Theor Appl Genet (2004) 108:800–807
DOI 10.1007/s00122-003-1491-x

ORIGINAL PAPER

K. Gu · D. Tian · F. Yang · L. Wu · C. Sreekala · D. Wang · G.-L. Wang · Z. Yin

High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L.

Received: 22 May 2003 / Accepted: 25 September 2003 / Published online: 31 October 2003

Abstract Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Ishyama) Dye, is one of the serious diseases prevalent throughout Asia. In a previous study, a resistance (*R*) locus was transferred from the tetraploid wild rice *Oryza minuta* to the cultivated rice species, *Oryza sativa* L. Here, we report the fine genetic mapping of the *R* locus, tentatively designated as *Xa27(t)*. We performed disease evaluation with an *Xa27(t)* near-isogenic line, IRBB27, testing 35 *Xoo* strains collected from 11 countries. The *Xa27(t)* locus conferred a high level of resistance to 27 strains and moderate resistance to three strains. Resistance of the *Xa27(t)* gene was developmentally regulated in IRBB27 and showed semi-dominant or a dosage effect in the cv. CO39 genetic background. As a prelude to cloning *Xa27(t),* a molecular mapping strategy was employed with a large mapping population consisting of 3,875 gametes. Three molecular markers, M336, M1081, and M1059, closely linked to *Xa27(t),* were identified to facilitate the mapping of *Xa27(t)* to the long arm of chromosome 6. The *Xa27(t)* locus was confirmed by chromosome landing of M1081 and M1095 markers on the rice genome. Markers derived from the genomic sequence of *O. sativa* cv. Nipponbare were used to further saturate the *Xa27(t)* genomic region. *Xa27(t)* was finally located within a genetic interval of 0.052 cM, flanked by markers M964 and M1197, and co-segregated with markers M631, M1230, and M449.

Communicated by D.J. Mackill

K. Gu · D. Tian · F. Yang · L. Wu · C. Sreekala · D. Wang · Z. Yin (✉)
Temasek Life Sciences Laboratory, 1 Research Link,
The National University of Singapore,
117604 Singapore, Republic of Singapore
e-mail: yinzc@tll.org.sg
Tel.: +65-68727420
Fax: +65-68727007

G.-L. Wang
Department of Plant Pathology,
The Ohio State University,
Kottman Hall, 2021 Coffey Road, Columbus,
OH 43210-1087, USA

Introduction

Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Ishyama) Dye, is one of the serious diseases prevalent throughout Asia (Gnanamanickam et al. 1999). Use of resistant varieties is the most effective method to control the disease. Race-specific interaction between rice and *Xoo* is thought to follow the classic gene-for-gene concept (Flor 1971), in which the plant resistance (*R*) gene can recognize or interact with an elicitor molecule, presumably encoded by an avirulence gene (*Avr*) from the pathogen. The recognition and interaction lead to activation of a battery of defense responses and effectively inhibit pathogen invasion. Currently, more than two dozen *R* genes or loci against *Xoo* have been identified in rice, most of them providing complete, race-specific resistance (Kinoshita 1995; Lin et al. 1996; Zhang et al. 1998; Khush and Angeles 1999; Gao et al. 2001; Chen et al. 2002; Yang et al. 2003). 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, two of the bacterial blight *R* genes in rice, *Xa21* and *Xa1*, have been isolated by map-based cloning approaches (Song et al. 1995; Yoshimura et al. 1998). Two dominant *R* genes, *Xa4* (Sun et al. 2003; Wang et al. 2001) and *Xa26(t)* (Yang et al. 2003), and two recessive *R* genes, *xa5* (Yang et al. 1998; Blair et al. 2003) and *xa13* (Sanchez et al. 1999), have been physically mapped.

In order to broaden the pool of germplasm for breeding of disease resistance to *Xoo*, a wide hybridization project to transfer *R* genes from wild species *Oryza minuta* was initiated at the International Rice Research Institute (IRRI) in the late 1980s. A novel resistance locus against bacterial blight was identified from a progeny of inter-specific hybrids of *O. sativa* cv. IR31917-45-3-2 and *O. minuta* Acc. 101141 (Amante-Bordeos et al. 1992). Disease evaluation and segregation of the resistance locus suggested that a single gene was, or closely linked genes conferring race-specific resistance to *Xoo* strain

PXO99 (race 6) were, introgressed into the cultivated rice line. However, no further study was conducted to determine its resistance spectrum and chromosomal location in the rice genome. In this study, we tentatively designated the resistance locus as *Xa27(t)* and initiated fine-scale genetic mapping with the following objectives: (1) to further evaluate the resistance of *Xa27(t)* to diverse *Xoo* strains, (2) to identify molecular markers which are closely linked to the *Xa27(t)* locus, (3) to integrate *Xa27(t)*-linked markers onto the rice linkage map, and (4) to further saturate the *Xa27(t)*-containing region with the markers derived from the rice genomic sequence of cv. Nipponbare.

Materials and methods

Plant materials and mapping populations

The F_1 hybrid seeds of 78-1-5 BC_2F_3 and IR24 were obtained from IRRI (R. Nelson and G. Khush). Plants of 78-1-5 BC_2F_3 were derived from a cross between *O. sativa* cv. IR31917-45-3-2 and *O. minuta* Acc. 101141 (Amante-Bordeos et al. 1992). Highly resistant plants from the backcross 78-1-5 BC_2F_3#169 were selected as male to backcross with susceptible cultivars IR24 and CO39. Ten generations of backcrosses were carried out to transfer *Xa27(t)* into IR24 genetic background, and one of the $BC_{10}F_3$ plants showing similar morphological phenotype as IR24 was designated as IRBB27 for *Xa27(t)* near-isogenic line. Seven backcrosses were made to transfer *Xa27(t)* into the CO39 genetic background. The *Xa27(t)* mapping populations consisted of 2,369 plants from both F_2 and BC_1 populations derived from the above crosses, which are summarized in Table 1. The doubled haploid (DH) mapping population, wild species *O. minuta* Acc. 101141, and other cultivars used in the study, i.e., CO39, IR24, and IRBB21, were kindly provided by N. Huang and H. Leung from IRRI. Rice plants, including those inoculated with *Xoo* strains, were grown in the greenhouse at a temperature of 30°C for 13 h (light) and 26°C for 11 h (dark).

Bacterial inoculation and disease scoring

Bacterial blight inoculation was carried out using the leaf-clipping method described by Kauffman et al. (1973). Briefly, *Xoo* strains were grown on PSA medium (10 g/l peptone, 10 g/l sucrose, 1 g/l glutamic acid, 16 g/l bacto-agar, and pH 7.0) for 2–3 days at 28°C. The bacterial cells were suspended in sterile water at an optical density of 0.5 at OD600. The bacterial cell suspension was applied to the two youngest, but fully expanded, leaves of each tiller by clipping 5–6 cm from the tip of the leaf using a pair of scissors dipped in the inoculum. Lesion length (LL) was measured 2 weeks after inoculation. The disease symptom was scored as resistant (R, LL$\leq$3.0 cm), moderately resistant (MR, 3.0 cm<LL$\leq$6.0 cm), moderately susceptible (MS, 6.0 cm<LL$\leq$9.0 cm) and susceptible (S, LL>9.0 cm) (Amante-Bordeos et al. 1992).

Table 1 Populations used in the genetic fine mapping of the *Xa27(t)* gene for resistance to bacterial blight disease of rice

Populations[a]	Phenotype[b]	Genotype[c]	Plants	Gametes
BC_1 populations				
BC_1(IR24)	R	*Rr*	809	809
BC_1(CO39)	R	*Rr*	54	54
F_2 populations				
F_2(IR24)	R	*RR, Rr*	196	392
F_2(IR24)	S	*rr*	1,106	2,212
F_2(CO39)	R	*RR, Rr*	65	130
F_2(CO39)	S	*rr*	139	278
Total			2,369	3,875

[a] BC_1 and F_2 populations from crosses with different susceptible parents (IR24 and CO39)
[b] *R* resistant, *S* susceptible
[c] *Rr Xa27(t)xa27(t)*, *RR Xa27(t)Xa27(t)*, *rr xa27(t)xa27(t)*

Molecular techniques

RAPD analysis was carried out according to the procedures as described by Williams et al. (1990). Equal amounts of genomic DNA (10 ng/μl) from ten resistant F_2 plants and ten susceptible F_2 plants from an *Xa27(t)* segregating population in the IR24 genetic background were selected to make the resistant and susceptible pools, respectively, for bulked segregant analysis (BSA) (Michelmore et al. 1991). Approximately 10 ng of the DNA pool was used for each RAPD reaction. Random primers were obtained from Operon Technologies (Alameda, Calif.). The PCR reaction was performed as follows: 94°C for 120 s; followed by 40 cycles of 94°C for 60 s, 37°C for 45 s, and 72°C for 90 s; and then 72°C for 5 min. About 0.1μl of [33P-α]dCTP (3,000 Ci/mmol, Amersham Biosciences) was added to each reaction mixture to label PCR products. The PCR products were denatured and then separated on a 4.5% polyacrylamide gel using the Sequi-Gen sequencing cell from Bio-Rad (Hercules, California). The gel was vacuum dried and then exposed to Biomax XR film (Eastman Kodak, Rochester, N.Y.) for 2–3 days. Primers that revealed polymorphism between pools were tested further using the 20 individual DNA samples in the two pools.

DNA samples from 18 resistant and 18 susceptible plants derived from six M336 recombinant segregating populations were used for AFLP (Vos et al. 1995) analysis. AFLP analysis was performed according to the AFLP instruction manual of AFLP™ Analysis System II and AFLP Small Genome Primer Kit (GIBCO BRL). The AFLP pre-amplified products were obtained through the use of the E-0/M+C primer pairs. For selective amplification, the [33P-γ]ATP-labeled *Eco*RI primers were utilized in combination with *Mse*I primers. All 64 pairwise combinations of *Eco*RI and *Mse*I primers were used to screen for polymorphism between individual plants. The amplified DNA fragments were denatured and separated on a 4.5% polyacrylamide gel as described above.

TAIL-PCR was performed according to Liu et al. (1995), with the minor modification of 15 supercycles in the secondary reaction and 30 reduced-stringency cycles in the tertiary reaction. The nested primers for M1059 were A1F1 (5′TAACAACATGAGAATTACTAATCCG3′), A1F2 (5′CATGTATCCAAGTTCGTAGCTAG3′), and A1F3 (5′TTGGTTTTTTTGAATGAA-GGGTATAT3′). The nested primers for M1081 were A2F1 (5′AATTCATGCCCACAAGTACAGTAC3′), A2F2 (5′CTGAAACACAGGAA-AAATCCCGTT3′), and A2F3 (5′TGCATAGGCCCTGTTTAGTTCTAA3′). The arbitrary degenerate primer was AD3 (5′(A/T)GTGNAG(A/T)ANCANAGA3′) (Liu et al. 1995).

Mapping of *Xa27(t)*-linked markers on the rice linkage map

To map the *Xa27(t)*-linked markers on the rice genetic linkage map, the DH population consisting of 111 lines developed from a cross between the *indica* variety IR64 and the *japonica* variety Azucena was used (Huang et al. 1994). The genetic linkage map constructed in the DH mapping population contained 179 RFLP markers. Chromosomal locations of the identified markers in this study were determined using the program MAPMAKER (Macintosh version 2.0) (Lander et al. 1987). The maximum-likelihood map order for markers was determined with a LOD score threshold of 3.0, and all map distances (cM) were reported in Kosambi units.

DNA sequence analysis

The publicly available BAC (Bacterial Artificial Chromosome) or PAC (P1 Artificial Chromosome) sequences of *O. sativa* cv. Nipponbare were downloaded from Rice Genome Sequence Program (RGP) Web site (http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/stattable.pl?chr=6&lab=RGP) and Genbank. DNA sequence was aligned and analyzed using Sequencher 3.0 program. Sequence alignment was also carried out using Pairwise BLAST (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html).

Results

Phenotypic analysis

The resistance spectrum of *Xa27(t)* was evaluated by inoculating IRBB27 with 35 *Xoo* strains collected from 11 countries around the world. IRBB21, a near-isogenic line of *Xa21* (Wang et al. 1996), was used as a control for disease evaluation in this study. Table 2 summarizes the lesion length and the phenotype of disease reaction. Among the 35 *Xoo* strains tested, IRBB27 conferred a high level of resistance to 27 strains, moderate resistance to three strains (HB17, JW89011, and PXO71), and was susceptible to five strains (1947, C4, ZHE173, K202, and 2). *Xa27(t)* and *Xa21* shared most of the incompatible strains tested. IRBB27 conferred resistance to A3842 and moderate resistance to JW89011, while IRBB21 was moderately susceptible and susceptible, respectively. Neither IRBB27 nor IRBB21 could confer resistance to the African strain 1947. In most of the incompatible reactions between *Xa27(t)* or *Xa21* and *Xoo* pathogens, IRBB27 had shorter lesions than that of IRBB21. The lesions observed in incompatible reactions (R and MR in Table 2) of *Xa21* measured in this study were longer than that of the previous study (Wang et al. 1996), which may partially be attributed to the higher temperature used for inoculation in this study. Strains A3842 and Thailand 2 were found to be incompatible with *Xa21* in the previous study (Wang et al. 1996), but were compatible in this study. In most of the incompatible reactions between *Xa27(t)* and *Xoo* strains, brown lesions were observed 3–4

Table 2 Comparison of the resistance spectrum of IRBB27 and IRBB21 to different *Xoo* strains

Xoo strain[a]	Origin	Lesion length and resistance score[b]		
		IRBB27	IRBB21	IR24
1947	Africa	21.5±2.2 (S)	18.1±4.4 (S)	21.2±3.3 (S)
Aust-2031	Australia	0.3±0.2 (R)	2.4±0.9 (R)	5.8±1.4 (MR)
Aust-R3	Australia	0.2±0.1 (R)	2.5±0.4 (R)	18.1±5.4 (S)
C1	China	0.2±0.1 (R)	2.9±1.0 (R)	29.9±3.1 (S)
C2	China	1.0±0.7 (R)	2.6±0.8 (R)	21.3±2.4 (S)
C3	China	0.2±0.1 (R)	5.3±2.4 (MR)	25.4±3.3 (S)
C4	China	16.6±3.7 (S)	1.3±0.3 (R)	18.2±2.5 (S)
C5	China	0.3±0.1 (R)	1.2±0.5 (R)	16.6±3.5 (S)
C6	China	0.3±0.1 (R)	1.3±0.3 (R)	22.9±3.0 (S)
C7	China	0.2±0.1 (R)	1.9±0.4 (R)	21.9±4.4 (S)
GD1358	China	0.2±0.0 (R)	2.7±0.8 (R)	13.7±3.3 (S)
HB17	China	4.5±3.9 (MR)	2.2±0.6 (R)	20.7±3.3 (S)
HB21	China	0.3±0.2 (R)	2.2±0.4 (R)	33.4±2.1 (S)
HLJ72	China	1.3±1.0 (R)	1.9±0.5 (R)	21.8±3.8 (S)
JS49-6	China	0.4±0.1 (R)	1.8±0.4 (R)	21.7±4.1 (S)
LN57	China	0.1±0.0 (R)	3.4±0.6 (MR)	25.2±4.5 (S)
NX42	China	0.4±0.1 (R)	2.0±0.8 (R)	20.4±3.4 (S)
ZHE173	China	17.2±3.4 (S)	1.6±0.6 (R)	21.7±3.7 (S)
CIAT1185	Columbia	0.2±0.2 (R)	2.0±1.0 (R)	13.8±3.3 (S)
A3842	India	0.2±0.1 (R)	7.0±2.7 (MS)	20.9±2.8 (S)
A3857	India	0.2±0.1 (R)	3.0±1.0 (R)	24.2±2.7 (S)
IXO56	Indonesia	0.2±0.1 (R)	4.4±1.9 (MR)	28.8±5.0 (S)
H75373	Japan	2.4±1.2 (R)	2.1±0.4 (R)	23.3±3.4 (S)
T7174	Japan	0.5±0.5 (R)	2.0±0.7 (R)	27.6±3.2 (S)
JW89011	Korea	5.7±3.5 (MR)	21.8±2.4 (S)	28.6±4.6 (S)
K202	Korea	24.1±2.8(S)	3.1±1.6 (MR)	28.3±3.0 (S)
NXO260	Nepal	0.2±0.1 (R)	5.6±1.6 (MR)	18.0±3.3 (S)
PXO86 (R2)	Philippines	0.1±0.0 (R)	1.3±0.6 (R)	20.0±3.5 (S)
PXO79 (R3)	Philippines	0.2±0.1 (R)	1.7±0.5 (R)	14.4±3.2 (S)
PXO71 (R4)	Philippines	5.5±3.5 (MR)	2.6±0.8 (R)	20.8±4.1 (S)
PXO113 (R4)	Philippines	1.5±0.3 (R)	2.6±1.1 (R)	14.8±2.2 (S)
PXO112 (R5)	Philippines	0.1±0.0 (R)	3.4±0.6 (MR)	14.9±3.5 (S)
PXO99 (R6)	Philippines	0.2±0.1 (R)	1.3±0.3 (R)	26.1±3.8 (S)
R-7	Thailand	1.6±0.7 (R)	2.3±0.6 (R)	12.9±2.7 (S)
2	Thailand	9.6±3.7 (S)	12.8±3.1 (S)	25.7±5.7 (S)

[a] Six-weeks-old plants were inoculated with *X. oryzae* pv. *oryzae*. For each strain, at least 16 leaves from four individual plants were inoculated

[b] The lesion length (LL) is the average of 16 infected leaves. The standard deviation of the mean is indicated. For score: *R* resistant, 0 cm≤LL≤3.0 cm, *MR* moderately resistant, 3.0 cm<LL≤6.0 cm, *MS* moderately susceptible, 6.0 cm<LL≤9.0 cm, *S* susceptible, LL>9.0 cm

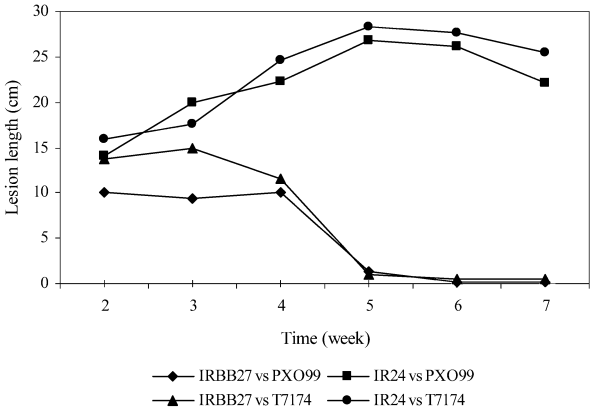

Fig. 1 Resistance of *Xa27(t)* to *Xoo* strain PXO99 and T7174 at different developmental stages as measured by mean lesion length. Bacterial inoculation was carried out as described in the text. Only the youngest, fully expanded leaves of the main culm (from seedling stage to active tillering stage) or each tiller (after active tillering stage) were selected for inoculation

days after inoculation around the cut area. Brown lesions were much more obvious at the infection sites of those leaves that showed highly resistant phenotype with mean lesion length being less than 0.5 cm 2 weeks after inoculation. The browning reaction against pathogens was also observed in the interaction of *Xa3* and its incompatible *Xoo* strains (Kaku and Ogawa 2000). No browning reaction was observed at the early stage of infection in the interaction between *Xa21* and its incompatible *Xoo* strains. The difference in browning reaction between *Xa27(t)* and *Xa21* against the bacterium suggested that the resistance mechanism of these two genes might be different at the molecular level.

The resistance of *Xa27(t)* to bacterial blight at different developmental stages was evaluated with two different *Xoo* strains, PXO99 and T7174. PXO99 is a representative strain of Philippines race 6, while T7174 is a representative strain of Japanese race 1 (Song et al. 1995; Yoshimura et al. 1998). IR24 was highly susceptible to the two *Xoo* strains from seedling stage (before 4–5 weeks) to adult plants (Fig. 1). IRBB27 plants were also susceptible to the two *Xoo* strains at seedling stage up to 4 weeks, even though their lesions were shorter than that of IR24 (Fig. 1). However, the resistance of IRBB27 to *Xoo* strains was increased drastically after the age of 4 weeks and reached almost complete resistance at the age of 5 weeks. Therefore, the resistance conferred by *Xa27(t)* was influenced by the developmental stage of the host plant and appeared to be induced during the late stage of vegetative growth.

The resistance of *Xa27(t)* to bacterial blight in the CO39 genetic background was evaluated by inoculating 38 BC_7F_2 plants with PXO99. The genotypes of the 38 plants were determined by co-dominant RFLP markers flanking the *Xa27(t)* locus. Three *Xa27(t)* homozygous plants [*Xa27(t)Xa27(t)*] were resistant (lesion length = 1.6±1.4 cm) while 12 recessive homozygous plants [*xa27(t)xa27(t)*] were susceptible to PXO99 (lesion length = 22.6±5.8 cm). However, 23 heterozygotes [*Xa27(t)-xa27(t)*] showed a great deviation in disease phenotype from moderately resistant and moderately susceptible to fully susceptible. The mean lesion length of the 23 plants was 13.5±7.0 cm. By comparing the mean lesion length of the heterozygotes with that of two kinds of homozygotes, we concluded that *Xa27(t)* conferred semi-dominant resistance or showed dosage effect for resistance in the CO39 genetic background. The inheritance of *Xa27(t)* as

Table 3 List of the forward (*F*) and reverse (*R*) primers for the molecular markers obtained during this study

Marker	PCR primer (5′-3′)[a]	Size (bp)	Enzyme[b]
M336[c]	F: AACTGTCAAACAACAGCTTCCATGGA R: TGCCTAGAAATGGTGGTATGTCACC	336	*Hin*dIII
M1059[d]	F: TTGGTTTTTTTGAATGAAGGG R: GCCTGGTGCTTTCATTGTTT	1,059	*Ava*I or *Hae*II
M1081[d]	F: TAGCTAAATAAAAGCAATTTTACGA R: GCCCTTACATATCGATGTTTATTG	1,081	*Xba*I or *Spe*I
M964[e]	F: TGTGCAATGCAGGATTTCAGTTACT R: TTTCACCTGCATAATGCAAAAGCTAA	964	*Pst*I
M631[e]	F: CTGCATCCATGCCGGTGGCCG R: AAACGTCACATGAAGACTCCAATTGT	631	*Nde*I
M1230[e]	F: AGGGATGTCGAGATGAGAGCTTC R: GGTGTCCTTCTTTACGGGCCTCC	1,230	*Eco*RI
M499[e]	F: CTCTGCCGGCCGGCCAAGAAAGC R: AAGCTTATAACACATACGTGAC	499	*Acc*I or *Hae*II
M1197[e]	F: GCTGTGAAGTGCCGGGTGTCC R: TGGACAGGACGATGCCGGTGG	1,197	*Bcl*I

[a] Primers used to amplify markers from RAPD- or TAIL-PCR-derived clones as well as from IRBB27 or cv. Nipponbare
[b] Restriction endonuclease that reveals polymorphism between resistant- and susceptible-associated alleles of the marker in RFLP analysis
[c] Marker obtained from RAPD
[d] Marker derived from AFLP and TAIL-PCR analyses
[e] Marker amplified from cv. Nipponbare

a semi-dominant *R* gene was also observed in the genetic background of five parent lines of Chinese hybrid rice when *Xa27(t)* was used during a marker-assisted breeding program (unpublished results). These results suggested that the resistance of *Xa27(t)* could be affected by other genetic factors in some genetic backgrounds when plants are heterozygous at the resistance locus.

Identification of molecular markers and linkage analysis for *Xa27(t)*

Both RAPD and AFLP techniques were used to screen for *Xa27(t)*-linked markers. In RAPD analysis, of 1,200 random primers screened, only primer BE05 (5′GGAACGCTAC3′) was found to reproducibly detect a resistance-associated polymorphic band between individuals. The 336-bp polymorphic band was cloned and designated as M336 (Table 3). RFLP analysis revealed that M336 had four bands in IRBB27 when digested by *Hin*dIII, one of which was linked to the *Xa27(t)* locus.

In AFLP analysis, two pairwise combinations of *Eco*RI and *Mse*I primers, E-AT (5′AGACTGCGTACCAATTCAT3′) / M-CAA (5′GATGAGTCCTGAGTAACAA3′) and E-AT (5′AGACTGCGTACCAATTCAT3′) / M-CTA (5′GATGAGTCCTGAGTAACTA3′), were found to detect resistance-associated polymorphic bands between individuals. The two bands were cloned and they were 130 bp and 212 bp in length, respectively. However, both the fragments were too short to be used as RFLP probes and, moreover, the 212-bp fragment contained repetitive sequences. We therefore used TAIL-PCR to obtain their flanking sequences from IRBB27. A 1,059-bp TAIL-PCR product flanking the 130-bp fragment was amplified from IRBB27 with the nested primer A1F3 and the arbitrary degenerate primer AD3. The single-copy 1,059-bp fragment was designated as M1059 (Table 3). M1059 served as a co-dominant marker being polymorphic between resistant and susceptible alleles of *Xa27(t)*, as revealed by RFLP analysis. Similarly, a 1,081-bp TAIL-PCR product flanking the 212-bp fragment was amplified from IRBB27 with the nested primer A2F3 and AD3. The 1,081-bp fragment was designated as M1081 (Table 3) and confirmed as a co-dominant marker that showed polymorphism between resistant and susceptible alleles of *Xa27(t)*, as revealed by RFLP analysis.

M336, M1081, and M1059 were then developed into the corresponding RFLP probes for co-segregating analysis with the *Xa27(t)* locus. Linkage analysis showed that M336 (20 recombinants identified from 1,110 gametes) and M1081 (17 recombinants identified from 1,750 gametes) were 1.8 cM and 0.97 cM, respectively, from the *Xa27(t)* locus on one side, while M1059 (12 recombinants identified from 3,875 gametes) was found to be 0.31 cM on the other side (Fig. 3).

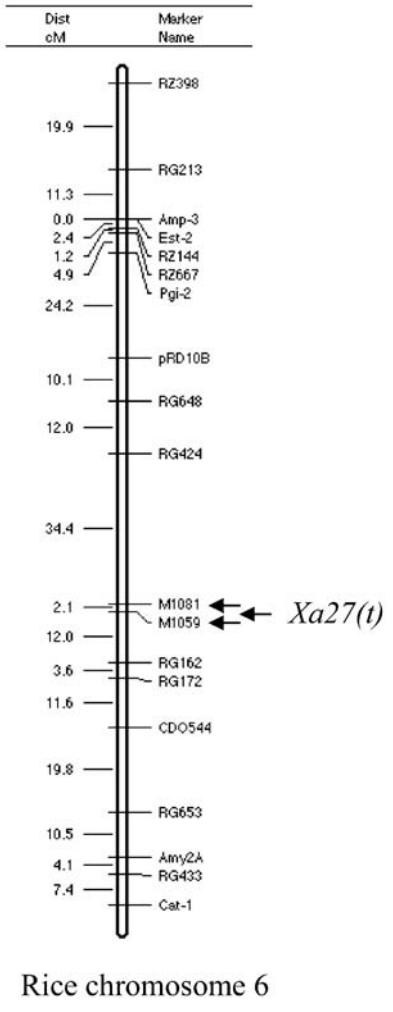

Fig. 2 Mapping of *Xa27(t)*-linked markers to the rice genetic linkage map with the DH population. M1059 (*arrow*) and M1081 (*arrow*) mapped to rice chromosome 6. Other markers on the map are RFLP markers from McCouch et al. (2001). *Boldface arrowhead* indicates the position of *Xa27(t)*. The maximum-likelihood map order for markers was determined with a LOD score threshold of 3.0, and all map distances (cM) are reported in Kosambi units

Integration of M1059 and M1081 into the rice linkage map

M1059 and M1081 were used to map *Xa27(t)* on the rice linkage map. For this purpose, genomic DNA from the two parents (IR64 and Azucena) of the DH mapping population (Huang et al. 1994) was digested by 30 different restriction enzymes and blotted for parental survey of polymorphism using M1059 and M1081 as RFLP probes. Both markers detected polymorphisms in combination with at least 1 of the 30 restriction enzymes tested. The two *Xa27(t)*-linked markers mapped to the long arm of rice chromosome 6 (between marker RG424 and RG162) with M1081 as a proximal, and M1059 as a distal, marker (Fig. 2). The genetic distance between M1059 and M1081 was 2.1 cM according to the rice linkage map (Fig. 2), which was comparable to 1.28 cM

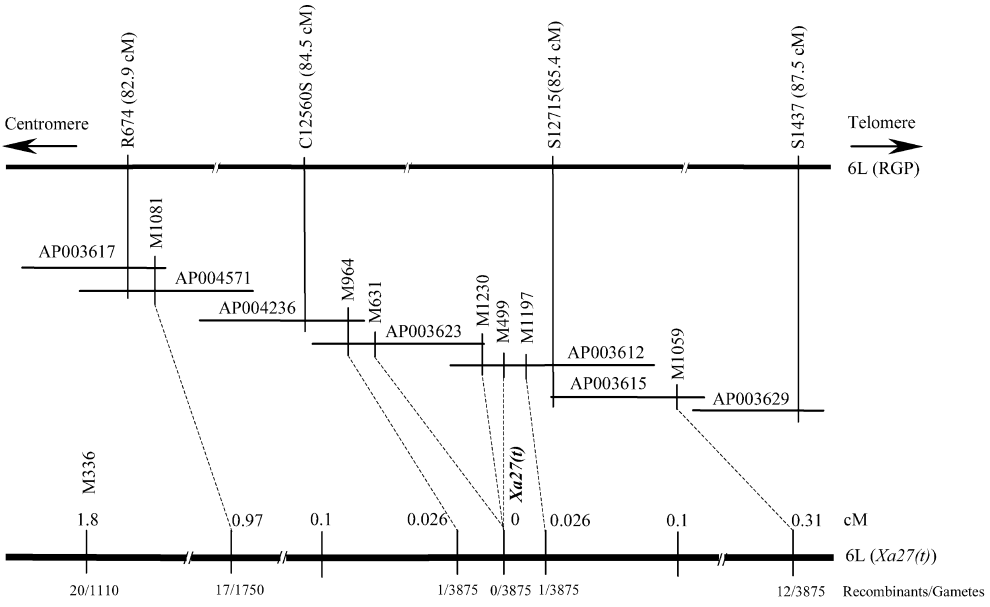

Fig. 3 High-resolution genetic map of the *Xa27(t)* locus. Genetic map of *Xa27(t)* based on linkage analysis is shown at the *bottom* and recombinants/gametes are indicated. Genetic and physical maps of RGP at the syntenic position of the *Xa27(t)* locus are also shown. Genetic distance is given in centiMorgans. The accession numbers of cv. Nipponbare sequences are indicated for each BAC/PAC clone. The *vertical line* denotes the position of the respective marker and the *dashed line* designates the relative position of the corresponding marker

(0.31 cM+0.97 cM) obtained from our linkage analysis (Fig. 3). The proximal marker M1081 was positioned 34.4 cM from the Cornell marker RG424. The distal marker M1059 was mapped 12.0 cM from the Cornell marker RG162 (Fig. 2). The genetic interval between RG424 (70.4 cM on chromosome 6) and RG162 (104.6 cM on chromosome 6) was 34.2 cM on the Cornell map (McCouch et al. 2001), which covers almost half of the long arm of chromosome 6.

Landing of M1081 and M1059 on the rice genome and high-resolution genetic mapping

BLASTN (http://www.ncbi.nlm.nih.gov/blast/index.html) was utilized to search for matching sequences of the M1081 and M1059 markers in the Unfinished High Throughput Genomic Sequences (HTGS) of rice. A sequence matching M1059 was found in the sequence AP003615 of RGP PAC clone P0486H12 at S12715 (85.4 cM on RGP map) locus on chromosome 6 [Identities = 999/1008 (99%) for position 19–1026 on M1059]. M1081 identified matching sequences in AP004571 (RGP PAC clone P0652A05), AP004327 (Monsanto BAC clone OJ1378_E04), AP003941 (Monsanto BAC clone OJ1111_E06), and AP003617 (RGP PAC clone P0502H06) [Identities = 412/416 (99%) for position 161–575 and 462/494 (93%) for position 589–1081 on M1081]. Sequence analysis indicated that the latter four BAC or PAC clones are overlapping clones at the R674 locus (82.9 cM on the RGP map) on chromosome 6. We then downloaded the genomic sequence in addition to genetic markers from R674 to S1437 loci from RGP Web site. A genetic map and a physical BAC/PAC contig of RGP were generated as shown in Fig. 3. The physical distance between M1081 and M1059 was 480 kb on the RGP BAC/PAC contig.

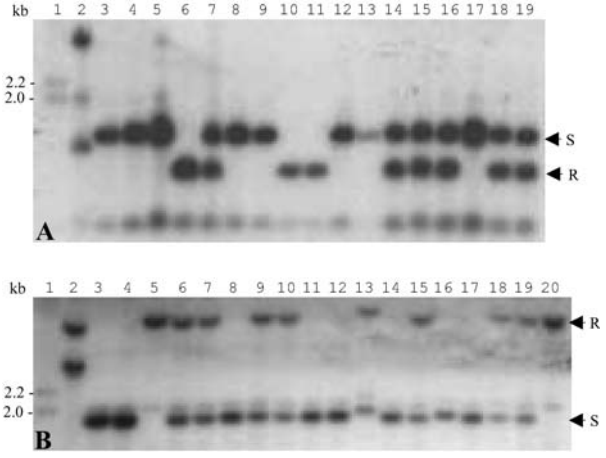

Fig. 4 Confirmation of recombinants 24-2-2-943 (**A**) and 24-10-3-2-147 (**B**). The Southern filters were detected with marker M964 (**A**) or M1197 (**B**) (*R* resistance-associated allele, *S* susceptible-associated allele). Samples in **A**: *1* λDNA-digested *Hin*dIII, *2 O.minuta* Acc. 101141, *3* cv. IR31917-45-3-2, *4* cv. CO39, *5* cv. IR24, *6 Xa27(t)* homozygote, *7* M964 recombinant 24-2-2-943 (susceptible), *8–19* 24-2-2-943 F_3 progenies (susceptible). Samples in **B**: *1* λDNA-digested *Hin*dIII, *2 O. minuta* Acc. 101141, *3* cv. IR31917-45-3-2, *4* cv. IR24, *5 Xa27(t)* homozygote, *6* M1197 recombinant 24-10-3-2-147 (resistant), *7–20* 24-10-3-2-147 F_3 progenies (resistant)

To perform fine-scale genetic mapping of the *Xa27(t)* locus, we designed markers based on available expressed sequence tags (ESTs) and unique sequences in the M1081-M1059 interval. Putative markers were amplified from cv. Nipponbare (Table 3), surveyed for polymorphisms among the *Xa27(t)* parents, and tested for linkage with the *Xa27(t)* locus. *Xa27(t)* was finally localized to the 0.052-cM interval flanked by markers M964 (1 recombinant) and M1197 (1 recombinant), and co-segregated with markers M631, M1230, and M499 (Fig. 3). Figure 4 shows the confirmation of the last two recom-

binants, 24-2-2-943 from the proximal side identified by M964 and 24-10-3-2-147 from the distal side identified by M1197 in the F_3 generation.

Discussion

Although a majority of bacterial blight *R* genes were identified from cultivated rice, three of these genes, i.e., *Xa21*, *Xa23*, and *Xa27(t)*, were introgressed from wild rice (Amante-Bordeos et al. 1992; Song et al. 1995; Zhang et al. 1998). These three *R* genes confer resistance to multiple *Xoo* strains. The cloned *Xa21* gene conferred resistance to 29 diverse *Xoo* strains from eight countries (Wang et al. 1996). The *Xa23* gene conferred resistance to 20 *Xoo* strains, including ten Philippine races, seven Chinese pathotypes, and three Japanese races (Zhang et al. 2001), and seedling resistance to nine Philippines races (Zhang et al. 1998). Interestingly, the blast *R* gene*, Pi9(t),* transferred from *O. minuta* Acc. 101141 to cultivated rice from the same study of alien introgression (Amante-Bordeos et al. 1992), conferred resistance to 43 blast isolates (Liu et al. 2002). Multiple resistance specificity from a single locus is highly desirable in breeding programs for durable resistance. Currently, two hypotheses could explain the molecular basis of this resistance. One hypothesis suggests that these loci are composed of a cluster of tightly linked functional genes, each of which recognizes unique pathogen elicitors produced by pathogen avirulence genes. The majority of *R* genes reside at complex loci, and the structure of these may influence the rate of *R*-gene diversification as well as resistance specificity (Hulbert et al. 2001). Another hypothesis speculates that these loci carry single resistance genes capable of recognizing diverse pathogen elicitors. The latter possibility can be explained by the recently proposed "guard" hypothesis that R proteins might guard a limited set of key cellular targets of pathogen virulence factors (Dangl and Jones 2001). From this study, we are still unclear whether the *Xa27(t)* locus contains multiple functional members with each possessing different resistance specificity or whether it harbors a single gene that confers broad resistance.

The mechanism of alien introgression is still unclear. Cytogenetic and molecular analyses indicate genetic recombination between chromosomes of cultivated and wild species as the cause of alien gene transfer (Brar and Khush 1997). In this scenario, the homologues of resistance genes at syntenic positions are required in the cultivated varieties for double-crossovers during alien introgression. For example, the *Cf-0* locus in susceptible *Lycopersicon esculentum* contains a homologue of *Cf-4* and *Cf-9* introgressed from wild species *L. hirsutum* and *L. pimpinellifolium*, respectively (Parniske et al. 1997; Thomas et al. 1997). The *Mla* locus derived from the susceptible cultivar Morex contains three RGH families of the NBS-LRR class that represent homologues of *Mla6* and *Mla14* introgressed from the wild barley *Hordeum spontaneum* nigr (Wei et al. 1999). In rice, *Pi9*(t) maps to an approximately 100-kb DNA interval and is thought to be either allelic or tightly linked to another blast *R* gene, *Pi2*(t) (Liu et al. 2002). A third blast *R* gene, *Pi-z*, also maps to the same region (Inukai et al. 1992), which makes it possible that *Pi9*(t) has been introgressed as part of a cluster of blast *R* genes (Liu et al. 2002).

Xa27(t) was mapped to a 0.052-cM interval between M964 and M1197. This 0.052-cM interval is flanked by the RGP markers C12560S and S12715, which cover a 0.9-cM genetic distance (Fig. 3). Another bacterial blight *R* gene, *Xa7*, was also mapped at the distal end of the long arm of chromosome 6 with a recombination frequency of 8.8% to marker G1091 (Nagato and Yoshimura 1998). G1091 is 22.1 cM away from marker S12715 (Harushima et al. 1998). *Xa7* was originally identified from rice cultivar DZ78 and controlled resistance only at flowering and later stages with specific resistance to PXO61 (race 1), PXO86 (race 2), and PXO79 (race 3) (Sidhu and Khush 1978). Based on the resistance specificity and genetic location, we believe that *Xa27(t)* is a new bacterial blight *R* gene and not allelic to *Xa7*.

The availability and utilization of the genomic sequence of cv. Nipponbare greatly accelerated the fine-scale genetic mapping of the *Xa27(t)* locus. We found that there was a good syntenic relationship for the marker location and order on the genetic map at the *Xa27(t)* locus between IRBB27 and cv. Nipponbare. The high-resolution genetic map at the *Xa27(t)* locus and closely linked markers obtained in this study will facilitate the isolation of the broad spectrum *R* gene by positional cloning. Identification of *Xa27(t)* will be a valuable resource for marker-assisted breeding and to deduce signal transduction cascade(s) conferring resistance in rice.

Acknowledgements The authors would like to thank N. Naqvi, Y. Hong, and W.-C. Yang for critical reading of the manuscript; G. Khush, H. Leung, and R. Nelson for providing the rice cultivars; N. Huang for providing the DH mapping population; and Q. Zhang and J. Leach for providing *Xoo* strains. Our research was supported by Temasek Life Sciences Laboratory.

Reference

Amante-Bordeos A, Sitch LA, Nelson R, Dalmacio RD, Oliva NP, Aswidinnoor H, Leung H (1992) Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theor Appl Genet 84:345–354

Blair MW, Garris AJ, Iyer AS, Chapman B, Kresovich S, McCouch SR (2003) High resolution genetic mapping and candidate gene identification at the *xa5* locus for bacterial blight resistance in rice (*Oryza sativa* L.). Theor Appl Genet 107:62–73

Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35:35–47

Chen H, Wang S, Zhang Q (2002) A new gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. Phytopathology 92:750–754

Dangl J, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–96

Gao DY, Xu ZG, Chen ZY, Sun LH, Sun QM, Lu F, Hu BS, Liu YF, Tang LH (2001) Identification of a new gene for resistance

to bacterial blight in a somaclonal mutant HX-3 (*indica*). Rice Genet Newsl 18:66–68

Gnanamanickam SS, Pryiyadarasani V, Narayanan NN, Vasudevan P, Kavitha (1999) An overview of bacterial blight disease of rice and strategies for management. Curr Sci 77:1435–1444

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2,275 markers using a single F_2 population. Genetics 148:479–494

Huang N, McCouch S, Mew T, Parco A, Guiderdoni E (1994) Development of an RFLP map from a doubled haploid population in rice. Rice Genet Newsl 11:134–137

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol 39:285–312

Inukai T, Mackill DJ, Bonman JM, Sarkarung S, Zeigler R, Nelson R, Takamure I, Kinoshita T (1992) Blast resistance genes *Pi2(t)* and *Pi-z* may be allelic. Rice Genet Newsl 9:90–92

Kaku H, Ogawa T (2000) The relationship between browning reaction and bacterial blight resistance gene *Xa3* in rice. Rice Genet Newsl 17:76–77

Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance to rice varieties of *Xanthomonas oryzae*. Plant Dis Rep 57:537–541

Khush GS, Angeles ER (1999) A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. Rice Genet Newsl 16:92-93

Kinoshita T (1995) Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newsl 12:9–115

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ. Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q (1996) identification and mapping of a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86:1156–1159

Liu G, Lu G, Zeng L, Wang GL (2002) Two broad-spectrum blast resistance genes, *Pi9*(t) and *Pi2*(t), are physically linked on rice chromosome 6. Mol Genet Genomics 267:472–480

Liu YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junction by thermal asymmetric interlaced PCR. Plant J 8:457–463

McCouch SR, Temnykh S, Lukashova A, Coburn J, DelClerkck G, Cartinhour S, Harrinton S, Thomson M, Septiningsih E, Semon M, Moncada P, Li J (2001) Microsatellite markers in rice: abundance, diversity, and applications. In: Khush GS, Brar DS, Hardy B (eds) Rice Genetics IV. Science Publishers, Enfield, N.H., pp 117–135

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: rapid method to detect markers in specific genomic regions by using segregating population. Proc Natl Acad Sci USA 88:9828–9832

Nagato Y, Yoshimura A (1998) Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newsl 15:13–74

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BBH, Jones JDG (1997) Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the *Cf-4/9* locus of tomato. Cell 91:821–932

Sanchez AC, Ilag LL, Yang D, Brar DS, Ausubel F, Khush GS, Yano M, Sasaki T, Li Z, Huang N (1999) Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. Theor Appl Genet 98:1022–1028

Sidhu GS, Khush GS (1978) Dominance reversal of a bacterial blight resistance gene in some rice cultivars. Phytopathology 68:461–463

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804–1806

Sun X, Yang Z, Wang S, Zhang Q (2003) Identification of a 47-kb DNA fragment containing *Xa4*, a locus for bacterial blight resistance in rice Theor Appl Genet 106:683–687

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JDG (1997) Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in *Cf-4* and *Cf-9*. Plant Cell 9:2209–2224

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Freijters A, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang GL, Song WY, Ruan DL, Sideris S, Ronald PC (1996) The cloned gene *Xa21*, confers resistance to multiple *Xanthomonas oryzae pv. oryzae* isolates in transgenic plants. Mol Plant-Microbe Interact 9:850–855

Wang W, Zhai W, Luo M, Jiang G, Chen X, Li X, Wing RA, Zhu L (2001) Chromosome landing at the bacterial blight resistance gene *Xa4* locus using a deep coverage rice BAC library. Mol Genet Genomics 265:118–125

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Yang D, Sanchez A, Khush GS, Zhu Y, Huang N (1998) Construction of a BAC contig containing the *xa5* locus in rice. Theor Appl Genet 97:1120–1124

Yang Z, Sun X, Wang S, Zhang Q (2003) Genetic and physical mapping of a new gene for bacterial blight resistance in rice. Theor Appl Genet 106:1467–1472

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1,* a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Zhang Q, Lin SC, Zhao CL, Wang CL, Yang WC, Zhou YL, Li DY, Chen CB, Zhu LH (1998) Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv *oryzae*) from *O. rufipogon*. Rice Genet Newsl 15:138–142

Zhang Q, Wang CL, Zhao KJ, Zhao YL, Caslana VC, Zhu XD, Li DY, Jiang QX (2001) The effectiveness of advanced rice lines with new resistance gene *Xa23* to rice bacterial blight. Rice Genet Newsl 18:71–72