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Effects of high temperatures on UV-B/visible irradiation induced postharvest anthocyanin accumulation in 'Yunhongli No. 1' (*Pyrus pyrifolia* Nakai) pears

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ABSTRACT

Red Chinese sand pears (*Pyrus pyrifolia* Nakai) have seen increased cultivation in China in recent years, prized for their attractive market value and nutritional benefits. However, poor fruit coloration has been a noticeable problem. Postharvest ultraviolet-B (UV-B)/visible irradiation has been used to improve anthocyanin accumulation and thus the coloration of fruit skin in apple and other fruits. In this study, the efficacy of UV-B/visible irradiation was evaluated under high- (27 °C) and low-temperature (17 °C) conditions using red Chinese sand pear 'Yunhongli No. 1' as a model. The results showed that UV-B/visible irradiation was more effective in inducing anthocyanin synthesis in peel tissues and improving fruit coloration at 27 °C than at 17 °C. PAL activity was markedly higher at 27 °C than at 17 °C. Expression of *PyMYB10* and five anthocyanin structural genes, *PpPAL*, *PpCHI*, *PpCHS*, *PpF3H*, and *PpANS*, was also higher in fruit irradiated at 27 °C than in fruit irradiated at 17 °C. For *PpUFGT*, transcription reached a maximum at 48 and 240 h after the onset of irratiation at 27 °C and 17 °C, respectively, but the peak value was lower at 27 °C than at 17 °C. There was no difference in expression of *PpDFR* between 17 °C and 27 °C irradiation temperatures. These results indicated that high temperatures (27 °C) enhanced UV-B/visible irradiation induced postharvest anthocyanin accumulation in 'Yunhongli No. 1' pears by up-regulating *PyMYB10* and anthocyanin structural genes and increasing the activity of phenylalanine ammonia-lyase.

1. Introduction

Like other Asian pears, Chinese sand pears (*Pyrus pyrifolia* Nakai) are eaten at a firm and crisp stage right after harvest or storage. Traditionally, the pear cultivars have green, yellow, or russet-brown skins. In recent years, red pear cultivars have rapidly become more popular in China for their attractive skin color and potential nutritional value. However, the development of this characteristic red color is not always uniform and can vary by cultivar, region, and agriculture practices (Tao et al., 2004).

Red coloration of pear skin is known to depend mainly on the composition and concentration of anthocyanin (Steyn et al., 2005). The main cyanidin pigments accounting for the red color in pear skin are cyanidin 3-galactoside and peonidin 3-galactoside (Dayton, 1966; Dussi et al., 1995). The accumulation of anthocyanin in red Chinese sand pears growing in Yunnan, China, increases constantly with fruit maturation under both natural and bagging-treatment conditions (Huang et al., 2009a, 2010). In this way, they differ from European pears (*P. communis* L.), in which red coloration decreases toward harvest and anthocyanin accumulation reaches maximum about midway between anthesis and harvest (Steyn et al., 2005). However, Feng et al. (2008, 2010) showed that the pattern of anthocyanin accumulation in 'Mantianhong' and 'Aoguan' pears (bud sport mutated from 'Mantianhong') in Shandong, China was similar to that in European pears. This indicated that anthocyanin biosynthesis in pear skin was regulated not only genetically but also by environmental factors and agricultural management.

The biosynthetic pathways related to anthocyanin in petunias (Holton and Cornish, 1995), temperate rosaceous fruits (Han et al., 2010), and grapes (Bogs et al., 2006) are all well established. The structural and regulatory genes related to anthocyanin biosynthesis have also been identified, cloned, and functionally analyzed. Recently, many studies have revealed that anthocyanin biosynthesis in apples and grapes is controlled by a distinct clade of R2R3 MYB transcription factors (Takos et al., 2006; Ban et al., 2007; Espley et al., 2007, 2009; Kobayashi et al., 2004; Walker et al., 2007; Bogs et al., 2007). Lately, the function of *F3′H* and *F3′5′H* genes in grapes (Bogs et al., 2006) and *F3′H* genes in apples (Han et al., 2010) has been demonstrated via their ectopic

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expression in petunia line Skr4 × Sw63, tobacco and *Arabidopsis tt7* mutants. Most of the structural and regulatory genes in the anthocyanin biosynthetic pathway in pears have also been identified and cloned (Fischer et al., 2007; Feng et al., 2010; Zhang et al., 2010). Five anthocyanin biosynthesis enzymes, namely phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), and anthocyanidin synthase (ANS), as well as UDP-glucose: flavonoid 7-O-glucosyltransferase (F7GT), were isolated from young leaves of 'Conference' and 'Pyrodwarf' (*P. communis*) pears (Fischer et al., 2007). Feng et al. (2010) reported a light-induced *PyMYB10* gene in *P. pyrifolia* that could regulate anthocyanin biosynthesis. Recently, *DFR* and *ANS* genes in *P. pyrifolia* have been considered the limiting factors for the skin coloration of the mildly colored pear 'Zaobaimi' (Zhang et al., 2010).

Anthocyanin synthesis in pears is also influenced by environmental factors, especially light and temperature (Dussi et al., 1995; Steyn et al., 2004a, 2004b). Light shows two opposing effects in pears: it is a prerequisite for anthocyanin synthesis and also an important factor for the loss of red color through anthocyanin degradation (Steyn et al., 2004b). The extent of light-responsive red coloration in apples is dose-dependent (Gould et al., 2009). Huang et al. (2009a, 2009b) found that anthocyanin biosynthesis in red Chinese sand pears is highly light-dependent and requires intense light. In addition to light intensity (quantity), light wavelength (quality) is also one of the major factors affecting anthocyanin synthesis in pears (Dussi et al., 1995). Wavelengths that transmit above 600 nm showed the largest effects on fruit coloration in European pear cultivars (Dussi et al., 1995). Light in the ultraviolet-B (UV-B) region (280–315 nm) was most effective for fruit coloration in apples (Arakawa et al., 1985; Arakawa, 1988; Ubi et al., 2006). Temperature also has a profound effect on the accumulation of anthocyanin in pears (Steyn et al., 2005). Steyn et al. (2004a, 2004b) reported that low temperatures increased anthocyanin accumulation in European pear cultivars and high temperatures decreased it, possibly through regulation of PAL and UFGT. In apples and grapes, the increased anthocyanin accumulation seen at low temperatures has been confirmed to be caused by induction of the expression of genes related to anthocyanin biosynthesis (Dong et al., 1995; Ubi et al., 2006; Ban et al., 2007; Mori et al., 2005). However, no investigation into the effects of temperature on the expression of genes related to anthocyanin synthesis in pears has yet been published.

UV-B irradiation has been tested as a postharvest treatment to improve color and/or nutritional benefits of apples, grapes, cherries, blackberries, and mushrooms (Ubi et al., 2006; Li et al., 2009; Kataoka et al., 2005; Basiouny, 1998; Roberts et al., 2008). Generally, the effects of UV-B radiation depend mainly on the crop, cultivar, UV-B dosage, irradiation time, temperature, and humidity conditions; it can also affect physiological processes at the gene-expression level. In apples, UV-B/visible irradiation up-regulated the structural and regulatory genes related to anthocyanin biosynthesis under low temperature conditions (17 °C) (Ubi et al., 2006; Ban et al., 2007). Li et al. (2009) observed a higher level of trans-resveratrol in the skins of UV-B-irradiated grapes kept in cold storage (−1 °C). Basiouny (1998) suggested that blackberry fruits should be stored under UV-B irradiation for only 1–2 weeks, depending on the time of harvest and cultivar. Roberts et al. (2008) conducted several commercial-scale UV-B treatments to enhance vitamin D production in mushrooms.

The objectives of this study were to investigate the regulatory mechanism of anthocyanin accumulation induced by postharvest UV-B/visible irradiation and to understand how temperatures influence the efficacy of UV-B/visible irradiation on anthocyanin accumulation on the gene-expression and enzyme-activity levels.

2. Materials and methods

2.1. Plant materials and experimental treatments

Samples of 'Yunhongli No. 1', a type of red Chinese sand pear (*P. pyrifolia*), were obtained from a commercial orchard (25°N, 102°E) in Kunming, Yunnan Province, China. Six uniform trees were selected. One hundred fruitlets per tree were bagged using waxed paper 20 days after full bloom to prevent skin russet and coloring. Fruit with bags were harvested at commercial maturity and transported to the laboratory immediately after harvest. Three hundred uniform and defect-free fruits were pooled and randomly divided into two groups for 17 °C and 27 °C UV-B/visible irradiation treatments, respectively.

UV-B/visible irradiation was applied using UV-B plus white light based on the method described by Ubi et al. (2006), with minor modifications. UV-B was generated by one UV lamp (PHILIPS TL 20W/12 RS, 290–315 nm, Amsterdam, Holland) and white light by three fluorescent tubes (FSL T_8 36W/765, Foshan, Guangdong, China). The photon flux density (PFD) at the floor of the incubators (2 m from the light source) was 4.56 μmol m^{-2} s^{-1} as measured by *Li-COR Li-188B* quantum meter (LI-COR, Lincoln, NE, USA). After the bags were removed, fruits were kept in temperature-controlled incubators in the dark overnight to equilibrate fruit temperature prior to application of UV-B/visible irridiation. The treatments were maintained continuously for 10 days. Treated fruits were sampled after 12, 24, 48, 124, and 240 h of irradiation.

2.2. Fruit color measurement

Pear skin color was measured on the most deeply colored part of fruit with a colorimeter (MiniScan XE Plus, HunterLab, USA), which provided CIE L^*, a^*, and b^* value. These values were converted to a hue angle degree ($h^\circ = \arctan[b^*/a^*]$) and chroma ($C = [(a^*)^2 + (b^*)^2]^{1/2}$), which quantified the intensity or purity of the hue (McGuire, 1992). Twenty fruits were used per treatment.

2.3. Fruit sampling for anthocyanin, enzyme activity, and gene expression assays

Peel tissues (including the epidermis and 1–2 mm of hypodermal cortex) were excised with a potato peeler from the colored sides of the fruits and immediately frozen in liquid N_2. They were then stored at −80 °C for further analysis of anthocyanin contents, enzyme activity, and gene expression. Each treatment included three replicates, and each replicate covered five randomly selected fruits.

2.4. Extraction and measurement of total anthocyanin content

One gram of tissue was extracted using methanol–HCl (99.99:0.01) solution. Anthocyanin content was quantified by the pH differential method and was presented as mg cyanidin-3-galactoside per 100 g fresh tissue (Dussi et al., 1995). Absorbance of each extraction (100 μl) was measured using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA) at 510 nm and 700 nm in buffers of pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}]$ with a molar extinction coefficient of cyanidin-3-galactoside of 3.02×10^4.

2.5. Assay of phenylalanine ammonia-lyase activities

Phenylalanine ammonia-lyase (EC 4.3.1.5) activity was assayed by the procedure described by Huang et al. (2009a). The assay mixture contained 0.8 ml crude enzyme and 1 ml of 50 mM