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High-resolution mapping and gene prediction of *Xanthomonas Oryzae* pv. *Oryzae* resistance gene *Xa7*

Shen Chen · Zhanghui Huang · Liexian Zeng · Jianyuan Yang · Qiongguang Liu · Xiaoyuan Zhu

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Abstract Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a devastating disease in rice worldwide. The resistance gene *Xa7*, which provides dominant resistance against the pathogen with avirulence (*Avr*) gene *AvrXa7*, has proved to be durably resistant to BB. A set of SSR markers were selected from the "gramene" database based on the *Xa7* gene initial mapping region on chromosome 6. These markers were used to construct a high-resolution genetic map of the chromosomal region surrounding the *Xa7* gene. An F_2 mapping population with 721 highly susceptible individuals derived from a cross between the near isogenic lines (NILs) IRBB7 and IR24 were constructed to localize the *Xa7* gene. In a primary analysis with eleven polymorphic SSR markers, *Xa7* was located in approximately the 0.28-cM region. To walk closer to the target gene, recombinant F_2 individuals were tested using newly developed STMS (sequence tagged microsatellite) markers. Finally, the *Xa7* gene was mapped to a 0.21-cM interval between the markers GDSSR02 and RM20593. The *Xa7*-linked markers were landed on the reference sequence of cv. Nipponbare through bioinformatics analysis. A contig map corresponding to the *Xa7* gene was constructed. The target gene was assumed to span an interval of approximately 118.5-kb which contained a total of fourteen genes released by the TIGR Genome Annotation Version 5.0. Candidate-gene analysis of *Xa7* revealed that the fourteen genes encode novel domains that have no amino acid sequence similar to other cloned *Xa*(*xa*) genes.

Keywords Bacterial blight · Candidate genes · High-resolution mapping · Marker-assisted selection (MAS) · *Xa7* · *Xanthomonas oryzae* pv. *oryzae* (*Xoo*)

Shen Chen and Zhanghui Huang are contributed equally to this work.

S. Chen · L. Zeng · J. Yang · X. Zhu (✉)
The Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
e-mail: zhuxy@gdppri.com

Z. Huang · Q. Liu
Laboratory of Plant Bacteria, College of Resources and Environmental Sciences, South China Agricultural University, Guangzhou 510642, China

Present Address:
Z. Huang
The Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

Introduction

Bacterial blight (BB), a vascular disease caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) resulting in a systemic infection that produces tannish-grey to white lesions along the veins, is one of the most serious rice diseases throughout the world

(Nelson et al. 1994). For the sake of food security, application of variety resistance has been, and undoubtedly will continue to be, the major method of disease control for rice BB. The rice-*Xoo* pathosystem has become the genetic model for understanding host–pathogen interactions and co-evolution for cereals (Dai et al. 2007). In this host pathosystem, race-specific resistance shows the gene-for-gene relationship (Mew 1987; Dai et al. 2007).

A clear understanding of the molecular mechanisms in host resistance to pathogens is the essential prerequisite for a better design of control strategies for rice BB (Dai et al. 2007). Large-scale and long-term cultivation of varieties carrying one single resistance gene resulted in a significant shift in pathogen race frequency with consequent breakdown of resistance in these cultivars. To tackle the problem of resistance breakdown, pyramiding of resistance genes into different varieties is indispensable. Identification and cloning of BB resistance genes has therefore become very important. During the last decade, significant achievements have been made in elucidating the molecular basis of rice–pathogen interactions (Dai et al. 2007). So far, more than 30 BB resistance genes have been identified and designated in a series from *Xa1* to *xa32*(t), etc. (Lin et al. 1996; Nagato and Yoshimura 1998; Zhang et al. 1998; Khush and Angeles 1999; Chen et al. 2002; Lee et al. 2003; Tan et al. 2004; Xiang et al. 2006; Singh et al. 2007). Of the identified genes, seven BB resistance genes *Xa1*, *Xa3*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27* have been cloned and characterized (Song et al. 1995; Yoshimura et al. 1998; Iyer and McCouch 2004; Sun et al. 2004; Gu et al. 2005; Chu et al. 2006; Jiang et al. 2006a, b; Xiang et al. 2006).

Previous studies have made clear that *Xa7* is a dominant BB resistance gene initially identified in rice cultivar DV85 (International Rice Research Institute accession number 8839) (Sidhu et al. 1978). The *Xa7* gene of DV85 was transferred to cv. IR24, and the near-isogenic line (NIL) IRBB7 with *Xa7* was created by backcross with cv. IR24 as a recurrent parent (Ogawa et al. 1991). This gene was previously mapped in an interval of 2.7-cM between M1 and M3 with a gap region between M3 and co-segregated marker M5 (Porter et al. 2003). To date, the *Xa7* gene has not been isolated, but the avirulence (*Avr*) gene *AvrXa7* corresponding to resistance gene *Xa7* has been cloned (Yang et al. 2000). It has been proved that the *Xa7* gene would be a durable resistance gene because of a fitness penalty in *Xoo* associated with adaptation to *Xa7* (Vera Cruz et al. 2000). Thus isolation of the *Xa7* gene can facilitate understanding of the interactive mechanism between durable BB resistance gene and *Xoo* pathogen.

The underlying objective of this study was to construct a high-resolution map of the *Xa7* gene, in an effort to clone it using the map-based cloning method. We have developed an F_2 mapping population with 721 highly susceptible individuals derived from the cross between the resistant parent IRBB7 and highly susceptible cv. IR24 using Chinese *Xoo* race 4 (strain SCB4-1) and have identified a great deal of SSR and STMS (sequence tagged microsatellite) tightly linked markers of *Xa7* and have constructed a BAC/PAC contig containing the target gene with overlapping clones, which will accelerate future marker-assisted selection (MAS) breeding of *Xa7* and, finally, isolation of the gene.

Materials and methods

Plant materials and disease evaluation

The *indica* rice cv. IRBB7, which is the NIL with the *Xa7* gene, was used as the donor parent, and crossed with the *indica* susceptible cv. IR24 which is a non-*Aus* variety of isozyme group I developed at the International Rice Research Institute (IRRI) in the Philippines. Chinese *Xoo* race 4 (strain SCB4-1) which is compatible with IR24 and incompatible with IRBB7 was selected to evaluate the resistance segregation of the F_2 population derived from the cross between IR24 and IRBB7 by the leaf-clipping method at the booting stage (Kauffman et al. 1973).

Thirty-nine major rice varieties in production and experimental lines from Guangdong province, South China, including Qilisimiao, Yuexiangzhan, Xianxiaozhan, Yesizhan, Yuefengzhan, Texianzhan25, Qishanzhan, Jingxian89, Lvhuangzhan, Zhongerruanzhan, Meixiangzhan2, Z1, GD9501, Texianzhan13, Fengaizhan1, Fengbazhan, Fengfuzhan1, Fenghuazhan, Fengsizhan, Fuqingzhan4, Huasizhan, Huanghuazhan, Huangxinzhan, Meisizhan, Meixiangzhan, Wushanyouzhan, Yuetaisimiao, Zhen-sizhan, Molisimiao, Molixinzhan, Wufengzhan2, BL122, 28zhan, R7, R8, R9, R10, R11, and R17, were used for the *Xa7* MAS breeding.

BB resistance to strain SCB4-1 was evaluated by scissors-clipping three of the youngest leaves of each plant approximately 2 cm below the leaf tips with a bacterial suspension having an OD600 = 0.6. The inoculum was prepared from bacteria revived from glycerol stocks and grown for 72 h in nutrient yeast sucrose broth at 30°C. After inoculation the plants were maintained in a growth chamber with 12 h of light, relative humidity above 80%, night temperatures of 28°C and day temperatures of 32°C. The plants were scored as resistant or susceptible through the average lesion length which was measured for the three inoculated leaves after 20 days of inoculation (Kauffman et al. 1973). Leaf tissue of these plants was stored at −70°C under refrigeration for DNA extraction from a portion of the uninoculated tissue, which was harvested at the time of bacterial blight inoculation. The controls used during inoculation were both parents of the F_2 population.

Marker development and detection

Plant DNA was prepared from frozen leaves of rice plants using the CTAB method (Murray and Thompson 1980). Only SSR and STMS (prefix GDSSR××) markers were used in this study. For SSR markers, the primer sets were adopted from the "gramene" database (http://www.gramene.org) based on the known *Xa7* gene target region (Porter et al. 2003). The detection procedures were followed by Blair and McCouch (1997). For further linkage analysis, STMS markers were developed in the target region through bioinformatics analysis using the publicly available reference sequences of the entire rice genome of two subspecies, i.e. *japonica* (cv. Nipponbare; http://rgp.dna.affrc.go.jp) and *indica* (cv. 93–11; http://www.genomics.org.cn). Particularly, primer sets for STMS markers were designed by the reference genome sequences of cv. Nipponbare using software tools, SSRIT (http://www.gramene.org/microsat/ssrtool) and FastPCR 4.0 (www.biocenter.helsinki.Fi/bi/programs/fastpcr.htm). The detection procedures and conditions of STMS markers are shown in Table 1.

Genetic and physical map construction

According to the initial genomic location of *Xa7* (Porter et al. 2003), five DNA markers from M1 to M5 were selected for chromosome landing. Thirty-two SSR markers from the "gramene" database were used for fine mapping of the *Xa7* gene. The recessive-class analysis (RCA; Zhang et al. 1994) was used to identify polymorphic molecular markers linked to the resistance gene. After determining the accurate chromosomal location of the target gene, chromosome walking to the resistance gene was initiated from both sides with SSR and STMS markers ulteriorly. Linkage analysis was conducted with Mapmaker/Exp (version 3.0) with a threshold LOD score of 3.0 (Lincoln et al. 1992). The recombination frequency was transformed into centimorgans according to the Kosambi function (Kosambi 1944).

The physical map of the target gene was constructed by bioinformatics analysis using BAC (bacterial artificial chromosome) and PAC (P1-derived artificial chromosome) clones of cv. Nipponbare released by the International Rice Genome Sequencing Project (IRGSP). In other words, these clones were anchored with the target gene-linked markers and then alignment of sequences was carried out using the software tool, Pairwise BLAST (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html).

Candidate gene annotation

According to the physical map of the target gene, the publicly available BAC or PAC sequences of *O. sativa* cv. Nipponbare in the target gene region were downloaded from Rice Genome Sequence Program (RGP) web site (http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/) and Genbank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). The open reading frames (ORFs) and potential exon/intron boundaries were predicted for the sequences described above using FgenesH (http://genomic.sanger.ac.uk), RiceGAAS (http://ricegaas.dna.affrc.go.jp/), and GeneScan (http://genes.mit.edu/GENSCAN) software. The candidate genes were analyzed through BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and confirmed by the TIGR Rice Genome Annotation Version 5 (http://www.tigr.org/tigr-scripts/osal_web/gbrowse/rice/).

Results

Construction of mapping population

The parental lines F_1 and the F_2 plants, showed clear reactions to Chinese *Xoo* race 4 SCB4-1. One of the

Table 1 Primer sequence of sequence tagged microsatellite (STMS) markers developed in the study

Marker name	Forward (5′–3′)	Reverse (5′–3′)	PCR[a]	Gel[b]	Product size (bp)
GDSSR01	GCTGATGCATACATACCTTGACG	GTGGACTCCGCAAAGCCGTC	A	I	206
GDSSR02[c]	TGCCCACCGTCGAACTCGTGG	AGCTAGCAATTCGCATGATTGC	A	I	207
GDSSR03	CACGGCGATGGCGTACATCG	TGGGCGTTCATCGAGTTCTG	A	I	93
GDSSR04	CACGGCGATGGCGTACATCG	TGTACTAGCCATCGCGAACG	A	I	223
GDSSR05	TCAGGTGATCGTGCTACTTGG	TCTTCTCGTAGACCTAGCCTC	A	I	118
GDSSR06	TCGTCGGCCATATCACCGACAC	CGGAGCAAGTGGAGCTTCTC	A	I	115

[a] A: After preheating 4 min at 94°C, 35 PCR cycles (30 sec at 94°C, 45 sec at 58°C, 1 min at 72°C), followed by 5 min at 72°C

[b] I = 8% acrylamide

[c] Polymorphic marker corresponding to the parents

parents of the F_2 population, IRBB7 carrying *Xa7*, was resistant to pathogen strain SCB4-1 with an average lesion length of 0.46 ± 0.17 cm 20 days after inoculation. The other parent of the population, IR24, was highly susceptible to SCB4-1 with an average lesion length of 24.6 ± 2.62 cm. The distribution of the lesion length for SCB4-1 inoculation in the 3201 F_2 plants was bimodal with a valley at 5 to 6 cm (Fig. 1). Using a lesion length of 5.0 cm as the dividing point (R or S), all of the F_1 plants from the cross IRBB7/IR24 were resistant to SCB4-1 (data not shown). Of the F_2 progenies, segregation of resistant and susceptible plants fitted a 3:1 ratio (2398R:803S, Chi-Square = 0.13, Asymp. Sig. = 0.91), which indicated that the resistance of IRBB7 to SCB4-1 was controlled by a dominant resistance gene. Because the efficiency of mapping with a recessive class is two or three times higher than that with a random population per assayed plant (Zhang et al. 1994), a population consisting of 721 highly susceptible F_2 individuals (with lesion length ≥ 8.0 cm) was selected for genetic mapping of the target gene.

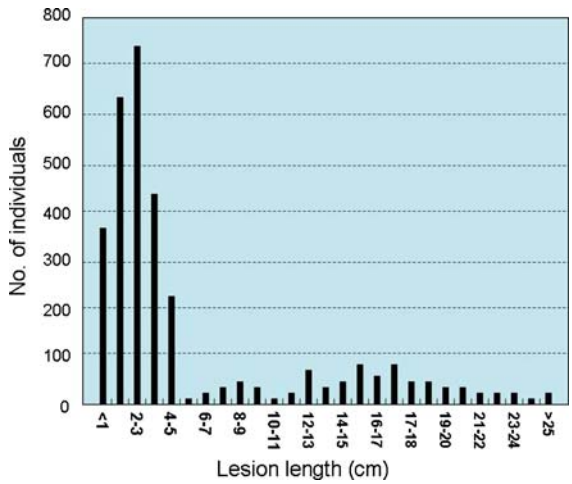

Fig. 1 Distribution of lesion length after inoculation with Chinese *Xoo* race 4 (strain SCB4-1) in a sample containing 3,201 individuals from a F_2 population derived from a cross between IRBB7 and IR24

Genetic map of the *Xa7* locus region

Based on the preliminary mapping result of *Xa7* (Porter et al. 2003), three publicly available BAC sequences of *O. sativa* cv. Nipponbare, AP005610, AP006454 and AP006055, were downloaded from the RGP web site and Genbank in the gap between clone AP005192 and AP004989. For fine mapping of the *Xa7* gene, thirty-two sets of SSR primers were adopted from the "gramene" database based on the above mentioned BAC clones for parents polymorphism assay. Of the thirty-two sets of SSR primers tested, eighteen primer sets were polymorphic between two parents IRBB7 and IR24 (data not shown). Eleven sets of SSR primers which showed clear polymorphism between two parents were selected to detect the F_2 mapping population. In the first screening, the *Xa7* gene was defined in a genetic interval of 5.3-cM between SSR markers RM20573 and RM20612, i.e. 34 susceptible F_2 recombinants were identified in the 5.3-cM interval. In the second screening the other nine SSR markers were used to reduce the number of recombinants. The results showed that five and two recombinants were screened out by two markers RM20580 and RM20582 which were flanked on the side of RM20573. On the other hand, sixteen, eight, five, three, and two recombinants

were screened out by five markers RM20608, RM20603, RM20601, RM206595, and RM20593 flanked on the side of RM20612. The remaining three markers RM20589, RM20590, and RM20591 were co-segregated with *Xa7* (Fig. 2). Thus *Xa7* was mapped in a genetic interval of 0.28-cM between SSR markers RM20582 and RM20593 (Fig. 2).

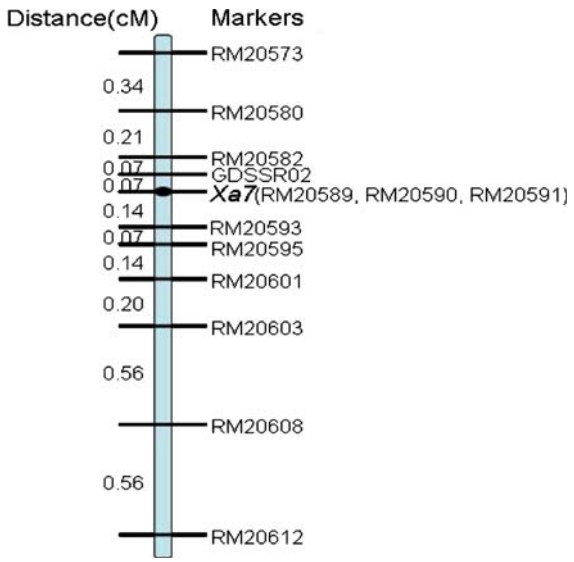

Fig. 2 High-resolution genetic map of the *Xa7* region on the short arm of chromosome 6. The chromosome is represented as a *filled bar*. SSR and STMS markers used in this phase of the study are indicated to the right of the bar; the distance between markers is indicated to the left

To further narrow the region covering *Xa7*, six new STMS markers were developed through the reference sequences of cv. Nipponbare by bioinformatics analysis in the 0.28-cM region (Table 1). Among the six STMS markers developed, only GDSSR02 showed polymorphism to both parents (Table 1). One recombinant derived from RM20573 was detected in the region. Therefore the genetic region spanning the *Xa7* gene was ultimately estimated as 0.21-cM in genetic length (Fig. 2).

Physical map construction in silico

All the anchor markers used in chromosome walking to the *Xa7* gene were landed on the reference sequences of cv. Nipponbare by bioinformatics analysis using a software tool BLASTN. Sequences matching showed that RM20573 and RM20580 were found in the BAC clones AP005192, RM20582 in AP005610, GDSSR02 in AP006454, three markers RM20589, RM20590, and RM20591 in AP006055, four markers RM20593, RM20595, RM20601, and RM20603 in AP004989, RM20608 in AP003728, and RM20612 in AP003723 (Fig. 3). The BAC/PAC clones landed were downloaded from the RGP web site and then aligned as a contig map covering the *Xa7* gene through Pairwise BLAST analysis. In combination with the genetic mapping region, the physical distance between markers GDSSR02 and RM20593 in 0.21-cM genetic interval is 118.5-kb on the RGP BAC/PAC contigs (Fig. 3).

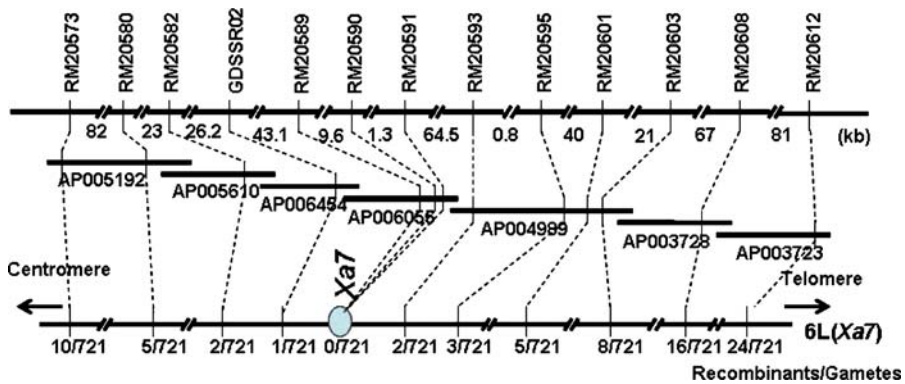

Fig. 3 Physical map of the *Xa7* gene. Twelve SSR and one STMS markers were used in this study. The *long horizontal lines* indicate the region containing the *Xa7* gene. The *short horizontal lines* represent the BAC/PAC clones of cv. Nipponbare, which were released by IRGSP and assembled by the corresponding markers linked to the *Xa7* gene. The *numbers* below the *lower long horizontal line* are the recombination events in the mapping populations (recombinants/gametes are indicated). The *digits* between markers are physical distances in kilobase (kb). The *vertical and dashed lines* denote the relative positions of the corresponding markers

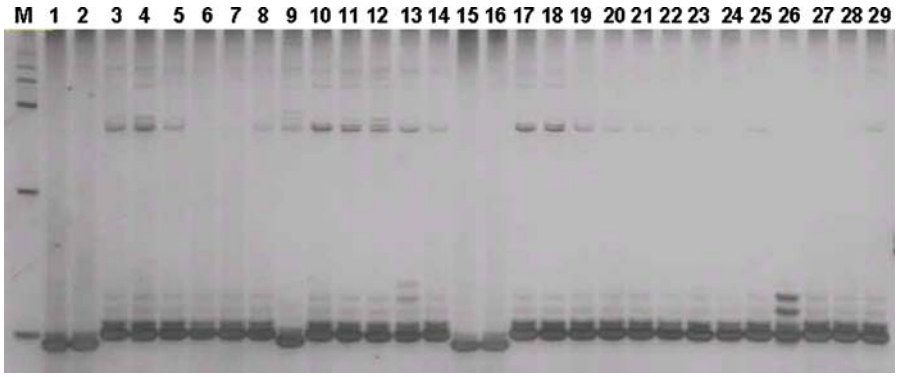

Fig. 4 Polymorphic analysis of partial major rice varieties from Guangdong province, South China, with SSR marker RM20580. *1* and *2* represent resistant control cvs IRBB7 and DV85 carrying the *Xa7* gene; *3–29* represent susceptible control varieties from Guangdong province, South China, which are Qilisimiao, Yuexiangzhan, Xianxiaozhan, Yesizhan, Yuefengzhan, Texianzhan25, Qishanzhan, Jingxian89, Lvhuangzhan, Zhongerruanzhan, Meixiangzhan2, Z1, GD9501, Texianzhan13, Fengaizhan1, Fengbazhan, Fengfuzhan1, Fenghuazhan, Fengsizhan, Fuqingzhan4, Huasizhan, Huanghuazhan, Huangxinzhan, Meisizhan, Meixiangzhan, Wushanyouzhan, and Yuetaisimiao, respectively; *M* represents DNA Marker DL2000

Candidate gene prediction of *Xa7*

Xa7 had been mapped within a 118.5-kb interval flanked by markers GDSSR02 and RM20593 (Fig. 3). Analysis of the 118.5-kb corresponding reference genome sequences of Nipponbare revealed that this region contains fourteen genes released by the TIGR Rice Genome Annotation Version 5. Among the fourteen predicted genes, six encode hypothetical protein unclassified, two encode expressed protein without resistance function, two encode putative retrotransposon protein unclassified, the other four encode putative and expressed alpha-glucosidase precursor, metal transporter Nramp6, BTB/POZ, and NAC domains containing protein.

Polymorphism of *Xa7* linked markers between *Xa7* donors and local varieties

On the basis of fine mapping of *Xa7*, four *Xa7* tightly linked markers (RM20580, GDSSR02, RM20593, and RM20595) and three *Xa7* co-segregated markers (RM20589, RM20590, and RM20591) were used for MAS breeding of *Xa7* in Guangdong province, South China. These seven markers were used to analyze thirty-nine major rice varieties released in the local region. IRBB7 and DV85 harboring *Xa7* were used as resistant control cvs, the other thirty-nine major varieties as susceptible control varieties. The results indicated that six markers (RM20580, GDSSR02, RM20593, RM20595, RM20590, and RM20591) except for RM20589 showed good polymorphism between resistant control cvs and almost all the susceptible control varieties (Fig. 4 lists the result for RM20580 only).

Discussion

Much evidence has indicated that *Xa7* is a broad-spectrum and durable resistance gene. The result of interactions between IRBB7 and almost all of *Xoo* strains from Guangdong province, South China, during 1991–2006 showed that IRBB7 maintained good, stable resistance to the *Xoo* pathogen (unpublished work). Rice lines with *Xa7* prevented bacterial blight epidemics with the presence of virulent *Xoo* strains in the Philippines from 1993 to 1995 (Vera Cruz et al. 2000). After planting cv. IRBB7 (*Xa7*) in field sites for 10 years (20 crops), the *Xa7* gene remained highly effective despite the *Xoo* population structure changing to one with an increased proportion of strains virulent to IRBB7 and higher aggressiveness to rice without *Xa7* (Leach et al. 2007).

Utilization of the horizontal resistance genes is more significant than use of the vertical resistance genes in genes pyramiding. Because the horizontal resistance gene *Xa7* has not been used in MAS breeding in South China, the *Xa7* gene can be

pyramided into elite varieties combined with other BB resistance genes. As by-products of our fine mapping, a number of tightly linked markers for the *Xa7* gene had been developed; these could provide a useful tool for the marker-assisted transfer of this R gene in rice improvement programs. Eight markers (RM20573, RM20580, RM20582, RM20595, RM20601, RM20603, RM20608, and RM20612) tightly linked to *Xa7* (genetic distance < 2 cM) can be used in MAS breeding. Specifically, two tightly linked markers GDSSR02 (0.07 cM) and RM20593 (0.14 cM) on both sides of *Xa7* gene and three *Xa7* co-segregated markers (RM20589, RM20590, and RM20591) should be more available than the previously identified markers (Porter et al. 2003), because of their much tighter linkages or co-segregation with *Xa7* than the previous markers (M1–M5), more convenient operation than for the AFLP-based markers (M1–M5), and high level of polymorphism in rice germplasm.

Of the fourteen predicted genes of *Xa7*, only three genes which encode putative and expressed metal transporter Nramp6, BTB/POZ, and NAC domains containing protein have been reported to have plant-resistance function (Cao et al. 1997; Collinge and Boller 2001; Mysorel et al. 2002; Richer et al. 2003; Oh et al. 2005; Jiang et al. 2006a, b; Lin et al. 2007a, b; Nakashima et al. 2007). Which of the candidate genes really is the *Xa7* gene must be identified by using a long-range PCR approach in combination with *Agrobacterium*-mediated transformation technology (Feuillet et al. 2003; Horvath et al. 2003; Song et al. 2003). Candidate genes analysis indicated that the *Xa7* gene perhaps represents a new class of plant resistance gene, because it has no protein similar to other cloned BB resistance genes (*Xa1*, *Xa3*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27*) (Song et al. 1995; Yoshimura et al. 1998; Iyer and McCouch 2004; Sun et al. 2004; Gu et al. 2005; Chu et al. 2006; Jiang et al. 2006a, b; Xiang et al. 2006). From these reports we found it interesting that the encoded products of four resistance genes (*Xa1*, *xa5*, *xa13*, and *Xa27*) are unique and different in cloned *Xa*(*xa*) resistance genes except for the fact that only three genes *Xa3*, *Xa21*, and *Xa26* encode similar receptor-like proteins, which showed that the structure diversity of BB resistance genes is more abundant. But for the eight cloned rice blast resistance genes (*Pi2*, *Pi9*, *Pi36*, *Pi37*, *Pib*, *Pid2*, *Pita*, and *Piz-t*; Wang et al. 1999; Bryan et al. 2000; Qu et al. 2006; Zhou et al. 2006; Chen et al. 2006; Liu et al. 2007; Lin et al. 2007a, b), only *Pid2* encodes a novel receptor-like kinase protein with a predicted extracellular domain of a B-lectin and an intracellular serine–threonine kinase domain, the encoded products of the other seven genes are all related to NBS-LRR domains. Therefore, the isolated BB resistance genes structure showed more protein diversity than the rice blast resistance genes, which might be very useful for the stable balance between resistance genes and *Avr* genes in the BB pathosystem.

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References

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial blight resistance gene *xa5*. Theor Appl Genet 95:174–184

Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B (2000) tA single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pita*. Plant Cell 12:2033–2046

Cao H, Glazebrook J, Clarke JD, Volko S, Dong X (1997) The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88:57–63

Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu GZ, Xu JC, Ling ZZ, Cao G, Ma BT, Wang YP, Zhao XF, Li SG, Zhu LH (2006) A Blectin receptor kinase gene conferring rice blast resistance. Plant J 46:794–804

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

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. Gene Dev 20:1250–1255

Collinge M, Boller T (2001) Differentiation induction of two potato genes, *Stprx2* and *StNAC*, in response to infection by *Phytophthora infestans* and to wounding. Plant Mol Biol 46:521–529

Dai LY, Liu XL, Xiao YH, Wang GL (2007) Recent advances in cloning and characterization of disease resistance genes in rice. J Integr Plant Biol 49:112–119

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum*

aestivum L.) genome. Proc Natl Acad Sci USA 100:15253–15258

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

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von-Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. Proc Natl Acad Sci USA 100:364–369

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

Jiang YX, Dong HT, Zhuang JY, Zheng KL, Li DB (2006a) A preliminary molecular characterization and functional investigation on *OsBTB* gene induced by *Magnaporthe grisea* in Rice. Chinese J Rice Sci 20:577–582

Jiang GH, Xia ZH, Zhou YL, Wan J, Li DY, Chen RS, Zhai WX, Zhu LH (2006b) Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog *TFIIA*γ*1*. Mol Genet Genomics 275:354–366

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

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

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Leach JE, Webb KM, Bai J, Ona I, Bruce M, Manosalva P, Lee S, Stephens J, Garrett K, Mew TW, Vera Cruz CM (2007) Understanding *Xanthomonas oryzae*/rice interactions to guide disease control. In: The 2nd International Conference on Bacterial Blight of Rice, Nanjing, China, October 1–3, pp 2

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

Lin F, Chen S, Que ZQ, Wang L, Liu XQ, Pan QH (2007a) The blast resistance gene *Pi37* encodes an NBS–LRR protein and is a member of a resistance gene cluster on rice chromosome 1. Genetics 177:1871–1880

Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q (1996) Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86:1156–11593

Lin RM, Zhao WS, Meng XB, Wang M, Peng YL (2007b) Rice gene *OsNAC19* encodes a novel NAC-domain transcription factor and responds to infection by *Magnaporthe grisea*. Plant Sci 172:120–130

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with Mapmaker/Exp 3.0. 3rd ed. Whitehead Institute Technical Report, Cambridge, Massachusetts, USA

Liu XQ, Lin F, Wang L, Pan QH (2007) The in silico map-based cloning of *Pi36*, a rice CC-NBS-LRR gene which confers race-specific resistance to the blast fungus. Genetics 176:2541–2549

Mew TW (1987) Current status and future prospects of research on bacterial blight of rice. Annu Rev Phytopathol 25:359–382

Murray MG, Thompson WK (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Mysorel KS, Crasta OR, Tuori RP, Folkerts O, Swirsky PB, Martin GB (2002) Comprehensive transcript profiling of *Pto*-and *Prf*-mediated host defense responses to infection by *Pseudomonas syringae* pv. *tomato*. Plant J 32:299–315

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

Nakashima K, Tran L-SP, Nguyen DV, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. Plant J 51:617–630

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

Ogawa T, Yamamoto T, Khush GS, Mew TW (1991) Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). Japan J Breed 41:523–529

Oh SK, Lee S, Yu SH, Choi D (2005) Expression of a novel NAC domain-containing transcription factor (CaNAC1) is preferentially associated with incompatible interactions between chili pepper and pathogens. Planta 222:876–887

Porter BW, Chittoor JM, Yano M, Sasaki T, White FF (2003) Development and mapping linked to the rice bacterial blight resistance gene *Xa7*. Crop Sci 43:1484–1492

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang GL (2006) The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide binding site-leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 172:1901–1914

Richer E, Courville P, Isabelle Bergevin I, Cellier MF (2003) Horizontal gene transfer of "prototype" Nramp in bacteria. J Mol Evol 57:363–376

Sidhu GS, Khush GS, Mew TW (1978) Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice, *Oryza sativa* L. Theor Appl Genet 53:105–111

Singh K, Vikal Y, Mahajan R, Cheema KK, Bhatia D, Sharma R, Lore JS, Bharaj TS (2007) Three novel bacterial blight resistance genes identified, mapped and transfer to cultivated rice *O. sativa* L. In: The 2nd International Conference on Bacterial Blight of Rice, Nanjing, China, October 1–3, pp 76–77

Song JQ, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang HH, Austin-Phillips S, Buell CR, Helgeson JP, Jiang JM (2003) Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. Proc Natl Acad Sci USA 100:9128–9133

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, 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

Tan GX, Ren X, Weng QM, Shi ZY, Zhu LL, He GC (2004) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Acta Genet Sin 31:724–729

Vera Cruz CM, Bai JF, 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

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T (1999) The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J 19:55–64

Xiang Y, Cao YL, Xu CQ, Li XH, Wang SP (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

Yang B, Zhu WG, Johnson LB, White FF (2000) The virulence factor *AvrXa7* of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. Proc Natl Acad Sci USA 97:9807–9812

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kuruta 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 BY, 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. ruWpogon*. Rice Genet Newsl 15:138–142

Zhang QF, Shen BZ, Dai XK, Mei MH, Maroof MAS, Li ZB (1994) Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. Proc Natl Acad Sci USA 91:8675–8679

Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G, Bellizzi M, Wang GL (2006) The eight amino acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. Mol Plant Microbe Interaction 19:1216–1228