Mol Gen Genomics (2004) 271: 111–120
DOI 10.1007/s00438-003-0964-6

ORIGINAL PAPER

Z. Chu · Y. Ouyang · J. Zhang · H. Yang · S. Wang

Genome-wide analysis of defense-responsive genes in bacterial blight resistance of rice mediated by the recessive *R* gene *xa13*

Received: 3 July 2003 / Accepted: 18 November 2003 / Published online: 17 January 2004

Abstract Defense responses triggered by dominant and recessive disease resistance (*R*) genes are presumed to be regulated by different molecular mechanisms. In order to characterize the genes activated in defense responses against bacterial blight mediated by the recessive *R* gene *xa13*, two pathogen-induced subtraction cDNA libraries were constructed using the resistant rice line IRBB13—which carries *xa13* —and its susceptible, near-isogenic, parental line IR24. Clustering analysis of expressed sequence tags (ESTs) identified 702 unique expressed sequences as being involved in the defense responses triggered by *xa13*; 16% of these are new rice ESTs. These sequences define 702 genes, putatively encoding a wide range of products, including defense-responsive genes commonly involved in different host-pathogen interactions, genes that have not previously been reported to be associated with pathogen-induced defense responses, and genes (38%) with no homology to previously described functional genes. In addition, *R* -like genes putatively encoding nucleotide-binding site/leucine rich repeat (NBS-LRR) and LRR receptor kinase proteins were observed to be induced in the disease resistance activated by *xa13*. A total of 568 defense-responsive ESTs were mapped to 588 loci on the rice molecular linkage map through bioinformatic analysis. About 48% of the mapped ESTs co-localized with quantitative trait loci (QTLs) for resistance to various rice diseases, including bacterial blight, rice blast, sheath blight and yellow mottle virus. Furthermore, some defense-responsive sequences were conserved at similar locations on different chromosomes. These results reveal the complexity of *xa13* -mediated resistance. The information obtained in this study provides a large source of candidate genes for understanding the molecular bases of defense responses activated by recessive *R* genes and of quantitative disease resistance.

Keywords Defense-related genes · *xa13* · Disease resistance · Resistance QTL · Rice

The first two authors contributed equally to this work
Communicated by R. Hagemann

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00438-003-0964-6

Z. Chu · Y. Ouyang · J. Zhang · H. Yang · S. Wang (✉)
National Key Laboratory of Crop Genetic Improvement,
National Center of Crop Molecular Breeding,
Huazhong Agricultural University,
430070 Wuhan, China
E-mail: swang@mail.hzau.edu.cn
Tel.: +86-27-87283009
Fax: +86-27-87287092

Introduction

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious diseases of rice worldwide. Resistance to bacterial blight is regulated by two classes of genes—major disease resistance (*R*) genes, and defense-related or defense-responsive genes. More than 20 *R* genes which confer resistance to various strains of *Xoo* have been identified, and have been named in sequence from *Xa1* to *Xa28* (Lin et al. 1996; Nagato and Yoshimura 1998; Khush and Angeles 1999; Chen et al. 2002; Lee et al. 2003; Yang et al. 2003). Three dominant *R* genes, *Xa1*, *Xa21* and *Xa26*, have been isolated. The products of these three genes have characteristic structural domains found in most of the known plant R proteins. The genes *Xa21* and *Xa26* encode leucine-rich repeat (LRR) receptor kinase-like proteins (Song et al. 1995; Sun et al. 2004), while *Xa1* encodes a nucleotide-binding site (NBS)-LRR protein (Yoshimura et al. 1998).

The products of dominant *R* genes are presumed to function as receptors, which interact with elicitors produced by pathogens to initiate disease resistance, or act to protect cellular machinery (Tang et al. 1996; Baker et al. 1997; Jia et al. 2000; Dangl and Jones 2001; Nimchuk et al. 2001). However, recessive *R* genes

appear to function differently from most of the known dominant *R* genes that encode conserved domains. Thus recessive alleles (*mlo*) resulting from mutations of the *Mlo* locus confer broad-spectrum resistance to powdery mildew in barley. The *Mlo* gene encodes a plasma membrane protein with seven transmembrane domains, and acts as a negative regulator in defense against powdery mildew (Buschges et al. 1997; Kim et al. 2002). A recessive mutant in Arabidopsis, *edr1*, also displays enhanced disease resistance to powdery mildew (Frye and Innes 1998). The dominant *EDR1* gene encodes a MAPKK kinase that also acts as a negative regulator of defense responses (Frye et al. 2001). Another recessive *R* gene in Arabidopsis, *RPS1-R*, which is associated with resistance to bacterial wilt, codes for a novel NBS-LRR-WRKY protein, which is presumed to function in the nucleus (Deslandes et al. 2002; Lahaye 2002).

Other lines of evidence also support the hypothesis that recessive and dominant *R* genes function differently. Genetic studies of bacterial blight resistance mediated by two dominant *R* genes, *Xa4* and *Xa21*, and two recessive *R* genes, *xa5* and *xa13*, have shown that the qualitative and quantitative components of resistance are associated with the properties of the *R* genes, suggesting that these four genes are involved in different defense pathways in rice (Li et al. 2001).

The proteins encoded by defense-responsive genes are components of the signal transduction pathways that lead to defense responses of the host plant after the recognition event triggered by an *R* gene product. Recent studies have shown that some defense-responsive genes are located in the vicinity of quantitative trait loci (QTLs) for disease resistances in different plant species (Faris et al. 1999; Wang et al. 2001; Xiong et al. 2002; Ramalingam et al. 2003; Wen et al. 2003). QTLs for quantitative disease resistance are generally considered to be valuable sources of broad-spectrum and durable resistance in rice breeding programs (Roumen 1994). However, such resources have not yet been exploited for plant improvement, because the properties of the QTLs are unknown and their locations are not known precisely. Thus the establishment of correlations between the chromosomal locations of defense-responsive genes and resistance QTLs will facilitate the characterization of genes underlying quantitative resistance to disease.

In rice, the recessive *R* gene *xa13* specifically confers resistance to the Philippine *Xoo* race 6 (PXO99) that is compatible with most of the bacterial blight resistance genes. It was first characterized in the rice variety BJ1, and mapped on the long arm of rice chromosome 8 (Ogawa et al. 1987; Zhang et al. 1996; Sanchez et al. 1999). Unlike other recessive genes for resistance to bacterial blight—such as *xa5*, which shows partial dominance to the avirulent *Xoo* races— *xa13* is a completely recessive gene (Li et al. 2001). Our recent results suggest that the predicted product of a candidate for *xa13* does not contain the conserved domains observed in most of the dominant *R* genes so far isolated (Chu et al., unpublished data). Small-scale gene expression studies have also revealed that some of the defense-responsive genes that are activated in *xa13*-mediated resistance are not involved in dominant *R* gene (*Xa4*, *Xa10* and *Xa26*)-mediated resistance (Zhou et al. 2002; Wen et al. 2003). These results suggest that *xa13*-mediated resistance may be regulated by a different molecular mechanism. Thus, large-scale analysis of the defense-activated genes in recessive *R* gene-mediated resistance should help to elucidate another aspect of the molecular mechanisms of disease resistance. The objectives of the present study were to characterize the genome-wide reaction of defense-responsive genes in *xa13*-mediated bacterial blight resistance, and to investigate their possible relations with quantitative resistance by associating their locations with QTLs for resistance to various diseases. The results provide a rich resource for the study of the defense pathways triggered by recessive *R* genes, and for improving the resistance of crops in breeding programs.

Materials and methods

Construction of pathogen induced subtraction cDNA libraries

Leaf tissue from IRBB13 (*Oryza sativa* sp. *indica*), a blight-resistant line carrying *xa13*, and its susceptible near-isogenic parental line IR24 (*O. sativa* sp. *indica*) were used for cDNA library construction. At the booting stage the plants were inoculated by the leaf-clipping method (Kauffman 1973) with Philippine *Xoo* strain PXO99 (race 6), which is incompatible with IRBB13 but compatible with IR24. Total RNA was isolated with TRIzol reagent (Invitrogen, Carisbad, Calif., USA) from leaf blades (4–5 cm in length) next to inoculation sites, at 12, 24 and 72 h after inoculation. Our previous studies had shown that expression of most of the rice defense-responsive genes was induced by 24 h after inoculation with the pathogen (Zhou et al. 2002; Wen et al. 2003). Thus, mRNA was purified from total RNA collected 24 h after inoculation, using Dynabeads oligo dT_{25} (Dynal A. S., Oslo, Norway), and then employed for the construction of cDNA libraries. Two subtraction cDNA libraries were constructed using pGEM-T as the vector and the Clontech PCR-Select cDNA Subtraction Kit (Clontech, Palo Alto, Calif., USA) according to the manufacturer's instructions. In brief, cDNAs from two types of tissue were employed as tester and driver, respectively. The tester cDNA was ligated with cDNA adaptors, and hybridized with an excess of driver cDNA. The non-hybridized tester molecules were therefore enriched for differentially expressed sequences, and were cloned into the vector. Library I was constructed using RNA isolated from inoculated-IRBB13 as tester and inoculated-IR24 as driver, and library II was constructed using inoculated-IRBB13 as tester and non-inoculated-IRBB13 (without any treatment) as the driver source. Thus, only pathogen-induced genes that showed up-regulated expression in IRBB13 were retained in the two libraries.

Sequence analysis

The cDNA clones were sequenced using the universal T7 primer and the BigDye Terminator Cycle Sequencing v2.0 (Applied Biosystems, Foster City, Calif., USA). Only sequences longer than 100 bp were collected. Clustering of the expressed sequence tags (ESTs) was analyzed using the computer program ESTClustering (Zhang et al. 2003). Briefly, the EST sequences were assembled based on the following criteria: (1) the overlapping region of two sequences must be larger that 40 bp, and (2) the degree of nucleotide sequence identity in the overlapping region must be larger than 94%. DNA sequence similarity analysis was performed using

BLASTN and BLASTX (Altschul et al. 1997). The ESTs were classified according to their putative functions as revealed by BLASTX.

The EST sequences were mapped onto the rice chromosomes, with a threshold E value of $\leq 10^{-5}$, by BLASTN analysis against homologous rice genomic sequences with known chromosomal locations (http://rgp.dna.affrc.go.jp and http://www.genome.clemson.edu).

RNA gel blot analysis

Aliquots (20 μg) of total RNA were fractionated by electrophoresis in a 1% agarose gel containing formaldehyde, and transferred to a Hybond-N^+ nylon filter. The filter was hybridized at 65°C overnight in hybridization buffer containing 0.14 M NaH_2PO_4, 0.36 M Na_2HPO_4, 7% SDS and 1 mM EDTA. The probe was prepared using the hexamer labeling method (Feinberg and Vogelstein 1983). The filter was washed once in 2×SSC and 0.1% SDS for 10 min at 65°C, and once in 0.5×SSC and 0.1% SDS for 15 min at 65°C.

Results and discussion

Analyses of the sequences and expression patterns of defense-responsive genes

Previous studies had revealed that some defense-responsive genes are involved in both incompatible and compatible interactions (Maleck et al. 2000; Wen et al. 2003). Therefore, cDNA library I may not include some defense-responsive genes that are also involved in compatible interactions. Thus unequal numbers of cDNA clones from the two libraries were sequenced. A total of 1152 cDNA clones, 384 randomly picked from cDNA library I and 768 randomly picked from cDNA library II, were sequenced, and 1050 ESTs, ranging in length from 100 to 802 bp, were generated. Sequence analysis of the ESTs using the program ESTClustering identified 702 unique sequences, of which 591 were singletons and 111 (designated as C, such as C3 and C12) were contigs made up of 2–16 overlapping ESTs (GenBank Accession Nos. CD150758–CD151455 and CD285455–CD285458) (Supplementary Table 1). In all, 233 (designated with the prefix 1; e.g., 1B05 and 1P17) of the 702 unique sequences were from cDNA library I and another 451 (designated as 2 or 3; e.g., 2I18 and 3H04) were from cDNA library II. The remaining 18 unique sequences (C4, C19, C21, C23, C24, C27, C33, C36, C38, C62, C65, C72, C77, C79, C80, C85, C90 and C91) were generated from contigs consisting of sequences from the both libraries. The average length of the 702 ESTs was 363 bp. Analysis of all 702 ESTs using BLASTN programs revealed 113 (16%) new rice ESTs that had no match or only a poor match (E value $>10^{-5}$) to any of the 201,747 rice EST entries in GenBank.

Based on sequence homology of the predicted products of the ESTs with sequences already present in databases, the 702 ESTs were classified into 13 groups (Table 1). The groups "Unknown Function" and "No Hits" accounted for 38% of the ESTs studied, and more than half of the new rice ESTs fell into one or other of these two groups. These results suggest that a large proportion of the genes activated in *xa13* -mediated bacterial blight resistance are novel genes.

About 6% of the ESTs show some sequence similarity to known or putative *R* -like genes and defense-responsive genes (Table 1). Analysis of these ESTs using the BLASTX program identified 14 *R* -gene homologs. Four of the 14 ESTs (1B05, 2I18, 1O21 and 2I04) display various degrees of sequence homology to putative *R* genes encoding NBS-LRR type proteins; the putative products of another 10 ESTs (C3, C12, C16, C33, C38, C97, 1P17, 1D10, 1M21 and 3H04) were homologous to rice XA21-like proteins (Supplementary Table 1). There are also ESTs which show homology to defense-responsive genes involved in host-pathogen interactions reported in various plant species, such as genes coding for pathogenesis-related proteins, peroxidases, WRKY-type transcription factors, glutathione S-transferases, RNA helicases, proteasome components, ubiquitins, ankyrin-like protein, cytochrome P450, catalase, and metallothionein-like protein (Supplementary Table 1) (Tenhaken et al. 1995; Choi et al. 1996; Seehaus and Tenhaken 1998; Takemoto et al. 1999; Yang et al. 1999;

Table 1 Classification of defense-responsive sequences in bacterial blight resistance mediated by *xa13* gene

Group	Number of ESTs[a]			Number of loci that co-localized with resistance QTLs
	Total	Mapped[b]	Novel[c]	
Disease-resistance-like and defense-responsive activity	44	34	9	15
DNA binding	20	15	3	7
RNA binding	9	9	0	4
Other binding activity	35	30	5	18
Chaperone	16	13	0	2
Apoptosis regulator	2	1	0	1
Enzyme	175	149	19	83
Signal transducer	20	18	3	11
Structural molecule	17	15	1	6
Transporter	26	23	0	11
Other function	70	56	11	25
Unknown function	215	182	32	88
No hits found	53	23	30	11
Total	702	568	113	282

[a]Supplementary Table 1 includes detailed information on all the ESTs
[b]ESTs mapped on rice molecular linkage map (Fig. 2)
[c]New rice ESTs

Yu et al. 1999; Etienne et al. 2000; Austin et al. 2002; Azevedo et al. 2002; Yan et al. 2002; Zhou et al. 2002). These results indicate that recessive gene-mediated disease resistance utilizes some components which also participate in other host-pathogen interactions.

The expression patterns of some of the ESTs in rice line IRBB13 after inoculation with the incompatible and compatible *Xoo* strains PXO99 and PXO61, respectively, were analyzed by Northern hybridization. Fifty-three ESTs were examined. Twenty-three of these showed various levels of differential expression after inoculation with the pathogen (Fig. 1; the data for three ESTs are not shown because the hybridization signal was very weak). No hybridization signal could be detected for another 30 of the ESTs, implying very low levels of expression. All 20 ESTs in Fig. 1 showed increased expression after inoculation with *Xoo* strain PXO99, although the level of increased expression varied greatly. In agreement with previous reports (Maleck et al. 2000; Wen et al. 2003), the expression of some of the ESTs was also induced by inoculation with the compatible *Xoo* strain PXO61 (Fig. 1). However, the expression patterns of the ESTs, in terms of the time of onset of induced expression and the level of expression, usually differed between incompatible and compatible interactions.

Among the ESTs that showed differential expression after inoculation with the incompatible pathogen, 2I18 and 1B05 are homologous to NBS-LRR-type *R* genes, and 1M21 and C38 are homologous to *Xa21* -type *R* genes. The induced expression of the four ESTs was detected at 12 or 24 h after inoculation with *Xoo* strain PXO99, and the elevated expression levels were maintained until 72 h after inoculation. The mapping data (Fig. 2) indicate that none of these four ESTs is a candidate for the *xa13* gene itself. Thus, the differential expression of 2I18, 1B05, 1M21 and C38 before and after inoculation provides molecular evidence to suggest that genes encoding R-like proteins can serve as defense components in recessive gene-mediated disease resistance. The result also further supports genetic observations on the additive action of *R* genes in rice (Li et al. 1999, 2001). The sequences of four ESTs (1A09, 2A13, 2P03 and C65) are highly similar to those of defense-responsive genes, and their expression levels were increased 12–24 h after inoculation, suggesting that these genes may function in different defense pathways, or in the same pathway but with up- and downstream relationships. The predicted products of four ESTs (2A07, 3A07, 3A17 and 1A01) are of unknown function. The other eight ESTs (2A01, 3A21, 1B06, 3A19, 1C07, 2H16, 3I04 and 3C06) show various degrees of sequence similarity to genes or putative genes encoding enzymes, senescence-associated proteins, keratins, membrane-binding proteins, protein kinases, and salt-inducible proteins that have not been reported to be involved in host-pathogen interaction of plants. Thus, they may be useful leads in the study of novel defense pathways activated by recessive *R* genes.

Co-localization of defense-responsive genes and disease resistance QTLs

Quantitative disease resistance is an important feature of a plant's defenses against pathogen invasion. Many QTLs for quantitative resistance to various rice diseases, including blast (Wang et al. 1994; Chen et al. 2003), sheath blight (Li et al. 1995), yellow mottle virus (Albar

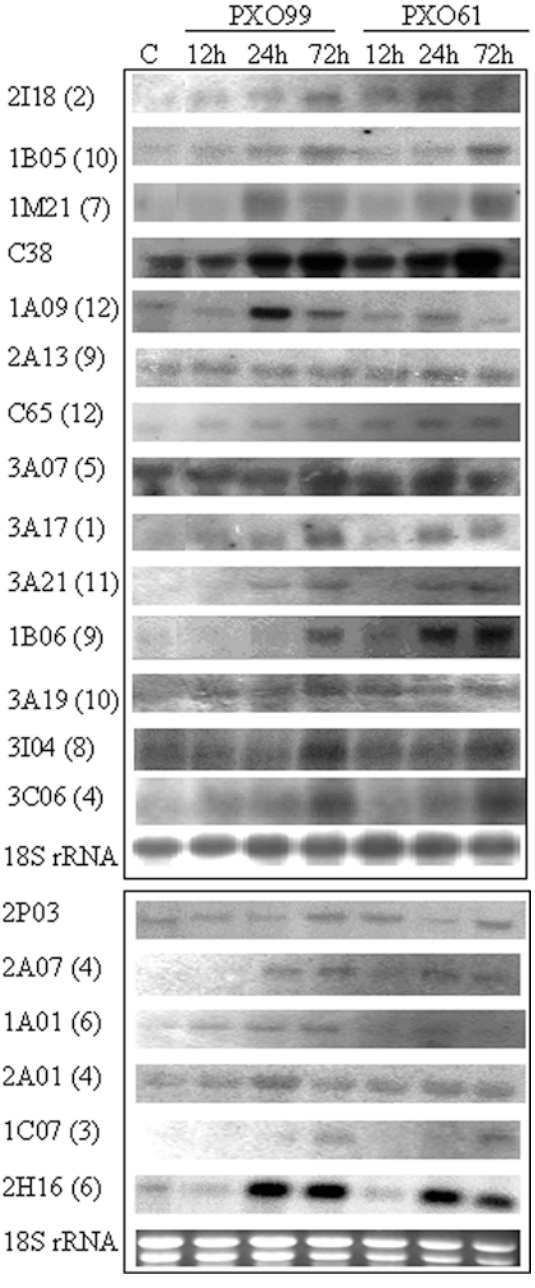


Fig. 1 Expression patterns of defense-responsive ESTs as revealed by RNA gel blot analysis. The samples were isolated from the rice line IRBB13 at 12, 24 and 72 h after inoculation with the incompatible *Xoo* strain PXO99 or the compatible *Xoo* strain PXO61. Lane C was loaded with RNA from a non-inoculated control 12 h after inoculation of the other plants with pathogen. The chromosomal location of each EST is indicated by the number in *parentheses*

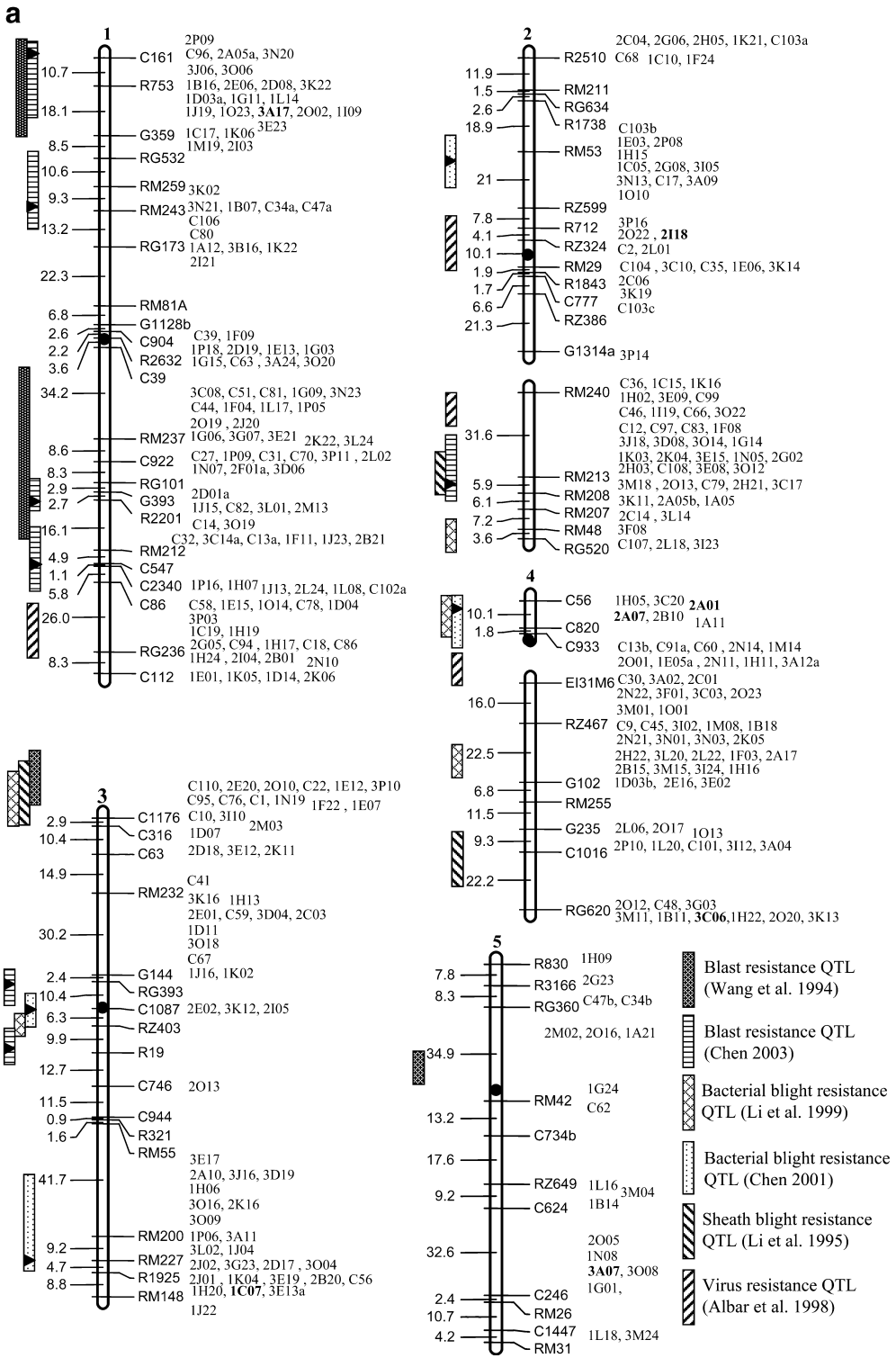
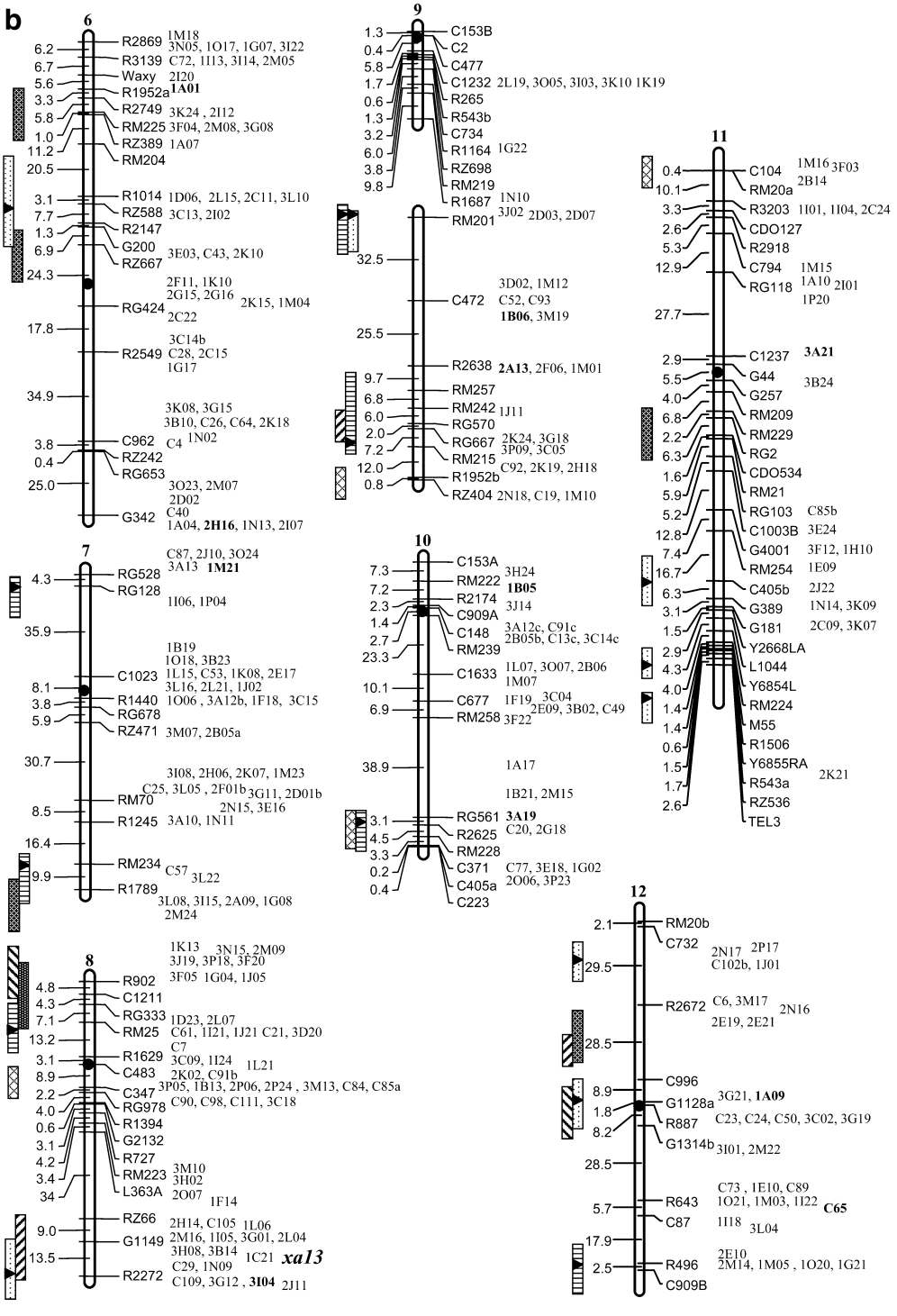


Fig. 2a, b Distribution of defense-activated ESTs on rice chromosomes 1–6 (**a**) and 7–12 (**b**). The loci placed on the *right* side of each chromosome were mapped based on homologous sequence analysis. The ESTs indicated in *bold face* are those whose expression patterns were studied (see Fig. 1). A *lower case letter* (a, b or c) following the name of an EST locus indicates that multiple loci were detected by the same EST. The *triangles* in the bacterial blight resistance and blast resistance QTLs, detected using the same population as was used to construct the framework map, indicate the logarithm-of-odds peaks of the resistance effects. The *filled circles* represent the centromeric regions identified previously (Wang et al. 2000a)

et al. 1998), and bacterial blight (Li et al. 1999; Chen 2001), have been identified. However, for none of the resistance QTLs has the molecular basis of the resistance been elucidated, because of the complexity of the quantitative effects. One way to characterize genes underlying QTLs is to construct near-isogenic lines of target QTLs and study the QTLs as single Mendelian factors (Yano and Sasaki 1997). The rice QTL *Hd1* for heading date has been characterized using this strategy (Yamamoto et al. 1998; Yano et al. 2000). Although the isolation of *Hd1* provides a successful example of the genetic dissection of rice QTLs, this approach is both time-consuming and labor-intensive. Furthermore, this strategy is not applicable to QTLs that explain only small fractions of phenotypic variance. Recent studies have revealed that some defense-responsive genes

Fig. 2a, b (Contd.)



co-localize with resistance QTLs identified in barley, bean, rice and potato (Faris et al. 1999; Geffroy et al. 2000; Trognitz et al., 2002; Xiong et al. 2002; Ramalingam et al. 2003). Thus, the candidate-gene approach may provide a means for pinpointing the genes that underlie resistance QTLs.

Altogether 618 ESTs were assigned to 639 loci distributed on all 12 rice chromosomes by bioinformatic analysis (Table 1, Supplementary Table 1). Most (602) of the ESTs could be assigned to single loci, 11 ESTs were each assigned to two loci and 5 were each assigned to three loci. A molecular linkage map containing 221 RFLP (restriction fragment length polymorphism) and SSR (simple sequence repeat) markers and covering the whole rice genome (Xing et al. 2002) was used as the framework map for mapping of the ESTs. The map was constructed with a segregating population including 241 recombinant inbred lines developed by single-seed

Table 2 Two- and three-copy defense-responsive sequences conserved at similar locations on different chromosomes

EST	Chromosome			Functional analysis (BLASTX)	
	Locus 1	Locus 2	Locus 3	Homolog	E value
C34	1	5	-	Aspartic proteinase (BAA02242)	1e-104
C47	1	5	-	S-Adenosyl methionine synthetase (CAA81481)	8e-61
2F01	1	7	-	Histone H1-like protein (BAB92342)	4e-39
2D01	1	7	-	Chlorophyll *a*/*b* binding protein (AAC15992)	1e-30
C85	8	11	-	GF14-d protein (AAB07458)	3e-66
2B05	7	10	-	Putative vacuolar sorting receptor protein (AAN05373)	2e-62
1D03	1	4	-	Putative peptide transporter (AAG46153)	1e-27
C102	1	12	-	Similar to transposon MuDR mudrA protein isolog (BAA92402)	8e-09
2A05	1	2	-	Unknown protein (BAB16327)	2e-22
C13	1	4	10	ORF (NP_817268)	8e-07
C91	4	8	10	Hypothetical protein (AAF09840)	1e-21
3A12	4	7	10	ORF16-LacZ fusion protein synthetic construct (AAA72562)	8e-07
3C14	1	6	10	Ribulose 1,5-bisphosphate carboxylase large subunit (AAA85330)	5e-46

descent from a cross between Zhenshan 97 (*Oryza sativa* ssp. *indica*) and Minghui 63 (*indica*). Resistance QTLs against bacterial blight and blast had been identified using this population in previous studies (Chen 2001; Chen et al. 2003). A total of 588 of the 639 loci represented by 568 ESTs were localized on the map, based on the linkage information for the genomic sequences that were homologous to the ESTs and molecular markers used in published rice maps (Fig. 2; Causse et al. 1994; Harushima et al. 1998; Xiong et al. 1998; Xing et al. 2002). For example, the EST 1G07 was homologous (E value = 2e-56) to a genomic sequence (GenBank Accession No. AP001389) based on BLASTN analysis; AP001389 encompasses the sequence of an RFLP marker, S1515, according to the information obtained during physical mapping of the rice genome (http://rgp.dna.affrc.go.jp and http://www.genome.clemson.edu); S1515 is tightly linked to another RFLP marker, R2869, as revealed by a published rice molecular linkage map (Harushima et al. 1998); thus, the EST 1G07 was mapped adjacent to R2869 on the terminal end of the short arm of chromosome 6 (Fig. 2). The distribution of the mapped loci was as follows: 19%, 13%, 10%, 11%, 3%, 9%, 7%, 9%, 5%, 5%, 4%, and 5% could be assigned to chromosomes 1–12, respectively.

Altogether 282 (48%) of the mapped EST loci fell in the vicinity of QTLs for resistance to various rice diseases, including blast, sheath blight, virus and bacterial blight (Wang et al. 1994; Li et al. 1995, 1999; Albar et al. 1998; Chen 2001; Chen et al. 2003) (Fig. 2). Thus, this class of ESTs may mainly include the genes commonly involved in quantitative disease resistance in rice. About half (15) of the mapped ESTs in the group of disease-resistance-like and defense-responsive activity co-localized with resistance QTLs against bacterial blight, blast, sheath blight or virus on chromosomes 1, 2, 3, 8, 9, 10, 11 and 12 (Table 1, Fig. 2). Fourteen of the 15 ESTs represent defense-responsive genes reported in various plant-pathogen interactions, such as some putatively encoding peroxidases, glutathione S-transferases, RNA helicases, proteasome components, ubiquitin, and cytochromes P450. These ESTs provide a resource for studying the architectures of resistance QTLs commonly involved in plant resistance to different diseases. Large numbers of mapped ESTs from 12 other groups also co-localized with rice QTLs for disease resistance. Although some of the ESTs may be associated with resistance QTLs just by chance (because some of the QTLs cover a large chromosomal region), these ESTs provide a wide range of choice to study the roles of different types of genes in quantitative disease resistance.

The resistance gene *xa13* co-localized with QTLs for resistance to *Xoo* and virus on the long arm of chromosome 8 (Fig. 2). A few ESTs (1C21, 3H08, 3B14, C29 and 1N09) were also assigned to this region, and none of these encodes the conserved domains found in most of the dominant *R* genes (Supplementary Table 1). This may indicate that *xa13* indeed functions differently from most of the dominant *R* genes.

Although the chromosomal locations of about 52% of the mapped ESTs showed no correspondence to known resistance QTLs, it is most likely that these ESTs include the genes that are involved specifically in *xa13* - mediated resistance. This hypothesis is supported by the following lines of evidence. First, genetic studies using near-isogenic lines carrying different *R* genes against bacterial blight have shown that the quantitative components of the disease resistance were associated with the properties of dominant *R* genes, suggesting that *xa13* has different regulatory roles in rice defense pathway(s) or that different defense pathways are involved in dominant *R* gene- and recessive *R* gene-mediated resistance (Li et al. 2001). Second, gene expression studies indicate that different defense-responsive genes are involved in different host-pathogen

interactions in rice, revealing three types of defense-responsive genes, pathogen non-specific, pathogen specific but race non-specific, and race specific, in bacterial blight and blast resistance (Zhou et al. 2002; Wen et al. 2003).

Some defense-responsive sequences are conserved at similar locations on different chromosomes

Another interesting feature of the distribution of the defense-responsive ESTs in the rice genome is that the loci detected by 13 of the 14 mapped two- and three-copy ESTs were distributed on different chromosomes. The locations of the loci detected by most of the multicopy ESTs on different chromosomes relative to their centromeres are consistent (Table 2, Fig. 2). For example, 2A05 was mapped to two loci in the terminal regions of chromosomes 1 and 2; the three loci detected by 3A12 were located in the peri-centromeric regions of chromosomes 4, 7 and 10. Simultaneous occurrence of the loci detected by different multicopy ESTs across different chromosomes was also observed. For example, loci detected by 2F01 and 2D01 occurred in the central regions of the long arms of chromosomes 1 and 7, as was the case for loci detected by C34 and C47 in the middle regions of the short arms of chromosomes 1 and 5. Similar distribution patterns were also observed when rice retrotransposons and multicopy RFLP markers were used as probes, which led to the hypothesis that chromosome duplication and diversification may have been involved in the origin and evolution of the rice chromosomes (Wang et al. 1999, 2000b). The present results further support the chromosomal duplication-diversification hypothesis. These data also suggest that, in addition to the conservation of homologous genomic sequences at similar locations on different rice chromosomes, there are defense-responsive-like genes located at similar loci on different chromosomes. It will be interesting to study the possible role of this feature in the complexity of disease resistance.

Conclusions

Defense responses against bacterial blight mediated by the *xa13* gene are complex processes which are regulated by many defense-responsive genes, including many novel genes (38%) and genes that have not previously been reported to be involved in host-pathogen interactions. In addition, *R*-like genes serve as defense components in the disease resistance activated by the recessive *R* gene. These results provide a rich resource for the study of the molecular mechanisms of disease resistance triggered by recessive *R* genes. About half of the defense-responsive genes, which code for a wide range of products, co-localize with disease resistance QTLs. This information provides a wide choice of candidate genes for understanding the molecular basis of quantitative disease resistance.

Acknowledgements We sincerely thank the International Rice Research Institute for providing the near-isogenic resistance lines of rice, and the Philippine strains of *Xoo*. This research was supported by grants from the National Natural Science Foundation of China (39970450) and the National Program of research and Development of Transgenic Plants of China (JY03A0502)

References

Albar L, Lorieux M, Ahmadi N, Rimbault I, Pinel A, Sy AA, Fargette D, Ghesquiere A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology. Theor Appl Genet 97:1145–1154

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JDG, Parker JE (2002) Regulatory role of *SGT1* in early *R* gene-mediated plant defenses. Science 295:2077–2080

Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of *R* gene-triggered disease resistance. Science 295:2073–2076

Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP (1997) Signaling in plant-microbe interactions. Science 276:726–733

Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelen R, van der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley *Mol* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chen H (2001) Population structure of *Pyricularia grisea* from Central and Southern China and comparative mapping of QTL for blast- and bacterial blight-resistance in rice and barley (in Chinese). PhD Thesis. Huazhong Agriculture University, Wuhan, China

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

Chen H, Wang S, Xing Y, Xu C, Hayes PM, Zhang Q (2003) Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. Proc Natl Acad Sci USA 100:2544–2549

Choi D, Kim HM, Yun HK, Park JA, Kim WT, Bok SH (1996) Molecular cloning of a metallothionein-like gene from *Nicotiana glutinosa* L. and its induction by wounding and tobacco mosaic virus infection. Plant Physiol 112:353–359

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

Deslandes L, Olivier J, Theulieres F, Hirsch J, Feng DX, Bittner-Eddy P, Beynon J, Marco Y (2002) Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RPS1-R* gene, a member of a novel family of resistance genes. Proc Natl Acad Sci USA 99:2404–2409

Etienne P, Petitot AS, Houot V, Blein JP, Suty L (2000) Induction of *tcI 7*, a gene encoding a beta-subunit of proteasome, in tobacco plants treated with elicitins, salicylic acid or hydrogen peroxide. FEBS Lett 466:213–218

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS (1999) Candidate gene analysis of quantitative disease resistance in wheat. Theor Appl Genet 98:219–225

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragment length polymorphisms to high specific activity. Anal Biochem 132:6–13

Frye CA, Innes RW (1998) An Arabidopsis mutant with enhanced resistance to powdery mildew. Plant Cell 10:947–956

Frye CA, Tang D, Innes RW (2001) Negative regulation of defense responses in plants by a conserved MAPKK kinase. Proc Natl Acad Sci USA 98:373–378

Geffroy V, Sevignac M, de Oliveira JCF, Fouilloux G, Skroch P, Thoquet P, Gepts P, Langin T, Dron M (2000) Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. Mol Plant-Microbe Interact 13:287–296

Harushima Y, et al (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J 19:4004–1014

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 Newslett 16:92–93

Kim MC, Panstruga R, Elliott C, Muller J, Devoto A, Yoon HW, Park HC, Cho MJ, Schulze-Lefert P (2002) Calmodulin interacts with MLO protein to regulate defence against mildew in barley. Nature 416:447–451

Lahaye T (2002) The Arabidopsis *RPS1* -R disease resistance gene—uncovering the plant's nucleus as the new battlefield of plant defense? Trends Plant Sci 7:425–427

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

Li Z, Pinson SRM, Marchetti MA, Stansel JW, Park WD (1995) Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet 91:382–388

Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol Gen Genet 261:58–63

Li ZK, Sanchez A, Angeles E, Singh S, Domingo J, Huang N, Khush GS (2001) Are the dominant and recessive plant disease resistance genes similar? A case study of rice *R* genes and *Xanthomonas oryzae* pv. *oryzae* races. Genetics 159:757–765

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

Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nature Genet 26:403–410

Nagato Y, Yoshimura A (1998) Report of the Committee on Gene Symbolization, Nomenclature and Linkage Groups. Rice Genet Newslett 15:13–74

Nimchuk Z, Rohmer L, Chang JH, Dangl JL (2001) Knowing the dancer from the dance: *R* -gene products and their interactions with other proteins from host and pathogen. Curr Opin Plant Biol 4:288–294

Ogawa T, Lin L, Tabien RE, Khush GS (1987) A new recessive gene for resistance to bacterial blight of rice. Rice Genet Newsl 4:98–100

Ramalingam J, Vera Cruz CM, Kukreja K, Chittoor JM, Wu J-L, Lee SW, Baraoidan M, George ML, Cohen MB, Hulbert SH, Leach JE, Leung H (2003) Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. Mol Plant-Microbe Interact 16:14–24

Roumen EC (1994) A strategy for accumulating genes for partial resistance to blast disease in rice within a conventional breeding program. In: Zeigler RS, Leong SA, Teng PS (eds) Rice blast disease. CAB International, Wallingford, UK, pp 245–265

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 gene bacterial blight resistance gene in rice. Theor Appl Genet 98:1022–1028

Seehaus K, Tenhaken R (1998) Cloning of genes by mRNA differential display induced during the hypersensitive reaction of soybean after inoculation with *Pseudomonas syringae* pv. *glycinea*. Plant Mol Biol 38:1225–1234

Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, Holsten T, Gardner J, Wang B, Zhai W-X, Zhu L-H, 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, encoding an LRR receptor kinase-like protein. Plant J, in press

Takemoto D, Hayashi M, Doke N, Nishimura M, Kawakita K (1999) Molecular cloning of a defense-response-related cytochrome P450 gene from tobacco. Plant Cell Physiol 40:1232–1242

Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. Science 274:2060–2063

Tenhaken R, Levine A, Brisson LF, Dixon RA, Lamb C (1995) Function of the oxidative burst in hypersensitive disease resistance. Proc Natal Acad Sci USA 92:4158–4163

Trognitz F, Manosalva P, Gysin R, Nino-Liu D, Simon R, Herrera MR, Trognitz B, Ghislain M, Nelson R (2002) Plant defense genes associated with quantitative resistance to potato late blight in *Solanum phureja* × Dihaploid *S. tuberosum* hybrids. Mol Plant-Microbe Interact 15:587–597

Wang G-L, Mackill DJ, Bonman M, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421–1434

Wang S, Liu N, Peng K, Zhang Q (1999) The distribution and copy number of *copia* -like retrotransposons in rice (*Oryza sativa* L.) and their implications in the organization and evolution of the rice genome. Proc Natl Acad Sci USA 96:6824–6828

Wang S, Wang J, Jiang J, Zhang Q (2000a) Mapping of centromeric regions on the molecular linkage map of rice (*Oryza sativa* L.) using centromere-associated sequences. Mol Gen Genet 263:165–172

Wang S-P, Liu K-D, Zhang Q-F (2000b) Segmental duplications are common in rice genome. Acta Botanica Sinica 42:1150–1155

Wang Z, Taramino G, Yang D, Liu G, Tingey SV, Miao G-H, Wang G-L (2001) Rice ESTs with disease-resistance gene- or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol Genet Genomics 265:302–310

Wen N, Chu Z, Wang S (2003) Three types of defense-responsive genes are involved in resistance to bacterial blight and fungal blast diseases in rice. Mol Genet Genomics 269:331–339

Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs in the genetic basis of yield traits in rice. Theor Appl Genet 105:248–257

Xiong L-Z, Wang S-P, Liu K-D, Dai X-K, Saghai Maroof MA, Hu J-G, Zhang Q-F (1998) Distribution of simple sequence repeat and AFLP markers in molecular linkage map of rice. Acta Botanica Sinica 40:605–614

Xiong M, Wang S, Zhang Q (2002) Coincidence in map positions between pathogen-induced defense-responsive genes and quantitative resistance loci in rice. Science China (Series C) 45:518–526

Yamamoto T, Kubiki Y, Lin SY, Sasaki T, Yano M (1998) Fine mapping of quantitative trait loci Hd-1, Hd-2 and Hd-3, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44

Yan J, Wang J, Zhang H (2002) An ankyrin repeat-containing protein plays a role in both disease resistance and antioxidation metabolism. Plant J 29:193–202

Yang P, Chen C, Wang Z, Fan B, Chen Z (1999) A pathogen- and salicylic acid-induced WRKY DNA-binding activity recognizes the elicitor response element of the tobacco class I chitinase gene promoter. Plant J 18:141–149

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

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35:145–153

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) Hd1, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12:2473–2484

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

Yu D, Xie Z, Chen C, Fan B, Chen Z (1999) Expression of tobacco class II catalase gene activates the endogenous homologous gene and is associated with disease resistance in transgenic potato plants. Plant Mol Biol 39:477–488

Zhang G, Angeles ER, Abenes MLP, Khush GS, Huang N (1996) RAPD and RFLP mapping of the bacterial blight resistance gene *xa13* in rice. Theor Appl Genet 93:65–70

Zhang L-D, Yuan D-J, Zhang J-W, Wang S-P, Zhang Q-F (2003) A new method for EST clustering. Acta Genetica Sinica 30:147–153

Zhou B, Peng K, Chu Z, Wang S, Zhang Q (2002) The defense-responsive genes showing enhanced and repressed expression after pathogen infection in rice (*Oryza sativa* L.). Science China (Series C) 45:449–467