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RNASeq reveals differential regulation of genes in resistant and susceptible rice varieties during early phases of infection with *Rhizoctonia solani*

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Abstract

Sheath blight disease caused by *Rhizoctonia solani* is one of the most destructive diseases of the rice and causes significant yield loss worldwide. To gain insight into the transcriptome dynamics that are associated with host-pathogen interaction, comparative studies of transcriptome profiling of sheath blight resistant (Tetep) and sheath blight susceptible (HP2216) rice lines after challenge with *R. solani* at early stages of infection (12 dpi) were conducted. A total of 319 and 252 differentially expressed genes (DEGs) were identified in Tetep and HP2216, respectively. MapMan pathway analysis revealed expression changes within the functional groups between the two genotypes post infection with *R. solani*. Molecular functional enrichment analysis showed that DEGs related to the hormone metabolism, cell wall modification, respiratory burst, signalling, transcription factors and secondary metabolites were significantly upregulated in sheath blight resistant cv. Tetep. A notable difference in gene expression was observed in biotic stress metabolic pathway with majority of the genes in signalling, hormone metabolism and transcription factors getting upregulated in resistant line Tetep. In case of TFs, higher expression of WRKY at 12 dpi in Tetep supports an important role in disease resistance. This study would be helpful in identification of early induced genes and understanding molecular componentsand factors which contribute to plant disease resistance.

Key words: Sheath blight, transcriptome, necrotrophic fungus, DEGs

Introduction

Rice is one of the major staple food crops of the world which feed almost half of the world population. In India, rice is consumed by 65% of the Indian population. Sheath blight is one of the most threatening diseases of the rice which can cause 10-30% yield loss annually. It is caused by soil borne necrotrophic fungus *Rhizoctonia solani* which exist either as sclerotia or vegetative mycelium. The sclerotia attached to the plant and cause lesions on leaf sheath.

Absolute resistance to *R. solani* is not available in any of the rice germplasm grown worldwide. Although partial genetic resistance to sheath blight in some rice lines such as Tetep, Tadukan, Teqing, Jasmine 85, ZYQ8, Minghui 63, LSBR-5 and LSBR-33 has been reported, but no major gene responsible for resistance has been found (Kumar et al. 2003; Xie et al. 1990; Groth and Nowick 1992; Li et al. 1995; Khush 1977; Pan et al. 1999; Channamallikarjuna et al. 2010). So far, much of the research has been emphasized on the "gene-for-gene" interaction diseases, such as rice blast disease and rice bacterial leaf blight disease. But in case of necrotroph fungus such as *R. solani*, the molecular mechanisms of the interaction between necrotrophic fungi and plants are complex and involve sophisticated recognition and signaling networks. In case of rice sheath blight, pathogenesis related proteins (PR-proteins) like β-1,3-glucanases, and β-1,3-glucan were found to be expressed upon pathogen infection (Datta et al. 1999). Molecular and functional analysis of six chitinase genes associated with the major *R. solani*-resistance QTLqSBR11-1 in Tetep rice line showed higher antifungal activity with a clear inhibitory effect on the growth of *R. solani* mycelium under *in vitro* condition (Kamboj et al. 2016). Recently, two PATHOGENICITY MAP KINASE1 homologues,

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RPMK1 and *RPMK2* has been targeted from *R. solani* using host delivered RNA interference (HD-RNAi) technology via a hybrid RNAi construct. A significant decrease in fungal infection in transgenic rice lines was observed compared to non transformed and delay in disease symptoms were also reported (Tiwari et al. 2017).

For the understanding host pathogen interaction and pathogenesis processes, recent advances in expression profiling tools such as microarray and RNA-seq have provided new opportunities to decode the complexity of interactions. Expression profiling using microarray has been performed to identify differentially expressed genes in rice during early stages of interaction with *Magnaporthe oryzae* (Vijayan et al. 2013). Transcription profiling using RNA-seq has become a powerful tool not only for better understanding of the physiological changes during host-pathogen interaction but also in revealing hidden molecular mechanisms and key regulatory networks involved in the process. A detailed RNA-seq time-course study for a susceptible and a resistant wheat host infected with *Puccinia striformis f.* sp. *tritici* identified clusters of differentially expressed genes in the host and pathogen and visualised the effect of pathogen infection on the expression of various defence components and host immune receptors (Dobon et al. 2016).

The aim of the present study was to compare and identify genes which are expressed in early stages of infection by *R. solani*in resistant line, Tetep and susceptible line, HP2216. Though, there are reports of transcriptome analysis of sheath blight infected rice lines using SSH and microarray approaches, but no studies have been reported to utilize RNASeq for transcription profiling of rice sheath tissue and comparing the pattern of gene expression with a susceptible genotype.

Materials and methods

Fungal pathogen and growth conditions

Rhizoctonia solani, the causal organism of rice sheath blight disease (AG1-1A isolate, obtained from Kapurthala, Punjab) was used in this study. The funguswas grown on potato dextrose agar (PDA) medium at 28^{o}C in dark for 5-6 days. Mycelial agar discs of 3 mm diameter were taken from 6-day-old fungal cultureswith a sterile cork borer. These mycelial discs were used as fungalinocula in the infection assays.

Inoculation of rice sheath with R. solani culture

Rice (*O. sativa* L. ssp. *indica*) line Tetep (resistant) and HP2216 (susceptible) were grown in a net house at NRCPB, New Delhi. Plants were grown under field conditions at 30-35^{o}C temperature and 60% relative humidity upto heading stage.Leaf sheaths were inoculated with agar plugs containing fungal mycelia keeping proper controls. Mock inoculation was done with plain agar plugs of same size. After the inoculation samples were collected from both resistant and susceptible genotypes of rice. Samples were collected after 12 hours after inoculation of *R. solani*.

Total RNA extraction and transcriptome analysis

Total RNA was isolated from sheath tissue of resistant (Tetep) and susceptible (HP2216) cultivars of rice at 12 hours post-inoculation of *R. solani* infection in three biological replicates using the QiagenRNeasy Plant Mini kit according to manufacturer's instructions. The total extracted RNA samples (5µg) from each biological replicate were used to isolate poly(A) mRNA and to prepare cDNA libraries using TruSeq RNA preparation protocol v.2 (Illumina, Inc., San Diego, CA, USA). Quality and size of cDNA library (three biological replicates) was confirmed on Agilent 2100 Bioanalyzer using High sensitivity DNA Kit.

Illumina Sequencing and Processing of RNA-Seq Reads

The TruSeq PE Cluster Kit v3-cBot-HS (Illumina, Inc., California, USA) was used for cluster generation using 2 pM of pooled normalized libraries on the cBOT. Sequencing was performed on the Illumina HiSeq 1000 paired-end (2×101bp read length) using the TruSeq SBS Kit v3-HS (Illumina, Inc.) at NRCPB, New Delhi. RNA-Seq reads were serrated from multiplexed data and converted from bcl to fastq files using CASAVA software. Quality of RNA-Seqfastq reads was checked with NGSQC Toolkit v2.3 which computes various quality matrices for raw reads.

The analysis of RNA-Seq data was further carried out on CLC Genomics Workbench v.7.0.4. (CLC bio, Aarhus N, Denmark), following the manufacturer's instructions. Paired fastq files were trimmed for low quality read ends, adapter contamination and ambiguous nucleotides to obtain information on distribution of average sequence quality scores, read length distribution and GC content. The resulting high quality reads were then mapped against annotated rice reference genome (MSU Rice Genome Annotation

Project Version 7.0). The insert size for paired-end reads was set between 180 and 250 bp keeping other parameters at default levels. Experiment was set up taking three biological replicates of each for treated and untreated samples. Total read counts was considered as expression value for further analysis and resulting data was log transformed and normalised for expression value. Differential expression of gene(s) was estimated based on the number of reads mapped against unique locus under both treated and untreated conditions using CLC Genomics Workbench v.7.0.4. (CLC bio, Aarhus N, Denmark). Exact test which utilises EdgeRBioconductor package version 3.4.0 was performed to calculate FDR corrected p value and fold change.

Functional enrichment analysis and pathway analyses

The significant genes were filtered on the criteria of FDR corrected p value ≤ 0.05. The resulting significant genes list was utilised for finding differentially expressed genes among resistant vs susceptible samples. A Gene Ontology (GO) functional enrichment analysis of screened DEGs was performed to understand their biological function. Pathway analysis was also performed to check the role of DEGs in metabolic pathways.

Results and discussion

Transcriptome analysis of rice sheath infected with R. solani

To identify genes regulated in rice sheaths in response to *R. solani* infection, paired end libraries were constructed from infected and mock inoculated rice sheaths from two rice cultivars, Tetep and HP2216. Infected plants showed water soaked lesions only after 12 hours of infection which were distinct in HP2216. Mock inoculated plants showed no symptoms of disease. In order to identify differential transcriptional changes during early stages of *R. solani* infection in resistant vs susceptible genotype, transcriptome sequencing was performed for pathogen infected sheath tissue. We identified 319 DEGs in resistant rice line out of which 220 were up-regulated (fold change ≥2, FDR corrected value ≤0.05) and 99 were down-regulated. While, in susceptible line, we found252 DEGs out of which 48 were up-regulated (fold change ≤2, FDR corrected value ≥0.05) and 204 were down-regulated. Twenty one DEGs were found to be common among two lines at 12hpi (Fig. 1A). These genes were associated with various developmental and regulatory functions of the plant though expressions were more in case of resistant line (Fig. 1B) and (Table 1).

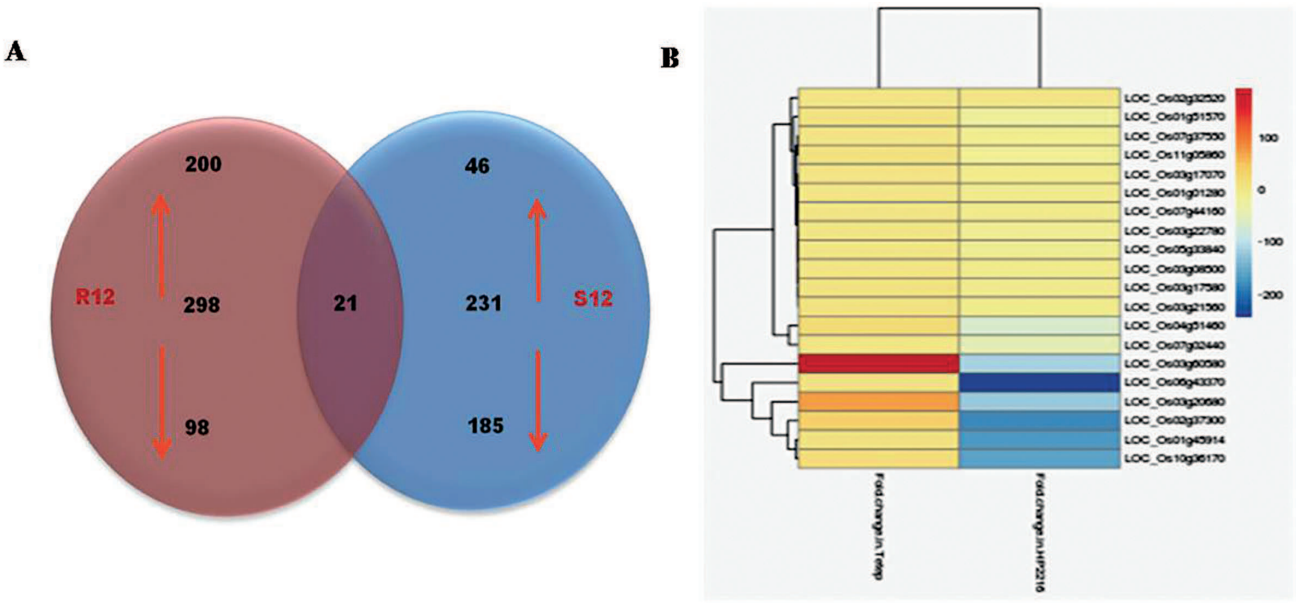

Fig. 1. Differentially expressed genes (DEGs) in sheath blight resistant and susceptible rice genotypes.(A) Venn diagram illustrating DEGs after *R. solani* infection in rice genotypes Tetep (R12) and HP2216 (S12) at 12hours post infection (B)Heat map of common set of DEGs between Tetep (R12) and HP2216 (S12)

Table 1. Common set of differentially expressed genes in the sheath of rice cvs. Tetep and HP2216 at 12 hours post infection with *R. solani*

LOCUS ID	Putative function	Fold change*	
		Tetep	HP2216
LOC_Os01g01280.1	Expressed protein	5.02	–4.29
LOC_Os01g45914.1	Expressed protein	9.66	–169.57
LOC_Os01g51570.1	Glycosyl hydrolases family 17, putative	9.62	–18.34
LOC_Os02g32520.1	ERD1 protein, chloroplast precursor	5.31	4.58
LOC_Os02g37300.1	Heavy metal associated domain protein	36.13	–185.74
LOC_Os03g08500.2	AP2 domain containing protein, expressed	5.84	–6.51
LOC_Os03g17070.1	ATP synthase B chain, chloroplast precursor	6.44	–4.70
LOC_Os03g17580.1	Ribosomal protein L10, putative, expressed	6.90	–6.49
LOC_Os03g20680.1	Late embryogenesis abundant protein 1, putative	72.61	–123.45
LOC_Os03g21560.1	Photosystem II 11 kD protein, putative	6.66	–6.00
LOC_Os03g22780.1	DVR, putative, expressed	4.95	–7.14
LOC_Os03g60580.1	Actin-depolymerizing factor, putative, expressed	195.30	–115.59
LOC_Os04g51460.1	Glycosyl hydrolases family 16, putative	21.31	–67.25
LOC_Os05g33840.1	Transketolase, putative, expressed	5.82	–7.52
LOC_Os05g34700.1	GDSL-like lipase/acylhydrolase, putative	–926.61	–313.44
LOC_Os06g43370.1	Cytochrome P450, putative, expressed	10.59	–243.49
LOC_Os07g02440.1	Peroxidase precursor, putative, expressed	6.34	–42.92
LOC_Os07g37550.1	Chlorophyll A-B binding protein, putative,	5.18	–9.30
LOC_Os07g44160.1	Retrotransposon protein, putative, unclassified,	5.26	–4.77
LOC_Os10g36170.1	LTPL160 - Protease inhibitor/seed storage/LTP	15.78	–164.30
LOC_Os11g05860.1	Harpin-induced protein 1 domain protein	7.70	–11.40

*Log_2 Fold change (compared to mock inoculated)

Gene Ontology (GO) Enrichment analysis of differentially expressed genes

TheGO enrichment analysis revealed an over-representation of genes involved in molecular function such as catalytic activity, hydrolase activity, transcription factor binding activity, transferase activity, transporter activity and kinase activity in both the rice lines (Fig. 2). It is interesting that most of DEGs involved in biological process such as response to stress, multicellular organismal process and signal transduction. Similar categories of genes were reported by conducting genome expression profile analysis of maize sheath in response to infection of *R. solani* (Gao et al. 2014).

DEGs under cellular component category include cytochrome P450, ethylene responsive transcription factor (ERF), AP2 domain containing protein, MADs box family protein, chitinases and WRKY. The WRKY transcription factors are crucial regulatory components of plant responses to pathogen infection. WRKY30 has been reported to increase the endogenous jasmonic acid (JA) accumulation, PR gene expression and in turn enhance resistance to fungal pathogens in rice (Peng et al. 2012). ERF have also been reported to increase activities of lipoxygenase (LOX) and phenylalanine ammonia lyase (PAL) in sheath blight infected rice to provide resistance to plant (Pan et al. 2014).In present study, involvement of genes in molecular function category constituted 35-43% of the total differentially expressed transcripts (Fig. 3).

Among defense response genes, in addition to thaumatin like protein, plant disease resistance response protein, bHLH domain containing protein,

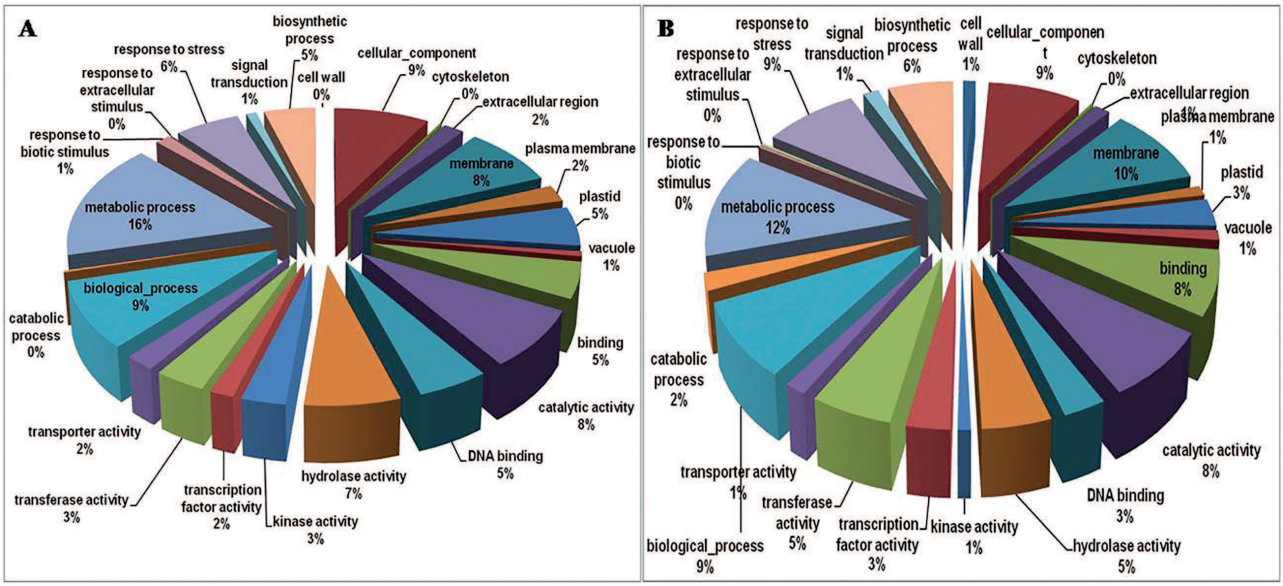

Fig. 2. GO enrichment of differentially expressed genes. Functional categorization of DEGs in Tetep(A) and HP2216 (B)

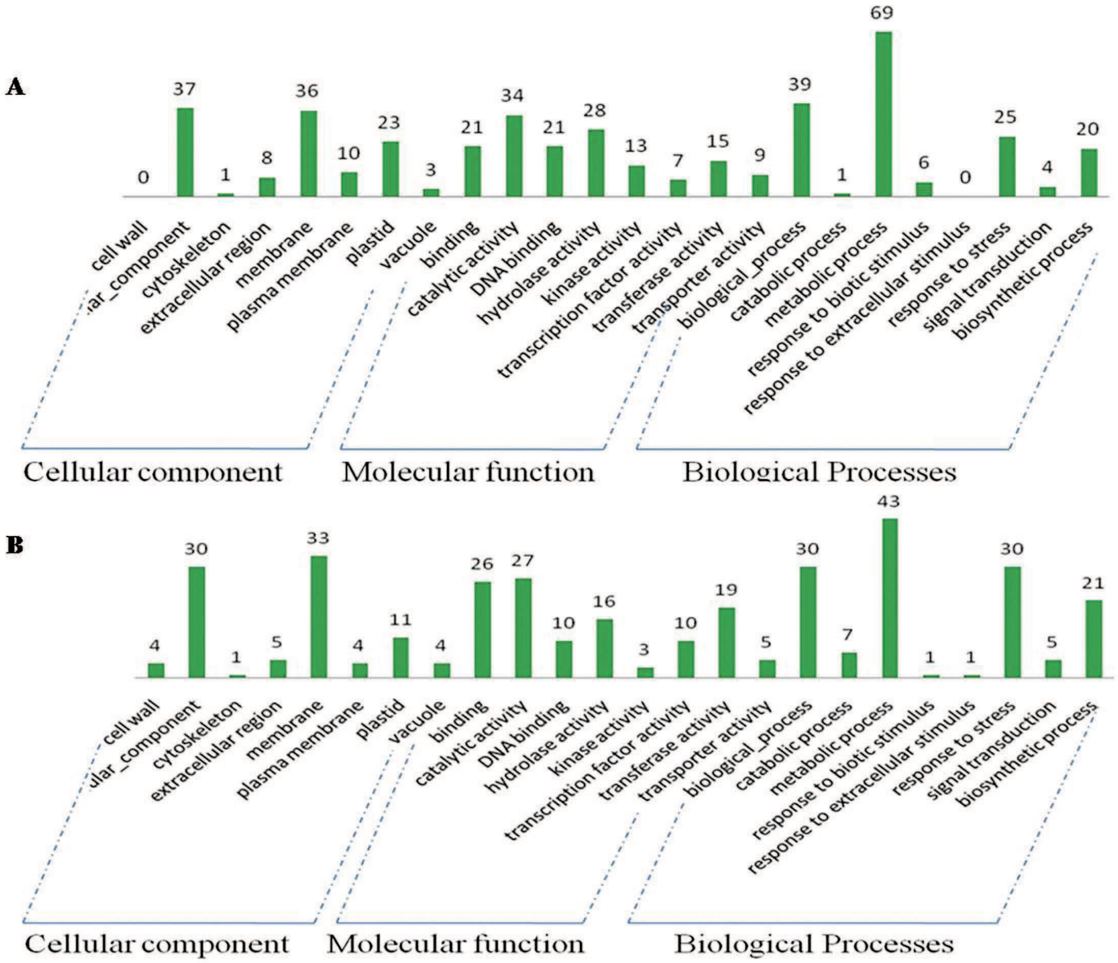

Fig. 3. Functional annotation of expressed genes Number of enriched GO DEGscategorized into Cellular component,Molecular Function andBiologicalProcesses in Tetep (A) and HP2216 (B)

ERF027, WRKY and glutathione S tranferase were enriched in the present study. Comparing GO enrichment analysis in both the lines has not shown much difference in the differential regulation of early responsive genes. Surprisingly, when we performed Mapman analysis to identify DEGs involved in biotic stress, we landed upon major difference in the expression of genes in two lines. We found significant upregulation of the genes involved in JA and ethylene biosynthesis in Tetep while HP2216 depicted downregulation of these genes. Another major difference was obtained in expression of genes in relation to signalling and transcription factors. Resistant line showed high expression of these genes compared to susceptible line.

Hormone metabolism

An increase in transcript expression in Tetep following *R. solani* infection was obtained for genes associated with hormone metabolism, such as genes encoding enzymes involved in the ethylene, jasmonic acid and ABA (Fig. 4). Among phytohormones, JA has a central role in imparting resistance to necrotrophic pathogens and cross talk between JA, ET and SA is key regulator of defense response. A role in SA–JA crosstalk has also been suggested for OsWRKY13. Functioning upstream of OsNPR1 and OsWRKY45, this TF positively regulates SA-mediated rice defenses while suppressing the JA pathway (Qiu et al. 2007, 2008, 2009; Tao et al. 2009). We obtained 3-5 fold upregulation of genes encoding 9-cis-epoxycarotenoid dioxygenase, aminotransferases, AP2 domain containing protein, ERF, LOX and cytochrome P450 in Tetep (Supplementary Table 1). ABA biosynthesis pathway involves the oxidative cleavage of 9-cis-epoxycarotenoids, which may be the key regulatory step in the pathway catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED) (Han et al. 2004). In general, the impact of ABA on plant defense seem to be plant-pathogen interaction-specific, rather than to rely on the lifestyle or infection strategy of the pathogen. In *Arabidopsis*, for instance, ABA both positively and negatively regulates resistance to the necrotrophic fungi *Alternaria brassicicola* and *Botrytis cinerea*, respectively (Adie et al. 2007). Two aminotransferases in *Arabidopsis* that appear to play important roles in disease resistance and plant development have been identified (Song et al. 2004). The AP2/ethylene response factor (ERF) TFs are considered to be one of the largest groups of TFs expressed in response to both biotic and abiotic stresses (Thirugnanasambantham et al. 2014). In addition, genes encoding ERF were induced by chemical inducers that are capable of inducing disease resistance (Cao et al. 2006). A single ERF gene from *V. vinifera* is involved in ABA, ethephon (ET), and

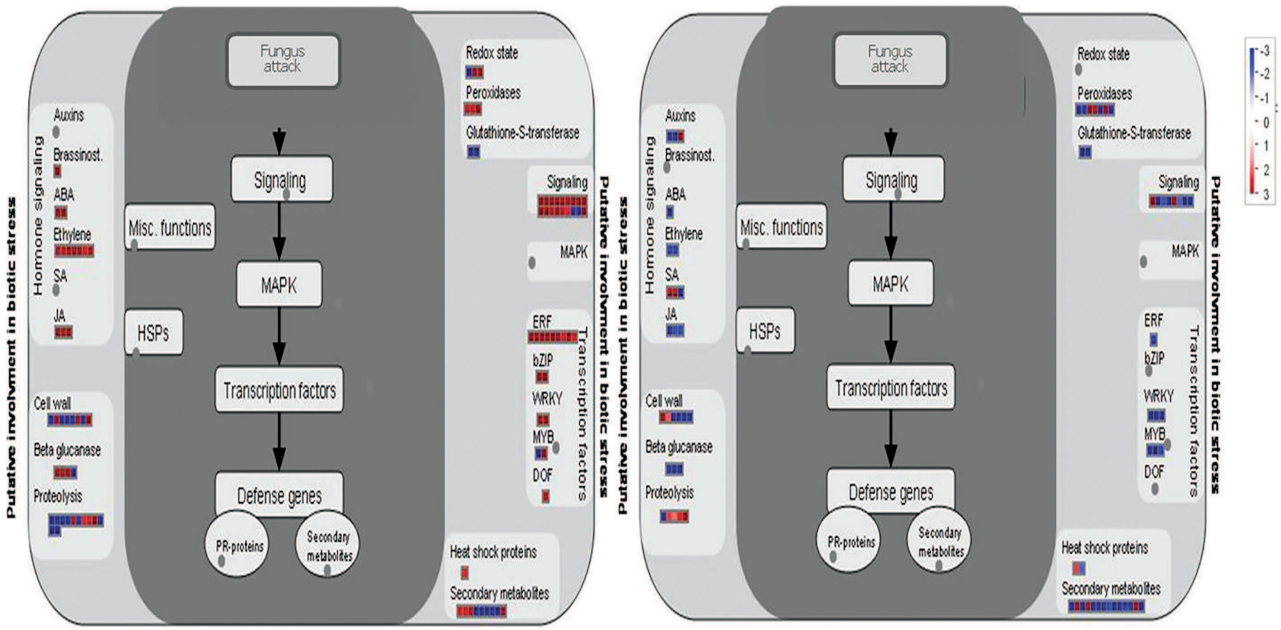

Fig. 4. Mapman biotic stress overview maps showing differences in transcript levels after infection with *R. Solani*in Tetep (A) and HP2216 (B). On the logarithmic color scale, blue represents downregulated transcripts and red represents upregulated transcripts

salicylic acid (SA) signal pathways (Zhu et al. 2013). *LOX* gene expression is regulated by the pathogen attack (Melan et al. 1993). Induction of *LOX* genes during plant-pathogen interactions has been reported in several species. Plant cytochrome P450 enzymes are involved in a wide range of biosynthetic reactions, leading to various fatty acid conjugates, plant hormones, or defensive compounds. Hwang and Hwang (2010) illustrated that pepper CaCYP450A is required for defense responses to microbial pathogens in plants. In HP2216, mostly down-regulation of genes was obtained under this category (Supplementary Table 2).

Cell wall modification

We found five categories of genes encoding an uncharacterized protein At4g06744 precursor, glycosyl hydrolases, pectinesterases, endo-1,3-β-glucosidase and AAA family ATPase upregulation in Tetep in relation to cell wall modification (Supplementary Table S1). Fungal necrotrophs extensively destroy the integrity of the cell wall through the combined action of degrading enzymes and plants respond to defend the pathogen attack by degrading pathogen cell wall. Interestingly, OsFBX22 - F-box domain containing protein and ubiquitin containing protein were upregulated in case of HP2216 in present study while ubiquitin containing protein was down-regulated in Tetep. F-box proteins are used in the defense response by the host. An F-Box protein ACRE189/ACIF1 has been reported to regulate cell death and defense responses during pathogen recognition in tobacco and tomato (Burg et al. 2008).

ROS metabolism and signalling

We found that genes corresponding to respiratory burst were getting differentially regulated in both the lines by way of upregulation of mostly peroxidase precursor. In addition to peroxidase, glutathione S transferase (GST) was getting down-regulated in both the lines. Reactive oxygen species (ROS) are one of the most important plant defense responses to pathogens, although they lead to host cell death and facilitate necrotrophic colonization (Levine et al. 1994). Upregulation of peroxidases result in plant resistance to pathogens. GSTs play an essential role in the protection of fungal nectrotrophs against plant-derived toxic metabolites and ROS that accumulate at the host-pathogen interface during infection (Calmes et al. 2013). In this study, we also found that at early infection stage, GSTs are not getting activated and may be concluded that as infection progresses, their roles in defense activity can be documented.

We obtained clear cut difference in differential regulation of genes involved in signalling between resistant and susceptible rice lines at early stages of infection. In Tetep, increase in accumulation of transcripts encoding LRR kinase, phosphate induced protein, and other kinases genes were observed. We found two genes corresponding to lectin-like protein kinase that were showing 10 fold upregulation in Tetep at 12hpi (Supplementary Table S1). Bouwmester and Govers (2009) hypothesized lectin receptor kinases to participate in biotic stress tolerance due to the resemblance of the extracellular domain with lectin proteins known to bind to fungal andbacterial cell wall components. *Pi-d2*, a G-type lectin receptor kinase from rice, provides resistance against the fungal pathogen *Magnaporthe grisea*, the causal agent of rice blast (Chen et al. 2006). In HP2216, only two genes corresponding to receptor like kinase and transposon protein showed upregulation (Supplementary Table S2).

Transcription factor and secondary metabolites

Several well studied plant transcription factor families are associated with defense responses (Eulgem 2005). These include AP2/ERF, basic-domain leucine-zipper (bZIP), WRKY, MYB and members of zinc family. Remarkable difference in gene expression was obtained in this category between two lines. While we found upregulation of genes encoding AP2/ERF, basic-domain leucine-zipper (bZIP), WRKY and dof zinc finger protein in resistant line, the same were downregulated in susceptible line. We found two genes corresponding to WRKY transcription factors upregulated in *R. solani* infected sheath of Tetep (Supplementary Table S1). MAPK-mediated phosphorylation of WRKY8 has an important role in plant immunity through activation of downstream genes (Ishihama et al. 2011). WRKY62, WRKY76 and WRKY21 were found to be downregulated in HP2216 depicting absence of defense activity at early stages of infection (Supplementary Table S2).

We found that genes involved in the synthesis of secondary metabolites were differentially regulated in both Tetep and HP2216. Among these, terpene synthase and phytoene synthase found to be activated upon pathogen attack at initial stages. Although higher concentrations of secondary metabolites might result in a more resistant plant, the production of secondary metabolites is thought to be costly and reduces plant growth and reproduction (Siemens et al. 2002). This

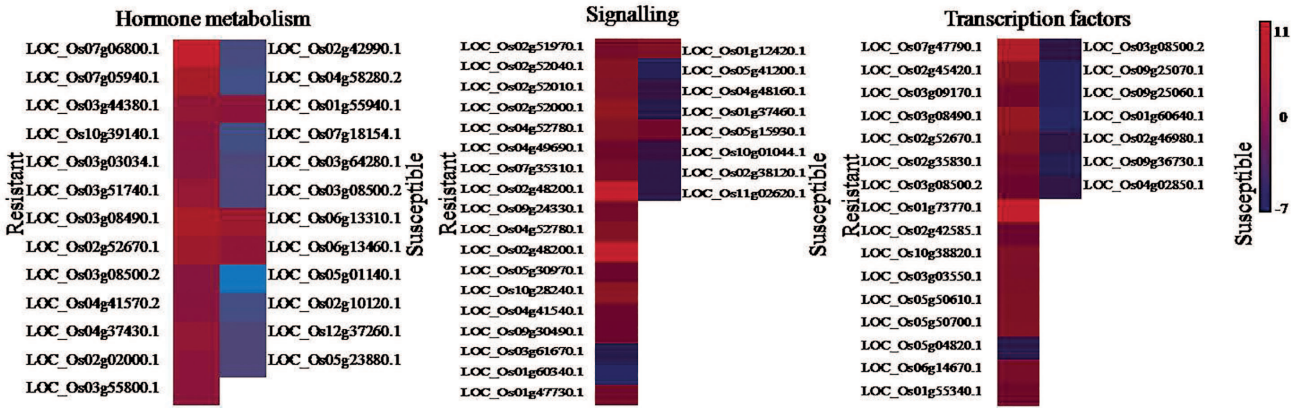

Fig. 5. Differential regulation of genes involved in hormone metabolism, signalling and TFs between resistant and susceptible rice sheath at early stages upon *R. solani* infection

invoked to explain why plants have not activated secondary metabolites production at early stages of infection and generally increase only when severe stress situation arrives.

Overall, based on our experiment at early infection stages, we report that though differential regulation of genes were initiated at early stages but it is not prominent and sufficient for disease resistance especially in case of susceptible line, HP2216. Set of transcripts containing genes involved in hormone metabolism especially JA and ethylene, signalling related genes and TFs found upregulated in resistant line, Tetep and simultaneously downregulated in HP2216 (Fig. 5). It suggests that these genes included might play central roles as master regulators for the disease resistance. The analysis provided an unprecedented global overview of the defence processes differentiating a resistant from a susceptible response to sheath blight infection that could ultimately be related to the resistant phenotype. Differential transcript levels for genes involved in hormone regulatory functions and signalling in the susceptible genotype ranged from lower gene expression to abolishment of gene transcription. This behaviour might be ascribed to the lack of effective pathogen sensing mechanisms and, possibly, to the suppressed basal defence responses in HP2216.In this study, we showed the transcriptome dynamics of resistant and susceptible genotypes at early stages of sheath blight infection to the sheath and first report of comparative transcriptome profiling of early response in sheath tissue of resistant vs susceptible genotypes. The data presented here might help to further clarify the mechanistic events underlying successful and resistance against *R. solani* and functional studies on the relevant genes are expected to prove useful in the improvement of sheath blight susceptible rice germplasm.

Authors' contribution

Conceptualization of research (TRS); Designing of the experiments (AK, TRS); Contribution of experimental materials (TRS); Execution of field/lab experiments and data collection (AK, RK); Analysis of data and interpretation (AK, PJ); Preparation of manuscript (AK, TRS).

Declaration

The authors declare no conflict of interest.

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References

Kumar K. K., Poovannan K., Nandakumar R., Thamilarasi K., Geetha C., Jayashree N., Kokiladevi E., Raja J. A. J, Samiyappan R., Sudhakar D. and Balasubramanian P. 2003. A high throughout functional expression assay system for adefence gene conferring transgenic resistance on rice against the sheath blight pathogen, *Rhizoctonia solani*. Plant Sci., **165**: 969-976.

Xie Q. J., Rush M. C. and Cao J. 1990. Somaclonal variation for disease resistance in rice *Oryza sativa* L. In: Grayson B. T., Green M. B. Copping L Geds

Pest management in rice. Elsevier, New York, 491-509.

Groth D. E. and Nowick E. M. 1992. Selection for resistance to rice sheath blight through number of infection cushion and lesion type. Plant Disease, **76**: 721-723.

Li Z. K., Pinson S. R. M., Marchetti M. A., Stansel J. W. and Park W. D. 1995. Characterization of quantitative trait loci (QTL) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theoretical and Applied Genetics, **91**: 382-388.

Khush G. S. 1977. Disease and insect resistance in rice. Adv. Agron., **29**: 268-341.

Pan X. B., Rush M. C., Sha X. Y., Xie Q. J., Linscombe S. D., Stetina S. R. and Oard J. H. 1999. Major gene, nonallelic sheath blight resistance from the rice cultivars Jasmine 85 and Teqing. Crop Science, **39**: 338-346.

Channamallikarjuna V., Sonah H., Prasad M., Rao G. J. N., Chand S., Upreti H. C., Singh N. K. and Sharma T. R. 2010. Identification of major quantitative trait loci qSBR11-1 for sheath blight resistance in rice. Mol. Breed., **25**: 155-166.

Datta K., Velazhahan N., Oliva I., Ona T., Mew G. S. and Kush S. 1999. Over-expression of the cloned rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhances environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease. Theor. Appl. Genet., **98**(2): 1138-1145.

Kamboj R., Tiwari I. M., Kumari M., Devanna B. N., Sonah H., Kumari A., Nagar R., Sharma V., Botella J. R. and Sharma T. R.2016. Functional characterization of novel chitinase genes present in the sheath blight resistance QTL: qSBR11-1 in rice line Tetep. Front Plant Sci., **7**: 244.

Tiwari I. M., Jesuraj A., Kamboj R., Devanna B. N., Botella J. R. and Sharma T. R. 2017. Host Delivered RNAi, an efficient approach to increase rice resistance to sheath blight pathogen (*Rhizoctonia solani*). Scientific Reports, **7**: 7521

Dobon A., Bunting D. C. E., Cabrera-Quio L. E., Uauy C., Saunders D. G. O. 2016. The host-pathogen interaction between wheat and yellow rust induces temporally coordinated waves of gene expression. BMC Genomics, **17**: 380.

Vijayan J., Jain S., Jain N., Devanna B. N., Rathour R., Variar M., Prashanthi S. K., Singh A. K., Singh U. D., Singh N. K. and Sharma T. R. 2013. Identification of differentially expressed genes in rice during its early phases of interaction with *Magnaporthe oryzae*. Indian J. Genet., **73**: 233-243.

Gao J., Chen Z., Luo M., Peng H., Lin H., Qin C., Yuan G., Shen Y., Ding H., Zhao M., Pan G. and Zhang Z. 2014. Genome expression profile analysis of the maize sheath in response to inoculation to *R. solani*. Mol. Biol. Rep., **41**(4): 2471-2483.

Peng X., Hu Y., Tang X., Zhou P., Deng X., Wang H. and Guo Z. 2012. Constitutive expression of rice WRKY30 gene increases the endogenous jasmonic acid accumulation, PR gene expression and resistance to fungal pathogens in rice. Planta, **236**: 1485-1498.

Pan X., Lia Y., Zhang H., Huang R., Liu W., Ming J., Liu S. and Lia X. 2014. Expression of signalling and defence-related genes mediated by over-expression of *JERF1*, and increased resistance tosheath blight in rice. Plant Pathology, **63**: 109-116.

Qiu D. Y., Xiao J., Ding X. H., Xiong M., Cai M. and Cao C. L. 2007. OsWRKY13 mediatesrice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol. Plant Microbe Interact., **20**: 492-499.

Qiu D., Xiao J., Xie W., Liu H., Li X. and Xiong L. 2008. Rice gene network inferred from expression profiling of plants over expressing OsWRKY13, apositive regulator of disease resistance. Mol. Plant., **1**: 538-551.

Qiu D., Xiao J., Xie W., Cheng H., Li X. and Wang S. 2009. Exploring transcriptional signaling mediated by OsWRKY13, a potential regulator of multiple physiological processes in rice. BMC Plant Biol., **9**: 74-85.

Tao Z., Liu H., Qiu D., Zhou Y., Li X. and Xu C. 2009. Apairofallelic WRKY genes play opposite roles in rice-bacteria interactions. Plant Physiol., **151**: 936-948.

Han S. Y., Kitahata N., Sekimata K., Saito T., Kobayashi M., Nakashima K., Yamaguchi-Shinozaki K., Shinozaki K., Yoshida S. and Asami T. 2004. A novel inhibitor of 9-cis-epoxycarotenoid dioxygenase in abscisic acid biosynthesis in higher plants. Plant Physiol., **135**: 1574-1582.

Adie B. A., Pérez-Pérez J., Pérez-Pérez M. M., Godoy M., Sánchez-Serrano J. J. and Schmelz E. A. 2007. ABA is an essential signal for plant resistance to pathogens affecting J Abiosynthesis and the activation of defenses in *Arabidopsis*. Plant Cell, **19**: 1665-1681.

Song J. T., Lu H. and Greenberg J. T. 2004. Divergent roles in *Arabidopsis* development and defense of two homologous genes, *AGD2* and *ALD1*, encoding novel amino transferases. Plant Cell, **16:** 353-66.

Thirugnanasambantham K., Durairaj S., Saravanan S., Karikalan K., Muralidaran S. and Islam V. I. H. 2014. Role of Ethylene Response transcription factor (ERF) and its regulation in response to stress encountered by plants. Plant Mol. Biol. Rep., **32**(5): 943-1102.

Cao Y., Song F., Goodman R. M. and Zheng Z. 2006. Molecular characterization of four rice genes encoding ethylene-responsive transcriptional factors and their expressions in response to biotic and

abiotic stress. J. Plant Physiol., **163**: 1167-1178.

Zhu Z., Shi J., Xu W., Li H.., He M., Xu Y., Xu T., Yang Y., Cao J. and Wang Y. 2013. Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways. J. Plant Physiol., **170**: 923-933.

Melan A. M., Dong X., Endara M. E., Davis K. R., Ausubel F. M. and Peterman T. K. 1993. An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid and methyl jasmonate. Plant Physiol., **101**: 441-450.

Hwang I. S. and Hwang B. K. 2010.Role of the pepper cytochrome P450 gene CaCYP450A in defense responses against microbial pathogens. Planta, **232**: 1409-1420.

Burg H. A., Tsitsigiannis D. I., Rowland O., Lo J., Rallapalli G., MacLean D., Frank Takken F. L. W. and Jones J. D. G. 2008. The F-Box Protein ACRE189/ACIF1 Regulates Cell Death and Defense Responses Activated during Pathogen Recognition in Tobacco and Tomato. Plant Cell, **20**(3): 697-719.

Levine A., Tenhaken R., Dixon R. and Lamb C. J. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell, **79:** 583-593.

Calmes B., GuillemetteT., TeyssierL., Siegler B., Pigne S., Landreau A., Iacomi B., LemoineR., Richomme P. and Simoneau P. 2013. Role of mannitol metabolism in the pathogenicity of the necrotrophic fungus *Alternaria brassicicola*. Front. Plant Sci., **4**: 1-18.

Bouwmeester K. and Govers F. 2009. *Arabidopsis* L-type lectin receptor kinases: Phylogeny, classification, and expression profiles. J. Exp. Bot., **60**: 4383-4396.

Chen X., Shang J., Chen D., Lei C., Zou Y., Zhai W., Liu G., Xu J., Ling Z., Cao G., Ma B., Wang Y., Zhao X., Li S. and Zhu L. 2006. A B-lectin receptor kinase gene conferring rice blast resistance. Plant J., **46**: 794-804.

Eulgem T. 2005. Regulation of the *Arabidopsis* defense transcriptome. Trends Plant Sci., **10**: 71-78.

Ishihama N., Yamada R., Yoshioka M., Katou S. and Yoshioka H. 2011. Phosphorylation of the *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defense response. Plant Cell, **23**: 1153-1170.

Siemens D. H., Garner S. H., Mitchell-Olds T. and Callaway R. M. 2002. Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. Ecology, **83**(2): 505-517.

Supplementary Table S1. Differentially expressed genes during early stages of infection with *R. solani* in Tetep

LOCUS ID	Putative function	Fold change*
Hormone metabolism		
LOC_Os07g06800.1	3-oxo-5-alpha-steroid 4-dehydrogenase, putative, expressed	7.9
LOC_Os07g05940.1	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor	5.36
LOC_Os03g44380.1	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor	3.55
LOC_Os10g39140.1	Flavonol synthase/flavanone 3-hydroxylase, putative	2.89
LOC_Os03g03034.1	Flavonol synthase/flavanone 3-hydroxylase, putative	2.69
LOC_Os03g51740.1	Aminotransferase, classes I and II, domain containing protein	3.79
LOC_Os03g08490.1	AP2 domain containing protein, expressed	5.69
LOC_Os02g52670.1	AP2 domain containing protein, expressed	4.53
LOC_Os03g08500.2	AP2 domain containing protein, expressed	2.55
LOC_Os04g41570.2	Ethylene-responsive protein related, putative, expressed	2.77
LOC_Os04g37430.1	Lipoxygenase protein, putative, expressed	3.59
LOC_Os02g02000.1	Cytochrome P450, putative, expressed	3.19
LOC_Os03g55800.1	Cytochrome P450, putative, expressed	3.07
Cell wall modification		
LOC_Os05g35200.2	Glycosyl transferase, putative, expressed	-2.87
LOC_Os07g07990.1	Uncharacterized protein At4g06744 precursor, putative	3.73
LOC_Os02g40260.1	Uncharacterized protein At4g06744 precursor, putative	-6.8
LOC_Os05g50260.1	Polygalacturonase, putative, expressed	-5.7
LOC_Os04g53950.1	Glycosyl hydrolases family 16 protein, protein, expressed	-9.33
LOC_Os04g51460.1	Glycosyl hydrolases family 16, putative, expressed	4.41
LOC_Os04g46740.1	Pectinesterase, putative, expressed	-7.35
LOC_Os09g39760.1	Pectinesterase, putative, expressed	3.44
LOC_Os04g33640.1	Glycosyl hydrolases family 17, putative, expressed	3.66
LOC_Os01g51570.1	Glycosyl hydrolases family 17, putative, expressed	3.27
LOC_Os03g57880.1	Glucan endo-1,3-beta-glucosidase precursor, putative	4.74
LOC_Os01g71474.1	Glycosyl hydrolases family 17, putative, expressed	-4.84
LOC_Os07g38590.1	Carboxyl-terminal peptidase, putative, expressed	-3.64
LOC_Os01g64860.1	OsSub11 - Putative Subtilisin homologue, expressed	-8.16
LOC_Os01g73980.1	Xylem cysteine proteinase 2 precursor, putative, expressed	-5.23
LOC_Os05g01810.1	Xylem cysteine proteinase 2 precursor, putative, expressed	-7.74
LOC_Os05g33400.1	Basic 7S globulin precursor, putative, expressed	3.37
LOC_Os01g44130.1	Aspartic proteinase oryzasin-1 precursor, putative, expressed	-9.11
LOC_Os02g32520.1	ERD1 protein, chloroplast precursor, putative, expressed	2.41
LOC_Os05g51130.1	Mitochondrial chaperone BCS1, putative, expressed	2.67
LOC_Os01g45450.1	AAA family ATPase, putative, expressed	5.25
LOC_Os04g55150.2	UBA/TS-N domain containing protein, expressed	-4.33
LOC_Os09g31031.1	Ubiquitin family protein, putative, expressed	-5.71
LOC_Os07g43260.1	SKP1-like protein 1B, putative, expressed	-6.67

Respiratory Burst		
LOC_Os05g11990.1	TTL1, putative, expressed	-6.13
LOC_Os09g38670.1	Thioredoxin, putative, expressed	2.99
LOC_Os01g63210.1	SOUL heme-binding protein, putative, expressed	3.04
LOC_Os07g02440.1	Peroxidase precursor, putative, expressed	2.66
LOC_Os01g22352.1	Peroxidase precursor, putative, expressed	2.69
LOC_Os01g73220.1	Peroxidase precursor, putative, expressed	7.27
LOC_Os03g04240.1	Glutathione S-transferase, putative, expressed	-6.17
LOC_Os03g04220.1	Glutathione S-transferase, putative, expressed	-10.3
Signalling		
LOC_Os02g51970.1	Phosphate-induced protein 1 conserved region	3.72
LOC_Os02g52040.1	Phosphate-induced protein 1 conserved region	4.99
LOC_Os02g52010.1	Phosphate-induced protein 1 conserved region	4.69
LOC_Os02g52000.1	Phosphate-induced protein 1 conserved region	5.85
LOC_Os04g52780.1	Leucine-rich repeat receptor protein kinase EXS precursor	4.89
LOC_Os04g49690.1	FERONIA receptor-like kinase, putative, expressed	3.15
LOC_Os07g35310.1	TKL_IRAK_DUF26-lc.12 - DUF26 kinases	3.8
LOC_Os02g48200.1	Lectin-like protein kinase, putative, expressed	11
LOC_Os09g24330.1	TKL_IRAK_DUF26-lg.2 - DUF26 kinases	3.44
LOC_Os04g52780.1	Leucine-rich repeat receptor protein kinase EXS precursor	4.89
LOC_Os02g48200.1	Lectin-like protein kinase, putative, expressed	11
LOC_Os05g30970.1	Copine, putative, expressed	2.78
LOC_Os10g28240.1	Calcium-transporting ATPase, plasma membrane-type	5.41
LOC_Os04g41540.1	OsCML22-Calmodulin-related calcium sensor protein	2.78
LOC_Os09g30490.1	EF hand family protein, expressed	2.57
LOC_Os03g61670.1	Calreticulin precursor, putative, expressed	-4.38
LOC_Os01g60340.1	NTMC2Type1.1 protein, putative, expressed	-8.43
LOC_Os01g47730.1	Ras-related protein, putative, expressed	3.33
Transcription Factor		
LOC_Os07g47790.1	AP2 domain containing protein, expressed	8.26
LOC_Os02g45420.1	AP2 domain containing protein, expressed	4.66
LOC_Os03g09170.1	Ethylene-responsive transcription factor, putative, expressed	3.08
LOC_Os03g08490.1	AP2 domain containing protein, expressed	5.69
LOC_Os02g52670.1	AP2 domain containing protein, expressed	4.53
LOC_Os02g35830.1	Extracellular ligand-gated ion channel, putative, expressed	3.26
LOC_Os03g08500.2	AP2 domain containing protein, expressed	2.55
LOC_Os01g73770.1	Dehydration-responsive element-binding protein, putative	9.97
LOC_Os02g42585.1	AP2 domain containing protein, expressed	2.57
LOC_Os10g38820.1	bZIP family transcription factor, putative, expressed	4.03
LOC_Os03g03550.1	bZIP family transcription factor, putative, expressed	3.95
LOC_Os05g50610.1	WRKY8, expressed	4.19
LOC_Os05g50700.1	WRKY111, expressed	4.25
LOC_Os05g04820.1	MYB family transcription factor, putative, expressed	-3.42

LOC_Os06g14670.1	ODORANT1, putative, expressed	3.6
LOC_Os01g55340.1	Dof zinc finger domain containing protein, putative	2.78
Secondary Metabolite		
LOC_Os05g05940.1	Stress-related protein, putative, expressed	2.48
LOC_Os05g33840.1	Transketolase, putative, expressed	2.54
LOC_Os09g38320.1	Phytoene synthase, chloroplast precursor, putative expressed	2.85
LOC_Os11g35710.1	Cycloartenol synthase, putative, expressed	-5.13
LOC_Os04g24530.1	AMP-binding domain containing protein, expressed	-11.6
LOC_Os02g56920.1	WAX2, putative, expressed	-7.28
LOC_Os10g34360.1	Stilbene synthase, putative, expressed	-10.6
LOC_Os01g03670.1	Dihydroflavonol-4-reductase, putative, expressed	-12.2
LOC_Os06g37150.1	L-ascorbate oxidase precursor, putative, expressed	2.92

*(Log_2fold change $\leq$2, FDR corrected value $\geq$0.05)

Supplementary Table S2. Differentially expressed genes during early stages of infection with *R. solani* in HP2216

LOCUS ID	Putative function	Fold change*
Hormone metabolism		
LOC_Os02g42990.1	OsSAUR11 - Auxin-responsive SAUR gene family member	-2.9
LOC_Os04g58280.2	Stem-specific protein TSJT1, putative, expressed	-3.6
LOC_Os01g55940.1	OsGH3.2 - Probable indole-3-acetic acid-amido synthetase	3.09
LOC_Os07g18154.1	Aldehyde oxidase 4, putative, expressed	-3.4
LOC_Os03g64280.1	1-aminocyclopropane-1-carboxylate oxidase homolog 1	-2.6
LOC_Os03g08500.2	AP2 domain containing protein, expressed	-2.7
LOC_Os06g13310.1	SAM dependent carboxyl methyltransferase, putative, expressed	4.36
LOC_Os06g13460.1	SAM dependent carboxyl methyltransferase family protein	3.36
LOC_Os05g01140.1	Methyltransferase, putative, expressed	-9.7
LOC_Os02g10120.1	Lipoxygenase, putative, expressed	-3.3
LOC_Os12g37260.1	Lipoxygenase 2.1, chloroplast precursor, putative, expressed	-2.3
LOC_Os05g23880.1	Lipoxygenase, putative, expressed	-2.4
Cell wall modification		
LOC_Os10g38040.1	LysM domain containing protein, putative, expressed	3.04
LOC_Os01g19170.1	Polygalacturonase, putative, expressed	2.03
LOC_Os06g48200.1	Glycosyl hydrolases family 16, putative, expressed	-4.6
LOC_Os06g48160.1	Glycosyl hydrolases family 16, putative, expressed	-4.8
LOC_Os04g51460.1	Glycosyl hydrolases family 16, putative, expressed	-6.1
LOC_Os04g35770.1	Pectinesterase, putative, expressed	-2.9
LOC_Os03g51240.2	Glycosyl hydrolases family 17, putative, expressed	-2.8
LOC_Os01g51570.1	Glycosyl hydrolases family 17, putative, expressed	-4.2
LOC_Os01g71810.1	Glycosyl hydrolases family 17, putative, expressed	-6
LOC_Os06g02780.1	Aspartic protease, putative, expressed	-6.8
LOC_Os02g32520.1	ERD1 protein, chloroplast precursor, putative, expressed	2.2
LOC_Os05g48390.1	Ubiquitin conjugating enzyme protein, putative, expressed	1.89

LOC_Os10g40490.1	U-box domain containing protein, expressed	2.43
LOC_Os01g52970.1	OsFBX22 - F-box domain containing protein, expressed	4.24
Respiratory Burst		
LOC_Os01g18970.1	Peroxidase precursor, putative, expressed	-3
LOC_Os09g29490.1	Peroxidase precursor, putative, expressed	-2.8
LOC_Os03g13210.1	Peroxidase precursor, putative, expressed	5.62
LOC_Os07g48010.1	Peroxidase precursor, putative, expressed	3
LOC_Os07g02440.1	Peroxidase precursor, putative, expressed	-5.4
LOC_Os10g02070.1	Peroxidase precursor, putative, expressed	6.58
LOC_Os05g04500.1	Peroxidase precursor, putative, expressed	-10
LOC_Os01g49710.1	Glutathione S-transferase, putative, expressed	-6.6
LOC_Os10g38140.1	Glutathione S-transferase, putative, expressed	-4.3
Signalling		
LOC_Os01g12420.1	Receptor-like protein kinase, putative, expressed	4.39
LOC_Os05g41200.1	OsCML9 - Calmodulin-related calcium sensor protein	-6.3
LOC_Os04g48160.1	IQ calmodulin-binding motif family protein, putative, expressed	-2.3
LOC_Os01g37460.1	Zinc finger family protein, putative, expressed	-4.2
LOC_Os05g15930.1	Transposon protein, putative, unclassified, expressed	3.21
LOC_Os10g01044.1	Isoflavone reductase, putative, expressed	-2
LOC_Os02g38120.1	BTBN3 - Bric-a-Brac, Tramtrack, Broad Complex BTB domain	-3.6
LOC_Os11g02620.1	BTBN21 - Bric-a-Brac, Tramtrack, Broad Complex BTB	-3.5
Transcription factor		
LOC_Os03g08500.2	AP2 domain containing protein, expressed	-2.7
LOC_Os09g25070.1	WRKY62, expressed	-6.5
LOC_Os09g25060.1	WRKY76, expressed	-6.5
LOC_Os01g60640.1	WRKY21, expressed	-7.3
LOC_Os02g46980.1	Pentatricopeptide, putative, expressed	-2.9
LOC_Os09g36730.1	MYB family transcription factor, putative, expressed	-4.7
LOC_Os04g02850.1	Pentatricopeptide, putative, expressed	-2.6
Secondary metabolites		
LOC_Os05g33840.1	Transketolase, putative, expressed	-2.9
LOC_Os04g01810.1	Terpene synthase, putative, expressed	4.55
LOC_Os02g04760.1	Cycloartenol synthase, putative, expressed	-7.1
LOC_Os04g27190.1	Terpene synthase, putative, expressed	4.03
LOC_Os04g43800.1	Phenylalanine ammonia-lyase, putative, expressed	-4.4
LOC_Os05g35290.1	Phenylalanine ammonia-lyase, putative, expressed	-3.3
LOC_Os02g41680.1	Phenylalanine ammonia-lyase, putative, expressed	-3.1
LOC_Os09g23550.1	Dehydrogenase, putative, expressed	-2.4
LOC_Os07g23150.1	Transferase family protein, putative, expressed	-7.8
LOC_Os07g34260.1	Chalcone and stilbene synthases, putative, expressed	-3
LOC_Os04g53780.1	Leucoanthocyanidin reductase, putative, expressed	-2.7
LOC_Os04g53800.1	Leucoanthocyanidin reductase, putative, expressed	-6.5
LOC_Os01g13590.1	Isoflavone reductase homolog IRL, putative, expressed	4.1
LOC_Os01g63190.1	Laccase precursor protein, putative, expressed	-3.8

*(Log_2 fold change ≤ 2, FDR corrected value ≥ 0.05