# Transcriptome analysis of rice leaves in response to Rhizoctonia solani infection

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# Abstract

Background: Sheath blight disease (ShB) is one of the important diseases that severely affects rice production. However, the mechanism of defense against ShB remains unclear. To understand the molecular mechanism of rice defense to ShB, an RNA-sequencing analysis was performed using Rhizoctonia solani AG1-IA-inoculated rice leaves.

Results: After 48 hours of inoculation, 6,838 genes were differentially expressed in rice leaves (>2 fold, $P<0.05$). Among them, 3,802 genes were upregulated, while 3,036 were downregulated compared to the control group. In addition, the differentially expressed genes were classified via GO, KEGG, and Mapman analyses. Thirty GO terms, including biological process, molecular function, and cellular component, were significantly enriched, and 30 KEGG pathways included ribosome, carbon metabolism, and biosynthesis of amino acids. A Mapman analysis demonstrated that the phytohormone and metabolic pathways were significantly altered. Interestingly, the expression levels of 359 transcription factors, including WRKY, MYB, and NAC family members, as well as 239 transporter genes, including ABC, MFS, and SWEET, were significantly changed upon R. solani AG1-IA inoculation. An additional genetic study showed that OsWRKY53 negatively and OsAKT1 positively regulate rice defense to R. solani, respectively. In addition, interestingly, many differentially expressed genes contain R. solani-responsive cis-elements in their promoter region.

Conclusions: Taken together, our analyses provide valuable information for the additional study of rice defense mechanisms to ShB, and the genes identified could be useful in the future to breed resistant rice.


# Background

Rice (*Oryza sativa)* is one of the most globally important staple crops, which feeds 50% of the global human population (1, 2). As the population grows, rice production needs to be increased, but plant diseases threaten the yield of rice. As one of the three major diseases of rice, sheath blight (ShB) infects rice in a wide area and causes particularly severe yield losses (3). It causes lesions on the rice sheath, leaf and even panicle, resulting in withered leaves and sheaths, as well as a decreased seed setting rate, and can reduce the yield by more than 50% when the disease is severe (4). Currently, treatment with fungicides is the major approach to protect rice from ShB (5). However, fungicides are harmful to the environment, and the long-term use of fungicides could increase the risk of fungicide resistance. Therefore, alternate manners of control, such as breeding disease resistance varieties, have been attempted.

Breeding crops with durable resistance to pathogens is an ideal strategy to manage plant diseases, but disease resistance breeding against ShB has lagged far behind, and the primary reason is a lack of donors that exhibit proper resistance (6). The resistance to ShB is thought to be a quantitative trait that is controlled by multiple genes, and some of these QTLs (quantitative trait locus) have been mapped and functionally characterized (7–9). Extensive studies have analyzed the molecular basis of resistance to

ShB. *PR* (pathogenesis-related) genes are major contributors to the plant defense of pathogens, and the overexpression of *OsOSM1,* which belongs to the PR5 family, could enhance resistance to ShB (10). The overexpression of *OsACS2* promoted rice defense to blast and ShB (11). Our previous research showed that brassinosteroids (BRs) negatively regulate, while ethylene enhances the resistance to ShB. The BR signaling transcription factor RAVL1 activates the key BR and ethylene signaling pathways to modulate the rice defense to ShB (12). *OsWRKY4, OsWRKY13, OsWRKY30,* and *OsWRKY80* have been reported to positively regulate the resistance to ShB (13–16), and more recently, we identified that sugar will eventually be exported via transporter 11 (SWEET11), which negatively regulates the rice resistance to ShB (17). Although much progress has been made, the knowledge about how rice defend themselves against ShB is still fragmented and limited.

Plants rapidly reprogram their transcription profile to respond to external stimuli. Monitoring transcriptome changes provides insights to understand how plants manage adverse conditions (18). Numerous reports illustrate that RNA-Seq research plays an important role in understanding many aspects of pathogen-plant interactions. A set of time-scale transcriptome analysis revealed that functional ETI (effector triggered immunity) responses rely on an appropriate time point for transcriptional reprogramming, which is mediated by jasmonate, ethylene, salicylic acid, and *PAD4* signaling (19). Transcriptome analysis provided useful information for understanding the resistance mechanism of *Brassica napus* to clubroot disease (20) and was performed to investigate the regulatory mechanism of *Pi9* and *Pi21,* two *R* (resistance genes) that control blast fungus (21, 22). The differences in the molecular basis between the interaction of resistant and susceptible cultivars or species with the pathogens (23, 24) suggests that RNA-Seq is an ideal tool to dissect the resistance mechanism of host plants (25).

In this study, we performed an RNA-Seq assay using rice leaves with or without *Rhizoctonia solani* AG1-IA inoculation for 48 hours. A total of 6,838 differentially expressed genes depict the transcription landscape of the rice response to *R. solani* infection, which were further classified via GO, KEGG, and Mapman analyses. In addition, differentially expressed transcription factors and transporters were collected and analyzed. Our results provide new insights into the mechanism of rice defense against *R. solani* and may contribute to resistance breeding.

## Results

# Transcriptome analysis of *Rhizoctonia solani*-infected rice leaves

ShB is one of the severe diseases that affects rice production. However, the molecular mechanism of how rice defends itself against ShB remains unclear. To analyze the rice defense mechanism to ShB, RNA-Seq analysis was performed. Before performing the sequencing, the optimized time point for the response of rice to *R. solani* AG1-IA was tested. One-month-old rice plants inoculated with *R. solani* AG1-IA and the

expression of the two PR genes *PBZ1* and *PR1b* were examined after 0, 24, 48, and 72 hours post inoculation (hpi). The results showed that *OsPBZ1* and *OsPR1b* exhibited the highest expression at 48 hpi (Figure 1). Therefore, 48 hpi was chosen for the RNA-sequencing analysis.

The RNA-sequencing data was established by 150 bp pair-end sequencing, and $2.86*10^8$ reads of three biological replicates. The Q20 and Q30 values were 98% and 95%, respectively, indicating that the RNA-Seq quality is high enough for further evaluation. On average, 95% of the reads were mapped to the Nipponbare reference genome (Os-Nipponbare-Reference-IRGSP–1.0; GenBank assembly accession: GCA_001433935.1). A total of 92.73%, 3.86%, and 3.41% of these reads were mapped to the exon, intergenic, and intron regions, respectively. Ht-Seq software was used to perform the gene expression assay based on a calculation of the FPKM (fragments per kilo bases per million reads) values of the genes (26). The volcano diagram and heatmap displayed the differentially expressed genes from RNA sequencing (Figure 2a). The genes whose expression variation are more than 2-fold between the control and inoculated leaves ($Log_2FC$ = >2, $P \leq 0.05$) were considered as differentially expressed genes (DEGs), and 6,838 genes were significantly changed, including 3,802 upregulated and 3,036 downregulated genes (Figure 2b, Table S1).

# Verification of the DEGs by qRT-PCR

To validate the RNA sequencing results, seven upregulated and two downregulated genes were randomly chosen and further confirmed by qRT-PCR. The qRT-PCR results indicated that seven genes, including *OsSWEET2a, OsSWEET14, OsWRKY108, OsERF096, OsNAC3, OsPR1b,*and *Os07g0550600,* were induced, while *OsMST1* and *Os03g0363500* were repressed by inoculation with *R. solani* at 48 hpi (Figure 3), suggesting that the RNA-Seq and qRT-PCR results are consistent.

# GO, KEGG, and Mapman analyses of the DEGs

To classify the DEGs, Gene Ontology (GO) and KEGG enrichment assays were performed. The DEGs were divided into 30 GO terms, and the most enriched GO terms are catalytic activity, binding, cell part, organelle, metabolic process, and cellular process (Figure 4a). The DEGs are classified into different biochemical or signaling transduction pathways through the KEGG analysis. The results showed that the most enriched pathways are ribosome, carbon metabolism, and biosynthesis of amino acids among the 30 KEGG pathways (Figure 4b).

To obtain additional insights into the DEGs, Mapman analysis was performed. The Mapman data indicated that the pathogen/pest attack, amino acid, sugar, and lipid metabolism, as well as the transporters, were enriched. In the pathogen/pest attack overview, phytohormones, abiotic stress, redox state, secondary metabolites, and cell wall-related were enriched. Among them, abscisic acid, redox-related, cell wall, and secondary metabolic associated genes were the most highly enriched (Figure 5a). In

metabolism, particularly the light reactions, fermentation, and the photorespiration-related genes were enriched (Figure 5b). The transporter overview showed that 20 types of transporters, including ABC transporters, nitrate and ammonium transporters, and lipid transfer proteins were classified. The lipid transfer proteins contained the highest number among them (Figure 5c).

# Identification of differentially expressed transcription factors and transporters

Transcription factors (TFs), such as the WRKY family and transporters and the SWEET genes, are well known for their function in plant defense. Therefore, the differentially expressed transcription factors and transporters from the RNA-Seq data were analyzed and classified. To isolate the TFs and transporters that expressed a differential response to *R. solani* inoculation, the Rice TF Database (https://ricephylogenomics.ucdavis.edu/tf/index.shtml) and the Rice Transporter Database (https://ricephylogenomics.ucdavis.edu/transporter/) were employed (27, 28). Accessed 9 July 2019. In general, 359 differentially expressed TFs with 45 types were identified. Among these TFs, WRKY, AP2-EREBP, bHLH, MYB, and TIFY were the most enriched TF families, and showed 40, 39, 31, 28, and 11 members in each type, respectively (Figure 6a, Table S2). Also, a qRT-PCR analysis was performed to verify the expression patterns of the TFs in response to *R. solani.* The qRT-PCR results indicated that three WRKY members (*OsWKRY28, OsWRKY32,* and *OsWRKY53)* and three TIFY family genes (*OsJAZ5, OsJAZ6,* and *OsJAZ9)* were induced by infection with *R. solani.* Among them, *OsWRKY28* exhibited the highest induction rate (approximately 20-fold) of the three WRKYs, while *OsJAZ6* exhibited the highest expression (approximately 20-fold) among the JAZ genes at 48 hpi (Figure 6b, f)

In addition, 239 differentially expressed transporters with 53 types were isolated. Since the recently characterized SWEET sugar transporters (29) were not included in the Rice Transporter Database, we manually added SWEET to the list of transporters. Among the differentially expressed transporters, ABC, AAAP, POT, and F-ATPase were the most enriched superfamilies, accounting for 22, 21, 19, and 18 members, respectively (Figure 7a). A qRT-PCR analysis was performed to verify the RNA-Seq data. The results showed that *OsAKT1, OsSWEET2b,* and a monosaccharide transporter (*OsMST4)* were significantly induced, while *OsMST8* was obviously suppressed by *R. solani* infection (Figure 7b-e). In addition, *OsSWEET2a* and *OsSWEET14* were induced, while *OsMST1* was repressed by *R. solani* inoculation (Figure 3), and the expression patterns of the seven transporters were similar to the results observed in the RNA-Seq results.

# *OsWRKY53* and *OsAKT1* regulate rice resistance to ShB

Since 40 WRKY genes differentially responded to *R. solani* infection, a genetic study was performed to evaluate their functions in the defense of rice against ShB. *OsWRKY53* was reported to be involved in BR signaling (30) and was significantly induced by *R. solani* infection. However, its function in rice defense is unclear. The *R. solani* AG1-IA detachment assay showed that the *Oswrky53* genome editing mutant was less susceptible, while the *OsWRKY53* overexpressor (OE) was more susceptible to ShB compared to the wild-type control Longjing11 (Figure 7a). The lesion area was 37.29% in Longjing11, 9.97% in *Oswrky53,* and 77.48% in the *OsWRKY53 OE* leaves, respectively (Figure 8b).

Interestingly, potassium transporter *OsAKT1* was significantly induced by *R. solani,* and the rice blast fungus *Magnaporthe grisesa* secreted the effector protein AvrPiz-t, which targets OsAKT1 to inhibit the interaction between CIPK23 and OsAKT1 and partially block the potassium influx to promote virulence (31). However, the function of OsAKT1 in the defense of rice to ShB has not been examined. The results of inoculation with *R. solani* AG1-IA showed that the *Osakt1* mutant (32) was less susceptible to ShB compared with its corresponding wild-type Dongjin (DJ) (Figure 8c). The lesion area was 34.02% in DJ and 30.76% in *Osakt1* leaves, respectively (Figure 8d). These results suggest that *OsWRKY53* negatively regulates the defense of rice to ShB, while *OsAKT1* positively regulates it.

# Identification of *Rhizoctonia solani* responsive *cis*-elements in the promoters of the DEGs

A previous study identified that the promoter of the *R. solani*-induced genes commonly harbors four types of *cis*-element sequences (GCTGA, TATAT, GTTGA, and TATTT) (33, 34). To test whether the upregulated genes also carry the *cis*-element motifs, the 1.5 kb of promoter sequences from 31 *OsWRKYs,* 4 *SWEET*s, and 5 *MST*s were searched. The results indicated that all the genes that were analyzed harbor the putative *cis*-elements. Among them, *OsWRKY75* contains the highest number of *cis*-elements with 36 in its promoter region, and *OsWRKY70* contains the lowest number of the putative *cis*-elements with only one (Table 1). Among the 40 gene promoters examined, 53 GCTGA, 186 TATAT, 61 GTTGA, and 169 TATTT *cis*-element sequences were identified, indicating that the numbers of TATAT and TATTT motifs are higher than those of GCTGA and GTTGA.

## Discussion

ShB is one of the major rice diseases that severely affects yield production, but the defense mechanism behind this disease remains elusive (4). In this study, we performed RNA-Seq-based transcriptome analysis to dissect the molecular mechanism of how rice defends itself against ShB. Several previously reported genes that contribute resistance to ShB, such as *OsACS2* (11), *14–3–3GF14f* (35), *OsPAL4* (36), *OsPR4b* (37), *OsMYB4* (38), and *OsASR2* (39), are present in our DEGs dataset. These results confirmed the effectiveness of our RNA-Seq experiment. In this condition, a total of 6,838 genes were differentially significantly expressed, including 3,802 upregulated and 3,036 downregulated genes. These genes were further enriched into 30 GO terms. including biological process, molecular function, and cellular

component. In addition, the KEGG analysis showed that the differentially expressed genes were classified into ribosome, carbon metabolism, and biosynthesis of amino acids. In addition, a large number of TFs and transporters were significantly changed. The promoter sequence analysis of the WRKYs, SWEETs, and MSTs revealed that many differentially expressed genes harbor *R. solani*-responsive *cis*-elements in their promoters. However, there is no correlation between the number of *cis*-elements and the induction fold.

# Phytohormone-related genes were significantly changed

The Mapman analysis using the DEGs revealed that phytohormones, particularly the auxin, BR, abscisic acid (ABA), and jasmonic acid (JA)-related genes, changed significantly. Our previous study identified that BR signaling negatively regulates rice defense to ShB (12), and exogenously treated auxin promotes rice defense to ShB, as well as having identified that the auxin polar transporter *OsPIN1a* positively regulates the defense of rice to ShB (40). JA regulates plant defense against necrotrophic fungi (41), and the JAZ family proteins are the key regulator of JA signaling. In our results, the expression of 11 JAZ genes was significantly altered by *R. solani* inoculation. A large number of ABA-related genes was also enriched. Some research demonstrated that ABA is involved in pathogen-plant interactions as a regulator of immunity (42–44). These results suggest that infection by *R. solani* rapidly influences the net phytohormone signaling, which might be important for the defense of rice to ShB. Of note, additional experiments would be valuable to investigate the detailed role of phytohormone signaling in the rice defense to ShB.

# Cell wall and redox state-related genes were significantly changed

The cell wall is a natural physical barrier that plants rely on to build a mechanical support and defend against the invasion of pathogens (45, 46). *R. solani* penetrates into the host cells using hyphae. Therefore, it requires enzymes that can be secreted. A previous finding showed that the *R. solani* AG1-IA genome contains an expanded set of cell wall degrading genes, such as those encoding pectinase, xylanase, and laccase, suggesting that cell wall degradation is also an important process for infectionby *R. solani* AG1-IA (47). Secondary cell wall deposition, a burst of reactive oxygen species (ROS), and the expression of PR genes are important parts of plant immunity. Secondary cell wall deposition will reinforce this barrier to prevent further invasion of pathogens or block the infected cell to stop the spread of pathogens. Therefore, cell wall-related enzymes are major contributors to defense. The Mapman analysis illustrated that cell wall-related genes are significantly enriched in the DEGs, suggesting that alteration of the cell wall is a common strategy for defense to pathogens. In addition, another significantly enriched Mapman term is redox state related genes. The redox state tightly correlates with

ROS production and salicylic acid (SA) signaling (48, 49). ROS play important roles in plant immunity, since ROS are signal molecules that induce programmed cell death (PCD) to protect the host from pathogen infection (50). These results suggest that the cell wall and redox state-related genes might play important roles in the defense of rice to ShB.

## *Rhizoctonia solani* infection significantly alters carbon and nitrogen metabolism

Nitrogen and carbon sources are necessary for living organisms, and serve as the food for pathogens that need to be obtained from the host plants. The KEGG analysis indicated that photosynthesis, pyruvate metabolism, and amino acid metabolism-related genes were significantly enriched. In addition, the Mapman overview showed that a large number of starch, sucrose, TCA, and amino acid metabolism-related genes were significantly changed. Comparative proteomic and metabolomic analyses illustrated that altering the energy and primary metabolism contributes to the resistance of rice to ShB (35). These results suggest that plants might rapidly reprogram the carbon and nitrogen metabolism to provide energy and metabolic sources for defense when they are infected by pathogens.

## TFs might play a function in rice defense to ShB

In the RNA-Seq data, 359 transcription factors among the 6,838 genes, comprising 5.3%, were identified to be significantly altered by *R. solani* infection. These TFs include 40 WRKY, 39 AP2-EREBP, 28 MYB, 11 TIFY, and several indeterminate domain (IDD) family genes. WRKYs are well known for their pivotal roles in plant responses to stress conditions. Several studies illustrated that *OsWRKY4, OsWRKY13, OsWRKY30,* and *OsWRKY80* positively regulates the resistance of rice to ShB (13–16). In this study, we identified that *OsWRKY53* was induced by *R. solani,* and further genetic study revealed that *OsWRKY53* overexpression in the plant rendered it more susceptible to ShB, while *Oswrky53* rendered it less susceptible. *OsWRKY53* was previously reported to positively regulate BR signaling downstream of the BR receptor *OsBRI1* (30). Our previous study showed that BR signaling negatively regulates the defense of rice to ShB (12), implying that *OsWRKY53* might activate BR signaling to suppress the ability of rice to defend against ShB. A few IDD family genes were changed, including *OsIDD14/LPA1,* in the RNA-Seq data. Our recent result has shown that LPA1 activates the expression of *OsPIN1a* to promote rice defense to ShB (40), and the RNA-Seq data further suggests that *LPA1* activation uf *R. solani* might be important to promote rice defense. In addition, *OsWRKY4, OsWRKY13, OsWRKY30,* and *OsWRKY80* regulate the resistance of rice to ShB (13–16). These results suggest that the TFs might play key roles during the defense of rice to ShB and will be interesting to examine in additional experiments.

# Diverse types of transporters are involved in rice defense against ShB

The nature of the cell membrane is its function as a selective-permeable physical barrier that separates the cell components from the external environment. To communicate with other cells or respond to external stimuli, plant cells employ membrane-anchored proteins, such as receptors, transporters, and enzymes. In plant-pathogen interaction systems, transporters play crucial roles in the defense of the plant against invading microbes. In total, 239 transporters were isolated from 6,838 DEGs, including ABC, AAAP, AMT, and SWEET family members. In *Arabidopsis,* the pleiotropic drug resistance transporters PEN3 and PDR12 mediate camalexin export that induces resistance to *Botrytis cinerea* (51). In wheat, a broad spectrum resistance gene *Lr34* was reported to be an ATP-binding cassette (ABC) transporter, which transports ABA (52). In addition, the expression levels of sugar, nitrate, ammonium, amino acids, and peptide transporters were also obviously changed. The SWEET family, sugar transporters that have been recently identified, play key roles in phloem loading, seed filling, nectar secretion, and feeding pathogens (29, 53–55). In rice, several *SWEET* members are transcriptionally activated by TAL effectors, which are secreted by *Xanthomonas oryzae* pv. *oryzase* (*Xoo),,* the causal agent of bacterial leaf blight, for susceptibility (56–58). *OsSWEET11* is a type of SWEET sugar transporter, which negatively regulates the rice defense to ShB (17). In the RNA-Seq data, we identified that *OsSWEET2a* and *OsSWEET2b* were dramatically induced by *R. solani,* suggesting that other SWEET members beside *OsSWEET11* might also play roles in rice and the *R. solani* interaction. In Arabidopsis, *AMT1;1* changes the basal defense, inducing resistance against *Pseudomonas syringe* and *Plectosphaerella cucumerina (*59), and *TaAMTs* were induced by the infection of *Puccinia graminis* f. sp. *tritici* in wheat (60). Our RNA-Seq data and qRT-PCR analysis showed that the rice potassium influx transporter *OsAKT1* transcriptionally activated a response to *R. solani* infection. OsAKT1 facilitates potassium absorption, and a high level of potassium concentration *in planta* confers resistance to rice blast disease, while potassium inhibits the growth of *M. grisesa* on media (31). In response to *R. solani, Osakt1* has improved resistance to this pathogen. This data suggested that potassium may differentially regulate the resistance of rice to different types of fungal pathogens. These data strongly suggest that many transporters might be involved in the regulation of rice defense to ShB.

## Conclusions

ShB severely threatens rice yield production, but the defense mechanism of rice is largely unknown. In this study, RNA-Seq was performed to investigate the changes in expression following the inoculation of *R. solani* in rice. The expression of almost 70,000 genes that are involved in diverse aspects of biological processes was significantly altered. Further bioinformatic and molecular analyses confirmed that phytohormones, metabolism, TFs, and transporters were differently expressed. Following a genetic study using an *OsWRKY53* mutant and overexpressor, as well as an *OsAKT1* mutant, we observed a new regulatory mechanism by which rice control defense to ShB. Taken together, our analyses provided useful information to further analyze the defense mechanism against ShB in rice.

## Methods

# Plant growth and inoculation

Wild-type (WT) rice (*Oryza sativa* L. Japonica. cv. Dongjin and Longjing 11), *OsWRKY53* overexpression plants, and *Oswrky53* and *Osakt1* mutants were grown in a greenhouse at Shenyang Agriculture University. One-month-old plants were used for *R. solani* inoculation using the method described previously (12).

# RNA extraction and quantitative real-time PCR assay

Rice leaves were inoculated with *R. solani* for 0, 24, 48, and 72 hours, and then the leaves were collected for RNA extraction. Total RNA was isolated from one-month-old seedlings using the RNAiso Plus reagent (Takara, Dalian, China) and then treated with RQ-RNase free DNase (Promega, Madison, WI, USA) to remove genomic DNA contamination. Complementary DNA was synthesized using the Reverse Transcription kit (Takara), and all these experiments were conducted according to the manufacturer's instructions. The qRT-PCR assays were performed using the BIO-RAD CFX96 Real-time PCR system (Bio-Rad, Hercules, CA, USA) with ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). The gene expression levels were normalized against *Ubiquitin.* The primers used in the qRT-PCR assays are listed in Supporting Information Table S5.

# mRNA sequencing and data analysis

Three biological replicates were performed for the RNA-Seq analysis. The Total RNA of each sample was extracted using the TRIzol reagent (Invitrogen). The total RNA of each sample was quantified and qualified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), NanoDrop (Thermo Fisher Scientific Inc., Waltham, MA, USA) and a 1.0% agrose gel. One microgram of total RNA with a RIN value above 7 was used for following library preparation. Next-generation sequencing library preparations were constructed according to the manufacturer's instructions (NEBNext® Ultra™ RNA Library Prep Kit for Illumina®, San Diego, CA, USA).

The poly(A) mRNA isolation was performed using NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs, Ipswich, MA, USA). The mRNA fragmentation and priming was performed using NEBNext First Strand Synthesis Reaction Buffer and NEBNext Random Primers. First strand cDNA was synthesized using ProtoScript II Reverse Transcriptase, and the second-strand cDNA was synthesized using Second Strand Synthesis Enzyme Mix. The purified double-strand cDNA by AxyPrep Mag PCR Clean-up (Corning, Corning, NY, USA) was then treated with the End Prep Enzyme Mix to repair both ends and add a dA-tailing in one reaction, followed by a T-A ligation to add adaptors to both ends. Size selection of the Adaptor-ligated DNA was performed using an AxyPrep Mag PCR Clean-up kit (Corning),

and fragments of ~360 bp (with the approximate insert size of 300 bp) were recovered. Each sample was then amplified by PCR for 11 cycles using P5 and P7 primers, with both primers carrying sequences that can anneal with the flow cell to perform bridge PCR and P7 primer carrying a six-base index allowing for multiplexing. The PCR products were cleaned up using AxyPerp Mag PCR Clean-up (Axygen), validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).

Then libraries with different indices were multiplexed and loaded on an Illumina Hi-Seq instrument according to the manufacturer's instructions (Illumina). Sequencing was performed using a 2x150 bp paired-end (PE) configuration; image analysis and base calling were conducted by the Hi-Seq Control Software (HCS) + OLB + GAPipeline–1.6 (Illumina) on a Hi-Seq instrument.Quality control, mapping, expression analysis, differential expression analysis, GO and KEGG enrichment analysis, novel transcripts prediction, alternative splicing, differential exon usage, principal component analysis, and protein-protein interaction analyses were performed using standard methods (61–65). The sequencing data analysis was processed and analyzed with GENEWIZ.

# Mapman analysis

The experiment was performed according to the instructions. Briefly, a table of RNA-Seq results, including gene ID and fold change data, was imported to generate a schematic diagram that show enriched pathways or processes (66).

# Identification of differential expressed transcription factors and transporters

Utilizing the Rice TF Database (https://ricephylogenomics.ucdavis.edu/tf/index.shtml) and Rice Transporter Database (https://ricephylogenomics.ucdavis.edu/transporter/) (27, 28), the genes listed in these databases were searched in our RNA-Seq data. Isolated differential expressed TFs and transporters were classified according to their families, respectively. The nomenclature and locus number of the rice SWEET genes refer to a previous publication (29).

## Abbreviations

ShB: Sheath blight disease; *R. solani: Rhizoctonia solani;* BRs: Brassinosteroids; ABA: Abscisic Acid; JA: Jasmonic acid; SWEET: Sugar will eventually be exported transporter; HPI: Hours post inoculation; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PR: Pathogenesis-related; qRT-PCR: Real-time quantitative PCR.

## Declarations

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# Authors' contributions

DPY, CZ and YHX designed the experiments. DPY, CZ, and STW performed the experiments. YL, SL, HNW, YW, SL, SHW and YHX analyzed the data. DPY, SHW and YHX wrote the manuscript. All the authors have read and approved the manuscript.

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# Availability of data and materials

Not applicable.

# Ethics approval and consent to participate

Not applicable.

# Consent for publication

Not applicable.

# Competing interests

The authors declare that they have no competing interests.

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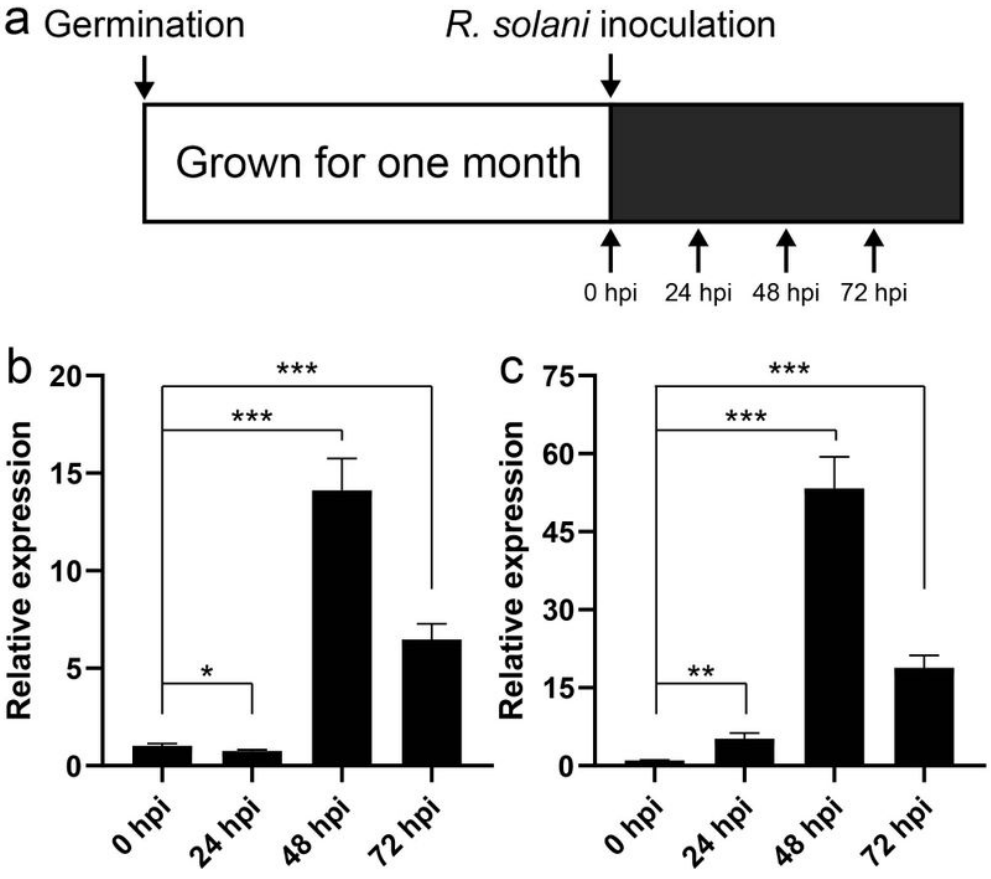
# Table 1

Table 1. The list of *Rhizoctonia solani*-induced *cis*-elements in the promoters

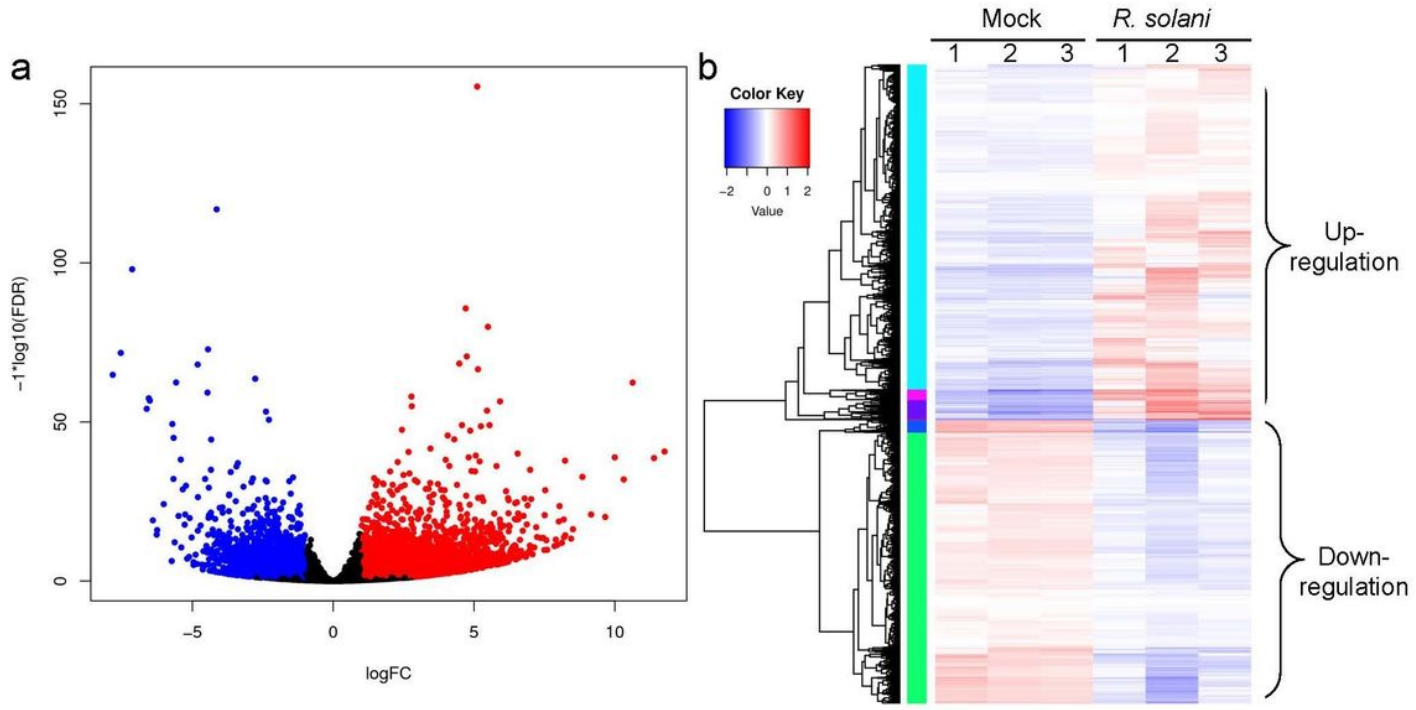
| Gene ID | Anno-tation | UP/ DOWN | $Log_2FC$ | Numbers of cis-elements within 1.5 kb promoters | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | GCTGA | TATAT | GTTGA | TATTT | Total |
| Os05g0537100 | *WRKY7* | UP | 3.198282 | 1 | 3 | 0 | 6 | 10 |
| Os05g0583000 | *WRKY8* | UP | 2.090289 | 0 | 6 | 1 | 6 | 13 |
| Os01g0289600 | *WRKY9* | UP | 1.744841 | 1 | 6 | 1 | 7 | 15 |
| Os01g0186000 | *WRKY10* | UP | 4.289374 | 3 | 5 | 1 | 3 | 12 |
| Os05g0571200 | *WRKY19* | UP | 4.751344 | 1 | 1 | 3 | 1 | 5 |
| Os01g0821600 | *WRKY21* | UP | 3.353674 | 2 | 8 | 1 | 4 | 15 |
| Os01g0826400 | *WRKY24* | UP | 4.046747 | 1 | 2 | 0 | 3 | 6 |
| Os01g0714800 | *WRKY26* | UP | 4.494687 | 1 | 6 | 0 | 14 | 21 |
| Os06g0649000 | *WRKY28* | UP | 5.709364 | 1 | 1 | 0 | 5 | 7 |
| Os02g0770201 | *WRKY32* | UP | 3.703045 | 1 | 10 | 6 | 2 | 19 |
| Os11g0117500 | *WRKY40* | UP | 4.561835 | 3 | 6 | 3 | 0 | 12 |
| Os11g0116900 | *WRKY46* | UP | 1.498728 | 0 | 2 | 4 | 5 | 11 |
| Os04g0287400 | *WRKY51* | UP | 1.090485 | 1 | 1 | 4 | 6 | 12 |
| Os05g0343400 | *WRKY53* | UP | 1.376023 | 1 | 6 | 2 | 1 | 10 |
| Os03g0321700 | *WRKY55* | UP | 2.625267 | 0 | 4 | 0 | 2 | 6 |
| Os09g0417800 | *WRKY62* | UP | 3.772405 | 1 | 4 | 0 | 7 | 12 |
| Os12g0116700 | *WRKY64* | UP | 1.641784 | 1 | 7 | 2 | 1 | 11 |
| Os05g0183100 | *WRKY67* | UP | 2.340772 | 0 | 2 | 0 | 9 | 11 |
| Os08g0386200 | *WRKY69* | UP | 1.885488 | 4 | 6 | 1 | 4 | 15 |
| Os05g0474800 | *WRKY70* | UP | 2.355018 | 1 | 0 | 0 | 0 | 1 |
| Os02g0181300 | *WRKY71* | UP | 3.281913 | 1 | 4 | 3 | 18 | 26 |
| Os11g0490900 | *WRKY72* | UP | 2.696032 | 0 | 4 | 1 | 4 | 9 |
| Os09g0334500 | *WRKY74* | UP | 1.654339 | 2 | 2 | 3 | 7 | 14 |
| Os05g0321900 | *WRKY75* | UP | 2.885381 | 0 | 28 | 1 | 7 | 36 |
| Os01g0584900 | *WRKY77* | UP | 2.038999 | 2 | 6 | 3 | 6 | 17 |
| Os12g0597700 | *WRKY94* | UP | 2.005375 | 1 | 0 | 2 | 0 | 3 |
| Os12g0116600 | *WRKY95* | UP | 2.384793 | 1 | 0 | 1 | 0 | 2 |
| Os12g0116400 | *WRKY97* | UP | 1.536944 | 1 | 4 | 0 | 8 | 13 |
| Os11g0117400 | *WRKY104* | UP | 9.995043 | 0 | 5 | 0 | 2 | 7 |
| Os01g0821300 | *WRKY108* | UP | 4.131182 | 3 | 4 | 0 | 2 | 9 |
| Os06g0157600 | *WRKY113* | UP | 3.048136 | 1 | 0 | 1 | 2 | 4 |
| Os01g0541800 | *SWEET2a* | UP | 5.78317 | 0 | 1 | 5 | 3 | 9 |
| Os01g0700100 | *SWEET2b* | UP | 6.993259 | 2 | 2 | 4 | 5 | 13 |
| Os02g0574500 | *MST1* | UP | 2.290565 | 4 | 1 | 0 | 2 | 7 |
| Os03g0218400 | *MST4* | UP | 4.860401 | 2 | 7 | 0 | 3 | 12 |
| Os07g0106200 | *MST3* | UP | 1.135706 | 1 | 15 | 2 | 6 | 24 |
| Os07g0559700 | *MST6* | UP | 1.758687 | 3 | 1 | 2 | 2 | 8 |
| Os11g0508600 | *SWEET14* | UP | 1.398355 | 3 | 7 | 0 | 0 | 10 |
| Os12g0476200 | *SWEET13* | UP | 1.390425 | 0 | 8 | 1 | 4 | 13 |
| Os04g0453500 | *MST1-2C* | UP | 1.865579 | 0 | 0 | 1 | 1 | 2 |
| Os02g0724500 | *STA72* | UP | 2.535833 | 2 | 1 | 3 | 1 | 7 |
| | | | Total | 53 | 186 | 61 | 169 | 469 |

# Figures




## Figure 1

Expression of OsPBZ1 and OsPR1b in response to inoculation with Rhizoctonia solani AG1-IA. (a) One-month-old seedlings were inoculated with R. solani AG1-IA, and the leaves were sampled after 0, 24, 48, and 72 hours post inoculation (hpi). R. solani-infection mediated expression patterns of OsPBZ1 (b) and OsPR1b (c) were analyzed by qRT-PCR. Data are the means ± standard error (SE) of three repeated experiments. Significant differences between different time points compared to 0 hpi were shown (**P<0.01, ***P<0.001).




## Figure 2

Volcano and heat map diagrams of RNA-sequencing data. (a) The distribution of the DEGs was shown. Red and blue spots represent up- and downregulated DEGs, respectively. (b) Hierarchical clustering map exhibited DEGs with or without Rhizoctonia solani inoculation. The numbers ‘1, 2, and 3’ above the map indicate the three replicates of RNA-sequencing. Up- and downregulated genes were marked in the right side of the map. Color key image indicate up- and downregulation of the DEGs.

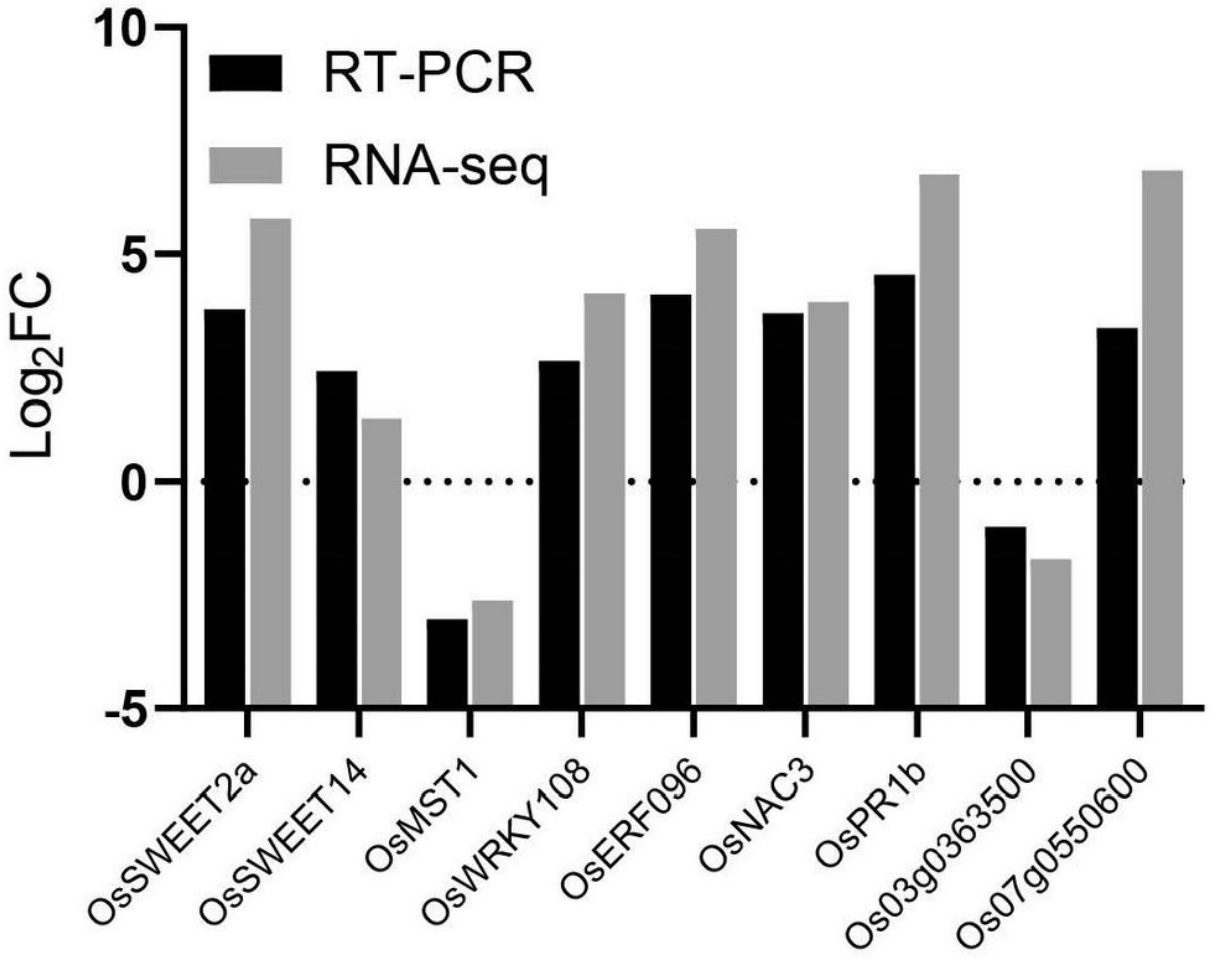
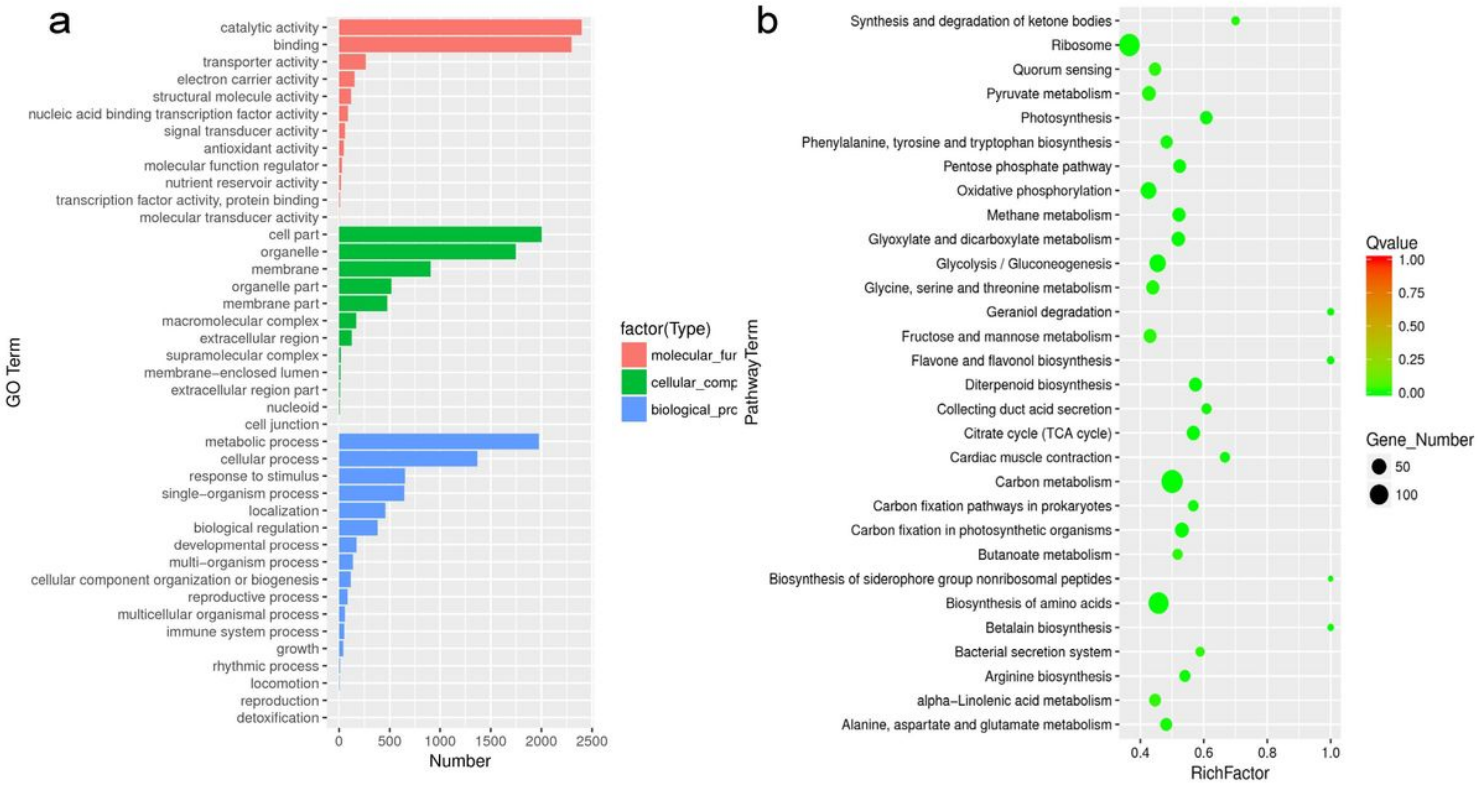


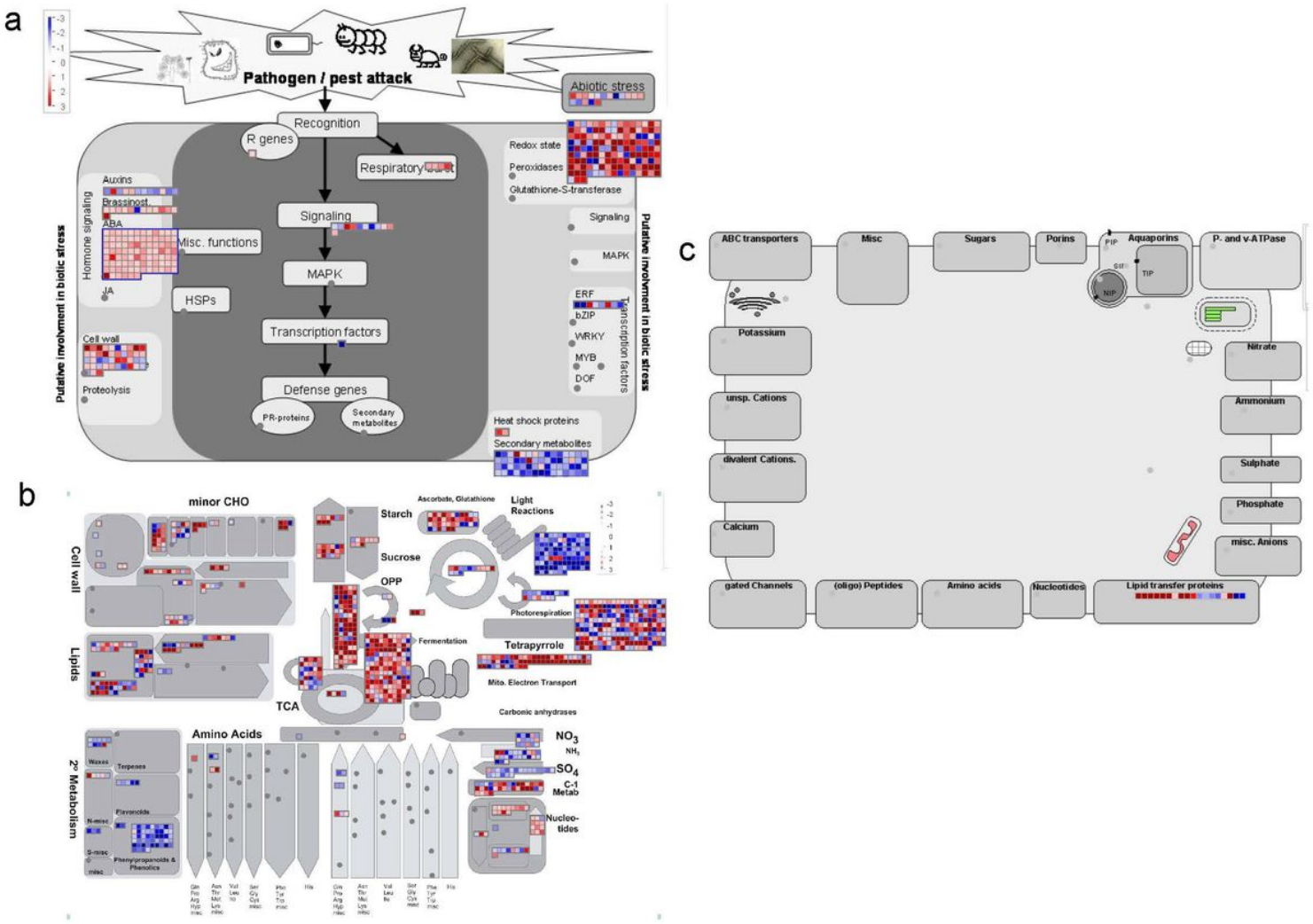

**Figure 3**

Validation of the DEGs by qRT-PCR analysis. Seven upregulated (OsSWEET2a, OsSWEET14, OsWRKY108, OsERF096, OsNAC3, OsPR1b, and Os07g0550600) and two downregulated genes (OsMST1 and Os03g0363500) were evaluated by qRT-PCR. White and black bars indicate qRT-PCR and RNA-Seq results, respectively.



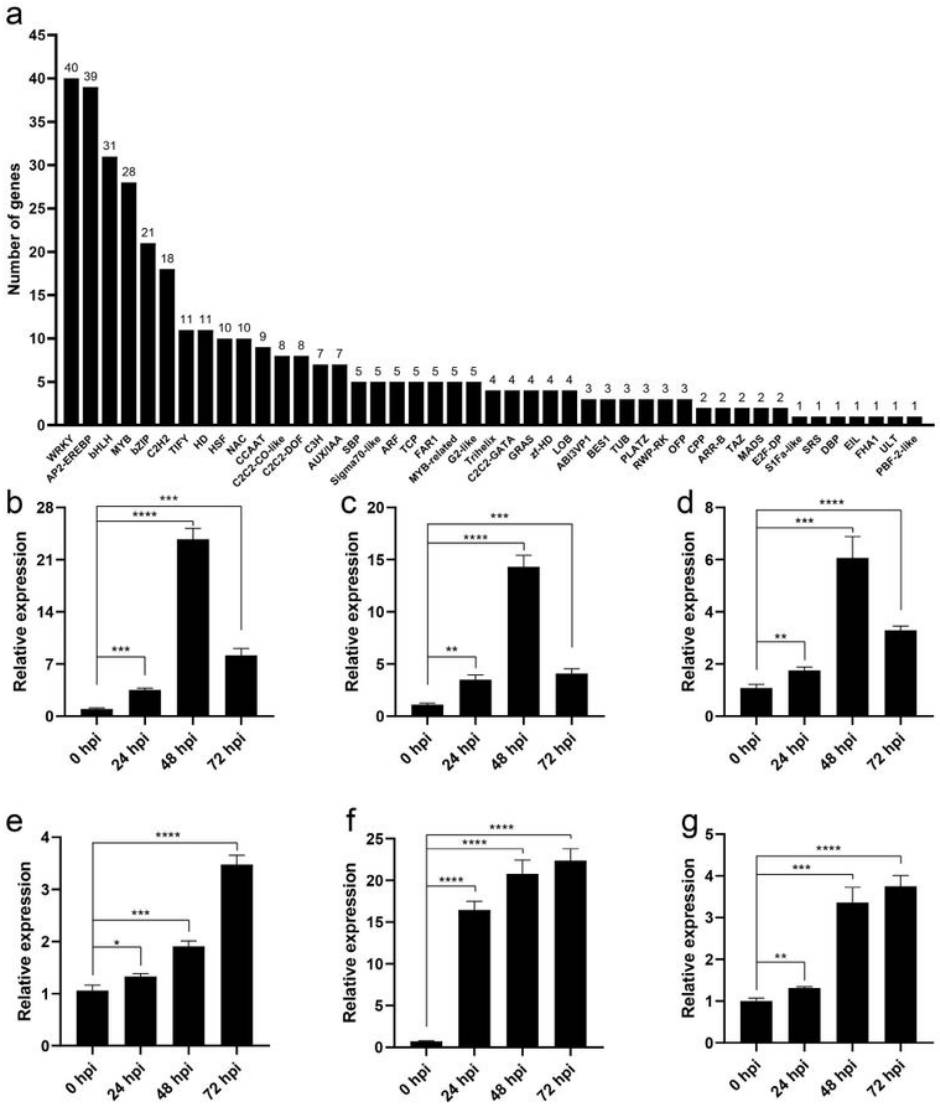

## Figure 4

The GO and KEGG analyses of the DEGs. (a) DEGs were classified into 30 GO terms, including catalytic activity, binding, metabolic process, and cell part. The number of DGEs belonging to each category was represented by pink, green, and blue bars. (b) DEGs were divided into 30 KEGG pathway, including ribosome, carbon metabolism, biosynthesis of amino acids. Different sizes of green ovals indicate the number of DGEs in each pathway.



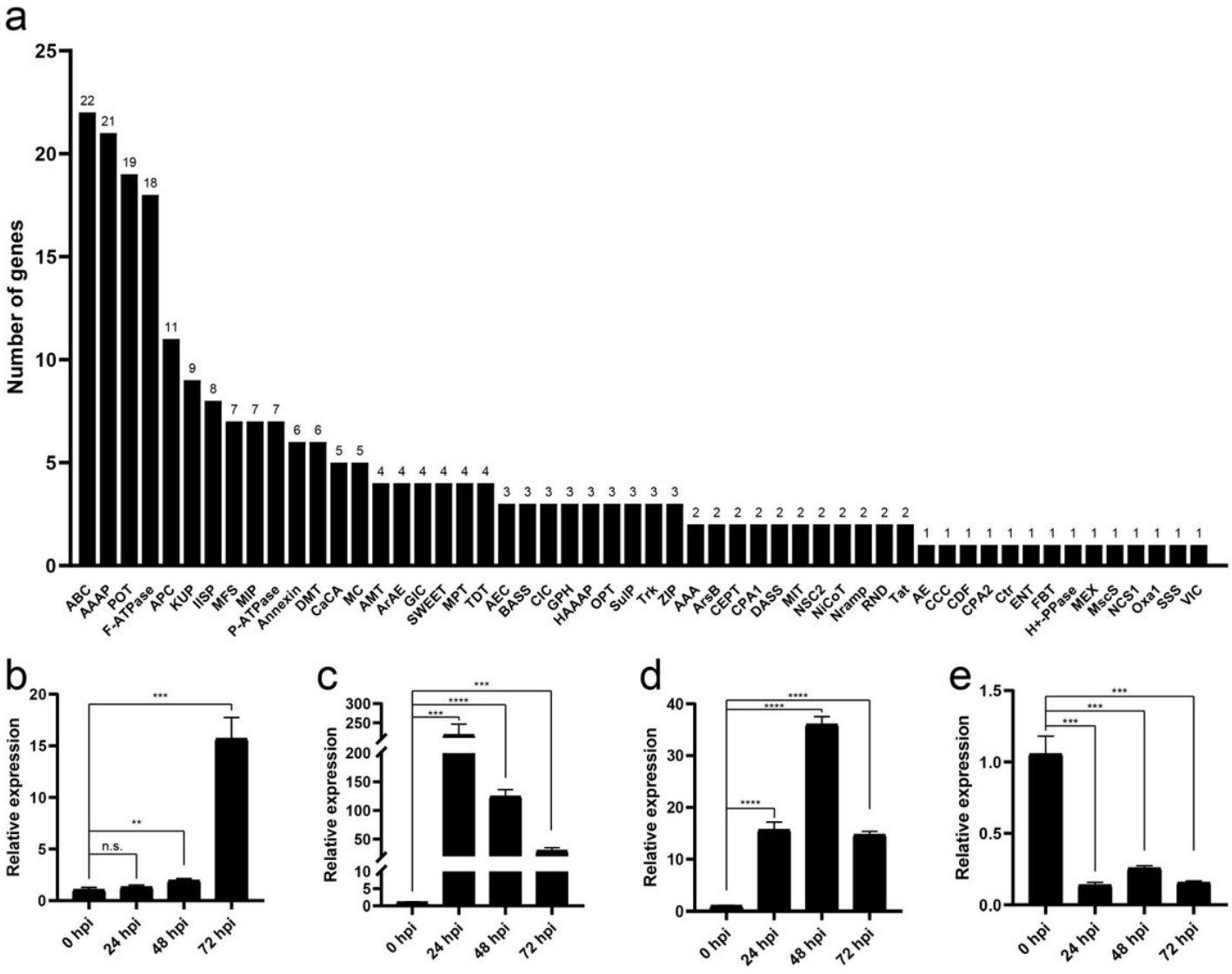

## Figure 5

Mapman analysis of the DEGs. (a) The pathogen/pest attack overview showed that phytohormone, abiotic stress, redox state, secondary metabolites, and cell wall-related genes were enriched. Abscisic acid, redox-related, cell wall, and secondary metabolic-associated genes were highly enriched. (b) DEGs involved in metabolism, including light reactions, fermentation, and photorespiration-related, were classified. (c) Differentially expressed transporters, including ABC transporters, nitrate and ammonium transporters, and lipid transfer proteins, were summarized.



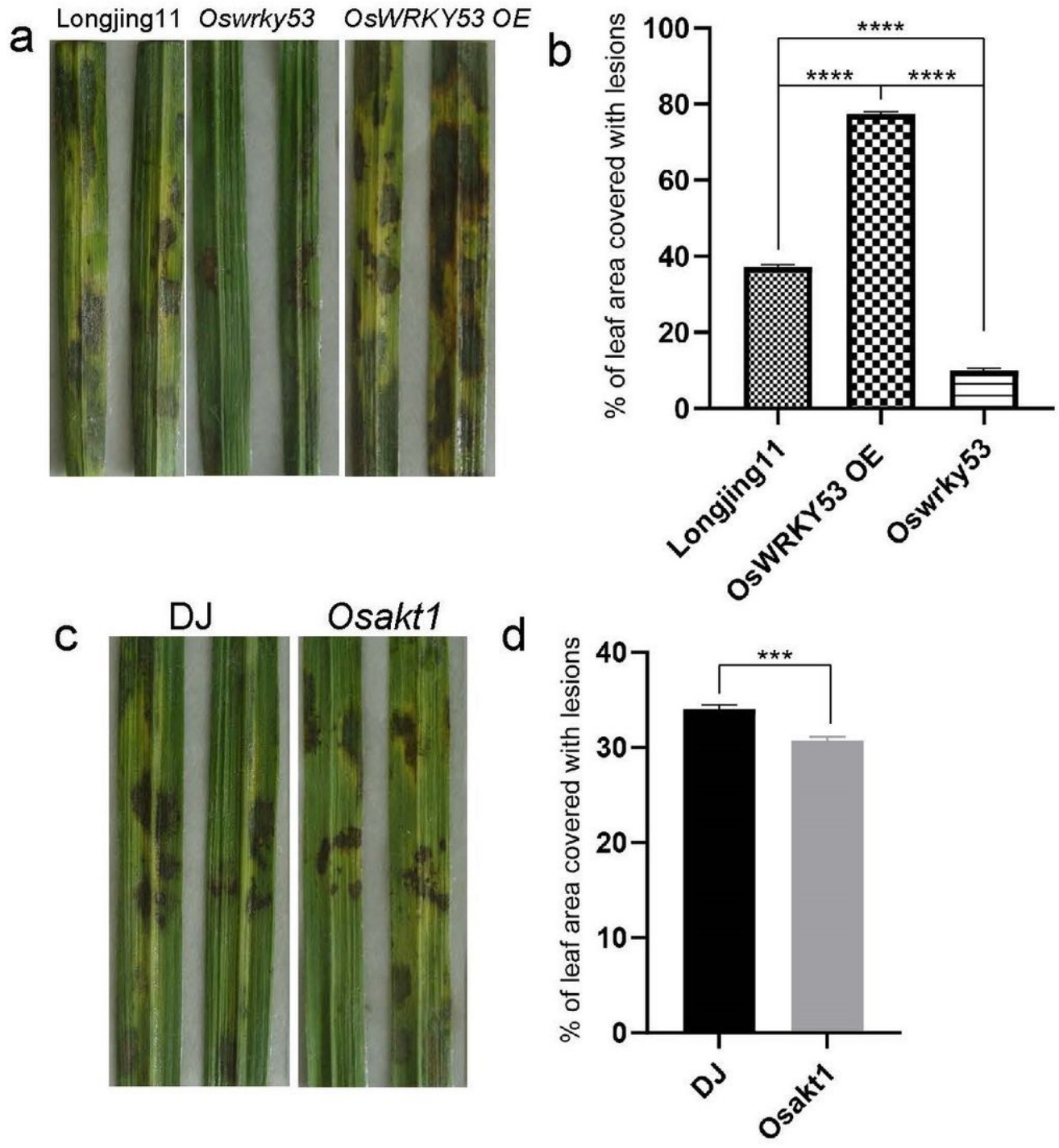

## Figure 6

Isolation of differentially expressed transcription factors and qRT-PCR verification. (a) Among the DEGs, differentially expressed transcription factors were collected. A total of 40 types of transcription factors, including WRKY, MYB, AUX/IAA, and C2H2 zinc finger families, were enriched. The Rhizoctonia solani-dependent expression of OsWKRY28 (b), OsWRKY32 (c), OsWRKY53 (d), OsJAZ5 (e), OsJAZ6 (f), and OsJAZ9 (g) was analyzed by qRT-PCR. Data are the means ± standard error (SE) of three repeated experiments. Significant differences between different time points compared to 0 hpi are shown (*P<0.05, **P<0.01, ***P<0.001).




## Figure 7

Isolation of differentially expressed transporters and qRT-PCR verification. (a) Transporters belong to 53 types of superfamilies, including ABC, AAAP, POT, and F-ATPase, were enriched. (b) The expression levels of OsAKT1 (b), OsSWEET2b (c), OsMST4 (d), and OsMST8 (f) were verified by qRT-PCR. Data are the means ± standard error (SE) of three repeated experiments. Significant differences between different time points compared to 0 hpi are shown (**P<0.01, ***P<0.001, ****P<0.0001).




## Figure 8

Evaluation of OsWRKY53 and OsAKT1 mutants in response to sheath blight disease compared with the wild type control. (a) Response of Oswrky53 and OsWRKY53 overexpressor (OE) to R. solani AG1-IA compared with the WT and parental lines. (b) Percentage of leaf area covered with lesions in Oswrky53 and the OsWRKY53 overexpressor (OE) compared with the WT and parental lines. (c) Response of Osakt1 to R. solani AG-1 compared with the WT and parental lines. (d) Percentage of the leaf area covered with

lesions in Osakt1 compared with the WT and parental lines. Data are the means ± standard error (SE) (n>15). Significant differences between different time points compared to 0 hpi are shown (***P<0.001, ****P<0.0001).

## Supplementary Files

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- SupplementalTables.xlsx