Transcriptome Profiling of the Rice Blast Fungus *Magnaporthe oryzae* and Its Host *Oryza sativa* During Infection

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Abstract

The rice blast (fungal pathogen: *Magnaporthe oryzae* and host: *Oryza sativa*) is one of the most important model pathosystems for understanding plant–microbe interactions. Although both genome sequences were published as the first cases of pathogen and host, only a few in planta transcriptome data during infection are available. Due to technical difficulties, previously reported fungal transcriptome data are not highly qualified to comprehensively profile the expression of fungal genes during infection. Here, we report the high-quality transcriptomes of *M. oryzae* and rice during infection using a sheath infection-based RNA sequencing approach. This comprehensive expression profiling of the fungal pathogen and its host will provide a better platform for understanding the plant–microbe interactions at the genomic level and serve as a valuable resource for the research community.

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Keywords

fungus–plant interactions, *Magnaporthe oryzae*, *Oryza sativa*, plant responses to pathogens, transcriptome

Transcriptome Announcement

Rice blast disease, caused by the hemibiotrophic fungus *Magnaporthe oryzae*, is the most devastating disease in the rice-growing area worldwide and can lead to losses of 10 to 30% of annual rice yield (Ebbole 2007; Kawahara et al. 2012; Talbot 2003). Along with the agronomic importance of rice, this pathosystem has been considered as a model to study plant–microbe interactions. Due to socioeconomic and scientific significance, this fungal pathogen has been extensively studied using molecular genetics to identify genes involved in fungal development and pathogenicity. Efforts to identify resistant genes and immune responses of rice against *M. oryzae* infection are also actively conducted (Chen and Ronald 2011). The reference genomes of the host plant and fungal pathogen were published in 2002

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and 2005, respectively (Dean et al. 2005; Goff et al. 2002; Yu et al. 2002), and many cultivars and strains have been resequenced. In addition to their genome sequences, their transcriptomes derived from different conditions have become available. However, only a few studies have described the transcriptome profiles of rice and *M. oryzae* during infection (Bagnaresi et al. 2012; Dong et al. 2015; Kawahara et al. 2012). In addition, the fungal transcriptome data are not highly enriched to decipher expression profiles at the genomic level. This was due to technical difficulties in the enrichment of fungal RNAs in infected samples, the portion of which is significantly smaller than the host RNAs. Therefore, only a few fungal sequence reads were profiled from the infected rice samples during pathogenesis. The completion of the *M. oryzae* infection cycle on rice takes more than 6 days (Ribot et al. 2008). This fungal pathogen switches its lifestyle from biotrophy to necrotrophy during infection. Expression profiling from biotrophic to necrotrophic stages of the fungal pathogen is a prerequisite to understanding the molecular mechanism of pathogenesis and would provide new insights into the switching of lifestyles.

In this study, we present high-quality transcriptome data of *M. oryzae* and rice during infection using a sheath infection-based RNA sequencing (RNA-Seq) analysis. We maximized the enrichment of fungal RNAs by collecting infected rice samples employing microscopic observations in a rice sheath assay. *M. oryzae* strain KJ201 used in this study was obtained from the Center for Fungal Genetic Resources at Seoul National University, Korea. The rice cultivar Nakdong, which contains no known resistance gene, was grown in a growth chamber at 27°C with a photoperiod of 16 h of light and 8 h of darkness. To collect the infected rice samples for RNA-Seq, excised rice sheaths (10 to 15 cm long) from 4- to 5-week-old plants (five- to six-leaf stage) were prepared as previously described (Kankanala et al. 2007; Koga et al. 2004; Mosquera et al. 2009). The conidial suspension (1×10^5 conidia/ml) was infiltrated into one end of the rice sheath placed on the adaxial side. The infected rice sheaths were placed on plastic supports in a moistened box. Representative infection stages were determined based on observation under a microscope. These included appressorium development (prepenetration, 18 h postinoculation [hpi]), penetration and development of primary invasive hyphae (biotrophic stage, 27 hpi), development and growth of invasive hyphae (biotrophic stage, 36 hpi), active growth of invasive hyphae into neighboring host cells (necrotrophic stage, 45 hpi), and extensive proliferation and killing of host cells (necrotrophic stage, 72 hpi). Forty inoculated rice sheath samples were used for RNA extraction at each time point. Only the infected side of the rice sheath was cut, immediately frozen in liquid nitrogen, and then stored at -80°C. For the control, rice sheaths were inoculated with sterilized distilled water and the mycelial sample was incubated in liquid complete media.

Total RNA was extracted by TRIzol reagent (Molecular Research Center, Inc., OH, U.S.A.) and an Easy-Spin Total RNA Extraction Kit (iNtRON Biotechnology, Korea). Total RNAs were inspected with a Pico RNA chip (Agilent Technologies, CA, U.S.A.) using a

Table 1. Transcriptome statistics of *Magnaporthe oryzae* and rice (*Oryza sativa*) during infection

Species, reads	Sample source ^a						Rice sheath
	Mycelia	Infected rice sheath					
		18 hpi	27 hpi	36 hpi	45 hpi	72 hpi	
<i>M. oryzae</i>							
Total reads ^b	64,370,558	239,715,825	207,173,947	230,328,075	233,971,030	221,236,255	249,377,027
Mapped reads ^c	63,220,201	22,826,910	12,465,910	13,946,600	22,910,441	58,846,821	...
Mapping CDS ^d	11,274	11,979	11,686	11,602	11,863	12,404	...
Ref CDS (%) ^e	86.78	92.21	89.95	89.31	91.32	95.48	...
<i>O. sativa</i>							
Mapped reads ^c	...	221,110,688	198,377,520	206,254,982	210,628,458	149,271,358	249,377,027
Mapping CDS ^d	...	37,237	36,331	36,605	36,984	36,052	38,048
Ref CDS (%) ^f	...	88.20	86.05	86.70	87.60	85.39	90.12
Accession ^g	8259727	8259732	8259731	8259730	8259729	8259728	8259726

^a The sequencing platform for all sources was HiSeq 2000; hpi = hours postinoculation.

^b Total number of reads.

^c Number of mapped reads to the genome.

^d Number of coding sequences (CDS) mapped by reads.

^e Percentage to the reference CDS (12,991).

^f Percentage to the reference CDS (42,220).

^g Sequence Read Archive accessions, prefix "SRR" for each number.

Bioanalyzer (Agilent Technologies). Poly-A-containing mRNA transcripts were purified using poly-T oligo magnetic beads and fragmented randomly by divalent cations treatment. cDNA synthesis was performed using Superscript II reverse transcription (Invitrogen, CA, U.S.A.) and adenylation of cDNA was followed. The cDNA was ligated with adapters of random hexamers (Invitrogen). This library construction protocol was performed using TruSeq RNA Sample Prep Kit (Illumina, CA, U.S.A.). A sequencing library was selected by the length of 300 to 400 bp. Paired-end RNA sequencing of each sample was performed by Illumina HiSeq 2000 (Illumina), which was conducted at the National Instrumentation Center for Environmental Management, Seoul National University, Korea. The quality of sequencing reads was assessed by MultiQC v1.7 (Ewels et al. 2016). The adaptor sequences, low-quality reads, and bases interrupting the analysis were removed by Cutadapt v1.13 (Martin 2011). Because the filtered reads from each sample were a mixture of *M. oryzae* and *O. sativa*, the alignment program BWA v0.7.16 was used to separate them (Li and Durbin 2009). The read mapping of each sample from the fungus and rice was performed using HISAT2 v2.1.0 (Kim et al. 2015) with the reference genomes of *M. oryzae* 70-15 (MG8) and *O. sativa* L. subsp. *japonica* (IRGSP-1.0) downloaded from Ensembl release 42 (Hubbard et al. 2002). Finally, the read counting for each annotated gene was performed with StringTie v1.3.5 (Pertea et al. 2015).

Because we used the rice sheath assay, the coverage of fungal transcriptome was particularly enriched in this analysis. Specifically, the average number of mapped reads in these in planta transcriptomes increased 64.9 and 19.1 times more than the previously reported fungal and plant transcriptome data, respectively (Dong et al. 2015) (Table 1). In addition, 86.8 to 98.5% of total fungal coding sequences (CDS) were counted as fragments per kilobase of exon model per million reads mapped (FPKM) across the in planta time points and 88.5 to 93.4% of total rice CDS were counted as values at the same time points (Table 1). This result indicates that the average mapping CDS of in planta fungal transcriptome more than doubled from the previous report (Dong et al. 2015). Principal component analysis (PCA) using log₂-transformed FPKM values showed a separation of the control and infected rice sheath samples. The multivariate analysis of the fungal samples showed that in planta transcriptomes were notably different from the mycelial transcriptome. The time points for in planta conditions (18, 27, 36, and 45 hpi) were clustered and these were distinguished from 72 hpi. The expression of candidate effector genes was profiled from 348 small secreted proteins (Kim et al. 2016). Among them, the expression of 303 genes was increased in at least one in planta condition. These transcriptomes could assist in evaluating expressed genes in plant-microbe interactions such as effector families. The transcriptome data were validated by quantitative reverse-transcription PCR (qRT-PCR), with 31 fungal and 8 rice genes from the same biological samples used for RNA-Seq and independent experiments. Pearson correlation analysis of transcriptome and qRT-PCR data showed a positive correlation ($r = 0.6712$), indicating the validity of transcriptome data.

The transcriptome sequence data in this study are deposited in the NCBI Sequence Read Archive with the accession numbers SRR8259726 to SRR8259732. The FPKM values of each gene for *M. oryzae* and *O. sativa* from all time points, the PCA information (including a phenotypic figure of each time point), the expression of the candidate effector genes, qRT-PCR validation, and the correlation analysis data of qRT-PCR and RNA-Seq are available in Figshare (Jeon et al. 2019). These data will help provide better understanding of fungus-plant interactions and further provide a valuable resource for the rice blast research community and beyond.

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