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ORIGINAL PAPER

Multiple gene loci affecting genetic background-controlled disease resistance conferred by *R* gene *Xa3/Xa26* in rice

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Abstract The function of bacterial-blight resistance gene *Xa3/Xa26* in rice is influenced by genetic background; the *Oryza sativa* L. ssp. *japonica* background can increase *Xa3/Xa26* expression, resulting in an enhanced resistance. To identify whether *Xa3/Xa26* transcript level is the only factor contributing to genetic background-controlled resistance, we screened an F_2 population that was developed from a cross between *Oryza sativa* L. ssp. *indica* and *japonica* rice lines and was segregating for *Xa3/Xa26*, and compared the expression profiles of a pair of *indica* and *japonica* rice lines that both carried *Xa3/Xa26*. Eight quantitative trait loci (QTLs), in addition to *Xa3/Xa26*, were identified as contributing to the bacterial resistance of this population. Four of the eight QTLs were contributed to the *japonica* line. The resistance of this population was also affected by epistatic effects. Some F_2 individuals showed significantly increased *Xa3/Xa26* transcripts, but the increased transcripts did not completely correlate with the reduced disease in this population. The analysis of the expression profile of *Xa3/Xa26*-mediated resistance using a microarray containing approximate 7,990 rice genes identified 44 differentially expressed genes. Thirty-five genes were rapidly activated in the *japonica* background, but not in the *indica* background, during disease resistance. These results suggest that multiple factors, including the one resulting in increased *Xa3/Xa26* expression, may contribute to the enhanced resistance in the *japonica* background. These factors can cause a variation in gene expression profile that differs from that in the *indica* background during disease resistance.

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Introduction

A large number of disease resistance (*R*) genes that confer race-specific resistance to diverse pathogens have been characterized from dicotyledonous and monocotyledon plants; most of these *R* genes encode proteins that harbor conserved domains and motifs (Martin et al. 2003). Among the characterized *R* genes encoding conserved domains, *Xa3/Xa26* from rice, encodes leucine-rich repeat (LRR) receptor kinase-type protein (Sun et al. 2004; Xiang et al. 2006). *Xa3/Xa26* mediates resistance to bacterial blight, a disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which is a significant agronomic problem in rice-growing regions. Asian-cultivated rice (*Oryza sativa* L.) consists of two major groups known by the subspecies names *indica* (*O. sativa* L. ssp. *indica*) and *japonica* (*O. sativa* L. ssp. *japonica*). *Xa3/Xa26* was first identified in the *indica* rice variety Minghui 63; it was mapped to the long-arm of chromosome 11 and named *Xa26* (Yang et al. 2003). The characterization of *Xa26* showed that Minghui 63 and *japonica* transgenic lines carrying *Xa26* regulated by its native promoter had a different resistance spectrum to *Xoo*; the transgenic lines showed a higher level and wider spectrum of resistance compared with Minghui 63 (Sun et al. 2004), indicating that *Xa26* functions better in the *japonica* background than in the *indica* background. Further study revealed that an *indica* near-isogenic line

IRBB3, which carries only one *R* gene, *Xa3*, for *Xoo* resistance (Ogawa et al. 1991), also carried *Xa26* (Sun et al. 2004). Fine genetic mapping of the *R* gene in IRBB3, DNA fingerprinting, and phenotypic comparison showed that *Xa3* and *Xa26* were the same gene, and they were renamed *Xa3*/*Xa26* (Xiang et al. 2006). IRBB3 has a higher level and wider spectrum of resistance to *Xoo* than that of Minghui 63, although the latter carries another gene, *Xa25*(*t*), in addition to *Xa3*/*Xa26*, for *Xoo* resistance (Chen et al. 2002; Sun et al. 2004), indicating that different *indica* backgrounds have different effects on the function of *Xa3*/*Xa26*. Further study confirmed that different *japonica* backgrounds also had different effects on *Xa3*/*Xa26*-mediated resistance, but, in general, the *japonica* background facilitates the function of *Xa3*/*Xa26* more than the *indica* background does (Cao et al. 2007).

Genetic background-influenced *R* gene-mediated resistance has been observed in many plant species using classic genetic analyses (Crute and Pink 1996, for review). The molecular mechanisms of host background effects are largely unknown, although reports from a few studies have revealed some clues (Banerjee et al. 2001; Cao et al. 2007; Van Poecke et al. 2007). The function of *Arabidopsis R* gene *RPS2* is influenced by genetic background, and the LRR domain determines the effectiveness of the interaction between RPS2 and other host factors in *RPS2*-mediated resistance (Banerjee et al. 2001). Further study has demonstrated that the variation in gene expression profiles is associated with genetic background-controlled disease resistance conferred by *RPS2* (Van Poecke et al. 2007). Rice *Xa3*/*Xa26*-mediated resistance is dosage dependent: as *Xa3*/*Xa26* expression increases, the plant's resistance increases (Cao et al. 2007). Genetic background influences *Xa3*/*Xa26* expression. The transcript level of *Xa3*/*Xa26* is markedly higher in *japonica* rice than in *indica* rice (Cao et al. 2007). In addition, *Xa3*/*Xa26*-mediated resistance to some *Xoo* races is influenced by developmental stage in *indica*, but not *japonica* rice. The *indica* plants carrying *Xa3*/*Xa26* were highly susceptible in four-leaf stage, but become resistant or moderately resistant to these *Xoo* races at booting stage. The development-controlled *Xa3*/*Xa26* activity is also associated with its expression level (Cao et al. 2007). However, it is unknown whether the expression level of *Xa3*/*Xa26* is the only factor that regulates genetic background-controlled resistance conferred by this gene.

To answer the question, we combined the analyses of quantitative trait locus (QTL) using an F_2 population segregating for *Xa3*/*Xa26*, which was developed from crossing between an *indica* line and a *japonica* rice line, and gene expression profile using a pair of *indica* and *japonica* rice lines that both carried *Xa3*/*Xa26*.

Materials and methods

Disease assays in different genetic backgrounds and developmental stages

Four rice lines, Minghui 63 (*O. sativa* ssp. *indica*), Rb1 (*O. sativa* ssp. *japonica*), Rb49 (*O. sativa* ssp. *japonica*), and Mudanjiang 8 (*O. sativa* ssp. *japonica*), were used to ascertain whether the resistance conferred by an *R* gene was influenced by genetic background and developmental stage. Minghui 63 carries two *R* genes, *Xa3*/*Xa26* and *Xa25*(*t*) against *Xoo* (Chen et al. 2002; Yang et al. 2003; Xiang et al. 2006). Rb1 and Rb49 are transgenic lines carrying a single copy of *Xa3*/*Xa26* regulated by its native promoter in the genetic background of susceptible Mudanjiang 8 (Sun et al. 2004; Cao et al. 2007). Rice lines were grown by staggered planting, so that all the lines with different genetic background and at designated development stages could be inoculated with pathogens at the same time.

Plants were inoculated with Chinese *Xoo* strain JL691 or Philippine *Xoo* strains PXO71 (race 4) or PXO99 (race 6) by the leaf-clipping method (Sun et al. 2004). The eight plants in the middle of each row were used for disease scoring. The disease was recorded as lesion area (lesion length/leaf length × 100%) and lesion length (cm) at 2–3 weeks after inoculation. A plant with lesion area of <15% was considered as highly resistant, with the lesion area of equal and larger than 15% and less than 30% as moderately resistant, with the lesion area of equal and larger than 30% and less than 50% as moderately susceptible, and with the lesion area of equal and larger than 50% as highly susceptible.

Mapping of resistance QTL

An F_2 population, consisting of 146 individuals from a cross between Mudanjiang 8 and Minghui 63, was used to detect QTLs that influence the function of *Xa3*/*Xa26*. The population was inoculated with Philippine *Xoo* strain PXO61 (race 1) when most of the plants were at the booting stage and rest of the plants were at the maximum-tillering or heading stages. Approximately, 2–6 leaves per plant were measured for lesion length and lesion area. For population genotyping, 136 polymorphic markers relatively evenly distributed on 12 rice chromosomes, including 135 simple sequence repeat (SSR) markers and a dominant PCR marker for *Xa3*/*Xa26*, were used. The primers of the marker of *Xa3*/*Xa26* were Rkb3R (5′-TCAGTGTCAAGACCACATCG-3′) and Rkb3F (5′-CTTCGTGCAACCGATGATTT-3′). The SSR markers of the RM series were designed according to Temnykh et al. (2000, 2001), and those of the MRG series were designed according to McCouch et al. (2002). Mapmaker/Exp 3.0

(Lincoln et al. 1992) was used for linkage analysis. The Kosambi function was used to calculate genetic distance. QTL analysis was conducted with the use of the program Windows QTL Cartographer Version 2.0 for composite interval mapping at a threshold of LOD 2.0 (Wang and Zeng 2003). The PCR primers used to identify F_2 individuals that carried *Xa3*/*Xa26* or its susceptible allele were Rkb3R and Rkb3F.

The entire genome was searched for digenic interactions for each trait with two-way analyses of variance (ANOVA) using all possible two-locus combinations of marker genotypes based on the unweighted cell means (Yu et al. 1997). The statistical significance for each term was assessed using an orthogonal contrast test with the statistical package STATISTICA (StatSoft 1991).

Gene expression analysis

The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis was conducted as described previously (Qiu et al. 2007). The PCR primers of *Xa3*/*Xa26* gene were RKb3F and RealR (5′-CCTAATTGCTT CCCTTGTTCTGA-3′). The expression level of actin gene was used to standardize the RNA sample for each qRT-PCR. The PCR primers of the actin gene were Actin120F (5′-TGTATGCCAGTGGTCGTACCA-3′) and Actin120R (5′-AGTCTGGAGTGTGTGGCTCAAG-3′). qRT-PCR assays were repeated at least twice, with each repetition having three replicates; similar results were obtained in repeated experiments.

cDNA microarray analysis

The rice cDNA microarray containing 9,198 unique EST sequences (Huang et al. 2006) was used to identify candidate genes involved in the regulation of genetic background-controlled *Xa3*/*Xa26* function. The cDNAs of the EST sequences were randomly chosen from a normalized whole lifecycle cDNA library of rice variety Minghui 63 (Chu et al. 2003). The design and the cDNA sequences of the arrays can be found on the Website (http://redb.ricefgchina.org/mged/hy/).

Rice lines Minghui 63, Rb49, and Mudanjiang 8 were inoculated with *Xoo* strain PXO71 or water (mock inoculation as control) at the booting stage. The 5-cm leaves below the inoculation site were harvested, respectively, at 2, 12, 24, and 72 h after inoculation. The experiments for each rice line were repeated four times on two independently grown sets of plants to prepare RNA samples used for independent hybridization to the microarrays. Total RNA was extracted with RNAex reagent (Huashun, Shanghai, China), and mRNA was purified with Dynabeads oligo$(dT)_{25}$ from Dynal A.S. (Oslo, Norway). The mRNA samples of treatment and control from each independently grown set of plants were reverse transcripted and labeled with Cy3 and Cy5 fluorescent dyes, respectively, in one repeat, and with Cy5 and Cy3, respectively, in another repeat as described previously (Lian et al. 2006). Hybridization and data collection and analysis were performed by the procedures described previously (Lian et al. 2006). All data were quantified with the use of the ImaGene 4.2 software (BioDiscovery, Los Angeles, CA). For normalization of the data, the scanning parameters were adjusted to make the sums of signal intensities of Cy3 and Cy5 nearly equal. Spots that were flagged "Bad" by ImaGene 4.2 and that exhibited a fluorescent intensity level two times below the local background were excluded from further analysis. The signal mean ratio of stressed/control was generated based on the normalized signals and used to measure the relative level of gene expression.

To obtain significant differentially expressed genes between PXO71 and mock inoculation, a "yellow" experiment was conducted, in which the same mRNA sample was labeled with Cy3 and Cy5, respectively (Lian et al. 2006). According to this experiment, the upper and lower 2.5 percentiles of the spots in the distribution curve were −1.19 and 1.25. In the analysis, more stringent criteria (average 1.4 and −1.4) were used as the critical points for declaring up- or down-regulations. Furthermore, to reduce the probability of false-positive results, a sequence was regarded as a differentially expressed gene when the ratios of four replicates were simultaneously larger than 1.25 or smaller than −1.19, and the log-transformed normalized signals of stress compared with that of control using the *t* test was at the 0.005 probability level (Dudoit et al. 2002).

Results

Genetic background-influenced *Xa3*/*Xa26* function throughout rice developmental stages

The growing stage of the two parents, Minghui 63 and Mudanjiang 8, of the F_2 population used for QTL analysis are 135–140 and 90–95 days, respectively. The growing stage of the F_2 plants in this population was segregated. To determine which growth stage would be used to detect loci that putatively influenced *Xa3*/*Xa26*-mediated resistance and to analyze *Xa3*/*Xa26*-influenced gene expression profiles, we first examined the pattern of development-controlled *Xa3*/*Xa26* activity (Fig. 1). The *indica* variety Minghui 63, the donor of *Xa3*/*Xa26*, is highly resistant to *Xoo* strain JL691, moderately resistant or susceptible to *Xoo* strain PXO71, and highly susceptible to *Xoo* strain PXO99 at the adult stage (Chen et al. 2002; Yang et al. 2003). The *japonica* transgenic lines Rb1 and Rb49, which

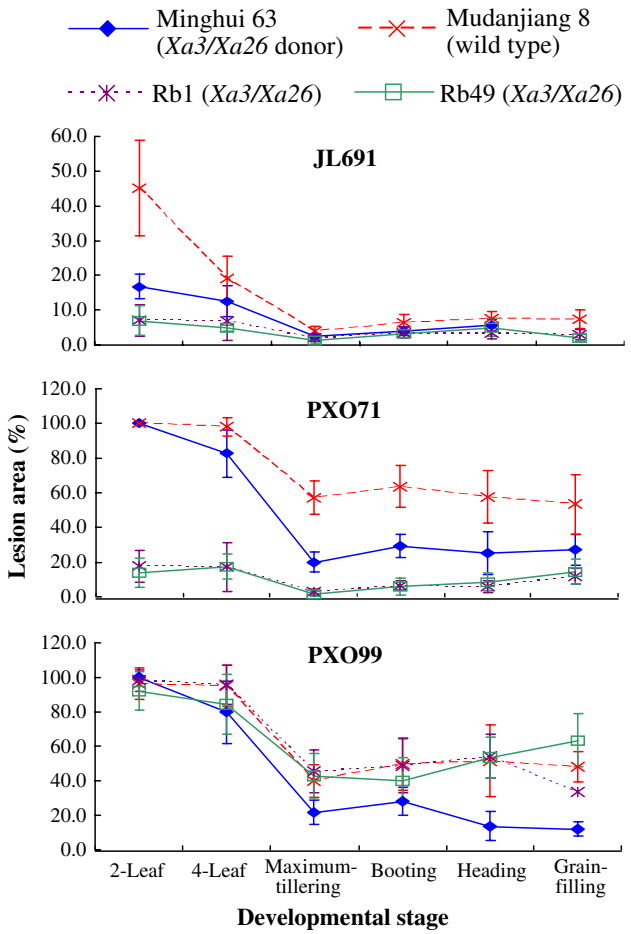

Fig. 1 Genetic background and developmental stages influence the function of *Xa3/Xa26* gene. Transgenic lines Rb1 and Rb49, susceptible *japonica* Mudanjiang 8, and *Xa3/Xa26* donor, *indica* Minghui 63, were inoculated with *Xoo* strains JL691, PXO71, and PXO99. Disease was scored at 14 days after inoculation. Each point represents mean (8 replicates) ±standard deviation

carry a single copy of *Xa3/Xa26* driven by its native promoter, is highly resistant to JL691 and PXO71, but susceptible to PXO99 at the adult stage (Sun et al. 2004). The analysis of the performance of different rice lines in response to *Xoo* infection showed that Rb1 and Rb49 had similar level of resistance to JL691 and PXO71 from the two-leaf stage to the grain-filling stage (Fig. 1). In contrast, Minghui 63 was moderately resistant to JL691 and highly susceptible to PXO71 at the two-leaf stage, and became highly resistant to JL691 and moderately resistant to PXO71 at the maximum-tillering stage and maintained the similar level of resistance until grain-filling stage. *Xa3/Xa26* could not mediate resistance to PXO99 throughout the rice growth stage (Fig. 1). An interesting feature was that even in the compatible (susceptible) interaction, the rice lines showed a gradual reduction in lesion area as the plants grew, and this reduced lesion area reached a relatively stable level at the maximum-tillering stage (Fig. 1). These results suggest that the influence of genetic background on the function of *Xa3/Xa26* is not restricted to a particular developmental stage; *Xa3/Xa26*-mediated resistance is more efficient in the background of *japonica* Mudanjiang 8 than in *indica* Minghui 63 throughout the rice growth stage. However, development stage influences the response of both resistant and susceptible plants to *Xoo* infection; plants at maximum-tillering stage or older appear more resistant to *Xoo* than the plants at two- and four-leaf stages. Based on these results, most of the F_2 plants were inoculated at booting and rest of the plants were inoculated at maximum tillering or heading stages for QTL analysis and inoculated at booting stage for gene expression profile analysis, which are described in the following text.

Multiple loci affected *Xa3/Xa26*-mediated resistance

An F_2 population developed from a cross between *indica* Minghui 63 and *japonica* Mudanjiang 8 was used to study genetic background-controlled resistance conferred by *Xa3/Xa26*. Minghui 63 is moderately resistant to *Xoo* strain PXO61 as to PXO71 at adult stage, which is mediated by *Xa3/Xa26* (Yang et al. 2003; Cao et al. 2007). Transgenic plants carrying a single copy of *Xa3/Xa26,* which was regulated by its native promoter in the genetic background of susceptible Mudanjiang 8, are highly resistant to PXO61 (Sun et al. 2004; Cao et al. 2007). This F_2 population was inoculated with PXO61. The distribution of lesion area or lesion length caused by PXO61 infection in the 146 individuals showed a continuous distribution at 14 and 21 days after infection (Fig. 2) suggesting the involvement of multiple genes for resistance to PXO61 in addition to the major resistance gene *Xa3/Xa26* in this population.

The disease measurements produced at 14 and 21 days after infection with PXO61 varied greatly in the two traits (lesion area and lesion length). Transgressive segregations were observed for all the trait/time combinations (Table 1). Some of the F_2 individuals showed less disease than their resistant parent, whereas other F_2 individuals showed more disease than their susceptible parent. Even six F_2 individuals carrying *Xa3/Xa26* showed similar or more susceptibility than that of their susceptible parent. These results further suggest that disease resistance conferred by *Xa3/Xa26* is influenced by some host factors.

To dissect the loci that might influence *Xa3/Xa26*-mediated resistance, we constructed a linkage map consisting of 136 markers spanning a total of 1,631 cM (Fig. 3). For the four trait/time combinations, lesion length/14 days, lesion area/14 days, lesion length/21 days, and lesion area/21 days, a total of nine marker intervals showing effect on resistance to *Xoo* were detected (Table 2 and Fig. 3). The marker interval on chromosome 11, which was detected by all four trait/time combinations and explained 31.5–57.7%

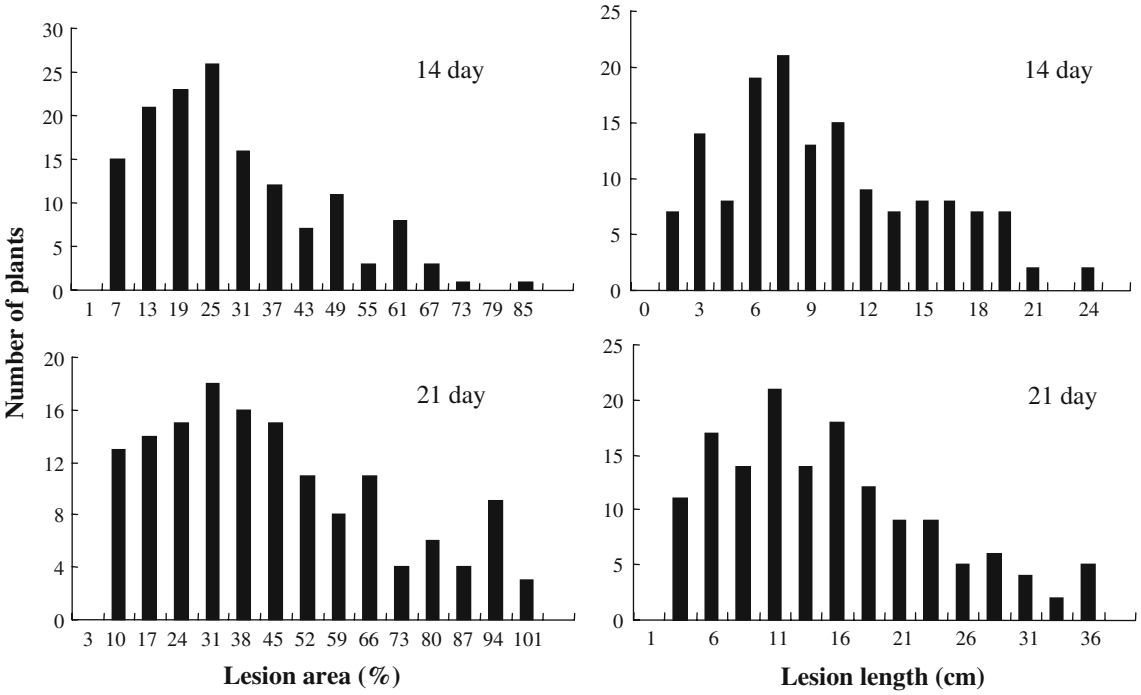

Fig. 2 The distribution of lesion area and lesion length after PXO61 infection in a sample containing 146 individuals from an F_2 population developed from the cross between moderately resistant Minghui 63 and susceptible Mudanjiang 8. Each datum represents mean (2–6 replicates) ± standard deviation at 14 or 21 days after infection

Table 1 The lesion area (%) of an F_2 population and its parent in response to infection with *Xoo* strain PXO61

Trait[a]	Parent		All F_2		F_2 carrying X*a3*/*Xa26*[b]	
	Minghui 63	Mudanjiang 8	Mean	Range	Mean	Range
14A	22.27 ± 7.36	56.62 ± 13.49	26.10	1.41–83.14	21.85	1.41–83.14
14L	10.73 ± 3.48	13.52 ± 2.83	9.02	0.25–22.67	7.38	0.25–20.50
21A	32.62 ± 9.35	86.32 ± 12.99	41.45	3.41–100.00	33.44	3.41–100.00
21L	15.86 ± 4.52	20.99 ± 4.21	14.16	1.10–35.83	11.01	1.10–29.42

[a] 14A and 21A, lesion area (%) at 14 and 21 days after *Xoo* infection; 14L and 21L, lesion length (cm) at 14 and 21 days after *Xoo* infection

[b] 115 F_2 individuals are *Xa3*/*Xa26* positive as identified by the dominant PCR maker

of phenotypic variation, corresponds to *Xa3*/*Xa26* gene. The phenotypic variations of resistance accounted for by the eight QTLs varied greatly among the different trait/time combinations, ranging from 6.6 to 20.5% (Table 2). The major QTLs, *XR3b* and *XR5* on chromosomes 3 and 5, respectively, were also detected by all four trait/time combinations. The rest of the minor QTLs, which explained less than 10% of phenotypic variation, was detected by one or two trait/time combinations (Fig. 3). Among the eight QTLs, the resistance alleles at four QTLs, *XR3a*, *XR3b*, *XR7*, and *XR9*, on chromosomes 3, 7, and 9 were from the moderately resistant parent Minghui 63, and the resistance alleles at another four QTLs, *XR1a*, *XR1b*, *XR4*, and *XR5*, on chromosomes 1, 4, and 5 were from the susceptible parent Mudanjiang 8 (Table 2). These results further confirm that multiple loci contributed by both the resistant and susceptible parent influence *Xa3*/*Xa26*-mediated *Xoo* resistance.

One hundred and twenty-nine co-dominant markers forming 8,756 possible two-locus combinations were used for testing digenic interactions in this segregating population. No epistatic effect between *Xa3*/*Xa26* and QTL allele from Mudanjiang 8 that increased resistance or between *Xa3*/*Xa26* and any allele from Minghui 63 that decreased resistance was detected. However, four digenic interactions were detected to significantly affect resistance (Supplemental Table 1). When the allele at locus RM254 flanking *Xa3*/*Xa26* (Table 2) was homozygous for Minghui 63, the allele at locus RM71 (chromosome 2) that was homozygous for Mudanjiang 8 increased resistance significantly. Another three digenic interactions at loci RM323/RM475, RM323/RM440, and RM475/RM6 also increased resistance

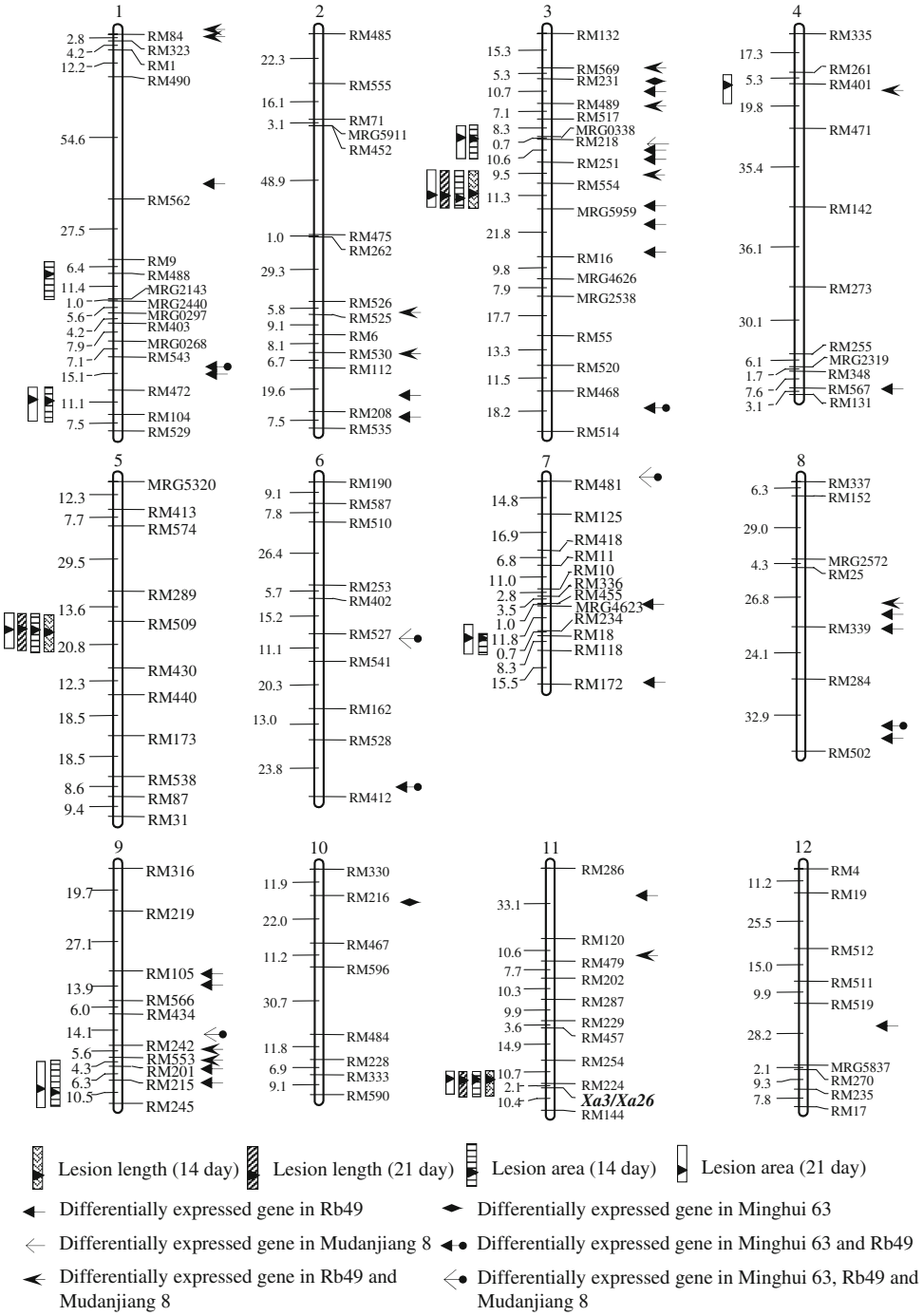

Fig. 3 Co-mapping of resistance QTLs against *Xoo* and differentially expressed genes after *Xoo* infection. The *bars* indicate the 1-LOD support intervals of the QTLs identified. *Small triangle* in each bar indicates the peak of the LOD contour. *Different arrows* represent differentially expressed genes identified in different rice lines after *Xoo* infection. The gene information is presented in Supplemental Table 4

when the alleles were homozygous for Mudanjaing 8 (Supplemental Table 1). These results suggest that *Xa3*/*Xa26*-mediated resistance was also affected by epistatic effects.

F_2 plants showed variable *Xa3*/*Xa26* transcript levels

Twenty-four F_2 plants and 21 of them carrying *Xa3*/*Xa26* were randomly chosen to analyze the expression level of *Xa3*/*Xa26*. Some F_2 plants showed a significantly ($P < 0.01$) higher expression level of *Xa3*/*Xa26* than that of the gene donor Minghui 63. The transcript levels of *Xa3*/*Xa26* in these F_2 plants were 1.5–4.4-fold higher than that in Minghui 63 as revealed by qRT-PCR analysis (Fig. 4). Most of the examined F_2 plants that significantly increased *Xa3*/*Xa26* expression showed enhanced resistance to *Xoo* strain PXO61 when compared with their resistant parent Minghui 63, whereas a few F_2 plants with significantly increased *Xa3*/*Xa26* expression showed a resistance level to PXO61 that was similar to or even slightly more susceptible than that of

Table 2 QTLs and *R* gene detected for resistance to PXO61 in the F_2 population

Trait	QTL or *R* gene	Marker interval[a]	LOD	Add[b]	Dom[c]	Var (%)[d]	Colocalization of gene[e]
Lesion length (14 days)	*XR3b*	RM554–MRG5959	4.31	–4.0873	–3.6706	20.5	*Os03g21640*
							Os03g24920
	XR5	RM509–RM430	2.65	1.9980	–2.3240	11.1	
	Xa3/Xa26	RM254–RM224	15.16	–4.7314	–3.3821	41.7	
Lesion area (14 days)	*XR1a*	RM488–MRG2143	2.89	2.9103	8.9965	8.7	
	XR1b	RM472–RM104	2.31	6.2111	3.1665	7.0	
	XR3a	MRG0338–RM218	2.50	–8.5348	–5.1687	7.5	*Os03g16170*
							Os03g16334
							Os03g17030
	XR3b	RM554–MRG5959	3.02	–12.5084	–11.5640	17.7	*Os03g21640*
							Os03g24920
	XR5	RM509–RM430	3.34	7.7849	–5.8282	13.2	
	XR7	RM18–RM118	2.43	–4.7495	5.6868	7.3	
	XR9	RM215–RM245	2.48	–8.0972	2.6701	8.7	*Os09g33910*
							Os09g36300
							Os09g37230
	Xa3/Xa26	RM254–RM224	10.27	–13.1779	–8.1989	31.5	
Lesion length (21 days)	*XR3b*	RM554–MRG5959	3.95	–6.0127	–6.3953	19.5	*Os03g21640*
							Os03g24920
	XR5	RM509–RM430	4.08	3.3391	–4.8450	15.3	
	Xa3/Xa26	RM254–RM224	25.44	–8.4853	–6.8206	57.7	
Lesion area (21 days)	*XR1b*	RM472–RM104	2.30	9.6014	2.8789	7.0	
	XR3a	MRG0338–RM218	2.63	–13.0776	–10.1732	7.9	*Os03g16170*
							Os03g16334
							Os03g17030
	XR3b	RM554–MRG5959	2.62	–16.4365	–17.5690	15.1	*Os03g21640*
							Os03g24920
	XR4	RM401–RM471	2.19	5.9434	14.1133	6.6	*Os04g25190*
	XR5	RM509–RM430	4.51	12.3706	–12.1572	17.0	
	XR7	RM18–RM118	2.40	–8.1756	6.1673	7.2	
	XR9	RM215–RM245	2.37	–12.1145	3.3560	8.6	*Os09g33910*
							Os09g36300
							Os09g37230
	Xa3/Xa26	RM254–RM224	17.08	–22.8999	–17.1740	45.5	

[a] The SSR markers were flanking the peak of LOD contour

[b] Additive effect; the positive and negative values indicate that the allele from Mudanjiang 8 or Minghui 63 decreases the trait score, respectively

[c] Dominance effect; positive values of the dominance effect indicate that the heterozygotes have higher phenotypic values than the respective means of two homozygotes

[d] Variation explained by the QTL

[e] The differentially expressed genes colocalized in the 1-LOD supported interval of QTL

Minghui 63. In addition, a few F_2 plants with similar or significantly lower level of *Xa3/Xa26* transcripts showed enhanced resistance when compared with Minghui 63; this disassociation of *Xa3/Xa26* expression level and enhanced resistance may be due to the complex genetic backgrounds of F_2 plants, such as No. 5 and No. 30 plants may carry the major resistance QTL *XR3b* (Fig. 4). Analyzing the relationship of *Xa3/Xa26*-transcript level and lesion area indicated that increased *Xa3/Xa26* expression was correlated ($r = -0.456$, $n = 24$, significant at $\alpha = 0.05$) with the enhanced resistance in these F_2 plants. These results suggest that genetic background does influence *Xa3/Xa26* expression;

furthermore, combining the results of both expression and QTL analyses suggest that other genetic background-associated factors may also influence *Xa3*/*Xa26* function.

Genetic background affects gene expression profiles in *Xa3*/*Xa26*-mediated resistance

The rice cDNA microarray containing 9,198 unique expressed sequence tags (ESTs), which correspond to approximately 7,990 genes according to the annotation of The Institute for Genomic Research (TIGR; http://rice.tigr.org), was used to compare gene expression profiles initiated in *Xa3*/*Xa26*-mediated resistance in *indica* Minghui 63 and *japonica* Rb49 backgrounds. To distinguish the differentially expressed genes in *R* gene-mediated resistance from basal immunity, we used the gene expression profile of susceptible Mudanjiang 8 after inoculation with compatible *Xoo* strain as a control. To assess the reproducibility of the hybridization data, we calculated correlations between technical repeats and biological repeats based on the signal intensities of *Xoo* inoculation and mock inoculation (control) (Supplemental Tables 2 and 3). Correlation coefficients between technical repeats ranged from 0.81 to 0.99, with an average of 0.95. Correlation coefficients between biological repeats varied from 0.77 to 0.97, with an average of 0.91.

We examined a total of 8,738, 8,835, and 8,422 sequences that produced hybridization signals in all four repeats of at least one time point in Minghui 63, Rb49, and Mudanjiang 8, respectively. A total of 51 sequences, representing 44 genes, showed differential expression in at least one rice line and at one time point after *Xoo* infection compared with mock inoculation (Supplemental Table 4). Among the 44 genes, three expressed differentially in all three rice lines, 13 in both resistant Rb49 and susceptible Mudanjiang 8, and four in both Rb49 and resistant Minghui 63, but these genes were frequently upregulated in one rice line and downregulated in another line (Fig. 5). The remaining 24 genes showed differential expression in only one rice line (Fig. 5). Most of the differentially expressed

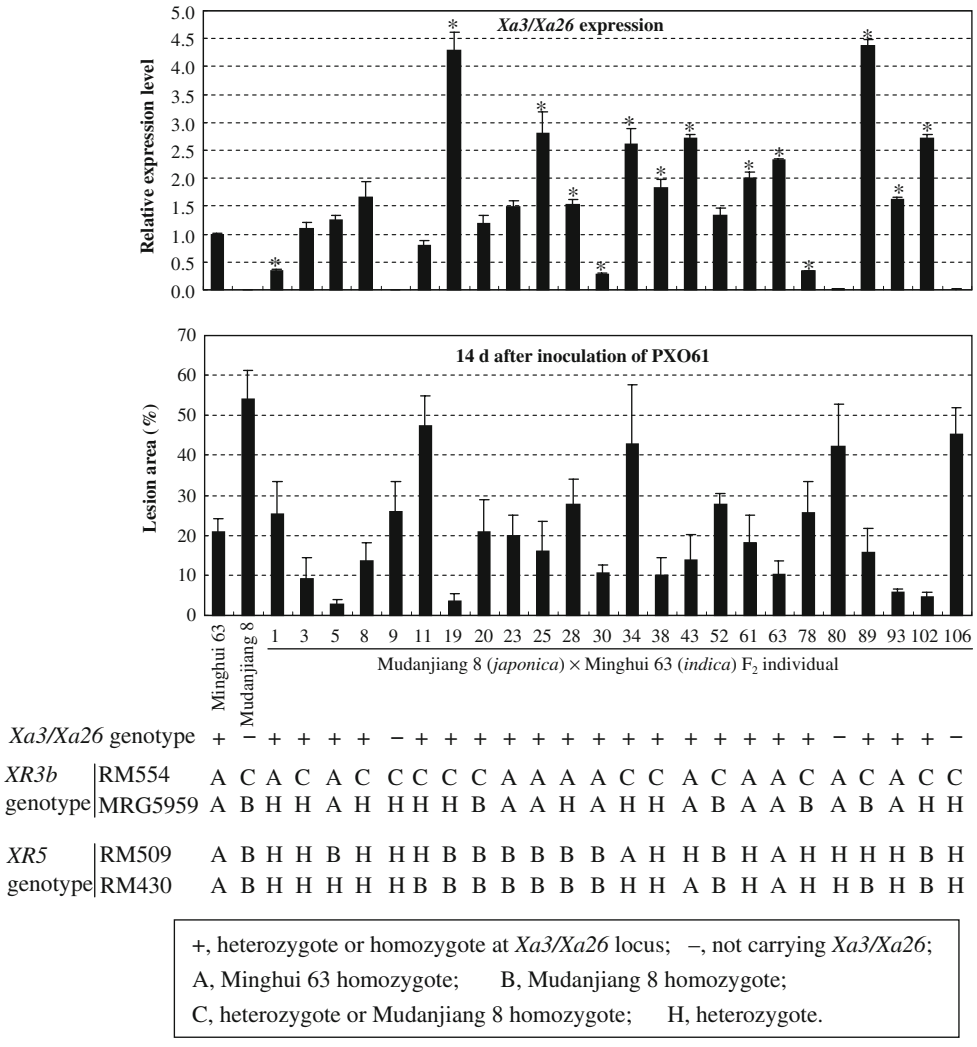

Fig. 4 Comparative analysis of disease resistance and expression level of *Xa3*/*Xa26* in F_2 individuals. Each datum represents mean (3 replicates) ± standard deviation. *Asterisks* indicate that a significant difference ($P < 0.01$) was detected between an F_2 individual and its resistant parent Minghui 63. The genotypes of the molecular markers flanking major resistance QTLs, *XR3b* and *XR5*, in each F_2 plants were shown

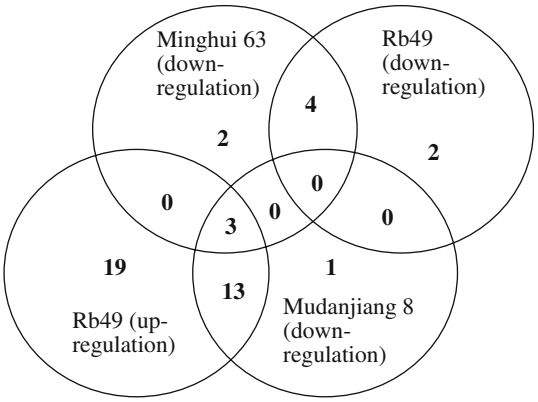

Fig. 5 Numbers of differentially expressed genes in Minghui 63, Rb49 and Mudanjiang 8 after pathogen infection

genes were detected at 12 h after infection; five and three genes were detected at 2 and 72 h after infection, respectively (Supplemental Table 4). In summary, 9 and 17 genes were differentially expressed in Minghui 63 and Mudanjiang 8 after *Xoo* infection, respectively, and they were all downregulated; 41 genes were differentially expressed in Rb49 after infection, and 35 of the 41 were upregulated and six were downregulated (Table 3; Fig. 5). These results indicate that the *Xa3*/*Xa26*-mediated highly resistant response in Rb49 is associated with the rapid activation of a subset of genes, when compared with the *Xa3*/*Xa26*-mediated moderately resistant response in Minghui 63 and the susceptible response in Mudanjiang 8.

Among the 44 differentially expressed genes, 28 have annotation in the "biological process" and "molecular function" ontologies of the Gene Ontology (GO) database (Supplemental Table 5). Some of the differentially expressed genes are included in more than one category or subcategory of the GO classification. The three genes, *Os01g58420*, *Os03g60080*, and *Os06g46950*, which are included in the "response to stimulus" subcategory in the GO term "biological process" and whose expression was suppressed in rice lines Minghui 63 and Rb49 during defense responses (Supplemental Table 4), also showed suppressed expression in OsWRKY13-activated rice lines (Qiu et al. 2008). OsWRKY13 is a positive regulator of pathogen-induced defense responses by activation of the SA-dependent pathway (Qiu et al. 2007). *Os01g58420*, *Os03g60080*, and *Os06g46950* putatively encode ethylene-responsive transcription factor, NAC domain-containing protein, and EF-hand Ca^{2+}-binding protein, respectively.

Chromosomal relationship of differentially expressed genes and resistance QTLs

To identify the candidate genes underlying resistance QTLs, we mapped the 44 differentially expressed genes after *Xoo* infection onto the molecular linkage map according to their physical locations with regard to the SSR markers used for construction of the map. The genes distributed on all rice chromosomes except chromosome 5 (Fig. 3). Nine genes were located on the 1-LOD support intervals of QTLs *XR3a*, *XR3b*, *XR4,* and *XR9* on chromosomes 3, 4, and 9 (Table 2; Fig. 3). The locations of three genes, *Os03g16170*, *Os03g16334*, and *Os03g17030*, corresponded to QTL *XR3a* (Table 2). After *Xoo* infection, *Os03g16170* expression was suppressed in susceptible *japonica* Mudanjiang, *Os03g16334* expression was upregulated in *Xa3*/*Xa26*-mediated resistance in *japonica* transgenic line Rb49 and downregulated in *Xa3*/*Xa26*-mediated moderate resistance in *indica* Minghui 63 and susceptible Mudanjiang 8, and *Os03g17030* expression was induced in Rb49 (Table 3, Supplemental Table 4). Two genes, *Os03g21640* and *Os03g24920*, were associated with QTL *XR3b* (Table 2); both genes were induced in Rb49, and *Os03g21640* was also suppressed in Mudanjiang after *Xoo* infection (Table 3, Supplemental Table 4). *Os03g24920* putatively encodes a ubiquitin-like protein. Ubiquitination-mediated protein degradation is associated with plant–pathogen interactions (Zeng et al. 2006). *Os04g25190* was located corresponding to QTL *XR4* (Table 2); it was upregulated in Rb49 and downregulated in Mudanjiang after infection (Table 3, Supplemental Table 4). Three genes, *Os09g33910*, *Os09g36300*, and *Os09g37230*, were associated with QTL *XR9* (Table 2). All the three genes were induced in Rb49, and *Os09g33910* was also suppressed in Mudanjiang 8 after *Xoo* infection (Table 3, Supplemental Table 4). *Os09g36300* putatively encodes a calcium-dependent protein kinase; this type of protein has been reported to function in barley–pathogen interaction (Freymark et al. 2007).

Discussion

The analysis of transgenic plants has revealed that *japonica* background facilitates *Xa3*/*Xa26* expression, and increasing *Xa3*/*Xa26* expression can further enhance *Xa3*/*Xa26*-mediated *Xoo* resistance, indicating *Xa3*/*Xa26* has dosage effect (Cao et al. 2007). The present results further confirmed these findings. Analysis of F_2 plants generated from a cross between *indica* Minghui 63 and *japonica* Mudanjiang 8 showed that genetic background did influence *Xa3*/*Xa26* expression and *Xa3*/*Xa26*-mediated disease resistance. However, the present results also suggest that the expression level of *Xa3*/*Xa26* does not appear to be the only factor contributing to the genetic background-influenced *Xa3*/*Xa26* function. This notion can be supported by the following evidence. First, four resistance QTLs from *japonica* Mudanjiang 8, which reduced disease phenotype,

Table 3 Upregulated genes in the microarray after infection of *Xoo* strain PXO71

TIGR homolog	Chr	Functional description[a]	Flanking marker[b]
Os01g05430	1	Putative membrane protein	Terminal–RM84
Os01g08320	1	OsIAA1	RM84–RM323
Os01g64360	1	Putative DNA binding protein	RM543–RM472
Os02g46180	2	Expressed protein	RM526–RM525
Os02g50174	2	Caleosin related protein	RM530–RM112
Os02g56140	2	Putative protein SPATULA	RM112–RM208
Os02g57650	2	Putative NAC domain-containing protein 78	RM208–RM535
Os03g04410	3	Putative aconitate hydratase	RM569–RM231
Os03g07360	3	Expressed protein	RM231–RM489
Os03g09830	3	Expressed protein	RM489–RM517
Os03g16334	3	Putative transferase	RM218–RM251
Os03g17030	3	Putative polyadenylate-binding protein 2	RM218–RM251
Os03g21640	3	Expressed protein	RM251–RM554
Os03g24920	3	Putative ubiquitin-like protein	RM554–MRG5959
Os03g27019	3	Expressed protein	MRG5959–RM16
Os03g39610	3	Putative chlorophyll a/b-binding protein	MRG5959–RM16
Os04g25190	4	Putative pollen allergen Phl p2 precursor	RM401–RM471,
Os04g59190	4	Putative peroxidase 2 precursor	RM567–RM131
Os06g18830	6	Putative protein kinase G11A	RM527–RM541
Os07g05360	7	Putative photosystem II 10 kDa polypeptide	Terminal–RM481
Os07g37550	7	Putative chlorophyll a-b binding protien of LHCII type III	RM455–MRG4623
Os07g48750	7	Putative alpha-*N*-arabinofuranosidase 1 precursor	RM118–RM172
Os08g26870	8	Putative wound responsive protein	RM25–RM339
Os08g28680	8	Putative NEDD8-conjugating enzyme Ubc12-like	RM25–RM339
Os08g31510	8	Expressed protein	RM339–RM284
Os08g41440	8	Putative NAD-dependent epimerase/dehydrase family protein	RM284–RM502
Os09g20390	9	Expressed protein	RM105–RM566
Os09g21919	9	Expressed protein	RM105–RM566
Os09g28230	9	Putative Gibberellin receptor GID1L2	RM434–RM242
Os09g31482	9	Putative splicing factor U2af 38 KDa subunit	RM242–RM553
Os09g33910	9	Putative calcium-dependent protein kinase	RM553–RM201
Os09g36300	9	Putative lon protease homolog 1	RM201–RM215
Os09g37230	9	Putative ATP binding protein	RM215–RM245
Os11g02840	11	Putative ATP binding protein	RM286–RM120
Os11g13890	11	Putative chlorophyll a/b-binding protein M9	RM120–RM479

TIGR The Institute for Genomic Research (http://rice.tigr.org), *Chr* chromosome

[a] TIGR annotation

[b] The SSR markers presented in Fig. 3 and flanking the gene

have been identified. Although the present results cannot determine which locus contributes to the enhanced expression of *Xa3*/*Xa26* in the *japonica* background, they suggest that multiple loci from the highly susceptible *japonica* background-influenced *Xa3*/*Xa26*-mediated resistance. Second, *Xa3*/*Xa26* function was also affected by the epistatic effect. The marker of *Xa3*/*Xa26* used in this study was a dominant marker. We used the co-dominant markers RM254 and RM224 that flanked *Xa3*/*Xa26* (Table 2) for the analyses of digenic interaction. The genotypes of the two markers were supposed to represent the genotype of *Xa3*/*Xa26* locus. The co-presence of the RM71 (*japonica* allele)/RM254 (*indica* allele) genotypes significantly increased resistance (Supplemental Table 1). No epistatic effect between the alleles of QTL from *japonica* rice was detected. The possible explanation may be that the complicated genetic background of this population and the effects of the QTLs independently in disease resistance masked the digenic interaction. Third, not all F_2 plants with increased *Xa3*/*Xa26* transcripts showed

enhanced resistance compared with the gene donor of *indica* Minghui 63. This result may be explained that other negative factor(s) from Minghui 63 masked the effect of increased *Xa3*/*Xa26* expression on disease resistance. Thus, the alleles of the negative factors from Mudanjiang 8 may contribute to the enhanced function of *Xa3*/*Xa26* in the *japonic*a background.

Although the cDNA microarray used in this study only represented approximately one-seventh of the loci in rice genome according to the 56,797 loci annotated by TIGR release 6, different expression profiles resulted by *Xa3*/*Xa26*-mediated resistance were clearly detected in *indica* and *japonica* backgrounds. A set of genes were rapidly activated in the *japonica* background, but these genes showed either no change in expression level or suppressed expression in *indica* background during disease resistance. The phenotypic responses of different *Arabidopsis* accessions to bacterial infection conferred by *RPS2*, an *R* gene encoding a nucleotide-binding site-LRR protein, are correlated with the expression patterns examined with the use of a 571-gene microarray (Van Poecke et al. 2007). These results suggest that the variation in gene expression profiles may be the cause of genetic background-controlled disease resistance conferred by some *R* genes. The differential expression of defense-response genes in different genetic background could be due to the presence of polymorphisms at the promoters of at least some genes. Furthermore, this differential expression could be also caused by rapid, slow or lack induction of defense signaling on pathogen infection. This explanation is supported by the following evidences. First, enhancing *Xa3*/*Xa26*-initiated defense signaling by increasing its expression resulted in increased and rapid expression of defense-response genes *OsWRKY13* and *NH1* (Cao et al. 2007). Second, the transgenic plants, which has the genetic background of susceptible line Mudanjiang 8, had more genes differentially expressed during rice–*Xoo* interaction compared with Mudanjiang 8 (Table 3, Supplemental Table 4). The differentially expressed genes in *Xa3*/*Xa26*-mediated resistance are putatively involved in different biological processes and putatively have different molecular functions, indicating that they may function in different signal transduction pathways. This hypothesis also supports that the genetic background-controlled disease resistance conferred by *Xa3*/*Xa26* may be regulated by multiple factors.

The expression of *Xa3*/*Xa26* is developmentally controlled in both *indica* and *japonica* backgrounds; its expression level is low at the two-leaf stage, gradually increases with development, and reaches the highest level at the maximum-tillering stage, suggesting that developmentally controlled resistance conferred by *Xa3*/*Xa26* is related to its differential expression during development (Cao et al. 2007). The present results confirmed this notion. The responses of both *indica* and *japonica* rice lines carrying *Xa3*/*Xa26* to incompatible *Xoo* strains through development are perfectly consistent with the expression patterns of *Xa3*/*Xa26* in the two types of genetic background (Cao et al. 2007). In other words, the present results demonstrate that genetic background influences *Xa3*/*Xa26* function throughout rice development stages.

Plant disease resistance QTLs are generally considered race-nonspecific and durable (Roumen 1994). Characterization of rice resistance QTL genes has confirmed that some QTL genes can mediate broadspectrum resistance to even different types of pathogens (Hu et al. 2008). Comparing the locations of the QTLs identified in this study with those reported previously reveals that five of the eight QTLs have chromosomal association with previously identified resistance QTLs. The QTL *XR1b* on chromosome 1 colocalizes with QTLs for resistance against yellow mottle virus and sheath rot (Albar et al. 1998; Srinivasachary et al. 2002). *XR1a*, *XR5,* and *XR7* on chromosomes 1, 5, and 7 colocalize with QTLs for blast resistance (Chen et al. 2003; Wang et al. 1994; Wisser et al. 2005). In addition, *XR9* overlaps with a previously identified QTL for bacterial blight resistance (Li et al. 1999). These results indicate that these QTLs may mediate a broad-spectrum resistance. A candidate gene strategy, which integrates linkage map, expression profile, and functional complementation analyses, has been proven to be applicable for identifying the genes underlying minor resistance QTLs (Hu et al. 2008). The colocalization of some differentially expressed genes with QTLs in *Xa3*/*Xa26*-mediated resistance will help to characterize the genes underlying these QTLs and involved in the regulation of genetic background-controlled resistance.

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