The durably resistant rice cultivar Digu activates defence gene expression before the full maturation of *Magnaporthe oryzae* appressorium

WEITAO LI\(^{1,2,3,†}\), YA LIU\(^{1,2,3,†}\), JING WANG\(^{1,2,3,†}\), MIN HE\(^{1,2,3,†}\), XIAOGANG ZHOU\(^{1,2,3,†}\), CHAO YANG\(^{1,2,3}\), CAN YUAN\(^{1,2,3}\), JICHUN WANG\(^{1,2,3}\), MAWSHENG CHERN\(^4\), JUNJIE YIN\(^{1,2,3,†}\), WEILAN CHEN\(^{1,2,3}\), BINGTIAN MA\(^{1,2,3}\), YUPING WANG\(^{1,2,3,5}\), PENG QIN\(^{1,2,3}\), SHIGUI LI\(^{1,2,3,5}\), PAMELA RONALD\(^4\) AND XUEWEI CHEN\(^{1,2,3,5,*}\)

\(^{1}\)Rice Research Institute, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
\(^{2}\)Key Laboratory of Major Crop Diseases, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
\(^{3}\)State Key Laboratory of Hybrid Rice, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
\(^{4}\)Department of Plant Pathology and the Genome Center, University of California, Davis, CA 95616, USA
\(^{5}\)Collaborative Innovation Center for Hybrid Rice in Yangtze River Basin at Sichuan, Chengdu, Sichuan 611130, China

SUMMARY

Rice blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most destructive diseases worldwide. Although the rice–*M. oryzae* interaction has been studied extensively, the early molecular events that occur in rice before full maturation of the appressorium during *M. oryzae* invasion are unknown. Here, we report a comparative transcriptomics analysis of the durably resistant rice variety Digu and the susceptible rice variety Lijiangxintuanheigu (LTH) in response to infection by *M. oryzae* (5, 10 and 20 h post-inoculation, prior to full development of the appressorium). We found that the transcriptional responses differed significantly between these two rice varieties. Gene ontology and pathway analyses revealed that many biological processes, including extracellular recognition and biosynthesis of antioxidants, terpenes and hormones, were specifically activated in Digu shortly after infection. Forty-eight genes encoding receptor kinases (RKs) were significantly differentially regulated by *M. oryzae* infection in Digu. One of these genes, LOC\(_{\text{Os08g10300}}\), encoding a leucine-rich repeat RK from the LRR VIII-2 subfamily, conferred enhanced resistance to *M. oryzae* when overexpressed in rice. Our study reveals that a multitude of molecular events occur in the durably resistant rice Digu before the full maturation of the appressorium after *M. oryzae* infection and that membrane-associated RKs play important roles in the early response.

Keywords: blast disease, durable resistance, *Magnaporthe oryzae*, receptor kinase, rice, transcriptional profiling.

INTRODUCTION

Rice blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most destructive diseases of rice, reducing global yields annually by 10%–15% (Dai et al., 2007; Skamnioti and Gurr, 2009; Talbot, 2003). Advancing knowledge of the molecular events governing the rice–*M. oryzae* interaction will contribute to methods for improving resistance to this serious disease (Chen and Ronald, 2011).

*Magnaporthe oryzae* is able to infect all rice tissues, including roots, leaves, stems and panicles (Ribot et al., 2008; Sesma and Osbourn, 2004). Infection is initiated by conidia that attach firmly to rice leaves and germinate within a few hours. After the germ tube ceases polar growth, the tip begins to swell, 2–4 h post-inoculation (hpi), and then forms a mature appressorium, which penetrates the underlying tissue within 24 hpi (Ebbole, 2007; Howard and Valent, 1996; Talbot, 2003). The primary hyphae differentiate into bulbous invasive hyphae in the cells at 32–36 hpi. The fungus then spreads into neighbouring cells to form necrotic lesions throughout the next several days (Kankanala et al., 2007).

To withstand *M. oryzae* attack, rice has evolved two main immune systems, namely pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (Chisholm et al., 2006; Dodds and Rathjen, 2010; Jones and Dangl, 2006). PTI is mediated by pattern recognition receptors (PRRs) through the recognition of PAMPs. Because PAMPs are widely conserved in pathogens and are often essential for pathogen virulence or survival, PTI is predicted to confer durable and broad-spectrum resistance. ETI is typically mediated by nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins that recognize effectors secreted into the plant cell by the pathogen. Unlike PAMPs, effectors are highly variable and thus the disease resistance mediated by ETI is generally race specific (Dodds and...
Table 1 Summary of previous transcriptomic data from the analysis of the rice–Magnaporthe oryzae interaction.

| Reference | Rice cultivars (resistant/susceptible) | Magnaporthe oryzae race | Time point after Magnaporthe oryzae inoculation |
|-----------|----------------------------------------|-------------------------|-----------------------------------------------|
| Li et al. (2006) | Near-isogenic lines H7R(Pi-4)-/H75 | ZB1 | 24 h |
| Vergne et al. (2007) | Near-isogenic lines R-I-64/PI3/S-IR64 | PHI4 | 24 h and 48 h |
| Bagnaresi et al. (2012) | Gigante Vercelli/Vialone Nano | it2, it3 and it10 (mixed) | 24 h |
| Vijayan et al. (2013) | None/HR-12 | Mo-si-63 | 6 h |
| Wei et al. (2013) | LTH/IRBL18 (P/1) and IRBL22 (P/9) | CH63 | 24 h |
| Wang et al. (2014a) | Jinheung/Jinheung | KJ401 (incompatible) and KJ301 (compatible) | 12 h and 48 h |

In rice, a well-studied PTI system is mediated by the LysM domain-containing receptor CEBiP (chitin elicitor-binding protein) and its co-receptor OsCERK1 (Kishi-Kaboshi et al., 2010). On recognition of the synthetic or pathogen-derived chitin, CEBiP and CERK1 form a receptor complex on the rice cell membrane and induce a series of defence responses, including the activation of mitogen-activated protein kinases (MAPKs) (Kishimoto et al., 2016), reactive oxygen species (ROS) production, defence gene expression, phytoalexin production and the accumulation of phosphatidic acid (PA), a signal molecule important for the plant response to both biotic and abiotic stresses (Kaku et al., 2006; Testerink and Munnik, 2005; Yamaguchi, 2004, 2005). A chimeric receptor, CRXA, consisting of the extracellular portion of CEBiP and the intracellular portion of the rice XA21 PR, is also able to perceive the chitin signal and gains the ability to confer resistance to *M. oryzae* (Kishimoto et al., 2011).

In ETI, rice NBS-LRR proteins interact directly or indirectly with *M. oryzae* effectors (Liu et al., 2013). The interactions trigger downstream defence reactions, such as increased production of antifungal secondary metabolites (Peters, 2006), cell wall thickening (Huckelhoven, 2007), pathogenesis-related (PR) protein expression (Iwa et al., 2006) and programmed cell death at sites of invasion (Greenberg and Yao, 2004). Although PTI and ETI use different receptors at the early stages of infection, they share common molecular processes, such as the activation of MAPK cascades, utilization of a shared set of transcription factors (TFs) and induced expression of PR genes (Eulgem and Somssich, 2007; Mishra et al., 2006).

Previous transcriptional profiling studies have been performed on the rice–*M. oryzae* interaction using many approaches, including expressed sequence tag (EST) sequencing (Jantasuriyarat et al., 2005), robust-long serial analysis of gene expression ( Gowda et al., 2007), proteomics (Kang et al., 2009; Kim et al., 2004), RNA-seq (Bagnaresi et al., 2012) and microarray (Vergne et al., 2007; Wei et al., 2013). These studies identified diverse biological processes and defence-related genes responsive to *M. oryzae* (Bagnaresi et al., 2012; Wei et al., 2013). However, almost all of these studies focused on the molecular events occurring in the host at 24 hpi or later, at which time the appressorium is already well developed. The transcriptome results obtained from previous studies are shown in Table 1. Very recently, a transcriptomic study on rice with the earlier time point (12 hpi) included after infection with *M. oryzae* has been performed using microarray. Although this study found some TFs and receptor-like kinases up-regulated at 48 hpi, and probably associated with the defence response in incompatible rice, it did not identify significant gene expression changes at an earlier time point (12 hpi) (Wang et al., 2014a). To date, the molecular events triggered at the early stages (before 24 hpi) of the rice–*M. oryzae* interaction, before the full formation of the appressorium, are still unknown.

Digui, a Chinese indica rice variety, confers robust and durable resistance to all of the tested blast isolates, including approximately 1000 *M. oryzae* strains collected from China, Japan, the Philippines, the UK and USA. It has been long and widely used as an important genetic resource for blast resistance breeding in China and south Asia (Chen et al., 2004). Previous studies have identified three blast resistance genes, *Pid1*, *Pid2* and *Pid3*, from Digui using three different *M. oryzae* strains as inocula: ZB13, ZB15 and Zhong-10-8-14, respectively (Chen et al., 2004; Shang et al., 2009). Of these, *Pid2* and *Pid3* have been isolated. *Pid2* encodes a B-lectin receptor kinase (RK) and *Pid3* encodes a typical NBS-LRR protein (Chen et al., 2006; Shang et al., 2009). Because each of these three identified resistance genes confers race-specific disease resistance (Chen et al., 2004, 2006; Shang et al., 2009), whereas Digui confers strong, broad-spectrum and durable resistance to *M. oryzae*, we hypothesized the presence of additional resistance mechanism(s), distinct from those mediated by these three genes, in Digui.

In this study, we examined the infection-associated development of *M. oryzae* in rice and found that the development of both the penetration peg and the primary invasive hyphae were inhibited at 24 hpi in Digui compared with the susceptible rice Lijiangxintuanheigui (LTH). We then performed comparative transcriptional profiling analyses between Digui and LTH at early stages of infection (5, 10 and 20 hpi). Our study reveals significant transcriptomic changes as early as 5 hpi that differ between these two rice varieties. Gene ontology (GO) and pathway analyses of the microarray data revealed that several biological processes,
including extracellular recognition and biosynthesis of antioxidants, terpenes and hormones, were specifically activated in Digu in response to *M. oryzae*, suggesting that these processes might be involved in blocking fungal penetration peg formation and inhibiting invasive hyphal development. We also identified 48 genes encoding predicted RKs whose expression was specifically up- or down-regulated by *M. oryzae* infection in Digu. One of these RK genes, LOC_Os08g10300, conferred enhanced resistance to *M. oryzae* when overexpressed in the susceptible rice variety, TP309. Our study reveals that the molecular interaction between pathogen and rice plant occurs hours before full development of the appressorium, and that RKs play important roles in the durably resistant rice cultivar Digu in the early defence response against infection by *M. oryzae*.

**RESULTS**

**Differences in *M. oryzae* development between compatible and incompatible interactions with rice are apparent within 24 hpi**

The invasion processes of *M. oryzae* in compatible rice have been well studied (Ebbole, 2007; Howard and Valent, 1996; Talbot, 2003). To explore whether the development of *M. oryzae* is different when grown on compatible and incompatible rice, we examined the development of *M. oryzae* during the first 52 h after infection on the durably resistant rice Digu and the susceptible rice LTH. To facilitate the observation, we used an *M. oryzae* strain carrying green fluorescent protein (GFP) as a marker. We used rice sheath tissue for microscopic observations of *M. oryzae* penetration of the rice cuticle because this tissue has reduced autofluorescence when compared with green leaves. By 5 hpi, the formation of the appressorium was evident as a dome-shaped structure at the tip of the germ tube of *M. oryzae* on both Digu and LTH (Fig. S1, see Supporting Information). By 10 hpi, the appressorium had initiated a cell wall melanization process, which is essential for the accumulation of the enormous turgor pressure needed to rupture the rice leaf cuticle. The autophagic fungal conidial cell death necessary for appressorium maturation was also detected on both Digu and LTH, as indicated by the disappearance of GFP fluorescence within the conidial cells at 10 hpi. At 16 hpi, the appressorium was fully melanized on both rice varieties (Figs 1 and S1). These results demonstrate that, before penetration of the host cuticle, the early invasion processes of *M. oryzae* are similar between the incompatible and compatible interactions, including conidial germination, germ tube extension, appressorium formation and maturation, and autophagic conidial cell death.

We found significant differences in the development of *M. oryzae* 16 h after inoculation on Digu and LTH. At 20 and 24 hpi, the penetration peg and primary invasive hyphae emerged frequently from the appressorium on LTH, but rarely on Digu (Fig. 1). At 36 hpi, the secondary invasive hyphae of *M. oryzae* on the LTH sheath expanded into rice cells neighbouring the cell ruptured by the appressorium. In striking contrast, *M. oryzae* on Digu only showed the initial emergence of a short penetration peg (Fig. S1). These results indicate that differences in *M. oryzae* infection on LTH and Digu could be observed at the penetration and post-penetration stages. Based on these results, we hypothesize that the molecular events that differentiate the compatible and
incompatible interactions of rice with *M. oryzae* would occur before 20 hpi when the fungal penetration peg forms and invasive hyphae begin to develop.

Several PR genes in rice have been reported to be up-regulated in response to biotic stress as a result of an enhanced immune response (van Loon et al., 2006). To investigate whether the rice immune response is activated before 20 hpi, we compared the transcriptional expression levels of PR genes between Digu and LTH challenged with *M. oryzae*. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on rice leaf samples collected at 5, 10 and 20 hpi for expression of the following PR genes: *PR10* (*LOC_Os12g36830*) and *BETV1* (*LOC_Os12g36850*). The expression levels of *PR10* and *BETV1* increased over time and reached a peak at 20 hpi in both Digu and LTH. Importantly, the expression of both PR genes reached higher levels in Digu compared with LTH during fungal infection (Fig. S2, see Supporting Information). These results reveal that the durably resistant rice Digu and the susceptible rice LTH respond to *M. oryzae* differently in the activation of PR genes.

Based on these findings, we hypothesize that the rice immune response to *M. oryzae* infection is activated within 20 h and is differentially regulated in the durably resistant rice Digu compared with the susceptible rice LTH. These differences result in reduced penetration peg formation and invasive hyphal growth on Digu relative to LTH, as observed at approximately 1 day post-inoculation (dpi).

Identification of rice genes differentially or specifically expressed in early compatible or incompatible interaction with *M. oryzae*

To understand the molecular events underlying the early response of Digu to *M. oryzae*, we performed a transcriptomic study to compare the responses between the durably resistant rice Digu and the susceptible rice LTH on *M. oryzae* infection. RNA samples were prepared from rice leaf tissues harvested from Digu and LTH at 5, 10 and 20 hpi following *M. oryzae* or mock treatment (Fig. S3A, see Supporting Information). After quality validation, the RNA samples were reverse transcribed and subjected to microarray hybridization for transcriptional profiling analysis. Three independent biological replicates were performed. Pearson’s correlation coefficients among these replicates ranged from 0.80 to 0.99 with an average of 0.93, showing high consistency among these biological replicates (Table S1, see Supporting Information). Genes whose expression levels increased or decreased by more than 0.5-fold in either Digu or LTH compared with mock inoculation were identified as DEGs. Only those DEGs present in all three independent biological duplicates were considered as real DEGs in our study. Top panel: DEGs with up-regulated expression; bottom panel: DEGs with down-regulated expression. (A) Venn diagram of DEGs in Digu and LTH within 20 h post-inoculation (hpi). (B) Venn diagram of DEGs in both Digu and LTH at 5, 10 and 20 hpi. (C) The column diagram shows the numbers of DEGs with up- and down-regulated expression in DG and LTH at 5, 10 and 20 hpi.

A total of 2226 DEGs were identified from rice Digu and LTH in response to *M. oryzae* infection (Fig. 2A and Table S2, see Supporting Information). Thirteen of 14 randomly selected DEGs were verified by qRT-PCR analysis (Fig. S4 and Table S3, see Supporting Information), suggesting that most of the DEGs (more than 92%) could be validated and the microarray data were highly reliable. Among all DEGs, 403 were commonly shared between Digu and LTH, whereas 820 were Digu specific and 1003 were LTH specific.
response to 

showing that more GO terms were used in the resistant rice in terms were present at 5 hpi, 32 at 10 hpi and 15 at 20 hpi, 
specific GO terms enriched from the up-regulated DEGs, 71 GO 
the down-regulated DEGs (Fig. 3A). Interestingly, of these Digu- 
DEGs, and five (5 hpi), 13 (10 hpi) and zero (20 hpi) GO terms for 
(10 hpi) and 122 (20 hpi) enriched GO terms for the up-regulated 
regulated DEGs in both Digu and LTH. At 5 hpi, Digu and LTH 
shared one from the common down-regulated DEG. Four LTH-
specific GO terms and no Digu-specific GO terms were observed 
for down-regulated DEGs. At 10 hpi, there were 16 in common, 12 
specific for Digu and seven specific for LTH. At 20 hpi, there were 
none in common, 30 specific for Digu and none specific for LTH 
(Fig. 3B). These results indicate that the down-regulated DEGs are 
less important than the up-regulated DEGs, in both resistant and 
susceptible rice, in response to M. oryzae. Collectively, these 
results suggest that many molecular pathways are activated 
instead of inhibited in Digu in defence against M. oryzae. These 
results also reveal that the molecular events activated at the early 
time point (at 5 hpi) are more important than those at later time 
points for resistance in Digu.

Among the GO terms associated specifically with the 
up-regulated DEGs in Digu at 5 hpi, we identified the GO term 
‘response to stimulus’ in biological process, the GO terms ‘mole-
ular transducer activity’ and ‘transporter activity’ in molecular 
function, and the GO term ‘extracellular region’ in cellular component. Because these processes have been reported to be involved in the perception and transduction of extracellular signals (Chasis et al., 1988; Nürnberger et al., 1994; Karnchanaphanurch et al., 2009),

Specific molecular pathways in durably resistant rice 
are involved in the early response against M. oryzae

To identify the specific molecular pathways involved in the early 
response against M. oryzae, we performed GO analysis on all 
DEGs. We identified 169 (at 5 hpi), 122 (10 hpi) and 84 (20 hpi) 
enriched GO terms for the up-regulated DEGs, and one (5 hpi), 18 
(10 hpi) and 30 (20 hpi) enriched GO terms (Fig. 3A) for the 
down-regulated DEGs in Digu. In LTH, we identified 110 (5 hpi), 113 
(10 hpi) and 122 (20 hpi) enriched GO terms for the up-regulated 
DEGs, and five (5 hpi), 13 (10 hpi) and zero (20 hpi) GO terms for 
the down-regulated DEGs (Fig. 3A). Interestingly, of these Digu-
specific GO terms enriched from the up-regulated DEGs, 71 GO 
terms were present at 5 hpi, 32 at 10 hpi and 15 at 20 hpi, 
showing that more GO terms were used in the resistant rice in 
response to M. oryzae at the earlier time points than at the later 
points. In contrast, among the LTH-specific GO terms, only 12 GO 
terms were present at 5 hpi, whereas 23 were present at 10 hpi and 
53 were present at 20 hpi (Fig. 3B), showing that less GO 
terms were used at the earlier time points than at the later points 
in LTH. These results suggest that the GO terms associated with 
compatible interaction are different from those associated with 
incompatible interaction, and that the earliest time point (5 hpi) is 
important for the Digu-specific response against M. oryzae inva-
sion. However, fewer GO terms were associated with the down-
regulated DEGs in both Digu and LTH. At 5 hpi, Digu and LTH 
shared one from the common down-regulated DEG. Four LTH-
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![Gene ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in Digu and Lijiangxintuanheigu (LTH). GO full assignments to the DEGs were retrieved from the BINGO database (Maere et al., 2005). (A) GO term enrichment analysis of up- and down-regulated DEGs in Digu and LTH at 5, 10 and 20 h post-inoculation (hpi). The number of GO terms enriched from the up-regulated DEGs was greater than those enriched from the down-regulated DEGs in both the durably resistant rice Digu and the susceptible rice LTH at each time point post-inoculation. (B) Venn diagram of GO terms in both DG and LTH at 5, 10 and 20 hpi.](image-url)
Table 2  Enrichment analysis of functional gene ontology (GO) categories of the Digu-specific and Lijiangxintuanheigu (LTH)-specific differentially expressed genes (DEGs) during rice early responses to Magnaporthe oryzae.

| GO-ID | Enriched GO terms     | Digu-5 h | Digu-10 h | Digu-20 h | LTH-5 h | LTH-10 h | LTH-20 h |
|-------|------------------------|----------|-----------|-----------|---------|---------|---------|
| 23052 | Signalling             | —        | UP        | —         | —       | —       | —       |
| 60089 | Molecular transducer activity | UP     | —         | —         | —       | —       | —       |
| 30234 | Enzyme regulator activity | UP      | —         | —         | —       | —       | —       |
| 43190 | ATP-binding cassette (ABC) transporter complex | UP     | —         | —         | —       | —       | —       |
| 5576  | Extracellular region   | UP       | —         | —         | —       | —       | —       |
| 9987  | Cellular process       | —        | —         | DOWN      | —       | —       | —       |
| 8152  | Metabolic process      | —        | —         | DOWN      | —       | —       | —       |
| 3824  | Catalytic activity     | —        | DOWN      | DOWN      | —       | —       | —       |
| 43231 | Intracellular membrane-bound organelle | —      | DOWN      | —         | —       | —       | —       |
| 43227 | Membrane-bound organelle | —      | DOWN      | —         | —       | —       | —       |
| 43229 | Intracellular organelle | —        | DOWN      | —         | —       | —       | —       |
| 43226 | Organelle              | —        | DOWN      | —         | —       | —       | —       |
| 44424 | Intracellular part      | —        | DOWN      | —         | —       | —       | —       |
| 5622  | Intracellular          | —        | —         | —         | —       | —       | —       |
| 44444 | Cytoplasmic part       | —        | DOWN      | —         | —       | —       | —       |
| 5737  | Cytoplasm              | —        | DOWN      | —         | —       | —       | —       |
| 5364  | Nucleus                | —        | —         | —         | —       | —       | UP      |

Note: GO full assignments to these Digu- (normal) and LTH-specific (bold) DEGs were retrieved from the BiNGO database. 'UP' indicates that the enriched GO terms are up-regulated at the time points post-inoculation, whereas 'DOWN' indicates that the enriched GO terms are down-regulated. ‘—’ shows that the enriched GO terms are not significantly regulated.

our finding indicates that the early response in the durably resistant rice Digu is probably activated through rice receptor(s) perceiving extracellular signal molecules derived from M. oryzae.

To better understand the molecular pathways associated with the GO terms, we analysed the GO categories with enrichment analysis. We found 19 up-regulated and 12 down-regulated GO categories in Digu, as well as 15 up-regulated and two down-regulated GO categories in LTH (Table S4, see Supporting Information). Among these, 16 GO categories were Digu specific. Interestingly, of these 16 GO categories, five were up-regulated in Digu specifically and LTH after M. oryzae inoculation. We identified a total of 38 Digu-specific and up-regulated metabolic pathways (29 at 5 hpi, nine at 10 hpi and 11 at 20 hpi) within 20 hpi (Table S5, see Supporting Information). Interestingly, most of these pathways are associated with metabolic processes, including carbon source (10 pathways), antioxidant (five pathways), nitrogen source (three pathways), terpene biosynthesis (three pathways) and hormone biosynthesis (two pathways) (Tables 3 and S5), suggesting that these metabolic processes are involved in the Digu response to M. oryzae invasion. Because most metabolic processes (29 of 38) were activated within 5 hpi, we hypothesize that these metabolic process are critical for the early defence response of Digu against M. oryzae.

Specific metabolic processes in Digu are involved in the early response against M. oryzae

The above GO-based analyses suggest that many 'metabolic processes' are part of the response of Digu to M. oryzae infection. To identify specific metabolic processes in Digu, we used the RiceCyc (http://pathway.gramene.org/) database to analyse the metabolic profiles of the up- and down-regulated genes in Digu and LTH after M. oryzae inoculation. We identified a total of 38 Digu-specific and up-regulated metabolic pathways (29 at 5 hpi, nine at 10 hpi and 11 at 20 hpi) within 20 hpi (Table S5, see Supporting Information). Interestingly, most of these pathways are associated with metabolic processes, including carbon source (10 pathways), antioxidant (five pathways), nitrogen source (three pathways), terpene biosynthesis (three pathways) and hormone biosynthesis (two pathways) (Tables 3 and S5), suggesting that these metabolic processes are involved in the Digu response to M. oryzae invasion. Because most metabolic processes (29 of 38) were activated within 5 hpi, we hypothesize that these metabolic processes are critical for the early defence response of Digu against M. oryzae.

We also identified eight pathways that were specifically activated in Digu, but inhibited in LTH, during the rice–M. oryzae interaction. These pathways are mainly associated with energy metabolic processes, including energy carriers (two pathways), nitrogen source (one pathway), and detoxification and nitrogen utilization (one pathway) (Tables 3 and S5). This result indicates that these energy metabolic processes are more active in Digu in response to M. oryzae invasion, as compared with LTH, and might contribute to durable resistance to M. oryzae.
suggests that the biosyntheses of lysine, UDP-galactose and haem in rice might facilitate *M. oryzae* infection in the susceptible rice LTH and are highly repressed in Digu, leading to the inhibition of *M. oryzae* invasion.

TFs are up-regulated at early stages in response to *M. oryzae* infection

TFs can directly regulate the expression of defence-related genes. In our study, we identified 37 TFs whose expression was responsive to *M. oryzae* specifically in the durably resistant rice Digu. These TFs belong to WRKY, AP2, MYB, bHLH, GRAS, NAC, ARF, C2H2, E2F, GATA, MADS, ARR-B, CAMTA and CO-like subfamilies. Among these TF genes, most were up-regulated at early time points (Fig. 4A). Four of these TF families were specific to the defence response of Digu: ARF (LOC_Os01g13520 up-regulated and LOC_Os12g41590 down-regulated), ZF-HD (LOC_Os09g24810 down-regulated), GATA (LOC_Os02g56250 up-regulated) and E2F (LOC_Os12g06200 up-regulated) (Fig. 4B). GATA is associated with the hypersensitive response (Dangl et al., 1996), programmed cell death and callus differentiation (Wang et al., 2005). ARF regulates the expression of auxin-responsive genes (Tiwari et al., 2003; Ulmasov et al., 1997). ZF-HD is expressed in the mesophyll specifically (Windhovel et al., 2001). E2F is important for cell proliferation (van den Heuvel and Dyson, 2008). Taken together, our results suggest that the hypersensitive response, programmed cell death, callus differentiation, inhibition of rice growth and development might be associated or required for the Digu defence response against *M. oryzae*, prior to fungal penetration peg formation and invasive hyphal development.

RKs play key roles in defence against *M. oryzae* infection leading to durable resistance

RKs play important roles as PRRs and regulators of PTI (Chen and Ronald, 2011; Ronald and Beutler, 2010; Zipfel et al., 2006). For instance, the LRR XII RKs, such as FLAGELLIN-SENSITIVE 2 (FLS2) (Gomez-Gomez and Boller, 2000), elongation factor-Tu receptor (EFR) (Zipfel et al., 2006) and XA21 (Song et al., 1995), function with the LRR II subfamily regulatory RKs, BAK1 (SERK3), BKK1 (SERK4) and OsSERK2, to perceive the signal molecules derived from bacteria and activate the defence response (Chen et al., 2014; Chinchilla et al., 2007; Roux et al., 2011; Wang et al., 2014).

To determine whether RKs are involved in the early response against *M. oryzae* in Digu and, if so, which RKs, we searched for RKs in the DEGs. We found 48 RK genes belonging to the subfamilies of LRR, WAK, SD, DUF26, L-LEC, LRK10L and RKF3, which were specifically up- or down-regulated in Digu post-inoculation (Fig. 5). We verified the *M. oryzae* responsiveness of these DEGs by qRT-PCR analysis on eight genes randomly selected from these genes.
Fig. 4  Transcription factors encoded by Digu-specific differentially expressed genes (DEGs). The gene IDs of the transcription factors were obtained from the database of Plant TFDB (Jin et al., 2014). The family names of the DEGs are shown next to their Locus IDs. (A) Transcription factors encoded by Digu-specific DEGs. The subfamilies WAKY, AP2, MYB, bHLH, GRAS, NAC, ARF, C2H2, E2F, GATA, MADS, ARR-B, CAMTA and CO-like are indicated. (B) Four transcription factor families activated specifically in rice Digu on Magnaporthe oryzae inoculation.

Fig. 5  Receptor kinase (RK) genes encoded by Digu-specific differentially expressed genes (DEGs). The IDs of the RK genes were retrieved from the rice kinase database (Dardick et al., 2007). The information on the subfamily and class of the genes was also obtained from the rice kinase database. The RKs encoded by these genes belong to LRR (leucine-rich receptor), WAK (cell wall-associated kinase), SD (S-domain receptor-like protein kinase), DUF26 (domain unknown function 26), L-LEC (lectin-like receptor kinase), LRK10L and RKF3 subfamilies. Up- or down-regulated expression of these genes at each time point post-inoculation is indicated. Among these genes, most are up-regulated, specifically in Digu overall, especially at the point of 5 hpi.
RK genes. The expression patterns of the eight DEGs were consistent when compared between the qRT-PCR and microarray results (Fig. S5 and Table S3, see Supporting Information). Forty of these RK genes were up-regulated, including 13 from the LRR subfamily, eight from the WAK subfamily, eight from the SD subfamily, seven from the DUF26 subfamily, one from the L-LEC subfamily, three from the LRK10L subfamily and one from the RKF3 subfamily (Fig. 5). These results suggest that most of these RK genes play positive regulatory roles in Digu in response to M. oryzae. Most of these RK genes (35 of 40) were rapidly up-regulated as early as 5 hpi, suggesting that these RKs are important for the early signal perception or signal transduction. Only eight RKs were specifically down-regulated in Digu, indicating that a small number of RK genes might negatively regulate the early defence response of Digu.

We identified two RK genes that were specifically up-regulated in Digu at all three time points: LOC_Os07g35410, encoding an RK belonging to the DUF26 subfamily, and LOC_Os08g10300, encoding an RK with eight extracellular LRRs belonging to the VIII-2 subfamily. One well-characterized representative of the VIII-2 subfamily is the sugarcane SHR5 gene. SHR5 expression is down-regulated in the interaction between sugarcane and endophytic, N2-fixing bacteria and is up-regulated in response to infection with pathogenic bacteria and fungi (Vinagre et al., 2006).

To assess the biological function of LOC_Os08g10300, we overexpressed this gene in the susceptible rice variety TP309 (Fig. S6, see Supporting Information) and assayed the resulting transgenic plants for resistance to M. oryzae. We inoculated seven independently transformed rice lines and the TP309 control. We found that all seven independently transformed rice lines (Os08g10300ox) conferred enhanced disease resistance to five different M. oryzae strains (WJ9, WJ10, YC7, YC6 and Zhong-10-8-14). We further analysed their T1 and T2 progeny plants. The expression levels of PR genes were up-regulated in the transgenic plants in comparison with that in TP309 (Fig. S7, see Supporting Information). We found that the progeny carrying the transgene exhibited much smaller disease lesion spots on leaves, whereas null segregants showed clear disease lesion spots, similar to those of the susceptible parental control TP309 to these strains (Figs 6 and S8, see Supporting Information). These results indicate that the observed resistance co-segregated with the transgene Os08g10300ox and that this gene confers enhanced resistance to M. oryzae.

**DISCUSSION**

Molecular interaction between rice and M. oryzae occurs within 5 h after pathogen inoculation

Previous studies have suggested that 24 hpi is a critical point for M. oryzae invasion (Talbot, 2003), and thus many transcriptional profiling studies have focused on this time point for studies of the rice response to M. oryzae (Kim et al., 2013; Wei et al., 2013). However, recent studies have also demonstrated that M. oryzae forms a mature appressorium in order to develop a penetration peg and invasive hypha prior to 24 hpi (Kankanala et al., 2007; Wilson and Talbot, 2009). No studies have analysed the molecular events that occur prior to this early time point. In our study, we used microarray-based expression profiling to assess the molecular events underlying the rice–M. oryzae interaction before 24 hpi. We identified more than 2000 DEGs before 24 hpi in Digu and LTH. The DEGs in Digu are largely different from those in LTH. Of the 586 DEGs at 5 hpi, 356 are Digu specific and 256 are LTH specific. DEGs specific to the durably resistant rice Digu encode RKs, signal transducers, TFs and PR proteins, involved in cell wall organization, signal transduction and the downstream defence response. Some of the DEGs also encode enzymes involved in sulfation pathways, such as sulfate reduction II (assimilatory) and sulfite oxidation III, which have also been shown previously to be associated with defence responses. Some of the Digu-specific DEGs identified at 5 hpi...
encode TFs of GATA and E2F types with known function in the defence response against biotic stress. In summary, our results indicate that the key molecular events that govern the Digu defence response occur very early (as early as 5 hpi) on *M. oryzae* infection. We hypothesize that these early responses inhibit key aspects of fungal development, including full maturation of the appressorium, formation of the penetration peg and the spread of invasive hyphae (Fig. 1), leading to durable resistance in Digu to *M. oryzae*.

**Durably resistant rice Digu employs a set of defence responses that are distinct from those mediated by LysM and NBS-LRR proteins**

In rice, the characterized immune systems against *M. oryzae* are mediated either by LysM motif-containing proteins (CEBiP, OsLYP4 and OsLYP6), a lectin motif-containing RK (Pid2) or NBS-LRR proteins. The receptors CEBiP, OsLYP4 and OsLYP6 perceive chitin molecules derived from *M. oryzae* (Liu et al., 2012; Liu and Zhao, 2012) to initiate immune signalling. The signal is then transmitted downstream through a MAPK cascade, leading to the activation of the immune response. In this immune system, the OsLYP4 and OsLYP6 receptor genes and MAPK cascade genes (OsMAPK5 and OsMAPK12) are rapidly induced on challenge by chitin or *M. oryzae* (Liu et al., 2012; Liu and Zhao, 2012). Despite their importance in the CEBiP-mediated defence response, none of these genes are present among our identified DEGs from Digu at 5, 10 or 20 hpi (Table S6, see Supporting Information). Further, qRT-PCR analysis also revealed that the expression of the genes CEBiP, OsLYP4 and OsLYP6 in rice Digu was not induced or repressed at the early time points post-inoculation with *M. oryzae* (Fig. S9, see Supporting Information). These results suggest that the molecular events in Digu underlying the early interaction between rice and *M. oryzae* before 20 hpi are unlikely to be dependent on LysM motif proteins, and that Digu may employ defence mechanisms distinct from those mediated by LysM motif proteins.

Previous analyses of transcriptional profiles mediated by NBS-LRR proteins (Vergne et al., 2007; Wei et al., 2013) have demonstrated that the responses of resistant and susceptible plants are quite similar overall, but with more genes prominently regulated in incompatible interactions. In contrast, our study reveals that the transcriptional profiles are largely different between the durably resistant rice Digu and the susceptible rice LTH at the early stages (5, 10 or 20 hpi) (Fig. 2 and Table S2). We also noted several activated molecular processes in Digu that were not detected in resistance mediated by NBS-LRR proteins. For example, we found that the GO terms ‘extracellular region’ and ‘peroxidase activity’ were associated with Digu in response to *M. oryzae*, but not with the transcriptional responses observed in NBS-LRR-mediated, race-specific resistance.

Previous studies have also demonstrated that some NBS-LRR genes are induced after pathogen infection. These include *Pib* (Wang et al., 1999), *Pi5-1* (Lee et al., 2009) and *Pi-k* (Sharma et al., 2005). We thus analysed whether NBS-LRR genes were up-regulated in DEGs. However, we did not identify any NBS-LRR genes that were up-regulated specifically in Digu on *M. oryzae* inoculation. Although we found five NBS-LRR genes whose transcriptional expression was induced in the susceptible rice LTH (Fig. S10, see Supporting Information), these NBS-LRR genes are unlikely to contribute to resistance to *M. oryzae*, because LTH is fully susceptible to the tested *M. oryzae* strains. Collectively, these findings indicate that rice Digu employs resistance mechanisms that are distinct from those employed by characterized NBS-LRR proteins (Shang et al., 2009).

**Plasma membrane kinases are expressed early in Digu after *M. oryzae* infection**

In our study, we identified 48 RK genes that were significantly differentially regulated in Digu at the early stages of infection; most were activated as early as 5 hpi. These RK genes encode proteins belonging to LRR, WAK, SD, DUF26, L-LEC, LRK10L and RKF3 subfamilies (Fig. 4). The up-regulation of these RKs was not observed in NBS-LRR-mediated resistance (Wei et al., 2013) (Fig. S11, see Supporting Information) or other race-specific resistance (Wang et al., 2014a) to *M. oryzae*. Thus, these RK genes appear to be induced specifically in the durably resistant rice Digu. Alternatively, these RKs may be expressed in other resistant rice varieties, but are only activated early in the rice response to infection (e.g. 5 hpi). In this case, such changes would not have been identified in studies that examined only later time points. We found that 23 of these RKs fall into the non-RD kinase subclass, a molecular signature tightly associated with the immune response in both plants and animals (Dardick and Ronald, 2006). Transgenic rice plants overexpressing LOC_Os08g10300, encoding an RK, conferred enhanced resistance to five strains of *M. oryzae* (Fig. 6), including strain Zhong-10-8-14. Because previous studies have shown that rice plants carrying Pid2 confer resistance to *M. oryzae* strain ZB15, but not to strain Zhong-10-8-14 (Chen et al., 2006), our results demonstrate that LOC_Os08g10300 employs a distinct mechanism of resistance or perception of *M. oryzae* when compared with Pid2. However, we could not rule out the possibility that this RK may function as an important partner instead of resistant protein in defence against the invasion of *M. oryzae*. Further loss-of-function studies would help to uncover the mechanism mediated by this RK gene. The protein structure of LOC_Os08g10300 is similar to that of sugarcane SHR5, which belongs to the VIII-2 subclass of LRR RKs. Sugarcane SHR5 is induced by infection with pathogenic bacteria and fungi, and is thus predicted to be involved in the defence response (Vinagre et al., 2006). Thus, we
hypothesize that VIII-2 subclass LRR RKs may serve as important regulators of the immune response.

In addition to LRR RKs, RKs from subfamilies WAK, SD and DUF26 were differentially regulated in Digu in response to *M. oryzae* infection. Of the eight WAK RK genes specifically regulated in Digu, six (LOC_Os09g38840, LOC_Os08g29040, LOC_Os09g38850, LOC_Os04g30160, LOC_Os09g29510 and LOC_Os07g31190) responded to the invasion of pathogens within 5 hpi (Fig. 5). Among the 12 SD RK genes, eight were up-regulated and four were down-regulated after *M. oryzae* infection (Fig. 5), indicating that the SD subfamily plays dual roles early in defence against the invasion of *M. oryzae* through both positive and negative regulation. Of the eight DUF RKs, seven were up-regulated on *M. oryzae* infection, suggesting that these DUF RKs positively regulate the immune response. Our results are consistent with previous studies showing that members of the Arabidopsis WAK subfamily (WAK1, WAK2, WAK3 and WAK5) (He et al., 1998, 1999), the SD subfamily [ARK1, ARK3, Brassica S family receptor 2 (SFR2) and SFR3] (Pastuglia et al., 1997, 2002) and the DUF26 subfamily (Czernic et al., 1999; Du and Chen, 2000; Ohtake et al., 2000) are implicated in pathogen defence responses.

In summary, our study reveals the activation of molecular events in Digu before the full formation of the appressorium of *M. oryzae* in response to pathogen infection, and demonstrates that many of these events are distinct from those observed in the susceptible control or those mediated by CEBIP, Pic2 or NBS-LRR proteins. Our study also identifies a large set of RKs that are up-regulated in *M. oryzae* before the appressorium is well developed, suggesting that these RKs play important roles in the durably resistant rice Digu in defence against the invasion of *M. oryzae* before the formation of the appressorium.

**EXPERIMENTAL PROCEDURES**

**Fungal growth on rice sheath**

The durably resistant indica rice (*Oryza sativa*) Digu and the susceptible rice LTH varieties were grown in a growth chamber at 28 °C in a 12-h light/12-h dark photoperiod with 75% humidity. Two-week-old rice plants in tube I were used for inoculation with mixed isolates of *M. oryzae* strains (W9, W10, YC7 and YC26). These isolates carry high virulence and are prevalent in Sichuan, China. The spore concentration was adjusted to 5 × 10⁵ conidia/mL with 0.2% Tween-20. Two-week-old rice plants in tube II were used for mock inoculation with Tween-20 (0.2%) lacking *M. oryzae* spores. The fungal- and mock-inoculated rice seedlings were kept in a dark chamber at 95% humidity and 28 °C. After 24 hpi, the plants were maintained in the growth chamber at 28 °C in a 12-h light/12-h dark photoperiod with 95% humidity. Leaves were harvested at 5, 10 and 20 hpi for experimentation. To ensure that the inoculation was successfully performed, the remaining seedlings were kept for another 8 dpi for disease evaluation (Fig. S3A). The harvested leaves were ground in liquid nitrogen and immediately stored at −80 °C until RNA extraction. The experiment was repeated three times with 1-month intervals, representing three independent biological replicates.

**Blast infection procedures**

The Digu and LTH varieties were grown in two tubs in a growth chamber at 28 °C in a 12-h light/12-h dark photoperiod with 75% humidity. Two-week-old rice plants in tub I were used for inoculation with mixed isolates of *M. oryzae* strains (W9, W10, YC7 and YC26). These isolates carry high virulence and are prevalent in Sichuan, China. The spore concentration was adjusted to 5 × 10⁵ conidia/mL with 0.2% Tween-20. Two-week-old rice plants in tube II were used for mock inoculation with Tween-20 (0.2%) lacking *M. oryzae* spores. The fungal- and mock-inoculated rice seedlings were kept in a dark chamber at 95% humidity and 28 °C. After 24 hpi, the plants were maintained in the growth chamber at 28 °C in a 12-h light/12-h dark photoperiod with 95% humidity. Leaves were harvested at 5, 10 and 20 hpi for experimentation. To ensure that the inoculation was successfully performed, the remaining seedlings were kept for another 8 dpi for disease evaluation (Fig. S3A). The harvested leaves were ground in liquid nitrogen and immediately stored at −80 °C until RNA extraction. The experiment was repeated three times with 1-month intervals, representing three independent biological replicates.

**Microarray analysis**

We used Affymetrix Genechip Rice Genome Arrays (Affymetrix, Santa Clara, CA, USA) in this transcriptomic study. RNA quality assessment, RNA labelling and microarray hybridization were performed at Capitalbio Ltd. (Beijing, China) following the manufacturer's instructions. After normalization, a non-specific filtering step was carried out (Wei et al., 2013). We identified those genes with an expression level increased or decreased by 0.5-fold in either Digu or LTH compared with mock inoculation as DEGs, according to the method described previously (Liu and Zhao, 2012). Only those DEGs present in all three independent biological replicates were considered as real DEGs in our study (Fig. S3B). The probe sets without gene annotations of MSU Rice Genome Annotation Project database release 7.0 (http://rice.plantbiology.msu.edu) (Kawahara et al., 2013) were removed. Hierarchical clustering was performed using cluster version 3.0 (http://www.falw.vu/huik/cluster.htm) with the average linkage clustering method, and was illustrated by TreeView software (Saldanha, 2004).

**Computational gene function analysis**

GO analysis was performed on DEGs based on gene annotations. GO full assignments to these genes were retrieved from the BiNGO database (Maere et al., 2005). Rice metabolic pathway analysis was performed using RiceCyc version 3.2 (Jaiswal et al., 2006). The plant TFDB (Jia et al., 2014) database was used for analyses of rice TFs; the rice kinase database (Dardick et al., 2007) was used for the analysis of rice kinase; the MSU database was used for the determination of rice gene families.

**qRT-PCR analysis**

Total RNA was extracted using TRIzol reagent (Invitrogen Life Technologies, Shanghai, China) according to the manufacturer's protocols. cDNA
was synthesized using an RNA reverse transcription kit (Invitrogen Life Technologies, Shanghai, China). The PCR program was run as follows: 5 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 30 s, 30 s of annealing at 60 °C, polymerization at 72 °C for 50 s and a final extension step at 72 °C for 3 min. The expression of the reference UBQ5 gene was used for the normalization of all qRT-PCR data (Jain et al., 2006). The 2^ΔΔCT method was used to calculate the relative expression levels with three experimental repeats (Livak and Schmittgen, 2001). Primer sequences for qRT-PCR are listed in Table S7 (see Supporting Information).

**Generation of transgenic plants**

The full-length cDNA of RK LOC_Os08g10300 (abbreviated as Os08g10300) was cloned into pENTR/D to yield pENTR-Os08g10300, which was then recombined with the Ubi-C1300GTW vector to generate the over-expression construct, Ubi-C1300-Os08g10300 (abbreviated as Os08g10300ox). The construct, Os08g10300ox, was introduced into the blast susceptible rice TP309 variety through Agrobacterium-mediated transformation according to the method described previously (Chen et al., 2005). The regenerated transgenic plants carrying Os08g10300ox were selected by hygromycin. PCR-based genotyping was performed to determine whether the transgenic plants contained the transgene, as described previously (Chen et al., 2010). Overexpression of Os08g10300 in the transgenic lines carrying Os08g10300ox was confirmed by qRT-PCR (Fig. S6).

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Rice early molecular response against *Magnaporthe oryzae*. Inoculation, sampling and expression analyses were carried out as described in Fig. S1A. The relative expression of genes after *M. oryzae* inoculation was normalized to the expression with mock inoculation. All quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data were normalized to the expression of the reference *UBQ5* gene before the determination of relative expression. The expression of PR genes *PR10* and *BETV1* was included for this assay. The 2^\(-\Delta\Delta CT\) method was used to calculate the relative expression levels with three repeats (Livak and Schmittgen, 2001). SD represents the standard deviation of three technical replicates. This experiment was biologically repeated three times with similar results.

**Fig. S3** The experimental design for screening differentially expressed genes (DEGs) from rice Digu and Lijiangxintuanheigu (LTH). (A) Preparation of seedling samples of rice Digu and LTH. The samples in tub I were inoculated by spraying with *Magnaporthe oryzae* spores. The concentration of spores was 5 × 10^7/ml with 0.2% Tween-20. The samples in tub II were sprayed with 0.2% Tween-20 without spores for mock inoculation. Sampling was performed at the time points 5, 10 and 20 h post-inoculation (hpi), as indicated. The seedling samples marked with ‘left’ were kept for disease evaluation to ensure that the inoculation was successfully performed. The disease evaluation was performed at day 8 post-inoculation. (B) The formula used for the determination of DEGs. Genes whose expression on *M. oryzae* inoculation was increased or decreased by 0.5-fold or more at each time point were selected as DEGs. Only those DEGs present in all three independent biological replicates were selected as DEGs in our study.

**Fig. S4** Validation of randomly selected differentially expressed genes (DEGs) by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). qRT-PCR was performed on 14 randomly selected genes using the leaf samples collected from rice Digu and Lijiangxintuanheigu (LTH) at the time points 5, 10 and 20 h post-inoculation (hpi). The experimental designs of inoculation and sampling were the same as described in Fig. S3A. The expression comparison was performed between *Magnaporthe oryzae* inoculation and mock inoculation at each time point of 5, 10 and 20 hpi for rice Digu or LTH after the qRT-PCR data had been normalized to the expression of the reference *UBQ5* gene. The 2^\(-\Delta\Delta CT\) method was used to calculate the relative expression levels with three repeats (Livak and Schmittgen, 2001). SD represents the standard deviation of three technical duplicates. This experiment was biologically repeated three times with similar results.

**Fig. S5** Validation of differentially expressed, Digu-specific receptor kinase (RK) genes using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The leaf tissues of Digu and Lijiangxintuanheigu (LTH) were harvested at the time points 5, 10 and 20 h post-inoculation (hpi) from *Magnaporthe oryzae*-inoculated and mock-inoculated plants. qRT-PCR experiments and
analyses were performed in the same way as described in Fig. S3. SD represents the standard deviation of three technical duplicates. This experiment was biologically repeated three times with similar results.

**Fig. S6** Determination of expression of Os08g10300 in the transgenic plants carrying Os08g10300ox. The leaf samples were collected at the seedling stage for RNA extraction, and real-time quantitative reverse transcription-polymerase chain reactions (qRT-PCRs) were performed using the extracted RNA samples. The 2^ΔΔCT method was used to calculate the relative expression levels with three repeats (Livak and Schmittgen, 2001). SD represents the standard deviation of three technical duplicates.

**Fig. S7** Determination of the relative expression levels of pathogenesis-related (PR) genes in transgenic plants carrying Os08g10300ox. Inoculation and expression analyses were carried out as described in Fig. S1A. The leaf samples were collected at the seedling stage for RNA extraction, and real-time quantitative reverse transcription-polymerase chain reactions (qRT-PCRs) were performed using the extracted RNA samples. The 2^ΔΔCT method was used to calculate the relative expression levels with three repeats (Livak and Schmittgen, 2001). SD represents the standard deviation of three technical duplicates.

**Fig. S8** Determination of the blast disease resistance of the transgenic plants carrying Os08g10300ox. The seedlings from 2-week-old plants were inoculated by spraying with *Magnaporthe oryzae* strains (WJ9, WJ10, YC7 and YC26). Disease resistance was determined on two representative transgenic lines overexpressing Os08g10300 (abbreviated as 10300ox-1 and 10300ox-2, respectively) in the TP309 genetic background.

**Fig. S9** Determination of the relative expression levels of CEBIP, OsLYP4 and OsLYP6 in Digu. Inoculation, sampling and expression analyses were carried out as described in Fig. S1A. The relative expression of genes after *Magnaporthe oryzae* inoculation was normalized to the expression with mock inoculation. All quantitative reverse transcription-polymerase chain reaction (qRT-PCR) data were normalized to the expression of the reference *UBQ5* gene before the determination of relative expression. The 2^ΔΔCT method was used to calculate the relative expression levels with three repeats (Livak and Schmittgen, 2001). SD represents the standard deviation of three technical duplicates.

**Fig. S10** Nucleotide-binding site and leucine-rich repeat (NBS-LRR)-containing genes encoded by differentially expressed genes (DEGs). Four NBS-LRR genes in Lijiangxintuanheigu (LTH), but none in Digu, are responsive to infection with *Magnaporthe oryzae*.

**Fig. S11** Comparative analyses of the receptor kinases (RKs) encoded by the Digu-specific differentially expressed genes (DEGs) [compared with Lijiangxintuanheigu (LTH)] and those expressed differentially in other resistant rice lines in response to *Magnaporthe oryzae*. The RK genes of the resistant rice Gigante Vercellior were retrieved from the study reported previously (Bagnaresi et al., 2012), whereas the RKs of the resistant rice Pi1 and Pi9 were from the study reported previously (Wei et al., 2013). GV, Gigante Vercelli; VN, Vialone Nano; IRBL18, LTH carrying Pi1; IRBL22, LTH carrying Pi9.

**Table S1** Spearman correlations among Digu and Lijiangxintuanheigu (LTH) samples.

**Table S2** The differentially expressed genes (DEGs) identified from Digu and Lijiangxintuanheigu (LTH) during rice early responses to *Magnaporthe oryzae*.

**Table S3** Comparison of the expression patterns of differentially expressed genes (DEGs) using microarray and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses.

**Table S4** The enrichment analysis of functional gene ontology (GO) categories associated with differentially expressed genes (DEGs) in response to *Magnaporthe oryzae*.

**Table S5** Pathways specifically present in the durably resistant rice Digu in response to *Magnaporthe oryzae*.

**Table S6** Expression evaluation of the genes associated with CEBIP (chitin elicitor-binding protein)-mediated pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) for their responsiveness to *Magnaporthe oryzae* in Digu and Lijiangxintuanheigu (LTH) at 5, 10 and 20 h post-inoculation (hpi).

**Table S7** Primers of pathogenesis-related (PR) genes, randomly selected differentially expressed genes (DEGs) and other genes for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses.