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Accumulation of carotenoids and expression of carotenogenic genes in peach fruit

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ABSTRACT

To understand better the regulatory mechanism of the carotenoid accumulation, the expression profile of relevant carotenoid genes and metabolites were compared between two peach cultivars with different colors during fruit development. Meanwhile, the change pattern of carotenoid content and expression of carotenoid metabolic genes in peaches after harvest in response to blue light were also investigated. As compared to the yellow fleshed-cultivar 'Jinli', lower carotenoid levels were observed in skin and pulp in white peach cultivar 'Hujing', which might be explained by differentially expression of *PpCCD4* gene. With respect to 'Jinli', the carotenoid accumulation during fruit development in fruit skin was partially linked with the transcriptional regulation of *PpFPPS*, *PpGGPS*, *PpLCYB* and *PpCHYB*. However, in the pulp, the accumulation might be also associated with the increased transcriptions of *PpPDS*, along with the above four genes. Blue light treatment induced carotenoid accumulation in 'Jinli' peaches during storage. In addition, the treated-fruit displayed higher expression of all the eight genes analysed with a lesser extent on *PpCCD4*, which suggested that the much more increased carotenoid synthesis rate could result in the higher carotenoid content in blue light-treated fruit. The results presented herein contribute to further elucidating the regulatory mechanism of carotenoid accumulation in peach fruit.

1. Introduction

Carotenoids are natural pigments involved in the photosynthetic process, which play indispensable roles in providing pigmentation for flowers and fruits to attract pollinators and seed dispersers (Nisar, Li, Lu, Khin, & Pogson, 2015). Some carotenoid compounds have been reported to exert beneficial effects on prevention of certain cancer cardiovascular diseases (Yuan, Zhang, Nageswaran, & Li, 2015). In view of the commercial and nutritional values of carotenoids, their biosynthetic and catabolic pathways have been widely investigated during the past two decades (Fig. S1) (Fraser & Bramley, 2004; Taylor & Ramsay, 2005).

Researches on the regulation of carotenoid accumulation during fruit development have shown that the transcriptional level of carotenogenic genes is principal factor controlling carotenoid accumulation in most plant species. The increased expression of upstream genes such as phytoene synthase (PSY) and phytoene desaturase (PDS) in carotenoid biosynthesis causes lycopene accumulation during tomato ripening (Fray & Grierson, 1993; Isaacson, Ronen, Zamir, & Hirschberg, 2002). The massive accumulation in α-carotene is consistent with increased transcripts of PSY and β-carotene hydroxylase (CHYB) in pepper during maturation (Hornero-Méndez, Gómez-Ladrón de Guevara, & Mínguez-Mosquera, 2000; Hugueney et al., 1996). The accumulation of β-carotene is well correlated with the expression of lycopene β-cyclase (LCYB) in different kiwifruit species (Ampomah-Dwamena et al., 2009). The huge accumulation in total carotenoids in red-fleshed loquat during maturation is found to be correlated with the increase in gene expressions of *PSY*, *LCYB* and *CHYB* (Fu et al., 2012). Therefore, differential expression of carotenogenic genes plays key roles in determining the amount and type of specific carotenoids.

Abbreviations: CCD, carotenoid cleavage dioxygenase; CHYB, β-carotene hydroxylase; DAFB, days after full bloom; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; FW, fresh weight; GGPP, geranylgeranyl diphosphate; GGPS, geranylgeranyl diphosphate synthase; IPP, isopentenyl pyrophosphate; LCYB, lycopene β-cyclase; LED, light-emitting diode; PCR, polymerase chain reaction; PDS, phytoene desaturase; PSY, phytoene synthase; q-PCR, quantitative real-time PCR; ZDS, ζ-carotene desaturase.

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It is well documented that the content and composition of carotenoid is developmentally regulated and affected by environmental stimuli (Cazzonelli & Pogson, 2010). Light has been reported to be an important environmental factor which can regulate carotenoid metabolism in plants (Wu et al., 2007; Zhang et al., 2012, 2015). In tomato irradiated with red light, the accumulation of lycopene, as well as an increase in total carotenoid content, was observed (Alba, Cordonnier-Pratt, & Pratt, 2000; Schofield & Paliyath, 2005). Blue light treatment induced carotenoid accumulation effectively in the juice sacs of Satsuma mandarin and Valencia orange (Zhang et al., 2012, 2015).

The flesh color of yellow-fleshed peach fruit (*Prunus persica* L. Batsch) is produced by a group of carotenoids, which is an important factor of nutritional quality and market acceptance (Cazzonelli & Pogson, 2010; Gil, Tomás-Barberán, Hess-Pierce, & Kader, 2002). Recently, it has been reported that the carotenoid cleavage dioxygenase (*PpCCD4*) was the major factor in determining carotenoid degradation in white peaches but no correlation was observed between carotenoid accumulation and the expression levels of carotenoid biosynthetic genes (Adami et al., 2013; Brandi et al., 2011). Despite recent efforts to understand the molecular biology of carotenogenesis takes place in peaches, several gaps remain in our understanding of the signals and mechanisms involved in carotenoid metabolism. To investigate further how carotenoid accumulation in peach fruit, in this study, firstly the concentration and composition of carotenoids and the expression of several carotenoid biosynthetic genes as well as *PpCCD4* in fruit peel and pulp were comparatively analysed in two different colored-peaches ('Jinli' and 'Hujing') during fruit development and maturation. Then, the effect of blue light on carotenoid content and composition, and the expression of genes related to carotenoid biosynthesis and catabolism were investigated in both peach cultivars after harvest.

2. Materials and methods

2.1. Plant materials, sample collection, and blue light treatment

Two peach cultivars (*Prunus persica*), 'Hujing' and 'Jinli', were obtained from the experimental farm of Fenghua Peach Fruit Research Institute (Ningbo, China). Trees were subjected to standard horticultural practices. Fruit ripening stages were defined according to Tonutti, Bonghi, Ruperti, Tornielli, and Ramina (1997) and Gabotti, Negrini, Morgutti, Nocito, and Cocucci (2015). At the desired times, fruit (ten fruit per each of the five plants of each cultivar considered per each ripening stage) of 'Hujing' and 'Jinli' were picked and quickly transferred to the laboratory, and fruit tissues at 98 (S1), 105 (S2), 120 (S3), 127 (S4), and 134 (S5) DAFB were sampled during the spring-summer season of 2015 (Fig. S2).

For blue light treatment, fruit of each cultivar at commercial maturity stage were selected for uniform size and color, and then divided into two groups randomly. The blue light treatment was the same as that in our previous publication (Gong et al., 2015). The fruit stored at 10 °C in the dark (90% relative humidity) was considered as the control. There were three replicates of eighty fruit each per treatment, and samples were taken initially and at 5-day intervals during storage. All samples were immediately frozen in liquid nitrogen and then stored at −80 °C for RNA extraction.

2.2. Extraction and HPLC analysis of carotenoids

Extraction and purification of carotenoids in tissue samples (2 g) were performed according to a previously described method (Tuan et al., 2013; Wright & Kader, 1997). To determine the carotenoids content in each sample, the HPLC analyses were carried out as previously described by Taylor and Ramsay (2005).

2.3. Total RNA extraction and cDNA synthesis

Total RNA was isolated using a Plant Total RNA Extraction Kit (Genotheramics, Suzhou, China) according to the manufacturer's instructions. Extracted RNA was treated with RNase-free DNase (Omega, Norcross, GA) to remove any genomic DNA according to the instruction manual. An aliquot (2 μg) of total RNA was reverse-transcribed with the SuperRT First Strand cDNA Synthesis Kit (CWBIO, Beijing, China), following the manufacturer's instructions.

2.4. Quantitative real-time PCR (q-PCR) analysis

Q-PCR analysis was performed using the Mx3000P q-PCR System (Agilent Stratagene, Santa Clara, CA, USA) and the DyNAmo™ ColorFlash SYBR Green qPCR kit (Thermo Scientific, Pittsburgh, PA) following the manufacturer's instructions. Amplifications were performed using a total volume of 12.5 μL reaction containing 0.5 μL of the synthesized cDNA, 0.25 μL of 10 μM each forward and reverse primers, 6.5 μL of the SYBR Green PCR Master Mix and 5 μL of RNase-free water. The data were analysed and normalized to *PpTEF2* to minimize variation in cDNA template levels. All gene expression analyses were performed with three independent biological replicates, and primer sequences used for real time PCR are listed in Supplementary Table S1.

2.5. Data processing and statistical analysis

The contents of carotenoids and transcript abundance of carotenoid metabolic genes of two peach cultivars were displayed with GraphPad Prism (v.5.01). All values are shown as the mean ± standard errors. Statistical analysis was performed using the SPSS package program version 16.0 (SPSS Inc., Chicago, IL). Student's unpaired *T* test was used to compare the means at $P < 0.05$.

3. Results

3.1. Carotenoid content and composition analysis in fruit peel of two peach cultivars during fruit development

The total carotenoid content in the peel of the yellow fleshed-peaches (Jinli) and white cultivar (Hujing) at five developmental stages was analysed. At early ripening stage S1, peel of 'Jinli' and 'Hujing' had similar total carotenoid levels and accumulated only a few carotenoid compounds at about 0.04 μg g^{-1} fresh weight (FW) in both cultivars. However, a significant increase in the carotenoid content of 'Jinli' was observed from the S2 stage, while carotenoid content in 'Hujing' remained low. At the S5 stage, the total carotenoid content in peach peels rose to 1.3 μg g^{-1} FW in 'Jinli' but only 0.10 μg g^{-1} FW 'Hujing' (Fig. 1). HPLC measurements showed that lutein, zeaxanthin, β-carotene and β-cryptoxanthin were predominant in both two cultivars during ripening. All of these carotenoids progressively increased during fruit development in 'Jinli' but nearly remained constant in 'Hujing' during the whole process of fruit development (Fig. 1).

3.2. Expression pattern of carotenoid metabolic genes in fruit peel of two peach cultivars during fruit development

The possibility that differences in carotenoid accumulation in our peach samples could be related to the expression of carotenoid metabolic genes was investigated. Most of the genes showed an