Scientia Horticulturae 237 (2018) 201–206

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Carotenoid accumulation and expression of carotenoid biosynthesis genes in mango flesh during fruit development and ripening

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ARTICLE INFO

Keywords:
Mango
Carotenoid
Gene expression
Development
Ripening

ABSTRACT

In order to investigate the regulation mechanisms of carotenoid biosynthesis in mango flesh, carotenoid content and the expression patterns of 15 carotenogenic gene in flesh of two mango cultivars 'Tainong1'and 'Hongyu' were analsyed during fruit development and ripening. Carotenoid were increasingly accumulated during fruit growth and sharply incaresed during postharvest ripening in both cultivars. In ripe fruit, total carotenoid content in the flesh of 'Tainong1' was 177.16 ug/g (Fresh weight) FW, and for 'Hongyu' 36.24 ug/gFW. The major carotenoids in the flesh were β-Carotene and α-carotene,with minor carotenoids being β-cryptoxanthin, zeaxanthin, lutin and neoxanthin. RT-PCR analysis revealed that the expression of carotenogenic genes *CRTISO*, *PSY*, *ZDS*, *BCH* and *ZEP* were up-regulated in flesh, whereas the transcript levels of *LCYB*, *LCYE* and *NCED* were down-regulated in two cultivars and during fruit development and ripening. *CRTISO*, *PSY*, *ZDS*, *BCH* and *ZEP* transcripts were significantly positively correlated with the total carotenoids content, while no differences were detected for *CRTISO* expression between cultivars. These results suggested that *PSY*, *ZDS*, *BCH* and *ZEP* coordinately contribute to carotenoid accumulation during mango fruit development and ripening.

1. Introduction

Fruit quality is a key determinant of commercial value in the horticulture industry. In addition to traditional properties such as fruit shape and sugar and acid content, properties related to secondary metabolites such as color, flavor, and nutritional and health-promoting properties can have important effects on consumer preferences. Carotenoids are a class of 40-carbon hydrocarbon compounds that are essential for plant survival and involved in many biological processes including photosynthesis, antioxidant protection, hormone synthesis, and pollination (Rock and Zeevaart, 1991; Zhou et al., 2007). They also have beneficial effects on human health: it has been shown that they can help to destroy free radicals, retard the aging process, prevent cardiovascular and eye diseases, and enhance the immune system (Hadley et al., 2002).

The only known non-plant organisms that can synthesize carotenoids are certain microorganisms – animals can only obtain carotenoids from external sources through feeding. Previous studies on carotenoid biosynthesis have shown that, like chlorophyll, cytokinins, gibberellins, and abscisic acid, carotenoids are synthesized via the methylerythritol phosphate (MEP) pathway in the plastids of most higher plants and then become part of a complex metabolic network.

Studies have shown that the carotenoid metabolic pathway in plants is highly conserved, but plant species differ widely in their carotenoid accumulation behavior. In some plants, carotenoid accumulation patterns differ across genotypes. For example, tomato fruit (Howitt and Pogson, 2006), yellow chrysanthemums (Zhu et al., 2010), carrot roots (Clotault et al., 2008), and maize endosperm (Zhu et al., 2008) mainly accumulate lycopene, lutein, β-carotene, and zeaxanthin, respectively. The red and yellow carrot cultivars contained high levels of lutein and lycopene in the root, but carotenoids were not detected in white carrot (Clotault et al., 2008).

Nearly all of the structural genes of carotenoid biosynthetic pathway have been cloned in a range of plant species. There have been extensive studies on various aspects of carotenoid biosynthesis in plants, including the expression and regulation of relevant genes, and the structure and function of diverse metabolic enzymes (Kita et al., 2007; Isaacson et al., 2002; Zhang et al., 2012). Previous researches have shown that the difference of carotenoid accumulation in fruit were determined primarily by differential levels of carotenogenic gene

Abbreviations: DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate-synthase; GGPS, Geranylgeranyl diphosphate synthase; PDS, Phytoene desaturase; ZDS, ζ-carotene desaturase; CRTISO, Carotenoid isomerase; LCYB, Lycopene β-cyclase; LYCE, Lycopene ε-cyclase; PSY, Phytoene synthase; BCH, β-Ring hydroxylase; ZEP, Zeaxanthin epoxidase; NXS, Neoxanthin synthsase; VDE, Violxanthin de-epoxidase; NCED, 9-cis-Epoxycarotenoid dioxygenase; CCD, 1,-4,-7 and -8 Carotenoid ccleavage dioxygenase

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https://doi.org/10.1016/j.scienta.2018.04.009
Received 27 December 2017; Received in revised form 4 April 2018; Accepted 4 April 2018

transcripts, and often these differential expression includes interaction of up- or down-regulation of multiple structural genes.

Mango (*Mangifera indica* Linn.) is a widely grown horticulture crop in many tropical and subtropical countries and is the fifth largest fruit industry in the world after citrus, banana, grape, and apple. The flesh color of ripening mango fruit ranges from light yellow to dark yellow, which results from the content of carotenoid, which is an important factor of nutritional quality and market acceptance (Pott et al., 2003; Vásquez-Caicedo et al., 2006).

Previous studies have reported carotenoid contents in mature fruits from a range of mango germplasm (Shi et al., 2015). However, not much work has been reported studying the molecular basis of carotenoid accumulation patterns during mango fruit development. In order to gain the regulatory mechanisms for carotenoid accumulation in the flesh of mango, two cultivars ('Tainong 1' and 'Hongyu') with varying flesh colours were used to compare the pattern of carotenoid accumulation and the expression of 15 carotenogenesis genes including *DXS*, *DXR*, *GGPS*, *PSY*, *PDS*, *CRTISO*, *ZDS*, *LCYB*, *LCYE*, *BCH*, *ZEP*, *NXS*, *NCED*, *VDE* and *CCD*, during fruit development and ripening.

2. Materials and methods

2.1. Materials

In 2016, 'Tainong 1' and 'Hongyu' fruits of five development stages were collected from orchard of the South Subtropical Crops Research Institute in Zhangjiang, China (Fig. 1). Because of differing flowering dates and maturity rates, each cultivar was sampled at five stages based on known developmental indices: 30(S1), 65(S2), 72(S3), 76(S4) and 80(S5) days after full bloom (DAFB) for 'Tainong 1'; and 45(S1), 70(S2), 105(S3), 111(S4) and 117(S5) DAFB for 'Hongyu'. S3 was the harvest stage when fruit had reached commercial maturity. S5 was the ripe stage. Fruits harvested at S3 were held at 25 °C for ripening.

Nine healthy medium-sized fruits from each cultivar were collected at each sampling point. The collected fruits were peeled immediately, and the flesh was quickly frozen in liquid nitrogen and stored at −80 °C.

2.2. Method

2.2.1. Carotenoid extraction, quantification, and HPLC analysis

Carotenoids in the flesh were extracted and analyzed as described by Xu et al. (2011) with some modification. Pigment extraction buffer (15 ml) was added to 10 g of flesh and the resulting suspensions were sonicated for 30 min followed by centrifugation at 8000 rpm for 15 min at 15 °C. The supernatant containing the pigments was then transferred to a 25-ml flask and the residual pellet was extracted twice using 15 ml pigment extraction buffer each time until it became colorless. The supernatants were combined, giving a solution with a total volume of 25 ml. The 25 ml was washed with saturated NaCl solution until it became neutral. Then, the extract was vacuum-dried and concentrated, dissolved in 2 ml methyl tertiary butyl ether (MTBE), and centrifuged at 12,000 rpm for 15 min. The supernatant was subjected to high-performance liquid chromatography with photodiode array detection (HPLC-DAD).

Chromatography was performed using the U.S. Waters 2695 HPLC system with a 2996 photodiode array detector. The column used was a YMC carotenoid (C30) column (Wilmington, NC, USA). HPLC elution conditions were as follows: mobile phase A: acetonitrile:methanol = 3:1 (containing 0.01% BHT); B: 100% MTBE (containing 0.01% BHT). The flow rate was 1 ml/min, and the injection volume was 20 µl. Gradient elution was performed as follows: 0–10 min, 95:5 A:B; 10–19 min, 86:14 A:B; 19–29 min, 75:25 A:B; 29–54 min, 50:50 A:B; 54–66 min, 26:74 A:B; and 67–76 min, 95:5 A:B. Carotenoid standards were purchased from Sigma Company. Qualitative determination of carotenoids was achieved by comparing the experimentally observed retention times and UV spectra to those of reference standards, while quantitative analysis was carried out using an external standard calibration curve.

2.2.2. Real-time quantitative PCR analysis

Total RNA was extracted from the flesh of 'Tainong 1' and 'Hongyu' fruits at different developmental stages as described by Wu et al. (2014). The transcript levels of carotenogenic genes were analyzed by means of quantitative real-time PCR using an iCycler instrument (Bio-Rad, USA). The reaction volume was 15 µl, which included 1.5 µl template, 1.2 µl RT-qPCR primer pairs, 4.8 µl water, and 7.5 µl of 2 × Platinum SYBR Green supermix (Bio-Rad, USA). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 5 s, 58 °C for 15 s, and 72 °C for 10 s. The gene-specific primers for real-time PCR were designed according to cDNA sequence of 15 carotenogenic genes by searching against the mango transcriptome database (Wu et al., 2014) (Table 1).

Melting peaks and dissociation curves were constructed for all of the primers used in this work to verify that each pair produced only a single product. The *actin* gene was used as an internal reference in the real-

Fig. 1. Photographs of flesh color of 'Tainong 1' and 'Hongyu' during fruit development and ripening. DAFB stands for Days After Full Bloom.