Theor Appl Genet (2015) 128:1933–1943
DOI 10.1007/s00122-015-2557-2

CrossMark

ORIGINAL PAPER

Identification and fine-mapping of a new resistance gene, *Xa40*, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.)

Suk-Man Kim[1,2] · Jung-Pil Suh[3] · Yang Qin[4] · Tae-Hwan Noh[5] · Russell F. Reinke[1,2] · Kshirod K. Jena[1]

Received: 11 February 2015 / Accepted: 5 June 2015 / Published online: 17 June 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Key message **A new bacterial blight resistance gene has been identified through fine-mapping, which confers high levels of resistance to all Korean *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) races, including the new *Xoo* race K3a.**

Abstract Rice bacterial leaf blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a serious constraint to rice production in Asia and Africa. The japonica advanced backcross breeding lines derived from the indica line IR65482-7-216-1-2 in the background of cultivar Junam are resistant to all Korean BB races, including K3a. To identify the gene(s) involved in resistance to Korean *Xoo* races, the association of genotypic and phenotypic variations was examined in two F_2 populations derived from the crosses between 11325 (IR83261-3-7-23-6-2-1-1-2-1-2)/Anmi and 11325/Ilpum. The segregation ratios of F_2 individuals from the crosses of 11325/Anmi and 11325/Ilpum were 578 resistant:209 susceptible and 555 resistant:241 susceptible, respectively, which is consistent with the expected allelic frequency of a 3:1 ratio. Genetic analysis using graphical mapping indicated that resistance (R) was controlled by a new resistance gene linked with the flanking markers RM27320 and ID55.WA18-5 within an approximately 80-kb region between 28.14 and 28.22 Mbp on chromosome 11. The eight candidate genes functionally predicted were included in the target region. Examination of the candidate genes by RT-PCR analysis only corroborated with the significant difference in transcript levels of the *WAK3* gene in the presence or absence of pathogen infection. Allelism tests performed with other known BB R-genes revealed that the allele was distinct from others having a similar chromosomal location.

Communicated by Y. Xu.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2557-2) contains supplementary material, which is available to authorized users.

✉ Kshirod K. Jena
k.jena@irri.org

[1] Plant Breeding, Genetics, and Biotechnology Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines

[2] C/o IRRI-Korea Office, National Institute of Crop Science, Rural Development Administration, Iseo-myeon, Wanju-gun, Jeollabuk-do 565-851, Republic of Korea

[3] Central Area Crop Breeding Research Division, National Institute of Crop Science, Rural Development Administration, Suwon 441-857, Republic of Korea

[4] Biosafety Division, National Academy of Agricultural Science, Rural Development Administration, Jeonju 560-500, Republic of Korea

[5] National Institute of Crop Science, Rural Development Administration, Iseo-myeon, Wanju-gun, Jeollabuk-do 565-851, Republic of Korea

Introduction

Bacterial leaf blight (BB) of rice caused *by Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease of tropical and temperate rice. The first report of rice BB was by Japanese farmers in 1884; however, it was not reported to cause serious damage to the rice crop until the appearance of new high-yielding varieties in the 1960s and 1970s (Mizukami and Wakimoto 1969; Mew et al. 1992; Adhikari et al. 1994). Since then, the incidence of BB has been widely reported throughout Asia and BB has become one of the three serious rice diseases causing significant yield

loss annually (Chen et al. 2011). Estimates of average yield losses are difficult to obtain because of the extensive geographic and seasonal variation in BB incidence. However, during disease severity, BB has been reported to cause yield losses of up to 80 % (Mew et al. 1993; Srinivasan and Gnanamanickam 2005). Enhancing host resistance is considered as the most effective strategy to achieve disease resistance in rice. However, the high degree of pathogenic variation in *Xoo* often causes the breakdown of resistance (Vera Cruz et al. 2000; Suh et al. 2013). Expanding genetic sources with novel resistance genes from wild relatives of rice, their deployment in breeding programs, and pyramiding two or more effective resistance genes are some of the approaches envisioned in developing rice cultivars with durable BB resistance to *Xoo* (Suh et al. 2009; Natraj Kumar et al. 2012). The race-specific interaction between rice and *Xoo* is thought to follow the classic gene-for-gene relationship (Flor 1971) and is similar to the host–pathogen model for understanding the molecular mechanisms in host resistance to pathogens (Dai et al. 2007). However, BB resistance mechanisms appear to have distinctive differences from other characterized R-genes. Although most of the reported R-genes in other crop pathogen systems are dominant in nature, almost one-third of the R-genes conferring resistance to *Xoo* have been reported as recessive (Verdier et al. 2012). Comparing the prevalent class of functionally defined R-genes that encode intracellular nucleotide-binding/leucine-rich repeat (NBS–LRR) proteins as immune receptors to initiate defense signaling, the R-genes to *Xoo* encode various types of proteins (Belkhadir et al. 2004; McHale et al. 2006; Xiang et al. 2006; Hammond-Kosack and Kanyuka 2007). To date, more than 39 R-genes conferring host resistance to various strains of *Xoo* have been identified and some of those have been characterized (Cheema et al. 2008; Wang et al. 2009; Guo et al. 2010; Miao et al. 2010; Verdier et al. 2012; Wang et al. 2014a; Zhang et al. 2014). A total of 14 recessive genes—*xa5, xa8, xa13, xa15, xa19, xa20, xa24, xa25, xa26b, xa28, xa31, xa32, xa33,*and *xa34*—in the series from *Xa1* to *Xa39* have been identified with the remainder dominant (Chen et al. 2011; Liu et al. 2011). Of these 39 R-genes, nine R-genes—*Xa1, Xa3/Xa26, xa5, xa13, Xa10, Xa21, Xa23*, *xa25* and *Xa27*—have been isolated and characterized as encoding five types of proteins, suggesting multiple mechanisms of R-gene-mediated *Xoo* resistance (Song et al. 1995; Yoshimura et al. 1998; Iyer and McCouch 2004; Sun et al. 2004; Gu et al. 2005; Chu et al. 2006; Tian et al. 2014; Wang et al. 2014b). Seven R-genes (*Xa4, Xa7, Xa22, Xa30, Xa31, Xa33*, and *xa34*) have been fine-mapped based on morphological and molecular markers. In some of them, however, there are discrepancies such as duplicate nomenclature of genes for the same gene without compelling evidence, for example, for the dominant R-genes *Xa25, Xa26, Xa32* and *Xa33*,and the recessive R-genes *xa25, xa26, xa32,* and *xa33* (Gao et al. 2001; Lee et al. 2003; Sun et al. 2004; Ruan et al. 2008; Korinsak et al. 2009; Zheng et al. 2009; Liu et al. 2011; Kumar et al. 2012). The R-genes are distributed among nine rice chromosomes and 12 of the R-genes are intensively clustered on chromosome 4 (*Xa1, Xa2, Xa12, Xa14, Xa30*, and *Xa31*) and chromosome 11 (*Xa3/26, Xa4, Xa10, Xa21, Xa22,* and *Xa23*), respectively.

In Korea, BB isolates were grouped into five races (K1–K5) based on the response of five rice cultivars to *Xoo* infection as a differential system, before the occurrence of the new BB race (K3a) in the southwestern coastal plain area in 2003 (Noh et al. 2003). Approximately 95 % of Korean BB isolates are concentrated in the four main races, K1, K2, K3, and K3a, and the races K4 and K5 have been less frequently reported (Noh et al. 2003; Jeung et al. 2006; Noh et al. 2013). In particular, the K3a race has been reported as the prevailing race in Korea because of fewer resistant cultivars (Jeung et al. 2006).

A series of japonica breeding lines, IR83261-3-7-23-6-2-1-1-2-1-2 (11325), IR83261-5-13-8-4-1-2-2-1-1-1 (11327), and IR83261-5-13-8-4-1-2-2-1-2-1 (11328), derived from indica donor line IR65482-7-216-1-2 were found to have high levels of resistance to the new *Xoo* race K3a in Korea. The present study was carried out to identify the genetic basis of the new BB R-gene conferring resistance to Korean *Xoo* race K3a in IR65482-7-216-1-2, to construct a physical map containing the region, and to identify candidate genes. The expression levels of candidate genes were confirmed by RT-PCR for isolation and characterization of this gene.

Materials and methods

Plant materials

Anmi, a japonica rice cultivar, was developed by backcrossing a donor line (IR65482-7-216-1-2) to the recurrent parent Junam. Anmi demonstrated a susceptible reaction to the Korean *Xoo* strain K3a isolate (HB01009). However, in contrast to Anmi, two of its sister lines (advanced backcrossed lines 11325: BC_3F_8 and 11327: BC_4F_8) demonstrated resistance to K3a (Supplementary Fig. 1). The F_1 progenies from crosses between 11325 and the highly susceptible cultivars Anmi and Ilpum were used to examine whether the BB resistance gene acted as a dominant or recessive trait. Two F_2 populations composed of 787 and 796 F_2 plants were created from the crosses Anmi/11325 and Ilpum/11325, respectively, for physical mapping of the target gene. Each F_2 individual was self-fertilized to obtain a set of F_3 lines. Another F_2 population, derived from a cross between IRBB4 (*Oryza sativa* sub sp. *indica*)

carrying *Xa4* and 11325 carrying the new gene, was used for an allelism test between *Xa4* and the target R-gene. The cultivar IR24, one of the progenitors of IR17494-32-3-1-1-3, and the near-isogenic lines IRBB1, IRBB3, IRBB5, IRBB8, IRBB10, IRBB13, IRBB21, and IRBB57 were used in haplotype analysis. Seeds of these lines were obtained from the Plant Breeding, Genetics, and Biotechnology Division of the International Rice Research Institute, Los Baños, Laguna, Philippines.

BB inoculation and evaluation of resistance

Four different virulent strains—K1(HP01013), K2(HP01014), K3(HP01015), and K3a(HP01009)—of *Xoo* from Korea were used for the BB evaluation. At maximum tillering stage, the F_1 progenies, the F_2 individuals, and F_3 lines derived from crosses 11325 (11327) and Anmi, Ilpum, and IRBB4 were inoculated with the strains using the leaf-clipping method (Kauffman et al. 1973) under field conditions in Suwon, Korea. Reaction to the pathogen was evaluated at 14 days after inoculation. Evaluation of leaf damage level by the pathogen was carried out by measuring the average lesion length of three leaves (highly resistant <1 cm, resistant 1–3 cm, moderately resistant 3–5 cm, susceptible 5–10 cm, highly susceptible >10 cm).

PCR conditions

Cultivar Anmi, the two sister lines (11325 and 11327), and the resistant donor (IR65482-7-216-1-2) line were used to determine the location of the new resistance gene conferring resistance to K3a. The lines were compared through PCR analysis using a total of 287 DNA markers (SSR 221 and STS 66) selected for an even coverage of the rice genome. PCR amplification was performed in 20-μl volumes of reaction mixture containing 10 pMol of each primer, 50 mM KCL, 1.5 mM $MgCl_2$, 25 ng template DNA, and 0.02 U/μl of GenDepot DNA polymerase. The PCR condition used consisted of one cycle of 8 min at 95 °C, followed by 35 cycles of 30 s at 55 °C, 1 min at 72 °C, and a final cycle of 10 min at 72 °C.

Development of DNA markers

To detect co-dominant DNA markers with the R-gene, additional DNA markers were developed in the region of the flanking markers RM1233 and RM5766. Primer sequences of published rice microsatellite markers (RM) located within the region were obtained from the Gramene database (http://www.gramene.org/; McCouch et al. 2002). Markers for detecting insertion-deletion (Indel) polymorphisms were developed using the DNA polymorphism database (http://shenghuan.shnu.edu.cn/Default.aspx?tabid=5641; Shen et al. 2004). Cleaved amplified polymorphic sequence (CAPS) markers were applied to construct high-resolution fine-mapping and haplotype tests using the sequencing data of gene-based STS-PCR products. Genomic DNA sequences for each bacterial artificial chromosome (BAC) clone were determined through primer walking based on the corresponding sequences in the Nipponbare genome by GreenGene Bio Tech, Inc. (Seoul, Korea). To design primers for Indel markers, Primer3 version 4.0 (http://frodo.wi.mit.edu/primer3/) was used (Rozen and Skaletsky 2000). Four gene-specific DNA markers, MP1+MP2, 10571.T17/*Hinf*1, and U1/I1, tightly linked to the resistance genes *Xa4, Xa3,* and *Xa21*, respectively, were used to analyze the gene validation.

Graphical mapping/gene annotation

F_2 populations were used to determine the exact position of the nearest recombination event to the target region on chromosome 11. The genetic fine-mapping of the target R-gene was constructed according to the physical distance of the DNA markers on BAC and P1-derived artificial chromosome (PAC) clones of cv. Nipponbare released by the International Rice Genome Sequencing Project (IRGSP). All genes with clear open-reading frames (ORFs) were analyzed based on the available rice genome sequence and annotation databases from NCBI (www.ncbi.nim.nih.org/unigene) and TIGR release 7.1 (http://rice.plantbiology.msu.edu/) and a putative function for each gene identified in the region of interest was annotated using BLAST-P utility (www.ncbi.nlm.nih.gov).

cDNA synthesis and semi-quantitative reverse transcription PCR (RT-PCR)

Total RNA was extracted from collected rice samples by using TRIzol reagent (Invitrogen, UK) according to the manufacturer's protocol. DNA was removed from RNA samples by using TURBO DNA-Free DNase (Ambion, Inc.). Total RNA was quantified by measuring absorbance at 260 and 280 nm using the Nanodrop ND 1000-spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, NC, USA).

Total RNA from leaves collected at 1, 4, 8, 24, and 48 h after inoculation was used to synthesize each pool of cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Briefly, cDNA synthesis from 10 μg of total RNA is incubated with 2 μl oligo (dT)18 primer. Subsequently, the buffer, dNTPs (final conc. 1 mM), 400 U M-MuLV Reverse Transcriptase, and 40 U RiboLock™ RNase Inhibitor were added and incubated for

60 min at 42 °C. The reaction was terminated through heating at 70 °C for 5 min. Semi-quantitative RT-PCR analyses were performed with 250 ng of cDNA for 28–35 cycles, with an annealing temperature of 55–62 °C. Rice *actin1* (Osactin) was used as an internal control, with the forward primer 5′-CTGCTATGTACGTCGCCATC-3′ and the reverse primer 5′-AGTCTCATGGATACCCGCAG-3′, and the same PCR conditions as described above.

Results

Resistance reaction to Korean BB races

The breeding lines and cultivars tested in this study were estimated for resistance levels against four BB isolates representing the spectrum of virulence in Korea (Table 1). All the plants, including International Rice Bacterial Blight (IRBB) NIL IRBB3, showed a resistant reaction to K1(HB01013), K2(HB01014), and K3(HB01015) isolates. However, in the case of K3a(HB01009), only three lines—11325, 11327, and IR65482-7-216-1-2 (containing the unknown BB R-gene)—showed strong resistance (Table 1).

Detection of substituted chromosomal segments

To ascertain the donor-derived segments conferring resistance to BB race K3a, a total of 261 DNA markers were used for background selection of Anmi along with two advanced backcross lines (ABLs), 11325 and 11327, and a graphical map constructed to determine the genomic position of the new BB R-gene, tentatively designated as *Xa40(t)* (Fig. 1). The substituted segments were distributed on chromosomal regions in the genetic background of two ABLs. On the graphical map, line 11325 had 10 substituted segments on chromosomes 1, 2, 6, 8, 11, and 12, whereas line 11327 had two substituted segments on chromosomes 4 and 11, respectively (Fig. 1). Substituted segments were not found on the remaining chromosomes in this genetic background survey. In the validation test with the F_2 population, including the new gene, we could exclude other candidate regions to narrow down the target region by fine-mapping. The analysis of introgression fragments positively showed the target region of *Xa40(t)* to be the telomeric region on the long arm of chromosome 11, as this section overlapped in the two R-lines, flanked by two polymorphic markers, RM 1233 and RM 5766.

Inheritance of the resistance gene

To analyze the inheritance of resistance associated with *Xa40(t)* derived from the R-donor line, four populations were constructed by crossing cultivars Anmi and Ilpum as susceptible parents and 11,325 and 11,327 as resistant parents. The isolate HB01009 of *Xoo* race K3a was selected to inoculate the parents along with F_1 progenies at the maximum tillering stage. The average lesion lengths of both susceptible parents (Anmi and Ilpum) were 15.45 ± 2.15 cm and 13.1 ± 1.53 cm at 14 days after inoculation (DAI), respectively (Supplementary Fig. 2). Resistant parents 11,325 and 11,327 were resistant to the K3a isolate, with an average lesion length of 0.51 ± 0.15 cm and 1.2 ± 0.35 cm at 14DAI. F_1 plants developed from each combination showed moderate resistance to the K3a isolate, with an average lesion length of 4.5 ± 2.15 cm. The two mapping populations, Anmi/11,325 and Ilpum/11,325, were selected for genetic analysis of the gene since 11,325 showed a relatively stronger resistance. A total of 1583 F_2 individuals from the two crosses were inoculated with K3a isolate at the maximum tillering stage under field conditions. The distribution of lesion lengths in the F_2 populations was bimodal with an apparent valley at approximately 5 cm (Supplementary Fig. 3). Using this value (lesion length <5.0 cm) to differentiate between resistant and susceptible, the segregation ratio of F_2 individuals from the cross Ilpum/11325 was 555 resistant and 241 susceptible, which is consistent with the expected allelic frequency of the 3:1 ratio ($X^2 = 1.10$, $P > 0.05$). Similarly, the segregation ratio of F_2 individuals from the cross Anmi/11325 was 578 resistant and 209 susceptible, which was also consistent with the expected 3:1 ratio ($X^2 = 1.03$, $P > 0.05$, Table 2). This evidence indicates that the resistance of 11325 to BB race K3a is controlled by a single gene with incomplete dominance.

Table 1 Leaf reaction of tested plants to four different BB isolates

Lines/cultivars	Reaction to four K races (isolate #)				R-gene
	K1(HB01013)	K2(HB01014)	K3(HB01015)	K3a(HB01009)	
11325, 11327, IR65482-7-216-1-2	R	R	R	R	Unknown
Ilpum	S	S	S	S	None
Anmi, Junam, Shindonjin, IRBB3	R	R	R	S	*Xa3*

R resistant (lesion length <3 cm), *MR* moderately resistant (lesion length 3–5 cm), *S* susceptible (lesion length >5 cm)

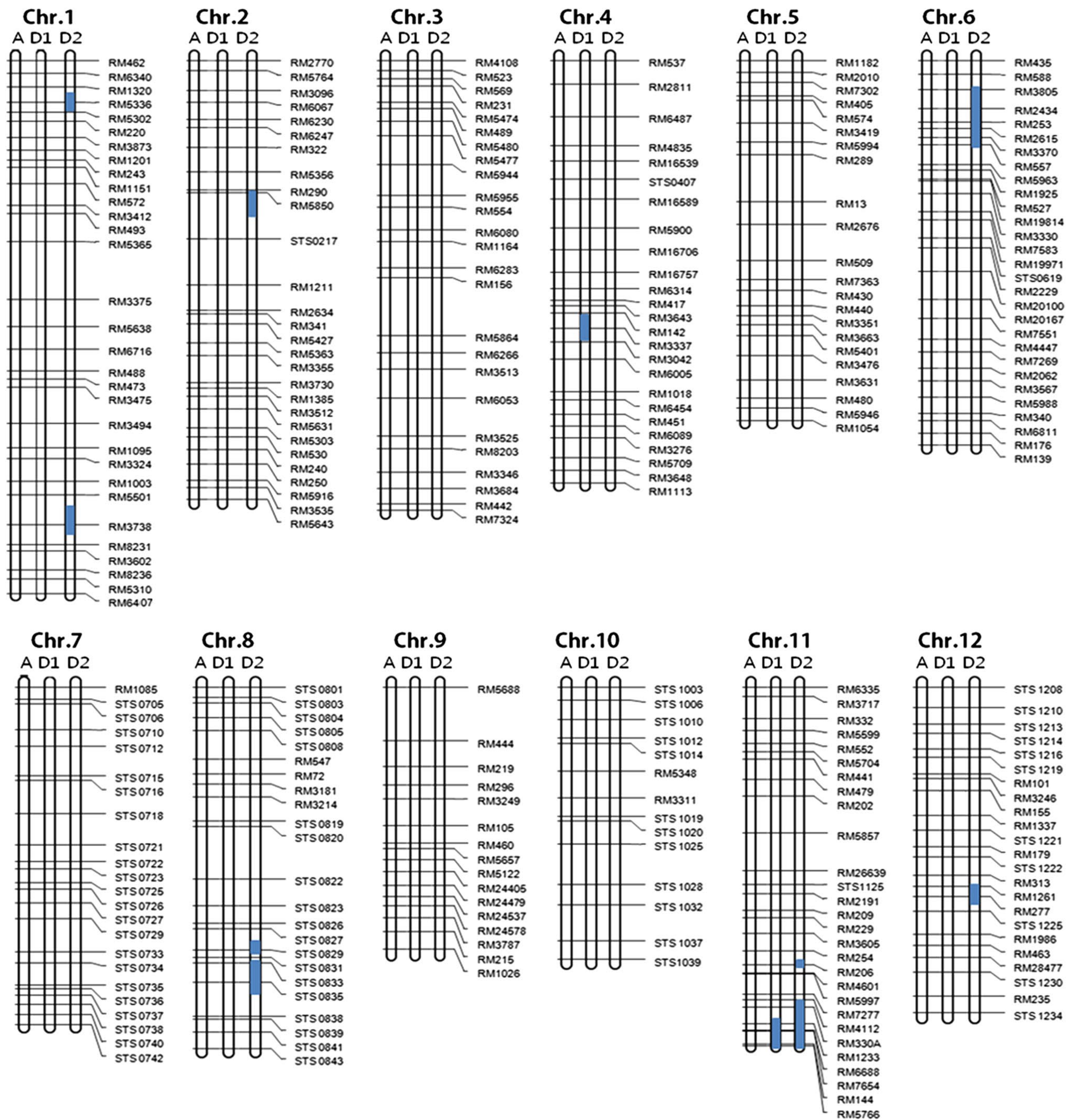

Fig. 1 Background selection of two tested lines in Anmi genetic background. *A* is Anmi, *D1* and *D2* are advanced backcross lines 11325 and 11327, respectively. *Blue bars* indicating introgressed DNA fragment from the R-donor plant show the polymorphic region between Anmi and the sister line (color figure online)

Construction of a physical map spanning the *Xa40* gene

A total of 54 molecular markers were selected and used to identify the markers linked to the target gene. A parental survey with R- and S-parents was carried out using PCR analysis. From the survey, 20 molecular markers were selected as anchor markers within the flanking region of RM1233 to RM5766 (Fig. 2a). A total of 80 F_2 individuals showing a highly susceptible reaction (lesion length >12 cm) to the K3a isolate were selected from both cross combinations (Ilpum/11325 and Anmi/11325) to position *Xa40(t)* within the region. On the reference sequences of cv.

Table 2 Resistance response of F_1 and F_2 crosses between Ilpum and Anmi against race K3a

Cross	Resistance response of parents		F_1 resistance response	F_2 resistance response					
	P1	P2		Resistant	Susceptible	Total	Seg.	X^2	*P*
Ilpum/11325	S	R	MR	555	241	796	3:1	1.10	0.05
Anmi/11325	S	R	MR	578	209	787	3:1	1.03	0.05

Seg. segregation ratio

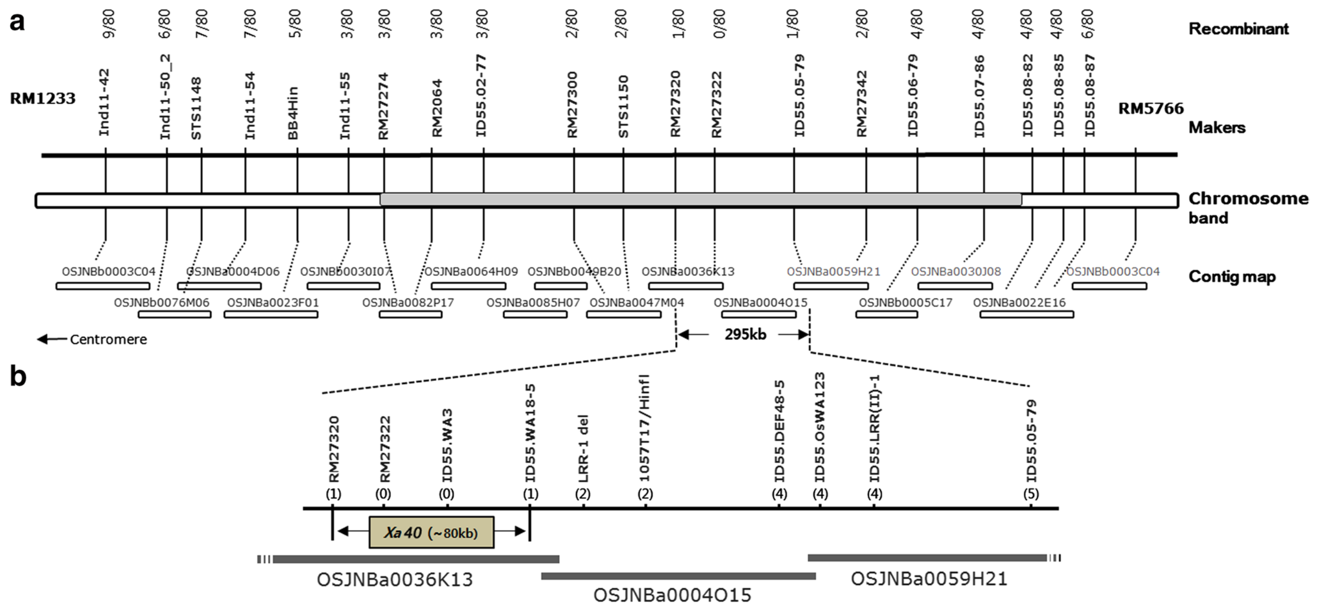

Fig. 2 Physical map of *Xa40(t)* conferring resistance to race K3a. **a** shows the 22 molecular markers located within the target region of 26.53–28.34 Mb on chromosome 11. Recombinant indicates a recombination event in the tested mapping population. The *gray bar* on the chromosome band corresponds to the physical region between 27 and 28 Mbp on chromosome 11. **b** is a fine map denoted by RM27320 and ID55.05-79 within a 295-kb physical interval. The *numbers in parentheses* indicate that the recombinants occurred at the corresponding marker loci derived in the resistant and susceptible plants. In the flanking region, a total 10 DNA markers are distributed. Based on these recombinants, *Xa40* was delimited to an 80-kb interval between RM27320 and ID55.WA18-5, which is spanned by the BAC clone OSJNBa0036K13

Nipponbare released by IRGSP, 17 BAC clones were listed in the Nipponbare rice genome sequence, which covered the flanking region with a physical interval of 1.81 Mbp (Fig. 2a; Supplementary Table 1). The anchor markers landed on all except three of the BAC clones (OSJNBa0085H07, OSJNBb0049B20, and OSJNBa0004O15) with an average physical distance of about 86 kb between markers. Analysis of the number of distinct recombinants using genotypic data and phenotypic reaction showed that, among the 80 F_2 individuals, nine and six distinct recombinants were identified within the region of interest. The marker RM27322, flanked by RM27320 and ID55.05-79, showed a co-segregation reaction in the population, allowing the position of the target gene to be narrowed down and mapped to a region within a 295-kb physical interval on chromosome 11.

To further narrow the interval containing *Xa40(t)*, we selected an additional 180 F_2 individuals showing a susceptible reaction (lesion length ≥8 cm) from the same populations. In addition, one SSR marker, 20 STS markers, and one CAPS marker between RM27320 and ID55.05-79 were developed according to the sequence information from the rice genome database. Of these, markers displaying polymorphism between parents were chosen. The 10 markers, including the flanking markers (RM27320 and ID55.05-79), were used to detect the number of distinct recombinants in the newly selected 180 susceptible F_2 individuals. Within the BAC clone OSJNBa0036K13, a total of one, zero, zero, and one recombinant from all the tested F_2 individuals were identified with markers RM27320, RM27322, ID55WA3, and ID55.WA18-5, respectively. For OSJNBa0004O15, a total of two, two, and four

Table 3 Annotation data of the putative ORFs in the target BAC clone (OSJNBa0036K13) region in the Nipponbare genome database

BAC	Locus identifier	Putative function	Predicted length (bp)	Exon	Full-length cDNA
AC148822 (OSJN-Ba0036K13)	LOC_Os11g46870	Protein kinase, putative, expressed	2040	4	
	LOC_Os11g46880	Protein kinase domain-containing protein, expressed	2133	4	
	LOC_Os11g46890	Expressed protein	597	1	
	LOC_Os11g46900	Wall-associated receptor kinase 3 precursor, putative, expressed	2121	4	
	LOC_Os11g46910	Transposon protein, putative, CACTA, En/Spm subclass, expressed	5266	9	AF121139
	LOC_Os11g46920	Retrotransposon protein, putative, unclassified, expressed	3051	2	
	LOC_Os11g46930	Transposon protein, putative, unclassified, expressed	5747	2	AK289114
	LOC_Os11g46940	Transposon protein, putative, CACTA, En/Spm subclass, expressed	1074	1	

recombinants were detected by markers LRR-1del, ID55. DEF48-5, and ID55.OsWa124, respectively. For OSJN-Ba0059H21, five recombinants were shown by ID55.05-79 (Fig. 2b). No recombinants were identified at markers RM27322 and ID55.WA3, which co-segregated with the *Xa40(t)* locus (Fig. 2b). Thus, based on the physical map of japonica rice Nipponbare, the *Xa40(t)* gene was defined by RM27320 and ID55.WA18-5 located on the BAC clone OSJNBa0036K13. The physical distance between the two markers is approximately 80 kb (Fig. 2b).

Candidate gene annotation

The intervening genomic region (~80 kb) of chromosome 11 where the *Xa40 (t)*gene was mapped contains at least eight gene models according to release 7 of the MSU Rice Genome Annotation Project Database and Resource (Table 3). The functionality of the genes predicted in the region includes one gene-encoding protein kinase, one encoding protein kinase domain-containing protein, one encoding expressed protein, one encoding wall-associated receptor kinase 3 precursor, three encoding transposon proteins, and one encoding retrotransposon protein. The region of *Xa40(t)* contains a gene (LOC_11g46900) encoding a wall-associated receptor kinase (WAK) 3 precursor. We considered *WAK3*, a promising candidate gene conferring resistance to BB race K3a.

Semi-quantitative RT-PCR analysis

To compare the expression level of the eight genes identified in the target region, a primer set (ID55.WA3) based on coding DNA sequence (CDS) was designed (Supplementary Table 2). The semi-quantitative PCR analysis of resistance to K3a race-related genes was performed using cDNA synthesized from the leaf of each sample collected at 0, 1, 4, 8, 24, and 72 h after BB inoculation. Expression patterns of the genes were observed in Anmi and 11325, respectively (Fig. 3). The accumulation of *WAK3* messenger RNA (LOC_11Os11g46900) was strongly promoted by inoculation in leaves of resistant 11325, but was not induced in the susceptible cultivar Anmi. The transcript levels of LOC_11g46880 were induced only in the susceptible line. Apart from these two genes, there were no significant differences between the resistant and susceptible lines in the transcript levels of the genes tested. The mRNA induction of LOC_11Os11g46900 was also pronounced during the procedure of leaf sampling for RNA extraction, indicating that the *WAK3* gene may be induced in response to wound stress by leaf-clipping.

Haplotype test

In the haplotype test using the STS primer ID55.WA3, only seven lines out of 24 cultivars/lines showed the same size of allele, A1, against 11325 with *Xa40(t)* (Table 4). The PCR products of the A1 type were recovered for sequencing by cloning and a single nucleotide polymorphism was found in A1 alleles, allowing classification into the G and A type. Among the four ancestors of IR65482-7-216-1-2, none showed 11325-type allele A1_A (Table 4). This SNP was confirmed by enzyme digestion using *HpCH4III* on 1.2 % agarose gel (Supplementary Fig. 4). Tracking the source of this gene could not be undertaken in this study as we did not have access to seeds of all of the ancestors in the pedigree.

Discussion

BB disease caused by *Xoo* is one of the major constraints for rice (*O. sativa* L.) production in most rice-growing

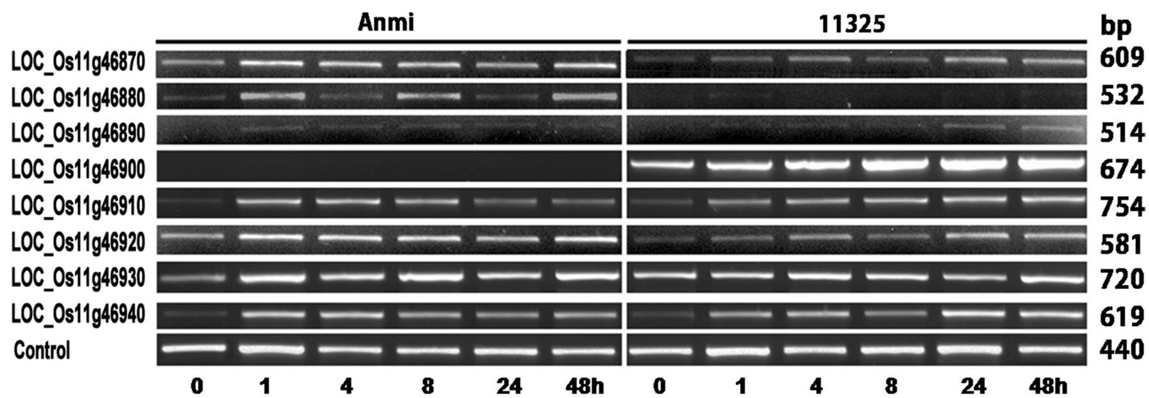

Fig. 3 Semi-quantitative RT-PCR assessment of gene transcript levels in the leaf tissue from resistant (11325) and susceptible (Anmi) genotypes following 0–48 h of inoculation. Rice *actin1* was used as an internal control in the same PCR condition

Table 4 Haplotypes of the *Xa40(t)* locus based on alleles of the *WAK3* gene in rice cultivars

Lines or cultivars	Phenotype to K3a	Subspecies	Allele type
IR65482-7-216-1-2	Resistant	Indica	A1_A
IRBB57, IR17494-32-3-1-1-3		Indica	A1_G
IRBB21		Indica	A2
11325, 11327, 11328		Japonica	A1_A
IRBB4	Moderately R.	Indica	A1_G
IR24, IRBB1, IRBB3, IRBB5, IRBB8, IRBB10, IRBB13	Susceptible	Indica	A2
Rathuheenati, BPI121-407		Indica	A6
Anmi, shindonjin, Ungwang, Gopum		Japonica	A3
Jinbu		Japonica	A4
Ilpum,		Japonica	A5
O. australiensis		Wild	Absent

IRBB1(*Xa1*), IRBB3(*Xa3*), IRBB4(*Xa4*), IRBB5(*xa5*), IRBB8(*Xa8*), IRBB10(*Xa10*), IRBB13(*Xa13*), IRBB21(*Xa21*), and IRBB57(*Xa4+5+21*) are BB monogenic lines. BPI121-407, Rathuheenati, IR17494-32-3-1-1-3, and *O. australiensis* are the ancestor lines of IR65482-7-216-1-2, which was used as the resistance R-donor line. Allele designations were based on the polymorphic amplicon by the *WAK3* gene-specific molecular marker (ID55.WA3)

areas worldwide. The disease is vascular, resulting in a systemic infection that produces gray to white lesions along the veins (Nelson et al. 1994). The most effective control method considered for rice BB disease is to use resistant cultivars. To date, around 39 BB resistance genes have been mapped on different rice chromosomes, and nine R-genes have been isolated using map-based cloning (Dai et al. 2007; Wang et al. 2014a). Many resistance genes have broken down following their extended use over wide areas under conditions conducive to disease development and intense selection pressure for virulent races of the pathogen. Therefore, new approaches using R-gene pyramiding, or a novel resistance gene with multiple-resistance specificity from a single locus, are necessary to provide stronger and more diversified levels of resistance as well as sustainable durable resistance (Gu et al. 2005; Chen et al. 2011). Here, we report evidence of the identification of a new BB gene conferring resistance to four BB isolates endemic to Korea.

Resistant lines 11325 and 11327 used in this study were developed from a backcross breeding program for the introgression of the *Bph18* gene to a japonica background using IR65482-7-261-2 derived from *O. australiensis* (Suh et al. 2011). To date, there are no known BB resistance genes in the two lines or the donor, even though the lines proved resistant to all races: K1, K2, K3, and K3a (Table 1). The R-gene *Xa3* is the main R-gene deployed against BB in resistant cultivars throughout Korea. The occurrence of a new BB pathotype underscores the need for new resistance genes.

Analysis of the inheritance of *Xa40(t)* in F_1 plants from three populations of Shindongjin/11325(data not shown) showed that the F_1 plants exhibited an intermediate resistance—more resistant than susceptible, but less

than the resistant parent (Table 2)—and thus the resistance was considered to be inherited by a gene with incomplete dominance. This inheritance pattern is similar to the case of the *xa33(t)* gene in rice (Korinsak et al. 2009). It is frequently reported in rice resistance studies that phenotype was controlled by a dominant or recessive gene with incomplete dominance (Ise et al. 2002; Zhang et al. 2006). The segregation ratio of F_2 individuals from both populations was shown to fit the expected 3(R):1(S) ratio when the resistance level was determined by lesion length <5.0 cm (Table 2). The R-gene designated as *Xa40(t)* was flanked by RM1233 and RM5766, covering around 27.0–28.9 Mbp of the physical region on the long arm of chromosome 11, along with 17 rice BAC clones (Fig. 2; Supplementary Table 1). According to previous reports, six BB R-genes (*Xa21, Xa4, Xa3/Xa26, Xa22, Xa10,* and *Xa23*) were already reported on chromosome 11L; in particular, *Xa4* and *Xa3/Xa26* were clustered together and located close to each other in that region (Song et al. 1995; Iyer and McCouch 2004; Sun et al. 2004; Verdier et al. 2012; Wang et al. 2014a). To discriminate these genes from *Xa40(t)*, a validation test was performed with DNA markers specifically able to detect the genes. In this PCR analysis, none of the lines showed amplicons relating to R-gene alleles of *Xa3/Xa26* and *Xa4* (data not shown). In phenotypic response to BB, the susceptible japonica parents Anmi, Shindongjin, and Junam, which include BB resistance gene *Xa3*, all showed a susceptible reaction to K3a isolate (Table 1) and the *Xa3* indica monogenic line IRBB3 also exhibited a susceptibility to K3a (Table 4). Both IRBB4 and japonica NILs, including the *Xa4* gene, showed moderate resistance to the isolate. Based on these observations, we decided to conduct an allelism test to confirm the difference between *Xa4* and *Xa40(t)*, even though the two genes had different responses to the K3a isolate, showing moderate resistance and resistance, respectively, in this study. A total of 867 F_2 plants from the cross between IRBB4 (with *Xa4*) and 11325 were inoculated with K3a isolate and the segregation pattern of resistant (lesion length below 5 cm) and susceptible plants had a ratio of 13R:3S ($X^2 = 5.76$, $P > 0.05$). Physically, both genes are closely linked with each other (approximately 0.5 Mbp). Given this fact, it is difficult to expect this many susceptible lines by chromosomal crossover. From the result, it is possible to assume that the occurrence of susceptible lines of high frequency resulted from a gene peculiar to *Xa4* depending on zygosity type. The response of the *Xa4* gene to K3a varied depending on allele type and gene combination (Suh et al. 2013). In particular, it has shown an unstable reaction to the race in heterozygous conditions.

Even though the allelism test carried out before fine-mapping did not give a clear answer because of the complexity caused by gene recombination, some evidence such as the difference in phenotypic reaction to the same race, a validation test using gene-specific markers (Supplementary Table 3), and haplotype analysis indicate that the R-gene in 11325 is a different allele from *Xa4*.

In this study, the region was narrowed down to the BAC clone regions flanked by markers RM1233 and RM5766 on chromosome 11L. Using recombinant analysis, we defined an interval of approximately 295 kb flanked by the ORF-based markers (Supplementary Table 2). Through additional analysis of recombination events within 180 F_2 plants, the *Xa40(t)* gene was further narrowed down to an 80-kb region on chromosome 11L. Sequence data from the region harboring the *Xa40(t)* gene indicated that this region contained eight candidate genes (Table 3), which all differed from other *Xoo* resistance genes reported to date. Given that the products of the other four BB R-genes that have been cloned are unique and not found in other plant species (*Xa21* and *Xa26* encode for similar receptor-like proteins; Dai et al. 2007), this result suggests a new type of BB resistance gene. In semi-quantitative PCR analysis confirming the gene associated with BB resistance, the transcript level of LOC_Os11g46900 in 11325 displayed a tendency to gradually increase over time after inoculation; whereas, this gene was not induced in the susceptible cultivar Anmi (Fig. 3). With reference to the mRNA induction of the gene at 0 h after inoculation, the patterns by mock inoculation were identical with each other regardless of the time after inoculation (data not shown). The wall-associated kinases, or WAKs, are receptor-like kinases that are linked to the pectin fraction of the cell wall and have a cytoplasmic protein kinase domain (Kohorn and Kohorn 2012). This ability to bind and respond to several types of pectin correlates with a demonstrated role for WAKs in both pathogen response and cell expansion during plant development. WAK expression is induced by wounding, pathogen infection, and by many stresses such as ozone and heavy metals (He et al. 1998; Anderson et al. 2001; Kohorn 2001; Wagner and Kohorn 2001; Sivaguru et al. 2003). We are not sure whether the *WAK3* gene confers resistance to BB in this study. Despite this, we consider that this study suggests a potential new BB R-gene, and results reported in this study, coupled with existing knowledge, provide evidence for this case. In a further study, we plan to identify the function of candidate genes through advanced methods, synthesis of the full-length cDNA of this gene and gene silencing.

In Korea, the resistance of the *Xa3* gene prevailing in most resistant Korean cultivars was broken down by the occurrence of a new BB race, K3a. This paper reports a new source of resistance to race K3a, the subsequent fine-mapping of the resistance gene to a region on rice chromosome 11, and the identification of a candidate gene underpinning resistance. The identification of *Xa40(t)* conferring

resistance to race K3a is of significant value to rice breeding programs through the expansion of the pool of R-genes conferring resistance to local races of *Xoo* and the use of advanced backcross lines with the gene for marker-assisted breeding and gene pyramiding strategies. Further characterization of *Xa40(t)* at the protein level will be helpful to elucidate the mechanisms of resistance.

Author contribution statement SMK (s.kim@irri.org) carried out the development of the mapping population, genetic analysis, data gene mining, and primer design, and drafted the manuscript. JPS (suhjp@rda.go.kr) participated in the phenotypic selection and development of the backcrossed lines. YQ (qinyang2013@korea.kr) performed cDNA synthesis and semi-quantitative reverse transcription PCR. THN (nohtw831@rda.go.kr) contributed to the isolation and culture of virulent strains of *Xoo* used in this study. RFR (r.reinke@irri.org) was involved in phenotypic selection for fine-mapping and data mining of candidates. KKJ (k.jena@irri.org) designed and coordinated the study, assisted with genetic analyses, and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgments This research was supported in part by a grant to the Agenda Program (Code No. PJ00926202) of the Rural Development Administration, Republic of Korea, and Global Rice Science Partnership program of IRRI. We thank the IRRI communication team for carefully editing the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments comply with the current laws of the country in which they were performed.

References

Adhikari TB, Mew TW, Teng PS (1994) Phenotypic diversity of *Xanthomonas oryzae* pv. *oryzae* in Nepal. Plant Dis 78:68–72

Anderson CM, Wagner TA, Perret M, He ZH, He D, Kohorn BD (2001) WAKs: cell wall-associated kinases linking the cytoplasm to the extra cellular matrix. Plant Mol Biol 47:197–206

Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signalling: NBS–LRR proteins and their partners. Curr Opin Plant Biol 7:391–399

Cheema K, Grewal N, Vikal Y, Sharma R, Lore JS, Das A, Bhatia D, Mahajan R, Gupta V, Bharaj TS, Singh K (2008) A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38 kb region on chromosome 4L and transferred to *Oryza sativa* L. Genet Res Camb 90:397–407

Chen S, Liu X, Zeng L, Ouyang D, Yang J, Zhu X (2011) Genetic analysis and molecular mapping of a novel recessive gene *xa34(t)* for resistance against *Xanthomonas oryzae* pv. *oryzae*. Theor Appl Genet 122:1331–1338

Chu Z, Yuan M, Yao J, Ge X, Yuan B, Xu C, Li X, Fu B, Li Z, Bennetzen JL, Zhang Q, Wang S (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes Dev 20:1250–1255

Dai L, Liu X, Xiao Y, Wang G (2007) Recent advances in cloning and characterization of disease resistance genes in rice. J Integr Plant Biol 49:112–119

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

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

Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, Yang F, Chu Z, Wang G, White FF, Yin Z (2005) R gene expression induced by a type-III effector triggers disease resistance in rice. Nature 435:1122–1125

Guo S, Zhang D, Lin X (2010) Identification and mapping of a novel bacterial blight resistance gene *Xa35(t)* originated from *Oryza minuta*. Sci Agric Sin 43:2611–2618

Hammond-Kosack KE, Kanyuka K (2007) Resistance genes (R genes) in plants. In: Encyclopedia of Life Sciences. Wiley, Chichester. www.els.net/. doi:10.1002/9780470015902.a0020119

He ZH, He D, Kohorn BD (1998) Requirement for the induced expression of a cell wall associated receptor kinase for survival during the pathogen response. Plant J 14:55–63

Ise K, Ishikawa K, Li C, Ye C (2002) Inheritance of resistance to rice stripe virus in rice line 'BL1'. Euphytica 127:185–191

Iyer AS, McCouch SR (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. Mol Plant Microbe Interact 17:1348–1354

Jeung JU, Heu SG, Shin MS, Vera Cruz CM, Jena KK (2006) Dynamics of *Xanthomonas oryzae* pv. *oryzae* populations in Korea and their relationship to known bacterial blight resistance genes. Phytopathology 96:867–875

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

Kohorn BD (2001) WAKs: cell wall associated kinases. Curr Opin Cell Biol 13:529–533

Kohorn BD, Kohorn SL (2012) The cell wall-associated kinases, WAKs, as protein receptors. Front Plant Sci 3(88):1–5

Korinsak S, Sriprakhon S, Sirithanya P, Jairin J, Korinsak S, Vanavichit A, Toojinda T (2009) Identification of microsatellite markers (SSR) linked to a new bacterial blight resistance gene *xa33(t)* in rice cultivar 'Ba7' Maejo. Int J Sci Technol 3:235–247

Kumar PN, Sujatha K, Laha GS, Srinivasa Rao K, Mishra B, Viraktamath BC, Hari Y, Reddy CS, Balachandran SM, Ram T, SheshuMadhav M, Shobha Rani N, Neeraja CN, Ashok Reddy G, Shaik H, Sundaram RM (2012) Identification and fine-mapping of *Xa33*, a novel gene for resistance *to Xanthomonas oryzae* pv. *oryzae*. Phytopathology 102(2):222–228

Lee KS, Rasabandith S, Angeles ER, Khush GS (2003) Inheritance of resistance to bacterial blight in 21 cultivars of rice. Phytopathology 93:147–152

Liu Q, Yuan M, Zhou Y, Li XG, Xiao J, Wang S (2011) A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. Plant Cell Environ 34:1958–1969. doi:10.1111/j.1365-3040.2011.02391.x

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

McHale L, Tan X, Koehl P, Michelmore RW (2006) Plant NBS–LRR proteins: adaptable guards. Genome Biol 7:212

Mew TW, Vera Cruz CM, Medalla ES (1992) Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. Plant Dis 76:1029–1032

Mew TW, Alvarez AM, Leach JE, Swings J (1993) Focus on bacterial blight of rice. Plant Dis 77:5–12

Miao L, Wang C, Zheng C, Che J, Gao Y, Wen Y, Li G, Zhao K (2010) Molecular mapping of a new gene for resistance to rice bacterial blight. Sci Agric Sin 43:3051–3058

Mizukami T, Wakimoto S (1969) Epidemiology and control of bacterial leaf blight of rice. Annu Rev Phytopathol 7:51–72

Natraj Kumar P, Sujatha K, Laha GS, SrinivasaRao K, Mishra B, Viraktamath BC, Hari Y, Reddy CS, Balachandran SM, Ram T, Sheshu Madhav M, Shobha Rani N, Neeraja CN, Ashok Reddy G, Shaik H, Sundaram RM (2012) Identification and fine-mapping of *Xa33*, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. Phytopathology 102:222–228

Nelson RJ, Baraoidan MR, Cruz CM, Yap IV, Leach JE, Mew TW, Leung H (1994) Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. Appl Environ Microbiol 60:3275–3283

Noh TH, Lee DK, Kang MH, Shin MS, Na SY (2003) Identification of new race of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in Korea. (Abstr.) Phytopathology 93(suppl.):S66

Noh TH, Lee DK, Shim HK, Seo SJ, Choi MY, Park JC, Paik CH, Kim HM (2013) Changes in distribution and disease occurrence of rice bacterial leaf blight on rice in Korea. J Agric Life Sci 44(1):5–10

Rozen S, Skaletsky H (2000) Primer 3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bio-informatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Ruan HH, Yan CQ, An DR, Liu RH, Chen JP (2008) Identifying and mapping new gene *xa32(t)* for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*, *Xoo*) from *Oryza meyeriana* L. Acta Agric Boreali-occidentalis Sin. 17:170–174

Shen YJ, Jiang H, Jin JP, Zhang ZB, Biao XB, He YY, Wang G, Wang C, Qian L, Li X, Yu QB, Liu HJ, Chen DH, GaoJH Huang H, Shi TL, Yang ZN (2004) Development of genome wide DNA polymorphism database for map-based cloning of rice genes. Plant Physiol 135:1198–1205

Sivaguru M, Ezaki B, He ZH, Tong H, Osawa H, Baluska F, Volkmann D, Matsumoto H (2003) Aluminum-induced gene expression and protein localization ofa cell wall-associated receptor kinase in *Arabidopsis*. Plant Physiol 132:2256–2266

Song WY, Wang GL, Chen L, Zhai W, Kim HS, Holsten T, Zhu L, Ronald PC (1995) A receptor kinase like protein encoded by the rice disease resistance gene *Xa-21*. Science 270:1804–1806

Srinivasan B, Gnanamanickam S (2005) Identification of a new source of resistance in wild rice, *Oryza rufipogon*, to bacterial blight of rice caused by Indian strains of Xanthomonas oryzae pv. oryzae. Curr Sci 88:25

Suh JP, Noh TH, Kim KY, Kim JJ, Kim YG, Jena KK (2009) Expression levels of three bacterial blight resistance genes against K3a race of Korea by molecular and phenotype analysis in japonica rice (*O. sativa* L.). J Crop Sci Biotechnol 12(3):103–108

Suh JP, Yang SJ, Jeung JU, Pamplona A, Kim JJ, Lee JH, Hong JC, Yang CI, Kim YG, Jena KK (2011) Development of elite breeding lines conferring *Bph18* gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in japonica rice. Field Crops Res 120:215–222

Suh JP, Jeung JU, Noh TH, Cho YC, Park SH, Park HS, Shin MS, Kim CK, Jena KK (2013) Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice 6:5

Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J 37:517–527

Tian D, Wang J, Zheng X, Gu K, Qiu C, Yang X, Zhou Z, Goh M, Luo Y, Murata-Hori M, White FF, Yin Z (2014) The rice TAL effector-dependent resistance protein *Xa10* triggers cell death and calcium depletion in the endoplasmic reticulum. Plant Cell 26(1):497–515

Vera Cruz CM, Bai J, Ona I, Leung H, Nelson RJ, Mew TW, Leach JE (2000) Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. Proc Natl Acad Sci USA 97:13500–13505

Verdier V, Vera Cruz C, Leach JE (2012) Controlling rice bacterial blight in Africa: needs and prospects. J Biotechnol 159:320–328. doi:10.1016/j.jbiotec.2011.09.020

Wagner TA, Kohorn BD (2001) Wall-associated kinases are expressed throughout plant development and are required for cell expansion. Plant Cell 13:303–318

Wang CT, Wen GS, Lin XH, Liu XQ, Zhang DP (2009) Identification and fine mapping of the new bacterial blight resistance gene, *Xa31(t)*, in rice. Eur J Plant Pathol 123:235–240

Wang C, Fan Y, Zheng C, Qin T, Zhang X, Zhao K (2014a) High-resolution genetic mapping of rice bacterial blight resistance gene *Xa23*. Mol Genet Genomics 289:745–753

Wang C, Zhang X, Fan Y, Gao Y, Zhu Q, Zheng C, Qin T, Li Y, Che J, Zhang M, Yang B, Liu Y, Zhao K (2014b) *XA23* is an executor R protein and confers broad-spectrum disease resistance in rice. Mol Plant 8:290–302

Xiang Y, Cao Y, Xu C, Li X, Wang S (2006) *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26*. Theor Appl Genet 113:1347–1355

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 J, Li X, Jiang G, Xu Y, He Y (2006) Pyramiding of *Xa7* and *Xa21* for the improvement of disease resistance to bacterial blight in hybrid rice. Plant Breed 125:600–605

Zhang F, Zhuoa DL, Zhang F, Huang LY, Wang WS, Xu JL, Vera Cruz C, Li ZK, Zhou YL (2014) *Xa39*, a novel dominant gene conferring broad-spectrum resistance to *Xanthomonas oryzae* pv. *oryzae* in rice. Plant Pathol 64:568–575. doi:10.1111/ppa.12283

Zheng CK, Wang CL, Yu YJ, Liang YT, Zhao KJ (2009) Identification and molecular mapping of *Xa32(t),* a novel resistance gene for bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice. Acta Agron Sin 35:1173–1180