechnologies Inc., Tokyo, Japan) to screen for polymorphisms between IR24 and IR-BB11. DNAs were extracted from fresh leaves according to Dellaporta et al.[4] Amplification reactions were performed in 25-µL reaction volumes containing 50 mM KCl, 10mM Tris·HCl (pH 9.0), 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 1 U *Taq* polymerase, 0.4 µM primers, and 25 ng genomic DNA in a PCR System-9700 (Applied Biosystems, Foster City, CA, USA). The PCR program was one cycle at 94°C for 4 min; 40 cycles of 1 min at 94°C, 1 min at 35°C, and 1 min at 72°C; and a final extension step at 75°C for 5 min before cooling to 4°C. Amplified products were resolved by electrophoresis at 35 V for 5 h in 1.5% agarose gels. The gels were stained with ethidium bromide, and the DNA fragments were visualized with UV light and photographed.

The CAPS marker KUX11 (F:5′-GTGATTCCGCGAAAGTGAAT-3′, R: 5′-AGTGTGAGGATGGGAAGCAC-3′) was designed and used to genotype the F_2 population. The PCR reaction mixture for the PCR-based markers contained 10–20 ng template DNA, 0.3 µM primers, 0.2 mM dNTPs, 50 mM KCl, 10mM Tris·HCl (pH 9.0), 2 mM $MgCl_2$, and 1 U *Taq* DNA polymerase in a final volume of 25 µL. The DNA was amplified in 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min. The amplified DNA was digested with restriction enzyme (*Sna*BI) and analyzed by electrophoresis in 4% agarose gels.

The SSR markers RM347 and RM1350 were also used for genotyping. The PCR mixture contained 10–20 ng template DNA, 0.2 µM primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 50 mM KCl, 10mM Tris·HCl (pH 9.0), and 1 U *Taq* DNA polymerase in a total volume of 15 µL. The PCR program was one denaturation step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The PCR products were resolved by electrophoresis at 200 V for 50 min in 4% agarose gels containing ethidium bromide in 0.5× TBE buffer.

3. DNA sequencing

Amplified products of the correct size were recovered from agarose gel with a Qiaquick gel extraction kit (Qiagen GmbH, Hilden, Germany). Recovered DNA fragments were cloned in the pGEM-T vector system

(Promega GmbH, Mannheim, Germany), following the manufacturer's instructions. A BigDye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems, Foster City, CA, USA) were used for sequencing. DNA sequence similarity analysis was performed by using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/).

4. Linkage analysis

For linkage analysis, CAPS and SSR genotypes of each plant were scored as codominant characters, and the disease reaction was scored as dominant or recessive. Recombination values were estimated by using the maximum likelihood equation, and the recombination values were converted into genetic map distance (cM) by using Kosambi's mapping function[11].

Results

To detect RAPD markers putatively linked to *Xa11*, we screened IR-BB11 and IR24 using 500 arbitrary primers. Although most of the amplified fragments were identical in both lines, one polymorphic fragment was identified: primer L19 (5′-GAGTGGTGAC-3′) yielded a 1200-bp fragment from IR-BB11 but not from IR24 (Fig. 2a). We named this fragment $L19_{1200}$. $L19_{1200}$ was cloned into the pGEM-T vector and sequenced. Polymorphism between IR-BB11 and IR24 was found at the *Sna*BI restriction site, so we designed the CAPS marker KUX11 (F: 5′-GTGATTCCGCGAAAGTGAAT-3′, R: 5′-AGTGTGAGGATGGGAAGCAC-3′) to amplify DNA containing that site. KUX11 amplified a 900-bp fragment from both IR24 and IR-BB11. Digestion of the fragment with *Sna*BI produced two fragments (~650 bp and ~250 bp) from IR24 and one fragment (no digestion) from IR-BB11 (Fig. 2b). We searched rice genome sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/) with the $L19_{1200}$ sequence as a query. The sequence was present in RGP contig AC097277 from chromosome 3. We then screened 32 SSR markers in the genomic region flanking AC097277 to detect polymorphisms between IR-BB11 and IR24. Two SSR markers—RM347 and RM1350—showed clear polymorphism.

To confirm the putative linkage among the loci of *Xa11*, *KUX11*, *RM347*, and *RM1350* on chromosome 3, we conducted linkage analysis. First we assessed disease reactions of 189 F_2 plants derived from IR24 × IR-BB11 to *Xoo*. The F_2 plants were clearly classified either as resistant or as susceptible (Fig. 1b): 138 plants were resistant and 51 were susceptible. The segregation of resistant and susceptible plants in the F_2 population fitted the 3:1 ratio ($\chi^2 = 0.397$, $P = 0.529$), which indicates that the resistance of IR-BB11 to T7156 is controlled by a single dominant gene, *Xa11*. Each plant was also genotyped with the three DNA markers and scored for codominance of the trait. The segregation of the three markers fitted the expected ratio of 1:2:1 (for RM347, $\chi^2 = 5.564$, $P = 0.062$; for RM1650, $\chi^2 = 5.500$, $P = 0.064$; for KUX11, $\chi^2 = 5.011$, $P = 0.082$), indicating their monogenic inheritance. The segregation pattern of *Xa11* with the three DNA markers revealed that three DNA markers were closely linked to *Xa11*. The cosegregation pattern of *Xa11* (resistant/susceptible) and KUX11 genotypes in F_2 is shown in Fig. 2b as an example. The arrangement of the four loci was *RM347–Xa11–KUX11–RM1350*. *Xa11* was located between *RM347* and *KUX11* at genetic distances of 2.0 and 1.0 cM, respectively (Fig. 3).

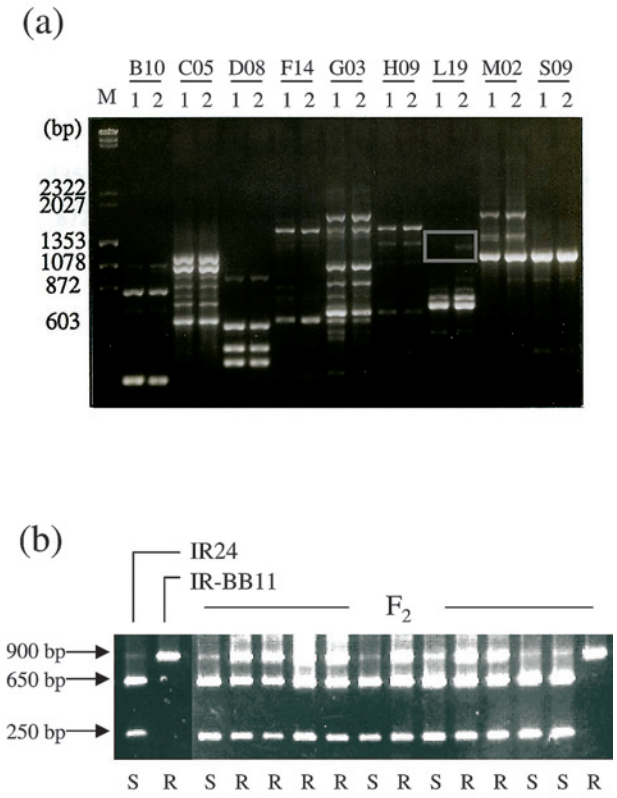

Fig. 2. Detection of polymorphisms between IR24 and IR-BB11

(a) Detection of random amplified polymorphic DNA (RAPD) by nine arbitrary primers. Amplification products were separated by electrophoresis. 1: IR24; 2: IR-BB11; M: Molecular marker. (b) Segregation of the CAPS marker KUX11 in F_2 population. A 900-bp fragment was amplified from both IR24 and IR-BB11 by KUX11. After digestion of this fragment with *Sna*BI, two fragments (~650 bp and ~250 bp) from IR24 and one fragment (900 bp, no digestion) from IR-BB11 were revealed by electrophoresis in agarose gel. R: resistant; S: susceptible.

Discussion

Ogawa and colleagues reported that IR8, a parent of IR-BB11, shares a dominant gene, *Xa11*, for resistance to Japanese races IB, II, IIIA, and V of *Xoo*[13,17]. However, the chromosomal location of the gene was not known. Our results reveal that *Xa11* is located on chromosome 3 between DNA markers RM347 (2.0 cM) and KUX11 (1.0 cM). These markers will provide a significant contribution to cloning and molecular characterization of *Xa11*.

Since RAPD fragment $L19_{1200}$ had been identified before the completion of the rice genomic sequence and the advent of SSR markers, the method we used to identify the chromosomal location of *Xa11* in this study was different from the current method of identifying the location of an unmapped gene. We detected the PCR product putatively linked to *Xa11* by RAPD analysis of a NIL and then identified its chromosomal location from the DNA sequence information. This method facilitated the mapping of *Xa11*.

A large number of disease resistance genes have been described in rice, and many have been roughly placed on genetic maps. However, no major disease *R*-genes have previously been located on chromosome 3, only some insect *R*-genes, such as *bph11* and *Grh4*[5,8]. Only quantitative trait loci for bacterial leaf streak, rice blast, and sheath blight resistance genes were known on chromosome 3[2,23,29]. Our results provide the first report of the location of a major resistance gene for BB on chromosome 3.

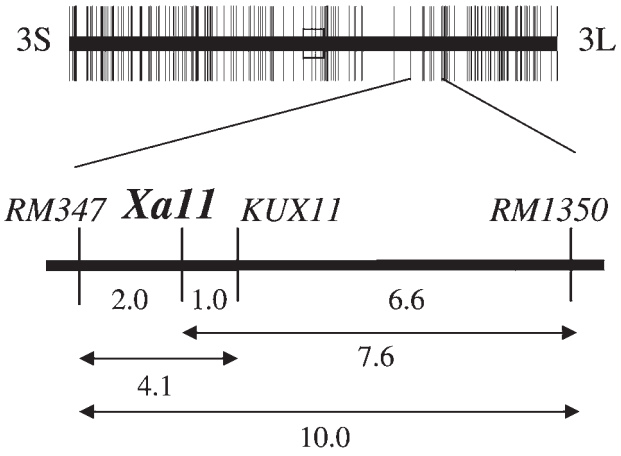

Fig. 3. Linkage map indicating the position of *Xa11* on chromosome 3

Vertical bars represent SSR and CAPS markers used for the linkage analysis. Framework maps were quoted from Harushima et al.[7]. Numbers indicate map distances (cM).

Most dominant *R*-genes encode structurally related proteins with predicted extracellular or intracellular leucine-rich repeat (LRR) motifs. *Xa21*, the first cloned *R*-gene for BB, is a member of a multigene family in rice, and encodes a receptor-like kinase protein with a predicted extracellular LRR domain, a transmembrane domain, and a cytoplasmic kinase domain[20,21]. *Xa26* encodes a protein structurally similar to that encoded by *Xa21*, but with a slightly larger LRR domain. Both genes are broad spectrum *R*-genes. *Xa1* encodes a nucleotide binding site (NBS)-LRR protein[27]. The rice blast *R*-genes *Pib*, *Pita*, *Piz-t*, and *Pi9* also encode NBS-LRR proteins[1,18,25,28]. Fewer clusters of NBS-LRR and related genes are located on chromosome 3 than on the other chromosomes[12]. This result is consistent with the fact that few major *R* genes are located on chromosome 3: most BB and rice blast resistance genes are closely associated with clusters of NBS-LRR genes. The *Xa11* candidate region flanked by markers RM347 and KUX11 in Nipponbare contains nine BAC clones (AC079830, AC079889, AC133335, AC084406, AC079736, AC087412, AC087851, AC092779, and AC097277). The Rice Genome Automated Annotation System (RiceGAAS, http://ricegaas.dna.affrc.go.jp/) and Rice Annotation Project Database (RAP-DB, http://rapdb.dna.affrc.go.jp/) predict two genes encoding putative NB-LRR proteins in AC097277. One of these genes may be an *Xa11* homolog.

Information on chromosome location and molecular markers linked to *Xa11* is important in breeding for BB resistance such as by gene pyramiding. Our results will support fine mapping and positional cloning of *Xa11*.

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