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Effects of exogenous auxin on pigments and primary metabolite profile of postharvest tomato fruit during ripening

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ABSTRACT

Auxin is an important plant hormone and plays crucial roles in regulating fruit ripening. The delay of ripening after auxin treatment has been found in tomato and other fleshy fruit. However, the influence of auxin on metabolites alteration during tomato ripening period has not been extensively studied. To investigate the impact of exogenous auxin on tomato fruit quality, pigment metabolism, primary metabolite profiling, and the expression of selected ripening-related transcription factor genes were analyzed. The results showed that exogenous auxin significantly interfered the accumulation and conversion of pigments, total phenolics and flavonoids but did not largely influence the final content of these compounds in full ripe tomato fruit. Dramatic changes on the content of primary metabolites were induced by auxin during tomato ripening period and the alterations were not able to be completely restored at the end of ripening. The contents of citric acid, threonic acid and succinic acid were increased whereas alanine and aspartic acid accumulation was repressed in auxin-treated fruit. The expression patterns of transcription factor genes related to ripening were also disturbed by exogenous auxin. The present study provided an overall insight on how auxin regulates pigment and primary metabolite accumulation during ripening stage and offered useful information for further investigation of auxin impact on fruit quality.

1. Introduction

Fruit ripening is a highly complex process, accompanying with massive biological and biochemical changes which lead to the final ripe fruit, and is precisely regulated by plant hormones such as ethylene, abscisic acid and other phytohormones (Kumar et al., 2014; Prasanna et al., 2007). Auxin, a key plant hormone in regulation of cell elongation and division, plays crucial roles in fruit setting and development (Rosquete et al., 2012; Srivastava and Handa, 2005). To date, more and more evidences have revealed that auxin also participates in the regulation of ripening process in its own manner and by interacting with other hormones (Chen et al., 2016; Trainotti et al., 2007; Ziliotto et al., 2012).

Fruit ripening process is largely affected by endogenous auxin content. High level auxin has been observed in tomato fruit of ripening-inhibitor (*rin*) mutant and the decrease of auxin level in fruit might be necessary for triggering ripening process (Liu et al., 2005; Rolle and Chism, 1989) .Transcriptional profiling studies showed that the expression levels of many genes involved in auxin signaling process significantly changed during ripening period, implying the participation of auxin in ripening process (Kumar et al., 2011; Liu et al., 2011). Beside modulating ripening process, auxin may also influence fruit quality. In apple, sweet berry and grape, the application of exogenous auxin at early stage of fruit development has been found to be able to improve the yield of fruit and change the level of volatile compounds (Bottcher et al., 2011; Yuan and Carbaugh, 2007; Zhang and Whiting, 2011). Recent studies have suggested that silence or overexpression of several auxin response genes, such as *SlARF4*, *SlARF2a* and *SlIAA27*, leads to the conspicuous changes in fruit pigment accumulation, sugar content, phenylpropanoid component and other fruit quality attributes (Bassa et al., 2012; Breitel et al., 2016; Sagar et al., 2013).

Chlorophyll degradation, lycopene accumulation and changes of primary (sugar, amino acids, organic acids) and secondary metabolites (phenolics and flavonoids) during ripening mainly contribute the final desirable fruit quality and are under the control of several key ripening-related transcription factors (Klee and Giovannoni, 2011). The metabolism pathway of carotenoid in tomato has been well described and the effect of exogenous auxin on it has also been investigated (Sandmann, 2001; Su et al., 2015). The influence of exogenous auxin on the metabolites alteration during ripening period and tomato fruit final quality, however, has not been exten-

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sively studied. Our previous transcriptome study has suggested that exogenous auxin retards tomato ripening process and interferes the normal expression patterns of many genes involved in metabolism pathway (Li et al., 2016). To investigate the impact of exogenous auxin on tomato fruit quality, pigment metabolism, primary metabolite profiling of tomato fruit through ripening period were analyzed in this study. Transcript levels of the key genes impacted pigment and primary metabolite accumulation were also investigated.

2. Materials and methods

2.1. Plant materials and hormone treatment

Four hundreds mature green cherry tomato fruits (cv. Xintaiyang) with uniform size and without injury were collected from a commercial standard greenhouse in Hangzhou, China. Fruits were washed by tap water and drained at room temperature after sterilization with 0.5% sodium hypochlorite aqueous solution, then were randomly divided into two groups and infiltrated with 0.45 mM 2, 4-dichlorophenoxyacetic acid or sterilized water under vacuum (35 KPa) for three minutes. After treatment, fruits were stored in darkness at constant temperature (20 ± 2 °C) and humidity (90 ± 5% RH). Samples of 5 fruits with 3 replicates were randomly taken at 1, 4, 7, 10, 13, 16, 20 and 25 day after treatment during the 25 days of storage for subsequent analyses.

2.2. Measure of chlorophyll and pigment content

Chlorophyll was extracted and measured as previously described (Bu et al., 2014; Camejo et al., 2005). Pericarp powders (1 g) pooled from five individual fruits were mixed with 3 mL 80% (v/v) acetone and kept at 4 °C for 12 h. After centrifuging at 6000 × *g* for 20 min, supernatant was collected and the absorbance at 663, 647 and 470 nm was measured using a UV Spectrophotometer (Shimadzu Corp., Kyoto, Japan). The contents of chlorophyll a and b were calculated according to the equations described by Camejo D et al. (Camejo et al., 2005). Three biological replicates were executed for each pigment measurement.

Pigment extraction was based on the method of Seírino et al. (Seírino et al., 2009). Frozen pericarps were ground into powder (0.5 g) and fully mixed with 6 mol L^{-1} NaCl aqueous solution (100 μL) and *n*-hexane (50 μL) using vortex (30 s). After centrifugation (13200*g*, 2 min) at 4 °C, the mixture was vortex-mixed (30 s) with dichloromethane (200 μL), followed by another vortex homogenization procedure (30s) with ethyl acetate (1000 μL). The supernatant was collected after centrifugation (13200*g*, 2 min, 4 °C) and filtered through a 0.45 μm membrane filter before high performance liquid chromatography (HPLC) assay. The assay was conducted according to the method previously described (Bu et al., 2013) using a HPLC with a Shimadzo LC2012A pump (Shimadzo Corp., Tokyo, Japan) and a Zorbas SB-C18 column (silica 5 μm, 4.6 mm × 250 mm, Agilent, USA). The mobile phase comprised solvent A of acetonitrile and H_2O (9:1) and solvent B of 100% ethyl acetate. The linear gradient between mobile phase A (acetonitrile/H_2O, v/v = 9:1) and B (ethyl acetate) was as follows: 0% B-100% B, 0–30 min, and the flow rate of mobile phase was 1 mL min^{-1}. The detection wavelength was 475 nm and the column temperature was 30 °C. The contents of lycopene, β-carotene and lutein were calculated according to the standard curve.

2.3. Total phenolics and flavonoids measurement

About 1 g pericarps were homogenized with 6 mL of 40% (v/v) ethanol solution, followed by the extraction at 60 °C for 1 h, and then centrifuged for 15 min at 9000*g*. The supernatant was used to determine the concentration of total phenolics and flavonoids according to the methods as previously described (Dewanto et al., 2002). The contents of total phenolics and total flavonoids were expressed as gallic acid equivalents and rutin equivalents, respectively.

2.4. Analysis of metabolite profiling of tomato fruit using Gas Chromatography–Mass Spectrometry

Primary metabolite profiling was performed based on the methods previously described (Lisec et al., 2006; Roessner et al., 2001) with slight modifications. Frozen pericarp powder (0.1 g) was vortex-mixed (10 s) with methanol (1.4 mL) and 60 μL ribitol (0.2 g L^{-1}, internal standard) and shaken (300 rpm) at 60 °C for 15 min. Then 1 mL water was added into the mixture and vigorously shaken. After centrifugation (1000*g*, 4 °C) for 15 min, the supernatant was dried under vacuum. The residue was redissolved and derivatized in 80 μL of 20 mg mL^{-1} methoxyamine hydrochloride in pyridine for 2 h at 40 °C. After that, the mixture was incubated with N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) (80 μL) at 40 °C for 0.5 h. The sample was filtered through a 0.45 μm filter before analysis by gas chromatography. Sample (1 μL) was injected under a split ratio of 25:1 with a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., USA). The analysis of monomer was conducted with the procedures described by Roessner et al. (2001). Values in control samples were normalized to one and the metabolites content in auxin-treated samples represented in relative-value (RV). Three replicates were performed for primary metabolite profiling measurement.

2.5. RNA extraction and assay of quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from pooled pericarps of five individual fruit using RNAiso Plus (TaKaRa, Japan) according to the description of protocol in the product manual. Single-strand cDNA was generated from 1 μg RNA using PrimeScript RT kit (TaKaRa, Japan). RT-qPCR assay was executed on ABI StepOne RT-PCR System (Applied Biosystems, USA) using SYBR® Premix Ex TaqTM (TaKaRa, Japan) as described previously (Bu et al., 2013). Primers used in RT-qPCR assay were listed in Table S1.

2.6. Statistics analysis

Significance of the data between each samples was analyzed by Student's *t*-test and principal component analysis was executed using SPSS version 20.0 (IBM Corp, Armonk, USA).

3. Results

3.1. Effect of exogenous auxin on pigment accumulation of tomato fruit during ripening process

The content of Chlorophyll a and b in control sample sharply dropped at 7 day after treatment (DAT) and reached to minimum at 13 DAT (Fig. 1a and b). In auxin-treated sample, this descent process of chlorophyll content was retarded for approximate 3 days (Fig. 1a and b). Lutein content also showed a similar downward trend during ripening stage but no significant difference of it was observed between control and auxin-treated sample (Fig. 1c). Lycopene content was undetectable in control sample until 10 DAT and then rapidly increased in the following period. The content of lycopene was markedly inhibited by exogenous auxin application during the transition from mature green stage to ripening stage. However, lycopene content in control- and auxin-treated sample was almost the same at the end of ripening (Fig. 1d). The content of β-carotene