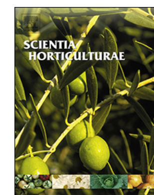
Scientia Horticulturae 245 (2019) 29–38

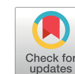
Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Comparative transcriptome analysis of genes involved in the response of resistant and susceptible peach cultivars to water stress



Qi Wang[a,b], Ke Cao[a], Gengrui Zhu[a], Weichao Fang[a], Changwen Chen[a], Xinwei Wang[a], Lirong Wang[a,*]

[a] Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, Henan 450009, China
[b] College of Horticulture & Forestry Sciences, Huazhong Agricultural University, Wuhan, Hubei 430070, China

ARTICLE INFO

Keywords:
Peach
Water stress
Next-generation sequencing
Differentially expressed genes
Auxin
Cytokinine

ABSTRACT

Plant growth and productivity are adversely affected by water stress. Several genotypes of peach have evolved features that impart tolerance to water stress and other abiotic stresses. The aim of this study was to identify differentially expressed genes (DEGs) between two peach cultivars, one resistant and one susceptible, at 0, 6, 12, 18 days without watering (dww), using the Illumina HiSeq 2000 platform. Transcriptome analysis indicated that plant response was vigorous at 12 dww, compared with other time points. Gene ontology (GO) analysis of the DEGs at 12 dww demonstrated that they were involved in metabolic and catalytic processes. Selecting of genes with obvious differences between the two cultivars revealed 48 genes involves in hormone signal transduction were up-regulated only in the resistant cultivar. Detailed analysis of gene expression profiles indicated auxin and cytokinine might exert a vital influence on signal transduction during water stress in the resistant cultivar. These results form a basis for understanding the mechanism of water stress resistance in peach and other plants.

1. Introduction

Water shortage was highlighted as one of the most pressing environmental issue facing agriculture today. The availability of water has been the biggest constraint to plant growth and, together with temperature, the main determinant of primary productivity of terrestrial biomes (Churkina and Running, 1998). For these reasons, the development of plant varieties with more efficient use of water is of prime importance in agriculture. In fact, plants have evolved various molecular mechanisms to reduce their consumption of resources and adjust their growth to adapt to adverse environmental conditions (Ahuja et al., 2010; Van Ha et al., 2014; Nishiyama et al., 2013; Skirycz and Inzé, 2010; Yuriko et al., 2011). These responses are mediated by plant growth regulators (phytohormones), compounds derived from plant biosynthetic pathways that can act either at the site of synthesis or following their transport, elsewhere in the plant.

Phytohormones regulate many aspects of plant life, including developmental processes and plant responses to biotic and abiotic stresses. Reviews on hormone action and signaling of abscisic acid (ABA) (Cutler et al., 2010; Klingler et al., 2010; Wan et al., 2009), cytokinin (CTK) (Argueso et al., 2010; Perilli et al., 2010; Werner and Schmülling, 2009), ethylene (Stepanova and Alonso, 2009), brassinosteroid (BR) (Divi and Krishna, 2009; Kim and Wang, 2010) and jasmonate (JA) (Wasternack, 2007), and on hormone cross-talk (Jaillais and Chory, 2010; Santner and Estelle, 2009) have been published recently (Peleg and Blumwald, 2011). A typical response to water stress is the production of ABA. ABA induces stomata closure and activates a signaling cascade that modifies the transcriptome and up-regulates genes encoding numerous proteins and enzymes involved in the drought response (Agarwal and Jha, 2010; Schroeder et al., 2001). Recent studies suggest that the role of auxins in drought tolerance was postulated; *TLD1/OsGH3.13*, encoding indole-3-acetic acid (IAA)-amido synthetase, was shown to enhance the expression of *LEA* (late embryogenesis abundant) genes, which correlated with the increased drought tolerance of rice seedlings (Zhang et al., 2009). Although CTK is an antagonist to ABA, the maximal expression of *IPT* (isopentenyl transferase, a gene encoding a key step in the biosynthesis of CTK) was attained during the drought episode and the transgenic plants displayed enhanced drought tolerance and superior yields (Zvi et al., 2011). Elevated CTK levels promoted survival under water-stress conditions, inhibited leaf senescence and induced increased proline levels (SOPHIE et al., 2007). Moreover exogenous application of BRs was reported in diverse plant species to induce drought tolerance (Divi et al., 2010). Therefore, phytohormones establish a drought-induced regulatory

* Corresponding author.
E-mail address: wlirong2009@sina.com (L. Wang).

https://doi.org/10.1016/j.scienta.2018.09.056
Received 30 January 2018; Received in revised form 18 September 2018; Accepted 20 September 2018
0304-4238/ © 2018 Elsevier B.V. All rights reserved.

network upon water deficit of plant roots.

Gene expression studies have become very important in the identification of genes that act directly in stress protection, signal transduction and gene expression regulation (Motoaki et al., 2002). Microarray analysis is a useful tool for discovering new genes and genetic pathways in various systems. Fabiana Aparecida Rodrigues et al. (2009) monitored gene expression profiles under water stress in tolerant and sensitive sugarcane plants by microarray methodology, verified 91 common genes between both cultivars, the majority of which were upregulated by water deficit and detected important stress-related pathways were repressed in sensitive plants. RNA-sequencing (RNA-seq) have been used for gene discovery and the estimation of overall gene expression in different conditions and tissues. Xueyan Zhang et al. (2013) investigated gossypium arboreum transcriptome response to PEG treatments in different tissues through mRNA-seq and identified tissue selective signalling and hormone crosstalk. Clara Pons Puig et al. (2015) compared pre-symptomatic transcriptome during cold storage of chilling sensitive and resistant peach cultivars and suggested that in sensitive fruit a cold response program is activated and regulated by auxin distribution and ethylene and these hormones have a role in sensitivity to CI even before fruit are cold stored.

In this study, a comparative transcriptome analysis was performed in two peach genotypes with contrast ability of drought tolerance at three time points without watering using RNA-seq in order to identify the relationship of water stress with specific phytohormones. Gene expression studies allowed us to better understand the molecular mechanisms of plant hormone signal transduction in response to water stress and formed a basis for future functional genomic studies in peach and other plants.

2. Materials and methods

2.1. Plant materials and stress conditions

Two peach genotypes with contrasting resistance to drought stress were used in this study. One was 'Taihangshantao' (T), variety of *Prunus davidiana* Franch and the other was 'Dongxuemitao' (D), cultivar of *Prunus persica* L.

Seeds were sown in seedling pots (12 cm in diameter, 15 cm in deep) filled with peat and perlite (4:1) after cold-stratifying at 4 °C for 60 days in damp vermiculite, which were watered every 6 days with 1/5 vol of the pot of water. Seedlings were grown in greenhouse at 24/18 °C (day/night temperature) under 16/8 h light/dark regime for three months. Water stress treatments were administered on a group of 24 plants (12 plants for each genotype) by withholding water for 18 days continuously; the other set of the plants including 18 plants (9 plants for each genotype) were watered normally and treated as controls. Samples of root tissue were collected from three independent plants of each genotype at 0, 6, 12, 18 days without watering (dww) and controls at every time point, then immediately frozen in liquid nitrogen, and stored at −80 °C until their use for RNA isolation.

2.2. Measurement of water status

Soil water potential was measured by a soil tension meter (TEN-30, Zhejiang Top Instrument Co., Ltd., Hangzhou, China). The soil tension meter, one per seedling, was buried 15 cm under the soil surface. Data was collected between 9:00 and 10:00 a.m.

2.3. RNA isolation and mRNA sequencing

Total RNA was extracted using the cetyl trimethylammonium bromide (CTAB) method and treated with RNase-free DNase I (Takara, Dalian, China). The total RNA content was quantified using an ND-1000 spectrophotometer (Thermo, Waltham, MA, USA). Oligo (dT)25 magnetic beads were utilized to isolate poly-(A) tails containing mRNAs from total RNA (20 ug) and then fragmentation buff ;er was added to interrupt mRNA to short fragments for 5 min at 70 ∘C. These short fragments were used as templates to synthesise first-strand cDNA using reverse transcriptase and random hexamer primers. Second-strand cDNA fragments were obtained using a buffer containing DNA polymerase I, dNTPs, and RNase H. The final cDNA library was obtained by ligating the cDNA fragments to sequencing adapters (Genomic DNA Sample Preparation Kit, Illumina, San Diego, CA, USA) and conducting PCR amplification (Illumina Genomic Sample Preparation Kit). The mRNA sequencing was performed using an Illumina HiSeq 2000 platform (Macrogen Bioinformatics Technology Co., Ltd., Shenzhen, China).

2.4. Transcript assembly and DEG analysis

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

HTSeq (Anders et al., 2015) was used to count the number of reads mapped to reference transcripts. Cufflinks (http://cufflinks.cbcb.umd.edu/) and RSEM (Li and Dewey, 2011) were used to calculate the reads per kilobase per million mapped reads and normalize values. DEGseq package was used to identify the DEGs between treated samples (6, 12 and 18 dww) and controls with P values of < 0.05 and fold change of > 2 or ≤ 2 (Anders et al., 2015).

WEGO software (http://wego.genomics.org.cn/cgi-bin/wego/index.pl) was used to perform GO functional classification.

2.5. Validation by qRT-PCR

The results from the RNA-seq experiment were validated by analyzing 5 significantly regulated genes involved in plant hormone signal transduction using qRT-PCR with 'Taihangshantao (T)' cDNA as template. The gene-specific primers were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and are listed in Supplementary Table 1. PCR was performed using the Roche Light Cycler 480 (Roche, Basel, Switzerland) with the following cycling conditions: 7 s at 95 °C, 10 s at 57 °C, and 15 s at 72 °C. RNA polymerase II was used as a housekeeping gene (Tong et al., 2009). Relative transcript levels for each sample were obtained using the comparative Ct method (Livak and Schmittgen, 2001).

3. Results

3.1. Soil water potential at different time points without watering

When watering normally (0 dww), the soil water potential was approximately the same in two genotypes, then decreased in the subsequent time points. In addition, the soil water potential in the resistant genotypes was obviously lower than that in the susceptible genotypes (Fig. 1). We also found the roots of the resistant genotypes were much more than the susceptible genotypes (Supplementary Fig. 1). These results suggest the resistant genotypes have a bigger capacity to absorb water.

3.2. Statistical analysis of transcriptome data

Illumina deep sequencing of the peach transcriptome yielded 412,380,524 high-quality reads of 101 bp, totaling 41.2 Gb. After unmapped reads were removed, 378,372,011 (91.8%) clean reads were obtained. More than 86.3% and 94.9% reads from 'Taihangshantao' and 'Dongxuemitao' were mapped to the peach genome (Table 1).

Download English Version:

https://daneshyari.com/en/article/11001871

Download Persian Version:

https://daneshyari.com/article/11001871

Daneshyari.com