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Effect of the combination of ethylene and red LED light irradiation on carotenoid accumulation and carotenogenic gene expression in the flavedo of citrus fruit

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ABSTRACT

The effects of ethylene and red light-emitting diode (LED) light (660 nm) on the accumulation of carotenoid and expression of genes related to carotenoid biosynthesis were investigated in the flavedo of Satsuma mandarin. The results showed that the contents of β-cryptoxanthin, all-*trans*-violaxanthin, 9-*cis*-violaxanthin and lutein were simultaneously increased along with the total carotenoid accumulation by the red LED light. With the ethylene treatment, the contents of β-carotene and β-cryptoxanthin were increased, while the content of lutein was decreased in the flavedo. The suppression of lutein accumulation by ethylene was inhibited when the ethylene treatment was performed under the red LED light. With the combination of ethylene and red LED light treatments, the contents of β-cryptoxanthin and lutein were simultaneously increased. Gene expression results showed that simultaneous increases in the expression of *CitPSY*, *CitPDS*, *CitZDS*, *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, and *CitZEP* contributed to the accumulation of β-cryptoxanthin and lutein in the treatment of ethylene combined with red LED light. The results presented might provide new strategies to enhance the commercial and nutritional value of citrus fruit.

1. Introduction

Carotenoids, important natural isoprenoid pigments, fulfill a variety of important functions in plants and play a critical role in human nutrition and health (Schwartz et al., 1997; Cunningham and Gantt, 1998; Havaux, 1998; Ledford and Niyogi, 2005). In citrus fruit, carotenoids are responsible for the external and internal coloration, and their contents and composition are important indexes for the commercial and nutritional quality of the fruit. The carotenoid accumulation in citrus fruit has been extensively investigated over the past decade (Kato et al., 2004, 2006; Rodrigo et al., 2004; Rodrigo and Zacarías, 2007; Kato, 2012; Zhang et al., 2012a; Ma et al., 2013). Moreover, genes encoding enzymes for the main steps of carotenoid biosynthesis have been isolated and their expression has been characterized in the flavedo and juice sacs of different citrus varieties (Kato et al., 2004, 2007; Alquézar et al., 2008, 2009; Fig. 1). In previous studies, we found that as fruit maturation progressed, a simultaneous increase in the expression of genes (*CitPSY*, *CitPDS*, *CitZDS*, *CitLCYb*, *CitHYb*, and *CitZEP*) led to massive β,β-xanthophyll accumulation in the flavedo and juice sacs of Satsuma mandarin and Valencia orange (Kato et al., 2004). In addition, the cyclization of lycopene by *CitLCYb1* and *CitLCYb2* played an important role in determining the profiles of carotenoids in the orange stage of the citrus fruit (Zhang et al., 2012b).

Light is an important environmental factor for plants. It is not only an essential energy source for plants, but also an important signal for plant growth and development (Chory et al., 1996; Clouse, 2001; Kim et al., 2002). In higher plants, sensing of light is carried out by various light photoreceptors (Briggs et al., 2001). Thus, plants exhibit different responses to different wavelengths of lights (Goins et al., 1997; Xu et al., 2011; Jung et al., 2013). Wu et al. (2007) reported that β-carotene content was much higher in the red light-treated group than blue light-treated group in leaves and stems of pea seedlings. In tomatoes, the accumulation of lycopene along with an increase in total carotenoid content, was also observed in response to red light treatment (Alba et al., 2000; Schofield and Paliyath, 2005; Liu et al., 2009). In citrus fruit, the red LED light was effective in enhancing carotenoid content, especially the content of β-cryptoxanthin, while blue LED light had no significant effect on the carotenoid content in the flavedo of Satsuma mandarin (Ma

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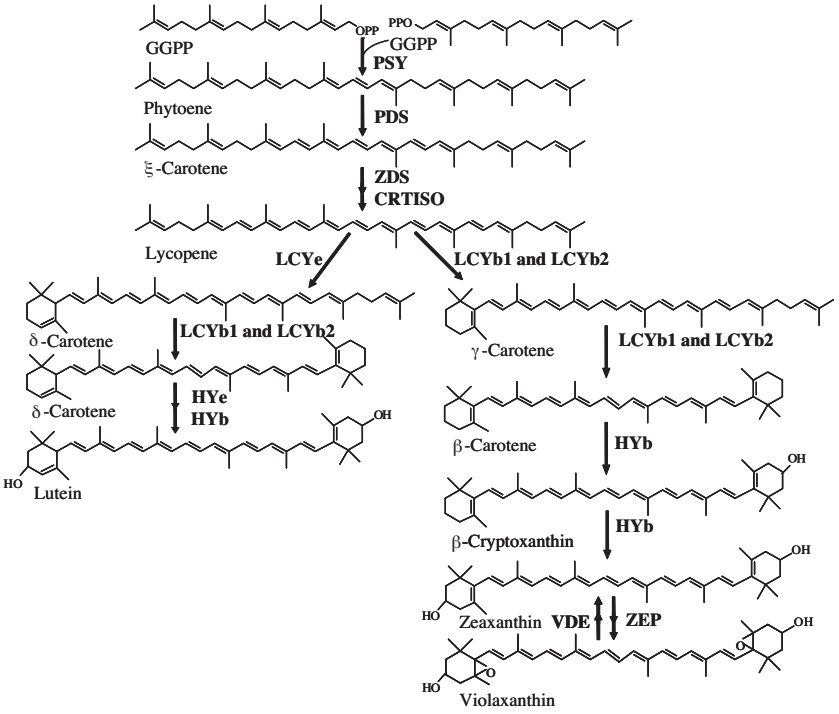

Fig. 1. Carotenoid biosynthetic pathway in citrus. GGPP, geranylgeranyl diphosphate. Enzymes are named according to the designation of their genes. PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LCYb, lycopene β-cyclase; LCYe, lycopene ε-cyclase; HYe, ε-ring hydroxylase; HYb, β-ring hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase.

et al., 2012). Even though citrus fruit are non-climacteric and produce a low level of ethylene during ripening, they are sensitive to exogenous ethylene. In recent years, exogenous application of ethylene has been widely employed to enhance the external coloration of citrus fruit. Rodrigo and Zacarías (2007) reported that exogenous ethylene treatment increased the content of carotenoids; as a result, the degreening process of citrus fruit was accelerated. To date, however, information on the effects of the combination of ethylene and the red LED light on carotenoid accumulation in citrus is still unknown. As citrus fruit pulp matures earlier than the peel, the pulp reaches maturity and is edible, while the peel is still green in October. In the present study, to promote peel degreening and improve the carotenoid content and composition in the flavedo of citrus fruit, the effects of red LED light (660 nm) and ethylene on carotenoid accumulation and the expression of genes related to carotenoid biosynthesis were investigated. The results presented herein might provide new strategies to enhance the carotenoid production in citrus fruit.

2. Materials and methods

2.1. Plant Materials

Fruit of Satsuma mandarin (*Citrus unshiu Marc.*) were harvested in October approximately 150 days after anthesis at the Fujieda Farm of Shizuoka University (Shizuoka, Japan). In this stage, the fruit peel just begins to degreen with the accumulation of carotenoids. Fruit 45–50 mm in diameter and light green in color were used as materials.

2.2. Treatment

Fruit were placed in 35-L sealed plastic chambers. The fruit were continuously treated for 6 days at 20 °C as follows: with 150 μmol m^{-2} s^{-1} red (660 nm) LED lights; with 50 μL L^{-1} ethylene in the dark; with 50 μL L^{-1} of ethylene under 150 μmol m^{-2} s^{-1} red LED lights. Fruit stored at 20 °C in the dark were used as the control. After each treatment, the flavedo was immediately frozen in liquid nitrogen, and kept at −80 °C until used.

2.3. Color measurement

Color measurement was carried out with a colorimeter (NR-11, Nippon Denshoku, Japan). The CIE 1976 L^*a^* b^* color scale was adopted. The hue angle [H° = arctangent (b^*/a^*) and Citrus color index (CCI) = [$1000 \times a^*/(L^* \times b^*)$] were calculated according to methods previously reported (Zhou et al., 2010).

2.4. Extraction and determination of carotenoids

The identification, extraction and quantification of carotenoids in citrus have been described previously (Kato et al., 2004). β-Carotene, β-cryptoxanthin, all-*trans*-violaxanthin, 9-*cis*-violaxanthin and lutein were quantified in the flavedo of the fruit during the experimental period. The content of carotenoids was expressed as μg g^{-1} fresh weight. Carotenoid quantification was performed in three replicates.

2.5. Total RNA extraction and real-time quantitative RT-PCR

Total RNA was extracted from the flavedo fruit according to a previously reported method (Kato et al., 2004). The total RNA was cleaned up with the RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase digestion. The reactions of reverse transcription (RT) were performed with 2 μg of purified RNA and a random hexamer at 37 °C for 60 min using TaqMan Reverse Transcription Reagents (Applied Biosystems).

TaqMan MGB probes and sets of primers for *CitPSY*, *CitPDS*, *CitZDS*, *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, *CitZEP* and *CitVDE* were designed according to Ma et al. (2013) (Table 1). For