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Full Length Research Paper

Different patterns of gene expression in rice varieties undergoing a resistant or susceptible interaction with the bacterial leaf streak pathogen

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The rice line carrying the nonhost gene *Rxo1*, which was cloned from maize, exhibits a rapid hypersensitive response (HR) to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*). In this study, a microarray experiment was carried out to analyze the genome-wide gene expression responses to *Xoc* at the early stage in the rice transgenic line and its wild type. 175 and 379 differentially regulated genes (DRGs) were detected in 9804-*Rxo1* and 9804 at 36 h post-inoculation (hpi), respectively. The patterns of DRGs induced by *Xoc* in the transgenic rice line and its wild type were distinctly different: 92.00% of the DRGs were up-regulated in inoculated 9804-*Rxo1* and 48.22% of the DRGs were sorted as defense-related genes. In contrast, 40.69% of the DRGs were up-regulated in inoculated 9804 and 40.00% were sorted as injury-induced genes. Some genes specifically up-regulated in infected 9804-*Rxo1* were defense-related, including the genes encoding pathogenesis-related protein, terpene synthase family, transcription factors (TFs) AP2 domain containing protein, myb-like deoxyribonucleic acid (DNA)-binding domain containing protein, and C_2H_2-type zinc-finger transcription factors. Six peroxidase (POD) genes were significantly up-regulated and POD activity consistently rose in the inoculated 9804-*Rxo1*, whereas seven POD genes were down-regulated in its wild type, indicating that POD may also play a role in the complex process of resistant response to *Xoc* in the transgenic line. Instead of focusing only on the *R*-gene-mediated defense, this study also discusses the susceptible response in the wild types to virulent pathogen infection in transcription level.

Key words: Rice bacterial leaf streak, nonhost resistance, expression profile, *Rxo1*.

INTRODUCTION

Plant defenses against pathogens are generally divided into host and nonhost resistance. Nonhost resistance is a type of nonspecific resistance that an entire plant species exhibits against all genotypes within a pathogen species. Mysore and Ryu (2004) proposed two types of nonhost resistance: Type 1, which does not result in visible cell death; and Type 2, in which a hypersensitive response (HR) occurs, resulting in cell death at the site of infection. Recently, more and more evidence has indicated that HR is a part of nonhost resistance (Heath, 2000; Komatsu et al., 2001; Niks and Marcel, 2009). Single resistance genes available in any one crop species are limited, and they tend to lose their effectiveness because of shifts in the pathogen population. Bacterial leaf streak (BLS) of rice, caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), was reported to be widely distributed in tropical and subtropical Asia, and has recently become a significant problem in western Africa (Pierzynski et al., 2007). To date, no BLS resistance gene (*R* gene) has been found in rice germplasm, and only a few quantitative resistance loci for the disease have been identified (Ochiai et al., 2000; Seo et al., 2008). *Rxo1* is a dominant nucleotide-binding site- leucine rich repeat (NBS- LRR)-type *R* gene identified in maize (Zhao et al. 2004; Zhao et al. 2005).

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Transgenic rice lines with cloned *Rxo1* exhibited a distinct HR symptom when inoculated with *Xoc* (Zhao et al. 2005; Xie et al., 2007; Zhou et al., 2010), demonstrating that certain *R* genes can be effectively transferred between distantly related cereals.

Although the molecular mechanisms of *R*-gene-mediated defenses against pathogens have been studied intensively in rice, there is little information on the molecular response mediated by nonhost genes derived from other plant species (Thordal-Christensen, 2003).

In this study, we primarily analyzed the resistance mechanism mediated by *Rxo1* in transgenic rice line at the earlier stage infected by *Xoc* and compared the result with that at 2 days post-inoculation (dpi) to identify the gene expression pattern in different stages of the disease resistance response. Our objective was to provide some information for understanding the molecular mechanisms that underlie disease resistance activated by nonhost resistance in heterologous plant species.

MATERIALS AND METHODS

Plant materials and artificial inoculation

The *Rxo1* gene was transformed into rice cultivar 9804 (*Oryza sativa* L. japonica) through the *Agrobacterium*-mediated transformation system (Xie et al., 2007). Homozygous T4 lines containing a single copy of *Rxo1*, designated as 9804-*Rxo1*, were used in our experiment. Four-week-old seedlings of 9804 and 9804-*Rxo1* were transplanted into an isolated field at a spacing of 25 × 20 cm with 1 seedling per hill in the greenhouse of the Fujian Academy of Agricultural Sciences.

The *Xoc* isolate FJR5 from Fujian province was revived on potato-sucrose agar and incubated at 30°C for three days. The *Xoc* cells were prepared by suspending the bacterial mass with sterile water to a concentration of approximately 10^8 cells/ml. Flag leaves of the 9801-*Rxo1* and 9804 plants were inoculated using the bacterial suspension by a pin-prick method at the booting stage (Tang et al., 2000) and each flag leaf was pricked at about 15 points. Three replicates of inoculated leaves from the transgenic and wild type lines were harvested at 36 h post-inoculation (hpi); meanwhile, three replicates of the untreated leaves from 9804-*Rxo1* and 9804 were collected as controls. The arrays, which were control samples of transgenic and wild type plants, were denoted by C and B, respectively, while *Xoc*-treated experimental samples of transgenic and wild type plants were denoted by A and D, respectively. The phenotypic response of inoculated leaves to *Xoc* was observed at 24, 36, 72, 96, and 132 hpi, respectively.

Extraction of total ribonucleic acid (RNA)

The collected leaves of 9804 and 9804-*Rxo1* were immediately frozen in liquid nitrogen, and then kept at -70°C. About 500 mg of leaf tissue from three leaves was ground to a fine powder in liquid nitrogen using a mortar and pestle. Total RNA was initially isolated using TRIzol reagent under conditions described by the supplier (Invitrogen) and treated with the Qiagen RNeasy MinElute Cleanup Kit (Cat. no. 74204), and then quality-checked and quantified using a Bioanalyzer 2100 (Agilent Technologies, Cheadle, UK).

RNA samples from each treatment were transcript to complementary deoxyribonucleic acid (cDNA) and complemenraey ribonucleic acid (cRNA), which was then used for hybridization to the Affymetrix Genechips® Rice Genome Array chips, which contain probes of 51,279 transcripts from two rice cultivars, including 48,564 japonica transcripts and 1,260 indica transcripts from Affymetrix (Santa Clara, CA). The sequence information for this array was derived from NCBI UniGene Build #52 (www.ncbi.nlm.nih.gov/Uni-Gene), GenBank mRNAs, and 59,712 gene predictions from TIGR's osa1 version 2.0.

Microarray experiment and data analysis

Hybridization to the arrays and quality control checks were carried out at CapitalBio Corporation, Beijing. The triplicate array data set was analyzed using GeneChip operating software (GCOS 1.2) and DChip (24) software. The scanned images of the hybridization chips were first examined by visual inspection and then satisfactory image files were analyzed to generate raw data files saved as CEL files using the default settings of GCOS 1.2 from Affymetrix. GCOS was used to evaluate the detection calls (present, absent, or marginal) for the probe sets.

Further analysis was done using DChip, which incorporates a statistical model for expression array data at the probe level. The differentially expressed genes were identified using the empirical criterion of more than 2-fold change and significant t-tests with q value < 0.05 based on three biological replicates. The fold change assigned to differentially expressed genes was used for ordering of up-regulated and down-regulated genes. The rice genome annotation project (http://rice.plantbiology.msu.edu/) and molecule annotation system (MAS) were used for the annotation, gene ontology (GO), and pathway analysis.

Real-time polymerase chain reaction and quantitative real-time polymerase chain reaction validation

In order to validate microarray results, several important expression profiles obtained from chip hybridizations were further validated by semi-quantitative real-time polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR) using transcript cDNA. Three replicates of total RNAs from different time points, prepared as described for the microarray analysis were used. The messenger ribonucleic acid (mRNA) levels for each gene in different tissue samples were calculated relative to the gene's expression in control seedlings. First-strand cDNA was prepared using a Promega Kit according to the manufacturer's instructions. A mixture of 1 µg total RNA, 0.5 µg oligo (dT) 15mer primers, and nuclease-free water to a total volume of 8 µl was incubated at 70°C for 10 min. 2 µl 10× reverse transcription buffer, 2 µl 10 mM deoxyribonucleoside triphosphates (dNTP) mixture, 15 U (0.76 µl)AMV reverse transcriptase, and 0.5 µl recombinant RNasin ribonuclease inhibitor were added into each tube, mixed mildly, and then incubated at 42°C for 1 h, followed by 95°C for 5 min. The reactions were kept at -20°C for use. 2 µL aliquots were used for agarose gel electrophoresis analysis. Then, 2 µl of 5× diluted first-strand reaction was subsequently used as a template for 25 µl PCR reaction in the presence of 2.5 units of Taq DNA polymerase (SBS Genetech Co., Ltd), 2 µl dNTPS, and 2 µl 10× PCR buffer. 1 µl 10 pmol primers was used to generate fragment amplifications (Table 1) and primers for a housekeeping actin gene (GenBank accession no. Os03g0718100) was used to generate a 505-bp product as an internal control. The PCR conditions were 5 min of pre-denaturation at 94°C followed by 29 cycles of denaturation at 94°C for 30 s, annealing at (T_m+3)°C for 30 s, and extension at 72°C for 45 s. 1.5 to 5 µl aliquot of PCR reactions was further used for agarose gel analysis.

For qRT-PCR, three replicates of the same template as RT-PCR were prepared and amplified using the RT-PCR kit (Takara SYBR

Premix Ex Taq™) according to the manufacturer's instructions. The qRT-PCR was performed in a 25 µl reaction in an ABIPrism7000 sequence detection system (Applied BioSystems). Primers (Table 1) designed by Primer Prime 5 were subsequently tested in a dissociation curve analysis and verified for the absence of nonspecific amplification (Luo et al., 2005). The obtained data were analyzed according to the methods described by Livak (Livak and Schmittgen, 2001) with minor modifications. The transcript level of each gene was normalized by *Actin* (Os03g0718100) as the internal control. The fold-change values in the treated sample as compared with its corresponding mock-treated control sample were used for analysis, and the relative magnitude of the change in expression was calculated based on the data for three biological replicates for each treatment.

Endogenous peroxidase extraction and peroxidase activity test

Three replicates of leaves samples collected from *Xoc*- or H_2O-inoculated 9804-*Rxo1* and 9804 at 12 hpi, 24 hpi, 48 hpi, 72 hpi, and 96 hpi were sampled and homogenized by a cold mortar and pestle into fine power with liquid nitrogen. The crude endogenous POD was extracted according to the method of Hammerschmidt et al. (1982). Data of absorbance at 470 nm were recorded in the first 6 min after mixing with the reaction buffer. Finally, POD activity was present as units of enzymatic activity (UE), which corresponds to the change of absorbance (OD_{470}), in 1 min, per gram of fresh weight.

RESULTS

Resistant and susceptible symptom of rice varieties response to *Xanthomonas oryzae pv. oryzicola*

The infected sites turned white in the first 36 hpi in both transgenic 9804-*Rxo1* and its wild type 9804 (Figures 1A, B, a, b), but susceptible water-soaked lesions were observed on the leaves of 9804 starting at 24 hpi and the water-soaked lesions kept spreading with time (Figures 1 c, d, e). Masses of bacterial ooze obviously accumulated on lesion surfaces at 84 hpi (Figures 1 d, e). In contrast, the transgenic plants were resistant to *Xoc*, showing typical light-brown edges around the pricked sites and restricted necrotic lesions on the inoculated leaves, which is the typical symptom of HR (Figures 1 C, D, and E).

Patterns of differentially regulated genes in 9804-*Rxo1* and 9804

175 and 379 differentially regulated genes (DRGs) were detected from the inoculated 9804-*Rxo1* and 9804, respectively, at 36 hpi using t-tests. Of the 175 DRGs in the inoculated 9804-*Rxo1* sample (A), 161 were up-regulated compared with the noninoculated transgenic lines (C); of the 379 DRGs in the inoculated 9804 sample (D), 164 were up-regulated compared with the non-inoculated 9804 (B). A much larger number of DRGs were found up-regulated in A/C (92.00%) than in D/B (43.16%). 47 DRGs were co-DRGs in both the A/C and D/B set; of these, four were co-down-regulated, 11 were co-up-regulated, 31 were up-regulated in 9804-*Rxo1* but down-regulated in 9804 and only one was converse. Among the DRGs, 48.22% in A/C and 40.00% in D/B were sorted as defense-related genes or injury-induced genes, respectively. Interestingly, 93.68% of the defense-related DRGs found in A/C were up-regulated vis-à-vis 32.11% in D/B.

Function classification of DRGs in infected 9804-*Rxo1* and 9804

Function analysis revealed that 95 of the 175 DRGs in A/C were related to biotic stress, abiotic stress, and stimulus-response genes; 18.78% were enzymes with catalytic activity; 10.15% were transport-related genes; and the functions of 34 DRGs were unknown (Figure 2A). Classification of the DRGs revealed that: four of the five differentially expressed AP2 domain containing transcription factors (TFs), four myb-like DNA-binding domain containing TFs, seven terpene synthase family genes, three genes coding protease inhibitor/seed storage/LTP family protein, six pathogenesis-related protein genes, six HR-related POD-coding genes, and six genes encoding disease resistance NB-ARC domain containing protein or LRR family protein were all up-regulated in the transgenic lines at 36 hpi. Two copies of the following proteins were also up-regulated in A/C: lipoxygenase 2.1, LysM domain containing protein, harpin-induced protein 1 containing protein, and nicotinamide adenine dinucleotide hydride (NADH) ubiquinone oxidoreductase (Supplementary Table 1).

Functional classification of the DRGs from D/B at 36 hpi indicated that genes related to defense response, transport, phytohormone signaling, catalytic activity, and physiological processes accounted for 40.00, 7.89, 7.63, 16.84, and 24.21%, respectively. Moreover, 7.11% of the identified proteins were functionally unknown (Figure 2B). In addition, 67.89% of the DRGs involved in defense response and 65.00% of the DRGs related to physiological processes were down-regulated, whereas 73.33% of the DRGs involved in transport and transferase activity were up-regulated. Significantly, three abscisic acid (ABA) and gibberellic acid (GA)-pathway-related F-box domain-containing proteins, three stress-activated protein kinase (SAPK) (SAPK7, SAPK9, and SAPK10), two pathogenesis-related proteins (PR) proteins (PR10 and PR Bet v I), four AP2 domain-containing proteins, three VQ motif family proteins, four wall-associated kinases (WAK1, WAK2, WAK3, and WAK87), and eight C_3HC_4-type family zinc-finger proteins were all down-regulated. In contrast, five ribosomal protein translational capacities, six retrotransposon proteins, four myb-like DNA-binding domain (SHAQKYF class family) proteins, and three UDP-glucoronosyl and UDP-glucosyl transferase family proteins were all up-regulated in 9804 at 36 hpi. Moreover, 21 of the 23 NBS-LRR, NB-ARC, or LRR domain-containing proteins, nine of the 11 stimulus- induced

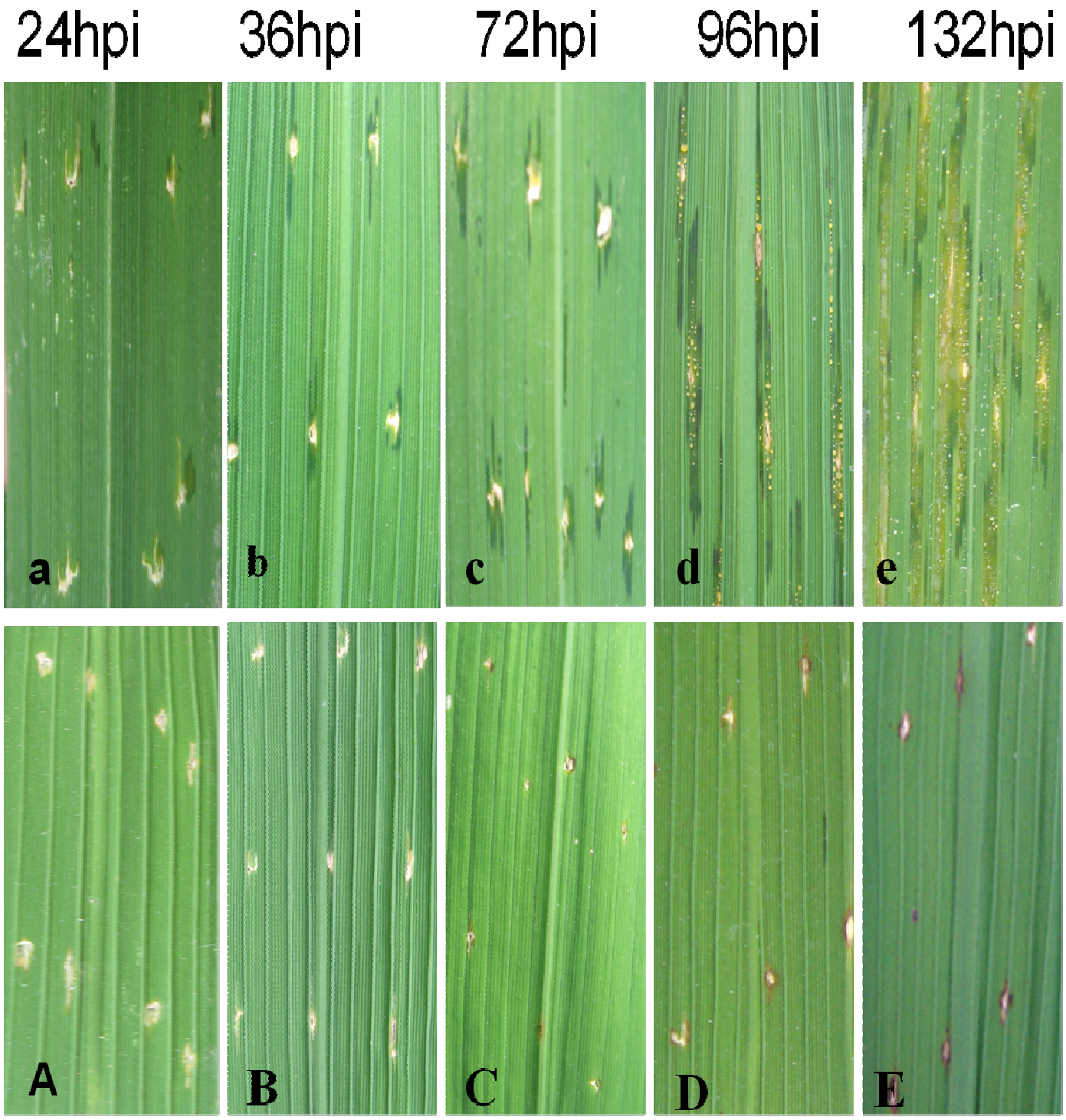

Figure 1. The phenotype of leaves after inoculation with *Xoc* in a transgenic line. A, B, C, D, and E, the sample of 9804-*Rxo1* at 24, 36, 72, 96, and 132 h after inoculation, respectively; a, b, c, d, and e, the sample of 9804 at 24, 36, 72, 96, and 132 h after inoculation, respectively..

proteins cytochrome P450, and seven of the nine HR-related POD-coding genes were found down-regulated at 36 hpi in inoculated 9804 (Os10g07978, Figure 3) (Supplementary Table 1). Besides, only one pentatricopeptide repeat (PPR) gene (Os01g0646000) was up-regulated in 9804-*Rxo1* while 49 of the 50 PPRs were detected down-regulated at 36 hpi in inoculated 9804.

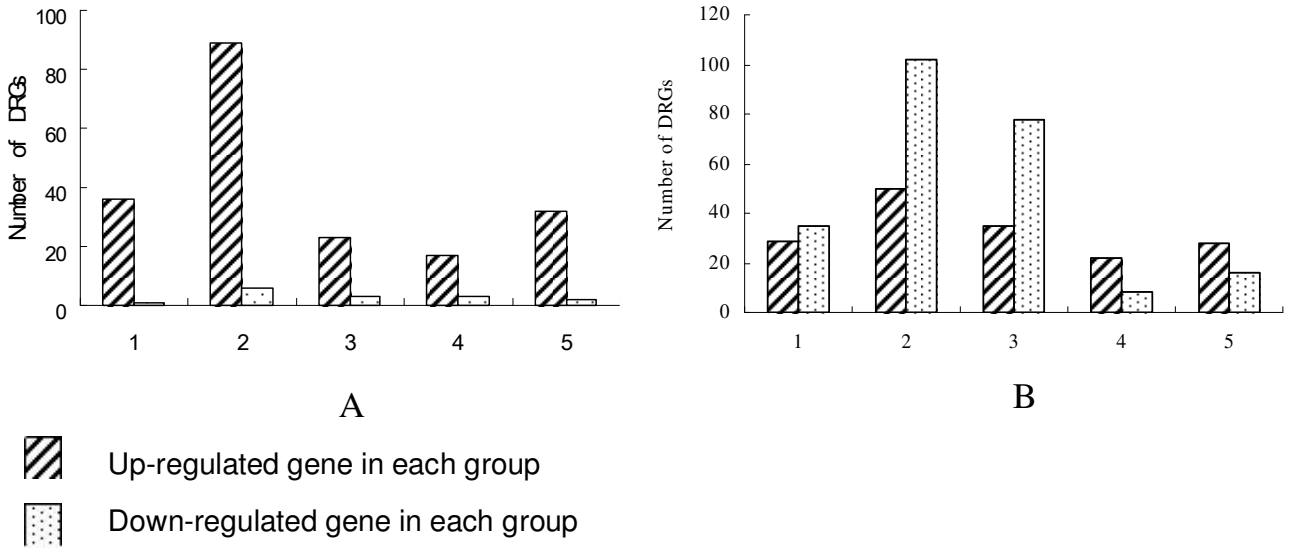

Figure 2. Function classification of DRGs in 9804-*Rxo1* and 9804 response to *Xoc*. A, Function of DRGs induced by *Xoc* in 9804-*Rxo1*; B, function of DRGs induced by *Xoc* in 9804. The numbers on the X axis indicate different function categories: 1, catalytic activity; 2, defense response; 3, physiological process; 4, transport and transferase activity; 5, others and unknown.

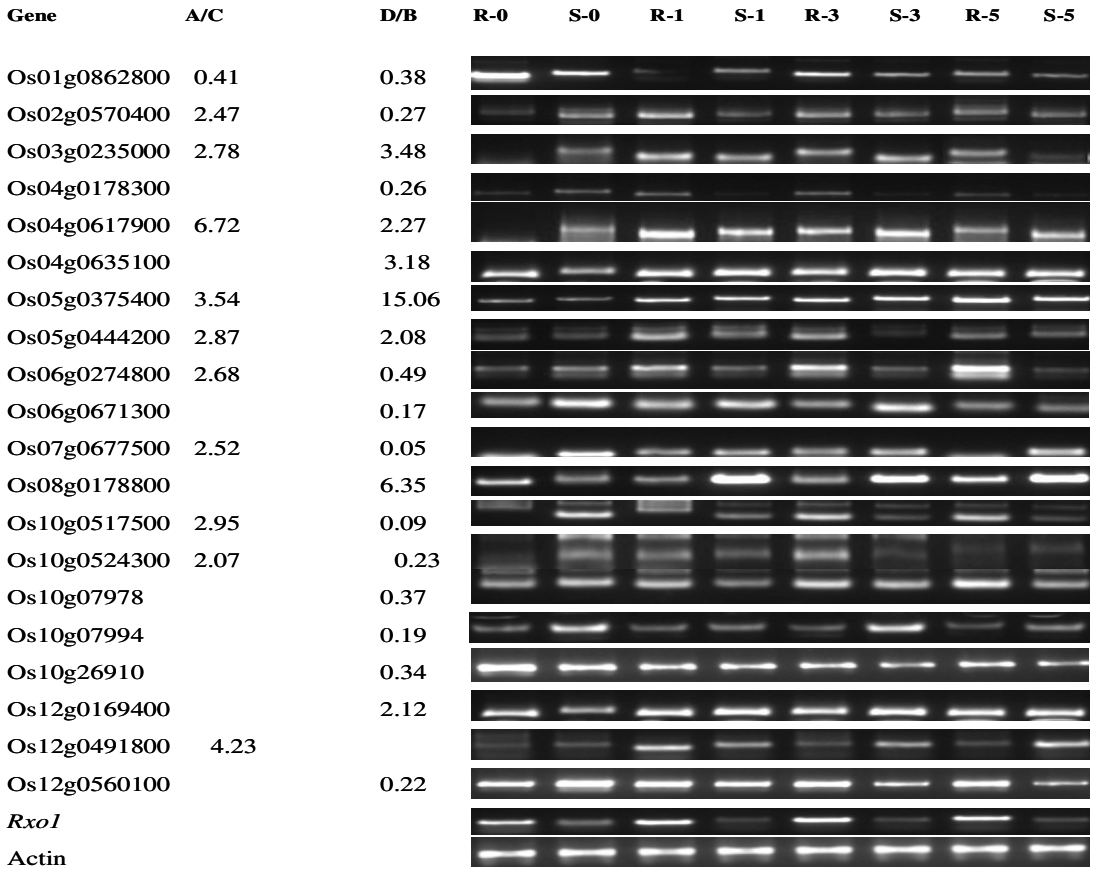

Figure 3. Expression profile of selected DEGs. A/C and D/B, expression ratio in *Xoc*-treated 9804-*Rxo1* and 9804, respectively. R-0, R-1, R-3, and R-5 represent the samples of 9804-*Rxo1* at 0, 24, 36, and 72 hpi, respectively; S-0, S-1, S-3, and S-5 were the samples of 9804 at the corresponding time points.

Table 1. Information on representative genes in PCR assays for the validation of microarray analysis.

Gene	Forward and reverse primer sequence (5′-3′)	A/C	D/B	Putative function	Transcript assignment
Os04g54300	GACTCTGCCGCTGGAACTACGC TCAACCCTCCTCGGCCTTCT	2.07	3.18	Wound-induced; response to stress	Wound-induced protein (fragment).
Os01g0862800	GGCGTCACCCGAGGAAA ACAGTTAGCACTACGAGCAAGAT	0.41	2.35	Response to stress; transcription factor activity	TF, NAC domain-containing protein 90
Os02g0570400	GCCAACACTGTATCCACTAA GGCTGCCTGACCAAGA	2.47	0.27	Response to stress; catalytic activity; signal transduction; response to endogenous stimulus	Terpene synthase family, metal binding domain-containing protein
Os03g0235000	CGCAACCGATGTTGTGGTC CCGAAGTCGCAGGAGAAGC	2.78	2.03	HR-related; response to stress; response to biotic stimulus; response to endogenous stimulus; catalytic activity	Peroxidase N precursor
Os04g0178300	CCGTCTCCTCTCACTTCAGG AACGTCTCCCGGGTTAGTCT	-	0.26	Phytohormone-related; response to endogenous stimulus; signal transduction	OsCyc1, terpene synthase, N-terminal domain-containing protein
Os04g0617900	AGATTTCTGCGTTGCTGACG GCTGGAAGTGGATGAGACCCT	6.72	2.27	Disease resistance	Germin-like protein precursor; response to stress
Os05g0375400	GCGTCGTGGGTGCGGAACAA CCCTGGCTGTAGGTGTAGGAGAAGTA	3.29	20.72	Kinase activity; phosphotransferase activity; carboxyl group as acceptor	Lichenase II precursor, kinase
Os05g0444200	CGGTCGGGAGTACAGGAAGG GACGTGGTCGTTGAGGGAGC	2.87	2.08	Transcription factor activity	TF, zinc finger, C2H2-type family protein, expressed
Os06g0274800	ACGACACTGCGACCCTGAT AGCACAACTGCGTCCCTC	2.68	0.49	HR-related; response to stress	Peroxidase 11 precursor
Os06g0671300	TTCCTCTTCCATGTCCAACC GCACCCCTACTTATGCCAAA	-	0.17	Phytohormone-related; response to stress; signal transduction	Cytochrome P450 family protein
Os07g0677500	CTTGCTTGTGGTCGTGGCT CGCATGAGACGGTCTGATTG	2.52	0.50	HR-related; catalytic activity; response to stress; response to endogenous stimulus; response to biotic stimulus	Peroxidase 2 precursor
Os08g0178800	GACTTATCACGCGGCCATCC GCCAGAACGCCTCATCCAAA	-	6.35	TF, signaling	B-box zinc-finger family protein, expressed
Os10g0517500	GGGTGGTGTACGTGGAGACGATG CCGCCGCTGATGAACTTGGA	2.95	0.09	Catalytic activity	Cys/Met metabolism PLP-dependent enzyme family protein
Os10g0524300	CACAGCATCAGCGACAGG GCATTCCGCCATACATT	2.07	0.23	Response to biotic stress	LysM domain-containing protein
Os10g07978	CTTCCGGTGTCTGAAGAAGC CATCGACGGATACTGTGTGG	-	0.37	Response to stress	NB-ARC domain-containing protein
Os10g07994	TGATCTGTGTCGAGCAGTCC CGGTTCACCACATAATGCAG	-	0.19	Immune response; physiological process	Autophagy protein 9, putative, expressed
Os10g26910	TCCATAGGGAAAGCCTCCTT GGGGATGTGGGTGAACATAG	-	0.34	Transport	Transposon protein, putative, CACTA, En/Spm subclass
Os11g0514400	CTGTCATCCGTGTGGATTTG GGTATGGTGCCAGTCAGGTT	2.46	0.04	HR-related; response to stress	BAK1; leucine-rich repeat

Table 1. Continues.

Os12g0169400	GCTATGCTGGACATTGCTAA TTTGTGCCCTCCGTGA	-	2.12	Stress response; tolerance and development	CBS domain-containing protein
Os12g0491800	TGATGCCCGAATGGTA AACGGTGGACAGACGAA	4.23	-	Response to stress; catalytic activity; signal transduction; response to endogenous stimulus	OsKS10, terpene synthase family, metal-binding domain-containing protein
Os12g0560100	TATCCACTCCCGAGAAGGTG GCCGAGGTCGTTGTAGACAT	-	0.22	Response to stress	Lipoxygenase
Rxo1	CCTTAGTTTAGATGGACG CTGCATATGTACGGGAT				
Os03g50890, Actin	CGCAGTCCAGGGGTATC TCCTGGTCATAGTCCAGGGC				

Supplementary Table 1. Some groups of genes involved in the susceptible response in infected 9804 at 36 hpi.

Gene	A/C	A/D	Putative function	Transcript assignment
Os03g0760500		5.33	Response to endogenous stimulus; response to external stimulus	cytochrome P450, putative, expressed
Os03g0594900		25.33		cytochrome P450, putative, expressed
Os06g0671300		0.17	Phytohormone-related;response to stress;signal transduction	Cytochrome P450 family protein
Os10g0440000		0.15		Cytochrome P450 family protein, expressed
Os12g0138800; Os11g0142400	2.06	0.52		Cytochrome P450 family protein;Strictosidine synthase family protein
Os01g0627900		0.16		cytochrome P450, putative, expressed
Os03g0760200		0.41		cytochrome P450, putative, expressed
Os01g0628900		0.43		cytochrome P450, putative, expressed
Os06g0129900		0.44		cytochrome P450, putative, expressed
Os06g0600400		0.55		cytochrome P450, putative, expressed
Os10g07978		0.37	Response to stress	NB-ARC domain containing protein
Os04g0514600		0.44	Response to stress	NB-ARC domain containing protein, expressed
Os11g15700		0.45	Disease resistance	NB-ARC domain containing protein, expressed
Os09g0517100		0.46	Disease resistance	NB-ARC domain containing protein, expressed
Os11g0226800		0.39	Disease resistance	NBS-LRR type disease resistance protein, putative, expressed
Os11g0227200		0.45	Disease resistance	NBS-LRR type disease resistance protein, putative, expressed
Os02g0615400		0.36	Disease resistance	Leucine Rich Repeat family protein
Os11g0229300; Os11g0227700		0.37	Disease resistance	Leucine Rich Repeat family protein
Os11g36140		0.06	Disease resistance	Leucine Rich Repeat family protein, expressed

Supplementary Table 1 continued.

Os02g0153900		0.22	Disease resistance	Leucine Rich Repeat family protein, expressed
Os12g0632800		0.26	Disease resistance	Leucine Rich Repeat family protein, expressed
Os02g0156800		0.30	Disease resistance	Leucine Rich Repeat family protein, expressed
Os01g41750		0.31	Disease resistance	Leucine Rich Repeat family protein, expressed
Os02g0615500		0.36	Disease resistance	Leucine Rich Repeat family protein, expressed
Os05g0522600; Os05g0522500		0.38	Disease resistance	Leucine Rich Repeat family protein, expressed
Os12g0182300		0.44	Disease resistance	Leucine Rich Repeat family protein, expressed
Os03g0668900		0.48	Disease resistance	Leucine Rich Repeat family protein, expressed
Os01g0742400		0.48	Disease resistance	Leucine Rich Repeat family protein, expressed
Os02g0609900		0.50	Disease resistance	Leucine Rich Repeat family protein, expressed
Os01g0818600; Os01g0818700		0.29	Kinase activity	Protein kinase domain containing protein; Leucine-rich repeat.
Os11g0514400	2.46	0.04	HR-related;response to stress	BAK1; Leucine-rich repeat
Os02g0227600		2.83	Disease resistance	Leucine Rich Repeat family protein, expressed
Os11g0172300		2.14	Signal transduction; response to stress	Leucine-rich repeat, plant specific containing protein.
Os07g0677100		2.69	Response to biotic stimulus; catalytic activity	Peroxidase 2 precursor, putative, expressed
Os03g0235000	2.78	3.48	HR related; response to stress; response to biotic stimulus;response to endogenous stimulus; catalytic activity	Peroxidase N precursor
Os06g0306300		0.32	HR related; response to stress	Peroxidase 1 precursor, putative, expressed
Os06g0274800	2.68	0.49	HR related; response to stress	Peroxidase 11 precursor
Os09g0471100		0.42		Peroxidase 17 precursor, putative, expressed
Os07g0677500	2.52	0.50	HR related; response to stress; response to biotic stimulus	Peroxidase 2 precursor
Os07g0677400		0.21	HR related; response to stress	Peroxidase 2 precursor, putative, expressed
Os02g0240100		0.35	HR- related; stress related	Peroxidase 52 precursor, putative, expressed
Os08g0522400		0.28	HR related; response to stress	Peroxidase family protein, expressed
Os12g0524700		5.47	Catalytic activity	Mitochondrial ribosomal protein S3, putative, expressed
Os10g0573700; Os10g0573800		4.76	Unknown	Mitochondrial carnitine/acylcarnitine carrier-like protein
Os.37083.1.S1_at		2.92	Physiological process	retrotransposon protein, putative, Ty1-copia subclass
Os06g0583500; Os06g38480		2.82	Physiological process	retrotransposon protein, putative, Ty1-copia subclass; Zn-finger, CCHC type domain containing protein
Os.38751.1.A1_at		4.31	Transport	retrotransposon protein, putative, Ty3-gypsy subclass
Os10g0159300; Os06g25770		2.88	Transport	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
Os11g0644600		2.13	Transporter activity	retrotransposon protein, putative, unclassified, expressed

Supplementary Table 1 continued.

Os12g0264500		2.86	Catalytic activity	retrotransposon protein, putative; CoA-thioester hydrolase CHY1 (3-hydroxyisobutyryl-coenzyme A hydrolase).
Os08g0558800		2.48	Translational capacity	60S ribosomal protein L10a-1, putative, expressed
Os04g16720		6.11	Translational capacity	ribosomal protein S15 containing protein
Os02g0680700		3.93	Response to stress	TF, myb family transcription factor
Os06g0348800		2.96	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os06g0728700		2.97	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os08g0157600		11.97	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os07g0510500		2.16	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os03g0757200		2.99	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os02g37690		2.01	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os12g0555000		0.35	Defence response; phytohormones (JA SA ET)	pathogenesis-related protein 10; RSOsPR10
Os12g0555200		0.40	Biotic stress	Pathogenesis-related protein Bet v I family protein, expressed
Os12g0555500		0.47	Biotic stress	Pathogenesis-related protein Bet v I family protein, expressed
Os03g0610900		0.45	Stress related	Serine/threonine-protein kinase SAPK10
Os04g0432000		0.30	Stress related	Serine/threonine-protein kinase SAPK7
Os12g0586000; Os12g0586100		0.05	Stress related	Serine/threonine-protein kinase SAPK9
Os02g0677300	0.16	0.21	Response to stress	TF, AP2 domain containing protein
Os01g0752500		0.29	Response to stress	TF, AP2 domain containing protein
Os09g0522000	0.26	0.14	Response to stress	TF, DREB1B, AP2 domain containing protein
Os02g0654700		0.23	Response to stress, ET	TF,AP2 domain containing protein, expressed; Ethylene-responsive element binding protein.
Os09g30454		0.12	Pathogen resistance; heavy-metal stress tolerance; cell expansion	OsWAK87 - OsWAK receptor-like protein kinase, expressed
Os08g0378000		0.45		Wall-associated kinase 2, putative, expressed
Os02g0111600; Os02g0111400; Os02g0111500		0.38		Wall-associated kinase 3, putative, expressed
Os12g0266200		0.30		Wall-associated kinase-like 1, putative, expressed
Os03g0712200		0.32	Unknown	Zinc finger, C3HC4 type family protein, expressed
Os12g0580700		0.22	Catalytic activity	Zinc finger, C3HC4 type family protein, expressed
Os05g0179000		0.23	Catalytic activity	Zinc finger, C3HC4 type family protein, expressed
Os03g17170		0.26	Catalytic activity	Zinc finger, C3HC4 type family protein, expressed
Os01g0822800		0.28	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein, expressed
Os10g0142100		0.34	Catalytic activity	Zinc finger, C3HC4 type family protein, expressed
Os05g0110000		0.35	Catalytic activity	Zinc finger, C3HC4 type family protein, expressed

Supplementary Table 1 continued.

Gene	A and C	A and D	Putative function	Transcript assignment
Os05g0211100	2.03		Signal transduction; response to endogenous stimulus; response to abiotic stimulus	Cytochrome P450 51
Os02g0323600	2.09		Phytohormone-related;response to stress	Cytochrome P450 51
Os02g0570500	2.00		Phytohormone-related;response to stress	Cytochrome P450 family protein
Os07g0520300	2.00		Phytohormone-related;response to stress	Cytochrome P450 family protein
Os02g0570700	2.46		Phytohormone-related;response to stress	Cytochrome P450 family protein
Os12g0268000	3.03		Phytohormone-related;response to stress	Cytochrome P450 family protein
Os12g0138800; Os11g0142400	2.06	0.53	Response to endogenous stimulus; response to external stimulus	Cytochrome P450 family protein;Strictosidine synthase family protein
Os09g0275400	2.06	0.52	Phytohormone-related;response to stress	Cytochrome P450 family protein
Os03g0760500		5.33	Phytohormone-related;response to stress	Cytochrome p450
Os03g0594900		25.33	Phytohormone-related;response to stress	Cytochrome p450
Os06g0671300		0.17	Phytohormone-related;response to stress	Cytochrome P450 family protein
Os10g0440000		0.15	Phytohormone-related;response to stress	Cytochrome P450 family protein
Os01g0627900		0.16	Phytohormone-related;response to stress	Cytochrome p450
Os06g0671300		0.40	Phytohormone-related;response to stress	Cytochrome p450
Os03g0760200		0.41	Phytohormone-related;response to stress	Cytochrome p450
Os01g0628900		0.43	Phytohormone-related;response to stress	Cytochrome p450
Os06g0129900		0.44	Phytohormone-related;response to stress	Cytochrome p450
Os06g0600400		0.55	Phytohormone-related;response to stress	Cytochrome p450
Os03g50150	2.09		Response to stress	NB-ARC domain containing protein, disease resistance protein
Os01g0721400	2.00		Response to stress	NB-ARC domain containing protein
Os07g0131000	2.02		Signal transduction; response to endogenous stimulus	lectin receptor kinase 7, Serine/threonine protein kinase family protein.
Os01g0152000	2.08		Response to biotic stimulus; response to abiotic stimulus;	LRR family protein
Os03g0397700	2.00		Signal transduction; response to endogenous stimulus	LRR transmembrane protein kinase 1, Serine/threonine protein kinase-like protein
Os07g0681100	2.01		Signal transduction; response to endogenous stimulus	LRR transmembrane protein kinase, MARK
Os10g07978		0.37	Response to stress	NB-ARC domain containing protein
Os04g0514600		0.44	Response to stress	NB-ARC domain containing protein
Os11g15700		0.45	Disease resistance	NB-ARC domain containing protein
Os09g0517100		0.46	Disease resistance	NB-ARC domain containing protein
Os11g0226800		0.39	Disease resistance	NBS-LRR type disease resistance protein
Os11g0227200		0.45	Disease resistance	NBS-LRR type disease resistance protein

Supplementary Table 1. continued.

Os02g0615400		0.36	Disease resistance	Leucine Rich Repeat family protein
Os11g0229300; Os11g0227700		0.37	Disease resistance	Leucine Rich Repeat family protein
Os11g36140		0.06	Disease resistance	Leucine Rich Repeat family protein
Os02g0153900		0.22	Disease resistance	Leucine Rich Repeat family protein
Os12g0632800		0.26	Disease resistance	Leucine Rich Repeat family protein
Os02g0156800		0.30	Disease resistance	Leucine Rich Repeat family protein
Os01g41750		0.31	Disease resistance	Leucine Rich Repeat family protein
Os02g0615500		0.36	Disease resistance	Leucine Rich Repeat family protein
Os05g0522600; Os05g0522500		0.38	Disease resistance	Leucine Rich Repeat family protein
Os12g0182300		0.44	Disease resistance	Leucine Rich Repeat family protein
Os03g0668900		0.48	Disease resistance	Leucine Rich Repeat family protein
Os01g0742400		0.48	Disease resistance	Leucine Rich Repeat family protein
Os02g0609900		0.50	Disease resistance	Leucine Rich Repeat family protein
Os02g0227600		2.83	Disease resistance	LRR family protein
Os11g0172300		2.14	Signal transduction; response to stress	LRR, plant specific containing protein.
Os05g0130100	2.06		Signal transduction; response to endogenous stimulus; biological process	Protein kinase domain containing protein
Os01g0548600	2.33			Protein kinase domain containing protein
Os11g0514400	2.46	0.04	HR-related;response to stress	BAK1; Leucine-rich repeat
Os01g0818600; Os01g0818700		0.29	Kinase activity	Protein kinase domain containing protein; Leucine-rich repeat.
Os07g0129300	2.73		Response to stress	Pathogenesis-related protein 1 precursor
Os01g0382000	2.08		Response to stress	Pathogenesis-related protein PRB1-3 precursor
Os01g0731100	2.47		Response to stress	Pathogen-related protein, putative
Os12g0555000	2.04	0.35	Defence response; phytohormones(JA SA ET) signaling	pathogenesis-related protein 10; RSOsPR10
Os12g0555500	2.00	0.47	Biotic stress	Pathogenesis-related protein Bet v I family protein, expressed
Os12g0555200	2.02	0.40	Response to stress	Pathogenesis-related protein Bet v I family protein, PBZ1
Os02g0570400	2.47	0.27	Response to stress; catalytic activity;signal transduction; response to endogenous stimulus	Terpene synthase family, metal binding domain containing protein
Os08g0167800	3.82	2.62	Response to stress; catalytic activity;signal transduction	Terpene synthase family, metal binding domain containing protein, expressed
Os10g0489500	2.00		Response to stress; catalytic activity;signal transduction	Terpene synthase family, metal binding domain containing protein, expressed

Supplementary Table 1. continued.

Os04g0179700	2.02		Response to stress; catalytic activity;signal transduction	Terpene synthase family, metal binding domain containing protein, expressed
Os04g0345400	2.15		Response to stress; catalytic activity;signal transduction	Terpene synthase family, metal binding domain containing protein, expressed
Os02g0571300	2.50		Response to stress; catalytic activity;signal transduction	Terpene synthase family, metal binding domain containing protein, expressed
Os02g0571100	2.29		Response to stress; catalytic activity;signal transduction	Terpene synthase, N-terminal domain containing protein, expressed
Os08g0113000	2.00		HR related; response to stress	Peroxidase 47 precursor
Os06g0547400	2.13		HR related; response to stress	Peroxidase 52 precursor, Peroxidase P7
Os01g0326000	2.00		HR related; response to stress	Peroxidase family protein, Peroxidase 1
Os06g0274800	2.68	0.49	HR related; response to stress	Peroxidase 11 precursor
Os03g0235000	2.78	3.48	Response to stress; catalytic activity	Peroxidase N precursor
Os07g0677500	2.52	0.50	Response to stress; catalytic activity	Peroxidase 2 precursor
Os07g0677100		2.69	Response to biotic stimulus; catalytic activity	Peroxidase 2 precursor
Os06g0306300		0.32	HR related; response to stress	Peroxidase 1 precursor
Os09g0471100		0.42		Peroxidase 17 precursor
Os07g0677400		0.21	HR related; response to stress	Peroxidase 2 precursor
Os02g0240100		0.35	HR- related; stress related	Peroxidase 52 precursor
Os08g0522400		0.28	HR related; response to stress	Peroxidase family protein
Os12g0560100	2.01		Response to stress	Lipoxygenase 2.1, chloroplast precursor
Os12g0559200	2.56		Response to stress	Lipoxygenase 2.1, chloroplast precursor
Os10g0524300	2.07	0.23	Response to biotic stress	LysM domain containing protein
Os10g0524300	2.09		Response to biotic stress	LysM domain containing protein, expressed
Os10g02380	2.57		HR-related; response to stress	NADH ubiquinone oxidoreductase, 20 Kd subunit family protein, expressed
Os02g24600	2.70		HR-related; response to stress	NADH ubiquinone oxidoreductase, 20 Kd subunit family protein, expressed
Os08g0102700	2.01		Response to stress	Harpin-induced protein 1 containing protein, expressed
Os04g0677300	3.09		Response to stress	Harpin-induced protein 1 containing protein, expressed
Os04g54300	2.00	2.09	Wound induced; response to stress	wound induced protein, putative, expressed
Os03g0793900	2.11	0.30	Transport	Protease inhibitor/seed storage/LTP family protein
Os03g0793800	3.03	0.39	Transport	Protease inhibitor/seed storage/LTP family protein
Os07g0175600	2.34		Transport	Protease inhibitor/seed storage/LTP family protein, expressed
Os03g15310		2.92	Physiological process	Retrotransposon protein, ty1-copia subclass
Os06g0583500; Os06g38480		2.82	Physiological process	Retrotransposon protein, ty1-copia subclass; zn-finger, cchc type domain containing protein

Supplementary Table 1. continued.

Os.38751.1.A1_at		4.31	Transport	Retrotransposon protein, ty3-gypsy subclass
Os10g0159300; Os06g25770		2.88	Transport	Retrotransposon protein, ty3-gypsy subclass
Os11g0644600		2.13	Transporter activity	Retrotransposon protein, unclassified
Os12g0264500		2.86	Catalytic activity	Retrotransposon protein; coa-thioester hydrolase chy1 (3-hydroxyisobutyryl-coenzyme a hydrolase).
Os08g0558800		2.48	Translational capacity	60s ribosomal protein l10a-1
Os04g16720		6.11	Translational capacity	Ribosomal protein s15 containing protein
Os04g16720		7.73	Translational capacity	Ribosomal protein s15 containing protein
Os02g0680700		3.93	Response to stress	TF, myb family transcription factor
Os06g0348800		2.96	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os06g0728700		2.97	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os08g0157600		11.97	Response to stress	TF, myb-like DNA-binding domain, SHAQKYF class family protein
Os07g0510500		2.16	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os03g0757200		2.99	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os02g37690		2.01	Physiological process	UDP-glucoronosyl and UDP-glucosyl transferase family protein
Os12g0555000		0.35	Defence response; phytohormones (JA SA ET) signaling	pathogenesis-related protein 10; RSOsPR10
Os12g0555200		0.40	Biotic stress	Pathogenesis-related protein Bet v I family protein
Os12g0555500		0.47	Biotic stress	Pathogenesis-related protein Bet v I family protein
Os03g0610900		0.45	Stress related	Serine/threonine-protein kinase SAPK10
Os04g0432000		0.30	Stress related	Serine/threonine-protein kinase SAPK7
Os12g0586000; Os12g0586100		0.05	Stress related	Serine/threonine-protein kinase SAPK9
Os04g0649100	2.02		Response to stress; response to abiotic stimulus	TF, AP2 domain containing protein
Os01g0165000	2.06		Response to stress; response to abiotic stimulus	TF, AP2 domain containing protein, DRE binding protein 2.
Os02g0655200	2.01		Response to stress; response to abiotic stimulus	TF, AP2 domain containing protein, Ethylene responsive element binding factor3 (OsERF3).
Os04g0398000	2.04		Response to stress; response to abiotic stimulus	TF, AP2 domain containing protein, Pathogenesis-related transcriptional factor and ERF domain containing protein.
Os04g0301500	0.41		Response to stress	TF, bHLH domain containing protein

Supplementary Table 1. continued.

Os07g0558100	2.14		Response to stress; signal transduction;	TF, Myb-like DNA-binding domain containing protein, expressed
Os12g0564100	2.19		Response to stress; signal transduction;	TF, Myb-like DNA-binding domain containing protein, R2R3MYB-domain protein
Os02g0139000	2.00		Response to stress; signal transduction;	TF, myb-like DNA-binding domain, SHAQKYF class family protein, expressed
Os04g0583900	2.00		Response to stress; signal transduction;	TF, myb-like DNA-binding domain, SHAQKYF class family protein, expressed
Os01g0752500		0.29	Response to stress	TF, AP2 domain containing protein
Os02g0654700		0.23	Response to stress, ET	TF,AP2 domain containing protein; Ethylene-responsive element binding protein.
Os02g0677300		0.21	Response to stress	TF, AP2 domain containing protein
Os09g0522000		0.14	Response to stress	TF, DREB1B, AP2 domain containing protein
Os09g30454		0.12	Pathogen resistance; heavy-metal stress tolerance; cell expansion	OsWAK87 - OsWAK receptor-like protein kinase
Os08g0378000		0.45	Pathogen resistance; heavy-metal stress tolerance; cell expansion	Wall-associated kinase 2
Os02g0111600; Os02g0111400;		0.38	Pathogen resistance; heavy-metal stress tolerance; cell expansion	Wall-associated kinase 3
Os12g0266200		0.30	Pathogen resistance; heavy-metal stress tolerance; cell expansion	Wall-associated kinase-like 1
Os05g0444200	2.87	2.08	Transcription factor activity	TF, Zinc finger, C2H2 type family protein
Os01g0839100	3.70		Transcription factor activity	TF, Zinc finger, C2H2 type family protein
Os03g0712200		0.32	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os12g0580700		0.22	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os05g0179000		0.23	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os03g17170		0.26	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os01g0822800		0.28	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os03g17170		0.33	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os10g0142100		0.34	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein
Os05g0110000		0.35	Response to stress; response to biotic stimulus	Zinc finger, C3HC4 type family protein

DRGs involved in defense response in 9804-*Rxo1* after inoculation

Among DRGs, the most genes classified as defense-related genes were only up-regulated in the A/C: two ABA-responsive GRAM domain containing proteins (Os12g0478200 and Os12g0478200), two genes encoding cystathionine beta-synthase (CBS) domain protein (Os04g0136700, and Os03g0737000), which was thought to be involved in stress-resistance or stress-tolerance signaling pathway were also up-regulated (Kushwaha et al., 2009). Moreover, a gene encoding LysM domain-containing protein (Os10g0524300) (Figure 3), nodulin MtN21 family protein (Os02g0768300), two harpin-induced proteins (Os08g0102700 and Os04g0677300), lipoxygenase 2.1-coding genes (Os12g0560100 and Os12g0559200), and ribonuclease T2 family protein (Os09g0537700 and Os09g0538000) were resistance related (Gimenez-Ibanez et al., 2009; Miya et al., 2007; Kottapalli et al., 2007; Irie and Ohgi, 2001). Other genes, including OsKS10, terpene synthase family (Os12g0491800, Figure 3), Hsp20/alpha crystalline family protein (Os10g0159700), and HR-related NADH ubiquinone oxidoreductase (Os10g28120 and Os02g24600) were also up-regulated only in 9804-*Rxo1 w*hereas three genes constrained in 9804: Os04g0178300 coding an OsCyc1, Cytochrome P450 gene Os06g0671300, and another programmed cell death (PCD) regulation protein (Os10g07994), autophagy protein 9, were further amplified (Figures 3 and 4).

Meanwhile, we also found that some genes were differentially up-regulated both in A/C and D/B: DRGs coding DNAJ heat shock N-terminal domain-containing protein (Os01g0606900, Os05g0427900, and Os11g0216000), Os04g54300 encoding a wound induced protein, C_2H_2-type family zinc-finger (Os05g0444200 and Os01g0839100) and germin-like protein precursor (Os04g0617900) (Figure 3).

Moreover, we found 5 PR: Os07g0129300, Os12g0555000, Os12g0555200, Os01g0382000, and Os01g0731100 encoding PR1, OsPR10, PRBetvI, PBZ1, PRB1-3, and PRN, respectively, were all up-regulated in A/C but not induced in D/B.

Strikingly, seven genes: Os02g0570400, Os08g0167800, Os10g0489500, Os04g0179700, Os04g0345400, Os02g0571300, and Os02g0571100, encoding terpene synthase (TPS) were identified up-regulated in 9804-*Rxo1* plants (Supplementary Table 1). The expression of a gene encoding TPS (Os02g0570400), which is involved in JA signaling pathway (Attaran et al., 2008), was highly (6.54-, 2.97-, 2.70-, and 3.55-fold) up-regulated in the infected 9804-*Rxo1* plants at 1, 3, 5, and 7 dpi but was down-regulated in the wild type plants at the same time points (Figure 3 and 4).

PODs belong to PR protein 9 family, conferring resistance to both biotic and abiotic stresses through lignification and suberization (Van Loon et al., 1994; Quiroga et al., 2000). In this study, we found that five genes encoding POD 11 precursor (Os06g0274800), POD 2 precursor (Os07g0677500), POD 47 (Os08g0113000), POD 52 (Os06g0547400), and POD 1 (Os01g0326000), respectively, were up-regulated in transgenic lines but were down-regulated or had no significant difference in expression in the wild type. In addition, only one POD N coding gene (Os03g0235000) was up-regulated in both inoculated transgenic and wild type plants. The expressions of Os03g0235000, Os06g0274800 and Os07g0677500 were further validated with RT-PCR and qRT-PCR (Figures 3 and 4). In contrast, the other five POD-coding genes, including POD 1 precursor (Os06g0306300), POD 17 precursor (Os09g0471100), POD 2 precursor (Os07g0677400), POD 52 precursor (Os02g0240100), and another gene encoding POD family protein (Os08g0522400), were all down-regulated in the wild type lines at 36 hpi (Supplementary Table 11).

In order to make clear the relationship between the activity of endogenous POD and the expression of POD-coding genes in different rice lines, extraction and quantification were applied. Good recoveries of this enzyme were obtained from 9804-*Rxo1* and 9804. The result demonstrated that POD activities in the leaves of *Xoc*-inoculated 9804-*Rxo1* and 9804 were higher than in the water (H_2O) mock lines at 12 hpi, but the trend was increased afterward in the pathogen-stressed transgenic line while it declined in the wild type inoculated by *Xoc*. The tendency of POD activities in H_2O-treated plants had no significant difference after 4 dpi (Figure 5).

Differential expression of transcription factors in 9804-*Rxo1* and 9804

The AP2/EREBP TFs play a central role in both ABA-dependent and ABA-independent pathways (Xiong et al., 2002). In this study, four AP2 domain containing protein genes, including Os04g0649100, Os01g0165000 (DRE binding protein 2), Os02g0655200 (OsERF3), and Os04g0398000 (ERF domain containing protein) were up-regulated in the transgenic lines at 36hpi.

The genome of *Oryza sativa* codes 189 C2H2 zinc-finger protein genes, which are considered to enhance rice tolerance of abiotic stress (Agarwal et al., 2007). We found that two genes in this family were up-regulated in the infected rice line with *Rxo1*. Figure 3 shows that the expression levels of a C2H2 zinc-finger-type family protein (Os05g0444200) were improved at 24, 36, 72, and 120 hpi in the transgenic plants (Fig 3.). The other C2H2 TF, Os01g0839100 was found 3.70-fold up-regulated only in the transgenic lines.

Two nascent polypeptide-associated complex (NAC) domain TFs were differentially expressed in this study: Os11g0126900 was up-regulated in both A/C and D/B,

while Os01g0862800 was depressed in A/C, but activeted in D/B (Figure 3). Another two genes encoding ZIM motif family protein with TF activity were only differentially regulated in A/C: Os10g0391400 was down-regulated but Os03g0180900 was up-regulated.

Moreover, four Myb-like domain TF genes were found up-regulated only in A/C, including Os07g0558100, Os12g0564100, Os02g0139000 and Os04g0583900. One gene Os10g0571600, encoding no apical meristem (NAM) protein with TF activity, was also up-regulated in 9804-*Rxo1*.

In the infected wild type (D/B), four MYB or MYB-like TFs (Os02g0680700, Os06g0348800, Os06g0728700, Os08g0157600), two TFs in B-box zinc-finger family (Os02g0176000, Os08g0178800), and five TFs of NAC or NAM (Os08g0200600, Os01g0672100, Os11g0126900, Os08g0200600, Os01g0862800) were up-regulated (Supplementary Table 1). The expression of B-box zinc-finger TF (Os08g0178800) was validated by RT-PCR and qRT-PCR analysis, which confirmed the microarray result (Figures 3 and 4). In contrast, four WRKY TFs, including Os08g0386200, Os01g0826400 (WRKY24), Os01g0246700 (WRKY1), and Os05g0474800 were down-regulated. Of them, WRKY24 was also down-regulated in A/C. Two TFs with AP2 domain (Os01g0752500 and Os02g0677300) were down-regualted in D/B, and Os02g0677300 was also down-regulated in A/C.

DRGs coding kinases in 9804-*Rxo1* and 9804 at 36 hpi

Ten kinases were up-regulated in infected 9804-*Rxo1* plants, whereas 60 kinases were differentially expressed in infected 9804, of these, 50 were down-regulated. Gene Os11g0514400 encoding a HR-related BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor (BAK1) was 2.46 times up-regulated in A/C, but 2.5 times down-regulated in D/B, which was confirmed by qRT-PCR (Figure 4). Four genes (Os07g0131000, Os03g0397700, Os01g0152000, and Os07g0681100) encoding serine/threonine protein kinase family lectin receptor kinase 7 or LRR transmembrane protein kinase mitogen-activated protein kinase (MAPK) were all up-regulated in 9804-*Rxo1*. Two protein containing protein kinase domain (Os01g0548600 and Os05g0130100) reported to have receptor activity and kinase activity, were induced in A/C.

A lichenase II precursor gene (Os05g0375400) was 3.54 and 15.06 times up-regulated in 9804-*Rxo1* and 9804, respectively (Figure 3.). Four DRGs encoding kinases in infected 9804, including CDPK (Os07g-0619800), which plays an important role in numerous-physiological processes (Komatsu et al., 2001), CIPK (Os03g0339900), APK1B (Os09g0442100), and STPK (Os01g0832900), were down-regulated. Intriguingly, genes encoding three SAPKs (SAPK7, SAPK9, and SAPK10) and four WAKs (WAK1, WAK2, WAK3, WAK87) were down-regulated in 9804 after inoculation with *Xoc*.

DISCUSSION

Prevalent in plant disease resistance mechanisms of two types: HR and systemic acquired resistance (SAR) was investigated by Devadas and Raina, (2002). SAR is a plant immune response that occurs following an earlier localized exposure to a pathogen, which is associated with accumulation of PR proteins (Sels et al., 2008). In our study, genes coding PR1, PR10, PRB1-3, and two PRBet vI, which were reported to have functions in response to pathogen infection and defense-related signal compounds (Mitsuhara et al., 2008; El-kereamy et al., 2009; Hashimoto et al., 2004; Chen et al., 2009), were significantly up-regulated only in the 9804-*Rxo1* lines during the early infection period. The evidence, that the non-host resistance gene *Rxo1* can specifically activate large numbers of genes involved in signaling pathways leading to HR, was reported in the former study (Zhou et al., 2010). In this study, significant up-regulation of PR proteins induced by BLS pathogen suggested that SAR was a part of defense mechanisms in the transgenic line with *Rxo1*.

In addition, several genes in POD and TPS families were induced by *Xoc* in the transgenic line. Six POD precursors were significantly up-regulated in the transgenic line with *Rxo1* but repressed in the wild type, and the other five POD-coding genes were down-regulated in the control. Meanwhile, endogenous POD activity in the *Xoc*-inoculated 9804-*Rxo1* increased significantly from 12 hpi to 96 hpi compared with the wild type. Also, seven TPSs were significantly up-regulated in the rice line with *Rxo1*.POD is a large protein family which consist genes contributing to self-defense, and acts an activator for polymerization and dehydro-genization (Quiroga et al., 2000; Wojtaszek, 1997; Hilaire et al., 2001; Kawaoka et al., 2003). The roles of TPSs in defense against pathogenic microbes is largely unknown (Karina et al., 2011). Nevertheless, a possible function of terpenoids which possess direct antimicrobial properties as phytoalexins was discussed, alternatively; products of TPS might participate in defense signaling (Soković and Griensven, 2006; Tholl, 2006; Kishimoto et al., 2006). Based on the above data, we speculated that PODs and TPSs may play important roles in the complex process of resistant response to *Xoc* in the transgenic line.

In this study, we found that DRG patterns in the transgenic rice line and its wild type induced by *Xoc* were distinctly different. An important characterization of DRGs from the infected wild type was that several gene families associated with the defense response were significantly down-regulated, including genes in SAPK and WAK/WAKL family. Kinases play key roles in signaling

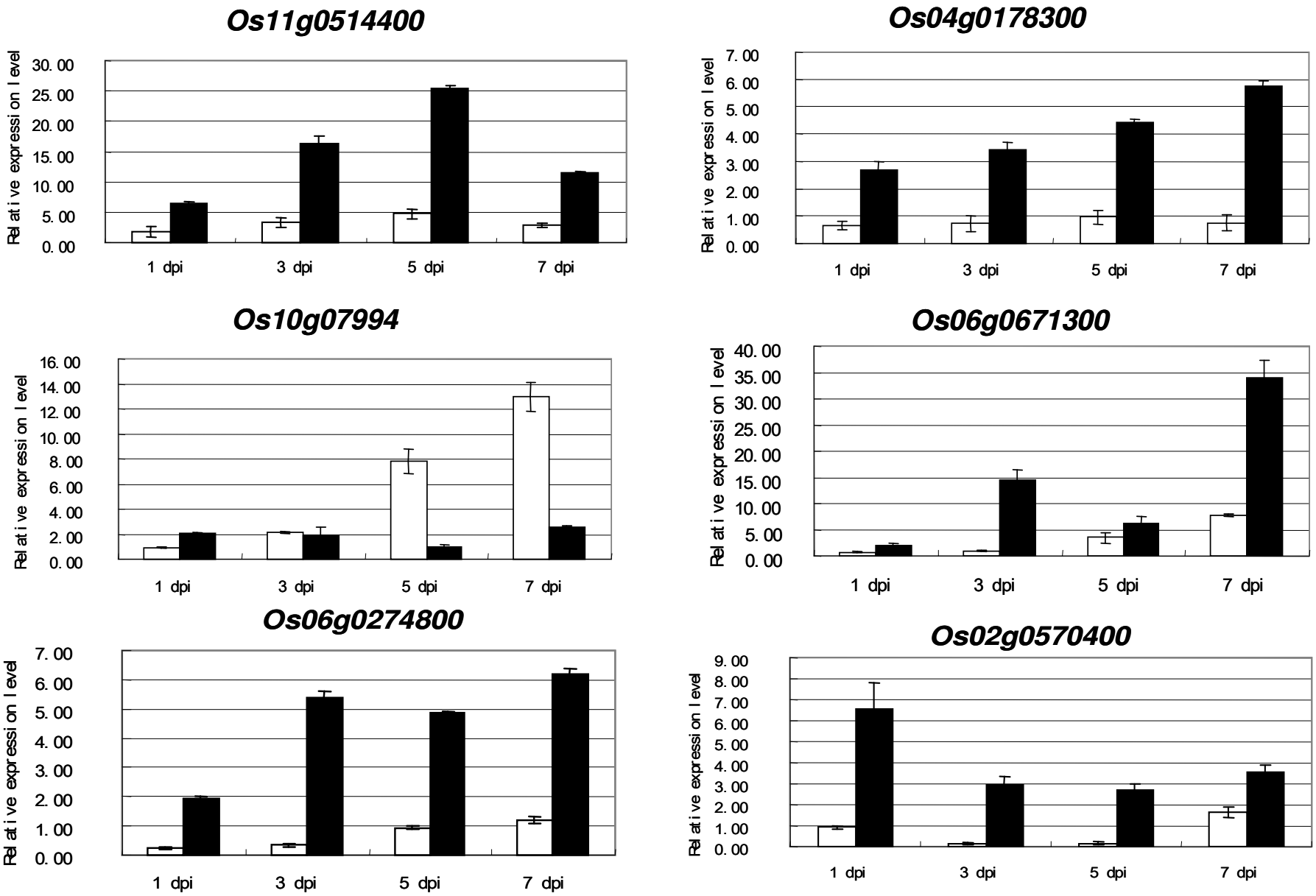

Figure 4. Quantitative reverse transcription-polymerase chain reaction assays of representative genes at four time points for the validation of microarray analysis (black columns, 9804-*Rxo1*; white columns, 9804).

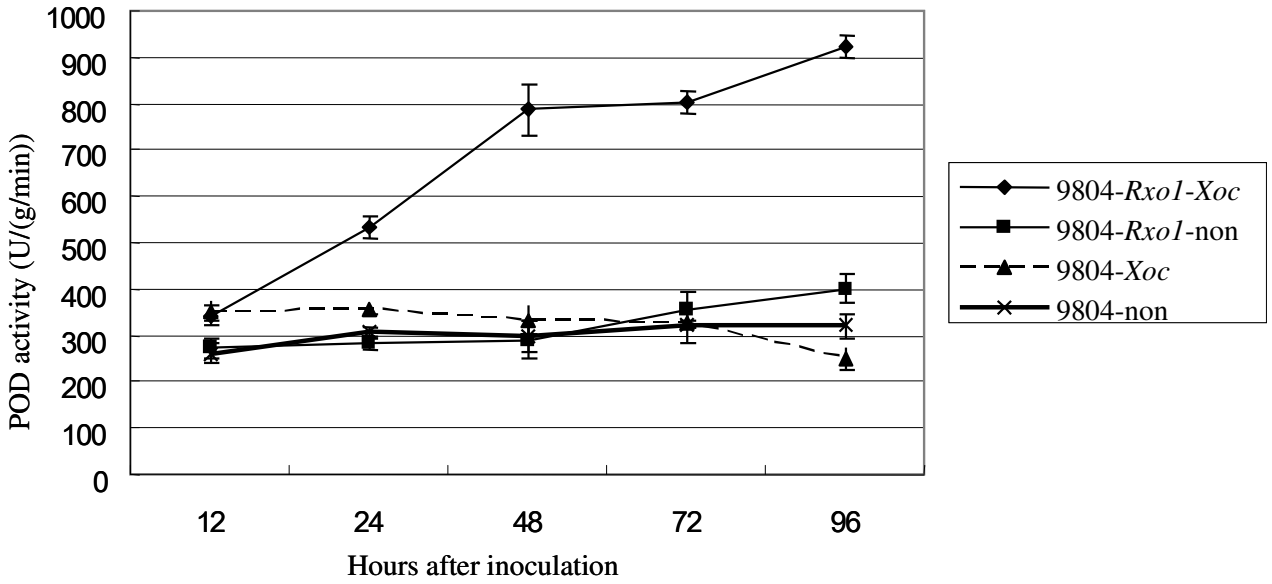

Figure 5. POD activities in *Xoc*- or H_2O-inoculated 9804-*Rxo1* or 9804 at different hours after inoculation. The numbers on the X axis indicate the different hours after inoculation, while the data on the Y axis show POD activity: changes in turbidity at 470 nm (A470) per gram of fresh leaf per minute.

pathways in the interaction between plants and their pathogens. Some WAK/WAKL members, such as OsWAK1, were reported to be involved in plant defense, mechanical wounding, and salicylic acid and methyl jasmonate (MeJA) signaling (He et al., 1999; Li et al., 2009). All *OsSAPK* family members were activated by hyperosmotic stress, and three (*OsSAPK8*, *OsSAPK9* and *OsSAPK10*) were induced by ABA (Kobayashi et al., 2004). In this study, four genes in WAK/WAKL family and three genes in SAPK family were all differentially down-regulated in the infected wild type rice plants, suggesting that these genes might negatively cause disease development in the compatible interaction of rice and *Xoc*.PPR genes have certain features and characters in common with disease *R* genes, which suggest that their evolutionary expansion in plants may have involved novel molecular processes and selective pressures (Geddy and Brown, 2007). At 48 hpi, 68 putative PPR-encoding genes were differentially regulated specifically in the infected 9804-*Rxo1* plants and 65 of them were significantly up-regulated, whereas no PPR genes were found differentially expressed in the wild type 9804 (Zhou et al., 2010).

In this study, one PPR gene (Os01g0646000) was up-regulated in 9804-*Rxo1* while 49 of the 50 PPRs differentially expressed in the infected 9804 plants were down-regulated. This result further indicates that the post-transcriptional modification might be an important feature in the incompatible and compatible interactions between rice and *Xoc*.Nonhost resistance to pathogens is highly effective and durable since it can refer to the immune responses of all members of a plant species to all members of a given pathogen species (Heath, 1991; Wojtaszek, 1997). With the advances in gene cloning technology and plant transformation techniques, accordingly, interest has been increasing in nonhost resistance because of its potential use in disease control of heterologous plant species. Our results provide some interesting information for a greater understanding of the molecular response mediated by nonhost resistance in heterologous plant species, which will shed more light on the co-evolutionary relationship between plant species and their pathogens.

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Abbreviations

HR, Hypersensitive response**; DRGs,** differentially regulated genes; ***Xoc***, *Xanthomonas oryzae pv. Oryzicola*; **BLS**, bacterial leaf streak; ***R* gene**, resistance gene; **POD**, peroxidase; **dpi**, days post-inoculation; **hpi**, hours post-inoculation; **BAK1**, BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor; **PPR**, pentatricopeptide repeat;; **TFs**, transcription

factors; **TPS**, terpene synthase; **WAK**, wall-associated kinases; **cDNA**, complementary deoxyribonucleic acid; **cRNA**, complemenraey ribonucleic acid; **GCOS**, GeneChip operating software; **MAS**, molecule annotation system; **RT-PCR**, real time-polymerase chain reaction; **qRT-PCR**, quantitative real time-polymerase chain reaction; **PR**, pathogenesis-related proteins; **SAR**, systemic acquired resistance; **NBS- LRR,** nucleotide binding site- leucine rich repeat; **dNTP**, deoxyribonucleoside triphosphates; **NADH**, nicotinamide adenine dinucleotide hydride; **ABA**, abscisic acid; **GA**, gibberellic acid; **SAPK**, stress-activated protein kinase; **CBS**, cystathionine beta-synthase; **NAC**, nascent polypeptide-associated complex; **NAM**, no apical meristem; **MAPK**, mitogen-activated protein kinase.

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