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Transcriptome analysis of aroma volatile metabolism change in tomato (*Solanum lycopersicum*) fruit under different storage temperatures and 1-MCP treatment

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ABSTRACT

Temperature control and ethylene inhibitor 1-methylcyclopropene (1-MCP) treatment are the main techniques for increasing the shelf life of tomato (*Solanum lycopersicum* L.) fruit. However, these techniques could strongly affect the aromatic flavor of tomato. In this study, RNA-sequencing was employed to characterize the transcriptomic profiles of cherry tomato fruit, harvested at breaker stage, during postharvest storage under different temperatures (25 °C, 10 °C, and 4 °C) and at 10 °C after 1-MCP treatment. Results showed that storage temperature remarkably affected the expression of numerous genes in tomato fruit, especially on several key genes associated with aroma volatile biosynthesis. It was found that 33 genes presented significantly different expression between 10 °C and 25 °C, and in particular, five genes expressed significantly lower at 10 °C than that at 25 °C, including *CCD1*, *GOT1*, *ADH2*, *PDC1-like1*, and *PDC1-like2*, mainly involved in the syntheses of pseudoionone, β-ionone, phenylacetaldehyde, phenylethylalcohol, *cis*-3-hexenol, and *trans*-3-Hexenol. The expression level of other 14 genes associated with aroma volatile biosynthesis was lower at 4 °C than that at 10 °C, among which, five genes, including *TPS24*, *PDS*, *ACOT9-like*, *ADH2* and *AAT* were directly related to the biosynthesis of terpenoids, alcohols and esters. Only few genes associated with aroma volatiles were affected by 1-MCP treatment at 10 °C. The presented results implied that the recommended storage temperature of 10 °C is able to result in a significant negative effect on the aromatic flavor of tomato at the gene transcriptional level, which could explain the flavor loss of tomato under market storage temperatures (8–12 °C) and household refrigerator temperatures (3–5 °C). To be mentioned, our results provide strong evidence that 10 °C, as the recommended storage temperature for tomato fruit, is not ideal to maintain the flavor quality of tomato, and 1-MCP treatment under 10 °C cannot further affect the flavor quality of tomato fruit compared with that at 10 °C alone.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop with a global yield of 164 million tons and a net value of $60 billion 2013 (Food and Agriculture Organization (FAO), 2015). Tomato fruit is an excellent source of antioxidants, vitamins, flavonoids, carotenoids (lycopene and beta-carotene) and phenolics (Causse et al., 2003; Yilmaz, 2001). In particular, carotenoids not only exhibit antioxidant and anticarcinogenic activities but also provide protection against chronic disease, different types of cancers, macular and cardiac vascular diseases (Beatty et al., 2004; Chen et al., 2001; Rao and Agarwal, 1998; Velioglu et al., 1998). However, tomato fruit features rapid ripening rate and high perishability, causing its shortened shelf life and rapid loss of qualities (Paul and Pandey, 2013; Yanuriati et al., 1999). Many management technologies, such as temperature control and chemical treatment, have been recently used to prolong the shelf life and maintain the quality of tomato fruit (EI Hadi et al., 2013; Fagundes et al., 2015; Zhang et al., 2016).

As an important non-visual quality parameter for fruit, aroma increases the appeal of fruit to different animals that spread their seeds (Borges et al., 2008). Aroma is also an important factor influencing the consumers' acceptance of fruit, thus, this non-visual quality affects the

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sales volume of fruit in the market (Awad and de Jager, 2003). In tomato, over 400 volatile compounds have been identified during ripening of an intact fruit (primary aroma compounds) and during tissue disruption (secondary aroma compounds) (Baldwin et al., 2000; Díaz de León-Sánchez et al., 2009). In higher plants, the carotenoid and terpenoid pathway, the fatty acid pathway, and the amino acid pathway have been associated with aroma volatile biosynthesis, where carotenoids, fatty acids and amino acids respectively act as the precursors (Song and Bangerth, 2003).

Many factors, including fruit maturity and ripeness, postharvest handling, and storage temperature, can affect the abundance of aromatic volatile compounds in fruit (EI Hadi et al., 2013). Among these factors, storage temperature mainly influences the composition and concentration of aroma volatiles (EI Hadi et al., 2013). Tietel et al. (2012) reported that low temperature significantly affects volatiles, including terpenes and their derivatives in chilling-sensitive mandarins. It was reported by de Leon-Sanchez et al. (2009) that refrigeration affects the expression of *trans*-2-hexenal, *trans*-3-hexenol, 3-methylbutanal, guiacol, linalool and hexanol in tomato. Meanwhile, heating or chilling treatment could reduce the C6 and C9 alcohol and aldehyde aroma volatiles in tomato and hinder the recovery of aldehydes even after repeated incubation at 20 °C (Bai et al., 2011; Zhang et al., 2016). Ethylene plays a critical role in fruit ripening and organ senescence, as well as in the metabolism of aroma flavor volatiles (Baldwin et al., 2000; Defilippi et al., 2005). Exogenous ethylene is able to significantly alter the level of some aroma volatiles in tomato fruit (McDonald et al., 1996; Zhu et al., 2005). Treatment with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, can delay fruit senescence and prolong the shelf life of tomato fruit (Serek et al., 1995). Many reports showed that 1-MCP treatment also significantly reduces volatile ester production in several fruit, including bananas (Golding et al., 2015), plum (Abdi et al., 1998), and apple (Defilippi et al., 2004; Lurie et al., 2002).

An appropriate low temperature and 1-MCP treatment affect the concentration and composition of aromatic flavor volatiles in tomato (Golding et al., 2015; Paul and Pandey, 2013), however, the mechanism remains unclear. Moreover, the relationships among aroma volatile synthesis, ethylene signaling, storage temperature, and aromatic-related gene expression have yet to be established. So it is necessary to inquire whether the expression of genes related to aroma synthesis in tomato under low temperature, including non-chilling and chilling low temperature, differs from that in room temperature, and how the use of an inhibitor of ethylene perception (1-MCP) affects their expression. In this study, a transcriptomics approach was performed to evaluate the expression of aromatic-related genes under different storage temperatures and under recommended storage temperature (10 °C) after 1-MCP treatment during the postharvest storage of tomato (Ponce-Valadez et al., 2016; Suslow and Cantwell, 2001). This study aims to investigate the effect of temperature and 1-MCP application under recommended storage temperature on the aroma volatile metabolism, especially the expression of related genes, during this process in tomato. It is hoped to provide some insights into the gene expression of tomato during postharvest storage in markets and households.

2. Materials and methods

2.1. Plant materials

Plants of cherry tomato (*Solanum lycopersicum*) variety “Taiya No.6” were grown on the field at Shapingba, Chongqing, China (106.29° E, 29.60° N). The fruit were harvested at the breaker stage, randomly sorted into four groups, and then stored at 25 °C (room temperature), 10 °C (non-chilling temperature, recommended storage temperature), and 4 °C (chilling temperature) for storage. Tomato fruit under 25 °C were treatment with 1-MCP (0.5 μL·L^{-1} 1-MCP for 24 h), to insure the inhibition of 1-MCP on ethylene signaling, then fruit group were treated with 0.5 μL·L^{-1} 1-MCP for 24 h, then stored at recommended temperature (10 °C), to investigate the effect of the usage of 1-MCP on ethylene production, along with different temperature. All the store conditions were maintained with 85–90% relative humidity. Fruit samples (25 per group) were collected on 7, 14, 21, and 28 d after storage at 25 °C, 10 °C, 4 °C, and 1-MCP treatment. The fruit with 0 d of storage were set as the control. The mixture samples (25 fruit) were used as an individual sequencing pool for each treatment.

2.2. Evaluations of fruit ripening

The fruit were placed in ten 100 mL gas collecting bottles, 1 fruit per bottle, and capped with a rubber stopper for 1 h. Ethylene was measured according to Zhang et al. (2009), with 1 mL of headspace gas from each gas collecting bottle sampled, and analyzed by gas chromatograph (CP 3800, VARIAN, USA) fitted with a GDX-502 column. Nitrogen was used as a carrier gas at 0.5 mL S^{-1}. The injector, detector and oven temperatures were 110 °C, 140 °C and 90 °C, respectively.

Fruit firmness was measured using a GY-4 digital fruit sclerometer (Aiwoshi, China), according to the procedure reported by Fan et al. (1999). The analyzer was equipped with a circular probe of diameter 7 mm, speed 10 mm·s^{-1} and depression 4 mm, and data were expressed in newtons per square centimeter (N cm^{-2}). Two measurements were made on opposite sides of each fruit after removal of a 1 mm thick slice of the skin, and the fruit number was set to 20.

In this study, juice samples were extracted from the pericarp and used to measure the soluble solids content (SSC), titratable acidity (TA), and pH value. Three fruit were analyzed per treatment per replicate, and two readings were recorded per fruit using the juice samples. The SSC was determined using a hand held refractometer, and expressed as gram per 100 g. TA was titrated using a standardized 0.1 M sodium hydroxide (NaOH) solution to a persistent pink end point (phenothalin was used as the indicator). TA was calculated as g L^{-1} of citric acid.

2.3. RNA extraction and Illumina sequencing

Total RNA from tomato was extracted and purified using QIAGEN RNeasy Plant Mini Kit and RNase-free DNase set (QIAGEN, Germany) in accordance with the manufacturer’s instructions. RNA-Seq was performed by technicians at Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China). A TruSeq™ RNA Sample Preparation Kit (Illumina, Inc.) was used to construct cDNA library. Poly (A) mRNA was enriched from 5 μg of total RNA via oligo (dT) magnetic beads and then cleaved using divalent cations. Double-stranded cDNA was generated and processed with end-repair, A-tailed and adapter ligation. The products enriched by PCR amplification were purified through 2% agarose gel electrophoresis and quantified by TBS380 (Picogreen). The cDNA libraries were subsequently sequenced using Illumina HiSeq™ 2000 platform.

2.4. Analysis of RNA-Sequence data

The raw sequences were filtered by removing the adapter sequences using SeqPrep (https://github.com/jstjohn/SeqPrep). Deteriorate quality bases at the end were trimmed using sickle (https://github.com/najoshi/sickle). Reads of less than 20 bp after processing were discarded. The tomato (*S. lycopersicum*) genome database (http://solgenomics.net/) was used as a reference to identify the gene expression signatures from the cherry tomato libraries. All clean reads were aligned to the reference sequences using Tophat (http://tophat.cbcb.umd.edu/) with default parameters (Trapnell et al., 2009). Transcript abundance was estimated using the fragments per kilobase of exon per million mapped reads (FPKM) values within a 95% confidence interval (Trapnell et al., 2010). Cuffdiff software (http://cufflinks.cbcb.umd.edu/) was used to calculate and analyze differentially expressed genes (DEGs) (Trapnell et al., 2013). The P-value denotes expression