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Global transcriptome profiling analysis of ethylene-auxin interaction during tomato fruit ripening

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ABSTRACT

Auxin-ethylene interactions are crucial for fruit ripening processes. However, the molecular basis of the regulatory network of auxin-ethylene interaction during ripening is still not very clear. To reveal the potential molecular mechanism of ethylene-auxin interplay in tomato (*Solanum lycopersicum* L.) fruit ripening, global transcriptome profiling analysis was performed on cherry tomato fruit treated with auxin, ethylene or the combination of the two hormones. The results showed that ethylene modulated auxin transport, metabolism and signaling processes by affecting the expression patterns of genes encoding auxin carrier proteins, aldehyde dehydrogenase and primary auxin-responsive proteins. Most genes involved in ethylene biosynthesis and signaling were regulated 7 days after treatment with exogenous auxin. Furthermore, the expression levels of mitogen-activated protein kinase (*MAPK*) and of ubiquitination-related genes were altered in auxin-treated fruit, suggesting that auxin regulates ethylene metabolism and signaling via complicated mechanisms. The potential interaction points in auxin-ethylene crosstalk were also identified and a model was proposed. Our analyses provide a global insight into the ethylene-auxin interaction and predict the potential regulators in the crosstalk of the two hormones during the fruit ripening process.

1. Introduction

Ethylene and auxin are two important plant hormones involved in regulating many plant developmental processes including the transition from maturation to ripening (Bapat et al., 2010; Kumar et al., 2014; Ziliotto et al., 2012). Ethylene, the most investigated plant hormone, is usually recognized as a trigger and promoter in the climacteric fruit ripening process and plays a role in non-climacteric fruit ripening (Bapat et al., 2010; Barry and Giovannoni, 2007; Chervin et al., 2004; Katz et al., 2004). In climacteric fruit, two systems of ethylene biosynthesis have been proposed to regulate different physiological processes. System 1 is auto-inhibitory and produces the basal ethylene in all tissues, and system 2, which is auto-catalytic, mainly operates during climacteric fruit ripening (Alexander and Grierson, 2002). Auxin is another crucial regulator in fruit development and mainly promotes fruit set and growth by influencing cell division and enlargement together with cytokinin, gibberellin, and other hormones (Kumar et al., 2014). Application of exogenous auxin to fruit usually leads to the delay of ripening and therefore auxin is commonly regarded as a negative regulator of fruit ripening (Chen et al., 2016; Jones et al., 2002; Li et al., 2016; Ziliotto et al., 2012). Moreover, recent studies have revealed that auxin may play more intricate roles in regulating the ripening process through interaction with ethylene and other plant hormones (Bottcher et al., 2013; Soto et al., 2012; Tadiello et al., 2016; Trainotti et al., 2007).

The interplay between auxin and ethylene varies in different tissues and physiological processes. A synergistic effect between the two hormones has been found in seedlings during root elongation, leaf development and many other physiological process (Ruzicka et al., 2007; Stepanova et al., 2008; Swarup et al., 2007; Takahashi et al., 2003; Wang et al., 2005). However, numerous biological and biochemical changes in the transition from fruit maturation to ripening are quite different from other development processes (Gapper et al., 2013), and the interaction between auxin and ethylene in this transition process seems more complex and elusive (Kumar et al., 2014). For instance, an antagonist effect between auxin and ethylene has been observed during the ripening of tomato, banana and grape (Choudhury et al., 2008; Su et al., 2015; Ziliotto et al., 2012) whereas high auxin levels were shown to stimulate more ethylene product in peach fruit (Tadiello et al., 2016; Tatsuki et al., 2013). The expression changes of some *ARF* (auxin

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response transcription factor) genes and *ERF* (ethylene transcription factor) genes in response to exogenous auxin or ethylene treatment are quite different between seedling and fruit (Breitel et al., 2016; Li et al., 2016; Pirrello et al., 2012; Wu et al., 2011), implying that molecular characterization of the ethylene-auxin crosstalk during ripening process is needed.

Previous studies have identified crucial gene changed during auxin-ethylene crosstalk, and several mechanisms to explain how these genes modulate the auxin-ethylene interaction during fruit ripening have been proposed (Breitel et al., 2016; Chaabouni et al., 2009; Hao et al., 2015; Liu et al., 2005). For instance, the tomato auxin response transcription factor gene *SlARF2a*, whose expression is up-regulated by ethylene, induces ethylene signaling components during tomato fruit ripening (Breitel et al., 2016). *SlIAA3* positively regulates the expression of ERF genes, and its expression is induced by ethylene during tomato ripening (Chaabouni et al., 2009). Our previous study also identified several ethylene-responsive genes that were under the regulation of auxin and provided overall insights on how auxin impacts ethylene biosynthesis and signaling during tomato ripening (Li et al., 2016). Although the function of some key elements in the auxin-ethylene interaction, such as *ARF2a* (Breitel et al., 2016) and *IAA3* (Chaabouni et al., 2009), have been well investigated, the complete regulatory network of auxin-ethylene interaction involved the ripening process is still not clear. Thus, we performed a global analysis on the transcriptome profile of tomato fruit in response to auxin, ethylene or the combined treatment of the two hormones to reveal the potential molecular mechanism of auxin-ethylene interplay during the ripening process. Comprehensive insights into the auxin-ethylene interaction at the transcriptional level may provide better understanding and pertinent information for further investigation.

2. Materials and methods

2.1. Fruit harvesting and hormone treatment

Cherry tomato (*Solanum lycopersicum* cv. Xin Taiyang) fruit were collected from a commercial greenhouse of Transfar Agribio Co., Ltd. (Xiaoshan County, Zhejiang Province, China). Mature green fruit without injury and disease were picked from fifty different plants and immediately transported to the lab.

Fruit were sterilized with 0.50% (m/v) sodium hypochlorite aqueous solution and washed twice with tap water. After air drying at room temperature and removing the pedicels, 400 fruit were randomly divided into four groups and treated with sterile water, 2,4-dichlorophenoxyacetic acid (2,4-D) (0.45 mM), ethephon (1 mM) and a mixted solution of 2,4-D (0.45 mM) and ethephon (1 mM), respectively. For the hormone treatment, fruit were fully immersed into sterile water or into a hormone solution in a vacuum dryer (inner diameter: 300 mm, volume: 15 L) and infiltrated under vacuum (35 kPa) for 3 min. Fruit treated with sterile water were used as control samples. Treated fruit were kept in darkness at 20 ± 2 °C with 90 ± 5% relativity humidity (RH) for 25 days. Samples (eight fruit) were taken every two days during storage and fruit pericarps were frozen and kept at −70 °C until used.

2.2. Measurement of plant hormone

Twenty fruit were sealed in a 2 L plastic jar and kept at 20 ± 2 °C for two hours. One milliliter of headspace gas was injected into a gas chromatograph (model SP 6800, Lunan Chemical Engineering Instrument Co., China) equipped with a GDX-502 column (JieDao TECH, China) and a flame ionization detector (Shimadzu GC-2014C, Schimadzu Corporation, Japan). The rate of ethylene production was represented as micromoles per kilogram per hour ($\mu mol\ kg^{-1}\ h^{-1}$).

Free indoleacetic acid (IAA) was extracted and purified according to the methods previously described (Chen et al., 2014) with some modifications. Frozen tomato pericarp tissues were powdered in liquid nitrogen. The IAA content of fruit in each group was obtained from three individual biological replicates and expressed in micrograms per kilogram on a fresh weight basis ($\mu g\ kg^{-1}$). For each biological replicate, total free IAA was extracted from 4 g powders with 40 mL 80% methanol (v/v, containing 1 mM butylated hydroxytoluene) at 4 °C in darkness for 12 h. The supernatant was collected after centrifugation (8000 × *g*, 5 min) and concentrated under vacuum at 35 °C to remove methanol. The concentrated extractions were pre-purified using a Sep-Pak cartridge (Waters Corporation, USA) before IAA measurement. Analysis of IAA in the samples was performed using an Agilent 6460 triple quad high-performance liquid chromatography-mass spectrometry system (Agilent Technologies Inc., CA, USA). Parameters for the analyses are described by Chen et al. (2014).

2.3. RNA isolation, library preparation and Illumina sequencing assay

Samples at 1 and 7 day after treatment (DAT) were selected for RNA-Seq assay and named CK1d (control samples at 1DAT), AX1d (auxin-treated samples at 1 DAT), ET1d (ethylene-treated samples at 1 DAT), ETAX1d (combination-treated samples at 1 DAT), CK7d (control samples at 7 DAT), AX7d (auxin-treated samples at 7 DAT), ET7d (ethylene-treated samples at 7 DAT), ETAX7d (ethylene-auxin combination treated samples at 7 DAT), respectively. Illumina sequencing assay were performed by Hangzhou 1gene Technology Co., Ltd. (Hangzhou, China). Two biological replicates for each treatment were used for RNA isolation, library preparation and sequencing assay. For each biological replicates, total RNA of each sample was extracted from the pooled pericarps of eight tomato fruit using RNAiso plus (Takara, Japan), as described in the protocol. Sequencing libraries were generated from 5 μg RNA using NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer's recommendations and index codes were added to the sequence identity of each sample. The clustering of the index-coded samples was performed using TruSeq PE Cluster Kit v4-cBot-HS (Illumina, USA) on a cBot Cluster Generation System according to manufacturer's instructions. Library preparations were sequenced on an Illumina Hiseq 2500 platform and 125-bp paired-end reads were generated.

2.4. Analysis of sequencing data

High quality clean data were obtained by filtering out low quality reads from the raw data and were mapped to the reference genome assembly SL2.50 (https://solgenomics.net/) using TopHat v2.0.12 (Kim et al., 2013). The value of RPKM (reads per kilobase of exon region in a gene per million mapped reads) was measured to estimate the expression level of each gene (Trapnell et al., 2009). The differential expression of the genes between two samples was analyzed using DESeq R package (1.18.0) based on the negative binomial distribution (Anders and Huber, 2010). A *Q*-value was used to adjust the *P*-value according to Benjamini and Hochberg's approach to control false discovery rate (FDR) value (Benjamini and Hochberg, 1995). The genes found by DESeq with a *Q*-value < 0.05 and a $|\log2Ratio| \geq 1$ were assigned as differentially expressed.

For gene function analysis, differentially expressed genes (DEGs) were annotated based on the non-redundant protein sequences in the NCBI database and tomato genome annotation (ITAG2.4). GO enrichment of DEGs was analyzed using topGO version 2.22.0 (Alexa and Rahnenfuhrer, 2010). KEGG pathway enrichment analysis was performed according to previously described methods (Kanehisa et al., 2008). GO terms and KEGG pathways with *Q*-value less than 0.05 were considered significantly enriched by DEGs.

2.5. Quantitative real-time PCR (RT-qPCR) assay

RNA extraction and cDNA preparation were performed using an