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Research article

Contribution of abscisic acid to aromatic volatiles in cherry tomato (*Solanum lycopersicum* L.) fruit during postharvest ripening

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ABSTRACT

Fruit aroma development depends on ripening. Abscisic acid (ABA) has been reported to be involved in the regulation of tomato fruit ripening. In the present study, the effects of exogenous ABA on aromatic volatiles in tomato fruit during postharvest ripening were studied. The results showed that exogenous ABA accelerated color development and ethylene production as well as the accumulation of carotenoids, total phenolics and linoleic acid in tomato fruit during ripening. Moreover, exogenous ABA increased the accumulation of volatile compounds such as 1-peten-3-one (2.06-fold), β-damascenone (1.64-fold), benzaldehyde (3.29-fold) and benzyl cyanide (4.15-fold); induced the expression of key genes implicated in the biosynthesis pathways of aromatic volatiles, including *TomloxC*, *HPL*, *ADH2*, *LeCCD1B* and *SlBCAT1* (the values of the $\log_2$ fold changes ranged from −3.02 to 2.97); and promoted the activities of lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH). In addition, the results of promoter analyses revealed that *cis*-acting elements involved in ABA responsiveness (ABREs) exist in 8 of the 12 key genes involved in volatile biosynthesis, suggesting that ABA potentially affects aromatic volatile emissions via the regulation of gene expression profiles.

1. Introduction

Tomato is one of the most widely cultivated and consumed horticultural crops worldwide. Owing to its versatile culinary use and rich content of antioxidants and vitamins, both overall consumption and per capita consumption of tomato fruit are high (Klee, 2010). Aromatic volatiles, which play an important role in tomato flavor, are considered sensory clues for the nutritional and health value of tomato fruit. Almost all of the important volatile compounds are derived from essential nutrients such as leucine, phenylalanine and linolenic acid (Goff and Klee, 2006). However, significant losses in characteristic flavor have long been a problem in commercially produced tomatoes (Klee, 2010). Currently, researchers are increasingly focusing on improving tomato fruit flavor.

In recent years, with the development of flavor chemistry and the availability of tomato genetic sequences, great progress has been made in elucidating the biosynthesis of tomato volatiles (Klee and Tieman, 2013). The accumulation of tomato aromatic volatiles occurs in a ripening-dependent manner. Most of the aromatic volatile compounds in tomato fruit accumulate at the onset of tomato ripening and peak at either full ripening or shortly before (Klee and Giovannoni, 2011). Several hundred volatile compounds in tomato fruit have been identified, whereas only approximately 30 of them, such as 1-penten-3-one, β-ionone, geranylacetone and β-damascenone, markedly influence tomato flavor (Baldwin et al., 2000). The volatile compounds that contribute to tomato fruit flavor are derived from a range of precursors including lipids, carotenoids and amino acids (Fig. S1) (Rambla et al., 2014). The synthesis of aromatic volatiles is multigenically regulated, and a number of key genes involved in aromatic volatile biosynthesis (*TomloxC*, carotenoid cleavage dioxygenases (*CCDs*), *AADCs*, *BCAT1*, etc.) have been identified and characterized (Ilg et al., 2014; Jiyuan et al., 2014; Tieman et al., 2006).

Because the metabolism of the tomato aroma occurs in a ripening-dependent manner, factors that influence the ripening of tomato fruit may also affect aromatic volatiles. In addition to environmental factors such as temperature, light and humidity, plant hormones also play important roles in fruit ripening (Fu et al., 2017; Liu et al., 2018). Tomato is a well-known climacteric fruit, and ethylene is required during the ripening process (Yun-Yi et al., 2013). The phytohormone abscisic acid (ABA) is primarily involved in the ripening of non-climacteric fruit (McAtee et al., 2013). *Cis*-acting elements involved in ABA responsiveness (ABREs) that exist as conserved sequences in the

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promoter of ABA-responsive genes were identified as targets for ABA signal transduction and play a vital role in the regulation ABA in plants (Kang et al., 2002). However, increasing numbers of studies have recently shown that ABA also plays a role in the ripening process of climacteric fruit (Mou et al., 2016; Wang et al., 2013). In climacteric fruit, ABA accumulation has been reported to precede or occur synchronously with ethylene production; ABA may act as an upstream modulator of the ethylene pathway (Leng et al., 2009). ABA may also stimulate the ripening of climacteric fruit by inducing ethylene-dependent processes and other ethylene-independent pathways (Kumar et al., 2014; Zaharah et al., 2013). The ripening and softening process of tomato fruit can be effectively delayed by ABA biosynthesis inhibitors (Zhang et al., 2009). RNA-mediated suppression of SlNCED1 increases carotenoid contents in tomato fruit (Sun et al., 2012). Studies about the effects of plant hormones on aromatic volatiles of tomato during fruit ripening, however, are scarce, and whether ABA can affect or how it affects tomato aromatic volatiles during the ripening process remain unknown.

In the present study, the roles of ABA in the biosynthesis of tomato aromatic volatiles were explored. The effects of exogenous ABA on the ethylene production, aromatic volatiles, the activities of some enzymes and the expression of key genes involved in the biosynthesis of tomato aromatic volatiles were analyzed. The practical implications of the present study are to provide valuable information for exploring the contribution of ABA to aromatic volatiles and further analyzing the molecular mechanism of ABA regulation in tomato fruit ripening.

2. Materials and methods

2.1. Materials

Cherry tomatoes (*Solanum lycopersicum* L. cv XinTaiyang) were grown in a commercial greenhouse (Transfer Agriculture Co. Ltd., Xiaoshan District, Hangzhou, Zhejiang Province, P. R. China) and harvested twice a year. Three biological replicates of tomato fruit used in the present study were harvested during the autumn of 2016 (Sep. 12, 2016; Oct. 13, 2016; and Nov. 10, 2016). Each time, four hundred intact mature green fruit were randomly collected from different plants at the same height and then immediately transported to the laboratory.

2.2. Treatment and storage

The harvested tomato fruit were randomly divided into two groups. After they were sterilized with 0.5% NaClO solution and washed with distilled water, the fruit were infiltrated with 1 mM ABA (98%, Aladdin, Shanghai, China) or with sterilized water under vacuum (60 kPa) for 3 min. The application approach and dose concentration of ABA was based on the discrepancy of the appearance and ethylene production between two groups in our preliminary experiments (0.1–2.0 mM). The tomatoes were then drained in a draught cupboard and stored in darkness at 20 ± 0.5 °C and 90 ± 2% RH for fifteen days. Three replicates of eight tomatoes were sampled every other day for subsequent analysis. Fresh fruit were directly subjected to color and ethylene production determination. For other assays, fruit seeds and sepals were removed, after which the pericarp tissues were frozen in liquid nitrogen and maintained at −80 °C for subsequent use. Fruit after 1, 7, 11 and 15 d, which corresponded to the mature green, breaker, turning and red ripe stages of the control fruit, were selected for fatty acid and volatile analyses. All measurements were performed in triplicate, and the results were expressed on a fresh weight (FW) basis. The entire experiment was repeated three times.

2.3. Color measurements and determination of chlorophyll, carotenoids and total phenolics

Five tomato fruit of each sample were used for color determination. The color of the tomato fruit was measured with a chromameter (CR-200, Konica Minolta, Tokyo, Japan) on three equatorial points of each fruit, and the a* value was used to depict the color development during fruit ripening.

The chlorophyll and total carotenoids contents were measured in accordance with the method of Zhu et al. (2014), with some modifications. Briefly, approximately 1.0 g of finely ground pericarp powder and 10 mL of hexane and acetone (6:4, v/v) were homogenized together and then extracted at 4 °C overnight. After centrifugation at 5000 * g for 10 min, the supernatant was collected, and its absorbance at 450, 643 and 647 nm was determined; hexane served as a blank. The results were evaluated in accordance with the following formulas: total carotenoid content (g L^{-1}) = (A450)/0.25 and total chlorophyll content (g L^{-1}) = 8.02 (A643) + 20.2 (A647).

The content of total phenolics was measured in accordance with the method of Lin et al. (2016). Approximately 1.0 g of finely ground pericarp tissue was extracted with 10 mL of 70% ethanol at 60 °C for 1 h. The mixture was then centrifuged at 8000 * g for 15 min, after which the supernatant was collected in a fresh tube. Afterward, 1 mL of the supernatant and an equal volume of freshly diluted Folin phenol reagent (water:Folin phenol, 1:1, v/v) were mixed together; the mixture was subsequently vortexed and placed for 3 min and then neutralized with 3.0 mL of 7.5% (w/v) sodium carbonate. The total volume was ultimately brought to 10 mL with 30% (v/v) ethanol. The reaction mixtures were homogenized for 20 s and then incubated in the dark for 2 h at 23 °C, after which the absorbance was measured at 765 nm. The content of total phenolics was evaluated via an external calibration curve of gallic acid concentrations.

2.4. Determination of ethylene production and ABA content

The ethylene production of tomato fruit was measured referred to the method of Bu et al. (2013), with slight modifications. Twenty fruit were weighed and sealed in a 2 L airtight container for approximately 1.5 h at 20 °C. One milliliter of headspace was subsequently sampled and analyzed with a gas chromatograph fitted with both a flame ionization detector (FID) and a 2000 × 3 mm column of aluminum oxide at 85 °C. The ethylene production was quantified with 10 μL L^{-1} standard ethylene gas, and the results were expressed as microliters per kilogram of FW per hour (μL kg^{-1} FW h^{-1}).

The ABA content in the tomato fruit was analyzed in accordance with the methods of Mou et al. (2016). Approximately 1.5 g of fully ground pericarp tissue and 20 mL of 80% methanol (v/v) was homogenized together and then extracted overnight at 4 °C. After concentrating and removing any polar compounds with an SPE-C18 cartridge (Waters, Milford, MA, USA), the collected solution was withdrawn and used for ABA analysis via HPLC. The mobile phase comprised acetic acid:H_2O (4:96) and 100% methanol, and the elution speed was 1 mL min^{-1}. The ABA content was evaluated by an external calibration curve of known ABA concentrations.

2.5. Fatty acids analysis

Fatty acids in tomato fruit were measured via gas chromatography-mass spectroscopy (GC-MS). The sample was extracted, methylated and analyzed as described by Roessner et al. (2001) and Lisec et al. (2006), with modifications. Specifically, 0.1 g of fully ground pericarp tissue, 1.4 mL of 100% methanol and 60 μL of 0.2 g L^{-1} ribitol (internal standard) were homogenized together. The mixture was incubated at 60 °C in a shaker at 300 rpm for 30 min, after which it was centrifuged at 11000 * g for 10 min. Afterward, 0.75 mL of chloroform and 1.4 mL of H_2O were added to the supernatant, and the mixture was then centrifuged at 2200 * g for 15 min. After centrifugation, the supernatant was transferred to a new reaction tube and dried with a termovap sample concentrator; the residue was subsequently methylated with 40 μL of 20 g L^{-1} methoxyamine hydrochloride in pyridine at 37 °C for 2 h. Afterward, the mixture was incubated together with 70 μL of N-