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Different Expression Analysis in Fruit Softening and Ethylene Biosynthetic Pathways in Peaches of Different Flesh Textures

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Abstract

The aim of our study was to assess differences in the expression of genes involved in fruit softening and ethylene biosynthetic pathways under different temperature storage conditions. Different peach cultivars of 'Xiacui' and 'Yumyeong', which are stonyhard, 'Yinhualu', which is soft-melting, 'Hujing Milu', which is hard-melting, and 'Baby Gold 6', which is non-melting at 80% ripening, were collected as test materials. The results showed that only slight ethylene production was detected after harvesting of 'Yumyeong' and 'Xiacui' under either a room temperature (25 °C) or low temperature (4 °C). The fruit firmness of stonyhard cultivars was retained at a high level under room temperature over time, whereas a low temperature induced 'Yumyeong' fruit to soften. Quantitative real-time PCR results indicated that the *PpACS1* gene was highly expressed in soft-melting, hard-melting and non-melting cultivars; however, expression was extremely low in stonyhard peaches. *PpACS2* or *PpACS3*, however, was not detected in all five cultivars. Interestingly, cold treatment significantly decreased firmness along with *endo-PG* expression obviously up-regulated in 'Yumyeong', but not in 'Xiacui' peaches. In conclusion, this study revealed that fruit softening of peaches with different flesh textures was closely related to ethylene biosynthesis during the storage period, which was controlled via regulating relevant gene expression levels under different storage temperatures.

Keywords: *Prunus persica*; ethylene; biosynthetic pathway; soften; gene expression

1. Introduction

Peach flesh textures are divided into four types; i.e., soft-melting, hard-melting, non-melting and cotton-like (Wang et al., 2005). Yoshida, a Japanese peach expert, proposed a new type of peach flesh texture; i.e., stonyhard, in which the fruit flesh does not soften in the developing period and after harvest, but changes color normally and has a good taste (Haji et al., 2001, 2004). Genetic analysis showed that stonyhard (*hd*) is a recessive genetic locus that is different from melting (*M*)/non-melting (*m*) (Yoshida, 1976; Haji et al., 2005). In general, the peach belongs to a climacteric fruit. During ripening, the fruit texture changes, resulting in a decrease in firmness accompanied by an increase of ethylene release and up-regulated expression of genes.

ACC (a direct precursor of ethylene biosynthesis) synthase (ACS) and ACC oxidase (ACO) are key enzymes in the regulation of ethylene biosynthesis (Yang and Hoffman, 1984; Xu et al., 1998; Yin et al., 2009). ACS and ACO are encoded by genes of multiple families and their expression levels are under the combined effects of developmental processes and environmental factors (Kende, 1993; Zarembinski and Theologis, 1994). Kan et al. (2012) found that soft-melting peach 'Yuhua 3' decreased its firmness rapidly after harvesting, significantly increasing ethylene production. Also, the expression levels of *ACS* and *ACO* genes increased first and then decreased. Instead, the firmness of non-melting 'Jianayan' peach fruits changed slowly during maturation and only decreased in late maturation, then maintained overall at a high level, and *ACS* and *ACO*

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expression levels were relatively low. Hayama et al. (2006) showed that the melting 'Akatsuki' fruits softened quickly after harvesting and *endo-PG* gene expression increased rapidly, while the stonyhard type peach 'Manami' showed no significant changes in fruit flesh firmness after harvesting and *endo-PG* gene expression stayed at a low level.

This study used gas chromatography to investigate the ethylene release mechanisms of various peach flesh textures after harvesting. We combined firmness changes and ethylene biosynthesis pathways with analysis of softening-related gene expression to further explore the mechanisms of peach fruit flesh softening. Thus, our results provide a theoretical basis for research on peach fruit flesh softening and ethylene biosynthesis mechanisms, offering a reference for peach preservation.

2. Materials and methods

2.1. Materials and processing

Five varieties of peach fruits were tested. Soft-melting peach 'Yinhualu' softens quickly after harvest and has a very short shelf life; i.e., only 2–3 d, and cannot tolerate storage. Hard-melting peach 'Hujing Milu' has a longer shelf life of 4–5 d. Non-melting peach 'Baby Gold 6' maintains a stable firmness after harvest. Even if it fully matures, its firmness does not decrease significantly. 'Yumyeong', of the stonyhard type, recovers softening ability by exogenous ethylene, a typical stonyhard melting (able to recover) type, while 'Xiacui' belongs to a stonyhard non-melting (not restored) type (Haji et al., 2005). Fruit fleshes of 'Yumyeong' and 'Xiacui' show a high level of firmness and soften slowly after picking, without a significant change in firmness, and the shelf life is up to about 15 d.

The five peach cultivars were grown in the test garden of Jiangsu Academy of Agricultural Sciences. The fruit trees were robust and naturally open center types. There were three experimental trees for each species. The tested plants were cultivated according to conventional management measures.

In 2014, we harvested fruits above the middle of the canopy in good lighting conditions and at 80% ripening. Immediately after the harvest, fruits were brought back to the lab and then pest-free fruits of uniform size and relatively uniform maturity were chosen for later tests.

Tests were performed as below. A single layer of fruits on a plastic tray were placed inside a polyethylene plastic bag with an inner-lining thickness of 0.04 mm. The bag was opened and placed at room temperature (25 ± 1) °C on a shelf (approximately 75% humidity). Another group of single-layer fruits on a tray was placed in a perforated polyethylene plastic bag with the inner-lining thickness of 0.04 mm. This bag was lightly tied and placed in a refrigerator (4 ± 0.5) °C at a relative humidity of 75%. Each group had 70 fruits and experiments were repeated three times, which included a total of 210 fruits. Each time 10 fruits were randomly chosen and the related parameters were measured. The measurements were repeated three times.

2.2. Measurement of ethylene release rate and firmness

Ten fruits were placed in a 5 L sealed container for 2 h and the extracted gas was measured for ethylene content; measurements were repeated three times. The ethylene release rate ($\mu L \cdot kg^{-1} \cdot h^{-1}$) was represented by the released amount of ethylene per unit time per unit fresh weight (FW) of fruits. Ethylene was measured with a GC-7890A gas chromatograph (Agilent) and chromatographic conditions were: FID detector, Hp-Plot q capillary column (20 m × 0.53 mm × 20 μm), split ratio 10, a carrier gas He, 40 °C column temperature, 220 °C detector temperature, and 1 mL injection volume. Fresh fruits were used for measurements and measurements were repeated three times.

A TA.XT. Plus Texture Analyzer was used to measure the firmness of peeled fruits in the middle of both sides of the fruit suture. The probe diameter was 8 mm and the test depth was 5 mm at a 1 $mm \cdot s^{-1}$ penetration rate. The average of two points was taken for the firmness of each peeled fruit.

2.3. Analysis of related gene expression

Total RNA was extracted using a modified CTAB method (Shi and Zhuo, 2006). DNA contamination was removed using DNase I (Promega Corporation) digestion. RNA reverse transcription was performed using a PrimeScript™ Double Strand cDNA Synthesis Kit (TaKaRa). All procedures followed the instructions of the manufacturers.

According to the recorded peach genomic sequences, Primer 5.0 was used to design specific primer sequences for *ACTIN*, *ACS1*, *ACS2*, *ACS3*, *ACO1* and *endo-PG* 6 genes (Table 1). Primers were synthesized by Shanghai Invitrogen Biotechnology. SYBR Green (TaKaRa) was used as a fluorescent dye. Fluorescence quantitative analysis was performed with an ABI 7500 quantitative real-time PCR (qRT-PCR) instrument. The amplification system and reaction procedures were similar to the method of Guo et al. (2013) and were repeated three times. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the gene expression levels and internal *ACTIN* expression (Tong et al., 2009) was used as a standard to determine the expression levels of target genes.

Table 1 Sequences of the primers used for qRT-PCR

Gene	Forward primer (5′—3′)	Reverse primer (5′—3′)	Fragment length/bp
PpACTIN	GTTATTCTTCATCGGCGTCTTCG	CTTCACCATTCCAGTTCCATTGTC	112
PpACS1	GGCAAGGTTCCTGGAGACAA	CACAATCACACGCCAAAGCA	187
PpACS2	TGCACAGCAGCAGGAGTAAA	CCAGGATCAGCCAAGCAGAA	203
PpACS3	ATGCTGGGTTGTTTTGCTGG	AACCTGGTTCAGAGCAGTGG	147
PpACO1	GCAACTACCCTCCTTGTCCC	TGGCCATCTTTGAGGAGCTG	127
endo-PG	ACAACATTGTGGTGAGTGGA	CCATCGGTGTTAGGGCTGTT	130

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