Gene Expression Analysis of Resistant and Susceptible Rice Cultivars to Sheath Blight after Inoculation with *Rhizoctonia Solani*

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Abstract

Background: Rice sheath blight, caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris*), is one of the most severe diseases in rice (*Oryza sativa* L.) worldwide. Studies on resistance genes and resistance mechanisms of rice sheath blight has mainly focused on indica rice. Rice sheath blight is a growing threat to rice production with the increasing planting area of japonica rice in Northeast China, and it is therefore essential to explore the mechanism of sheath blight resistance in this rice subspecies.

Results: In this study, RNA-seq technology was used to analyse the gene expression changes of leaf sheath inoculation with *R. solani* at 12, 24, 36, 48, and 72 h after inoculation of the resistant cultivar 'Shennong 9819' and susceptible cultivar 'Yueguang'. A total of 2275 differentially expressed genes (DEGs) were identified in our study. Gene Ontology (GO) annotation and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis of these DEGs showed that the metabolic pathways of the two rice cultivars were similar and approximately covered most of the biological processes after inoculation with *R. solani*.

Conclusion: The main differences between the resistant and susceptible cultivars were the activity of DEGs in response to *R. solani* and the enrichment of metabolic pathways involved in these genes. Upon experiencing external stress, the genes in the leaf sheath of Shennong 9819 showed a fluctuating response, whereas the genes in the leaf sheath of Yueguang had a higher activity. The phenylalanine metabolism and plant hormone signal transduction play important roles in disease resistance. Their response in the leaf sheath of Shennong 9819 was faster than that of Yueguang, and they were highly enriched at 12 h after inoculation.

Background

Rice (*Oryza sativa* L.) is one of the three most important crops worldwide, and rice sheath blight is one of its most destructive diseases [1–3]. The annual loss of rice products caused by rice sheath blight is as high as 50% worldwide [4–7]. *Rhizoctonia solani* is a soil-borne fungal plant pathogen [8]. The host range of the pathogen is wide, and the sclerotium of the pathogen has strong resistance to the external environment [9–10]. Due to the lack of resistant donors in cultivated cultivars [11–12], studies on the resistance mechanism of rice sheath blight are lacking [13–14]. For a long time, the resistance genes and mechanism of rice sheath blight have mainly focused on indica rice [15–17].

With the rapid development of molecular biology technology and the wide application of various omics techniques in the interaction between plants and pathogens, the identification of rice sheath blight resistance genes and the interaction mechanism between sheath blight pathogen and rice are becoming increasingly deep. Chitinases are the members of PR proteins responsible for the hydrolysis of chitin, a structural polysaccharide of the cell wall of many pathogens. Overexpression of the chitinase gene *CHI11* enhanced resistance to rice sheath blight [18–19]. *OsOSM1*, a gene mainly expressed in the leaf sheath at the booting stage in rice, encodes an osmotin protein belonging to the pathogenesis-related protein 5 family. Overexpression of this gene can enhance the resistance of rice to sheath blight [20]. Plant polygalacturonase-inhibiting protein (PGIP) is a structural protein that can specifically recognise and bind to fungal polygalacturonase (PG). PGIP plays an important role in antifungal activity in plants. Overexpression of PGIP-related genes such as *ZmPGIP3*, *OsPGIP1*, and *OsPGIP2* increases resistance to rice sheath blight in rice [21–23]. Lignin deposition can enhance plant cell walls against pathogens and provide structural barriers for pathogen infection [24]. Overexpression of the lignin-related gene *OsPAL4* increases resistance to rice sheath blight [25]. *OsWRKY4* is an important positive regulatory factor in the interaction between rice and pathogens. It participates in the defence response of rice sheath blight through the jasmonic acid (JA)/ethylene (ET)-dependent signalling pathway [26].

Due to its high throughput and sensitivity, RNA-sequencing (RNA-Seq) technology is increasingly being used in the research and analysis of gene function [27–28]. Transcriptome sequencing technology has been successfully used to study plant-pathogen interactions [29–30]. Bagnaresi et al. [31] used comparative transcriptome technology to analyse the early molecular interaction of resistant and susceptible rice cultivars infected with *Magnaporthe grisea*. They found that chitinase and WRKY transcription factors were involved in the resistance of rice blast. Strauss et al. [32] identified one major Bs4C candidate transcript from pepper by RNA-seq to regulate the transcription activator-like effector AvrBs4 of *Xanthomonas*. Kawahara et al. [33] analysed the mixed transcripts of rice and blast fungus in infected leaves at 24 h after inoculation using the RNA-Seq technique. It was found that in the interaction between host plants and pathogens, the transcripts of glycosyl hydrolase, cutinases, and LysM domain-containing proteins of *M. grisea* were up-regulated, including the pathogenesis-related and phytoalexin biosynthetic genes in rice. Xiao et al. used next-generation sequencing technology to study the gene expression profiles of *Fusarium* head blight-related genes in common wheat. It was found that pathogen-related proteins such as *PR5*, *PR14*, *ABC transporter*, and JA signalling pathway were the key to *Fusarium* head blight resistance [34].

Transcriptional changes in indica rice cultivars inoculated with *R. solani* were analysed using RNA-Seq technology [35]. The rice cultivars used in our study were japonica rice cultivars from Northeast China. This is the first study to compare the gene expression patterns of resistant and susceptible japonica rice inoculated with *R. solani*. In this study, the transcripts of resistant and susceptible cultivars were compared at 12, 24, 36, 48, and 72 h after inoculation with *R. solani*. The results showed significant differences in the expression of differentially expressed genes and genes related to metabolic pathways. The expression characteristics of metabolic pathways related to disease resistance were defined. The key genes related to rice sheath blight resistance were identified, which provided gene resources for molecular-assisted breeding of japonica rice in Northeast China.

Results

Symptoms of Leaf Sheath after Inoculation

The symptoms of leaf sheath after inoculation are shown in Figs.1. After inoculation for 24 h, evident brown spots appeared on the leaf sheath of Yueguang; 48 h after inoculation, they were grey. These spots appeared on the leaf sheath of Shennong 9819 at 36 h after inoculation. The expanded area of the spot of Yueguang was significantly larger than that of Shennong 9819 at 72 h after inoculation.

The inoculated leaf sheath was decolorized by chloral hydrate, stained with aniline blue, and observed under a light microscope. At 12 h after inoculation, hyphae were observed in the leaf sheaths of both cultivars, and more hyphae were found in the leaf sheath of Yueguang than in Shennong 9819. At 24 h after inoculation, infection cushions appeared in the leaf sheaths of both cultivars. The number and density of the infection cushions of Yueguang were greater than those of Shennong 9819 (Figs.2).

RNA-seq Results of Transcriptome Samples

To study the changes in gene expression of the leaf sheath of Shennong 9819 and Yueguang at the initial infection stage of *R. solani*, we used high-throughput sequencing technology to measure the transcription in rice leaf sheaths after inoculation. The transcriptome analysis of each cultivar included five time points with three biological repeats at each time point. A total of 342.24 Gb clean data was obtained from 36 samples; the clean data of each sample reached 8.08 Gb, and the percentage of Q30 base was 92.49% or more (Table S1). A total of 2,294,868,004 single-end clean reads (total records) were obtained after pre-processing the reads (Table S2). The clean reads of each sample were

sequenced with the designated reference genome, and the efficiency of alignment ranged from 80.43% to 92.33%. The correlation analysis among the samples showed that the three repeats of the two cultivars had a high correlation (Figs.3).

Differential Gene Analysis of Leaf Sheath after inoculation

To determine which gene expression had changed and the stage of these changes, we counted the number of different genes between the two rice cultivars at each time point after inoculation with *R. solani* (Table 1). A total of 2275 differentially expressed genes (DEGs) were identified in our study (Table S3). After inoculation with *R. solani*, the number of upregulated genes in the leaf sheath of Yueguang was higher than that of Shennong 9819 at all inoculation time points.

Table 1

Statistics of differentially expressed genes

DEG Set	DEG Number	up-regulated	down-regulated
SS12	2,403	1,242	1,161
SS24	190	129	61
SS36	2,127	815	1,312
SS48	790	658	132
SS72	1,120	818	302
YY12	2,817	1,419	1,398
YY24	3,392	1,983	1,409
YY36	4,873	2,438	2,435
YY48	1,303	661	642
YY72	1,766	881	885

At 12 h, 2,403 DEGs (1,242 upregulated and 1,161 downregulated) were identified in the leaf sheath of Shennong 9819, whereas 2,817 DEGs (1,419 upregulated and 1,398 downregulated) were identified in Yueguang, indicating that the Yueguang sheath was more susceptible to *R. solani* than Shennong 9819; the infection pressure on Yueguang plants was thus higher than that on Shennong 9819. From 12 to 72 h, the number of DEGs in the Yueguang leaf sheath was higher than Shennong 9819. The number of DEGs in the Yueguang leaf sheath was highest at 36 h (4,873 DEGs; 2,438 upregulated, 2,435 downregulated) after inoculation. The data show that the number of DEGs in the susceptible cultivar was higher than that in the resistant cultivar.

The DEGs at 12, 24, 36, 48, and 72 h after inoculation of *R. solani* were analysed for the two cultivars. The DEGs related to the infection response of *R. solani* in rice were further analysed.

In this study, the DEGs of the two cultivars at the same time point (SS12-YY12, SS24-YY24, SS36-YY36, SS48-YY48, and SS72-YY72) were compared (Fig.1), and the number of DEGs for each cultivar at different time points (SS12-SS24-SS36-SS48-SS72 or YY12-YY24-YY36-YY48-YY72h) were also compared (Fig.2). As shown in Fig.1, 1,347 and 1,269 DEGs were identified in the two cultivars at 12 h and 36 h after inoculation, respectively, and the number of these DEGs was higher than that at other time points.

In the leaf sheaths of Shennong 9819, 12 DEGs were continuously expressed at 12, 24, 36, 48, and 72 h after inoculation, including four upregulated genes and seven downregulated genes. At 12 h, 832 DEGs were identified (575 upregulated and 268 downregulated). There were 23 DEGs (15 upregulated and 14 downregulated) discovered at 24 h, and 693 DEGs (284 upregulated and 419 downregulated) were identified at 36 h. In the leaf sheath of Yueguang, 269 DEGs were expressed at 12, 24, 36, 48, and 72 h after inoculation (132 upregulated and 137 downregulated). At 12 h, 537 DEGs (417 upregulated and 172 downregulated) were identified, and 925 DEGs (619 upregulated and 359 downregulated) were identified at 36 h. At 36 h, 1,251 DEGs were identified (612 upregulated and 645 downregulated). In conclusion, the number of DEGs which were continuously expressed in the sheath of Yueguang was higher than that in Shennong 9819 after inoculation.

Gene Ontology (GO) Analysis of Differentially Expressed Genes

GO annotation was used to classify the enriched DEGs between the control and inoculation treatments. The results showed that these enriched DEGs were involved in many biological activities (Fig.3).

After annotating the GO database, all the DEGs were classified into three main categories: biological process, molecular function, and cellular component. In the biological process, “metabolic process”, “cellular process”, “single-organism process”, “response to stimulus”, and “biological regulation” were the five processes with the highest degree of DEGs enrichment. In terms of molecular function, most DEGs were concentrated in two processes: “catalytic activity” and “binding”. For the cellular component, the five most common processes were “cell”, “membrane”, “organelle”, “organelle part” and “cell part”.

The results showed that the GO terms in different cultivars showed completely different patterns of expression. Taking “response to stimulation” as an example, in the leaf sheath of Shennong 9819, the number of DEGs related to this process reached the maximum at 12 h. The number of DEGs was the least at 24 h after inoculation, indicating that plants responded most strongly to external stimuli at 12 h and were closest to the uninoculated state at 24 h. The results showed that the plants could repair themselves in response to external stimuli; at 36 h, the number of DEGs increased again, and at 48 h, the number of DEGs decreased again. Shennong 9819 exhibited a fluctuating response to external stress. However, the number of DEGs related to the process of “response to stimulation” increased rapidly in the leaf sheath of Yueguang after inoculation, reached a maximum at 36 h and then decreased sharply. The genes related to this process in the leaf sheath of Yueguang were always active after inoculation. The results showed that Shennong 9819 and Yueguang had different resistance patterns to *R. solani* infection, and the genes in the leaf sheath of Yueguang were always in a higher activity state.

Analysis of Metabolic Pathways of Two Rice Cultivars after Inoculation

To further study the specificity of pathways affected by *R. solani* infection in Shennong 9819 and Yueguang, Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis was performed on the upregulated genes ($|\log_2fc| > 1$, FDR < 0.05) at different times after inoculation (Table S4 and S5). The results showed that alanine, aspartate and glutamate metabolism was significantly enriched in both cultivars at 12 h after inoculation, although Shennong 9819 was more significant. The phenylalanine metabolism, plant hormone signal transduction, tropane, piperidine and pyridine alkaloid biosynthesis pathways were significantly enriched in Shennong 9819 at 12 and 36 h after inoculation; however, no significant difference was found in Yueguang. The tyrosine metabolism and isoquinoline alkaloid biosynthesis pathways were significantly enriched in Shennong 9819 at 24 and 36 h after inoculation, but not

in Yueguang. At 36 h after inoculation of Shennong 9819 and Yueguang with *R. solani*, glycine, serine and threonine metabolism and beta-alanine metabolism pathways were significantly enriched, whereas Yueguang was not.

Similarly, some pathways were specifically enriched in Yueguang. For example, ascorbate and aldarate metabolism pathway was significantly enriched at 12 and 24 h after inoculation in Yueguang. The linoleic acid metabolism pathway was significantly enriched 24 h after inoculation in Yueguang. Valine, leucine and isoleucine degradation pathway was significantly enriched at 24 and 36 h in Yueguang after inoculation. The arginine biosynthesis and 2-Oxocarboxylic acid metabolism pathways were significantly enriched at 36 h in Yueguang after inoculation. The propanoate metabolism pathway was significantly enriched at 24, 36, 48, and 72 h in Yueguang after inoculation. The above pathways were not significantly enriched at any time in Shennong 9819 after inoculation. At different times after inoculation, the upregulated genes of the two cultivars had specific enrichment pathways, suggesting that the resistance mechanisms of the two cultivars might be different.

To identify potential regulatory genes closely related to the phenylalanine metabolism pathway, DEGs involved in phenylalanine metabolism were identified by comparing the two cultivars. At 12 and 36 h, the expression levels of genes related to phenylalanine metabolism in the Shennong 9819 leaf sheath were significantly higher than those in Yueguang. The difference between the two cultivars is shown in the heatmap (Fig.4). Through gene analysis, a series of PAL genes was activated after inoculation with *R. solani*: *OsPAL1* (LOC_Os02g41630), *OsPAL2* (LOC_Os02g41650), *OsPAL3* (LOC_Os02g41670), *OsPAL4* (LOC_Os02g41680), *OsPAL6* (LOC_Os04g43800), and *OsPAL9* (LOC_Os12g33610). In this study, the expression of *OsPAL1* and *OsPAL6* in the leaf sheath of Shennong 9819 was higher than that in Yueguang (Fig.5).

Furthermore, we compared the DEGs involved in plant hormone signalling pathway between the two cultivars. We found that the expression of genes closely related to plant hormone signal transduction in the leaf sheaths of the two cultivars was similar to that of phenylalanine metabolism. At 12 and 36 h, the expression levels of genes related to plant hormone signal transduction in Shennong 9819 leaf sheaths were significantly higher than those in Yueguang (Fig.6). The resistance-related protein kinases *OsSAPK9* (LOC_Os12g39630) [36] and *OsSAPK10* (LOC_Os03g41460) [37] were upregulated in the leaf sheaths of Shennong 9819, and their expression levels were higher than those of Yueguang (Fig.5). In addition, *OsNPR1* (LOC_Os01g09800), a positive regulator related to resistance, was also detected and upregulated in both cultivars (Fig.5). In the early stage of infection with *R. solani*, the two cultivars initiated similar pathways in response to stimulation; however, the expression of resistance-related genes may be different in time and expression levels.

As previously mentioned, the two cultivars participated in similar metabolic pathways after inoculation with *R. solani*; however, the upregulated differential expression pathways were different. Among the pathways related to disease resistance, plant hormone signal transduction and phenylalanine metabolism pathways were significantly enriched in Shennong 9819, except in Yueguang. However, ascorbate and aldarate metabolism, and linoleic acid metabolism were significantly enriched in Yueguang, though not in Shennong 9819. It is necessary to further study the differentially expressed genes in the metabolic pathway to understand the different resistance mechanisms of the two cultivars after inoculation with *R. solani*.

Validation of DEGs by Quantitative RT-PCR (qRT-PCR)

Real-time quantitative PCR (qRT-PCR) was used to validate RNA-seq data. *OsPR1b* and other genes were selected for further validation [38]. The *Ct* values obtained by qRT-PCR were normalised. The fold change in gene expression of the two rice cultivars inoculated with *R. solani* was calculated. The results showed that the expression trend of qRT-PCR was consistent with that of the RNA sequence, indicating that Illumina data were relatively reliable (Figs.4).

Discussion

Plant-pathogen interaction

Pathogenic-related proteins (PR) are markers of plant defence responses related to plant resistance to pathogens [39-40]. PR1 proteins are the first pathogenesis-related proteins identified in the PR family [41]. In this study, *OsPR1b* and *OsPR1a* were detected, and their expression trends were quite different (Fig.5). The expression level of *OsPR1b* in the resistant cultivar Shennong 9819 was always higher than that in the susceptible cultivar Yueguang. In contrast, the expression level of *OsPR1a* in Yueguang was higher than that in Shennong 9819 at most time points, indicating that they may have different resistance mechanisms.

After pathogen infection, timely transcriptional regulation of defence genes in plants is very important [42-43]. WRKY family proteins are important regulators of this defence response pathway [44-45]. Different WRKY proteins have different regulatory effects on crop defence responses. For example, OsWRKY28, OsWRKY62, and OsWRKY76 are negative regulators of the rice defence response, and OsWRKY45 is a positive regulator of rice resistance to M. grisea and X. oryzae pv. oryzae(Xoo) [46]. In rice, overexpression of OsWRKY30 can improve the resistance of rice to sheath blight and blast [47]. In this study, OsWRKY24, OsWRKY30, OsWRKY53, and OsWRKY70 were detected (Table S6). The degree of gene expression variability was higher in the Shennong 9819 leaf sheath than in the Yueguang leaf sheath. The expression level of OsWRKY30 in Shennong 9819 was higher than that in Yueguang (Fig.5).

In plants, Ca^{2+} acts as a core signal transducer and regulator in adapting plants to environmental conditions. Calcium concentration changes in response to various stimuli, including hormones, pathogens, light, and abiotic stresses [48-49]. The major calcium-binding proteins include calmodulin (CaM), calmodulin-like proteins (CML), calcium-dependent protein kinases (CDPKs), and calcineurin-B-like proteins (CBL) [50]. This study detected some genes related to calcium-dependent signalling (Table S6). Among them, *OsCPK9* plays an important role in the signal transduction of rice blast [49].

Plant Signal Transduction in Plant Disease Resistance

In the natural environment, plants are constantly subjected to abiotic and biotic stresses, such as drought, salinity, and pathogen infection. Stress signals are recognised and transmitted to different cell compartments through special signalling pathways, in which protein kinases and phosphatases are the key components [51-52]. Members of the sucrose nonfermenting1-related protein kinase2 (SnRK2) gene family are plant-specific serine/threonine kinases involved in plant responses to abiotic stresses and abscisic stresses [36, 53]. All members of the SnRK2 protein kinase gene family encoded by the rice genome are activated by hyperosmotic stress, and have been designated as stress-activated protein kinases (SAPKs). Studies have shown that four *OsSAPKs* are significantly upregulated in *X. oryzae* pv. *oryzicola* (*Xoc*) infection in rice plants: *OsSAPK3*, *OsSAPK5*, *OsSAPK7*, and *OsSAPK9*; these are positive regulators of rice bacterial leaf streak resistance to *Xoc* [36]. It has also been reported that *OsSAPK10* promotes endogenous JA levels in rice and enhances the resistance of rice to *Xoo* [37] (Fig.5).

The *NON EXPRESSOR OF PR1* (*NPR1*) gene is a key factor in salicylic acid (SA)-mediated disease resistance, and *OsNPR1* overexpression enhances resistance to *Xoo* [54]. It has been reported that the Arabidopsis genome contains five *NPR1* homologous genes, among which *NPR3* and *NPR4* also play a role in plant disease resistance [55]. *NPR3* and *NPR4* negatively regulate PR gene expression and pathogen resistance through their association with TAG2 and their homologs [55]. In this study, *OsNPR1* was upregulated in both cultivars (Fig.5). *OsNPR3* was downregulated in the

susceptible cultivar Yueguang at 36 h, while rTGA2.1 was downregulated in the resistant cultivar at 12 h and 36 h (Fig.5).

Expression of OsPAL in Plant Disease Resistance

Phenylpropanoid metabolism is an important metabolic pathway in the secondary metabolism of plant disease resistance [56], which leads to the biosynthesis of a wide range of plant natural products, including hydroxycinnamic acids, flavonoids, coumarins, lignin, condensed tannins, and stilbenes, which have various biological functions, such as UV protectants, signal molecules, phytoalexins, and flower pigments [57]. L-phenylalanine ammonialyase (PAL; EC 4.3.1.5) plays a crucial role in phenylpropanoid metabolism by catalysing the deamination of L-phenylalanine to form *trans*-cinnamic acid [58], which is a precursor for the biosynthesis of lignin and other phenolics such as SA [59], which accumulate when infected [60-61]. Studies have shown that SA is essential for systemic acquired resistance (SAR) [62]. Moreover, it has been shown that when inoculated with the same pathogen, the lignin concentration obtained in the resistant tissues was higher than that of the susceptible tissues [63]. It has been reported that the transcripts of PAL genes accumulate in several incompatible host-pathogen combinations [64], and PAL participates in inducing plant defence responses [65-66]. Transgenic tobacco with suppressed expression of PAL genes showed reduced basal resistance to *Cercospora nicotianae* [67].

In this study, KEGG analysis showed that the expression level of the phenylalanine metabolism pathway in the leaf sheath of the resistant cultivar Shennong 9819 was significantly higher than that of the susceptible cultivar Yueguang at 12 and 36 h after inoculation. We speculated that in the initial stage of infection, Shennong 9819 might protect the host by increasing the secondary metabolites related to disease resistance, thus improving the resistance of plants.

Overexpression of PAL genes induces plant resistance in the interaction between plants and pathogens [64]. It has been proved that overexpression of *OsPAL 1* confers resistance to *M. grisea* in wild-type rice [68]. *OsPAL 6* regulates SA and lignin biosynthesis [25, 61]. The *OsPAL 4* mutant showed increased sensitivity to rice bacterial blight, blast, and sheath blight diseases [25]. *OsPAL 9* may be involved in plant defence [69]. In this study, the expression levels of *OsPAL 1* and *OsPAL 6* were significantly higher in the resistant cultivar than in the susceptible cultivar. Compared with *OsPAL 1* and *OsPAL 6*, *OsPAL 4* and *OsPAL 9* were highly expressed in susceptible cultivars (Fig.5). The improvement in resistance of resistant cultivars may be related to the overexpression of different PAL genes.

Conclusion

In conclusion, the RNA-seq technique was used to analyse the transcript levels of rice cultivars with different resistance levels after inoculation with *R. solani*. The results showed that in the early stage of infection, the difference in the DEGs activated in the leaf sheaths of different resistant cultivars was significant. After inoculation, the DEGs induced from the leaf sheath of resistant cultivar Shennong 9819 and susceptible cultivar Yueguang were classified by GO annotation. The results showed that these enriched DEGs participated in many biological activities. The patterns of expression of the GO process for different cultivars are completely different. Shennong 9819 exhibited a wave response to adapt to external stress. The genes in the sheath of Yueguang were constantly in a state of higher activity. KEGG analysis was conducted on the upregulated genes at different times after the inoculation of the two cultivars, and the expression of resistance-related pathways was significantly different between the two cultivars. The pathways of phenylalanine metabolism, plant hormone signal transduction, tropane, piperidine and pyridine alkaloid biosynthesis, and isoquinoline alkaloid biosynthesis were significantly enriched in Shennong 9819 after inoculation, although Yueguang was not evident. Similarly, ascorbate and aldarate metabolism, linoleic acid metabolism, arginine biosynthesis, and propanoate metabolism were significantly enriched in Yueguang after inoculation. These differences showed that Shennong 9819

and Yueguang initiated different resistance mechanisms after sheath blight infection. We believe that the phenylalanine metabolism pathway, plant hormone signal transduction, and plant pathogen interactions play important roles in the process of disease resistance. Further studies are needed to identify candidate genes to determine whether these DEGs are caused by *R. solani* infection in Shennong 9819 and Yueguang. The data obtained in this study can screen suitable candidate genes, improve the genetic improvement of susceptible rice cultivars, and cultivate japonica rice cultivars that are resistant to sheath blight.

Materials And Methods

Plant Growth

Shennong 9819, a japonica rice cultivar from the Rice Research Institute of Shenyang Agricultural University (Shenyang, China), is a rice cultivar resistant to rice sheath blight. Resistance was identified by the Rice Disease Research Office of the Shenyang Agricultural University (Shenyang, China)[70].

Yueguang is a japonica rice cultivar from Japanese, a cultivar susceptible to rice sheath blight.

All resistant and susceptible cultivars were planted at the experimental base of Jiamusi Branch of Heilongjiang Academy of Agricultural Sciences (Jiamusi, China).

Pathogen Inoculation

The strain of *R. solani* used in this experiment, R-36, was provided by the Rice Disease Research Office of Shenyang Agricultural University.

A short toothpick (1 mm diameter, 1.0–1.2 cm long) colonised by R-36 was used as the inoculum for pathogen infection. Sterilised short toothpicks were placed on non-coagulated PDA plates. After the culture medium solidified, the activated mycelium was inoculated in the culture dish, cultured at 28°C for 5 d, and a short toothpick with mycelium was selected for inoculation. At the late tillering stage, inoculation was performed using a short toothpick with mycelium. The inoculation site was the third leaf sheath from the top of the plant. The inoculation tool used was the forceps. When inoculated, the original state of the leaf sheath should be maintained. To maintain the same temperature and humidity as the inoculated leaf sheath, the inoculated leaf sheath was wrapped with a cling film.

Sample Collection Method and Time

The inoculated leaf sheaths were cut from the plant 12, 24, 36, 48, and 72 h after inoculation, and uninoculated rice leaf sheaths were collected at 0 h after inoculation as a control. Samples collected from plants were placed in cryotubes separately, immediately frozen in liquid nitrogen, and stored at –80°C. The transcriptome sequencing and cDNA library construction of all samples were completed by Beijing Biomarker Technology, Inc.(Beijing, China).

Quality Control, Analysis, Comparison and Quantification of Sequencing Data

Raw reads in fast format were first processed using internal Perl scripts. Clean reads were obtained by removing the reads containing adapter, poly-n, and low-quality reads from the raw reads. Q20, Q30, GC-content, and sequence duplication levels of the clean reads were calculated. All downstream analyses were based on high-quality, clean reads.

These clean reads were mapped to the reference genome sequence. The reference genome used was Nipponbare MSU_v7.0. The programme Hisat2 was used to compare the reads [71], and stringties were used to assemble, evaluate, and quantify the reads [72]. Fragments per kilobase of transcript per million fragments mapped (FPKM) was used to calculate gene expression [73].

Screening and Functional Annotation of DEGs

Differential expression analysis of the differential groups was performed using DEseq [74]. The resulting P values were adjusted using Benjamini and Hochberg's approach to control the false discovery rate. Genes with an adjusted P-value < 0.01 found by DEseq, were assigned as differentially expressed. The GO enrichment analysis of the DEGs was implemented using the Goseq R package [75], which can adjust for gene length bias in DEGs. We used KOBAS software to test the statistical enrichment of DEGs in the KEGG pathways [76].

Real-Time PCR Analysis

Eight genes that were co-expressed in the two cultivars were selected for real-time quantitative PCR. Specific primers were designed using Primer-BLAST of NCBI and are listed in Table S7.

The RNA was extracted using the TaKaRa MiniBEST Plant RNA Extraction Kit, and the 1st Strand cDNA Synthesis Kit (TaKaRa, Tokyo, Japan) was used for reverse transcription into cDNA. qRT-PCR experiments were performed using a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, Hercules, CAUSA) according to the manufacturer's instructions. Reactions were prepared using 20 µL of total volume, 10 µL of SYBR® Premix Ex Taq™ II, 1.0 µL of gene-specific primers (0.5 µL each primer), and 0.5 µL of cDNA.

The reaction procedure was run as follows: (1) 95°C for 30 s; (2) 95°C for 5 s, 60°C for 30 s, for 40 cycles. (3) 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. The actin gene was used as an internal control to normalise the data. Gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ algorithm [77].

Abbreviations

DEGs: differentially expressed genes; ET: ethylene; GO: gene ontology; JA: jasmonic acid; KEGG: Kyoto encyclopaedia of genes and genomes; *NPR1*: *nonexpressor of PR1* genes; PAL: L-phenylalanine ammonialyase; PG: polygalacturonase; PGIP: plant polygalacturonase-inhibiting protein; PR: pathogenic-related; qRT-PCR: real-time quantitative PCR; RNA-Seq: RNA-sequencing; SA: salicylic acid; SAR: systemic acquired resistance; TPM: transcripts per million; *Xoo*: *Xanthomonas oryzae* pv. *oryzae*; *Xoc*: *Xanthomonas oryzae* pv. *Oryzicola*.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Material

All datasets generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

YXH participated in the conception and design of the study, performed the experimental work and data analysis, and drafted the manuscript. WSH and FJF supervised the study and wrote the commentary. GX, and MQY participated in the experimental design. YLL, and DJJ participated in the conception and experimental design of the study, and assisted in biological interpretation. GXD, and ZMM assisted with computational data analysis and biological interpretation, and revised the manuscript. All of the authors reviewed and accepted the final version of the manuscript.

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References

1. Telesh-Sasani S, Soltani BM, Behmanesh M, Safaie N. A magnaporthe avr-pita gene orthologous in *Rhizoctonia solani* AG1-IA shows characteristic of an effector protein. *Australas Plant path.* 2015;44(5):567–574. <https://doi.org/10.1007/s13313-015-0372-5>.
2. Swain DM, Sahoo RK, Chandan RK, Ghosh S, Kumar R, Jha G, et al. Concurrent overexpression of rice G-protein β and γ subunits provide enhanced tolerance to sheath blight disease and abiotic stress in rice. *Planta*.

- 2019;250(5):1505–1520. <https://doi.org/10.1007/s00425-019-03241-z>.
3. Choudhary P, Rai P, Yadav J, Verma S, Chakda H, Goswami SK, et al. A rapid colorimetric LAMP assay for detection of *Rhizoctonia solani* AG-1 IA causing sheath blight of rice. *Sci Rep-uk*. 2020;10(1):22022. <https://doi.org/10.1038/s41598-020-79117-0>.
 4. Lee F N, Rush M C. Rice Sheath Blight: A Major Rice Disease, *Plant Dis*. 1983;67(7):829–832. <https://doi.org/10.1094/PD-67-829>.
 5. Liu G, Jia Y, Correa-Victoria FJ, Prado GA, Yeater KM, McClung A, et al. Mapping quantitative trait loci responsible for resistance to sheath blight in rice. *Phytopathology*. 2009;99:1078–1084. <https://doi.org/10.1094/PHYTO-99-9-1078>.
 6. Zheng A, Lin R, Zhang D, Qin P, Xu L, Ai P, et al. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. *Nat Commun*. 2013;4:1424. <https://doi.org/10.1038/ncomms2427>.
 7. Zhou Y, Bao J, Zhang D, Li Y, Li H, He H. Effect of heterocystous nitrogen-fixing cyanobacteria against rice sheath blight and the underlying mechanism. *Appl Soil Ecol*. 2020;153(5):103580. <https://doi.org/10.1016/j.apsoil.2020.103580>.
 8. Okubara PA, Dickman MB, Blechl AE. Molecular and genetic aspects of controlling the soilborne necrotrophic pathogens *Rhizoctonia* and *Pythium*. *Plant Sci*. 2014;228:61–70. <https://doi.org/10.1016/j.plantsci.2014.02.001>.
 9. Lin R, He L, He J, Qin P, Wang Y, Deng Q, et al. Comprehensive analysis of microRNA-Seq and target mRNAs of rice sheath blight pathogen provides new insights into pathogenic regulatory mechanisms. *DNA Res*. 2016;23, 415–425. <https://doi.org/10.1093/dnares/dsw024>.
 10. Singh P, Mazumdar P, Harikrishna JA, Babu S. Sheath blight of rice: a review and identification of priorities for future research. *Planta*. 2019;250(5):1387–1407. <https://doi.org/10.1007/s00425-019-03246-8>.
 11. Maeda S, Dubouzet JG, Kondou Y, Jikumaru Y, Seo S, Oda K, et al. The rice *CYP78A* gene *BSR2* confers resistance to *Rhizoctonia solani* and affects seed size and growth in *Arabidopsis* and rice. *Sci Rep-uk*. 2019;9(1):587. <https://doi.org/10.1038/s41598-018-37365-1>.
 12. Rao TB, Chopperla R, Methre R, Punniakotti E, Venkatesh V, Sailaja B, et al. Pectin induced transcriptome of a *Rhizoctonia solani* strain causing sheath blight disease in rice reveals insights on key genes and RNAi machinery for development of pathogen derived resistance. *Plant Mol Biol*. 2019;100:59–71. <https://doi.org/10.1007/s11103-019-00843-9>.
 13. Ghosh S, Kanwar P, Jha G. Alterations in rice chloroplast integrity, photosynthesis and metabolome associated with pathogenesis of *Rhizoctonia solani*. *Sci Rep-uk*. 2017;7, 41610. <https://doi.org/10.1038/srep41610>.
 14. Zhao C, Wang A, Shi Y, Wang L, Liu W, Wang Z, et al. Identification of defense-related genes in rice responding to challenge by *Rhizoctonia solani*. *Theor Appl Genet*. 2008;116:501–516. <https://doi.org/10.1007/s00122-007-0686-y>.
 15. Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, et al. Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). *Theor Appl Genet*. 2000;101(4):569–573. <https://doi.org/10.1007/s001220051517>.
 16. Pinson SRM, Capdevielle FM, Oard JH. Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. *Crop Sci*. 2005;45(2):503. <https://doi.org/10.2135/cropsci2005.0503>.
 17. Zuo S, Yin Y, Pan C, Chen Z, Zhang Y, Gu S, et al. Fine mapping of *qSB-11^{LE}*, the QTL that confers partial resistance to rice sheath blight. *Theor Appl Genet*. 2013;126(5):1257–1272. <https://doi.org/10.1007/s00122-013-2051-7>.
 18. Richa K, Tiwari IM, Devanna BN, Botella JR, Sharma V, Sharma TR. Novel chitinase gene *LOC_Os11g47510* from indica rice tetep provides enhanced resistance against sheath blight pathogen *Rhizoctonia solani* in rice. *Frontiers*

in Plant Sci. 2017;8:596. <https://doi.org/10.3389/fpls.2017.00596>.

19. Zhang C, Huang M, Sang X, Li P, Ling Y, Zhao F, et al. Association between sheath blight resistance and chitinase activity in transgenic rice plants expressing *McCHIT1* from bitter melon. Transgenic Res. 2019;28:381–390. <https://doi.org/10.1007/s11248-019-00158-x>.
20. Xue X, Cao ZX, Zhang XT, Wang Y, Zhang YF, Chen ZX, et al. Overexpression of *OsOSM1* enhances resistance to rice sheath blight. Plant Dis. 2016;100(8):1634–1642. <https://doi.org/10.1094/PDIS-11-15-1372-RE>.
21. Zhu G, Liang E, Lan X, Li Q, Qian J, Tao H, et al. *ZmPGIP3* gene encodes a polygalacturonase-inhibiting protein that enhances resistance to sheath blight in rice. Phytopathology. 2019;109(10):1732–1740. <https://doi.org/10.1094/PHYTO-01-19-0008-R>.
22. Wang R, Lu L, Pan X, Hu Z, Ling F, Yan Y, et al. Functional analysis of *OsPGIP1* in rice sheath blight resistance. Plant Mol Biol Rep. 2015;87:181–191. <https://doi.org/10.1007/s11103-014-0269-7>.
23. Chen X, Chen Y, Zhang L, He Z, Huang B, Chen C, et al. Amino acid substitutions in a polygalacturonase inhibiting protein (*OsPGIP2*) increases sheath blight resistance in rice. Rice. 2019;12(1):56. <https://doi.org/10.1186/s12284-019-0318-6>.
24. Kawasaki T, Koita H, Nakatsubo T, Hansegawa K, Wakabayashi K, Takahashi H, et al. Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is an effector of small GTPase Rac in defense signaling in rice. Proc Natl Acad Sci U S A. 2006;103(1):230–235. <https://doi.org/10.1073/pnas.0509875103>.
25. Tonnessen BW, Manosalva P, Lang JM, Baraoida M, Bordeos A, Mauleon R, et al. Rice phenylalanine ammonia-lyase gene *OsPAL4* is associated with broad spectrum disease resistance. Plant Mol Biol. 2015;87(3):273–86. <https://doi.org/10.1007/s11103-014-0275-9>.
26. Wang H, Meng J, Peng X, Tang X, Zhou P, Xiang J, et al. Rice *WRKY4* acts as a transcriptional activator mediating defense responses toward *Rhizoctonia solani*, the causing agent of rice sheath blight. Plant Mol Biol Rep. 2015;89:157–171. <https://doi.org/10.1007/s11103-015-0360-8>.
27. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews. Genetics. 2009;10(1):57–63. <https://doi.org/10.1038/nrg2484>.
28. Metzker M L. Sequencing technologies-the next generation. Nat Rev Genet. 2010;11:31–46. <https://doi.org/10.1201/9781420008876.pt1>.
29. Zhang H, Yang Y, Wang C, Liu M, Li H, Fu Y, et al. Large-scale transcriptome comparison reveals distinct gene activations in wheat responding to stripe rust and powdery mildew. BMC Genomics. 2014;15(1):898. <https://doi.org/10.1186/1471-2164-15-898>.
30. Han Y, Zhang K, Yang J, Zhang N, Fang A, Zhang Y, et al. Differential expression profiling of the early response to *Ustilagoideae virens*, between false smut resistant and susceptible rice varieties. BMC Genomics. 2015;16(1): 955. <https://doi.org/10.1186/s12864-015-2193-x>.
31. Bagnaresi P, Biselli C, Orrù L, Urso S, Crispino L, Abbruscato P, et al. Comparative transcriptome profiling of the early response to *Magnaporthe oryzae* in durable resistant vs susceptible rice (*Oryza sativa* L.) genotypes. Plos one. 2012;7(12):e51609. <https://doi.org/10.1371/journal.pone.0051609>.
32. Strauß T, Poecke RMP, Strauß A, Römer P, Minsavage G, Singh S, et al. RNA-seq pinpoints a *Xanthomonas* TAL-effector activated resistance gene in a large-crop genome. Proc Natl Acad Sci U S A. 2012;109:19480–19485. <https://doi.org/10.1073/pnas.1212415109>.
33. Kawahara Y, Oono Y, Kanamori H, Matsumoto T, Itoh T, Minami E. Simultaneous RNA-Seq analysis of a mixed transcriptome of rice and blast fungus interaction. Plos one. 2012;7:e49423. <https://doi.org/10.1371/journal.pone.0049423>.

34. Xiao J, Jin X, Jia X, Wang H, Cao A, Zhao W, et al. Transcriptome-based discovery of pathways and genes related to resistance against *Fusarium* head blight in wheat landrace Wangshuibai. BMC Genomics. 2013;14(1):1–19. <https://doi.org/10.1186/1471-2164-14-197>.
35. Zhang J, Chen L, Fu C, Wang L, Liu H, Cheng Y, et al. Comparative transcriptome analyses of gene expression changes triggered by *Rhizoctonia solani* AG1 IA infection in resistant and susceptible rice varieties. Front Plant Sci. 2017;8:1422. <https://doi.org/10.3389/fpls.2017.01422>.
36. Xu MR, Huang LY, Zhang F, Zhu LH, Zhou YL, LI ZK. Genome-wide phylogenetic analysis of stress-activated protein kinase genes in rice (OsSAPKs) and expression profiling in response to *Xanthomonas oryzae* pv. *oryzicola* infection. Plant Mol Biol Rep. 2013;31(4):877–885. <https://doi.org/10.1007/s11105-013-0559-2>.
37. Hou Y, Wang Y, Tang L, Tong X, Wang L, Liu L, et al. SAPK10-mediated phosphorylation on WRKY72 releases its suppression on jasmonic acid Biosynthesis and bacterial blight resistance. iScience. 2019;16:499–510. <https://doi.org/10.1016/j.isci.2019.06.009>.
38. Agrawal GK, Rakwal R, Jwa NS. Rice (*Oryza sativa* L.) *OsPR1b* gene is phytohormonally regulated in close interaction with light Signals. Biochem bioph res commu. 2000;278(2):290–298. <https://doi.org/10.1006/bbrc.2000.3781>
39. Mitsuhashi I, Iwai T, Seo S, Yanagawa Y, Kawahigashi H, Hirose S, et al. Characteristic expression of twelve rice *PR1* family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). Mol Genet Genomics. 2008;279(4):415–427. <https://doi.org/10.1007/s00438-008-0322-9>.
40. Wu Q, Hou MM, Li LY, Liu LJ, Hou YX, Liu GZ. Induction of pathogenesis-related proteins in rice bacterial blight resistant gene XA21-mediated interactions with *Aanthomonas oryzae* pv. *Oryzae*. J Plant Pathol. 2011;93(2):455–459.
41. Showmy KS, Yusuf A. Characterization of disease resistance in nine traditional rice (*Oryza sativa* L.) cultivars and expression of chennellu PR1 gene in response to *Xanthomonas oryzae* pv. *oryzae*. Indian Phytopathology. 2020;73(1). <https://doi.org/10.1007/s42360-020-00220-3>.
42. Nimchuk Z, Eulgem T, Holt BF, Dangl JL. Recognition and response in the plant immune system. Ann Rev Genet. 2003;37(1):579–609. <https://doi.org/10.1146/annurev.genet.37.110801.142628>.
43. Jones DA, Takemoto D. Plant innate immunity-direct and indirect recognition of general and specific pathogen-associated molecules. Curr Opin Immunol. 2004;16:48–62. <https://doi.org/10.1016/j.coi.2003.11.016>.
44. Ülker B, Somssich IE. WRKY transcription factors: from DNA binding towards biological function. Curr Opin Plant Biol. 2004;7(5):491–498. <https://doi.org/10.1016/j.pbi.2004.07.012>.
45. Zhang Y J, Wang L. The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. BMC Evol Biol. 2005;5:1. <https://doi.org/10.1186/1471-2148-5-1>.
46. Han M, Ryu HS, Kim CY, Park DS, Ahn YK, Jeon JS. *OsWRKY30* is a transcription activator that enhances rice resistance to the *Xanthomonas oryzae* pathovar *oryzae*. J Plant Biol. 2013;56(4):258–265. <https://doi.org/10.1007/s12374-013-0160-0>.
47. Peng X, Hu Y, Tang X, Zhou P, Deng X, Wang H, et al. Constitutive expression of rice *WRKY30* gene increases the endogenous jasmonic acid accumulation, *PR* gene expression and resistance to fungal pathogens in rice. Planta. 2012;236(5):1485–1498. <https://doi.org/10.1007/s00425-012-1698-7>.
48. Zeng H, Xu L, Singh A, Wang H, Du L, Poovaiah BW. Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. Front Plant Sci. 2015;6:600. <https://doi.org/10.3389/fpls.2015.00600>.
49. Asano, T. Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice. Plant Cell Physiol. 2005;46(2):356–66. <https://doi.org/10.1093/pcp/pci035>.

50. Reddy ASN, Ali GS, Celesnik H, Day IS. Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. *Plant Cell*. 2011;23(6):2010–2032. <https://doi.org/10.1105/tpc.111.084988>.
51. Stone JM, Walker JC. Plant protein kinase families and signal transduction. *Plant Physiol*. 1995;108:451–457. <https://doi.org/10.1104/pp.108.2.451>.
52. Agueci F, Rutten T, Demidov D, Houben A. Arabidopsis AtNek2 kinase is essential and associates with microtubules. *Plant Mol Biol Rep*. 2012;30(2):339–348. <https://doi.org/10.1007/s11105-011-0342-1>.
53. Wang P, Xue L, Batelli G, Lee S, Hou Y, Oosten MJV, et al. Quantitative phosphoproteomics identifies SnRK2 protein kinase substrates and reveals the effectors of abscisic acid action. *Proc Natl Acad Sci U S A*. 2013;110(27):11205–11210. <https://doi.org/10.1073/pnas.1308974110>.
54. Liu G, Holub EB, Alonso JM, Ecker JR, Fobert PR. An Arabidopsis *NPR1*-like gene, *NPR4*, is required for disease resistance. *Plant J*. 2005;41:304–318. <https://doi.org/10.1111/j.1365-313X.2004.02296.x>.
55. Zhang Y, Cheng YT, Qu N, Zhao Q, Bi D, Li X. Negative regulation of defense responses in Arabidopsis by two *NPR1* paralogs. *Plant J*. 2006;48:647–656. <https://doi.org/10.1111/j.1365-313X.2006.02903.x>.
56. Dixon RA, Achnine L, Kota P, Liu C, Reddy MSS, Wang L. The phenylpropanoid pathway and plant defence- a genomics perspective. *Mol Plant Pathol*. 2002;3(5):371–390. <https://doi.org/10.1046/j.1364-3703.2002.00131.x>.
57. Reichert AI, He XZ, Dixon RA. Phenylalanine ammonia-lyase (PAL) from tobacco (*Nicotiana tabacum*): characterization of the four tobacco *PAL* genes and active heterotetrameric enzymes. *Biochem J*. 2009;424(2):233–242. <https://doi.org/10.1042/BJ20090620>.
58. Liu Y, Liu L, Yang S, Zeng Q, He Z, Liu Y. Cloning, Characterization and Expression of the Phenylalanine Ammonia-Lyase Gene (*PaPAL*) from Spruce *Picea asperata*. *Forests*. 2019; 10(8):613. <https://doi.org/10.3390/f10080613>.
59. Mauch-Mani B, Slusarenko AJ. Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of Arabidopsis to *Peronospora parasitica*. *Plant Cell*. 1996;8(2):203–212. <https://doi.org/10.2307/3870265>.
60. Duan L, Liu H, Li X, Xiao J, Wang S. Multiple phytohormones and phytoalexins are involved in disease resistance to *Magnaporthe oryzae* invaded from roots in rice. *Physiol Plant*. 2014, 152(3):486–500. <https://doi.org/10.1111/ppl.12192>.
61. He J, Liu Y, Yuan D, Duan M, Liu Y, Shen Z, et al. An R2R3 MYB transcription factor confers brown planthopper resistance by regulating the phenylalanine ammonia-lyase pathway in rice. *Proc Natl Acad Sci*. 2019;117(1):201902771. <https://doi.org/10.1073/pnas.1902771116>.
62. Chen Z, Zheng Z, Huang J, Lai Z, Fan B. Biosynthesis of salicylic acid in plants. *Plant Signaling & Behavior*. 2009; 4(6):493–496. <https://doi.org/10.1073/pnas.1902771116>.
63. Bhuiyan NH, Selvaraj G, Wei Y, King J. Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J Exp Bot*. 2009;60(2):509–521. <https://doi.org/10.1093/jxb/ern290>.
64. Edwards K, Cramer CL, Bolwell GP, Dixon RA, Schuch W, Lamb C. Rapid transient induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells. *Proc Natl Acad Sci U S A*. 1985;82(20):6731–6735. <https://doi.org/10.1073/pnas.82.20.6731>.
65. Tanaka N, Che FS, Watanabe N, Fujiwara S, Takayama S, Lsogai A. Flagellin from an incompatible strain of *Acidovorax avenae* mediates H₂O₂ generation accompanying hypersensitive cell death and expression of *PAL*, *Chl-1*, and *PBZ1*, but not of *Lox* in rice. *Mol Plant Microbe Interact*. 2003;16(5):422–428. <https://doi.org/10.1094/MPMI.2003.16.5.422>.
66. Kim DS, Hwang BK. An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signalling of the defence response to microbial pathogens. *J Exp Bot*. 2014;65(9):2295–2306.

<https://doi.org/10.1093/jxb/eru109>.

67. Maher EA, Bate NJ, Ni W, Elkind Y, Dixon RA, Lamb CJ. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. *Proc Natl Acad Sci U S A*. 1994;91(16):7802–7806.
68. Zhou X, Liao H, Chern M, Yin J, Chen Y, Wang J, et al. Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. *Proc Natl Acad Sci U S A*. 2018;115:12. <https://doi.org/10.1073/pnas.1705927115>.
69. Yan J, Aboshi T, Teraishi M, Strichler SR, Spindel JE, Tung CW, et al. The tyrosine aminomutase tam1 is required for β -tyrosine biosynthesis in rice. *Plant Cell*. 2015;27(4):1265–1278. <https://doi.org/10.1105/tpc.15.00058>.
70. Yang X, Wei S, Gu X, Yao L, Gao X, Shen H, et al. A preliminary report on resistance of rice germplasm resources in the northeast of China to rice sheath blight disease. *Plant Protection*. 2020;46(6):205–208. (in Chinese with an English Abstract)
71. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat methods*. 2015;12(4):357–360. <https://doi.org/10.1038/nmeth.3317>.
72. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat biotechnol*. 2015;33(3): 290–295. <https://doi.org/10.1038/nbt.3122>.
73. Florea L, Song L, Salzberg SL. Thousands of exon skipping events differentiate among splicing patterns in sixteen human tissues. *F1000 Research*. 2013;2:188. <https://doi.org/10.12688/f1000research.2-188.v1>.
74. Wang L, Feng Z, Wang X, Wang X, Zhang X. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics*. 2010;26(1), 136–138. <https://doi.org/10.1093/bioinformatics/btp612>.
75. Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNAseq: accounting for selection bias. *Genome Biol*. 2010;11(2):1–14. <https://doi.org/10.1186/gb-2010-11-2-r14>.
76. Mao X, Cai T, Olyarchuk JG, Wei L. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. *Bioinformatics*. 2005;21(19):3787–3793. <https://doi.org/10.1093/bioinformatics/bti430>.
77. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using Real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*. 2001;25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>.

Figures

Figure 1

Venn diagram of DEGs discovered in both rice cultivars after inoculation with *Rhizoctonia solani* at the same time points. SS12, SS24, SS36, SS48, and SS72 represent DEGs sets discovered from leaf sheath of Shennong 9819 at 12, 24, 36, 48, and 72 h after inoculation; YY12, YY24, YY36, YY48, and YY72 represent DEGs sets discovered from leaf sheath of Yueguang at 12, 24, 36, 48, and 72 h after inoculation.

Figure 2

Venn diagram of DEGs in both rice cultivars after inoculation with *Rhizoctonia solani* at the different time points. SS12, SS24, SS36, SS48, and SS72 represent DEGs sets discovered from leaf sheath of Shenlong 9819 at 12, 24, 36, 48, and 72 h after inoculation; YY12, YY24, YY36, YY48, and YY72 represent DEGs sets discovered from leaf sheath of Yueguang at 12, 24, 36, 48, and 72 h after inoculation.

Figure 3

Functional classification of DEGs in the Shennong 9819 and Yueguang. SS12, SS24, SS36, SS48, and SS72 represent DEGs sets discovered from leaf sheath of Shennong 9819 at 12, 24, 36, 48, and 72 h after inoculation; YY12, YY24, YY36, YY48, and YY72 represent DEGs sets discovered from leaf sheath of Yueguang at 12, 24, 36, 48, and 72 h after inoculation. (A) Cellular Component (B) Molecular Function (C) Biological Process.

Figure 4

Expression profiles of genes related to phenylalanine metabolism pathways.

Figure 5

Expression of genes related to phenylalanine metabolism, plant-pathogen interaction, and plant signal transduction after inoculation based on transcripts per million (TPM).

Figure 6

Expression profiles of genes related to plant hormone signal transduction pathways.

Supplementary Files

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