The defense-responsive genes showing enhanced and repressed expression after pathogen infection in rice (*Oryza sativa* L.)

ZHOU Bin (周 斌), PENG Kaiman (彭开蔓), CHU Zhaohui (储昭晖), WANG Shiping (王石平) & ZHANG Qifa (张启发)

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China
Correspondence should be addressed to Wang Shiping (email: swang@mail.hzau.edu.cn)

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Abstract Despite large numbers of studies about defense response, processes involved in the resistance of plants to incompatible pathogens are still largely uncharacterized. The objective of this study was to identify genes involved in defense response by cDNA array analysis and to gain knowledge about the functions of the genes involved in defense response. Approximately 20000 rice cDNA clones were arrayed on nylon filters. RNA samples isolated from different rice lines after infection with incompatible strains or isolates of *Xanthomonas oryzae* pv. *oryzae* or *Pyricularia grisea*, respectively, were used to synthesize cDNA as probes for screening the cDNA arrays. A total of 100 differentially expressed unique sequences were identified from 5 pathogen-host combinations. Fifty-three sequences were detected as showing enhanced expression and 47 sequences were detected as showing repressed expression after pathogen infection. Sequence analysis revealed that most of the 100 sequences had various degrees of homology with genes in databases which encode or putatively encode transcription regulating proteins, translation regulating proteins, transport proteins, kinases, metabolic enzymes, and proteins involved in other functions. Most of the genes have not been previously reported as being involved in the disease resistance response in rice. The results from cDNA arrays, reverse transcription-polymerase chain reaction, and RNA gel blot analysis suggest that activation or repression of most of these genes might occur commonly in the defense response.

Keywords: bacterial blight, blast, defense-related genes, cDNA arrays.

The resistance of plants to incompatible pathogens is manifested as two biochemical and physiological responses, the hypersensitive reaction (HR) and the systemic acquired resistance (SAR). Large efforts have been put in recent years in the studies of major disease resistance or *R* genes and defense related genes, especially those encoding pathogenesis-related (PR) proteins. Many genes, other than *PR* genes, are also identified to be involved in the cascade induced by pathogens[1,2]. Information is also slowly accumulating about other elements involved in the defense cascade. Recent studies revealed that several types of DNA-binding proteins are involved in regulating the expression of *PR* genes[1,3—5]. In addition to the regulation at transcription level, translation regulating factors, such as the poly (A)-binding protein and RNA helicase also appear to be involved in the defense responses[6,7]. Emerging evidence shows that protein kinases, such as the mitogen-activated protein kinase, play a role in the signal transduction of defense responses[8].

Although the exact function of protein kinase in defense responses is unclear, phosphorylation of transcription and translation factors by protein kinase has been considered as being important in pathogen-induced defense response[9]. A common feature of *R* genes and defense-related genes as well as actively regulated signal transduction genes is that their expression in host is induced by incompatible pathogen infection[1,2]. Increased levels of encoding products of these genes are required for host defense responses.

However, limited information indicates that over-expression of *R* genes, defense-related genes, and some signal transduction genes may not present a complete picture of regulation of pathogen-induced defense responses. It is reported that the expression of catalase gene is repressed during hypersensitive reaction of tobacco plant[10]. The expression of anthocyanin biosynthesis genes encoding flavanone 3-hydroxylase, dihydroflavonol 4-reductase, and anthocyanidin synthase is also inhibited in sorghum during pathogen-induced defense responses[11]. The functions of genes encoding ribulose-1, 5-bisphosphate carboxylase/oxygenase are depressed in tobacco and potato plants after pathogen infection[12,13]. Some cell cycle-related genes, the histone gene families, p34cdc2 protein kinase gene, and mitotic cyclin gene, are transcriptionally repressed after fungal elicitor treatment in parsley[14]. The functions of a number of genes are also repressed during defense responses induced by pathogen and defense-related signaling molecules in *Arabidopsis*[15]. However, because most efforts are put on the studies of positive regulation of defense responses to the pathogen invasion, it is unclear how large ranges of cellular activities are repressed and what types of gene expression are inhibited in the defense cascade.

In addition, much still remains to be learned concerning the regulation of HR and SAR, as well as the signaling pathway between HR and SAR. One way to gain such understanding is to identify as many as possible genes that are involved in the defense response, and by understanding the roles of the genes in the resistance reaction, we may be able to obtain knowledge about the pathways underlying the defense response. The cDNA array technique, by displaying the expression of large numbers of genes, provides a powerful tool for studying the genes involved in the defense cascade at the whole genome level. The present study was undertaken to identify genes that are involved in the responses to pathogen infections and to deduce the functions of the identified genes in the defense response, with the long-term goal to characterize the processes and pathways underlying the resistance reactions of rice to important diseases.

1 Materials and methods

1.1 Experimental materials

Rice variety Minghui 63 (*Oryza sativa* ssp. *indica*) and rice lines, C101A51 (*indica*), IRBB10 (*indica*) and IRBB13 (*indica*), containing various bacterial blight resistance and blast resistance genes (table 1), were used as hosts for pathogen inoculation. The pathogens used for inoculation were 2 strains of *Xanthomonas oryzae* pv *oryzae* (*Xoo*), PXO86 (Philippine Race 2) and PXO99 (Philippine Race 6), that cause bacterial blight, and 2 isolates of *Pyricularia grisea*, one from the Philippines (V86013) and another from China (1366), that cause fungal blast. All of

the pathogens are incompatible with the corresponding rice lines listed in table 1. The Philippine strains of *Xoo* and isolate of *P. grisea* were kindly provided by the International Rice Research Institute. The Chinese isolate of *P. grisea* was from our laboratory[16].

Table 1 Pathogen-inoculated materials used for study

Material	Rice line	Resistance gene	Incompatible pathogen
1	IRBB10	*Xa10*	*Xoo*, PXO86
2	IRBB13	*xa13*	*Xoo*, PXO99
3	C101A51	*Pi2*	*P. grisea*, V86013
4	Minghui 63[a)]	Undefined	*P. grisea*, V86013
5	Minghui 63[a)]	undefined	*P. grisea*, 1366

a) There is at least one undetermined major gene for bacterial blight resistance and one undetermined major gene for blast resistance (unpublished data).

A normalized cDNA library from rice variety Minghui 63 (K. Peng et al., unpublished data) was used for preparation of cDNA arrays. This cDNA library was constructed using 15 different tissues including callus, whole plants (etiolated, 3-leaf stage, 5-leaf stage, young panicle development stage and heading stage), culms (flowering stage), roots, flag leaves, panicles (flowering stage and two different stages of grain-filling), flowers, leaves of 5-day after *Xoo* (Philippine strain PXO61, incompatible to Minghui 63) inoculation, and leaves of 5-day after *P. grisea* (Chinese isolate 1814, incompatible with Minghui 63) inoculation. This library contains about 62000 clones with average insert length of 1.2 kb. About 10000 clones of this library were single-pass sequenced, and approximately 63% of the sequences appeared to be non-redundant (L. Zhang et al., unpublished data).

1.2 Pathogen inoculation

The rice lines were inoculated with incompatible pathogens that induce the corresponding resistance reaction of the host plants (table 1). The procedures for blast infection including inoculum preparation, inoculation and incubation followed essentially as described by Chen et al.[16]. Bacterial blight inoculation was conducted on adult rice plants by the leaf clipping method. The preparation of bacterial suspension and inoculation were the same as described previously[17]. The viability of the bacteria and the fungi used for inoculation was confirmed by examining the lesion length and lesion numbers in susceptible varieties IR24 (*indica*) and CO39 (*indica*), respectively. Mock inoculated (control) plants were treated under the same conditions except that pathogen suspension was replaced by water for both bacterial blight and blast inoculations. The leaves of inoculated plants were collected 1, 3, 5, and 7 d after inoculation.

1.3 The cDNA array analysis

The plasmids of 21504 clones were extracted from the cDNA library and arrayed onto the Hybond-N^+ nylon filters with Biomek 2000 laboratory automation workstation (Beckman, Fullerton, CA). The filter, 8 × 12 cm in size, was arrayed into 384 grids each containing 8 dots of plasmid DNA with each sample replicated in symmetric positions within the grid. The DNA was fixed to the nylon filter by laying the nylon filter sequentially for 5 min each on filter paper saturated

with solutions I (0.5 mol/L NaOH and 1.5 mol/L NaCl), II (0.5 mol/L Tris·HCl, pH 7.5), and III (2X SSC, 0.1% SDS), respectively. After air-drying, the nylon filters were baked in a vacuum oven at 80℃ for 2 h.

Total RNA was isolated from the rice leaves with TRIzol Reagent (GIBCO-BRL) according to the manufacturer's instruction. The first strand cDNA was synthesized as the hybridization probe for cDNA array screening. The reverse transcription reaction was performed in a 50 μL volume containing 50—100 μg total RNA, 1 μg oligo $(dT)_{15}$ primer, 400 U Superscripts II reverse transcriptase (GIBCO-BRL), 1X first strand buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, 3 mmol/L $MgCl_2$), 100 μmol/L each of dATP, dGTP and dTTP, 50 μCi ^{32}P-dCTP (3000 Ci/mmol), and 0.5 μmol/L DTT. After incubation at 37°C for 1 h, the reaction was stopped and the RNA was degraded with 5 μL of 0.5 N NaOH and 5 μL of 100 mmol/L EDTA for 10 min at 70°C. The probe was purified using Sephadex G-50 column.

Up to 4 filters were prehybridized for 3 to 6 h at 65℃ in a plastic bag containing 15 mL of hybridization buffer (50 mmol/L phosphate buffer, pH 6.8, 2.5 mmol/L EDTA, pH 8.0, 5X SSC, 5X Denhart's, 0.01% denatured salmon sperm DNA, 0.4% SDS, and 5% dextran sulfate). The filters were hybridized overnight in the same hybridization buffer added with radioisotope-labeled first strand cDNA probe. After hybridization, the filters were washed twice in 0.5X SSC and 0.1% SDS for 15 min at 65℃. The analysis was subsequently carried out using PhosphorImager SI (Molecular Dynamics, Sunnyvale, CA). The intensity of the hybridization signal of each spot was measured using the computer program ArrayGauge Version 1.0 (Fuji Photo Film Co. LTD.).

Rice materials 1 to 4 in table 1 were used for cDNA array analysis. The probes prepared from tissues 5-day after pathogen inoculation and mock inoculation were hybridized with the cDNA arrays for the initial identification of defense-responsive sequences. Differentially expressed sequences were identified according to the following criteria: (1) the difference between the hybridization signals of the control tissue and the infected tissue could be clearly determined by the naked eyes, which corresponded to at least a 1.6 ratio difference according to the PhosphoImager reading; (2) both of the duplicated dots of the clone showed elevated (or repressed) expression compared to the control, and; (3) the intensity of the hybridization signals of clones around the putative positive clone was not higher (or lower) than the control (fig. 1). Although the criteria adopted may miss some of differentially expressed sequences, they provided assurance for the clones so identified to be truly differentially expressed. To further confirm the results, the differentially expressed clones identified by the first round of hybridization were picked out and arrayed on a new set of filters with each clone replicated in symmetric positions. The new filters were hybridized with probes prepared from tissues of 1-day and /or 5-day after pathogen inoculation and mock inoculation.

1.4 Reverse transcription-polymerase chain reaction (RT-PCR)

The RT-PCR was carried out as a two-step reaction. The reverse transcription step was per-

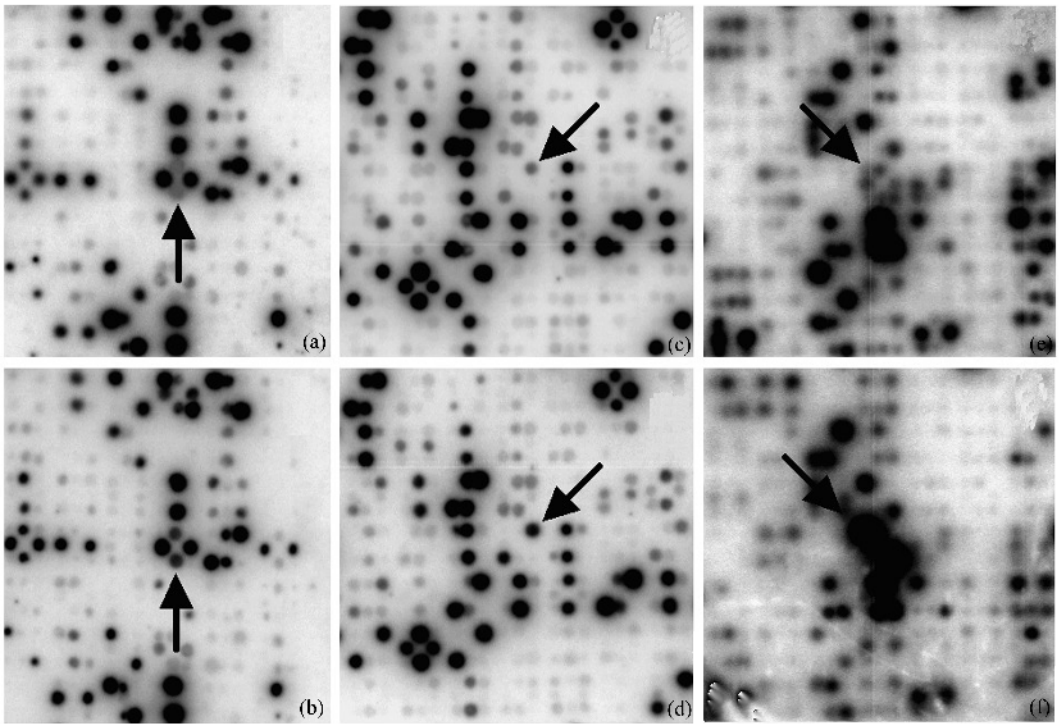

Fig. 1. Analysis of differentially expressed sequences by cDNA arrays. Each cDNA clone on the filters was arrayed twice on symmetric position in each of the 384 grids (arrows). The filters were hybridized with cDNA probes prepared from IRBB10 of mock inoculation ((a) and (c)) and 5-day after PXO86 inoculation ((b) and (d)) or from IRBB13 of mock inoculation (e) and 5-day after PXO99 inoculation (f), respectively. The arrows indicate differentially expressed cDNA clones, EI22C7 ((a) and (b)), EI31K10 ((c) and (d)), and EI1K8 ((e) and (f)).

formed in a 10 μL volume containing 0.5—1 μg total RNA pre-treated with DNase I (GIBCO-BRL), 50 ng oligo $(dT)_{15}$ primer, 100 U M-MLV reverse transcriptase (GIBCO-BRL), 1X first strand buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, 3 mmol/L $MgCl_2$), 20 mmol/L DTT, and 10 μmol/L each of dATP, dCTP, dGTP, and dTTP at 42℃ for 1.5 h. The reaction mixture was then diluted by adding 40 μL deionized water, and 1 μL of the diluted mixture was used for PCR. The PCR primers were as follows: EI12I1 forward 5′-CCCTACCCAAGGGGGTACTA-3′, reverse 5′-TTAAAGTTGGGGTTCCCATTC-3′; EI4J22/EI1K8 (partial overlap) forward 5′-CATCATCGGCTGCTGTTG-3′, reverse 5′-CTGCGTCTTGGAGTCCGT-3′; EI22F22 forward 5′-ACATTGCACACCATGCTCAT-3′, reverse 5′-CCAACACGGACTACAACGG-3′; EI35I3 forward 5′-TCCAGCCTCCTCAGGACCT-3′, reverse 5′-AGTCCTCCTGCTTCGTCGTA-3′; EI38P6 forward 5′-GCTGAAGAAGCAGGTGACATC-3′, reverse 5′-TGGGAAGGGTCATGAAGTTC-3′; EI28P15 5′-TTCGGATCCCCAATCTAG-3′, reverse 5′-TGAGAATGCTTGCCACAGAC-3′; EI2N8 forward 5′-GGACTCCACCAACAGAAACAA-3′, reverse 5′-CCTC-

AAACTCCTTGAGCTGG-3′. The actin primers, 5′-TATGGTCAAGGCTGGGTTCG-3′ (forward) and 5′-CCATGCTCGATGGGGTACTT-3′(reverse) were used as internal standard for each RT-PCR[18]. DNA contamination in the RNA sample was tested by replacing the reverse transcriptase with water in the RT-PCR.

1.5 RNA gel blot analysis

Twenty micrograms of total RNA was separated in an 1% agarose gel containing formaldehyde and transferred to Hybond-N^+ nylon filter. The filter was hybridized at 60℃ overnight in the hybridization buffer (0.07 mol/L NaH_2PO_4, 0.18 mol/L Na_2HPO_4, 7% SDS, 1% bovine serum albumin, fraction V, and 1 mmol/L EDTA). After hybridization, the filter was washed once in 0.5X SSC and 0.1% SDS for 15 min at 60°C.

2 Results and discussion

2.1 Differential expression of defense-responsive genes after pathogen inoculation

Hybridization of the filters with the probes prepared from tissues after pathogen inoculation and mock inoculation revealed a large number of differentially expressed sequences. Comparison of the images from the repeated hybridizations identified a total of 53 unique cDNA sequences as showing enhanced expression and 47 unique cDNA sequences as showing repressed expression after pathogen infection, with the level of differential expression ranging from 1.6- to 20.4-ratio of computer reading difference (fig. 1, tables 2 and 3).

As detected by the cDNA array analysis, the enhanced expression exhibited by 31 of the 53 sequences and the repressed expression exhibited by 31 of the 47 sequences respectively were not host-pathogen specific; their expression levels were increased or decreased in tissues inoculated with 2 to 4 isolates (strains) of both pathogens (tables 2 and 3). However, the same sequence usually varied in levels of differential expression in different pathogen-host combinations. Whereas differential expression of the remaining 38 sequences was each observed in only one pathogen inoculation in the corresponding host tissue according to the cDNA array analysis.

2.2 Confirmation and profiling of the defense-responsive sequences by RT-PCR and RNA gel blot analyses

Fourteen defense-enhanced sequences, EI12I1, EI22F22, EI14J22/EI1K8 (partially overlapped), EI35I3, EI38D7, BI26N5, EI19D23, EI10L24, EI28H1, EI10P9, EI13J2, EI5P11, EI35K2 and EI44N14, and 5 defense-repressed sequences, EI2N8, EI28P15, EI38P6, EI1E16 and EI6H23, showing various degrees of differential expression ranging from 1.7- to 20.4-ratio of difference 1 and 5 d after pathogen inoculation (tables 2 and 3), were further studied with RT-PCR or/and RNA gel blot analyses. As expected, all the 19 sequences were confirmed to be differentially expressed in the respective pathogen-host combinations 1 or 5 d after pathogen inoculation, consistent with the cDNA array analysis (figs. 2—4, only part of the data presented). However, the RT-PCR and RNA gel blot analyses also revealed differential expression of these sequences in other pathogen-

Table 2 Sequences showing increased expression after pathogen inoculation identified by cDNA array analysis

cDNA sequence	GenBank accession	Ratio of increase in expression level[a)b)]								BLAST analysis	
		IRBB10 PXO86		IRBB13 PXO99		C101A51 V86013		Minghui 63 V86013		homologous sequence	E value
		1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d		
EI12I1	BF108309	na	2.9	–	–	7.4	na	–	–	putative WRKY-type DNA-binding protein (AAC12823)	3e-18
EI38D7	BF108310	na	2.6	–	–	2.2	2.2	–	–	zinc finger protein (T48868)	5e-51
BI75E3	BF108311	na	2.3	–	–	–	–	–	–	ZF1 finger protein (AF332876)	1e-174
EI8H23	BF108312	–	–	1.0	3.5	na	2.2	–	–	putative homeodomain transcription factor (AAC69941)	2.9
BI26N5	BF108313	na	3.8	na	1.8	–	–	na	5.6	translation initiation factor (GOS2) mRNA (AF094774)	0.0
EI3G1	BF108314	2.0	2.6	3.3	1.1	–	–	2.1	1.0	poly(A)-binding protein mRNA (TAU81318)	1e-23
BI73F17	BF108315	1.7	1.7	6.6	4.8	–	–	1.2	4.4	glycine-rich RNA-binding protein (T04346)	8e-45
EI19D23	BF108316	–	–	na	8.6	–	–	–	–	RNA helicase (T51343)	1e-43
EI6G21	BF108317	1.6	1.6	2.2	2.1	–	–	–	–	putative nonsense-mediated mRNA decay protein (AAD24816)	5e-16
EI10L24	BF108318	–	–	6.6	6.4	–	–	3.1	3.1	similar to WEB1/SEC31-like transport protein (AAF27099)	4e-38
EI22F22	BF108319	na	1.7	na	3.1	na	2.1	–	–	putative sugar transporter protein (AAG46115)	1e-64
EI44L19	BF108320	–	–	–	–	–	–	1.1	5.2	similar to epoxide hydrolases (BAA84626)	1.7
EI28N12	BF108322	-	-	2.0	10.7	2.2	1.0	-	-	nucleoside diphosphate kinase (AF271362)	1e-126
EI39C8	BF108323	–	–	2.4	2.1	–	–	1.0	2.6	protein kinase (LAMMER) (P51567)	5e-39
EI39F4	BF108324	na	5.8	–	–	–	–	–	–	hydroxyproline-rich glycoprotein 1 (Q9FPQ6)	2e-07
EI31I7	BF108325	–	–	–	–	2.7	2.7	1.0	1.8	phospholipase like protein (CAB37511)	0.9
EI24B14	BF108326	–	–	na	2.3	–	–	–	–	pectin methylesterase (BAA89480)	9e-24
EI39P24	BF108327	–	–	1.0	2.3	–	–	–	–	putative pectinesterase (AAF26136)	3e-22
EI36C19	BF108328	–	–	na	1.6	4.5	3.8	–	–	S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase (AJ231133)	5e-71
EI10N21	BF108329	–	–	na	1.8	–	–	–	–	ferrochelatase precursor (P42045)	2e-66
EI11C3	BF108330	na	2.4	–	–	–	–	–	–	glyoxysomal malate dehydrogenase (P46488)	3e-55
EI28H1	BF108331	1.4	1.7	1.2	2.0	–	–	–	–	acylaminoacyl-peptidase like protein (CAB10236)	9e-09
EI35I3	BF108332	–	–	–	–	–	–	na	3.2	thiamine biosynthetic enzyme (thi1-1) mRNA (ZMU17350)	4e-97
EI36H4	BF108333	1.7	1.5	–	–	1.5	2.8	1.1	3.4	molybdopterin synthase sulphurylase (AAD18052)	3e-60
BI76J4	BF108321	–	–	–	–	1.0	3.5	na	3.0	zinc-induced protein (AF323612)	1e-109
BI74J19	BF108334	–	–	1.7	1.7	2.2	1.3	1.8	2.1	putative S-adenosylmethionine: 2-demethylmenaquinone methyltransferase (BAB84438)	3e-14
EI38M12	BF108335	–	–	na	2.2	–	–	–	–	disease resistance proteins (AAC35544)	0.9

(*To be continued on the next page*)

(*Continued*)

cDNA sequence	GenBank accession	Ratio of increase in expression level[a)b)]								BLAST analysis	
		IRBB10 PXO86		IRBB13 PXO99		C101A51 V86013		Minghui 63 V86013		homologous sequence	E value
		1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d		
EI3H3	BF108336	3.0	3.0	–	–	–	–	–	–	putative Mlo protein (O80580)	1.2
EI4J22 & EI1K8	BF108337 BF108338	na	1.9	19.3	20.4	–	–	–	–	light-induced mRNA (X68807)	0.0
EI31G5	BF108340	1.0	2.1	1.2	1.9	–	–	–	–	putative integral membrane protein (T34987)	2.0
EI39A7	BF108341	–	–	na	3.2	2.6	1.0	-	-	glycoprotein A (JE0120)	0.9
EI11K3	BF108342	–	–	na	2.0	–	–	–	–	alpha-tubulin (X91807)	0.0
BI76G3	BF108343	na	2.1	–	–	–	–	–	–	beta-expansin (EXPB3) (AF261271)	1e-156
EI10P9	BF108344	–	–	–	–	na	2.1	–	–	polyubiquitin (Rubq1) (X76064)	0.0
EI13J2	BF108345	na	2.2	–	–	–	–	–	–	proteasome component (T02089)	2e-52
EI5P11	BF108346	–	–	1.0	1.7	–	–	1.0	3.2	Nt-gh3 deduced protein (AAD32141)	1e-40
EI8A21	BF108347	na	2.8	–	–	1.8	1.1	2.2	2.0	LIP9 (low temperature induced protein) (AB011367)	e-135
EI13D12	BF108348	–	–	–	–	na	2.6	–	–	heavy metal associated domain (AAF82161)	2e-10
EI20C18	BF108349	–	–	1.1	3.4	–	–	–	–	mucin 2 precursor (Q02817)	2e-13
EI22C7	BF108350	1.7	1.1	–	–	na	1.6	–	–	putative calreticulin protein (AAL07169)	1e-61
EI23E13	BF108351	–	–	1.3	3.3	–	–	–	–	ATP/ADP translocator (D12637)	0.0
EI30O11	BF108352	–	–	na	2.6	2.9	1.6	–	–	thioredoxin h (D21836)	0.0
EI31K10	BF108353	na	2.3	2.5	1.3	–	–	–	–	putative glycine-rich protein (P13728)	2e-12
EI38J4	BF108354	–	–	–	–	na	2.1	na	3.2	metallothionein-like protein mRNA (AF009959)	1e-124
EI43O12	BF108355	na	1.9	–	–	–	–	–	–	Dreg-2 like protein (AAC79147)	3e-17
BI77K23	BF108361	1.1	2.0	1.0	2.8	–	–	–	–	aluminum-induced protein-like (BAB11312)	2e-65
EI8F24	BF108364	–	–	2.1	1.4	5.0	3.5	–	–	acrosin precursor (P08001)	7e-05
BI77C16	BF108357	–	–	1.5	6.1	3.1	2.9	1.1	2.7	unknown protein (AAD26478)	2e-24
EI35K2	BF108358	–	–	–	–	na	1.8	na	3.6	unknown protein (AAD42001)	4e-24
EI19I24	BF108360	–	–	na	2.2	–	–	–	–	unknown protein (AAK25977)	5e-75
EI44N14	BF108362	5.2	5.1	-	–	–	–	–	–	unknown protein (CAB66804)	0.8
EI31M6	BF108363	1.9	2.5	na	3.2	–	–	–	–	no significant similarity found	
BI77N18	BF108365	–	–	na	2.0	–	–	–	–	no significant similarity found	

a) Each number represents the average intensity of the hybridization signal of the duplicate dots of the positive clone relative to the mock-inoculated control; b) na, not applied; –, hybridization signal between mock and pathogen inoculation showed no visible difference and the intensity of the signal was not measured.

Table 3 Sequences showing decreased expression after pathogen inoculation identified by cDNA array analysis

cDNA sequence	GenBank accession	Ratio of decrease in expression level[a) b)]								BLAST analysis	
		IRBB10 PXO86		IRBB13 PXO99		C101A51 V86013		Minghui 63 V86013		homologous sequence	E value
		1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d		
EI35O16	BF145163	2.2	2.4	1.9	3.3	1.3	1.0	–	–	chloroplast ribulose-bisphosphate carboxylase large chain (T02958)	9e-89
EI36C5	BF145168	na	2.2	1.1	4.5	na	1.8	7.3	2.2	ribulose-1, 5-bisphosphate carboxylase small subunit (D00644)	0.0
EI38P6	BF145169	–	–	na	6.4	na	1.0	3.5	4.6	RuBisco activase large isoform precursor (AB034698)	0.0
EI6H23	BF145164	na	1.6	4.3	4.3	1.0	1.0	na	3.6	type I light-harvesting protein (D00641)	0.0
EI40J22	BF145212	–	–	1.0	1.0	3.4	11.3	2.6	2.9	photosystem II 10 kDa polypeptide mRNA (U86018)	0.0
EI12F17	BF145165	1.0	1.0	9.5	1.9	2.5	7.9	1.0	3.0	photosystem II 10 kDa polypeptide mRNA (U86018)	6e-24
EI19L22	BF145166	2.1	4.7	3.7	6.0	1.0	1.0	–	–	photosystem II 22 kDa protein mRNA (D84392)	0.1
BI27A2	BF145208	1.0	1.0	1.1	7.5	na	2.5	3.0	4.2	precursor of photosystem II subunit (CAA59007)	0.02
EI21G20	BF145206	1.0	1.0	na	6.8	1.0	1.0	1.0	1.0	chlorophyll a/b-binding protein mRNA (AF094775)	0.0
EI37F11	BF145210	na	1.6	na	2.5	1.2	1.0	5.8	2.1	chlorophyll a/b-binding protein type III precursor (T06411)	6e-31
EI35E22	BF145175	1.0	1.5	1.0	3.6	1.0	1.0	1.0	1.0	cytoplasmic malate dehydrogenase mRNA (AF353203)	0.0
EI4G10	BF145213	–	–	4.3	5.1	2.6	1.3	na	1.0	fructose-bisphosphate aldolase, chloroplast precursor (T03679)	3e-68
EI21F11	BF145216	–	–	2.6	8.6	5.2	6.2	–	–	fructose-1, 6-bisphosphatase (BAA25422)	1e-54
BI71C20	BF145214	1.0	1.0	1.0	5.4	3.7	1.5	1.4	1.0	glyceraldehyde-3-phosphate dehydrogenase subunit (AF022730)	0.2
EI34A18	BF145215	1.0	1.0	2.1	1.3	1.0	1.0	2.3	7.3	glycolate oxidase subunit D-like (BAB11407)	3e-06
EI40D10	BF145173	–	–	na	2.6	–	–	–	–	alanine aminotransferase (AB007405)	0.0
EI38C4	BF145211	–	–	–	–	na	1.6	–	–	complete chloroplast genome (NC_001320)	0.0
EI12K3	BF145167	1.0	1.0	1.2	1.0	na	4.6	1.0	1.0	monogalactosyldiacylglycerol synthase (CAA04005)	3e-56
EI21J20	BF145207	2.1	2.5	na	3.0	1.0	1.0	4.4	1.2	chloroplast channel forming outer membrane protein (CAB58442)	2e-5
EI34K15	BF145209	1.0	1.0	1.0	1.0	4.9	4.9	1.0	1.0	chloroplastic outer membrane protein (S55344)	2e-72
EI35O18	BF145176	1.0	1.5	1.3	2.0	na	2.0	2.6	2.4	glyoxalase I (AB017042)	0.0
BI71N2	BF145170	1.5	3.4	1.3	1.0	3.3	1.4	1.0	1.0	ubiquitin-conjugating enzyme (BAA96583)	6e-25
EI28P15	BF145171	1.0	1.0	1.1	2.1	3.0	1.0	2.4	1.0	ubiquitin conjugating enzyme 2 (AAL35400)	5e-36
EI21C23	BF145185	1.0	1.0	1.1	2.8	1.0	1.0	na	1.0	26S proteasome regulatory subunit 3 (BAB78499)	2e-69

(*To be continued on the next page*)

(*Continued*)

cDNA sequence	GenBank accession	Ratio of decrease in expression level[a) b)]								BLAST analysis	
		IRBB10 PXO86		IRBB13 PXO99		C101A51 V86013		Minghui 63 V86013		homologous sequence	E value
		1 d	5 d	1 d	5 d	1 d	5 d	1 d	5 d		
EI30L12	BF145177	1.3	1.9	1.4	9.1	na	1.9	2.3	1.7	novobiocin biosynthetic gene (AF170880)	1.6
EI2N8	BF145179	na	1.9	1.0	1.0	–	–	1.0	1.0	heat shock protein 82 mRNA (Z11920)	0.0
EI34M21	BF145180	1.0	1.0	na	2.0	na	2.1	4.2	1.8	prolamin (X17074)	0.0
EI16P6	BF145181	1.0	1.0	na	8.5	1.0	1.0	–	–	26 kDa globulin (D50643)	0.0
EI2G24	BF145182	1.0	1.0	1.0	1.0	Na	1.8	1.0	1.0	butyrophilin (BTF5) mRNA (U90552)	4e-12
EI17F17	BF145183	1.0	1.0	1.4	9.1	1.1	1.6	2.0	1.6	putative phosphatidylinositol / phosphatidylcholine transfer protein (AAD23696)	4e-44
EI18N8	BF145184	–	–	na	2.8	–	–	–	–	similar to maize transposon MuDR mudrA protein (BAA92521)	2e-07
BI26M22	BF145178	na	1.0	na	1.0	na	1.0	3.8	1.7	histone H2A mRNA (D38091)	1e-83
EI8A13	BF145186	na	3.5	na	1.0	1.0	1.0	1.0	2.6	histone H3 mRNA (AF093633)	0.0
BI75M9	BF145187	na	3.0	–	–	na	1.7	4.0	2.1	high mobility group protein (HMG) (AF093632)	1e-144
EI35K24	BF145188	1.5	1.8	1.0	1.0	na	1.6	1.0	1.0	putative bHLH DNA-binding protein (CAB77716)	2e-37
EI7O24	BF145189	1.0	1.0	na	1.7	1.0	1.0	1.0	1.0	DDX1 (putative RNA-binding protein) (AAC47310)	1.8
EI40O11	BF145190	2.4	3.1	1.0	1.0	1.9	1.5	1.0	1.0	unknown protein (AAF23194)	2e-40
EI21E9	BF145194	1.1	1.8	1.0	2.7	1.9	1.0	1.0	1.0	unknown protein (BAB10177)	2e-08
EI4F15	BF145195	na	1.6	1.0	4.0	1.0	1.0	1.0	1.0	hypothetical protein (T04790)	2e-36
EI10P4	BF145196	5.1	1.9	1.0	1.0	1.0	1.0	1.0	1.0	hypothetical protein (BAA81869)	2e-65
EI28D19	BF145197	na	1.7	1.1	2.0	1.0	1.0	1.0	1.0	hypothetical protein (BAA90620)	1e-8
EI28E7	BF145198	na	2.1	1.0	1.0	2.1	5.0	1.0	1.0	hypothetical protein (CAB38795)	1e-110
EI31G14	BF145199	2.5	3.2	2.7	2.1	1.0	1.0	1.0	1.0	hypothetical protein (T04215)	7e-5
EI1K6	BF145202	1.0	1.0	7.7	7.5	2.3	1.8	1.4	2.1	no significant similarity found	
EI1E16	BF145203	1.8	1.4	na	6.1	1.0	1.0	1.0	1.0	no significant similarity found.	
EI42N19	BF145204	1.0	1.0	1.0	1.0	1.0	1.0	2.1	1.1	no significant similarity found	
BI75E5	BF145205	na	1.0	na	1.7	1.0	1.0	–	–	no significant similarity found	

a) Each number represents the average intensity of the hybridization signal of the duplicate dots of the positive clone relative to the mock-inoculated control; b) na, not applied; –, no signal detected in both mock inoculation and pathogen inoculation.

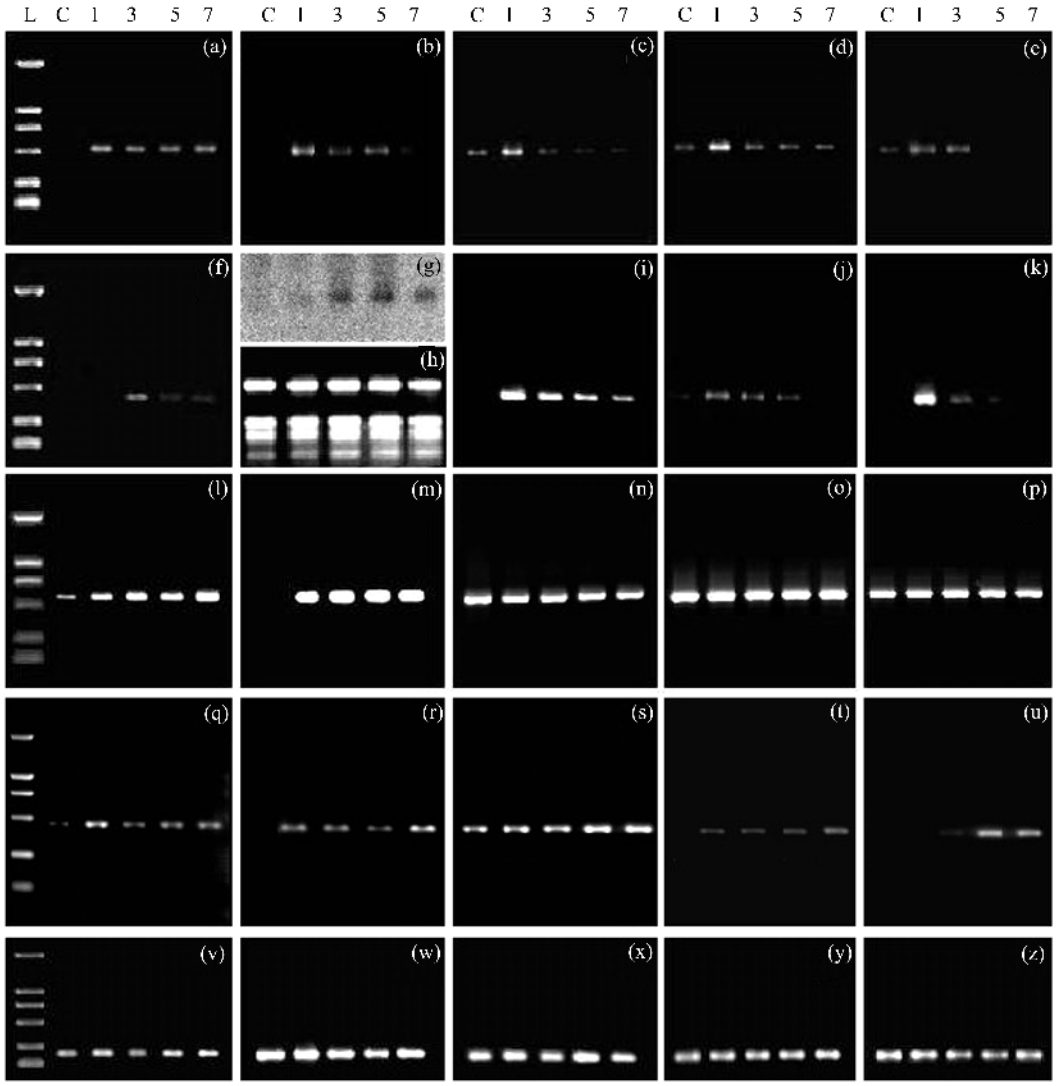

Fig. 2. The expression patterns of the defense-enhanced sequences. RT-PCR assay of the expression of sequences EI12I1 (row 1), EI22F22 (row 2, f, i, j, and k), EI14J22/EI1K8 (row 3), EI35I3 (row 4), and actin (row 5) in different pathogen-host combinations (from left to right: column 1, PXO86-IRBB10; column 2, PXO99-IRBB13; column 3, V86013-C101A51; column 4, V86013-Minghui 63; column 5, 1366-Minghui 63). The actin was amplified as the internal control for RT-PCR in each row. (g) RNA gel blot analysis of EI22F22; (h) the same RNA fractions as in G were electrophoresed on an agarose gel and stained with ethidium bromide. Lane L, DNA ladder (band sizes: 2000, 1000, 750, 500, 250, and 100 bp); lane C, mock inoculation; lanes 1, 3, 5, and 7, 1, 3, 5, and 7 d after pathogen inoculation.

host combinations at time courses other than the first and fifth day after inoculation.

To examine the consistency of the results obtained by RT-PCR and RNA gel blot analysis, the expression patterns of sequence EI38P6 in all the 5 pathogen-host combinations listed in table 1 were studied by both RT-PCR and RNA gel blot analyses. The results showed that both approaches revealed very similar expression profiling of EI38P6 in corresponding pathogen-host combinations and time courses (figs. 3(k)—(o)) and 4(k)—(o)).

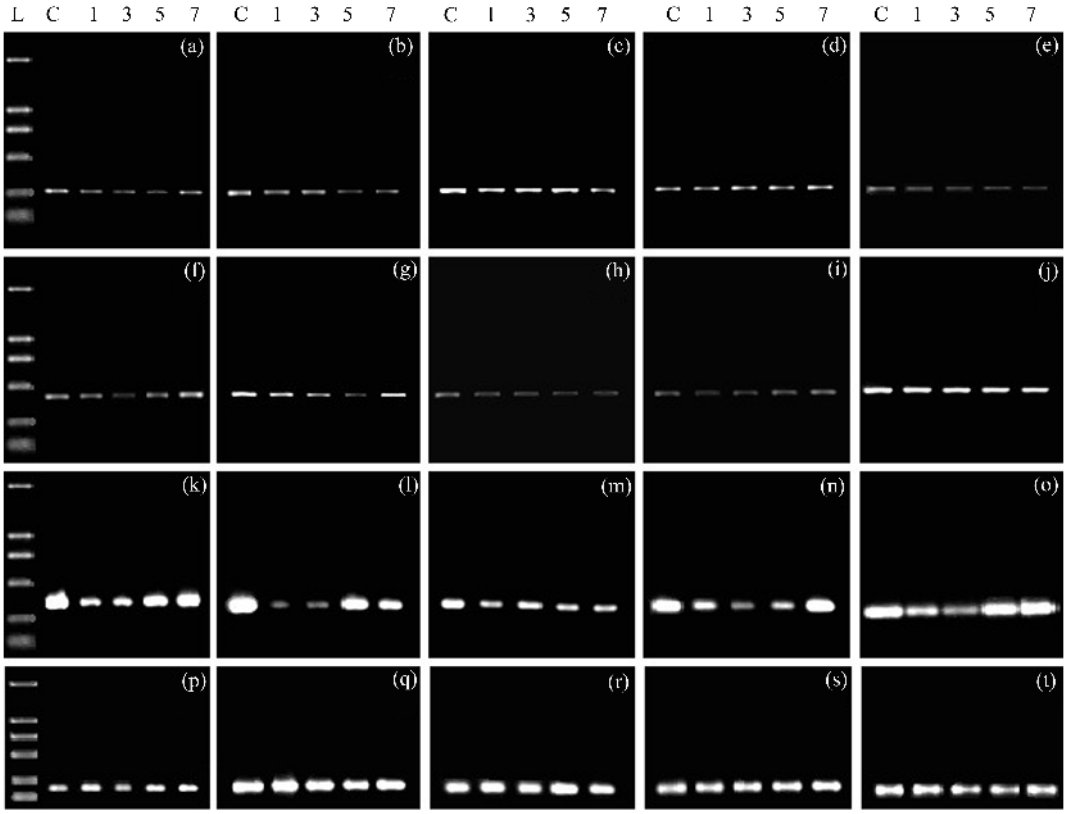

Fig. 3. Expression patterns of the defense-repressed sequences analyzed by RT-PCR. The expression of sequences, EI2N8 (row 1), EI28P15 (row 2), EI38P6 (row 3), and actin (row 4), was analyzed in different pathogen-host combinations (from left to right: column 1, PXO86-IRBB10; column 2, PXO99-IRBB13; column 3, V86013-C101A51; column 4, V86013-Minghui 63; column 5, 1366-Minghui 63). The actin was amplified as the internal control for RT-PCR in each column. Lane L, DNA ladder (band sizes: 2000, 1000, 750, 500, 250, and 100 bp); lane C, mock inoculation; lanes 1, 3, 5, and 7, 1, 3, 5, and 7 d after pathogen inoculation.

The detection of the differential expression of the above 19 sequences in pathogen-host combinations and time courses other than the ones used for cDNA array analysis indicates that many of the differentially expressed sequences identified by cDNA array analysis may be commonly involved in defense responses in rice, although the time course and the level of induction or repression may be different from one pathogen-host combination to another. These results further support the view that except for the specificity of *R* gene-mediated pathogen recognition, the downstream pathways of defense responses may be common[19]. The RT-PCR and RNA gel blot analyses showed that the enhanced or repressed expression of the above 19 sequences occurred 1 d after pathogen inoculation (figs. 2 and 3). The expression of another 21 up-regulated and 28 down-regulated sequences was also enhanced or depressed 1 d after pathogen inoculation as revealed by the cDNA array analysis (tables 2 and 3). These results indicate that induction for some of the up-regulated genes and inhibition for most of the down-regulated genes appeared at the early stage of the defense cascade.

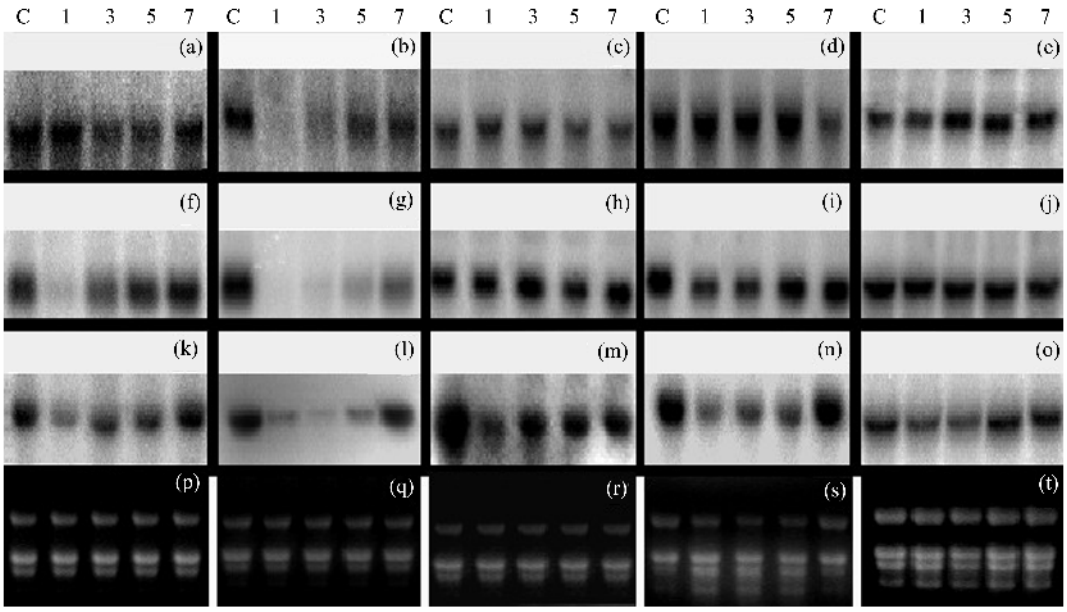

Fig. 4. Expression patterns of the defense-repressed sequences analyzed by RNA gel blot. The expression of sequences, EI1E16 (row 1), EI6H23 (row 2), and EI38P6 (row 3), was analyzed in different pathogen-host combinations (from left to right: column 1, PXO86-IRBB10; column 2, PXO99-IRBB13; column 3, V86013-C101A51; column 4, V86013-Minghui 63; column 5, 1366-Minghui 63). Row 4 shows the same RNA fractions as in each column electrophoresed on an agarose gel and stained with ethidium bromide. The variant intensity of the hybridization signals in (d) of row 1 was due to unequal amount of total RNA loaded. Lane C, Mock inoculation; lanes 1, 3, 5, and 7, 1, 3, 5, and 7 d after pathogen inoculation.

2.3 Putative roles of defense-enhanced genes in defense responses

Sequence comparison as well as BLASTN and BLASTX search[20] were conducted to examine the differentially expressed sequences. For the convenience of description, we will refer to these sequences as defense-responsive genes in discussing the putative functions of these sequences. Of the 53 sequences showing increased expression after pathogen induction, 46 had various degrees of similarity with plant genes, 4 had low degree of similarity with human or animal genes, and one had very low degree of similarity with *Streptomyces* gene in databases. The remaining 2 sequences had no homology with any of the genes or sequences in databases. The 50 sequences, showing various degrees of similarity with genes in databases, can be placed into the following groups according to their putatively encoded proteins: transcription regulating proteins, translation regulating proteins, transport proteins, kinases, metabolic enzymes, and proteins involved in other function (table 2). Since none of the 53 defense-enhanced sequences showed a high degree of similarity with any of the genes or sequences of microorganisms, it is unlikely that any of the differentially expressed sequences resulted from the contamination of pathogen RNA in the tissues used in the study.

According to the predicted functions of the differentially expressed sequences, 10 (represented by sequences EI12I1, EI3G1, EI19D23, EI36C19, EI44L19, BI76J4, EI13J2, EI3H3,

EI31K10, and EI10P9) of the 53 defense-enhanced genes were previously reported as being induced during defense responses in rice (1) and other plant species (9). Some of the 10 can be assigned to defense pathways known to be active during disease resistance responses. Whereas the remaining 43 defense-enhanced genes have not been reported previously to be involved in defense responses. However, the functions for many of these sequences can be speculated on the basis of their homology with known genes.

2.3.1 Regulation of the expression of defense-enhanced genes. Sequence EI12I1 may encode the WRKY type DNA-binding protein that has been found to be defense responsive in *Arabidopsis*, tobacco, and parsley (table 2). This transcription factor recognizes and binds to a special sequence in the promoter regions of certain *PR* genes of tobacco and parsley[5,21]. A group of *PR-1* regulon genes may also be regulated by WRKY type DNA-binding protein during disease resistance response in *Arabidopsis*[1]. At least one WRKY type gene in parsley can be induced by pathogen elicitor[21]. The elevated expression of the sequence EI12I1 by both pathogens (fig. 2) suggests that WRKY-like gene may play an important role in the regulation of both bacterial blight and blast resistance in rice. The predicted encoding products of another two sequences, EI38D7 and BI75E3, also show a high degree of sequence similarity with DNA-binding proteins (table 2). They may also be involved in the regulation of the expression of defense-related genes, because transcription factors other than the WRKY type have been identified to participate in the regulation of *PR* genes in different plant species[3,4,9].

In addition to regulation at the transcriptional level, the defense-related genes also appear to be regulated at the translational level. It has been reported that tobacco plants transformed with yeast poly (A)-binding protein gene are resistant to a range of plant pathogens such as virus, bacteria, and fungi, and the transgenic plants also showed elevated levels of expression of *PR* genes[7]. It was observed that *Nicotiana benthamiana* transformed with the mutated (defective) virus RNA helicase gene became virus resistant[6]. The present study identified two pathogen inducible sequences, EI3G1 and EI19D23, whose putative products show high degree of similarity with the wheat poly (A)-binding protein and *Arabidopsis* RNA helicase, respectively (table 2). It has been reported that the poly (A)-binding protein can increase the RNA helicase activity of translation initiation factors in wheat[22]. Thus, the concurrence of the enhanced expression of sequences EI3G1 and EI19D23 after *Xoo* inoculation suggests that they may function in the same pathway during defense responses. Another three sequences, BI26N5, BI73F17, and EI6G21, induced by both *Xoo* and *P. grisea* inoculation are also predicted to encode translation regulating factors, and they may play similar roles as the poly (A)-binding protein or the RNA helicase in defense responses.

2.3.2 Cell wall modification. The defense-enhanced sequence EI36C19 encodes putative S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase, an enzyme involved in lignification. The increased expression of this gene after pathogen induction may be an indication of strength-

ening cell wall during defense responses. A similar type of defense-enhanced gene that encodes cafeoyl-CoA 3-O-methyltransferase was also reported to be activated in disease resistance responses in parsley[23]. The expression levels of another two sequences, EI24B14 and EI39P24 encoding putative pectin methylesterase and pectinesterase, respectively, were also increased after the same *Xoo* strain inoculation as EI36C19. Although no data have been reported previously about the roles of the two putative genes in defense responses, the predicted functions of the two genes suggest that they may work together with the caffeic acid 3-O-methyltransferase in reinforcing the rice cell wall during defense against pathogen infection.

The expression of another cell wall-related sequence, BI76G3, was also enhanced after pathogen inoculation (table 2). However, BI76G3 encodes a putative expansin, an extracellular protein involved in loosing cell wall. A recent study revealed that one expansin region resembles the catalytic domain of endoglucanase[24], while it has been well studied that beta-1,3-glucanase, a PR protein, functions in the degradation of the cell walls of fungi in defense response of the host plants. Therefore, the target of the putative expansin may be the invading pathogen during host defense.

2.3.3 Defense signal transduction. Sequence EI28N12 shows high similarity to the gene encoding nucleotide diphosphate kinase, an enzyme catalyzing the transfer of γ-phosphate from ATP to NDP through autophosphorylation[25]. This enzyme is an important element for maintaining stable GTP levels in various metabolic pathways and GTP-mediated signal transduction pathways in animals and plants[26,27]. It has been reported that this enzyme can suppress tumor incidence and metastasis in animal[26], a process resembling the disease resistance responses in plant. The expression of nucleotide diphosphate kinase gene of tomato is rapidly elevated by wounding[28]. Wounding-induced signal transduction pathway appears to share some elements with the defense pathways caused by pathogens[29,30]. Therefore, this enzyme may function in the defense signal transduction through phosphorylation during pathogen-induced defense responses.

2.3.4 HR-related. Sequence EI44L19 showed 5.2-ratio increase in expression level after pathogen inoculation. Its encoding product has low degree of similarity to epoxide hydrolase, an enzyme that converts epoxides to diols. The expression of epoxide hydrolase gene was found to be inducible by virus infection in tobacco and the gene product may play a role in protection from oxidative damage associated with defense responses[31]. Oxidative burst is a key response of host plant during HR. It is thus likely that the elevated expression of EI44L19 after pathogen inoculation may be related to HR. This enzyme was also reported to be involved in both salicylic acid-dependent pathway and jasmonic acid-dependent pathway[31,32]; both pathways are known to be important in signal transduction in SAR[29].

The sequence EI22C7 putatively encodes calreticulin, a Ca^{2+}-binding protein. A recent study revealed that a sustained increase in cytosolic Ca^{2+} is necessary for the oxidative burst and the HR in *Arabidopsis*[33]. Thus, the enhanced expression of the EI22C7 after both *Xoo* and *P. grisea* in-

oculation may be related to the flux of the intracellular Ca^{2+}.

2.3.5 Other undefined roles. The sequence EI13J2 encodes a putative proteasome component. It was reported in tobacco that the expression of a subunit of proteasome can be induced by salicylic acid, one of signal molecules for SAR[34]. Proteasome is a multicatalytic proteinase complex that functions mainly in the ATP-dependent degradation of proteins that have been conjugated with polyubiquitin. Interestingly, the sequence EI10P9, that also showed enhanced expression, encodes polyubiquitin. A similar gene encoding putative ubiquitin is activated during HR in soybean after bacteria inoculation[35].

The BI76J4 had a high degree of sequence similarity to an unpublished zinc-induced protein isolated from rice leaves infected by blast fungus (GenBank accession number AF323612). The enhanced expression of BI76J4 was also confirmed after inoculation with isolate V86013 of *P. grisea* in both C101A51 and Minghui 63. The predicted encoding product of another infection-enhanced sequence EI31K10 is a glycine-rich protein. The expression of this type of genes is also pathogen-induced in barley and wheat[36,37]. However, the functions of the two predicted genes in defense responses need to be elucidated.

2.4 Predicted roles of the defense-repressed genes in defense responses

BLASTN and BLASTX analysis[20] showed that 11 of the 47 defense-repressed sequences had either no sequence similarity with entries in the databases (4 sequences) or various degrees of similarity with sequences in the databases (7 sequences) currently with unknown functions (table 3). Thus the functions for the 11 sequences could not be speculated in the analysis. The other 36 sequences showed various degrees of similarity with genes, mostly (33 sequences) with plant genes, in the databases that have identified or predicted functions.

2.4.1 Photosynthesis related. Of the 36 sequences showing similarity with genes in databases, 20 sequences (first 20 sequences in table 3), under the regulation of either nuclear genome or chloroplastic genome, were directly or indirectly related to photosynthesis. The depressed expression of the large numbers of photosynthesis-related and chloroplast-related sequences after pathogen infection suggests that one of the major repressed activities during host defense responses is photosynthesis. The expression of photosynthetic genes, encoding ribulose-1,5-bisphosphate carboxylase/oxygenase and chlorophyll a/b binding protein, was repressed in tobacco by soluble sugars, which could induce the expression of *PR* genes in salicylic acid-independent pathway *in vitro*[12]. The correlation between elevated levels of PR proteins as well as decreased photosynthetic capacity and local accumulation of sugars is also confirmed *in vivo* in tobacco and potato plants[13]. Therefore, sugar accumulation could be one of the factors causing the repressed expression of photosynthesis- and chloroplast-related sequences in the present study. This hypothesis can also be supported by the enhanced expression of EI22F22, encoding putative sugar transporter protein, in all the 5 pathogen-host combinations (fig. 2). The induced expression of EI22F22 suggests an increased level of sugar content in the tissues after pathogen inoculation.

2.4.2 Other functions. The remainder of the 36 sequences encoded putative enzymes and proteins with functions not apparently associated with photosynthesis or chloroplast (table 3). The sequence EI35O18 encoded glyoxalase I, an enzyme that acts coordinately with glyoxalase-II to convert toxic 2-oxo-aldehydes into less reactive 2-hydroxyacids and whose expression is enhanced in plant in responses to several types of stress, such as salt, water and heavy metal stresses[38]. However, the expression of this gene was decreased after both *Xoo* and *P. grisea* inoculations in different rice lines (table 3), which seemed to indicate an opposite role of the enzyme in defense responses to biotic and abiotic stresses.

It is reported that fungal infection in parsley is correlated with repression of cell cycle-related genes[14]. Glyoxalase I plays a role in cell cycle under the regulation of auxin[39]. Therefore, the decreased expression of glyoxalase I gene observed in the present study may be due to the repression of cell cycle caused by pathogen inoculation. The possibility of repression of host cell cycle after pathogen infection in this study can be further supported by the evidence of the repression of histone H2A and H3 genes (represented by sequences BI26M22 and EI8A13, respectively) after both *Xoo* and *P. grisea* infections (table 3). The expression of a group of cell cycle-related genes including the H2A and H3 is declined after fungal infection in parsley[14].

Although over-expression of a number of putative DNA- and RNA-binding sequences after pathogen infection was observed in this study (table 2), 3 defense-repressed sequences, BI75M9, EI35K24, and EI7O24, except of BI26M22 and EI8A13 were also predicted to encode DNA- or RNA-binding proteins (table 3). The opposite roles of these regulating proteins in defense responses indicate the likely complexity of the processes.

As discussed in the previous section, 2 sequences (EI10P9 and EI13J2) that encoded and were predicted to encode polyubiquitin and proteasome component, respectively, showed enhanced expression after pathogen inoculation (table 2). Interestingly, the expression of 2 different sequences, BI71N2 and EI28P15 putatively encoding ubiquitin-conjugating enzyme, the protein that ligates ubiquitin to intracellular target proteins, was repressed, accompanying the induction of the putative proteasome gene and polyubiquitin gene after *Xoo* and *P. grisea* inoculations (table 3). The expression of another sequence EI21C23 encoding putative proteasome regulating subunit was also decreased after *Xoo* inoculation (table 3). The functional correlation and regulation indicate that the 5 putative genes are probably interacting in the same pathway in defense responses.

The reduced expression of genes after the infection of incompatible pathogen indicates that plant must inhibit certain metabolic activities for the needs of the biochemical and physiological activities in defense cascade, and suggests that inhibiting the functions of certain genes may be as important as enhancing the functions of other genes during defense responses. Although the mechanism of repression of these defense-responsive genes in defense responses is not clear based on the results of this study, these findings may have brought about a new dimension for studying and understanding of pathogen-host interactions.

3 Conclusions

The present study identified 53 sequences with enhanced expression and 47 sequences with repressed expression after pathogen inoculation, which may represent 100 putative defense-responsive genes. Most of these defense-responsive genes have not been reported previously to be involved in response to pathogen infection in any plant species. The identification of induction and repression of about equal amount of genes suggests the complexity of the processes and pathways involved in plant defense against the infection of incompatible pathogens. Despite the limitations of the results produced by this cDNA array analysis using 21504 randomly chose cDNA clones (representing approximately 13000 unique sequences) and the tissues from plants 5 d after pathogen inoculation for initial identification of defense-responsive genes, the large amount of information can be useful in many ways in future studies. This includes identification and isolation of genes involved in response to pathogen invasions, molecular characterization of plant defense pathways, and also deployment of the genes for developing cultivars for disease resistance in plant breeding programs.

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