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Comparative RNA-Seq analysis of *Nicotiana benthamiana* in response to *Phytophthora parasitica* infection

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Abstract *Phytophthora parasitica* causes serious damage to a broad spectrum of agriculturally important crops and natural ecosystems. To investigate plant responses to *P. parasitica*, differential gene expressions between inoculated and mock-treated *Nicotiana benthamiana* leaves were analyzed by RNA-Seq approach. A total of 5375 and 3614 *N. benthamiana* genes were found to be upregulated and downregulated, respectively. Infection with *P. parasitica* triggered massive metabolic reprogramming in the inoculated tissues. Genes related to photosynthesis, starch biosynthesis, and nitrogen assimilation were suppressed while sucrose degrading genes were induced. Notably, plant defense responses were activated, reflected by larger number of upregulated JA and ET signaling genes, receptor-like kinases, pathogenesis-related genes, and transcription factors. Collectively, these results provide broad insights into *N. benthamiana* defense mechanisms against *P. parasitca* and advance our understanding of plant-*Phytophthora* interactions.

Keywords *Nicotiana benthamiana* · RNA-Seq analysis · Plant-*Phytophthora* interaction · Plant defense

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Introduction

Over the past several centuries, the agricultural productivity was severely threatened by pathogen diseases, leading to vast economic losses every year. Plants have evolved complex mechanisms to recognize and induce defense responses to protect themselves from the threats of pathogens (Jones and Dangl 2006). The induced defenses include two layers: the first layer is triggered upon recognition of pathogen-associated molecular patterns (PAMPs) (Boller and He 2009), and the second layer of defenses is activated by special recognition of effectors from pathogens (Jones and Dangl 2006). In both layers, plants can induce defense-related physical changes containing cytoskeletal reorganization, callose depositions and cell wall thickening. Meanwhile, some biochemical responses such as generation of reactive oxygen species, production of salycylic acid, jasmonic acid or ethylene signaling compounds, are activated to perturb infection (Jones and Dangl 2006; Chisholm et al. 2006). In addition, energy balance is also critical to fight off pathogens (Scheideler et al. 2002). Therefore, changes in the transcriptomes of plants are usually significant and distinct during interactions with different pathogens (Rojas et al. 2014).

Phytophthora is an important plant pathogen genus belonging to oomycetes, and most species cause severe threats to crops and natural ecosystems (Kroon et al. 2012; Kamoun et al. 2015). Among these species, *P. infestans* and *P. sojae* are two notorious pathogens with narrow host ranges, while many other *Phytophthora* species infect a broad spectrum of plants (Attard et al. 2008). As a consequence, an increasing attention has focused on a representative species, *P. parasitica* Dastur (syn. *Phytophthora nicotianae* Breda de Haan). This pathogen is capable of infecting many plant species, including *Arabidopsis*

thaliana and *Nicotiana tabacum* (Meng et al. 2014). It causes severe foliar, fruit, root and crown rots on solanaceous crops, as well as horticultural and fruit trees. *P. parasitica* has emerged as an ideal pathogen to study because of its economic importance and broad host ranges. However, its interactions with plants have not been extensively studied.

Nicotiana benthamiana is an important model plant for studying molecular plant-pathogen interactions (Goodin et al. 2008). Firstly, it was originally found to be infected by diverse plant viruses, leading to development of virus-induced gene silencing (VIGS), a widely used technology to decipher functions of plant genes (Purkayastha and Dasgupta 2009). Secondly, *N. benthamiana* is also susceptible to a vast number of other pathogens including bacteria, fungi and oomycetes, giving rise to a system to study plant innate immunity mechanisms (Kamoun et al. 1998; Bos et al. 2010). Thirdly, due to the large leaves and its susceptibility to diverse pathogens, *N. benthamiana* has been adopted as the host for transient expression using either engineered viruses or syringe-infiltration of *Agrobacterium tumefaciens* (Chapman et al. 1992; Ma et al. 2012). Recently, the draft genomic sequence of *N. benthamiana* was released (Bombarely et al. 2012), increasingly heightening its usefulness for plant-pathogen interaction researches.

Many researchers have focused on the pathosystems of interactions between *Phytophthora* and model plant *N. benthamiana*. Combined with great wealth of *N. benthamiana*, including its natural ecotypes, genomic resources, and vast genetic and molecular tools, researchers could excavate more basic knowledge on plant immune system in response to *Phytophthora* attack. The majority of basic knowledge about PAMP perception and PAMP-triggered immunity (PTI), effector-triggered immunity (ETI), transcriptional regulation networks, energy arrangement, and hormone pathway signaling could be greatly accelerated. On the other side, the functions of diverse *Phytophthora* genes have been studied by robust genetic tools in *N. benthamiana* (Dou et al. 2008; Liu et al. 2011; Chen et al. 2015), which also accelerates the functional study of virulence-related genes in *Phytophthora*. The study of this pathosystem is expected to facilitate understanding of *Phytophthora* pathogenesis and plant susceptibility or resistance responses, and ultimately lead to the development of novel strategies to control *Phytophthora* diseases.

In this study, RNA-Seq technology was applied to investigate the dynamic changes of the *N. benthamiana* transcriptome in response to *P. parasitica*. This approach allowed us to globally examine the expression changes of genes involved in primary and secondary metabolisms, defense-related pathways, and transcriptional regulation. These results could gain molecular insights into plant genes underlying the immune responses against *P. parasitica*.

Materials and methods

Materials and inoculation procedures

Phytophthora parasitica isolate 025 was grown on V8 agar and cultured at 25 °C in the dark. *N. benthamiana* seedlings were grown in a growth chamber at 25 °C under fluorescent white light in a 16:8-h light/dark cycle. Six-week-old *N. benthamiana* leaves were detached and immersed in suspensions containing 10^5 zoospores of *P. parasitica*. The leaves were collected at 3, 6, 9, and 12 hours post inoculation (hpi), and then *P. parasitica* mycelia and *N. benthamiana* cell death were visualized by staining with trypan blue staining method. Briefly, the trypan blue stock solution was prepared by mixing 10 g of phenol, 10 mL of glycerol, 10 mL of lactic acid, 10 mL of distilled water, and 0.02 g of trypan blue. The inoculated leaves in trypan blue solution were boiled in a water bath for 2 min and then incubated for 1 day. After destaining in saturated chloral hydrate solution (2.5 g chloral hydrate/1 mL water) for 2 days, the leaves were equilibrated with 70 % glycerol for photography.

Sample preparation and sequencing

The *N. benthamiana* leaves at 6 hpi and the mock-treated leaves were selected for RNA-Seq sequencing. Total RNA was isolated using the PureLink RNA mini kit (Invitrogen) according to the manufacturer's instructions and then treated with DNase I (RNase free, *TaKaRa*) to remove genomic DNA contaminations. The RNA samples from three biological replicates for each sample were pooled and sequenced with 100 bp paired-end reads on Illumina HiSeq 2000 platform.

Reads mapping and annotations

The produced clean reads were mapped to the *N. benthamiana* V0.4.4 reference genome (https://solgenomics.net/organism/Nicotiana_benthamiana/genome) and *P. parasitica* genome (https://www.broadinstitute.org) using TopHat (Trapnell et al. 2009) software with default parameters. Only the reads that could be uniquely mapped to the *N. benthamiana* genome were used for subsequent processing. The retained reads were quantified using Cufflinks v1.0.3 program (Trapnell et al. 2010), and expression level of each gene was calculated by normalizing to the fragment per kilobase of exon per million mapped reads (FPKM) value. To filter out weakly expressed genes, only genes with FPKM larger than one were included in the analysis. Differentially expressed genes were identified using the GFOLD algorithm (Feng et al. 2012), which was biologically meaningful for

single replicate experiments. Genes with four fold change (GFOLD > 1 or < −1) were considered differentially expressed between two samples. TransDecoder tool was applied to identify potential coding regions within novel transcripts. MapMan ontology tool was used to obtain an overview of tobacco genes involved in metabolic pathways, in which a plant-specific ontology classifies genes into well defined hierarchical categories and were denominated BINs (Thimm et al. 2004). Tobacco genes were assigned to BINs using the Mercator automated annotation pipeline. The enriched Mapman BINs were identified using Fisher's exact test.

Validation of RNA-Seq data by qRT-PCR

To validate the RNA-Seq results, qRT-PCR was performed for six transcription factors which were induced by more than four fold after *P. parasitica* infection. First strand cDNA was synthesized using an iScript cDNA Synthesis kit (Takara Bio DRR036A). *N. benthamiana ef1-α* (Genbank accession no. AF120093.1) was used as an endogenous control to normalize the expression data. Quantitative real-time PCR thermal cycler conditions and reaction mixtures were performed according to the manufacturer's instructions (SYBR® Premix Ex Taq™). The endpoint was used in the real-time PCR quantification (Livak and Schmittgen 2001), $\triangle$Ct (for cycle threshold) relative quantification with reference gene normalization was performed. At least three biologically independent experiments were carried out. Pearson correlation coefficient was calculated between the fold change value of the qRT-PCR analysis and RNA-Seq analysis.

Results

P. parasitica could infect *N. benthamiana*

Phytophthora parasitica is a pathogen with broad host ranges (Meng et al. 2014). To test whether it could infect the model plant *N. benthamiana*, the detached leaves of *N. benthamiana* were immersed in *P. parasitica* zoospore suspensions and phenotypes were observed. The necrosis symptoms was visually observed at 9 hpi. The leaves began to darken and wilt at 12 hpi. To investigate the infection processes, the trypan blue-stained leaves were observed. At 3 hpi, most of cysts germinated and the hyphae began to spread among plant cells. At 6 hpi, the hyphae extensively observed and could touch almost all the plant cells. Then, the necrosis occurred in only limited areas at 9 hpi and the majority of plant tissues developed necrosis at 12 hpi (Fig. 1). The results suggest that *P. parasitica* is able to infect *N. benthamiana*, making this pathosystem a good

model to study interactions of plants and *Phytophthora* pathogens.

Overview of the transcriptome

Two pool samples including the inoculated and mock-treated *N. benthamiana* leaves at 6 hpi were analyzed using RNA-Seq approach. Based on illumina HiSeq 2000 platform, approximately 65.6 and 52.8 million paired-end reads were produced from the two samples. The reads were then aligned onto the *N. benthamiana* V0.4.4 reference genome using TopHat software, resulting in 91.5 and 85.1 % mapped reads, respectively (Table 1). Most of the mapped reads (95.6 and 95.7 %) were uniquely aligned to the genome, and were used for further analysis. To get the expression value of each *N. benthamiana* gene in inoculated and mock-treated samples, the fragment per kilobase of exon per million mapped reads (FPKM) value was calculated for each gene. Of the 76,379 predicted genes in the *N. benthamiana* genome (Bombarely et al. 2012), 35,180 (46.1 %) genes were expressed using FPKM larger than 1 as the cutoff for gene expression, and 26,041 genes among these were detectable in the both samples.

To evaluate the gene expression, ten housekeeping genes including *tubulin*, *actin*, and *ef1-α* (elongation factor 1-α) (Supplementary Table 1) were selected as described previously (Liu et al. 2012). Based on the comparison of the two samples, the $\log_2$ fold changes for the housekeeping genes ranged from −0.20 (NbS00023178g0001 coding for ef1-α) to 0.21 (NbS00033002g0006 coding for TIP41-like protein) (Supplementary Table 1), indicating that none of these housekeeping genes was significantly altered. The results reflect that the transcript data meets the requirements for the following transcriptome analysis.

Identification of novel transcripts

We observed that a small number of reads could be mapped in regions of the *N. benthamiana* genome which has not yet been annotated, indicating that potential new genes could be predicted based on RNA-Seq data. In total, we identified 7547 novel transcripts based on the following filtering criteria: (1) transcript length longer than 300 bp; (2) The FPKM value was larger than 1 in at least one sample; (3) the novel transcript was at least 300 bp away from an annotated gene.

Based on previous studies, novel transcripts seem to have fewer exons and less protein-coding genes compared with the annotated genes (Bruno et al. 2010; Lu et al. 2013). Here, we found that 98.5 % of the novel transcripts had no more than three exons while this number was 57.7 % in the annotated genes (Fig. 2a). Averagely, the novel transcripts had ∼1.3 exons per transcript while the annotated genes were ∼4.3. The average size of the novel transcripts was 1048 bp. In total,
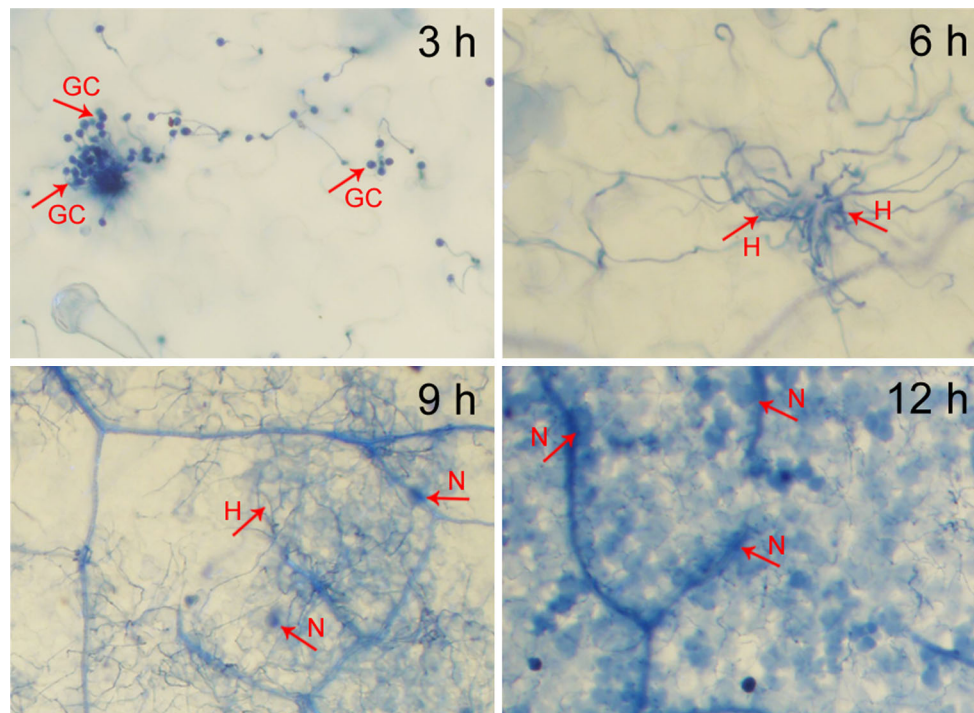
Fig. 1 Trypan blue-stained *N. benthamiana* leaves inoculated with *P. parasitica*. The leaf discs at 3, 6, 9 and 12 hpi were observed. *GC* germinating cyst, *H* hyphae, *N* necrosis

Table 1 Summary of RNA-Seq and mapping results

	Mock-treated	6 h inoculated
Total clean reads	65,630,706	52,829,642
Total nucleotides (bp)	6,563,070,600	5,282,964,200
Aligned reads	60,039,443	44,937,852
Aligned reads (%)	91.5	85.1
Uniquely aligned reads	57,400,789	43,013,984
Uniquely aligned reads (%)	95.6	95.7

only 1481 transcripts were predicted to encode open reading frames >100 amino acids, suggesting that others may be non-coding RNAs. As Fig. 2b shows, 1126 transcripts with >100 amino acids had blast hits in the NCBI NR database (E-value $\leq 1 \times 10^{-5}$) and could be considered as novel protein-coding genes. More than half of these were matched to uncharacterized proteins or hypothetical proteins, while the rest had sequence similarity to polyproteins, resistance proteins, ubiquitin proteins, auxin-induced proteins and other functional proteins. Thus, the results added a large amount of unpredicted protein-encoding genes and non-coding RNAs in *N. benthamiana*.

Differentially expressed genes

For further identification of differentially expressed genes (DEGs) between the two samples, four fold-change was

applied with GFOLD algorithm (GFOLD > 1 or GFOLD < −1), which gives more biologically meaningful results when no replicate is available. A total of 8989 (11.8 %) DEGs were identified, including 5375 upregulated and 3614 downregulated genes in the inoculated sample (Supplementary Table 2). We used the MapMan tool to investigate how *P. parasitica* infection affected the expressions of *N. benthamiana* genes. The results reflected dynamic transcript changes in photosynthesis, sucrose degradation, nitrogen assimilation and defense-related pathways (Supplementary Table 3), which will be described in the following parts.

Primary and secondary metabolisms

Phytopathogen infection usually alters plant photosynthesis process (Berger et al. 2007). Here, we found that 208 *N. benthamiana* genes associated with photosynthesis were found to be differentially downregulated while only one gene was upregulated (Supplementary Table 4). This phenomenon was particularly evident for genes encoding proteins in photosystem I and photosystem II reaction centres, including 58 downregulated genes participated in light harvesting complex and 53 downregulated photosystem subunit genes (Supplementary Table 4). Moreover, 43 DEGs were identified as significantly downregulated in calvin cycle, which referred to the light-independent

Fig. 2 Features of novel transcripts. **a** Comparison of exon numbers between novel transcipts and annotated genes. **b** Distribution of potential coding protein length associated with corresponding blast hits in NCBI NR database. The novel transcripts which encoded open reading frames greater than 100 amino acids were used for blast analysis against NCBI NR database. The longer potential proteins had higher probability to be homologs to the known proteins
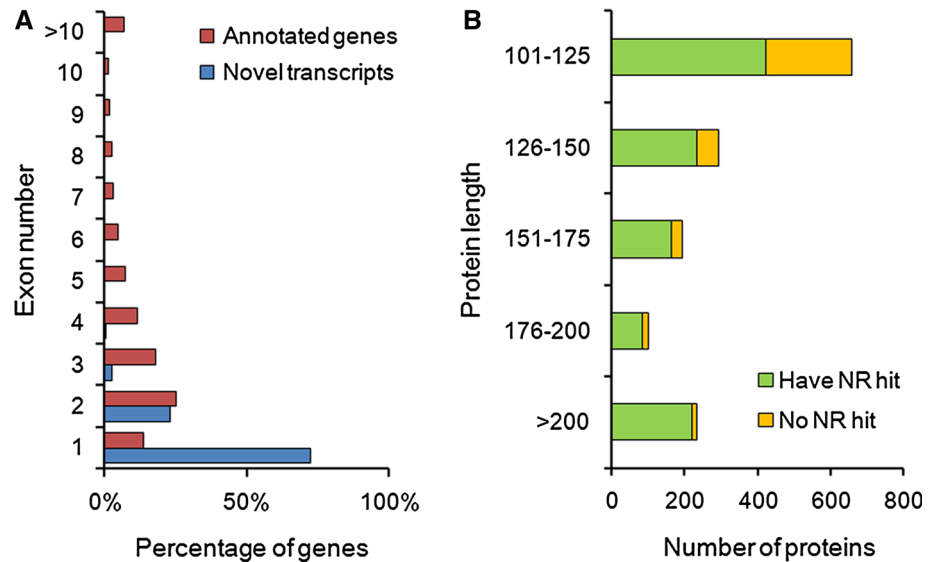
reactions in photosynthesis. These results indicated that the photosynthetic rates of *N. benthamiana* were largely reduced by the infection of *P. parasitica*.

The reduction of photosynthetic metabolism and the simultaneously increased cellular demands during the defense response often initiate the transition from source status to sink status in infected tissue (Bolton 2009). Eleven genes involved in sucrose degradation were differentially expressed, in which eight were upregulated (Supplementary Table 5). For examples, three genes encoding cell wall invertases were highly induced, especially for NbS00010282g0017, whose FPKM value was over 500-folds increased. Two genes encoding hexokinases were also induced, which were reported to sense soluble hexoses (Bolton 2009). At the same time, seven upregulated hexose transporters were identified, suggesting that the plants need to transport hexoses into the cells to fulfill the energy and carbon requirements for defense responses. Conversely, the expressions of genes associated with sucrose and starch biosynthesis were significantly reduced (Supplementary Table 5).

In response to *P. parasitica* infection, *N. benthamiana* amino acid metabolism pathways also seemed to be differentially regulated. Genes involved in biosynthesis of asparagine, methionine, serine, and tyrosine were upregulated (Supplementary Table 6). By contrast, other amino acids including proline, isoleucine, and histidine were reduced by the observation of upregulated degrading genes (Supplementary Table 6). It is likely that the above accumulated or reduced amino acids were rearranged to participate in plant defense, and more comprehensive studies are needed to prove it. The catabolism of some amino acids may result in the release of ammonium and intermediate compounds to energy-generating pathways such as the

tricarboxylic acid cycle. Nitrogen assimilation, where the inorganic nitrogen is assimilated into glutamine and glutamate, is important for plant development. However, this process was impaired in infected *N. benthamiana*. Genes encoding critical enzymes for nitrogen assimilation were reduced, presented by two nitrate reductase, three nitrite reductases, and two glutamine synthetases (Supplementary Table 6).

Despite the variation of primary metabolism, plant secondary metabolism also changes based on the induction of defense programmes. In this study, a cohort of DEGs involved in secondary metabolism were identified in infected *N. benthamiana*. Genes related to the biosynthesis of terpenoids, lignins and alkaloids were highly induced (Supplementary Table 7). It was previously reported that the lignin pathway was activated due to the importance of lignification and reinforcement of cell walls in the response of plants to pathgen infection (Miedes et al. 2014). Most *N. benthamiana* lignin biosynthetic genes, especially six phenylalanine ammonia-lyases which played an important role in cell wall lignification, were upregulated, indicating that lignin was deposited in infected regions to avoid the spread of the pathogen.

Defense signaling pathways

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are three major defense hormones, which mediate plant defense responses to pathogens (Lopez et al. 2008; Spoel and Dong 2008). We identified 55 DEGs involved in ET-signaling pathway, and 44 of these coding for ethylene response factors were upregulated after *P. parasitica* infection (Supplementary Table 8). Meanwhile, four 1-aminocyclopropane-1-carboxylate (ACC) oxidases and

one ACC synthase were identified as upregulated in infected *N. benthamiana*. The ACC oxidases and synthase are known to play important roles in ET biosynthesis (Wang et al. 2002). The hormone JA oftern acts synergistically with ET to trigger the expression of defense-related proteins. Four jasmonate ZIM-domain genes, which acted as negative regulators in the JA-signalling pathway, were identified to be downregulated. Meanwhile, the majority of JA biosynthesis genes, such as lipoxygenases and allene oxidase synthases, were upregulated (Supplementary Table 8). Paradoxically, no gene associated with SA-signaling pathway was differentially expressed, and genes involved in regulating synthesis of SA were downregulated. These results reflected that attack by *P. parasitica* induced constitutive activation of ET and JA, but not SA signaling pathway.

In response to pathogens, the ability to perceive extracellular molecules and then rapidly initiate defense responses is integral to the basal plant resistance (Goff and Ramonell 2007). *N. benthamiana* possesses a large superfamily of receptor-like kinases (RLKs) and receptor-like proteins (RLPs) to recognize pathogenic molecules, including PAMPs. Remarkly, 253 genes belonging to RLKs or RLPs were identified to be differentially expressed in our study, and 168 among these were upregulated (Supplementary Table 9). We discovered 56 upregulated LRR-RLKs, which comprise the largest class of RLKs. One of the best-characterized LRR-RLK was the FLAGELLIN SENSITIVE 2 (FLS2) protein, an RLK that was reported to be involved in the perception of bacterial flagellin in *Arabidopsis thaliana* (Gomez-Gomez and Boller 2000). Five homologs of FLS2 were identified to be highly induced in infected *N. benthamiana*, suggesting similar functions as in *A. thaliana*. In addition, other classes of RLKs including wall associated kinases, S-locus receptor kinases, wheat LRK10-like kinases, and legume lectin kinases were mainly upregulated after *P. parasitica* inoculation (Supplementary Table 9). These results indicated that different RLK classes may play important or overlapping roles in pathogen recognition and plant defense.

Defense-related genes

Pathogenesis-related (PR) genes are produced by plants to protect themselves against invading pathogens and accumulate mainly in the infected and surrounding tissues (Sels et al. 2008). Currently, PR genes were classified into 17 families based on their properties and functions. At least 140 PR genes belonging to 15 families were identified to be differentially expressed in infected *N. benthamiana* (Supplementary Table 9). Most PR family members, including chitinases, thaumatin-like proteins, β-1,3-glucanases, and

peroxidases, were induced. Chitinases and β-1,3-glucanases are two critical hydrolytic enzymes that play an important role in defense reaction against pathogen by degrading cell walls (Jongedijk et al. 1995). We found 13 induced β-1,3-glucanases from PR-2 family, and 16 induced chitinases from PR-3, PR-4, PR-8 and PR-11 families. Intriguingly, 22 upregulated PR-9 family genes coded for peroxidases, which were involved in cell wall lignification and enhanced resistance in plants. On the opposite, several PR families, involing subtilisin-like proteases, lipid transfer proteins and germin-like proteins, were downregulated after *P. parasitica* infection (Supplementary Table 9).

Other putative defense-related genes, such as two NADPH oxidases involved in reactive oxygen species production in response to pathogens, six kunitz trypsin inhibitors with antifungal activity, were upregulated (Supplementary Table 9). These results revealed that the *N. benthamiana* defense responses were active once infected by *P. parasitica*.

Transcriptional regulators

Identification of transcription factors (TFs) expressed in a specific stage provides a foundation for understanding the transcriptional regulatory networks underlying the development, structure and function of the stage. Based on family assignment rules on the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn), we identified 1428 TFs expressed in at least one of the two samples, and 504 of these showed differential expression, covering 40 families (Supplementary Table 10). Among the 504 TFs, 235 showed upregulation while the left 269 showed downregulation in infected *N. benthamiana* (Fig. 3). Ethylene responsive factors (ERFs), involving in biotic and abiotic stress responses, composed the largest TF family in *N. benthamiana*. A total of 68 ERFs were induced after *P. parasitica* infection, suggesting activation of the transcription of defense-related genes. WRKY transcription factors are the second large family of regulatory proteins, which participate in regulating defense gene expression (Jing et al. 2009). We identified 50 upregulated WRKY TFs, potentially had partly redundant functions in regulating resistance to *P. parasitica*. Furthermore, 30 downregulated genes were identified to encode bHLH TFs (Supplementary Table 10), acting as negative regulators of JA-mediated defense. In addition to the above three major TF families associated with plant defense, we also identified other induced TFs such as MYB, HSF, and GRAS TFs, although most of their functions were unclear.

To validate the differentially expressed TFs identified by RNA-Seq analysis, three ERF and three WRKY TFs involved in defense responses were evaluated by qRT-
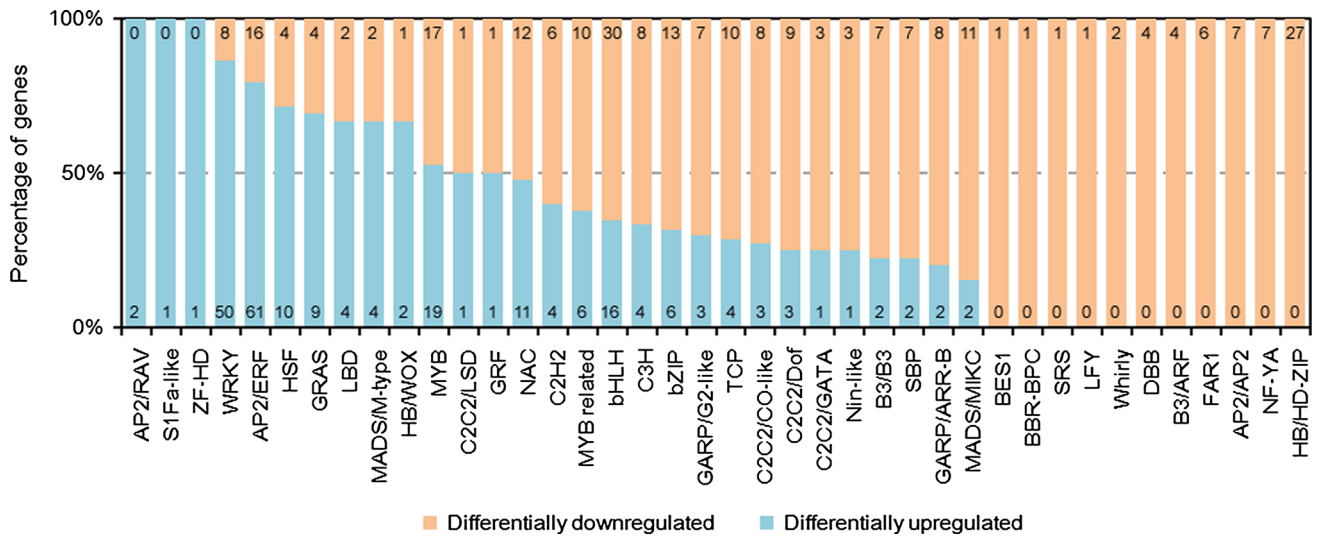
Fig. 3 Summary of differentially expressed transcription factors. The 504 differentially expressed transcription factors were distributed in 40 families according to family assignment rules from Plant Transcription Factor Database. The x-axis represented 40 transcription factor families. The proportions of upregulated and downregulated genes within a family were represented by blue and orange bars, respectively. The *number* on the *top* of *each bar* represented downregulated gene number while the *number* on the *bottom* represented upregulated gene number

PCR. All of these selected TFs were shown to be upregulated in infected *N. benthamiana* by RNA-Seq analysis (Supplementary Table 10). The validation results revealed that the genes' differential expressions were positively correlated with qRT-PCR (r = 0.8, Fig. 4), which highlighted the accuracy and reproducibility of the RNA-Seq analysis in this study.

Discussion

The molecular mechanisms related to the interactions between *Phytophthora* and plants are largely unknown. In this study, the model plant *N. benthamiana* was applied to investigate the plant responses triggered by the inoculation of *P. parasitica* using RNA-Seq technology. We found that expression profiles of *N. benthamiana* genes involved in metabolism and defense pathways were altered in the infection stage. The majority of genes involved in sucrose degradation, secondary metabolism, and defense-related pathways were upregulated. In contrast, genes related to photosynthesis, starch biosynthesis, and nitrogen assimilation were downregulated. These results expanded our general understanding of plant immune responses against *P. parasitica*.

Plants rely on intricate defense responses to protect themselves from the attack of pathogens. The induction of defense responses is cost-intensive, which causes increasing energy demands (Bolton 2009). Therefore, plants induce desense-related pathways while downregulate other
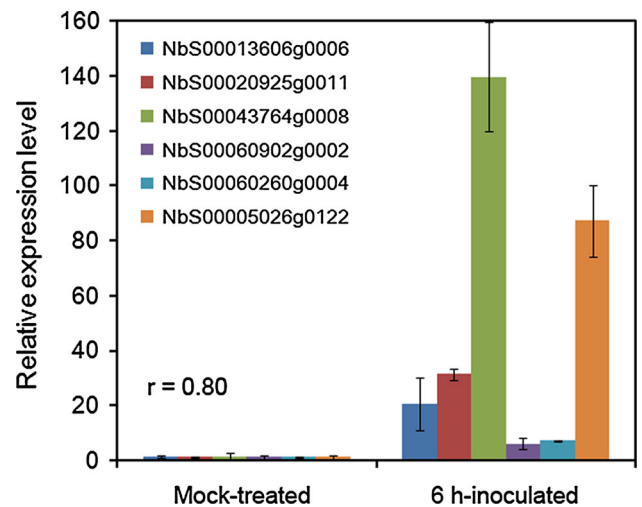

Fig. 4 Validation of RNA-Seq data by qRT-PCR. The relative expression levels of six transcription factors were verified by qRT-PCR. *Error bars* represented the SD for three independent experiments. The y-axis represented the relative expression level. 'r' refers to the Pearson correlation coefficient of gene expression changes between RNA-Seq and qRT-PCR

metabolic pathways to establish a favorable energy balance for defense. In agreement with this notion, the decrease in photosynthesis has been broadly reported upon the threats of pathogens (Teixeira et al. 2014; Chandran et al. 2010). In the current study, large number of *N. benthamiana* genes involved in photosynthesis were differentially downregulated after infection. Among them, most genes encoding proteins in photosystem I and photosystem II reaction

centres were repressed, suggesting impairment of photosynthesis in *N. benthamiana*. Moreover, it has been reported that the role of primary metabolism is to provide cellular energy demands for defense responses (Bolton 2009). Remarkably, we identified upregulated genes associated with sucrose degradation, which support energy and carbon requirements for defense responses. In this process, three cell wall invertases participated in cleaving sucorse into glucose and fructose were highly induced. Moreover, seven hexose transporters which transport the resulting hexoses into the plant cells were also upregulated. These results reflected that infected plant tissues appear to use alternative sources to obtain energy.

In general terms, plant resistance against biotrophic pathogens is mainly controlled by SA-dependent signalling pathways. On the other hand, resistance to necrotrophic pathogens involves JA and ET signalling pathways (Glazebrook 2005). In our study, *P. parasitica* was a hemibiotrophic pathogen and it was still in biotrophic stage during the early infection we investigated. However, we didn't find any difference in the expression of SA-signaling genes. In contrast, totally 55 genes involved in ET-signaling pathway were highly expressed, and majority of these were ethylene response factors. Meanwhile, most genes involved in JA biosynthesis were also upregulated. These results suggested that JA and ET signalling pathways were activated in inoculated *N. benthamiana* leaves. In addition, plants produce a large family of RLKs to perceive different signals in the presence of pathogens. Among these, LRR-RLKs are the best-studied RLKs which recognize pathogen elicitors and participate in plant defense. In current study, we identified 56 upregulated LRR-RLKs, and five of these were FLS2-type receptors. The upregulation of FLS2 receptors suggest the recognition of well-conserved protein flagellin from pathogens and activation of plant initiate immunity. In response to pathogen attack, plants produce another large family of PR proteins to protect themselves. Several PR subfamilies, including chitinases, thaumatin-like proteins, β-1,3-glucanases, and peroxidases, were found to be upregulated in our study. Notably, β-1,3-glucanases participate in degradation of pathogen cell walls while peroxidases contribute to plant cell wall lignification. The upregulation of these PR proteins reflects that *N. benthamiana* fights against *P. parasitica* infection in different ways.

In conclusion, we present the dynamic changes of the *N. benthamiana* transcriptome in response to *P. parasitica*, including metabolisms, defense-related pathways, and transcriptional regulation. The above results would provide a broader view of knowledge underlying plant immune responses against *P. parasitica*.

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