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ORIGINAL ARTICLE

Comparative Transcriptome Analysis of *Rhizoctonia solani*-resistant and -Susceptible Rice Cultivars Reveals the Importance of Pathogen Recognition and Active Immune Responses in Host Resistance

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Abstract Rice sheath blight (SB), caused by *Rhizoctonia solani* (*R. solani*), is a major threat to rice production worldwide. The molecular mechanisms of the SB resistance in rice are poorly understood. The transcriptomes of the SB-resistant rice cultivar YSBR1 and the susceptible cultivar Lemont were analyzed after *R. solani* infection. A total of 7624 differentially expressed genes (DEGs) were identified at one or more timepoints in a cultivar. 5526 and 5618 DEGs were differentially expressed in Lemont and YSBR1, respectively. YSBR1 exhibited stronger and earlier transcriptional response to *R. solani* than Lemont. Gene ontology enrichment analysis revealed that genes that encode cell wall-modifying and glycosyl-degrading enzymes or anti-microbial proteins were specifically induced in YSBR1 at 6 hpi. MapMan analysis revealed that more DEGs related with cell wall, β-glucanses, respiratory burst, phenylpropanoids and lignin were highly induced by *R. solani* in YSBR1 than in Lemont. The results also showed that receptor-like kinases and jasmonic acid signaling may play important roles in host resistance to *R. solani*. This study highlights potential candidate genes and signaling pathways involved in rice sheath resistance and can help to further clarify the mechanistic events underlying resistance and susceptibility to *R. solani*.

Keywords: Disease resistance, Jasmonic acid, Receptor like kinase, Rice sheath blight, *Rhizoctonia solani*, Transcriptome

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Introduction

Rice sheath blight (SB) is a major threat to rice production worldwide, especially in intensified production systems (Lee and Rush 1983; Banniza and Holderness 2001; Hossain et al. 2014). *Rhizoctonia solani* (*R. solani*), the causal agent of rice SB, is a necrotrophic fungal pathogen. *R. solani* has a wide host range, considerably high genetic diversity, and survives in the soil for years (Banniza and Holderness 2001). Rice germplasm that is highly resistant to *R. solani* has not been discovered yet (Eizenga et al. 2002; Willocquet et al. 2012; Hossain et al. 2014). Thus, rice SB management heavily relies on fungicides, which significantly increase production cost and cause serious environmental problems (Yellareddygari et al. 2014).

Plants have highly developed immune systems that defend themselves against infection (Chisholm et al. 2006; Jones and Dangl 2006; Liu et al. 2013). The plant immune system is composed of pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Chisholm et al. 2006; Jones and Dangl 2006). PTI is initiated by recognizing non-self molecular patterns, which may be slow-evolving signature-pattern molecules from pathogens or microbial degradation products of host cell components, by pattern recognition receptors (PRRs, Zipfel 2014). ETI is activated when a pathogen effector is directly or indirectly detected by the cognate resistance (R) protein (Boller and He 2009; Thomma et al. 2011). ETI often involves a hypersensitive reaction and is conceptually equivalent to the classical gene-for-gene resistance (Chisholm et al. 2006; Jones

and Dangl 2006). Examples of gene-for-gene resistance in nature are rare for necrotrophs/heminecrotrophs, such as *R. solani.* Thus, plants mainly use PTI to defend themselves against *R. solani* infection (Mengiste 2012; Lai and Mengiste 2013). Chitin, an N-acetyl-d-glucosamine polymer found in fungal cell walls, but not in plants, is the most well-known necrotrophic/heminecrotrophic fungi PAMP (Mengiste 2012; Lai and Mengiste 2013). Chitin fragments are recognized by a class of PRRs called LysM receptor-like proteins (Miya et al. 2007; de Jonge et al. 2010; Shimizu et al. 2010; Gust et al. 2012). Chitin recognition activates signaling proteins, such as mitogen-activated protein kinase (MAPK) signaling cascade components (Asai et al. 2002). MAPK3 and MAPK6 are involved in necrotrophic pathogen resistance in Arabidopsis (Han et al. 2010). MAPK3 and MAPK6 activate key enzymes involved in hormone synthesis, such as ACC synthase (Han et al. 2010) and transcription factors of the WRKY, ERF, MYB, and NAC families (Pitzschke 2015; Wang et al. 2015a). This activation results in highly orchestrated and co-operated transcriptome reprogramming, causing toxic compound production and plant cell wall reinforcement to prevent infection (Moore et al. 2011; Windram et al. 2012). Jasmonic acid (JA) and ethylene play important roles in PTI signal relay against necrotrophs/heminecrotrophs (Glazebrook 2005; van Loon et al. 2006; Mengiste 2012). Other phytohormones, such as salicylic acid (SA), abscisic acid, auxin, and gibberellin, are also implicated in plant resistance against necrotrophs/heminecrotrophs. However, the defense mechanism against these pathogens remains poorly understood and contradicting results are common among different systems (Mengiste 2012; Lai and Mengiste 2013; Kushalappa et al. 2016). PTI is important for basal plant resistance against necrotrophs/heminecrotrophs because the malfunction of several proteins involved in PTI signaling enhances susceptibility to necrotrophs/heminecrotrophs (Mengiste 2012; Lai and Mengiste 2013) and over-expression of pathogenesis-related proteins induced in PTI enhances plant resistance against necrotrophs/heminecrotrophs (Taheri and Tarighi 2011; Mengiste 2012; Lai and Mengiste 2013; Karmakar et al. 2016).

Gene-for-gene resistance to necrotrophs/heminecrotrophs is uncommon; however, different cultivars of the same plant species may have substantially different susceptibilities to a specific necrotrophic/heminecrotrophic pathogen (Mengiste 2012; Lai and Mengiste 2013). This type of resistance is usually controlled by quantitative trait loci (QTLs) and valuable in breeding programs for controlling damages caused by necrotrophs/heminecrotrophs (Kou and Wang 2010; Mengiste 2012). Several lines of rice showing varying resistance levels to *R. solani* have been identified (Srinivasachary et al. 2011; Hossain et al. 2014; Suharti et al. 2016). Dozens of QTLs that control SB resistance have been mapped on their corresponding chromosomes (Srinivasachary et al. 2011; Zuo et al. 2014a; Wen et al. 2015). Several rice genes were identified to be involved in resistance to *R. solani* (Srinivasachary et al. 2011; Molla et al. 2013; Chen et al. 2015; Wang et al. 2015a b; Karmakar et al. 2016; Xue et al. 2016). However, the molecular mechanisms underlying the resistance to *R. solani* in these rice cultivars are poorly understood (Srinivasachary et al. 2011; Karmakar et al. 2016; Xue et al. 2016).

Gene expression profiling is a promising approach for studying the regulatory mechanisms and signaling networks of plant defense responses and identifying the genes involved in disease response (Wise et al. 2007). Zhu et al. (2016) studied *Zoysia japonica* root response to *R. solani* AG1 IA strain invasion by pathogenesis-related proteins assay and transcriptome analysis. Zhao et al. (2008) constructed a subtractive hybridization cDNA library to enrich differentially expressed rice transcripts during *R. solani* infection and detected *PR1b*, *PBZ1*, and other defense-related gene expressions in rice. Some rice genes induced by *Magnaporthe grisea*, *Xanthomonas oryzae* pv. *oryze* (Xoo), and *X. oryzae* pv. *oryzicola* (Xoc) infections are differentially expressed, suggesting overlapping defense responses to different fungal and bacterial infections (Zhao et al. 2008). The Jasmine 85 rice cultivar, which has moderate SB resistance, was infected with *R. solani*, and its defense response was profiled using both RL-SAGE and microarray methods (Venu et al. 2007). Different metabolic processes are also correlated with rice resistance to *R. solani*. For example, accompanying *R. solani* infection, respiration, photorespiration, pectin synthesis, lignin accumulation, and metabolic processes were affected in resistant line 32R and nitrogen metabolism was changed in susceptible line 29S (Suharti et al. 2016). Many defense- and metabolism-related genes were identified in infected Jasmine 85 leaves, with either highly induced or suppressed expression. Many *R. solani*-induced rice genes interact with other pathogens, such as the rice blast fungus, *M. grisea* (Jantasuriyarat et al. 2005; Zhao et al. 2008).

YSBR1 is a rice line derived from the progeny of the hybrid between a *japonica* and an *indica* cultivar. YSBR1 shows high and consistent resistance to SB, which can be utilized for breeding resistant rice cultivars (Zuo et al. 2009). In this study, we performed microarray analysis of YSBR1 and the *R. solani*-susceptible Lemont cultivar and compared their transcriptomes to study the mechanisms underlying YSBR1 resistance to *R. solani*. We observed dynamic and different responses of the two rice cultivars during *R. solani* infection, and YSBR1 exhibited stronger and earlier transcriptional response to *R. solani* than Lemont. Our study has highlighted new candidate genes for better understanding of the host resistance and developing strategies to breed resistant rice cultivars against *R. solani*.

Results

Validation of Samples before Transcriptome Analysis

The resistant rice cultivar YSBR1 and the susceptible cultivar Lemont (Fig. S1A) were infected with *R. solani* or mock treated and sampled at 6, 10, and 20 hours post inoculation (hpi). The cDNA reverse-transcribed from the RNA of each sample was hybridized to the arrays, and the expression values for each gene were obtained as described in the Materials and Methods. Prior to the microarray analysis, RT-PCR analysis of *PBZ1* and *PR1* were validated in YSBR1 and Lemont infected with *R. solani* at the indicated time points. The expression levels of *PBZ1* and *PR1* increased over time and peaked at 20 hpi in Lemont, while both genes were strongly induced in YSBR1 from 6 hpi. Importantly, the expression of *PBZ1* and *PR1* reached higher levels in YSBR1 compared with Lemont as early as 6 hpi (Fig. S1B). These results indicate that the resistant cultivar YSBR1 and the susceptible rice cultivar Lemont respond to *R. solani* differentially in the activities of PR genes.

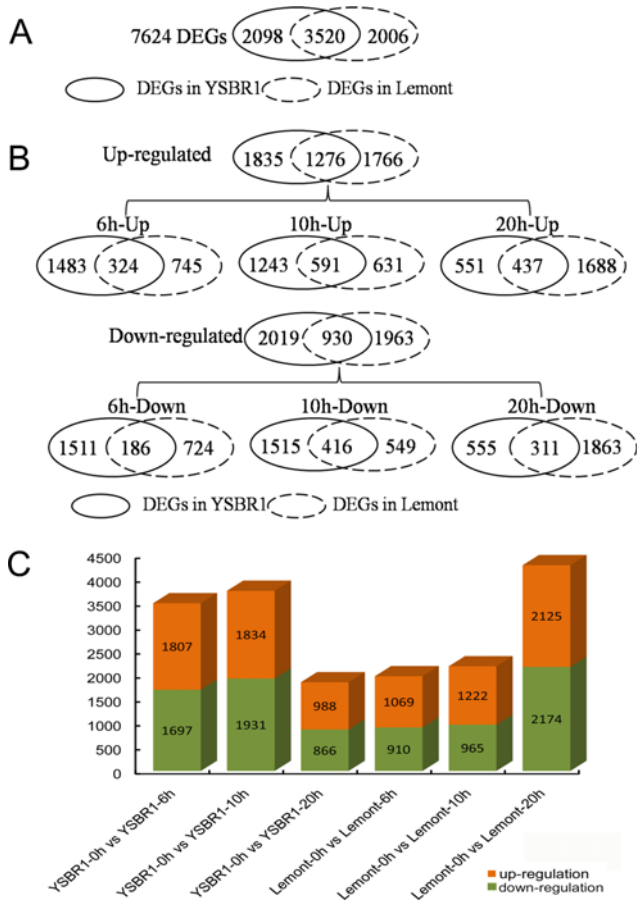


Fig. 1. Identification of DEGs in rice cultivars YSBR1 and Lemont infected with *R. solani* at 6, 10 and 20 hpi. Genes with expression levels increased or decreased by more than 2-fold in either YSBR1 or Lemont compared with mock inoculation were identified as DEGs. (A) Venn diagram of DEGs in YSBR1 and Lemont within 20 hpi. (B) Venn diagram of DEGs in YSBR1 and Lemont at 6, 10 and 20 hpi. (C) The numbers of up-regulated and down-regulated DEGs detected in either YSBR1 or Lemont compared with mock inoculation at 6, 10 and 20 hpi.

Dynamic and Different Responses of the Two Rice Cultivars During *R. solani* Infection

Genes that showed more than 2-fold change were identified as differentially expressed genes (DEGs). A total of 7624 genes showing differential expression at one point in one cultivar were identified (Table S3). To examine whether the observed differential expression genes in rice-*R. solani* interactions were potentially influenced by circadian regulation, we checked the meta-expression data (http://ricexpro.dna.affrc.go.jp/RXP_0002/index.php; Sato et al. 2013). By comparing the expression patterns of genes in resistant and susceptible rice cultivars with the circadian regulation data, we identified 425 differential expression genes that might be subject to circadian regulation (Table S4). For these genes, the potential influence of their circadian regulation could not be distinguished from that of *R. solani* infection. In spite of that, they were assumed to be also transcriptionally responsive to *R. solani* infection and thus were further analyzed.

Among the 7624 DEGs, 3520 were shared between YSBR1 and Lemont, while 2098 were YSBR1 specific and 2006 were Lemont specific (Fig. 1A). In detail, among the up-regulated DEGs, 1276 were commonly shared between YSBR1 and Lemont, whereas 1835 were YSBR1 specific and 1766 were Lemont specific. Similarly, among the down-regulated DEGs, 930 were commonly shared between YSBR1 and Lemont, whereas 2019 were YSBR1 specific and 1963 were Lemont specific (Fig. 1B). These results suggest that most DEGs are either YSBR1 or Lemont specific. Furthermore, we found that more DEGs in YSBR1 were up-regulated at the early time points (1807 and 1834 genes at 6 and 10 hpi, respectively) than at the later point (988 genes at 20 hpi). Similarly, among the down-regulated DEGs, more were present at the early time points than the later point (1697, 1931 and 866 genes at 6, 10 and 20 hpi, respectively) in YSBR1. In contrast, in Lemont, more genes were up-regulated or down-regulated at the latest time point (20 hpi) than at the earlier time points (6 and 10 hpi). There are 3504 and 3765 DEGs in YSBR1 at 6 and 10 hpi, respectively, whereas 1979 and 2187 in Lemont (Fig. 1C). These results suggest that the resistance cultivar YSBR1 exhibited stronger and earlier transcriptional response to *R. solani* compared to susceptible cultivar Lemont.

To examine the similarity and diversity of expression profiles in YSBR1 and Lemont infected with *R. solani* within 20 hpi, a hierarchical clustering analysis of all 7624

DEGs was generated using Cluster 3.0 software after loading log2 fold change values (Fig. 2A). The hierarchical clustering shows that samples infected with *R. solani* at different time points have been divided into two distinct cluster groups, one group characterized by genes expressed in YSBR1 and one group represented by genes expressed in Lemont. This result indicates that different gene expression patterns in YSBR1 and Lemont may be associated with their different resistant levels to *R. solani*. In each rice cultivar, the gene expression patterns of DEGs at 6 hpi and 10 hpi were clustered together and then with that at 20 hpi. Either in YSBR1 or Lemont, DEGs at early stages of *R. solani* infection (6 and 10 hpi) showed similar expression patterns, with relatively different patterns at later stage (20 hpi). However, while more DEGs were up-regulated or down-regulated at the time points 6 and 10 hpi than at 20 hpi in YSBR1, more genes in Lemont were up-regulated or down-regulated at 20 hpi than that at 6 and 10 hpi (Fig. 2A). These results indicate that the majority of gene expression changes in Lemont in response to *R. solani* are delayed relative to the rapid response of YSBR1. To further investigate the *R. solani*-induced gene expression patterns in the resistant and susceptible lines at different time points, the 7624 DEGs were sorted into 9 subclusters (Fig. 2B). DEGs in subclusters 1, 4, 5 and 9, showed elevated expression in YSBR1. These DEGs were up-regulated in YSBR1 but down-regulated or exhibited no significant changes in Lemont after *R. solani* infection; thus, these genes may positively regulate rice resistance to *R. solani*. Conversely, DEGs in subclusters 2, 3, 6, 7 and 8, were significantly activated in Lemont but decreased or showed minor changes in YSBR1; thus, these DEGs may negatively regulate rice immunity. In subclusters 1, 4, 5 and 9, 46 DEGs encoding known or putative PRRs showed induced expression in YSBR1, while 35 known or putative PRR genes remained unchanged or decreased at equivalent time points in Lemont (Table 1). PRRs are key elements for PTI, thus, these DEGs might function as PRRs triggering PTI response in YSBR1 before 6 hpi. Therefore, the activation of PTI immune responses in the early stage of *R. solani* infection is important for SB resistance in YSBR1.

To gain more insight into the functions of the genes that play important role in SB resistance, we focused on the genes in clusters 1, 4, 5 up-regulated in the resistant line and those in clusters 2, 3, 6 up-regulated in the susceptible line for GO enrichment analysis using the agriGO tool. SEACOMPARE revealed 20 biological process and 2 molecular function GO terms to be enriched in common between the two groups of genes ($p < 0.01$). A set of 26 GO terms were specifically enriched in clusters 1, 4, 5, while 63 GO terms were specifically enriched in clusters 2, 3, 6. From the biological process category, we identified six processes that were specifically enriched and up-regulated in YSBR1. These processes are mainly associated with responses to oxidative stress and chemical stimulus, carbohydrate metabolic process, protein ubiquitination and protein modification. We also identified 'antioxidant activity 'and 'peroxidase' in molecular function and 'extracellular region', 'apoplast' and 'cell wall' in cellular component specifically enriched in

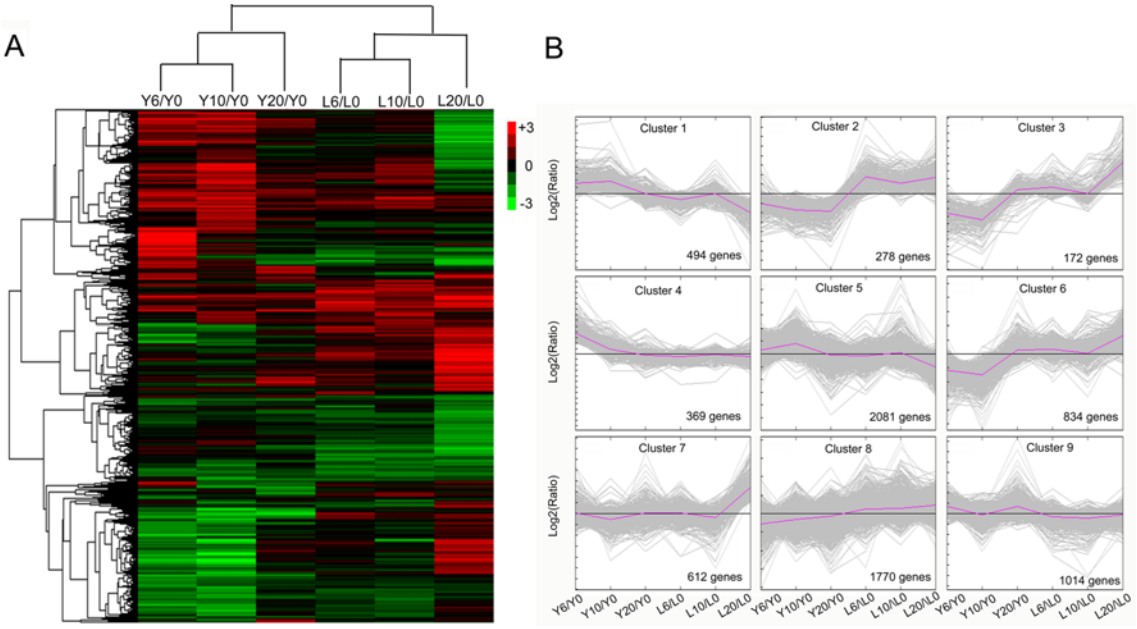


Fig. 2. Clustering analysis of time-course expression profiles. (A) Hierarchical clustering analysis of 7624 DEGs in YSBR1 and Lemont infected with *R. solani* within 20 hpi. (B) K-means clustering analysis of 7624 DEGs in YSBR1 and Lemont infected with *R. solani* within 20 hpi. Y, YSBR1; L, Lemont.

Table 1. Up-regulated PRRs in YSBR1 at 6 hpi after inoculation with *R. solani*

Gene ID	YSBR1	Lemont	Putative Function	Gene ID	YSBR1	Lemont	Putative Function
Os08g43470.1	2.352	1.713	ER lumen protein retaining receptor	Os10g35040.1	2.329	0.820	receptor kinase like protein
Os08g37030.1	5.499	0.719	gibberellin receptor GID1L2	Os01g57510.1	5.787	0.775	receptor protein kinase
Os03g14730.1	2.823	1.677	gibberellin receptor GID1L2	Os03g64030.1	6.823	0.300	receptor protein kinase
Os09g28690.1	23.273	0.190	gibberellin receptor GID1L2	Os01g12420.1	4.267	0.760	receptor-like protein kinase
Os09g28730.1	3.324	1.963	gibberellin receptor GID1L2	Os01g12430.1	3.400	0.991	receptor-like protein kinase
Os01g60330.1	2.209	0.716	inactive receptor kinase precursor	Os04g12600.1	2.980	1.039	receptor-like protein kinase
Os05g40200.1	2.616	1.033	inactive receptor kinase precursor	Os08g40990.1	3.462	0.852	receptor-like protein kinase 1
Os07g38810.1	2.326	1.264	lectin receptor-type protein kinase	Os02g12420.1	2.121	1.543	receptor-like protein kinase precursor
Os02g56380.1	3.635	0.210	OsWAK21	Os05g33690.1	4.778	0.264	receptor-like protein kinase precursor
Os04g24220.1	2.136	1.550	OsWAK32	Os01g66680.1	3.209	0.963	S-domain receptor-like protein kinase
Os04g24510.1	2.676	1.644	OsWAK36	Os01g02560.1	3.751	1.994	Ser/Thr receptor-like kinase
Os05g04460.1	2.581	0.972	OsWAK56	Os01g02440.1	3.181	0.603	Ser/Thr receptor-like kinase
Os09g29510.1	4.133	0.994	OsWAK80	Os05g16420.1	80.072	1.068	SHR5-receptor-like kinase
Os09g38840.1	6.031	1.043	OsWAK90	Os05g17604.1	2.797	1.164	SHR5-receptor-like kinase
Os10g07548.1	20.932	0.719	wall-associated receptor kinase-like 4 precursor	Os01g59570.1	2.315	0.419	senescence-induced receptor-like serine/threonine-protein kinase
Os06g47650.1	2.195	0.776	phytosulfokine receptor precursor	Os04g34370.1	3.138	0.433	serine/threonine-protein kinase receptor precursor
Os01g05960.1	4.169	1.004	receptor kinase	Os01g72800.1	2.753	1.203	signal recognition particle receptor
Os04g55620.1	3.800	0.352	receptor kinase	Os10g38040.1	2.865	0.801	lysM domain containing protein

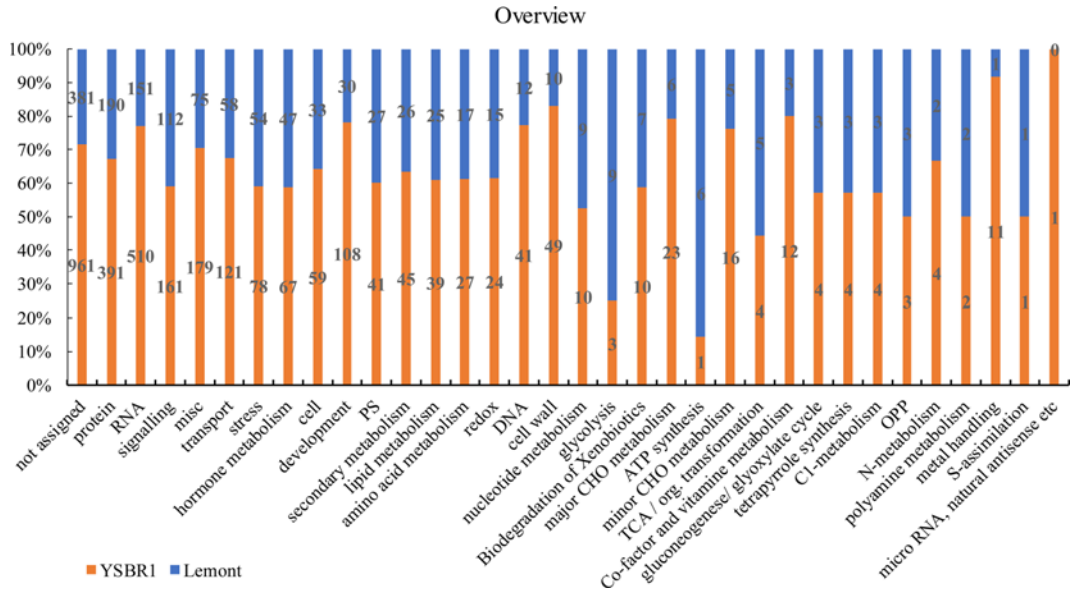


Fig. 3. An overview of DEGs in clusters 1, 4, 5 and clusters 2, 3, 6 assigned to 35 functional categories. Yellow, genes upregulated in YSBR1 in clusters 1, 4, 5. Blue, genes upregulated in Lemont in clusters 2, 3, 6.

YSBR1. Our finding suggests response to oxidative stress, metabolic process, protein ubiquitination and cell wall modification are critical for the defense response of YSBR1 against *R. solani*. For the upregulated genes in Lemont in clusters 2, 3, 6, the most specifically enriched biological process terms were those related to post-translational protein modification, phosphorylation, kinase activity, photosynthesis, oxidation reduction, generation of precursor metabolites and energy, signaling, and catabolic process. These processes might facilitate *R. solani* infection in Lemont and are highly repressed in YSBR1, leading to the inhibition of *R. solani* invasion. GO enrichment analysis of the six clusters of genes as above is summarized in Table S5.

To obtain functional classifications, 2944 upregulated genes in clusters 1, 4, 5, and 1284 upregulated genes in clusters 2, 3, 6 were assigned to 35 major pathways using MapMan tool. In clusters 1, 4, 5, 510 genes were assigned to the 'RNA' category, 391 to 'protein', 179 to 'miscellaneous

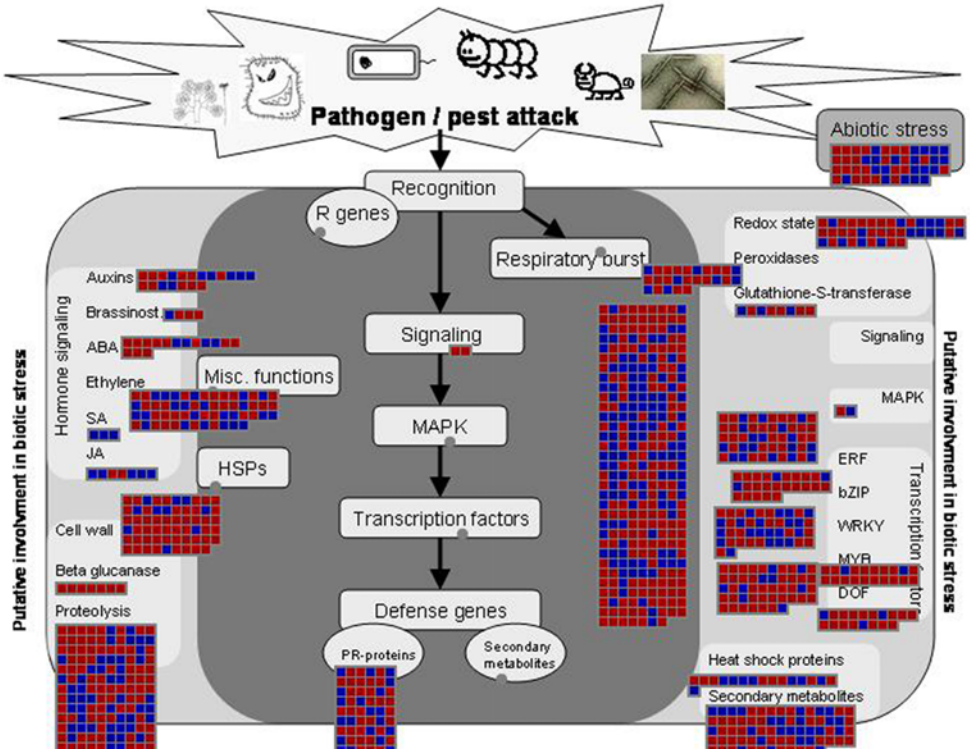


Fig. 4. MapMan overview of biotic stress genes associated with clusters 1, 4, 5 upregulated in YSBR1 and clusters 2, 3, 6 upregulated in Lemont. Red indicates genes in clusters 1, 4, 5; blue, genes in clusters 2, 3, 6.

function' ('misc'), 161 to 'signaling', 121 to 'transport', 78 to 'stress', 67 to 'hormone metabolism', 108 to 'development', 49 to 'cell wall', 45 to 'secondary metabolism', and a smaller number to other functional groups. In clusters 2, 3, 6, less genes were assigned to the corresponding pathways, for example, 190 genes were assigned to the 'protein' category, 151 to 'RNA', 112 to 'signaling', 58 to 'transport', 54 to 'stress', 47 to 'hormone metabolism', 10 to 'cell wall', 27 to 'photosynthesis', and 26 to 'secondary metabolism'. Genes involved in 'glycolysis' and 'ATP synthesis' were predominantly expressed in Lemont (Fig. 3). Detailed information is presented in Table S6.

In the biotic stress overview, 'protein degradation', 'signaling', 'cell wall' and 'secondary metabolism' were top four distinct pathways between YSBR1 and Lemont (Fig. 4). In 'cell wall' category, there were 45 genes showing continuous high expression in YSBR1, and only 10 genes in Lemont. Seven β-glucanases were identified as up-regulated genes only in YSBR1, suggesting unique role of β-glucanases for SB resistance. For the respiratory-burst pathway, 46 genes were detected in clusters 1, 4, 5 and 26 genes in clusters 2, 3, 6. We also found that more pathogenesis related proteins, MYB, bZIP TF genes were highly induced in YSBR1 than in Lemont (Fig. S2, Table S6). These observations indicate that protein degradation, signaling, cell wall modification, β-glucanses and secondary metabolism play important roles in the resistance of YSBR1 against *R. Solani*.

GO Enrichment Analysis of DEGs

Gene ontology was used to analyze global gene expression profiles and study which biological processes, molecular functions, and cellular components are affected by *R. solani* inoculation. The significantly enriched GO terms (with $P < 0.01$) in each gene set were identified using the web-based tool Rice Oligonucleotide Array Database (ROAD) (Cao et al. 2012). A total of 96 and 83 GO terms were enriched in at least one gene set for YSBR1 and Lemont, respectively (Table S7). The GO enrichment analysis also showed that the two cultivars differentially responded to *R. solani* infection. Dozens of GO terms were specific to either YSBR1 or Lemont. Only 5 among the 52 enriched GO terms shared by the two cultivars were in the same gene set at the same time-point, both in the up- and down-regulated genes.

GO terms that were enriched in the up-regulated genes in YSBR1 but not in Lemont are of particular interest. Related GO cellular components, including apoplasts (GO:0048046), extrinsic to the membrane (GO:0019898), cell wall (GO:0005618), and extracellular regions (GO:0005576), are most interesting (Table 2). The terms as above were significantly enriched in the up-regulated YSBR1 genes at 6 hpi, but not in Lemont. Cell periphery, where the host and

Table 2. Extracellular region-related GO terms enriched in the genes up-regulated exclusively in YSBR1 at 6 hpi

Gene ID	6 hpi		GO ID	Gene ID	6 hpi		GO ID
	YSBR1	Lemont			YSBR1	Lemont	
Os01g39830.1	13.73	0.62	GO:0048046; GO:0005576	Os06g21250.1	30.60	0.40	GO:0005576
Os01g62480.1	33.95	1.10	GO:0048046; GO:0005576	Os06g21270.1	18.11	0.18	GO:0005576
Os01g63190.1	2.57	0.18	GO:0048046; GO:0005576	Os06g47800.1	5.37	0.54	GO:0005576
Os01g65460.1	7.28	0.66	GO:0048046; GO:0005576	Os07g03279.1	24.02	1.01	GO:0005576
Os01g72290.1	669.06	0.88	GO:0048046; GO:0005576	Os11g03110.1	2.54	1.35	GO:0005576
Os02g32980.1	7.05	0.55	GO:0048046; GO:0005576	Os11g05190.1	4.70	1.16	GO:0005576
Os03g01800.1	11.43	0.94	GO:0048046; GO:0005618	Os07g03710.1	3.76	1.10	GO:0005576
Os05g35360.1	5.80	0.96	GO:0048046; GO:0005576	Os09g29710.1	4.25	1.11	GO:0005576
Os06g37560.1	8.26	0.55	GO:0048046; GO:0005576	Os01g28450.1	171.8	17.99	GO:0005576
Os06g48180.1	4.26	0.21	GO:0048046; GO:0005618	Os01g60770.1	23.86	0.17	GO:0005576; GO:0005618
Os07g29750.1	5.13	0.69	GO:0048046; GO:0005618	Os05g39990.1	8.49	0.09	GO:0005576; GO:0005618
Os08g08970.1	7.49	0.06	GO:0048046; GO:0005576	Os10g31330.1	5.74	0.94	GO:0005576; GO:0005618
Os08g35750.1	3.26	0.48	GO:0048046; GO:0005576	Os10g40720.1	10.14	0.18	GO:0005576; GO:0005618
Os08g09060.1	4.10	14.86	GO:0048046; GO:0005576	Os10g40730.1	7.33	0.28	GO:0005576; GO:0005618
Os11g33270.1	30.48	0.58	GO:0048046; GO:0005618	Os01g56320.1	3.00	0.35	GO:0005618
Os08g13920.1	9.87	0.10	GO:0048046; GO:0005576; GO:0005618	Os01g65790.1	3.93	0.95	GO:0005618
Os01g01650.1	203.02	1.11	GO:0005576	Os05g29790.1	20.90	1.09	GO:0005618
Os01g03340.1	6.55	1.91	GO:0005576	Os05g44600.1	17.02	1.26	GO:0005618
Os01g39150.1	2.03	1.00	GO:0005576	Os01g59090.1	3.79	1.17	GO:0019898
Os01g47400.1	26.89	0.10	GO:0005576	Os01g70820.1	2.16	0.97	GO:0019898
Os02g50490.1	2.27	1.70	GO:0005576	Os03g17174.1	9.39	1.50	GO:0019898
Os03g12990.1	2.96	0.50	GO:0005576				

the invading pathogen initially meet and interact, plays an important role in determining the outcome of the plant-pathogen interaction. The plant induces apoplastic defense for inhibiting microbial enzymes, cell wall strengthening, or pathogen poisoning after a pathogen is recognized at the cell periphery (Hückelhoven 2007; Hématy et al. 2009). Genes encoding cell wall-modifying enzymes, such as those encoding pectinesterase, laccase, and expansin precursors, genes encoding degrading enzymes, such as those encoding glycosyl hydrolases, and beta-galactosidases, and genes encoding anti-microbial products, such as those encoding defensin, defensin-like proteins, or trypsin inhibitor precursors, were included in these GO terms (Table 2). The induction of these genes strongly suggests that active immune responses have been triggered in YSBR1 before 6 hpi.

Pathway Analysis of DEGs

We next used the MapMan package to investigate the pathways involved in rice-*R. solani* interaction. The MapMan tool utilizes the input from several experts to curate specific biological processes by using information from the RICE Database. An overview of biotic stress showing the transcriptional changes in YSBR1 and Lemont plants at 6

hpi is generated (Fig. S3). In YSBR1 and Lemont, most of the genes associated with respiratory burst, signaling, cell wall, β-glucanases, proteolysis, TF, and secondary metabolites were showing similar trend of up-regulation (Fig. S3; Table S8). This reflects that in both YSBR1 and Lemont upon *R. solani* attack, unique set of biotic stress genes were activated. Despite the up-regulated trend in both cultivars, careful analysis of individual genes involved in biotic stress revealed substantial diversity between YSBR1 and Lemont. In YSBR1, 54 DEGs were identified as cell wall related genes, and 48 DEGs of them were highly induced upon *R. solani* inoculation. In Lemont, most of cell wall related genes (25 out of 36) were down-regulated. There were 9 of 10 β-glucanases genes showed up-regulated expression in YSBR1, while only 5 of 9 β-glucanases genes were up-regulated in Lemont. More up-regulated DEGs regarding respiratory burst such as redoxins, peroxidases and glutathione S transferases were detected in YSBR1 compared with Lemont. We also found that more secondary-metabolite related genes were induced in YSBR1 upon *R. solani* inoculation than that in Lemont. During interaction, the induction of the DEGs related with hormones, proteolysis, signaling, TF and PR were quantitatively higher in YSBR1 than in Lemont.

Secondary metabolites include phenylpropanoids, lignin, phenolics, waxes, terpens, and flavanoids and they are important in defense response. Secondary metabolism pathways were further analyzed (Fig. S4; Table S9). In YSBR1, 68 secondary metabolism genes were found to be differentially expressed. And fewer secondary metabolism genes (46 DEGs) were altered in Lemont upon *R. solani* attack. Compared with Lemont, the top four differential expressed pathways were related to flavanoids, phenylpropanoids, lignin, and waxes in YSBR1. Seven flavonoids related genes (Os11g35930.1, Os11g32650.1 Os01g01650.1, Os10g41020.1 Os03g08624.1, Os07g40974.1, Os11g26920.1), four phenylpropanoids related genes (Os09g31506.1, Os09g31514.1, Os07g28040.1, Os09g31502.1), two lignin biosynthesis genes (*PAL*, Os05g35290.1; *CCoAOMT*, Os08g38900.1) and one wax gene (Os02g56920.1) were specially induced in YSBR1 upon *R. solani* inoculation. Moreover, most DEGs related to shikimate pathway, simple phenols, carotenoids, and sulfur-containing compounds were higher expressed in YSBR1 than in Lemont. These results suggested that during *R. solani* infection, YSBR1 exhibited stronger transcriptional response to *R. solani* than Lemont, and these DEGs were related to cell wall, β-glucanses, respiratory burst, ERF, bZIP, DOF, and secondary metabolites such as flavanoids, phenylpropanoids, shikimate pathway, lignin, and waxes. These DEGs may be important for YSBR1 resistance to *R. solani*. These visual annotations provide a useful resource for the investigation of pathways involving rice-*R. solani* interaction.

JA- and SA-related Genes Show Contrasting Modulation Trends between the Cultivars

The genes related to SA and JA were retrieved from the RiceCyc database (http://pathway.gramene.org/) and the literature (Lyons et al. 2013). Up to 43 JA-related genes and 22 SA-related genes were detected in this study. SA and JA are signaling molecules; thus, their activities in the early stages of rice-*R. solani* interaction are important (Table S10). Therefore, only 6 hpi was chosen to compare the expression levels of the SA- and JA-related genes in YSBR1 and Lemont.

28 of the 43 JA-related genes showed contrasting modulation trends between the two cultivars (Table 3). Among these genes, Os03g08310 and Os03g28940 encode ZIM domain-containing proteins. ZIM domain-containing proteins negatively regulate JA-related genes (Chini et al. 2007; Thines et al. 2007). JA helps degrade ZIM domain-containing proteins to restore the expression of JA-modulated genes (Chini et al. 2007; Thines et al. 2007). The contrasting expression patterns of the two ZIM domain-containing proteins indicate that some JA-related genes are differentially modulated in *R. solani*-infected YSBR1 and Lemont.

16 of the 22 SA-related genes showed contrasting modulation trends between the two cultivars (Table 4). Four of these genes encode the BTB family E3 ligases, whose closest homologues in Arabidopsis are *NPR1* or *NPR1*-like proteins. *NPR1* is a crucial factor in the SA signaling pathway. Some SA-related genes are not properly expressed in *npr1* mutant plants (Fu et al. 2012; Fu and Dong 2013). The other 5 SA-related genes with contrasting expression patterns between the two cultivars encode bZIP transcription factors homologous to TGACG sequence-specific DNA binding proteins (TAGs). TAGs are redox-controlled regulators of systemic acquired resistance that are important factors for SA signaling downstream of *NPR1* (Fu and Dong 2013; Withers and Dong 2016). Decreased *NPR1* and TGA-related gene expression levels in YSBR1 indicate that SA signaling is repressed in this cultivar. On the contrary, increased *NPR1* and TGA-related gene expression levels in Lemont indicate that SA signaling is activated in this cultivar.

Validation of Microarray Data using Real-time Quantitative RT-PCR

Nine DEGs that might be essential for rice sheath blight resistance were selected to verify the DEGs identified by the microarray data using qRT-PCR. RNA was isolated from samples independent of those used for the microarray experiments to increase biological relevance. The nine genes analyzed through qRT-PCR included four genes (Os01g28450, Os09g29710, Os10g40730, and Os11g33270) that encode proteins functioning in extracellular regions, according to

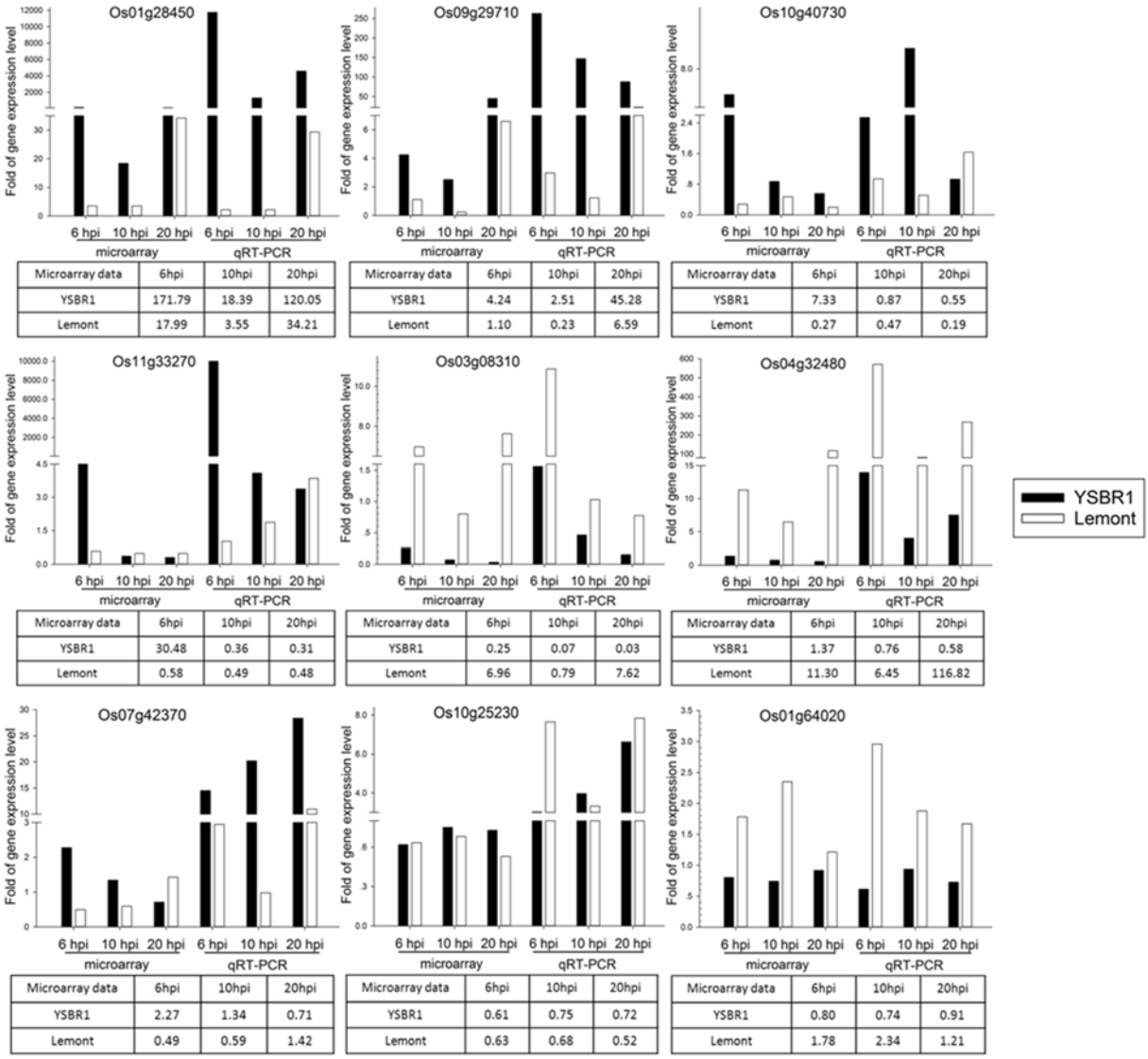
Table 3. A list of 28 JA-related genes differentially expressed between YSBR1 and Lemont induced by *R. solani*

Cultivars	6 hpi	10 hpi	20 hpi	Gene ID	Putative Function
YSBR1	10.74	1.56	7.10	Os06g11210.1	12-oxophytodienoate reductase, putative, expressed
Lemont	0.18	0.20	1.64		
YSBR1	2.72	6.24	4.53	Os06g11240.1	12-oxophytodienoate reductase, putative, expressed
Lemont	0.47	0.42	1.15		
YSBR1	0.82	1.18	0.76	Os08g35740.1	12-oxophytodienoate reductase, putative, expressed
Lemont	0.99	1.12	3.37		
YSBR1	0.72	0.92	0.94	Os03g50290.1	14-3-3 protein, putative, expressed
Lemont	1.38	1.42	2.83		
YSBR1	1.17	4.52	1.01	Os02g12690.1	cytochrome P450, putative, expressed
Lemont	2.46	1.30	0.69		
YSBR1	1.96	0.92	3.52	Os03g12500.1	cytochrome P450, putative, expressed
Lemont	8.00	3.39	5.34		
YSBR1	3.35	1.31	0.98	Os03g55800.1	cytochrome P450, putative, expressed
Lemont	1.61	0.89	3.96		
YSBR1	2.17	2.20	2.19	Os05g51520.1	expressed protein
Lemont	1.71	1.91	2.13		
YSBR1	2.42	3.36	1.06	Os09g04880.1	expressed protein
Lemont	1.48	1.30	0.51		
YSBR1	1.15	0.91	1.09	Os03g08220.1	lipoxygenase protein, putative, expressed
Lemont	1.33	1.03	3.76		
YSBR1	1.50	1.60	0.81	Os04g37430.1	lipoxygenase protein, putative, expressed
Lemont	1.42	1.39	2.09		
YSBR1	1.04	1.08	1.40	Os08g39840.1	lipoxygenase, chloroplast precursor, putative, expressed
Lemont	1.22	1.50	4.03		
YSBR1	2.87	2.23	3.30	Os08g39850.1	lipoxygenase, chloroplast precursor, putative, expressed
Lemont	23.46	8.10	49.84		
YSBR1	1.28	1.72	0.81	Os02g10120.1	lipoxygenase, putative, expressed
Lemont	2.11	10.63	6.18		
YSBR1	1.10	11.81	1.28	Os03g49260.1	lipoxygenase, putative, expressed
Lemont	0.52	1.18	6.78		
YSBR1	1.24	2.03	1.12	Os05g23880.1	lipoxygenase, putative, expressed
Lemont	3.11	2.89	2.40		
YSBR1	0.72	1.14	0.69	Os01g63420.1	OsFBL4 - F-box domain and LRR containing protein, expressed
Lemont	1.01	1.06	0.49		
YSBR1	2.11	0.53	0.06	Os06g40170.1	phospholipase D, putative, expressed
Lemont	0.48	0.35	16.24		
YSBR1	2.63	0.66	1.81	Os01g47330.1	ribosomal protein L7/L12 C-terminal domain containing protein, expressed
Lemont	1.02	1.11	0.63		
YSBR1	1.59	1.36	1.27	Os10g42430.1	transcription factor MYC7E, putative, expressed
Lemont	1.23	1.38	2.37		
YSBR1	0.26	0.07	0.03	Os03g08310.1	ZIM domain containing protein, putative, expressed
Lemont	6.97	0.80	7.62		
YSBR1	0.26	0.07	0.08	Os03g08330.1	ZIM domain containing protein, putative, expressed
Lemont	1.00	0.68	1.30		
YSBR1	0.80	0.96	0.57	Os03g28940.1	ZIM domain containing protein, putative, expressed
Lemont	0.64	0.75	2.15		
YSBR1	2.49	1.09	0.38	Os10g25290.1	ZIM domain containing protein, putative, expressed
Lemont	1.64	0.83	9.10		
YSBR1	0.57	0.57	0.70	Os08g33160.1	ZIM motif family protein, expressed
Lemont	0.44	0.63	0.36		
YSBR1	1.37	0.76	0.58	Os04g32480.1	zinc-finger protein, putative, expressed
Lemont	11.30	6.45	116.83		
YSBR1	2.28	1.34	0.71	Os07g42370.1	zinc-finger protein, putative, expressed
Lemont	0.50	0.59	1.43		
YSBR1	1.69	0.26	0.26	Os09g26780.1	zinc-finger protein, putative, expressed
Lemont	1.79	0.48	38.37		
Lemont	0.98	1.00	0.99		

Table 4. A list of 16 SA-related genes differentially expressed between YSBR1 and Lemont induced by *R. solani*

Cultivars	6hpi	10hpi	20hpi	Gene ID	Putative Function
YSBR1	3.034	2.617	1.414	Os01g09800.1	BTBA1 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region, expressed
Lemont	4.422	2.072	4.386		
YSBR1	0.770	1.184	1.376	Os01g72020.1	BTBA3 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region, expressed
Lemont	2.613	1.596	1.760		
YSBR1	0.350	0.330	0.646	Os03g46440.1	BTBA4 - Bric-a-Brac,Tramtrack, Broad Complex BTB domain with Ankyrin repeat region, expressed
Lemont	2.207	1.081	1.242		
YSBR1	0.462	0.975	1.269	Os02g10140.1	bZIP transcription factor domain containing protein, expressed
Lemont	0.609	1.025	0.492		
YSBR1	0.435	0.351	1.096	Os11g09010.1	lipase, putative, expressed
Lemont	1.006	0.671	2.810		
YSBR1	1.487	0.410	0.370	Os02g41650.1	phenylalanine ammonia-lyase, putative, expressed
Lemont	0.817	1.721	1.357		
YSBR1	0.455	0.152	0.055	Os02g41680.1	phenylalanine ammonia-lyase, putative, expressed
Lemont	3.284	2.884	1.097		
YSBR1	0.487	0.990	0.491	Os04g43800.1	phenylalanine ammonia-lyase, putative, expressed
Lemont	31.383	19.906	5.945		
YSBR1	2.630	0.570	1.764	Os05g35290.1	phenylalanine ammonia-lyase, putative, expressed
Lemont	0.772	0.616	6.523		
YSBR1	171.798	18.398	120.053	Os01g28450.1	SCP-like extracellular protein, expressed
Lemont	17.993	3.550	34.219		
YSBR1	1.012	1.000	1.805	Os03g20310.1	transcription factor HBP-1b, putative, expressed
Lemont	1.740	0.999	3.120		
YSBR1	0.803	0.742	0.918	Os01g64020.1	transcription factor, putative, expressed
Lemont	1.784	2.349	1.214		
YSBR1	5.172	3.320	0.947	Os06g15480.1	transcription factor, putative, expressed
Lemont	0.744	0.934	0.747		
YSBR1	0.624	0.885	1.000	Os06g41100.1	transcription factor, putative, expressed
Lemont	0.969	0.849	0.494		
YSBR1	1.099	1.562	1.522	Os07g48820.1	transcription factor, putative, expressed
Lemont	1.528	1.832	2.244		
YSBR1	1.679	0.843	2.020	Os09g31390.1	transcription factor, putative, expressed
Lemont	0.386	0.440	0.689		

their gene ontology annotation, four genes (Os03g08310, Os04g32480, Os07g42370, and Os10g25230) involved in JA signaling, and two genes (Os01g64020 and Os01g28450) involved in SA signaling. Fig. 5 shows that for the nine genes, qRT-PCR detected essentially the same expression tendency as the microarray data. *PR1b* (Os01g28450), the glycosyl hydrolase gene Os11g33270, and two extracellular region-related genes Os09g29710 and Os10g40730 showed higher expression levels in YSBR1 compared with Lemont as early as 6 hpi. JA ZIM-domain (JAZ) proteins generally act as repressor of JA signaling and two JAZ genes Os03g08310 and Os04g32480 continuously exhibited lower levels in YSBR1 than that in Lemont at 6, 10 and 20 hpi. The expression level of SA-related gene Os01g64020 was also lower in YSBR1 compared with Lemont from 6 dpi to 20 hpi. It should be noted that our transcriptome analyses were carried out without biological replicates and that could result in potentially biased interpretation of the transcriptome data. Nevertheless, the qRT-PCR data derived from another independent experiment is consistent with the results of transcriptome data, which partially validates the transcriptome analyses and increases the biological relevance. Also, YSBR1 is one of the most resistant rice cultivars to *R. solani* and Lemont is a typical *R. solani*-susceptible rice cultivar. In this study, comparative transcriptome analysis between YSBR1 and Lemont revealed distinctive regulation of defense responses against *R. solani* infection. A set of DEGs that show strongly and significantly differential expression between YSBR1 and Lemont have been identified, and these genes could serve as candidate genes for better understanding of the host resistance and breeding resistant rice cultivars against *R. solani*.



Microarray data	6hpi	10hpi	20hpi
YSBR1	171.79	18.39	120.05
Lemont	17.99	3.55	34.21

Microarray data	6hpi	10hpi	20hpi
YSBR1	4.24	2.51	45.28
Lemont	1.10	0.23	6.59

Microarray data	6hpi	10hpi	20hpi
YSBR1	7.33	0.87	0.55
Lemont	0.27	0.47	0.19

Microarray data	6hpi	10hpi	20hpi
YSBR1	30.48	0.36	0.31
Lemont	0.58	0.49	0.48

Microarray data	6hpi	10hpi	20hpi
YSBR1	0.25	0.07	0.03
Lemont	6.96	0.79	7.62

Microarray data	6hpi	10hpi	20hpi
YSBR1	1.37	0.76	0.58
Lemont	11.30	6.45	116.82

Microarray data	6hpi	10hpi	20hpi
YSBR1	2.27	1.34	0.71
Lemont	0.49	0.59	1.42

Microarray data	6hpi	10hpi	20hpi
YSBR1	0.61	0.75	0.72
Lemont	0.63	0.68	0.52

Microarray data	6hpi	10hpi	20hpi
YSBR1	0.80	0.74	0.91
Lemont	1.78	2.34	1.21

Fig. 5. Validation of microarray data using qRT-PCR. Nine differential expression genes identified by the microarray analysis were verified using qRT-PCR. For each rice genotype (YSBR1 or Lemont), the gene expression reads at 6hpi, 10hpi and 20hpi derived from microarray or qRT-PCR analysis were compared to the read at 0h, respectively, resulting in folds of gene expression level. The vertical axis in the histogram of each gene represents the fold of gene expression level based on microarray and qRT-PCR data, respectively. Also, the microarray data (folds of gene expression level) of each gene are listed in the table below the histogram. The microarray data and the qRT-PCR data were derived from independent rice sheath blight inoculation experiments.

Discussion

The *R. solani*-resistant cultivar YSBR1 has 3504 and 3765 DEGs at 6 and 10 hpi, respectively, whereas those for the susceptible cultivar Lemont were 1979 and 2187, respectively (Fig. 1C). Our results showed that *R. solani*-induced rice transcriptome changes were highly dynamic. GO analysis showed that cell wall modifying GO terms composed of genes encoding degrading enzymes, and anti-microbial products were increased earlier in YSBR1 than in Lemont. Two non-mutually exclusive possibilities may account for the significantly higher level of resistance in YSBR1 than in Lemont to *R. solani*. YSBR1 detects *R. solani* invasion earlier than Lemont, or YSBR1 contains other functional pathways that make it more resistant to *R. solani*. The results of the present study cannot rule out the second possibility, but clearly supports the first. YSBR1 evidently responds to *R. solani* earlier and stronger than Lemont, this consistent with the results of Zuo et al. (2014b) who studied on YSBR1 resistance to *R. solani*. Similarly, stronger and earlier responses to pathogen invasion at the transcriptome level have been found in the resistant grapevine cultivar *Vitis riparia* infected with *Plasmopara viticola* (Polesani et al. 2010). This result is consistent with other studies on different plant and pathogen

species and emphasizes the importance of time series analyses in understanding *R. solani* interaction mechanisms (Moore et al. 2011; Windram et al. 2012; Zhu et al. 2016).

After contact with the rice tissue surface, the mycelia of *R. solani* differentiates infection cushions and/or lobate appressoria to penetrate into the cell wall by penetration pegs and then gets into the inside of rice (Taheri and Tarighi 2011; Basu et al. 2016). Except for the mechanical support of the plant growth and development, the plant cell wall can be an important defensive structure that protects the plants against pathogens invasion (Hématy et al. 2009). Xylan is the skeletal component of hemicelluloses which is the one of the three components of the cell wall (Cosgrove 2005). Recently, a cupin-domain containing protein TaGLP was identified as key component of xylan synthase complex (Cosgrove 2005; Jiang et al. 2016). Germin-like oxalate oxidase enzymes belonging to the cupin superfamily can break down the oxalic acid, and generate active oxygen species, which triggers host defense response to pathogen invasion (Molla et al. 2013). Oxalic acid is usually secreted by *R. solani* and can weaken host cell walls at the infection site. It is reported that a germin-like protein gene family functions as a complex QTL and confers broad-spectrum disease resistance in rice (Manosalva et al. 2009). Overexpression of the rice *oxalate oxidase 4* gene increased tolerance to sheath blight pathogen in transgenic rice (Molla et al. 2013; Karmakar et al. 2016).

The oxidative enzymes laccases are spatially localized to secondary cell walls and involved in oxidative lignin polymerization in plant species (Schuetz 2014). The pathogen showed more hyphal growth in the xylem of the susceptible host than in the tolerant variety (Basu et al. 2016). In this study, genes encoding cell wall-modifying enzymes, such as those encoding pectinesterase (Os01g65790, Os05g29790, Os05g44600), laccase (Os01g62480, Os01g63190, Os05g38410), beta-galactosidase (Os01g39830, Os01g65460, Os05g35360, Os06g37560), and cupin domain-containing protein (*OsGLP8-10*, Os08g09060) were included in extracellular region-related GO terms (Table 2). These extracellular region-related genes that exhibited induced expression upon *R. solani* infection especially in YSBR1 but not in Lemont have been identified. Mapman analysis also showed that most cell wall related genes (48 out of 54) were highly induced upon *R. solani* inoculation in YSBR1, while most cell wall related genes (25 out of 36) were down-regulated in Lemont. These results suggest that compared with the susceptible Lemont, up-regulation of cell wall related genes may play an important role in YSBR1 on the enhanced resistance to *R. solani*.

Plants have evolved sophisticated innate immune system to defense against pathogens invading. They employ cell surface-localized immune receptors, which function as PRRs, to trigger PTI (Zipfel 2014). In this study, 46 DEGs encoding known and putative PRRs were detected elevated expression in YSBR1, among which 35 receptor-like kinases (RKs) and one LysM-domain receptor-like protein showed decreased expression or exhibited no significant changes in Lemont after *R. solani* infection at 6 hpi (Table 1). A well-studied PTI system in rice contains the LysM domain-containing receptor CEBiP and its co-receptor CERK1, and LysM is the function domain of rice and Arabidopsis chitin immune receptor (Gust et al. 2012; Sánchez-Vallet et al. 2015). Os10g38040.1 encoding a LysM domain-containing protein was specifically induced in YSBR1 at 6 and 10 hpi, implying a potential role on chitin immune recognition in YSBR1. The above-mentioned 35 RK DEGs belong to cell wall-associated kinase (WAKs), gibberellin receptor kinase, *SHR5* and Ser/Thr kinase subfamilies. WAKs have demonstrated critical roles in pathogen responses in Arabidopsis and rice. It is reported that several WAKs participated positively or negatively in basal resistance against *M. oryzae* (Delteil et al. 2016). In another study, OsWAK25-overexpressed rice plants display increased susceptibility to *R. solani* (Harkenrider et al. 2016). In this study, seven WAK genes were higher induced in YSBR1 than in Lemont, and these genes may be associated with YSBR1 resistance to *R. solani*. Overall, these RK DEGs might function as PRRs triggering PTI response in YSBR1 before 6 hpi, and the activation of PTI immune responses in the early stage of *R. solani* infection is important for YSBR1 resistance.

JA and SA are important phytohormones that regulate plant defense responses (Bari and Jones 2009; Pieterse et al. 2009). Generally, SA and JA signaling pathways are antagonistic, with SA acting against biotrophic pathogens and JA acting against necrotrophs, which benefit from the dead cells of a host plant (Glazebrook 2005; Pieterse et al. 2009). Crosstalk between SA and JA has been extensively reported and is believed to help the plant minimize fitness costs and to create a flexible signaling network that improves defense response. Many necrotrophs have adapted to use the antagonistic relationship between SA and JA. Necrotrophs activate SA signaling to repress JA-regulated defense responses (Oirdi et al. 2011; Rahman et al. 2012). *R. solani* is a necrotrophic pathogen (Okubara et al. 2014). Evidences have shown that JA plays an important role in controlling rice defense responses against *R. solani*. The chitin pathway is mediated by JA signal pathway (Glazebrook 2005; Van Loon 2006; Mengiste 2012), increasing the expression of chitinase could improve rice resistance to *R. solani* (Karmakar et al. 2016). Taheri and Tarighi (2010) reported that increasing the synthesis of JA could strengthen rice cell wall and enhance rice resistance to *R. solani*. Overexpression of *WRKR30* increased the expression of JA synthesis related genes *LOX*, *AOS2* and pathogenesis related genes, increased endogenous JA accumulation, and enhanced rice resistance to *R. solani*

(Peng et al. 2012). OsWRKY80-OsWAKY4 module functions as a positive regulatory circuit in rice resistance against *R. solani* (Peng et al. 2016). Overexpression of *WRKR4* in rice plants increases resistance to *R. solani*, concomitant with elevated expression of JA/ET responsive PR genes *PR1a*, *PR1b*, *PR5* and *PR10/PBZ1* (Wang et al. 2015a). In this study, up to 28 of the 43 JA related genes and 16 of the 30 SA related genes were detected, and these genes showed contrasting modulation trends between the two cultivars. Therefore, the contrasting expression patterns between YSBR1 and Lemont regarding SA-related genes and possibly JA-related genes are important and need to be studied further. Our study highlights potential candidate genes and signaling pathways involved in rice sheath resistance and can help to further clarify the mechanistic events underlying the resistance and susceptibility to *R. solani*.

Materials and Methods

R. solani Inoculation and Sample Collection

The rice cultivars Lemont (highly susceptible) and YSBR1 (resistant) were inoculated with *R. solani* strain YN-7 during the later tillering stage using toothpicks as inoculum carrier (Pan et al. 1997; Xue et al. 2016). The toothpicks were split into 0.8 cm to 1.0 cm segments, autoclaved, and then incubated with the YN-7 strain on PDB medium for 3 to 5 days at room temperature. A toothpick segment carrying the inoculum was inserted between the sheath and stem of the third fully expanded leaf from the top. Three main tillers of each rice plant were inoculated. *R. solani*-infected sheath tissues were sampled at 0, 6, 10, and 20 hpi. The lower sheath segment 1.0 cm away from the inoculation point was initially collected; then the tissue of the old leaf was removed, whereas that of the inner leaf that is in contact with the toothpick was sampled for RNA extraction.

Microarray Analysis

An Agilent-customized microarray representing 12,500 rice genes (Table S1), including disease-related transcription factors, receptor-like kinases, CaM kinases, and SNARE proteins, were used to study the temporal transcript profiles of YSBR1 and Lemont in response to *R. solani* infection (target genes, probe sequences and expression data are presented in Table S2). The YSBR1 and Lemont samples harvested at 0, 6, 10, and 20 hpi were stored in liquid nitrogen and transported to Shanghai Biotechnology Co., Ltd. (Shanghai, China) for RNA extraction and microarray analysis. Normalization signal values were obtained and linear scale data were converted into log scale, where logs were converted to base 2. The log data set was then subjected to significance analysis, fold change (Fold change = $2^{6hpi-0hpi}$; Fold change = $2^{10hpi-0hpi}$; Fold change = $2^{20hpi-0hpi}$), and direction of change (up- or down-regulation) for each gene. A gene with fold change ≥ 2 or ≤ 0.5 was considered significantly differentially expressed. Hierarchical clustering was performed with similarity metrics using average linkage clustering to calculate the distance of gene. We next used TIGR Multi-Experiment Viewer to carry out clustering analyses.

GO Enrichment Analysis

To determine the enriched gene ontologies, Singular Enrichment Analysis (SEA) for the genes in clusters 1, 4, 5 upregulated in the resistant line and the genes in clusters 2, 3, 6 upregulated in the susceptible line was performed in the agriGO database (Du et al. 2010), using Hypergeometric statistical test under adjusted P-values ≤0.01 using Yekutieli method. To compare the enriched GO terms of the upregulated genes in YSBR1 and Lemont more effectively, the results were compared using a cross comparison of SEA (SEACOMPARE).

MapMan Analysis

To obtain functional classifications, we present the Mapman tool with information of two groups at one time by assigning genes in clusters 1, 4, 5 to a value of '3' and clusters 2, 3, 6 to a value of '-3' in the fold change column of uploading data, referred as Jung and An (2012).

RNA Extraction and qRT-PCR

The total RNA of YSBR1 and Lemont rice cultivars was extracted from the *R. solani*-infected rice samples at 0, 6, 10, and 20 hpi using Trizol reagent (Invitrogen, USA) according to the instructions of the manufacturer. RNA quality and concentration were verified using NanoDrop 2000 spectrophotometer (Thermo, USA). RNA samples were reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with gDNA eraser (Takara, Japan) according to the instructions of the manufacturer. qRT-PCR reactions were carried out in a 384-well format with SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Japan) using the ABI PRISM7900®HT real-time PCR detection system (ABI, USA). The reactions were repeated three times, and the $2^{-\Delta\Delta CT}$ method was used to quantitatively analyze the qRT-PCR data. The fold change of the gene expression was determined as (gene expression)^{6hpi}/(gene expression)^{0hpi}, (gene expression)^{10hpi}/(gene expression)^{0hpi}, or (gene expression)^{20hpi}/(gene expression)^{0hpi} in Lemont and YSBR1. All gene-specific primers were designed using the NCBI primer designing tools: Primer3 and Primer-BLAST, to ensure their specificity to the target genes in rice. The primer sequences for the qRT-PCR validation of the microarray data of the 9 DEGs are listed in Table S11. EF-Tu (Os03g08020; forward primer: 5' CCACG-GGCCATC-TGATCTAC 3'; reverse primer: 5' AGTCAATGATGA-GCACGGCA 3') was used as internal control for the qRT-PCR reactions.

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Authors' Contributions

ZY contributed to microarray data analysis, qRT-PCR, and manuscript

preparation; YZ contributed to microarray data analysis (Venn diagram, HCL, K-means, WEGO and MapMan analysis) and manuscript preparation; GX contributed to microarray data analysis and qRT-PCR. DB, HQ, XZ, XG, HY, HH, LP, and ZX participated in sample collection, RNA extraction, and contributed to microarray data analysis; XW contributed to design the study and performed microarray data analysis; JB contributed to microarray preparation and data analysis; SZ and PX prepared the rice materials and participated in *R. solani* inoculation; BZ contributed in designing the study and performed microarray preparation; G-LW contributed in designing the study and assisted in drafting the manuscript; SQ conceived the study, and contributed in data analysis, result interpretation, and manuscript preparation. All authors read and approved the final manuscript.

Supporting Information

Fig. S1. Disease reactions of the *R. solani*-susceptible cultivar Lemont and moderately-resistant cultivar YSBR1 using toothpick inoculation method on field and expression patterns of PR genes in YSBR1 and Lemont infected with *R. solani* at four timepoints.
Fig. S2. A biotic stress overview of DEGs in clusters 1, 4, 5 and clusters 2, 3, 6 assigned to 35 functional categories.
Fig. S3. MapMan overview of biotic stress showing the transcriptional changes in YSBR1 and Lemont at 6 hpi, respectively.
Fig. S4. MapMan overview of secondary metabolism showing the transcriptional changes in YSBR1 and Lemont at 6 hpi, respectively.
Table S1. Rice gene categories for customized microarray.
Table S2. A total of 12,500 genes derived from 8 cDNA libraries.
Table S3. Table listing 7,624 differentially expressed genes (DEGs) identified from YSBR1 and Lemont during rice early response to *R. solani*.
Table S4. A list of 425 genes that might be partially affected by circadian regulation.
Table S5. GO enrichment of genes in clusters 1, 4, 5 upregulated in the resistant line YSBR1 and clusters 2, 3, 6 upregulated in the susceptible line Lemont using AgriGO SEACOMPARE.
Table S6. Summary of MapMan analysis of genes in clusters 1, 4, 5 upregulated in the resistant line YSBR1 and clusters 2, 3, 6 upregulated in the susceptible line Lemont.
Table S7. Significantly enriched GO terms in 5618 DEGs for YSBR1_Molecular Function and 5526 DEGs for Lemont_Molecular Function.
Table S8. Details of 42 and 38 branch pathways of biotic stress in YSBR1 and Lemont, respectively, at 6 hpi.
Table S9. Details of 8 and 7 branch pathways of secondary metabolism in YSBR1 and Lemont, respectively, at 6 hpi.
Table S10. A list of 28 DEGs involved in JA pathway and 22 DEGs involved in SA pathway in the results.
Table S11. Primers used for qRT-PCR in this study.

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