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Comparative transcriptome analysis of genes involved in the response of resistant and susceptible peach cultivars to nematode infection

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ABSTRACT

Root-knot nematode (RKN) is among the most devastating peach pathogens, since it establishes and maintains a permanent feeding site within the plant root system. The objective of this study was to identify differentially expressed genes between two peach cultivars, one resistant and one susceptible, at 0, 0.25, 0.5, 1.5, 2.5, and 3.5 d post-inoculation (dpi) with RKN, using the Illumina HiSeq 2000 platform. Transcriptome analysis indicated that plant response was vigorous at 0.5–1.5 dpi, compared with the other time points. A gene ontology analysis of the differentially expressed genes at 0.5 dpi demonstrated that they were involved in metabolic and cellular processes. Filtering of genes with no obvious differences between the two cultivars revealed 11 genes associated with phytohormone metabolism that were up-regulated only in the resistant cultivar, and two genes involved in ethylene biosynthesis in the susceptible cultivar. The detailed expression pattern of the genes indicated that abscisic acid and ethylene might play an important role in signal transport during RKN infection in the resistant cultivar. Overall, this study provided a platform for further functional genomic research on peach response to RKN infection.

1. Introduction

Root-knot nematodes (RKN, *Meloidogyne* spp.) are serious pathogens in agriculture (Fassuliotis, 1987). Grafting on resistant rootstocks is an economic and flexible solution for managing soilborne diseases (Nyczepir, 1991). There are many rootstocks with various levels of resistance to RKN, such as the Nemared, Juseitou, Shalil, and GF305 peaches (*Prunus persica* [L.] Batsch.), and the Myrobalan plum (*Prunus cerasifera* Ehrh.) (Esmenjaud and Dirlewanger, 2007). However, only the latter shows high resistance to four species of RKNs: *M. arenaria*, *M. incognita*, *M. javanica*, and *M. floridensis*. In a previous study, we identified the Honggengansutao peach (*Prunus kansuensis* L.) as being highly resistant to most RKN species, and more compatible with *P. persica* than the Myrobalan plum is (Cao et al., 2011). Furthermore, Honggengansutao is also reported to be resistant to *Agrobacterium tumefaciens* (Hao et al., 2015). In contrast with Myrobalan plum, which is regulated by resistance genes, the resistance of Honggengansutao to RKN is controlled by a single dominant gene located in linkage group 2 (Cao et al., 2014). However, further genetic studies are needed in order to decipher the resistance mechanism of this cultivar.

Phytohormones regulate many aspects of plant life, including developmental processes and plant responses to biotic and abiotic stresses. A number of studies have demonstrated that RKN alters the balance of phytohormones to achieve the appropriate cellular conditions for developing a feeding structure. For instance, the accumulation of cytokinin (Schmulling, 2002) and auxin (Hutangura et al., 1999) was found to be involved in nematode feeding-site formation (Goverse and Bird, 2011). Additionally, ethylene was found to modulate the attraction of RKN to its host in the early infection stage (Wubben et al., 2001; Fudali et al., 2013). Therefore, phytohormones establish a nematode-induced regulatory network upon the infection of plant roots.

The complex changes in plant gene expression due to nematode infection have been studied extensively. Large-scale microarray analysis is considered a useful tool for discovering new genes and genetic pathways in numerous systems. Using cDNA-array technology, Puthoff et al. (2007) studied the transcriptome of soybean infected by soybean cyst nematodes and identified numerous differential transcripts that could be assigned as cell wall structure groups. Schaff et al. (2007) investigated the root transcriptome of resistant and susceptible tomato cultivars during the time course of

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infection by *M. incognita* and *M. hapla*, and reported that 25% of the genes annotated as phytohormones were modulated by RKN. RNA-sequencing (RNA-seq) analyses based on deep sequencing have been used for gene discovery and the estimation of overall gene expression in different developmental stages and tissues. Wang et al. (2013) compared the expression of genes in the leaf, flower, and fruit of peach, and found that the top of chromosome 2 has a higher density of expressed single nucleotide polymorphisms than the rest of the peach genome; this study focused on overall tissue-specific gene expression.

In this study, we compared the root transcriptome of two peach cultivars, one resistant and one susceptible, at six different time points post-inoculation with RKN using RNA-seq in order to investigate the relationship of RKN infection with specific phytohormones. The profiles of differentially expressed genes (DEGs) allowed us to better understand the molecular mechanisms of phytohormone signal pathways in response to nematode infection and provide a foundation for future functional genomic studies in peach.

2. Materials and methods

2.1. Nematode culture

M. incognita was propagated in the greenhouse on tomato plants as described by Cao et al. (2011). The eggs were collected by root bleaching, and concentrated by 36% (w/v) sucrose flotation. Next, the eggs were placed in sterile water for 3 d for hatching, and the second-stage juveniles were collected from the concentrated fluid by discarding the supernatant after gentle centrifugation at 1208 × *g*.

2.2. Nematode infection and plant resistance assays

Nematode infection experiments were performed using the peach cultivars Honggengansutao (RKN-resistant) and Bailey (RKN-susceptible). One 10-d-old peach seedling was added to each well of a six-well tissue culture plate that contained 5 ml of 23% (w/v) Pluronic F-127 (Sigma-Aldrich, St. Louis, MO, USA) and 500 second-stage juveniles or H_2O (control) as described by Wang et al. (2009). The plates were incubated at 25 °C, with 70% humidity and a moderate photoperiod (12 h light: 12 h dark).

Root tip samples were collected from 10 seedlings of each cultivar at 0, 0.25, 0.5, 1.5, 2.5, and 3.5 d post-inoculation (dpi) and either used for stereoscopic observation or frozen in liquid nitrogen for transcriptome analysis. The former were stained, and the number of nematodes that had invaded the root tissue was counted.

2.3. Total RNA extraction

Total RNA was extracted using an extraction kit (Aidlab, Beijing, China) and treated with RNase-free DNase I (Takara, Dalian, China). The RNA quantity was analysed using a ND-1000 spectrophotometer (Thermo-Fisher Scientific, Waltham, MA, USA).

2.4. Library construction and transcriptome sequencing

mRNA was isolated from total RNA (20 μg) of each cultivar at six different time points post-inoculation using beads containing oligo $(dT)_{25}$. Purified mRNA was fragmented by incubation in an RNA fragmentation buffer for 5 min at 70 °C. Fragments digested by a restriction enzyme were used to synthesise first-strand and second-strand cDNA using random hexamer primers and reverse transcriptase. Next, the double-stranded cDNA molecules were ligated to the addition of an 'A' base and Illumina sequencing adaptors (Genomic DNA Sample Preparation Kit, Illumina, San Diego, CA, USA). Finally, cDNA fragments with a size of about 350 bp were gel-purified and used as templates in a PCR reaction (Illumina Genomic Sample Preparation Kit), and sequenced using the Illumina HiSeq 2000 platform (Macrogen Bioinformatics Technology Co., Ltd, Shenzhen, China).

2.5. Sequence alignment

RNA-seq reads were aligned against the *P. persica* genome 1.0 (http://www.rosaceae.org/node/355) using Tophat, Bowtie, and BWA (Trapnell et al., 2009; Langmead et al., 2009; Li and Durbin, 2009).

2.6. Analysis of DEGs

The number of reads mapped to each gene were counted using HTSeq (Anders et al., 2015), and the normalised values were calculated as Reads Per Kilobase per Million mapped reads (RPKM) using Cufflinks (http://cufflinks.cbcb.umd.edu/) and RSEM (Li and Dewey, 2011). Homologues were sequentially annotated according to blast results. Gene ontology (GO) enrichment analysis of DEGs was carried out using WEGO (Ye et al., 2006).

2.7. Validation of RNA-seq data by real-time quantitative PCR (qRT-PCR)

Ten genes, encoding 1-aminocyclopropane-1-carboxylate oxidase, 1-aminocyclopropane-1-carboxylate synthase, resistance protein, and L-lactate dehydrogenase, were selected to validate the RNA-seq results using qRT-PCR with Bailey cDNA as a template. The primer pairs used in qRT-PCR were designed by Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are listed in Supplementary Table 1. qRT-PCR was performed by the Roche LightCycler 480 (Roche, Basel, Switzerland) under the following cycling conditions: 95 °C for 5 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s. Elongation factor 1-alpha was used as a housekeeping gene (Tong et al., 2009). The gene expression levels were analysed by the comparative Ct method (Livak and Schmittgen, 2001).

3. Results

3.1. Overview of RNA-seq data

A total of 43.7 Gb reads with a length of 101 bp were generated from the 12 cDNA libraries. Raw reads were trimmed by removing adaptor sequences, empty reads, and low quality sequences, and the 90.56% of trimmed reads that mapped to the eight scaffolds of the peach genome was designated as clean reads (Table 1). The results showed that the mapping rate of Bailey was higher than that of Honggengansutao.

3.2. Plant response to RKN at different time points post-inoculation

A total of 10,492–10,719 genes were identified in the two cultivars at different time points post-inoculation (Fig. 1). Although the total number of expressed genes was lower in Honggengansutao than that in Bailey, the change of these expressed genes with the extension of time of inoculation present very low, at 0.5 and 1.5 dpi in both cultivars (Fig. 1a). Furthermore, DEGs with 2-fold changes between the control and different time points post-inoculation in both cultivars were screened. We found that the stage with the highest number of DEGs was observed in Bailey at 0.5 dpi and in Honggengansutao at 0.25 dpi (Fig. 1b).