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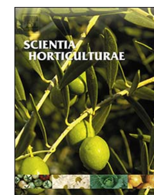

Transcriptome analysis of chrysanthemum in responses to white rust

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ABSTRACT

Chrysanthemum white rust (CWR), one of the primary diseases on chrysanthemum, has been considered as a quarantine disease in many countries. Few studies have been conducted on the molecular mechanisms of white rust resistance in chrysanthemum. In this study, we performed transcriptome and digital gene expression profiling to investigate the responses of chrysanthemum to white rust infection. By using RNA-Seq technology, we aimed to screen the differentially expressed genes (DEGs) for functional annotation and metabolic pathway analysis. The resistant (C029) and susceptible (C008) cultivars were sequenced by Illumina HiSeq™ 2000. 20,597,533, 19,519,160, 20,570,705 and 20,301,157 total reads were obtained from four libraries, respectively, and for all samples, clean reads were over 97.34% of the total. We obtained 392,137 unigenes through sequencing assembly and we gained 213,531 complete protein coding sequences by annotation. 433 significant DEGs were obtained between R0 (resistant cultivar control) and R1 (resistant cultivar with inoculation), and 787 DEGs were obtained between S0 (susceptible cultivar control) and S1 (susceptible cultivar with inoculation) through expression quantity analysis and functional annotation in 194,179 genes. There were 14 genes which had differential expressions in both two comparison groups. These DEGs were involved in the pentose phosphate pathway, phenylpropanoid biosynthesis pathway, carbon metabolism pathway and phenylalanine metabolism pathway. The results of the qRT-PCR indicated that the expression tendency of DEGs was consistent with that of the transcriptome sequencing results. Our transcriptome sequencing can help to fill the gaps in the literature on CWR transcriptome and enrich sequence information of chrysanthemum transcriptome. It also offers candidate genes that can be used to guide future studies attempting to breed resistant cultivars, and provides a theoretical basis for exploring the mechanism of response of chrysanthemum to white rust.

1. Introduction

Chrysanthemum (*Chrysanthemum* × *grandiflorum* (Ramat.) Kitam.), ranked as one of top ten most famous traditional Chinese flowers and top four in the world for cut flowers, plays an important role in the modern flower production and has both ornamental and economic value. CWR, one of the primary diseases of chrysanthemum, is caused by *Puccinia horiana* Henn. and is considered as a quarantine disease in many countries (Whipps, 1993). China, as a major producing and consuming country of cut chrysanthemum, has been potting and ground-covering chrysanthemum, which caused a large scale of occurrence about CWR in many provinces (Wang et al., 2006; Wang et al., 2009a). Recently studies about white rust in chrysanthemums are mainly focused on occurrence regularity (Zhu et al., 2010), control and prevention method (Backer et al., 2011), screen for disease resistance cultivars (Zhu et al., 2011), establishment of affinis chrysanthemums susceptible cultivars and disease index (Zeng et al., 2013), etc., however, there are not too many studies on molecular aspects. Huang et al. (2012) obtained nucleotide sequences of 51 chrysanthemum genes that are responsive to white rust using cDNA-AFLP technique, in which 18 genes have higher homology, and predicted to get macromolecular protein chaperone-associated genes that may play important roles in resistance to white rust. Alaei et al. (2009) adopted RT-PCR method and studied the method of rapidly determining the infection of *Pyracantha fortunei* by using the specific sequences in ribosomal DNA transcribed region. However, there is still no transcriptional study on chrysanthemums resistance to white rust.

In recent years, RNA-Seq has been widely used to study gene expression changes during plant and pathogen interaction process. Hao et al. (2016) found 2,666,783 (upregulated) and 2587 (down-regulated) differ-entially expressed genes in wheat seedlings and adult plants after *Puccinia striiformis* f. sp. *tritici* infection at 24, 48 and 120 h post-inoculation. And KEGG pathway analysis of the up-regulated unigenes showed that many biological processes which mainly control

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the mechanisms of lignification, reactive oxygen species and sugar are involved in adult plant resistance. Bruce et al. (2014) obtained 15 genes that are related to amino acid changes, and they found that one gene has significant similarity to the gene encoding fungal effector cerato-platanin, and another gene has similarity to tyrosinase-encoding gene which is involved in the synthesis of melanin. Cao et al. (2017) performed transcriptome analysising of resistant and susceptible peach cultivars with RNK infected at 0.5 dpi, the results indicated that 11 genes associated with phytohormone metabolism were upregulated only in the resistant cultivar, and 2 genes involved in ethylene biosynthesis in the susceptible cultivar. In the transcriptome analysis of the millet response to rust, 228 genes related to the defense mechanism and 5 genes related to the immune system process were identified. The up-regulated genes were involved in the biosynthesis of phenylpropanoid, Amino acid, tyrosine and tryptophan biosynthesis, and phenylpropane biosynthesis pathways which are associated with millet rust resistance (Li et al., 2015a, 2015b). In the process of resistance to black spot, Chinese roses identified the pathogens and conduction signals through the signal recognition and transmission system, and reactive oxygen is the reaction signal. The genes that are related to ROS elimination, light respiration pathway and phenylpropane metabolic pathway were all up-regulated, which indicated that these genes may play an important role in the process of resistance to black spot (Liu et al., 2015a, 2015b).

In this study, we used resistant and susceptible cultivars of chrysanthemum as experimental materials. We conducted transcriptome sequencing analysis on the response of the chrysanthemum leaf to *Puccinia horiana* Henn. inoculation, as well as the functional annotation and metabolic pathway analysis of differential genes. The results of this study should provide an important reference for the comprehensive understanding of the molecular mechanism of white rust resistance in chrysanthemum, and, in addition, provide a theoretical basis for the discovery of new resistance genes and the enhancement of disease resistance.

2. Materials and methods

2.1. Plant materials and treatments

The chrysanthemum susceptible cultivar ‘C008′ and the resistant cultivar ‘C029′ were used as experimental materials obtained from the Flower base of Shenyang Agricultural University. Cuttings were grown in 15 cm pots containing 1:2 mixture of vermiculite and garden soil without fertilizer. The growth conditions for these plants were 10–12 h of light a day, 40–50% humidity, and day/night temperature of 25/18 °C. Uniformed plants grown to the 6–8 leaf stage were selected for further experiment. Finger friction daubing method was applied for the saturated uniform inoculation of pathogen spores on both upper and lower epidermis of the second and third true leaves of the two cultivars. We collected the inoculated and non-inoculated leaves after inoculation for 24 h, and labeled as S1 (C008 with inoculation), R1 (C029 with inoculation), S0 (C008 without inoculation) and R0 (C029 without inoculation), respectively, then quick-froze the leaves in liquid nitrogen and stored them at −80 °C for use.

2.2. Total RNA extraction

Total RNA of four separate libraries (S1, R1, S0, R0) were extracted with RNAiso reagent (TaKaRa, Japan), following the manufacturer’s instructions. The integrity and quality of the total RNA were evaluated using a 2100 Bioanalyzer RNA Nano chip device (Agilent, Santa Clara, CA, USA) and agarose gel electrophoresis respectively, and the concentration was measured with a ND-1000 spectrophotometer (NanoDrop, Wilmington, DE).

2.3. cDNA library construction and illumina sequencing

2.3.1. RNA quantification and qualification

RNA degradation and contamination was monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using Qubit® RNA Assay Kit in Qubit®2.0 Flurometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

2.3.2. Library preparation for transcriptome sequencing

A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3′ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150 ∼ 200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

2.3.3. Clustering and sequencing

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) according to the manufacturer’s instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2500 platform and paired-end reads were generated.

2.4. RNA-seq data processing, de novo assembly and annotation

The raw reads were processed to remove adaptor sequences, repeat and low-quality reads ($Q \leq 5$) in order to obtain the clean reads. Trinity (http://trinityrnaseq.sourceforge.net/) was used to assemble the pair-end short reads into contigs. Unigene sequences were mapped to protein databases including Nr, Swissprot, KEGG and KOG via Blastx algorithm to obtain the protein with the highest similarity, thus we obtained the annotation information of protein function.

2.5. Differential gene expression analysis

RPKM method was used to compare the gene expressions of four samples, and DEGs were obtained. P-value ≤ 0.05 was considered as a threshold value, and it was corrected using FDR. FDR ≤ 0.001 was considered as a threshold value, and simultaneously a fold change of > 2 and a gene length of ≥500 bp were used as the screening criteria of DEGs. The obtained DEGs were compared in the GO and KEGG Pathway databases. From this, we gained the GO terms and Pathways with significant enrichment in the DEGs.

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