Transcriptome analysis of the rice response to blast fungus identified core genes involved in immunity

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Abstract

Rice blast disease caused by the filamentous Ascomycetous fungus *Magnaporthe oryzae* is a major threat to rice production worldwide. The mechanisms underlying rice resistance to M. oryzae, such as transcriptional reprogramming and signaling networks, remain elusive. In this study, we carried out an in-depth comparative transcriptome study on the susceptible and resistant rice cultivar in response to *M. oryzae*. Our analysis highlighted that a rapid, high-amplitude transcriptional reprogramming was important for rice defense against blast fungus. The ribosome- and protein translation-related genes were significantly enriched in differentially expressed genes (DEGs) at 12 hpi in both cultivars, indicating that the protein translation machinery is regulated in the activation of immunity in rice. Furthermore, we identified a core set of genes that are involved in the rice response to biotic as well as to abiotic stress. More importantly, among the core genes, we demonstrated that the metallothionein *OsMT1a* and *OsMT1b* genes positively while a peroxidase gene *Perox4* negatively regulated rice resistance to *M. oryzae*. Our study provides novel insight into transcriptional reprogramming and a valuable resource for functional studies on rice immune signaling components in resistance to blast disease.

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Abstract: Rice blast disease caused by the filamentous Ascomycetous fungus *Magnaporthe oryzae* is a major threat to rice production worldwide. The mechanisms underlying rice resistance to*M. oryzae* , such as transcriptional reprogramming and signaling networks, remain elusive. In this study, we carried out an in-depth comparative transcriptome study on the susceptible and resistant rice cultivar in response to *M. oryzae* . Our analysis highlighted that a rapid, high-amplitude transcriptional reprogramming was important for rice defense against blast fungus. The ribosome- and protein translation-related genes were significantly enriched in differentially expressed genes (DEGs) at 12 hpi in both cultivars, indicating that the protein

translation machinery is regulated in the activation of immunity in rice. Furthermore, we identified a core set of genes that are involved in the rice response to biotic as well as to abiotic stress. More importantly, among the core genes, we demonstrated that the metallothionein *OsMT1a* and *OsMT1b* genes positively while a peroxidase gene *Perox4* negatively regulated rice resistance to*M. oryzae.* Our study provides novel insight into transcriptional reprogramming and a valuable resource for functional studies on rice immune signaling components in resistance to blast disease.

Keywords: Rice blast disease, immunity, transcriptome, peroxidase, *Magnaporthe*

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Introduction

Rice (*Oryza sativa*) is one of the main staple food crops for over half of the world's population. Rice blast disease, caused by the blast fungus *Magnaporthe oryzae,* is the most devastating rice disease, causing from 10-30% annual yield losses worldwide (Deng et al., 2017; Liu et al., 2013; Liu, Liu, Triplett, Leach, & Wang, 2014). Due to its importance in rice production, the rice-*M. oryzae* interaction has been studied for decades and has become a model system in the study of plant-fungal interactions. The infection starts when fungal spores, called conidia, land on the surface of rice leaf (Talbot, 2003). Conidia attach to the leaf cuticle, germinate rapidly and form a dome-shaped infection cell called an appressorium within 8 h (Dagdas et al., 2012). The appressorium produces a specialized hypha, a penetration peg, pierces the leaf cuticles and invades epidermal cells, where the peg expands to form invasive hyphae by 24 h postinoculation (hpi) (Dagdas et al., 2012; Kankanala, Czymmek, & Valent, 2007). During fungal invasion, rice cells first mount a less specific immune response upon recognition of pathogen-associated molecular patterns (PAMPs), named PAMPs-triggered immunity (PTI). In the compatible interaction, *M. oryzae* secrete a large number of effector proteins to counteract the PTI in susceptible rice plants (Liu et al., 2014). While in the incompatible interaction, certain fungal effector/effectors is/are recognized by cognate nucleotide-binding, leucine-rich-repeat (NLR) proteins, resulting in robust immune responses, called effector-triggered immunity (ETI) in resistant rice plants (Jones & Dangl, 2006; Liu et al., 2014; Tang, Wang, & Zhou, 2017; W. Wang, Feng, Zhou, & Tang, 2020). PTI and ETI trigger many similar immune responses, including the activation of mitogen-activated protein kinases, transient calcium influx, a rapid burst of reactive oxygen species (ROS), deposition of callose, transcriptional reprogramming and phytohormone regulation (Cui, Tsuda, & Parker, 2015; W. Wang et al., 2020).

Currently, although more than 70 defense regulators in rice blast resistance have been identified, many more defense regulators in rice blast resistance remain to be identified (Li, Chern, Yin, Wang, & Chen, 2019). The immune signaling pathways and networks in rice are elusive. Large-scale approaches have been used to study the transcriptome profile of rice in response to *M. oryzae* , including microarrays and RNA-sequencing. Due to technical limits of microarrays, previous studies have only identified a small number of DEGs in the rice response to*M. oryzae.* For instance, Wei et al identified 551 and 131 DEGs at 24 hpi in resistant and susceptible rice cultivars, respectively (Wei et al., 2013). Within the limit number of DEGs, functional analysis indicated that genes involved in signaling pathways were upregulated during the rice early response to *M. oryzae* (Wei et al., 2013). In recent years, RNA-sequencing (RNA-seq) technology has provided a powerful and effective tool to study the transcriptome profile in plant-microbe interactions. A comprehensive transcriptome analysis using time-series RNA-seq on *Arabidopsis* challenged with virulent or avirulent *Pseudomonas syringae* strains discovered that*Arabidopsis* activates very similar transcriptome responses in compatible and incompatible interactions but with different speeds, and the phytohormone network is required for achieving high-amplitude transcriptional reprogramming within the early infection stage (Mine et al., 2018). However, to date, full time-series RNA-seq data on rice -*M. oryzae* interactions have not been reported.

In this study, we present a high-resolution time-series transcriptome data at 12, 24, 36 and 48 hpi of both compatible and incompatible rice-*M. oryzae* interactions using RNA-seq. We found that the resistant rice cultivar activated high-amplitude transcriptional responses at 12 hpi, which is much earlier than 24 hpi, as suggested in previously studies. A group of core genes involved in both compatible and incompatible rice-*M. oryzae* interactions were identified. Functional pathway analysis of DEGs revealed that the protein translation machinery was regulated at the early stage in rice immune responses to *M. oryzae* . Furthermore, we identified and verified three new genes that are involved in rice resistance to blast disease. Collectively, these findings provide a comprehensive overview of transcriptional reprogramming during rice immune responses to *M. oryzae* and potential gene resources for functional characterization of the rice immune system.

Materials and methods

Rice materials and *M. oryzae* strains

Nipponbare (*Oryza sativa* L. ssp. *japonica* cv.) and Zhonghua11 (*Oryza sativa* L. ssp. *japonica* cv.) were kept in the Rice Research Institute, Fujian Academy of Agricultural Sciences. Suxiu867, a *japonica* cultivar, was used as the recipient, and Minghui86, a restorer *indica* cultivar, was used as the donor. The F_1 plants were generated from Suxiu867 as female and Minghui86 as male. The F_1 plants were back crossed with Suxiu867 to produce the BC_1F_1generation. These BC_1F_1 plants were backcrossed to Suxiu867 to produce BC_2F_1, and these plants were self-pollinated to produce BC_2F_2 lines. These lines were self-interbred for 6 generations, and a stable line was obtained, named as Hui1586. Rice plants were grown in the natural field or in the green house at 28 *C under 12-h light and 12-h dark condition.

The *M. oryzae* isolates Guy11, 18SH-D527, FJ2011, 95085AZB and 18NH-16-3 were kindly provided by the State Key Laboratory for Ecological Pest Control of Fujian and Taiwan Crops, Fujian Agriculture and Forestry University; isolate Zhong 1 was provided by Sichuan Agricultural University; isolates KJ201, 501-3, RB22 and 20-15 were from Fujian Academy of Agricultural Sciences and isolates M409, MH86-1, and MH86-3 have been isolated and maintained at the Plant Immunity Center, Fujian Agriculture and Forestry University.

Disease resistance assays

Two-week-old Hui1586 and Nipponbare plants were used for inoculation with Guy11 as previously described (Y. B. Li et al., 2020) with minor modifications. The spore concentration was adjusted to 5×10^5 spores/mL with 0.02% Tween-20, and H_2O with 0.02% Tween-20 was used as the mock treatment. The fungal-inoculated rice seedlings were kept in a dark chamber at 25*C with 85% humidity for 24 hours, and then the plants were maintained in the growth chamber at 26/24*C under a 14-h light/10-h dark cycle with 85% humidity. Flag leaves were harvested at 12, 24, 36, and 48 hpi, and three biological replicates are collected for each treatment. On the seventh day, the plants were scored using the 0-9 scale of the standard evaluation system for rice (International Rice Research Institute 1996). The plants with a rating of 0-3 were considered resistant, and those with a rating of 4-9 were considered susceptible.

RNA extraction and quality testing

RNA extraction was performed using kits (Magen, IF210200) according to the manufacturer's protocols. Total RNA quality detection was performed with a Nanodrop for determining the RNA concentration; nondenaturing agarose electrophoresis for determining RNA integrity; and an Agilent 2100 Bioanalyser for determining the total RNA.

RNA-seq and data analysis

Library construction and RNA sequencing were conducted by the Beijing Genomics Institute (BGI) using the BGISEQ-500 platform, producing approximately 6.8 Gb data of 100-base long paired reads per sample. RNA-seq data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database under accession number GSE157400. All clean reads were mapped to the *Oryzae sativa* L. cv. Nipponbare reference genome using Hisat2 (Kim, Langmead, & Salzberg, 2015) and trans-

formed into a count per gene per library using HTseq (Anders, Pyl, & Huber, 2015). Statistical analysis of the RNA-seq data was performed in the R environment with the DEseq2 package (L. Wang, Feng, Wang, Wang, & Zhang, 2010). To extract significantly differentially expressed genes, a cut-off of $\log_2$ (fold change) [?] 1 or [?] -1 and adjusted P-value [?] 0.001 was applied. Heatmaps were generated with webtool Morpheus (https://software.broadinstitute.org/morpheus/) using one minus the Pearson correlation and average linkage clustering. Transcriptome similarity analysis and *cis* -regulatory motif enrichment were performed with the webtool Plant Regulomics (http://bioinfo.sibs.ac.cn/plant-regulomics) (Ran et al., 2020).

Gene Ontology and KEGG pathway enrichment analysis

The Gene Ontology (GO) function significant enrichment analysis provided a GO function term that was significantly enriched in candidate genes compared with the entire genetic background of the species. The analysis first maps all candidate genes to each term in the Gene Ontology database (http://www.geneontology.org/). Then, the software 'GO::TermFinder' (https://metacpan.org/pod/GO::TermFinder) was used to calculate the number of genes in each term, and then the hypergeometric test was applied to find GO terms that were significantly enriched in candidate genes compared with the background of all genes in this species. For KEGG pathway enrichment analysis, the candidate genes were first mapped to the KEGG pathway term in the public database (Kanehisa et al., 2008). Then, a hypergeometric test was applied to find pathways that were significantly enriched in the candidate gene map to the entire genome background. After the calculated P-value was corrected by Bonferroni (Abdi, 2007), the GO terms or KEGG pathway terms of which Q -value (corrected P -value) [?] 0.05 were defined as significantly enriched GO/KEGG pathway terms.

Generation of rice mutants using the CRISPR/Cas9 method

For the generation of *perox4* and *osmt1a/b* knockout lines, guide RNAs (gRNAs) (Supplemental Table 4) were designed to target exons of the targeted genes (Fig. S3). The synthesized oligo of gRNAs was annealed to form the oligo adaptors, which then was joined to the BGK03 CRISPR/Cas9 vector (Biogle Biotechnology, Hangzhou, China). The CRISPR/Cas9 plasmids were introduced into *Agrobacterium tumefaciens* EHA105 and then into rice through*Agrobacterium*- mediated transformation as described previously. The individual T1 plants were genotyped by sequencing the DNA products of PCR with primers (Supplemental table 4).

Evaluation of Agronomic Traits

According to standard commercial practices, mutants and their parents were grown in a paddy field under natural conditions at an experimental farm in Fuzhou, China. All plants were grown with a spacing of 13.3 cm between plants within each row and 26.4 cm between rows. Field management essentially followed normal agricultural practices, and the amounts of N, P_2O_5 and K_2O applied were 127.5 kg/hm^2, 45.0 kg/hm^2, and 30.0 kg/hm^2, respectively.

Ten random plants from each line were chosen to measure the plant height, panicle length, number of effective panicles, spikelet number per panicle, seed setting rate and 1000-grain weight, grain length, grain width, and grain yield per plant using conventional methods at maturity in 2020.

Results

Rice cultivar Hui1586 exhibits strong broad-spectrum resistance to blast fungi

To breed new rice restorer cultivar with broad-spectrum resistance to blast fungus *Magnaporthe oryzae* , a resistant *japonica*cultivar Suxiu867 was crossed with a restorer cultivar Minghui86. After two times backcross with Suxiu867 and six successive generations self-interbred, a new stable restorer line named Hui1586 was obtained (see methods). To assess the resistance of Hui1586 to rice blast fungi in natural conditions, we planted Hui1586 and susceptible*japonica* cultivar Nipponbare as control in the field at Jinggangshan, Jiangxi Province and Yichang, Hubei Province, two locations in China with a high incidence of rice blast disease. At both locations, Hui1586 exhibited strong resistance to blast disease compared with Nipponbare (Fig. 1A and 1B), indicating that Hui1586 has broad-spectrum resistance to blast fungus. To confirm this, two-week-old

plants growing in the greenhouse were inoculated with thirteen Nipponbare-compatible *M. oryzae* isolates (Table. 1) including Guy11, a widely used strain in studying rice–*M. oryzae*interactions. We observed that Hui1586 was highly resistant to all tested blast strains (Fig. 1C and Table 1). Together, the results showed that Hui1586 has broad-spectrum high resistance to rice blast fungi.

Dynamic changes in transcriptome in rice compatible and incompatible interactions with *M. oryzae* are different

To gain molecular insight into the mechanisms involved in the resistance to blast disease in Hui1586, in-depth comprehensive transcriptome profiles compared with susceptible Nipponbare were achieved through RNA-seq experiments. The two-week-old rice plants were spray inoculated with spores of *M. oryzae* Guy11 or H_2O (mock treatment), and then leaf samples with 3 biological replicates were collected at 12, 24, 36 and 48 hpi for RNA-seq. The mock for each time point was processed to ensure appropriate comparisons. In total, 48 samples were subjected to RNA-seq, resulting in 6.8-gigabyte 100-bp paired-end reads per sample on average.

To visualize the variation as well as the similarity for all samples, we performed a principal component analysis (PCA) on the normalized FPKM (fragments per kilobase of transcript per million mapped reads) values of all the detected genes. The PCA plot showed that the data for three biological replicates were clustered closely and were separated by the time point, treatments and genotypes (Fig. 2A). Among the data for the mock treatment, the 12, 24, 36, and 48 h samples were clustered away from each other, indicating that the circadian clock is a major factor affecting gene expression. At 12 h, the *M. oryzae* treatments were clustered away from the mock treatment in both susceptible Nipponbare and resistant Hui1586. However, at the late time points of 24, 36, and 48 h, the *M. oryzae* and mock treatments were clustered closely on Hui1586, while they were clustered away on Nipponbare, indicating*M. oryzae* induced large transcriptome changes in the susceptible Nipponbare at all time points but high-amplitude transcriptome changes only at the early infection stage in resistant Hui1586.

A hierarchical clustering analysis was conducted on the FPKM values of all detected genes. The samples were clustered into 4 larger groups according to the time point (Fig. 2B), again showing that the circadian clock is a major regulator in the rice transcriptome. In each large group, the H_2O treatments were clustered into the same subgroup, except the 36 hpi samples of H_2O-treated Hui1586, which were clustered with *M. oryzae*- treated Hui1586, suggesting the occurrence of small transcriptional changes in Hui1586 at 36 hpi with *M. oryzae* treatment. The 24, 36, and 48-hpi samples of *M. oryzae* -treated Nipponbare were clustered away from the mock samples (Fig. 2B), indicating that *M. oryzae* persistently induced strong transcriptional changes in the susceptible Nipponbare.

Then, we identified differentially expressed genes (DEGs; fold change >2; $P < 0.001$) between the fungal and mock treatment in Nipponbare or Hui1586 at each time point. A large number of DEGs in Nipponbare were identified, especially at 12 h (4680 upregulated and 2045 downregulated) and 36 h (3347 upregulated and 3653 downregulated). While Guy11 induced more dramatic transcriptional changes (6808 upregulated and 2895 downregulated genes) in the resistant Hui1586 at 12 h, the numbers of DEGs markedly decreased at 24, 36 and 48 h (Fig. 2C). In total, 12333 and 12147 DEGs, accounting for approximately 25% of the rice genes, were identified respectively in Nipponbare and Hui1586 in the 48 h time-series of the fungal infection. Among these DEGs, 8211 were commonly regulated in both cultivars (Fig. 2D and Supplemental dataset 1). Together, the data indicated that*M. oryzae* infection induced dramatic and dynamic transcriptional reprogramming in rice. Remarkably, the resistant Hui1586 mounted faster and stronger transcriptional reprogramming at the early infection stage than the susceptible Nipponbare, which likely restricted the fungal infection more efficiently and resulted in smaller transcriptional changes at the later time points. In contrast, Guy11 could successfully infect and constitutively multiply on the susceptible Nipponbare, inducing continuous dramatic transcriptional changes within all infection stages.

The basal expressions of immune genes in Nipponbare differs from which in Hui1586

As the mock samples of Nipponbare and Hui1586 were clearly separated from each other in the PCA analysis

(Fig. 2A), we questioned what genes were differently expressed at the basal level in Hui1586 compared with Nipponbare. There were 5328, 4508, 4215 and 3952 DEGs at 12, 24, 36 and 48 h between the two cultivars with mock treatment (Fig. 3A and Supplemental dataset 2). A Venn diagram analysis identified 1331 overlapped DEGs at all four time points (Fig. 3B and Supplemental dataset 3), indicating that these genes were steadily differentially expressed in the two cultivars. We then functionally analyzed the 1331 DEGs with their Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment to understand the biological relevance underlying these genes. Interestingly, only two pathway terms, "Plant-pathogen interaction" and "Fatty acid elongation", were significantly enriched in the KEGG pathway analysis (Fig. 3C). Consistently, only defense related GO terms, "defense response", "response to stress", "response to stimulus", "hormone biosynthetic process" and "signal transduction", were significantly enriched in the GO analysis (Fig. 3D). These results illustrated that defense related genes were differently regulated in the two cultivars, which might be the main driving force for the differential expression of other genes.

The plant immune system mainly relies on receptor-like kinases (RLKs) and NLR receptors to detect pathogen-related molecules and activate defense responses (Cui et al., 2015; Tang et al., 2017; W. Wang et al., 2020). The RLK and NLR receptor genes account for ˜1% of the rice genes (Vij, Giri, Dansana, Kapoor, & Tyagi, 2008; T. Zhou et al., 2004). Remarkably, nearly 2.3% and 4.4% of 1331 DEGs encoded RLK and NLR receptors (Fig. 3E and Supplemental dataset 3), respectively, indicating that two types of receptor genes were highly enriched in the 1331 DEGs. Together, these results suggest that the basal expression of many immune receptor genes and defense-related genes are differentially regulated in Nipponbare and Hui1586, which might be one factor contributing their resistant spectrum to *M. oryzae* .

Rapid and dramatic transcription reprograming occurs in the resistant rice cultivar Hui1586 challenged with *M. oryzae*

To gain more details about the *M.* oryzae-induced DEGs in Nipponbare and Hui1586, we compared DEG overlaps between them at each time point. Large DEG overlaps, especially upregulated DEGs, were observed between the two cultivars at all four time points (Fig. 4A), indicating that a large number of common genes were involved in the transcriptional reprograming between the susceptible and resistant cultivars. As the early time point is crucial for determining the interactions between plant and pathogens (Mine et al., 2018), we performed hierarchical clustering of the DEGs identified at 12 hpi in the two cultivars based on the $\log_2$-transformed fold-change value (Supplemental dataset 4). This analysis generated a global view of the expression levels of those DEGs at four time points in Nipponbare and Hui1586. As shown in Fig. 4B, a large portion of DEGs was induced more strongly at 12 h in Hui1586 compared with Nipponbare, suggesting that a faster and stronger activation of transcriptional reprogramming is important for rice efficient defense against *M. oryzae* .

Protein translation is regulated in rice early defense responses to *M. oryzae*

GO enrichment and KEGG pathway analysis were performed to determine the functional classification and pathway assignment of the shared DEGs at 12 h in both cultivars. Remarkably, among the 3398 *M. oryzae*-upregulated genes (Fig. 4A), the "ribosome" pathway was strikingly enriched in the KEGG analysis and GO terms related to protein translation, including "ribosome biogenesis", "ribosome assembly", "rRNA processing", "protein folding" and "tRNA metabolic" were significantly enriched in the GO analysis (Fig. 4C and Supplemental table 1). Within the rice genome, ribosome-related and rRNA-related genes accounted for ˜2% and less than 0.5%, respectively. However, nearly 10.0% and 1.7% of the 3398 genes were ribosome- and rRNA-related (Fig. 4D and Supplemental dataset 5), respectively, showing that the two GO terms were enriched in this group. However, ribosome- and protein translation-related pathways were not enriched in DEGs at 24, 36 and 48 hpi (Supplemental table 1). Hierarchical clustering of ribosome- and rRNA- related genes showed that most of them were upregulated at 12 h but not at later time points (Fig. 4E). Notably, the induction of these genes was much higher in the resistant Hui1586 than the susceptible Nipponbare (Fig. 4E). Together, these results indicated that the regulation of the protein translation is involved in rice early defense responses to *M. oryzae* .

Energy metabolism pathways were strongly suppressed by *M. oryzae* on susceptible rice

KEGG pathway enrichment analysis of *M. oryzae*- upregulated genes at 12, 24, 36, or 48 hpi in both Nipponbare and Hui1586 revealed that immunity-related pathway terms, such as "Diterpenoid biosynthesis", "Flavonoid biosynthesis", "Plant-pathogen interaction", "MAPK signaling pathway" and "Phenylpropanoid biosynthesis", were significantly enriched (Fig. S1B and Supplemental table 1), indicating the immune systems were activated in both susceptible and resistant rice cultivar in response to *M. oryzae* infection. On the contrary, energy metabolism pathways, such as "Photosynthesis", "Photosynthesis – antenna proteins" or "Carbon fixation in photosynthetic organisms" were enriched in the *M. oryzae*- suppressed genes on both cultivar at 12, 36 and 48 hpi (Fig. S1A and Supplemental table 1). The result is consistent with the trade-off between immunity and growth (Huot, Yao, Montgomery, & He, 2014; Smakowska, Kong, Busch, & Belkhadir, 2016). Notably, the "Photosynthesis" pathways were enriched in the Nipponbare specific, *M. oryzae* down regulated DEGs at 24, 36 and 48 hpi, but not in the Hui1586 specific DEGs, suggesting that Energy metabolism pathways were strongly suppressed by *M. oryzae* on susceptible rice.

Identification of core genes involved in rice defense responses to *M. oryzae*

To identify specific or common genes involved in rice defense responses to *M. oryzae* at different time point, we performed Venn diagram analysis of the DEGs from Nipponbare or Hui1586 at all four time points. The diagrams showed that the majority of rice DEGs were temporarily up- or down-regulated by *M. oryzae* at specific periods. For instance, 2899 of 6725 DEGs from Nipponbare, and 6678 of 9703 DEGs from Hui1586 were specifically regulated by *M. oryzae*only at 12 hpi (Fig. 5A). Relatively small amount of DEGs, 1464 (1119 upregulated and 293 downregulated) and 578 (448 upregulated and 89 downregulated) were overlapped at all time points in Nipponbare or in Hui1586, respectively (Fig. 5A and Supplemental dataset 6). We then performed KEGG pathway enrichment analysis on each group of DEGs in Fig. 5A (Supplemental table 2). Among the 2899 DEGs specifically regulated at 12 hpi on Nipponbare, only protein translation related pathways were significantly enriched (Supplemental table 2), which is consistent with our previous analysis (Fig. 4). Surprisingly, no KEGG pathway term was enriched in the 259, 682, 407, 551, 362, 126, 173, 1680, and 832 DEG groups. Moreover, plant defense related pathways were enriched mainly in the common 1464 DEGs that were regulated at all four time points on Nipponbare (Fig 5A and Supplemental table 2). Similarly, in Hui1586, protein translation pathways were only enriched in 6678 DEGs that specifically regulated at 12 hpi, and no pathway term was enriched in 711, 501, 284, 221, 523, 197,101, 694, and 186 DEG groups (Fig 5A and Supplemental table 2). However, different to Nipponbare, defense related pathways were enriched in all other DEG groups, especially with lowest Q -value in the common 578 DEGs overlapped by all four time points (Fig. 5A and Supplemental table 2). These results showed that the expressions of the majority of DEGs from Nipponbare or Hui1586 were regulated dynamically/temporarily in response to *M. oryzae* , except a common group of defense related genes, most of which were constitutively upregulated (Fig. 5A). Thus, we hypothesized that a group of common DEGs might forms the main part of the engine for immune transcriptional reprogramming.

To test above hypothesis, we focused on the 353 overlapped DEGs between the 1464 and the 578 common DEGs in Nipponbare and Hui1586 (Fig. 5A and Supplemental dataset 7). Among these DEGs, 321 were upregulated and 32 were downregulated genes (Fig. 5A). Consistently, hierarchical clustering of the FPKM values of those 353 genes showed that they fell into two groups: genes in the large group (A) were upregulated and genes in the small group (B) were downregulated by *M. oryzae* in both cultivars (Fig. 5B). A KEGG pathway analysis of the common 321 upregulated genes showed that four immunity-related pathways were significantly enriched, including "diterpenoid biosynthesis", "flavonoid biosynthesis", "plant-pathogen interaction" and MAPK signaling pathways (Fig. 5C and Supplemental dataset 7), suggesting that these 321 genes were important components in rice resistance against*M. oryzae.* The GO enrichment analysis also showed that "defense response" and "diterpene phytoalexin metabolic" were the most significantly enriched GO terms (Fig. S2). Furthermore, among the GO molecular function terms, "carbohydrate binding", "ion binding", "protein kinase activity", "phosphotransferase activity", "oxidoreductase activity", "kinase activity", "anion binding", and "pattern binding" were significantly enriched (Fig. S2). Carbohydrate binding

proteins are proteins that can interact with sugar chains (Someya et al., 2010). For example, lectins are carbohydrate-binding proteins that play numerous roles in biological recognition events. Several plant lectin receptor kinases are involved in plant innate immunity (Singh, Chien, Mishra, Tsai, & Zimmerli, 2013; Singh & Zimmerli, 2013). Among "ion binding" proteins, many different types are found in plants. Metal ions help stabilize protein and regulate protein catalytic activity (Lu, Lin, Lin, & Yu, 2012). For example, calcium, calcium channel and calcium-binding proteins play different and important roles in activating plant defense responses (J. M. Zhou & Zhang, 2020). No significant KEGG pathway terms or GO terms were found to be enriched in the common 32 downregulated genes. Taken together, these analyses indicated that most of the 321 unregulated genes were involved in rice immunity and are potential candidates for functional characterization in further studies. Thus, the 321 genes were termed as a "core" set of rice immune genes for further analysis.

The core genes are involved in rice resistance to other pathogens and drought stress

In a larger transcriptome comparison analysis with microarray or RNA-seq datasets deposited in the NCBI GEO database, we investigated the conditions in which the expressions of core genes were regulated. In this analysis, 2308 rice microarray and 355 RNA-seq datasets consisting of various perturbations, such as biotic, abiotic, hormone, nutrient and genetic background alterations, were screened. Consistent with our results, most of the core genes were regulated in the datasets of rice-*M. oryzae* interactions (Fig. 6A and Supplemental dataset 8). The core genes were also regulated in the rice response to other pathogens, such as the bacteria *Xanthomonas oryzae,* which causes rice blight disease, and the fungus *Ustilaginoidea virens,* which causes rice false smut (Fig. 6A, and Supplemental dataset 8). These findings suggested that these genes were common regulators of the rice immune system. Interestingly, more than 80% of the core genes were also regulated under rice drought stress (datasets GSE57950, GSE25176, GSE41647, GSE24048 and GSE92989), indicating the involvement of the core genes in drought stress responses (Fig. 6A and Supplemental dataset 8). Hierarchical clustering of the fold-change of the core genes during different perturbations was conducted to illustrate their regulation patterns (Supplemental dataset 9). The majority of the genes were upregulated in rice infected with *M. oryzae, X. oryzae* or *U. virens* , while they were downregulated during drought treatment (Fig. 6B). A similarity comparison showed that the dataset of *M. oryzae* treatment was similar to the datasets of *U. virens* and *X. oryzae* treatments but different from the dataset of drought treatment (Fig. 6C). These analyses indicated that the core genes were involved in both biotic and abiotic stress responses.

Transcription factors in the core gene list are important for rice stress resistance

Transcription factors (TFs) are master regulators of gene expression. Our RNA-seq detected the expression of 1573 putative TF genes that could be classified into 58 families. Among the *M. oryzae*- induced 8211 DEGs in both Nipponbare and Hui1586 (Fig. 2D), 313 genes encoded TFs (Supplemental dataset 10). These TFs were further classified into two large clusters in a hierarchical clustering based on the fold-change values (Fig. 7A). Most of the TFs in cluster I (205 TFs) were upregulated, while the majority of TFs in cluster II (108 TFs) were downregulated by *M. oryzae* (Fig. 7A). Among the cluster I TFs, the WRKY family contained 38 members, which was clearly enriched (Fig. 7B). The MYB family ranked second with 25 members, followed by the NAC, bHLH, and AP2-ERF families with 21, 20, and 19 members, respectively (Fig. 7B). The enrichment of WRKY TFs in upregulated DEGs is consistent with the well-established roles of the WRKY family in plant immunity. The NAC, bHLH and AP2-ERF families were slightly enriched in cluster I, suggesting that these TFs were also involved in rice resistance to blast fungi (Fig. 7B).

Among the 313 rice blast fungi-responsive TFs, 14 members were in the core gene list, including 5 WRKY (OsWRKY19, 28, 31, 45, and 77), 2 bHLH, 2 NAC, 2 MYB (OsMYB30 and OsMYB55), 2 G2-like and 1 C2H2 TFs (Supplemental dataset 7). Notably, 5 of them have been reported to regulate rice resistance to pathogens. For instance, *OsMYB30* ,*OsWRKY31* and *OsWRKY45* positively while *OsWRKY28* negatively regulates rice resistance to *M. oryzae* (Chujo et al., 2013; W. Li et al., 2020; Lv et al., 2017; Shimono et al., 2012; J. Zhang, Peng, & Guo, 2008). *OsMYB30* also plays a negative role in rice cold tolerance (Lv et al., 2017). In addition, *OsMYB55* enhances rice tolerance to high temperature (El-Kereamy et al., 2012). Thus, approximately 36% (5 of 14) of the TFs in core genes have been functionally characterized as being

important regulators of rice stress resistance, indicating that the core gene list contains important regulators in rice immunity and merit evaluation in further functional studies.

TFs regulate gene expression by binding to sequence-specific *cis* -regulatory elements of target genes. Therefore, we investigated the TF binding motifs that were overrepresented within the promoters of the 321 core genes. Motifs corresponding to DNA binding sites of WRKY, bHLH and MYB TFs were overrepresented in the core genes (Fig. 7C, 7D and Supplemental dataset 11). The DNA motifs for B3 and bZIP, especially AP2-ERF-type TFs, were markedly overrepresented among the core genes (Fig. 7C). AP2 (APETALA2) and ERF (ethylene-responsive factor) transcription factors are unique to plants, the distinguishing characteristic of which is their so-called AP2 DNA-binding domain (L. Wang, Ma, & Lin, 2019). AP2-ERF genes form a large multigene family and play a variety of roles in plant developmental processes, as well as in plant responses to various types of biotic and environmental stresses (Mizoi, Shinozaki, & Yamaguchi-Shinozaki, 2012; Rashid, Guangyuan, Guangxiao, Hussain, & Xu, 2012). The DNA binding motifs of many AP2-ERF TFs, such as ORA59 and CBF1 (Fig. 7D), are represented on the promoter of 111 and 87 core genes, respectively (Supplemental dataset 11). In *Arabidopsis* , ORA59 positively regulates resistance to necrotrophic pathogens by direct binding to the promoters of jasmonate- and ethylene-responsive genes (Catinot et al., 2015). CBF1 induces cold-regulated gene expression and increases plant freezing tolerance (Chinnusamy, Zhu, & Zhu, 2007). These findings suggest that AP2-ERF TFs are important regulators in rice resistance to blast fungi.

Identification of a peroxidase gene negatively regulating rice resistance to blast disease

Among the 321 core genes, a peroxidase gene, *Os07g0677200,* drew our attention, with the highest FPKM value at 12 hpi of *M. oryzae* in Nipponbare and the second highest FPKM value in Hui1586 (Supplemental dataset 7). To test the function of *Os07g0677200* (hereby named *Perox4* following the names of published peroxidase genes in rice) in rice blast disease resistance, we used CRISPR/Cas9 technology to knock out the *Perox4* gene in the rice cultivar Zhonghua 11 (ZH11). Japonica rice ZH11 is moderately susceptible to *M. oryzae* isolate Guy11 and has been used in a large-scale gene knockout project in our group. A 20-nt sequence in the third exon of the *Perox4* gene was designed as the target site for Cas9 cleavage, and multiple putative transgenic lines were generated and verified by sequencing. We found one line (named *perox4-1*) carrying a one-base insertion in the target site that truncates the *Perox4* open reading frame, and another line (named *perox4-2*) carrying a three-base deletion in the *Perox4* gene (Fig. S3A).

The *perox4* lines were challenged with the blast isolate Guy11. We found that the lesion numbers and lesion size were dramatically reduced in the *perox4* lines compared with ZH11 (Figs. 8A and 8B).We then measured the expression of defense-related genes, such as *OsPR4* and *OsPR* 5, in *perox4* and ZH11 plants with *M. oryzae* infection using qRT-PCR. We observed that the expression levels of these marker genes were elevated in *perox4* compared with ZH11 plants (Fig. 8C). Together, these results demonstrated that the *Perox4* negatively regulated rice resistance to *M. oryzae.*

Perox4 **regulates key agronomic traits**

We next monitored several key agronomic traits in the *perox4* mutant to assess its application potential in rice breeding. We compared agronomic-related traits, including plant height, panicle length, number of effective panicles, spikelets per panicle, seed setting rate, thousand-grain weight, grain length, grain width, and grain yield per plant between parents and knockout lines. The grain width of *perox4* was significantly smaller than WT ZH11 (Fig. 8D and 8E). Accordingly, the 1,000-grain weight and grain yield per plant were lower in *perox4* than ZH11 (Fig. 8F and Supplemental table 3).

OsMT1a **and** ***OsMT1b*** **positively regulate rice resistance to** ***M. oryzae***

The above RNA-seq analysis suggested that 12 hpi is a critical time point for rice defense against *M. oryzae.* Among 4680 fungal upregulated DEGs at 12 hpi in Nipponbare, the *type 1 metallothionein* (*OsMT1a* , *Os11g0704500*) gene had the highest expression value (FPKM). *OsMT1a* was also one of the top four genes in terms of the FPKM value among 6808 upregulated DEGs at 12 hpi in Hui1586 (Supplemental dataset 1).

In rice, OsMT1a has a very close protein homolog (with 84% identity and 89% positivity in Blastp) encoded by *Os03g0288000* (hereafter named *OsMT1b*), which was also upregulated by *M. oryzae* in Nipponbare and Hui1586 (Supplemental Dataset 1). To assess the involvement of *OsMT1a* and*OsMT1b* in rice blast resistance, we generated *osmt1a osmt1b* (*osmt1a/b* for short) double knockout lines in the Nipponbare background using CRISPR/Cas9 technology. We acquired the*osmt1a/b-1* and *osmt1a/b-2* double mutant, which contains a one-base deletion in *OsMT1a* and a five-base deletion or one-base deletion in *OsMT1b* (Fig. S3B).

We then inoculated *osmt1a/b* mutant and Nipponbare plants with the blast isolate Guy11. We found that the *osmt1a/b* plants were more susceptible to rice blast compared with Nipponbare (Figs. 9A and 9B). Consistent with the enhanced blast susceptibility in *osmt1a/b,*the expression of *OsPR5* and *OsPAL* were reduced in*osmt1a/b* compared with Nipponbare plants after *M. oryzae*infection (Fig. 9C). Thus, we concluded that *OsMT1a* and*OsMT1b* positively contributed to rice resistance to blast disease.

Discussion

In this study, we carried out a comprehensive transcriptome study of susceptible and resistant rice cultivars over a 48 h time course of infection with the blast fungus *M. oryzae* . Our data showed that high-amplitude transcriptional reprogramming was kicked off very quickly in rice-*M. oryzae* compatible and incompatible interaction. Large numbers of DEGs were identified, and a core set of genes involved in rice stress responses were defined. Furthermore, our study not only provided an overview of transcriptional reprogramming during rice-*M. oryzae* interactions but also identified and functionally validated three novel players in rice blast resistance.

A number of rice blast resistance genes and signaling regulators have been mapped and identified after decades of study (Li et al., 2019; Liu et al., 2014). However, the full regulatory network and immune signaling components remain elusive. Here we designed a comprehensive RNA-seq experiment to study the transcriptional profiles of rice-*M. oryzae* compatible (susceptible Nipponbare) and incompatible (resistant Hui1586) interactions. Four time points were included in the experiment, from very early at 12 hpi to later at 48 hpi with 12 h intervals between each point. Because the circadian clock controls the expression of ˜ 30% of the transcriptome in plants (Harmer, 2009), mock-treatment (H_2O) samples were included as controls at each time point to ensure a proper comparison for identifying DEGs. Our RNA-seq data showed that the circadian clock is the major factor determining the transcription dynamics (Fig. 1A, H_2O treatment). Thus, it was essential to compare *M. oryzae* treatment data with the mock data to identify DEGs during the rice response to blast fungi. In this way, thousands of DEGs were identified at each time point, far exceeding the numbers in previous reports (Wei et al., 2013; Y. Zhang et al., 2016). In total, the expression of ˜25% of the rice transcriptome was affected by *M. oryzae* treatment (Fig. 2C and 2D), indicating that great transcriptional changes occurred in rice challenged with blast fungus. Remarkably, the largest number of DEGs (6808 upregulated and 2895 downregulated, 9703 in total) was observed in the resistant Hui1586 at 12 hpi of blast fungus. Compared with the smaller number of DEGs in the susceptible Nipponbare (4680 upregulated and 2045 downregulated genes), we concluded that the resistant cultivar induced active defense more quickly and strongly than the susceptible cultivar. Unexpectedly, the number of DEGs declined in Hui1586 to 2849 (2224 upregulated and 625 downregulated) at 24 hpi, which was ˜1/3 of the DEG number at 12 hpi. This result suggested that the strongest transcriptional reprogramming occurred before 24 hpi, and the critical time period for *M. oryzae* invasion was probably less than 12 hours. RNA-seq data for more time points, earlier than 12 hpi, is needed to determine the critical time points for rice-*M. oryzae*interactions.

In addition to transcriptional reprogramming, global translational reprogramming has been shown to be another fundamental regulatory layer of plant immunity (Xu et al., 2017). A consensus sequence, R-motif, present on a large number of messenger RNA, regulates translation in response to PTI induction (Xu et al., 2017). A latest study has shown that plants protect stem cells against viral infection through impairing global protein synthesis and limiting the replication and spread of the virus (Wu et al., 2020). In our transcriptome analysis, we found that among the common upregulated DEGs at 12 hpi in both Nipponbare and Hui1586, "ribosome" was the most significantly enriched pathway in the KEGG analysis, and "translation" was

the most significantly enriched term in the GO analysis (Fig. 4C). These results strongly indicated that the protein translation machinery was regulated during the rice response to *M. oryzae* . Consistent with our results, a previous transcriptome study of rice *Pi21*- silenced plants infected by*M. oryzae* showed that "ribosome" is the third most enriched pathway compared with Nipponbare plants (Y. Zhang et al., 2016).*Pi21* encodes a cytoplasmic proline-rich protein that negatively regulates rice blast resistance, and silencing of this gene results in enhanced resistance to blast fungi (Fukuoka et al., 2009). However, enrichment of the "ribosome" pathway was only observed at 12 hpi, not 24, 36, or 48 hpi, indicating that the regulation of protein synthesis in rice responses to *M. oryzae* was transient. Protein synthesis is a high-energy consumption process in living cells. It consumes approximately two-thirds of the total energy produced by a rapidly growing *Escherichia coli* cell (Jewett, Miller, Chen, & Swartz, 2009). Plant defense also imposes a substantial demand of resources and energy, which negatively impact growth (Huot et al., 2014). Activation of defense would decrease the overall pool of energy reserves through diminishing photosynthesis. It is possible that the demand of energy during the quick activation of plant immunity might reduce energy for the ribosome, the protein translation factory, slowing down the translation globally. As a negative feedback, the transcription of ribosome-related genes was upregulated accordingly. Further biochemical experiments are required to examine whether the global protein translation was transiently suppressed during the activation of rice immunity. However, in agreement with this hypothesis, protein synthesis inhibitor cycloheximide treatment of *Arabidopsis* induces transcriptional reprogramming similar to pathogen treatment (Navarro et al., 2004).

Among more than 10 thousand DEGs identified from four time points, only 1464 and 578 DEGs were shared by all four time points in Nipponbare and Hui1586, respectively, indicating that the transcriptome in the rice response to blast fungus infection was changing dynamically. A small set of DEGs shared by Nipponbare and Hui1586 at all time points were identified. These common DEGs might be important for driving overall immune transcriptional reprogramming. Indeed, only defense-related signaling and metabolism pathways were enriched in the common DEG group, and therefore, these genes were considered the "core" genes in*M. oryzae*- induced transcriptional reprogramming. Expressions of most of the core genes were also induced by other pathogens, such as the fungal *U. virens* and bacterial *X. oryzae* (Fig. 6A). Furthermore, the core genes were largely repressed during drought stress (Fig. 6B). Thus, the core genes were likely common genes involved in rice defense against pathogens and abiotic stress responses. Supporting this conclusion, among 14 TFs from the core genes, 5 TFs have been functionally characterized as important regulators of disease resistance, and 2 TFs have been reported to be involved in rice tolerance to temperature stress. We speculate that the core gene set contains convergence points between biotic and abiotic stress signaling pathways.

In this study, we identified a peroxidase gene *Perox4* that plays a negative role in rice resistance to blast fungus (Fig. 8). The main role of peroxidase in plant immune responses is to aid in maintaining hydrogen peroxide (H_2O_2), the most stable ROS, at the appropriate level, which is toxic to plant cells at high concentrations (Qi, Wang, Gong, & Zhou, 2017). Three rice peroxidases (*Os05g0135200* , *Os10g0536700* , and*Perox3*) have been reported to contribute to BSR-D1, a C_2H_2-type transcription factor-mediated susceptibility to blast disease (Li et al., 2017; Zhu et al., 2020). BSR-D1 induces the expression of these peroxidase genes by direct DNA binding. An allele, *Bsr-d1,* in the rice cultivar Digu confers broad-spectrum resistance to *M. oryzae* (Li et al., 2017; Zhu et al., 2020). Notably, the expression of *Perox4* is compromised in *bsr-d1* knockout plants (Zhu et al., 2020), suggesting it is one of the target of BSR-D1. However, it remains inconclusive whether BSR-D1 can bind to the *Perox4* promoter (Zhu et al., 2020). In our RNA-seq data, *Perox3* was upregulated in Nipponbare and Hui1586, while the mRNA of *Os05g0135200* and*Os10g0536700* were barely detectable even with *M. oryzae* -treatment in both cultivars. In addition, another 4 peroxidase genes were upregulated by *M. oryzae* in Nipponbare and Hui1586 (Supplemental table 7). The peroxidase genes were proposed to be hijacked by *M. oryzae* through activation of the *BSR-D1*gene to counter the ROS burst induced by *M. oryzae* infection. It is reasonable to speculate that as an important susceptibility factor,*Perox4* might be hijacked by blast fungi to suppress host immune responses.

In addition to ROS-scavenging enzymes, low-molecular mass antioxidants, including glutathione, ascorbate, carotenoids and (MTs), are involved in ROS maintenance (Qi et al., 2017; Yang, Wu, Li, Ling, & Chu, 2009). MTs are small, cysteine-rich, metal-binding proteins that are involved in metal homeostasis and detoxification

in both plants and animals (Yang et al., 2009; Zimeri, Dhankher, McCaig, & Meagher, 2005). It has been shown that *OsMT1a* improves drought tolerance when overexpressed in rice by not only participating in ROS scavenging but also regulating the expression of zinc finger-type TFs via the regulation of Zn^{2+} homeostasis (Yang et al., 2009). However, unlike the ROS-scavenging enzyme *Perox4* , simultaneous knockout of*OsMT1a* and its close homolog *OsMT1b* in Nipponbare further enhanced susceptibility to blast fungus (Fig. 9). Thus, *OsMT1* positively regulated drought tolerance and blast disease resistance. DNA motifs of several zinc finger-type TFs were significantly enriched in the promoter of 321 core genes (Fig. 7C and Supplemental dataset 11), suggesting that *OsMT1* might regulated blast disease resistance in a manner similar to the regulation of drought stress resistance, partly through zinc finger-type TFs.

In summary, this study provides a high-quality, comprehensive RNA-seq data set for rice-*M. oryzae* interactions and enhances our understanding of the transcriptional networks in rice immune responses to the blast fungus, highlighting possible candidate genes that may play important roles in rice disease resistance and abiotic stress tolerance.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Figure legends

Figure 1. The disease symptoms on the rice cultivars Nipponbare and Hui1586 infected by blast fungi.

(A-B) Hui1586 exhibited strong leaf blast resistance in a natural nursery at two locations, Jinggangshan, Jiangxi Province (A) and Yichang, Hubei Province, China (B). (C) Blast resistance of Hui1586 plants using spraying inoculation in a greenhouse. Representative leaves obtained 7-day postinoculation of blast strain Guy11.

Figure 2. Overview of transcriptome data and differentially expressed genes (DEGs) in the rice response to blast fungus. (A) Principal component analysis of the time-series transcriptome data in Nipponbare (Nip) and Hui1586 treated with the blast isolate Guy11 (M.o) or H_2O. (B) Hierarchical clustering of the samples for the normalized FPKM (fragments per kilobase of transcript per million mapped reads) values of all detectable genes. (C) The numbers of up- and downregulated genes by *M. oryzae* in Nipponbare or Hui1586 compared with the mock treatment are shown. (D) Venn diagram of total DEGs in Nipponbare compared with Hui1586.

Figure 3. Analysis of differentially expressed genes (DEGs) between Nipponbare and Hui1586 in the mock treatment. (A) The numbers of DEGs between Nipponbare and Hui1586 at the indicated time points after H_2O treatment. (B) Venn diagram of DEGs in (A). (C-D) KEGG pathway (C) and GO (D) enrichment analysis of 1331 DEGs in (B). The y-axis represents the negative $\log_{10}$-transformed Q -value

(blue bars) and gene numbers (yellow dots). (E) Enrichment of the *LRR* and *NLR*genes in the 1331 DEGs compared with their ratio in the rice genome.

Figure 4. Pathway enrichment of DEGs in the rice response to blast fungus. (A) Venn diagram of DEGs induced by *M. oryzae* in Nipponbare and Hui1586 compared with mock treatment within 48 hpi. Up- and downregulated DEGs are indicated by upward and downward pointing arrows, respectively. (B) Hierarchical clustering of all the DEGs at 12 hpi based on the $\log_2$ fold change in transcript levels in Nipponbare (Nip) and Hui1586 (Hui) with *M. oryzae* treatment relative to the mock treatment. (C) KEGG pathway and GO enrichment analysis of 3398 upregulated DEGs at 12 h in (A). The y-axis represents the negative $\log_{10}$-transformed Q -value (blue bars) and gene numbers (yellow dots). (D) The enrichment of ribosome- and rRNA-related genes in the 3398 DEGs in (C) compared with its ratio in the rice genome. (E) Hierarchical clustering of ribosome- and rRNA-related genes in the 3398 DEGs in (C) based on the $\log_2$ fold change as in (B).

Figure 5. Identification and pathway analysis of a "core" set of DEGs in Nipponbare and Hui1586. (A) Venn diagram of DEGs at the four time points in Nipponbare (Nip) and Hui1586. The numbers in the blue circle represent the overlapped DEGs between the two linked groups. The upward and downward arrows indicate up- and downregulated DEGs, respectively. (B) Hierarchical clustering of the 321 + 32 DEGs in (A) based on normalized FPKM values in Nipponbare and Hui1586. (C) KEGG pathway enrichment analysis of 321 DEGs in (A). The y-axis represents the negative $\log_{10}$-transformed Q -value (blue bars) and gene numbers (yellow dots).

Figure 6. The core genes are involved in biotic and abiotic stress responses. (A) The bar plot shows that among the 321 core genes, the number of genes that were differentially regulated in rice treated with *Magnaporthe oryzae* (GSE83219), *Ustilaginoidea virens*(GSE39049), *Xanthomonas oryzae* (GSE36272) or drought (GSE57950) in polished transcriptome data sets. (B) Hierarchical clustering of the 321 core genes based on the $\log_2$–fold change with various treatments as in (A). (C) Similarity comparation among the data sets as in (A) based on the $\log_2$–fold change of the 321 core genes.

Figure 7. Characterization of transcription factors and*cis*-regulatory motifs in differentially regulated genes in rice in response to *M. oryzae*. (A) Hierarchical clustering of the total 313 transcription factors (TFs) that were differentially regulated in both Nipponbare (Nip) and Hui1586 in response to *M. oryzae*infection. The rows of collapse represent the average value of genes in the indicated groups in each column/condition. (B) Enrichment of the TF families of the 205 TFs (Cluster I in (A)) compared to their ratio in each family in the rice genome. (C) Overrepresentation of known TF DNA binding motifs within the 500-bp promoter of the 321 core genes. Rows indicate motifs of corresponding TF families and are colored according to the P- value for enrichment. (D) Sequence logo depiction of the key overrepresented TF DNA binding motifs in the 500-bp promoter of the 321 core genes.

Figure 8. Phenotypic characterization of the rice *perox4*knockout lines. (A) Blast resistance of two independent *perox4*knockout lines compared with the parental Zhonghua11 (ZH11) plants. Three representative leaves collected at 3 d postinoculation of the blast isolate Guy11 are shown. (B) Lesion numbers per cm^2 on the rice leaves (mean +- SD, n > 10 leaves) after inoculation with blast fungus as in (A). (C) Expression of defense marker genes (*PR2,* and *PR5*) in *perox4* and ZH11 at the indicated times after infection of Guy11 measured by qRT-PCR. The y-axis represents the relative expression value ($\log_2$ – transformed, mean +- SD, n = 3) normalized to*Osactin* (*Os03g0718100*). *, Statistical significance ($P < 0.01$) determined by the Student's t- test; ns, no significant difference. (D) Photograph of 10 seeds each for ZH11 and the *perox4* mutant lines. Scale bars, 0.5 cm. (E) and (F) Grain width and thousand-gain weight of the ZH11 and *perox4*mutant. Error bars indicate the mean +- SD (n=3 biological replicates, each replicate contains 10 seeds for G and 1000 seeds for H), *, statistical significance ($P < 0.01$) determined by the Student's t- test.

Figure 9. Disease resistance of the rice *osmt1a/b*knockout lines. (A) Blast resistance of two independent *osmt1a/b*knockout lines compared with the parental Nipponbare (Nip) plants. Three representative

leaves collected at 3 d postinoculation of the blast isolate Guy11 are shown. (B) Lesion numbers per cm^2 on rice leaves (mean +- SD, n > 10 leaves) after inoculation with blast fungus as in (A). (C) Expression of defense marker genes (*PR5,* and *PAL*) in *osmt1a/b* and Nipponbare at the indicated times after infection of Guy11 measured by qRT-PCR. The y-axis represents the relative expression value ($\log_2$ – transformed, mean +- SD, n = 3) normalized to *Osactin*(*Os03g0718100*). *, Statistical significance ($P < 0.01$) determined by the Student's *t*- test; ns, no significant difference.

Table 1. Inoculation experiment with different blast isolates showing that Hui1586 has broad spectrum resistance.

M. oryzae strain	Hui1586	Nipponbare
Guy11	R	S
18SH-D527	R	S
KJ201	R	S
501-3	R	S
FJ2011	R	S
95085AZB	R	S
Zhong1	R	S
MH86-1	R	S
MH86-3	R	S
RB22	R	S
20-15	R	S
18NH-16-3	R	S
M409	R	S

Supplemental Data

Figure S1. KEGG pathway and GO enrichment analysis of differentially regulated genes (DEGs) by *M. oryzae.* (A) KEGG pathway and GO enrichment of the 1115 downregulated DEGs in Nipponbare and Hui1586 at 12 hpi of *M. oryzae.* (B) KEGG pathway enrichment of the *M. oryzae*-upregulated DEGs in Nipponbare and Hui1586 at 24, 36, or 48 hpi.

Figure S2. KEGG pathway and GO enrichment analysis of differentially regulated genes (DEGs) by *M. oryzae.* (A-C) KEGG pathway enrichment of DEGs that were commonly regulated in Nipponbare or Hui1586 at 12, 24, 36, and 48 hpi of *M. oryzae.* There were 578 DEGs in Hui1586 (A), 1464 DEGs in Nipponbare (B), and 293 *M. oryzae* -downregulated DEGs in Nipponbare. (C) GO enrichment of the 321*M. oryzae*- upregulated core DEGs in Nipponbare and Hui1586 at 24, 36, and 48 hpi.

Figure S3. Information on the *perox4* and *osmt1a/b*knockout lines generated by CRISPR/Cas9 technology. (A) Two independent knockout lines of *Os07g0677200* (*Perox4*) were generated using the CRISPR/Cas9 system and verified by sequencing. (B) Two independent knockout lines of *Os11g0704500 Os03g0288000*(*OsMT1a OsMT1b*) were generated using the CRISPR/Cas9 system and verified by sequencing.

Supplemental dataset 1. The expression values for all differentially expressed genes between *M. oryzae* and mock treatment in the rice Nipponbare or Hui1586.

Supplemental dataset 2. The expression values for all differentially expressed genes between Nipponbare or Hui1586 with mock treatment.

Supplemental dataset 3. The expression values and KEGG pathway analysis for the common 1331 differentially expressed genes between Nipponbare or Hui1586 at 12, 24, 36 and 48 hpi with mock treatment.

Supplemental dataset 4. The expression values for differentially expressed genes between *M. oryzae* and mock treatment at 12 hpi in the rice Nipponbare or Hui1586.

Supplemental dataset 5. The expression values and KEGG pathway analysis for *M. oryzae*-upregulated genes at 12 hpi in both Nipponbare and Hui1586.

Supplemental dataset 6. The expression values for the common differentially expressed genes between *M. oryzae* and mock treatment at 12, 24, 36 and 48 hpi in Nipponbare or Hui1586.

Supplemental dataset 7. The expression values and KEGG pathway analysis for the common differentially expressed genes between *M. oryzae* and mock treatment at 12, 24, 36 and 48 hpi in both Nipponbare and Hui1586.

Supplemental dataset 8. Transcriptome comparison identified conditions in which the rice immune "core" genes were regulated.

Supplemental dataset 9. The expression values for the rice immune "core" genes in the three published datasets.

Supplemental dataset 10. The expression values for the differentially expressed transcription factors (TFs) induced by *M. oryzae* in Nipponbare and Hui1586.

Supplemental dataset 11. *Cis*-regulatory motif enrichment analysis of the promoters of the rice immune "core" genes.

Supplemental table 1. KEGG pathway enrichment in different groups of differentially expressed genes (DEGs) in rice induced by blast fungus.

Supplemental table 2. KEGG pathway enrichment in differentially expressed genes (DEGs) at different time points in rice induced by blast fungus.

Supplemental table 3. Comparison of the main agronomic traits between the knockout and parental lines.

Supplemental table 4. Primer sequences used for synthesizing gRNA spacers, genotyping CRISPR/Cas9-edited mutants and qPCR

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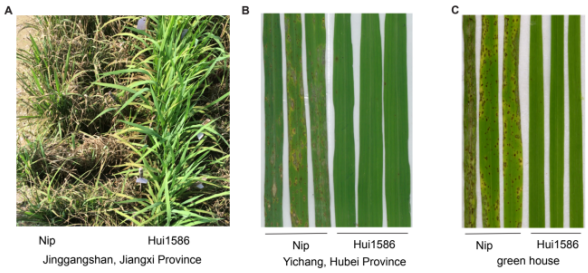

Figure 1. The disease symptoms on the rice cultivars Nipponbare and Hui1586 infected by blast fungi. (A-B) Hui1586 exhibited strong leaf blast resistance in a natural nursery at two locations, Jinggangshan, Jiangxi Province (A) and Yichang, Hubei Province, China (B). (C) Blast resistance of Hui1586 plants using spraying inoculation in a greenhouse. Representative leaves obtained 7-day postinoculation of blast strain Guy11.

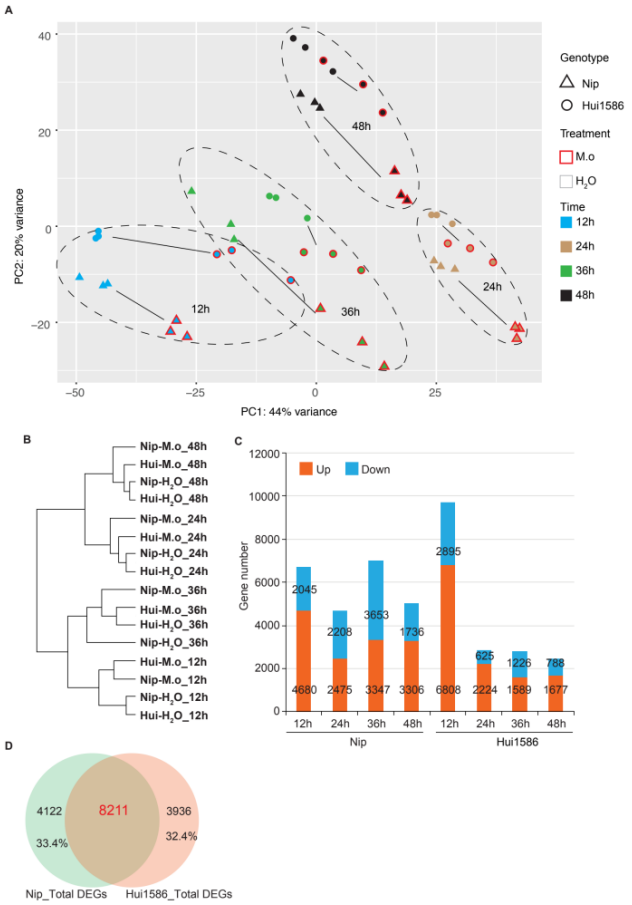

Figure 2. Overview of transcriptome data and differentially expressed genes (DEGs) in the rice response to blast fungus. (A) Principal component analysis of the time-series transcriptome data in Nipponbare (Nip) and Hui1586 treated with the blast isolate Guy11 (M.o) or H2O. (B) Hierarchical clustering of the samples for the normalized FPKM (fragments per kilobase of transcript per million mapped reads) values of all detectable genes. (C) The numbers of up- and downregulated genes by *M. oryzae* in Nipponbare or Hui1586 compared with the mock treatment are shown. (D) Venn diagram of total DEGs in Nipponbare compared with Hui1586.

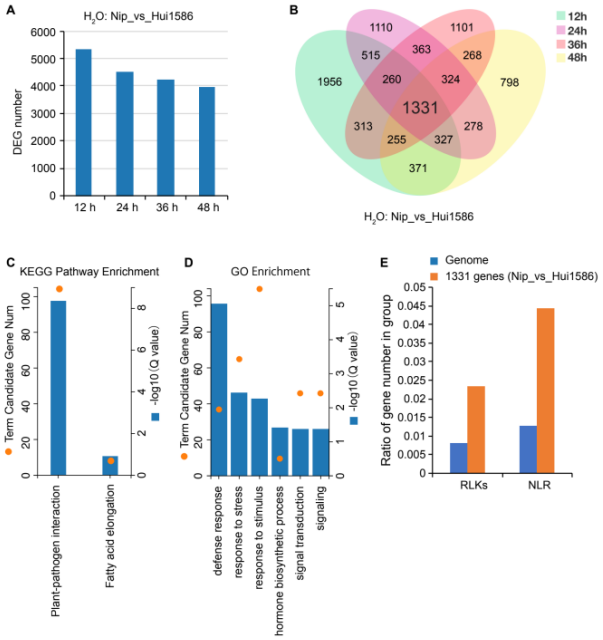

Figure 3. Analysis of differentially expressed genes (DEGs) between Nipponbare and Hui1586 in the mock treatment. (A) The numbers of DEGs between Nipponbare and Hui1586 at the indicated time points after H2O treatment. (B) Venn diagram of DEGs in (A). (C-D) KEGG pathway (C) and GO (D) enrichment analysis of 1331 DEGs in (B). The y-axis represents the negative $\log_{10}$-transformed *Q*-value (blue bars) and gene numbers (yellow dots). (E) Enrichment of the *LRR* and *NLR* genes in the 1331 DEGs compared with their ratio in the rice genome.

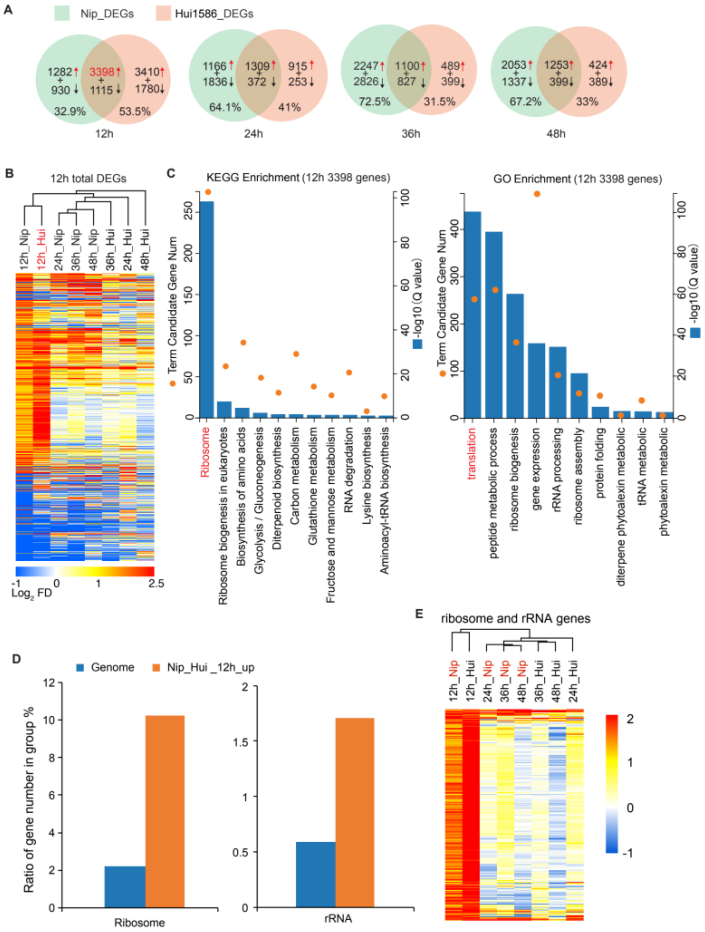

Figure 4. Pathway enrichment of DEGs in the rice response to blast fungus. (A) Venn diagram of DEGs induced by *M. oryzae* in Nipponbare and Hui1586 compared with mock treatment within 48 hpi. Up- and downregulated DEGs are indicated by upward and downward pointing arrows, respectively. (B) Hierarchical clustering of all the DEGs at 12 hpi based on the $\log_2$ fold change in transcript levels in Nipponbare (Nip) and Hui1586 (Hui) with *M. oryzae* treatment relative to the mock treatment. (C) KEGG pathway and GO enrichment analysis of 3398 upregulated DEGs at 12 h in (A). The y-axis represents the negative log10-transformed Q-value and gene numbers. (D) The enrichment of ribosome- and rRNA-related genes in the 3398 DEGs in (C) compared with its ratio in the rice genome. (E) Hierarchical clustering of ribosome- and rRNA-related genes in the 3398 DEGs in (C) based on the $\log_2$ fold change as in (B).

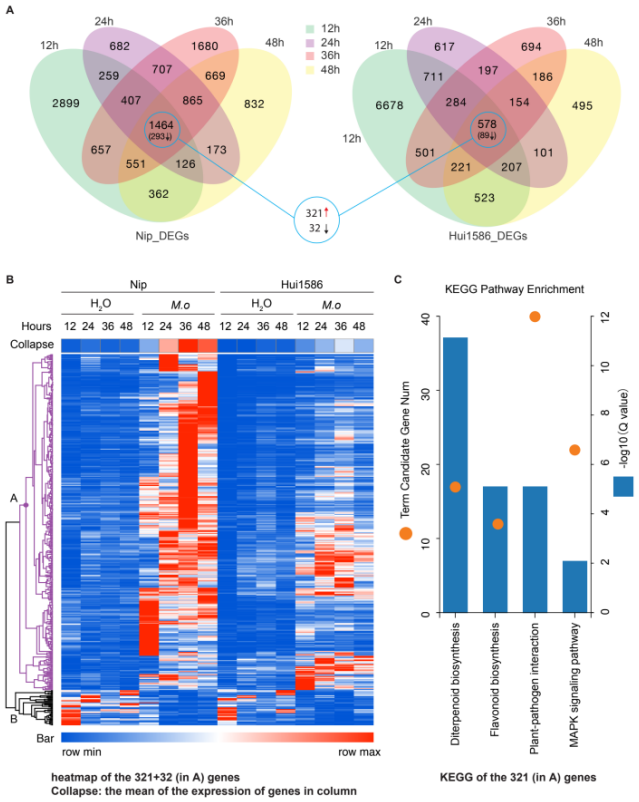

Figure 5. Identification and pathway analysis of a "core" set of DEGs in Nipponbare and Hui1586.
(A) Venn diagram of DEGs at the four time points in Nipponbare (Nip) and Hui1586. The numbers in the blue circle represent the overlapped DEGs between the two linked groups. The upward and downward arrows indicate up- and downregulated DEGs, respectively. (B) Hierarchical clustering of the 321 + 32 DEGs in (A) based on normalized FPKM values in Nipponbare and Hui1586. (C) KEGG pathway enrichment analysis of 321 DEGs in (A). The y-axis represents the negative $\log_{10}$-transformed Q-value and gene numbers.

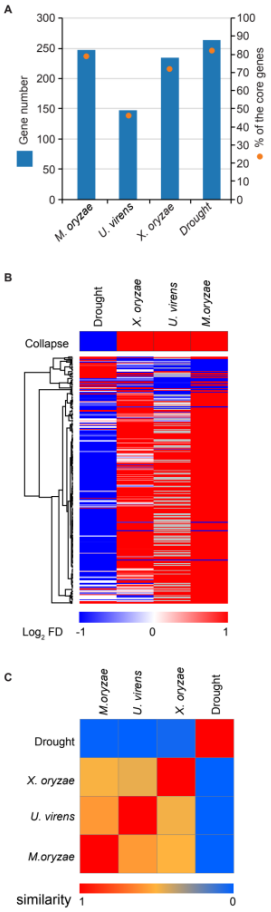

Figure 6. The core genes are involved in biotic and abiotic stress responses.
(A) The bar plot shows that among the 321 core genes, the number of genes that were differentially regulated in rice treated with *Magnaporthe oryzae* (GSE83219), *Ustilaginoidea virens* (GSE39049), *Xanthomonas oryzae* (GSE36272) or drought (GSE57950) in polished transcriptome data sets. (B) Hierarchical clustering of the 321 core genes based on the $\log_2$–fold change with various treatments as in (A). (C) Similarity comparation among the data sets as in (A) based on the $\log_2$–fold change of the 321 core genes.

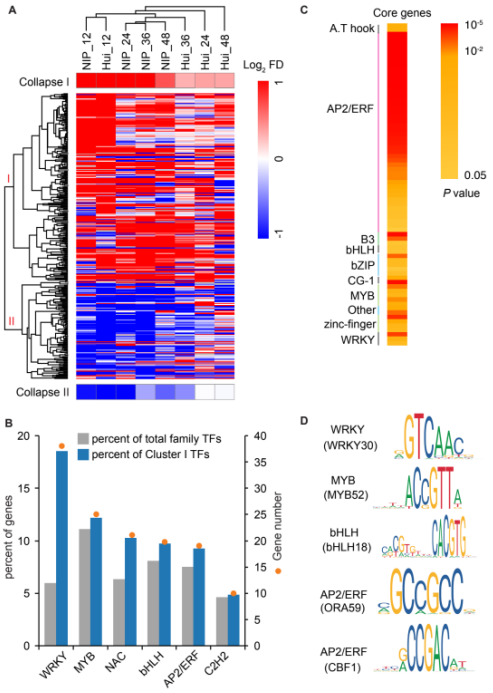

Figure 7. characterization of transcription factors and *cis*-regulatory motifs in differentially regulated genes in rice in responses to *M. oryzae*. (A) Hierarchical clustering of the total 313 transcription factors (TFs) that were differentially regulated in both Nipponbare (Nip) and Hui1586 in response to *M. oryzae* infection. The rows of collapse represent the average value of genes in the indicated groups in each column/condition. (B) Enrichment of the TF families of the 205 TFs (Cluster I in (A)) compared to their ratio in each family in the rice genome. (C) Overrepresentation of known TF DNA binding motifs within the 500-bp promoter of the 321 core genes. Rows indicate motifs of corresponding TF families and are colored according to the P-value for enrichment. (D) Sequence logo depiction of the key overrepresented TF DNA binding motifs in the 500-bp promoter of the 321 core genes.

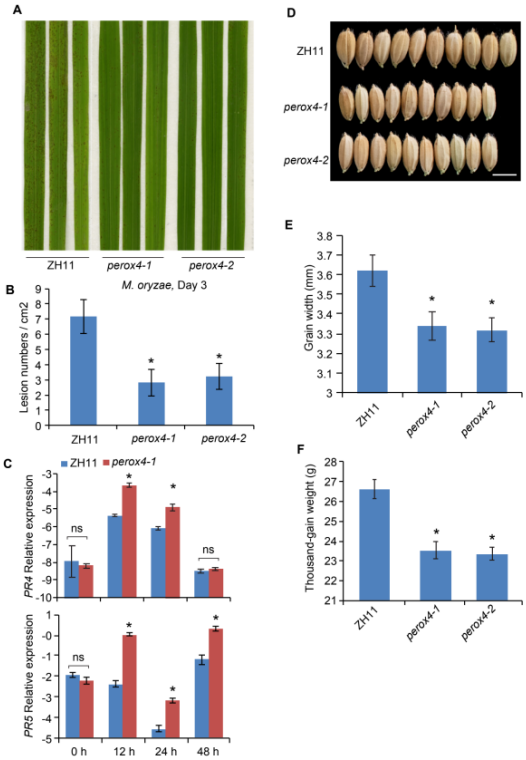

Figure 8. Phenotypic characterization of the rice *perox4* knockout lines.
(A) Blast resistance of two independent perox4 knockout lines compared with the parental Zhonghua11 (ZH11) plants. Three representative leaves collected at 3 d postinoculation of the blast isolate Guy11 are shown. (B) Lesion numbers per cm^2 on the rice leaves (mean ± SD, n > 10 leaves) after inoculation with blast fungus as in (A). (C) Expression of defense marker genes (*PR2*, and *PR5*) in *perox4* and ZH11 at the indicated times after infection of Guy11 measured by qRT-PCR. The y-axis represents the relative expression value (log_2 – transformed, mean ± SD, n = 3) normalized to *Osactin* (*Os03g0718100*). *, Statistical significance ($P < 0.01$) determined by the Student's *t*-test; ns, no significant difference. (D) Photograph of 10 seeds each for ZH11 and the *perox4* mutant lines. Scale bars, 0.5 cm. (E) and (F) Grain width and thousand-gain weight of the ZH11 and *perox4* mutant. Error bars indicate the mean ± SD (n=3 biological replicates, each replicate contains 10 seeds for G and 1000 seeds for H), *, statistical significance ($P < 0.01$) determined by the Student's *t*-test.

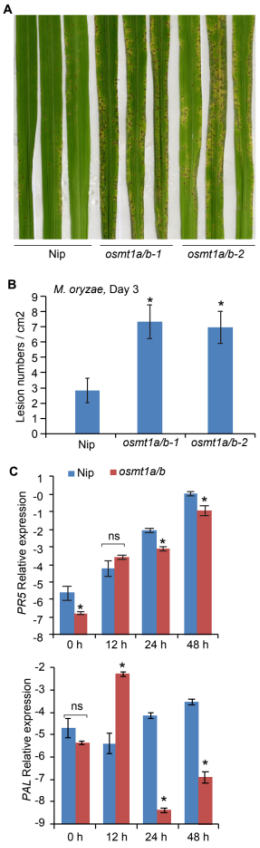

Figure 9. Disease resistance of the rice *osmt1a/b* knockout lines.
(A) Blast resistance of two independent *osmt1a/b* knockout lines compared with the parental Nipponbare (Nip) plants. Three representative leaves collected at 3 d postinoculation of the blast isolate Guy11 are shown. (B) Lesion numbers per cm^2 on rice leaves (mean ± SD, n > 10 leaves) after inoculation with blast fungus as in (A). (C) Expression of defense marker genes (*PR5,* and *PAL*) in *osmt1a/b* and Nipponbare at the indicated times after infection of Guy11 measured by qRT-PCR. The y-axis represents the relative expression value (log_2 – transformed, mean ± SD, n = 3) normalized to *Osactin* (*Os03g0718100*). *, Statistical significance ($P < 0.01$) determined by the Student's *t*-test; ns, no significant difference.