Effects of blue LED light irradiation on pigment metabolism of ethephon-degreened mandarin fruit

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ABSTRACT

To improve peel color of ethephon-degreened citrus fruit, in this study, the effects of blue light emitting diode (LED) light (450 nm) irradiation on chlorophyll and carotenoid metabolism of ethephon-degreened mandarin fruit were evaluated. Deeper and faster coloration were observed in blue LED light-irradiated fruit, accompanied by the accumulation of carotenoid and the degradation of chlorophyll. Blue LED irradiation could shorten the degreening time of ethephon-degreened mandarin fruit. The accumulation of total carotenoid, lutein, violaxanthin, β-cryptoxanthin and zeaxanthin could well explain the faster coloration of the blue LED-irradiated mandarin fruit, while the accumulation of β-cryptoxanthin could well explain the deeper color of the blue LED-irradiated mandarin fruit. For chlorophyll metabolism, the increased expression of chlorophyll catabolism related genes [CitChlase, CitPao and CitRCCR], especially the increased expression of CitChlase might partly explain the sharply decrease of chlorophyll a and b in blue LED-irradiated fruit. However, it seems that both the expression levels of genes in metabolism of carotenoid and chlorophyll could only partly explain the changes in carotenoid and chlorophyll contents, more complex mechanisms might be interacting. Blue LED light may be a potential technique for improving the external color and commercial value of ethephon-degreened citrus fruit.

1. Introduction

The external color of fruit is one of the primary attributes that determines the commercial quality and a major determinant of consumer acceptance in the fresh market (Alos et al., 2006; Rodrigo et al., 2004). Light is one of the most important environmental factors affecting the pigments metabolism of vegetables and fruits. The regulation of pigments by light has been extensively studied, and in general, light can change the color formation of fruits and provide a deeper color. Evidence accumulated these years indicated that citrus peel coloration is influenced by the light quantity and quality. Citrus fruits located inside the canopy developed a different peel color compared with those located in the outer canopy (Iwagaki and Kudo, 1977; Lado et al., 2015). Citrus fruits bagged with black-colored polyethylene-film showed a lower coloration (Uchida et al., 1985). Light can also affect the carotenoid content in citrus peel after harvest. Usually, the effectiveness of an artificial light treatment depends on the wavelength and the intensity of the light emitting diode (LED) light, as well as the exposure duration (Jiao et al., 2007). Tao et al. (2003) found that light treatment can increase the carotenoid content, especially the accumulation of β-cryptoxanthin (β-Cry) in citrus fruit peel. In addition, the effectiveness varies with citrus genotypes. Zhang et al. (2015) demonstrated that 100 mol m⁻² s⁻¹ blue light emitting diode (LED) light alone was effective for increasing carotenoid content, especially β-Cry in the juice sacs of Satsuma mandarins after in vitro culturing for four weeks. While in Valencia orange, 50 mol m⁻² s⁻¹ blue LED light treatment induced carotenoid accumulation by increasing the contents of all-trans-violaxanthin and 9-cis-violaxanthin and up-regulating the expression of related genes ([phytoene synthase (CitPSY), phytoene desaturase (CitPDS), ζ-carotene desaturase (CitZDS) and lycopene β-cyclase 2 (CitLCyB2)]).

Plants can detect different environmental light signal due to the existent of a complex system of photoreceptor molecules. Many blue light photoreceptors, including phototropins (phot), cryptochromes (cry) and ZEITLUPE (ZTL)/Flavin-binding Kelch repeat F-box protein (FKF1)/LOV Kelch Protein (LKP2) family are involved in diverse photomorphogenic responses induced by blue irradiation (Huché-Thélier et al., 2016). Quecini (2007) demonstrated that a large portion of the genes involved in light responses from model species (the phytochrome, cryptochrome and phototropin families) are present in citrus and they share extensive protein sequence conservation in several regions, including functionally characterized domains.
The color of citrus fruit peel is determined by the contents and composition of pigments. Chlorophylls (Chl) are one of the critical pigments that provide green colors. Chl a is the major component of Chl in citrus fruit, followed by Chl b (Rodrigo et al., 2013; Yamauchi et al., 1997). Carotenoids are also important citrus fruit pigments, and their content and composition are responsible for the characteristic color of most citrus fruits, ranging from yellow, orange to deep red (Alquezar et al., 2008; Kato et al., 2004; Peng et al., 2013b; Rodrigo et al., 2013; Rodrigue-Concepcion and Stange, 2013, Fig.51). Both chlorophylls and carotenoids have maximum absorption at blue regions. Chlorophyll a and b absorb strongly at 430 and 453 nm, respectively, and the carotenoid pigments lutein and β-carotene absorb strongly at 448 and 452 nm, respectively (Wright and Shearer, 1984).

In citrus fruit, the accumulation of carotenoids is partly regulated at the transcriptional level (Kato et al., 2006; Toledo-Ortiz et al., 2010), and the biosynthesis of carotenes was more sensitive to light than that of xanthophylls (Gao et al., 2011). Many studies have identified and characterized the carotenoid biosynthetic genes in citrus and correlated their expression with carotenoid profile and accumulation during fruit ripening (Costa et al., 2012; Kato et al., 2004). The gene PSY is a rate-limiting gene in the carotenogenesis in calluses of two sweet oranges and Murcott tangor, and CRTISO gene is a light-induced gene in citrus callus of four citrus genotypes (Gao et al., 2011). A light-response motif existed in the PSY promoter region of sweet orange (Zeng et al., 2013), and this motif might interact with transcription factors regulating the expression level of this rate-limiting enzyme. Besides, the light effects on the carotenoids biosynthesis and expression of related genes in callus of citrus largely varied with genotypes. However, the expression levels of carotenogenesis genes not always resulted in the corresponding expected changes in carotenoids accumulation, suggesting that more complex mechanisms may be interacting (Gao et al., 2011).

The influence of light on Chl levels in fruit has also been investigated, the Chl content decreased quickly, which resulted in its earlier color development (Tao et al., 2003). In citrus fruit, Chl catalysis is one of the main positive reason contributing to degreening, and Chlase plays a central role in Chl breakdown during citrus fruit color-break. Chl quantity at the cellular level was negatively correlated with plastid Chlase accumulation (Shemer et al., 2008). However, citrus Chlase initially accumulates as an approximately 35-kD precursor, which is subsequently N-terminally processed to approximately 33-kD mature forms (Shemer et al., 2008). The mature version of Chlase led to extensive Chl breakdown, suggesting that Chlase functions as a rate-limiting enzyme in Chl catalysis controlled via posttranslational regulation (Harraz-Saad et al., 2007). Besides, CitERF5, CitERF6, CitERF7, and CitERF13 showed negative correlations with Chl content (Xie et al., 2014). Yin et al., 2016 Yin et al. (2016) found that the transcriptional changes of CitERF13 were closely correlated with fruit peel degreening. Possibly, CitERF13 directly binds to the pheophytin pheophorbide hydrolase (CitPPH) promoter and enhances its activity.

In citrus cultivars harvested early in the season, such as satsuma mandarin and grapefruit, the internal maturity of the fruit does not always correspond to the external color. In general, their flesh becomes edible when the external peel is still green (Chaudhary et al., 2015; Yamauchi et al., 1997). However, for citrus, the marketing of ‘perfectly orange oranges’ has resulted in consumer associating green-colored peel with unripe fruit (Poole and Gray, 2002). Therefore, to increase the fruit marketability, a common commercial postharvest practice that involves ethylene or 2-chloroethylphosphonic acid (ethephon, an ethylene releasing chemical) degreening treatment (Chaudhary et al., 2015; Zhou et al., 2010) were conducted. The application of exogenous ethylene/ethephon can accelerate the external color change from green to the characteristic orange/yellow (Sdiri et al., 2012), while without impairment on internal fruit quality (Mayoumi et al., 2011).

Nevertheless, ethylene degreening is not a simple simulation of the ‘natural ripening process’, the color changes in ethylene-degreened citrus fruit differ from those fruit with on-tree maturation (Shimokawa et al., 1978; Yamauchi et al., 1997). Shimokawa et al. (1978) reported that the size of chloroplasts in the ethylene-treated satsuma mandarin became small, which indicated that the collapse of chloroplasts and the degradation pathway of Chls in ethylene-degreened satsuma mandarin fruit was different from that of on-tree matured fruit. Chlase a, a derivative of Chl, did not decrease in ethylene-treated fruit during storage, whereas it decreased significantly in on-tree matured fruit, and a small amount of Pyrophb a was found in the fruit with on-tree maturation, but not in ethylene-degreened fruit (Yamauchi et al., 1997). Visually, in our previous studies, we determined that the peel color of ethephon-degreened mandarin fruit were pale and yellow-colored compared to ‘on-tree’ matured fruit. Therefore, the finding of an alternative or assisted degreening method is important for improving the external quality of ethylene-degreened citrus fruit.

As mentioned above, light is one of the most important environmental factors affecting the pigment metabolism of vegetables and fruits. In the context of ethylene degreening treatment, the effect of light on the color of citrus peel was more complicated. Ma et al. (Ma et al., 2012) reported that red light induced the accumulation of β-Cry in harvested Satsuma mandarins (Citrus unshiu Marc.), but this accumulation was not affected by blue light. The possible underlying mechanism was illustrated in another publication by the same group (Ma et al., 2015). However, our previous research, which was conducted in 2014 and 2015, indicated that the blue LED light provided better improvement in the peel color of ethephon-degreened mandarin fruit compared to that obtained using red LED light (data not shown). In the current study, to well explain our results, the effects of blue LED irradiation on pigment (chlorophyll and carotenoid) metabolism as well as the expression of related genes in ethephon-degreened mandarin fruit were analyzed. These results could provide a new effective method to improve the visual appearance of postharvest degreening citrus fruit.

2. Materials and methods

2.1. Fruit materials

Early-season Satsuma mandarins (Citrus unshiu Marc.) were harvested from the Beibei District of Chongqing, China, and then transported to the laboratory. The fruit were placed at 5–8 °C for 24 h (pre-cooling). Disease- and damage-free fruit with a uniform size, color, and maturity were selected and used for this study.

2.2. Treatment and sampling

The fruit was immersed in a 1000 mg L⁻¹ ethephon solution for 1 min (Zhou et al., 2010) and dried in air, and then the fruit was continuously treated at 20°C for 10 h under 300 Lux blue LED light (450 nm), or in the dark (positive control). The distance from the lamp to the fruit is 40 cm, and the fruit were placed with the stem end up. After measurement of peel color, Each sampling consisted of ten fruit for each treatment, separated into 3 replicates, with ten fruit for each replicate. The fruit was continuously treated at 20°C for 10 h under 300 Lux blue LED light. Disease- and damage-free fruit with a uniform size, color, and maturity were selected and used for this study.
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