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# Effect of exogenous auxin on aroma volatiles of cherry tomato (*Solanum lycopersicum* L.) fruit during postharvest ripening

Qiong Wu[1], Xiaoya Tao[1], Xinzi Ai, Zisheng Luo, Linchun Mao, Tiejin Ying*, Li Li*

*College of Biosystems Engineering and Food Science, Fuli Institute of Food Science, Key Laboratory of Agro-Products Postharvest Handling of Ministry of Agriculture and Rural Affairs, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang University, Hangzhou 310058, Zhejiang Province, PR China*

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ABSTRACT

The phytohormone auxin has been proved to be involved in the regulation of quality traits in climacteric fruit ripening, but there was little information about the correlation between auxin and aroma volatiles in fruit. In the present study, the effect of auxin on aroma volatiles in tomato fruit during postharvest ripening was studied by treating detached tomato fruit at mature green stage with 2, 4-dichlorophenoxyacetic acid. The results showed that exogenous auxin delayed the ripening process of tomato fruit via inhibiting the ethylene production and the carotenoids accumulation, as well as the degradation of chlorophyll. In addition, exogenous auxin enhanced the accumulation of phenolic volatiles such as phenylacetaldehyde (1.57-fold), 2-phenylethanol (1.56-fold) and methyl benzoate (1.75-fold), and inhibited the production of 1-hexanol (56.59 %), 1-nitro-2-phenylethane (23.74 %), benzyl cyanide (45.69 %) and 2-isobutylthiazole (35.18 %). Exogenous auxin altered the expression of a number of key genes involved in the biosynthetic pathway of aroma volatiles, including induction of SlSAMT1, *LePAR1*and *LePAR2,* and inhibition of *TomloxC*, *HPL*, *ADH2*, *LeCCD1s*, *LePAR1*, *LePAR2*, *LeAADCs*, *SlBCAT1*. The $\log_2$ fold change of these genes ranged between -4.53 and 3.02 compared to that in the control group. Moreover, correlation analysis revealed that changes of apocarotenoids and amino acid-derived volatiles were positively correlated with the expressions of related genes in response to auxin. The present study provided valuable information for further elucidating the regulation of tomato fruit aroma as well as quality traits during ripening.

## 1. Introduction

Fruit play an important role in human diets as it provides a large portion of essential micronutrients as well as fiber and phytonutrients. The characteristic flavor of different fruits was made up by aroma and taste (Meilgaard et al., 2016). Fruit aroma is composed by a sophisticated group of volatile compounds and is momentous in determining human's perception and acceptableness of fruit products (Kader, 2008). However, over the past few decades, the characteristic flavor of new cultivars was less because of that the modern breeding focused in high yield, handling and storability, disease and pest resistance (Klee and Tieman, 2013). Nowadays, researchers are increasingly focused on the improvement of fruit flavor (Klee, 2010; Tieman et al., 2006b).

Tomato was regarded as a fantabulous model to study fruit quality at the molecular level owing to the availability of genome, well characterized chemical composition and short life cycle, (Klee and Tieman, 2013). Up to date, volatile compounds of tomato fruit have been studied by many researchers, and great progresses have been made in exploring the biosynthetic mechanism of important aromatic volatiles. The production of tomato fruit aroma is ripening-dependent, and most volatiles were accumulated at the onset of ripening and peak at full ripening (Klee and Giovannoni, 2011). More than four hundred of volatile compounds have been identified in tomato fruit (Tikunov et al., 2005), and only approximately thirty of them (*cis*-3-hexenal, 1-penten-3-one, *trans*-beta-ionone, and so on) were regarded as characteristic volatiles contributed to tomato flavor based on their concentrations and

*Abbreviations:* AADC, aromatic amino acid decarboxylase; ADH, alcohol dehydrogenase; BCAT, branched-chain aminotransferases; CCD, carotenoid cleavage dioxygenases; DAHP, 3-deoxy-d-arabino-heptulosonate 7-phosphate; DMAPP, dimethylallyl diphosphate; F-6-P, fructose-6-phosphate; FPP, farnesyl diphosphate; G-6-P, glucose 6-phosphate; GA-3-P, glyceraldehyde-3-phosphate; GGPP, geranyl diphosphate; GTase, glucosyltransferase; HPL, hydroperoxidelyase; IPP, isopentenyl diphosphate; LOX, lipoxygenase; MEP, 2-C-methyl-d-erythritol 4-phosphate; MeSA-Glue, MeSA glucosides; PAL, phenylalanine ammonialyase; PAR, phenylacetaldehyde reductase; PEP, phosphoenolpyruvate. SAMT salicylic acid methyltransferase

* Corresponding authors.
*E-mail addresses:* tjying@zju.edu.cn (T. Ying), lili1984@zju.edu.cn (L. Li).
[1] These authors contributed equally to this work.

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odour thresholds (Baldwin et al., 2000; Petro-Turza, 1986). Tomato aromatic volatiles were mainly classified as fatty acid volatiles (C5 and C6 volatiles), apocarotenoid volatiles, phenolic volatiles, branched-chain volatiles and others (Fig. S1) (Rambla et al., 2014). Currently, with the advances in genetic and molecular manipulation techniques, more and more genes (such as *AADC1 A, AADC1B, BCAT1, TomloxC* etc.) involved in tomato volatiles biosynthesis were identified and characterized (Maloney et al., 2010; Tieman et al., 2006a; Tieman et al., 2007).

Tomato is a typical climactic fruit, and ethylene is known to be necessary for tomato fruit ripening (Bapat et al., 2010). However, it is also known the ripening process of tomato fruit is sophisticated in response to exogenous phytohormone (Kumar et al., 2014; McAtee et al., 2013). Auxin, an important plant hormone involved in the regulation of cell elongation and division, plays pleiotropic roles in a wide range of developmental processes, such as embryogenesis, organ differentiation, leaf elongation, fruit setting and development (Rosquete et al., 2012; Srivastava and Handa, 2005), as well as in the regulation of fruit ripening (Trainotti et al., 2007). It was reported that the higher auxin content was observed in tomato *rin* (a ripening inhibitor) mutant, and the decrease of endogenous auxin contributed to initiating the ripening process of fruit (Liu et al., 2005; Rolle and Chism, 1989). In addition, it was reported that altered expression of auxin response factors (ARF2, ARF4, DR12 and Sl-IAA3) could lead to significant changes in tomato fruit maturation and the nutrient metabolism (Breitel et al., 2016; Chaabouni et al., 2009; Jones et al., 2002; Sagar et al., 2013). Moreover, transcriptomic profiling of tomato fruit in response to exogenous auxin demonstrated that auxin could influence the ripening process of tomato fruit by modulating genes expression involved in ethylene biosynthetic and signal pathway, as well as physicochemical traits (Li et al., 2016). The information of the correlation between auxin and tomato aroma, however, is rare and the effects of auxin on aromatic volatiles during tomato ripening remain obscure so far. This study focused on investing the volatiles profile of tomato in response to exogenous auxin.

## 2. Materials and methods

### 2.1. Plant materials

Cherry tomatoes (*Solanum lycopersicum* L. cv Xin Taiyang) were cultivated in a commercial greenhouse of Transfer Agriculture Co. Ltd (Xiaoshan, Hangzhou, Zhejiang, China). Three independent biological replicates of tomato fruit were used in this study and were harvested in three successive months (Apr 20$^{th}$ 2017, May 18$^{th}$ 2017 and Jun 17$^{th}$ 2017) in the spring of 2017, and each time four hundreds of intact mature green fruits with uniform size were randomly collected from about one hundred tomato plants at the second inflorescence (about 0.5 m above the ground), and transported to the laboratory in one hour.

### 2.2. Auxin treatment and storage

The harvested tomatoes were randomly divided into two groups. After they were sterilized with 0.5 % sodium hypochlorite (aqueous solution, m / v) for five minutes and washed three times with distilled water, the fruit were stemmed and infiltrated with 0.45 mM 2, 4-D (97 %, Macklin, Shanghai, China) aqueous solution or sterilized water under vacuum (35 K Pa) in a vacuum dryer (volume / 15 L, inner diameter / 0.3 m) for three minutes, respectively. The application method and concentration of 2, 4-D were applied based on our preliminary experiments. The tomato fruit were then air-dried and stored in dark at 20 ± 0.5 °C and 90 ± 2 % RH for twenty-two days. During the storage, three replicates of eight tomatoes in each group were sampled every three days. For color and ethylene determination, fresh fruit from all sampling days were directly subjected to analyze. For carotenoids, chlorophyll, volatiles determination and RNA extraction, the tomato pericarps after removing the placenta and seed were frozen in liquid nitrogen and kept at −80 °C for analysis. Tomatoes at 1, 7, 13 and 22 d, which corresponding to the mature green, breaker, turning or pink, and red ripe stages in the control group, were used for volatiles analysis. All measurements were carried out in triplicate, and the results were expressed on a fresh weight (FW) basis. The whole experiment was repeated three times.

### 2.3. Ethylene, color, chlorophyll and carotenoids contents determination

The fruit color was measured with five tomatoes in each sample using a Chromameter (Konica Minolta, CR-200, Japan). The value of a* was measured at three points on the equator of each fruit.

Ethylene determination of tomato fruit was conducted as described by Zhu et al. (2010) with modifications. Briefly, twenty tomato fruit were weighed and sealed in a 2 L airtight container and placed in an incubator for 2 h at 20 °C. Then one milliliter of headspace was 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 nanogram per kilogram of FW per second (ng $kg^{-1}$ $s^{-1}$).

Chlorophyll and total carotenoids were extracted and analyzed according to Zhu et al. (2014) with some modifications. Briefly, about 1.0 g of finely ground pericarp powder was homogenized and extracted with 10 mL extracting solution (60 % hexane and 40 % acetone, v / v) at 4 °C overnight. After centrifugation at 5000 × *g* for ten minutes, the supernatant was transferred to a fresh tube and the absorbance of it at 450, 643 and 647 nm were measured against hexane as blank. The results were evaluated based on the formulas: Chlorophyll (g $L^{-1}$) = 8.02 ($OD_{643}$) + 20.2 ($OD_{647}$), and total carotenoids content (g $L^{-1}$) = ($OD_{450}$) / 0.25.

### 2.4. Volatiles determination

Aromatic volatile compounds of tomato fruit were extracted by solid- phase micro extraction (SPME) and analyzed with gas chromatography-mass spectroscopy (GC-MS) according Li et al. (2013). Five grams of fully ground pericarp powder, 5 mL of saturated sodium chloride solution and 50 μL of 5.24 mg $L^{-1}$ 2-octanone (internal standard) were mixed together in a 20 mL sample vial for volatiles extraction, the extraction and sampling of volatile compounds were performed by a MultiPurpose Sampler (MPS-XT, Gerstel, Germany), and the volatile components were analyzed via an Aligent 7890B GC System (Agilent, Palo Alto, CA, USA) and an Aligent 5977 A quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA). GC was performed on an HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies Inc., Santa Clara, CA, USA) executing the following temperature program: a homothermal initial temperature at 35 °C for 5 min, a ramping period to 240 °C at a rate of 0.25 °C $s^{-1}$, and isothermal heating held at 240 °C for 4.5 min. Compounds were identified by matching the collected mass spectra to a mass spectral library (National Institute of Standards and Technology, NIST, version 2.3) and the retention time of the reference compounds. Quantities of identified volatiles were evaluated by comparing each peak area with the peak area of the internal standard or with the external calibration curves of corresponding reference compounds. Calibration curves of reference compounds were generated by measuring different known concentrations of reference compounds following the same determination procedure as that used for the samples. The detailed information for calibration curves of different reference compounds were listed in Supplementary Table 1.

### 2.5. Gene expression

The pericarp of each sampled tomato fruit was cut into eight small