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Blue light induced anthocyanin accumulation and expression of associated genes in Chinese bayberry fruit

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ABSTRACT

Effect of blue light-emitting diode light (470 nm) treatment on anthocyanin accumulation and expression of related genes in Chinese bayberry fruit was investigated. Bayberries hand-harvested when nearly 3/4 of the fruit turned red were exposed to blue light for 8 days at 10 °C. Anthocyanin accumulated in bayberries during storage, which can be drastically enhanced by blue light treatment. The blue light treatment also increased the expression of *MrMYB1* and structural genes involved in anthocyanin biosynthesis such as *MrCHI*, *MrF3H*, *MrF3′H*, *MrDFR1*, *MrDFR2* and *MrANS*. These findings suggested that the induction of anthocyanin accumulation by blue light was associated with the increased expression of anthocyanin biosynthetic and regulatory genes. Our results suggested that blue light treatment may be a useful technique to enhance commercial and nutritional value of Chinese bayberry fruit.

1. Introduction

Chinese bayberry (*Myrica rubra* Sieb. and Zucc.) is an economically important subtropical fruit crop native to southern China and other Asian countries (Zhang et al., 2008). It is a very popular berry fruit because of its unique sweet sour taste, attractive color and exquisite flavor. Anthocyanins are the major contributors to the red color pigment in Chinese bayberry fruit and are also used by consumers to judge its quality (Zhang et al., 2005; Yang et al., 2009).

Anthocyanins are synthesized via the flavonoid pathway (Fig. 1), a branch of the phenylpropanoid pathway. The genes encoding the enzymes of the flavonoid biosynthesis pathway in bayberry fruit have already been isolated. All the genes involved in the pathway except for chalcone synthase (*CHS*) and chalcone isomerase (*CHI*), were up-regulated during fruit ripening, which was consistent with color change (Niu et al., 2010). Additionally, Niu et al. (2010) reported that anthocyanin biosynthesis in bayberry fruit was controlled by a R2R3 MYB transcription factor, activating the structural genes in the flavonoid pathway.

The formation and accumulation of anthocyanins in fruit is affected by diverse developmental processes and enviromental cues (Winkel-Shirley, 2001). Light is an important factor regulating anthocyanin synthesis in plants. However, different types of lights might have distinct effects (Saure, 1990; Kataoka and Beppu, 2004; Feng et al., 2010). It was reported that among the different light wavelengths, blue light is one of the most effective regulating anthocyanin biosynthesis (Chen et al., 2006; Kadomura-Ishikawa et al., 2013). For example, Chen et al. (2006) found that blue light strongly induce anthocyanin accumulation in *Arabidopsis* seedlings and it was more effective than red and far-red light. In apple fruit, anthocyanin production had a strong dependence on both intensity and quality of light. Blue and UV lights were most effective and far red least effective, or even inhibitory (Saure, 1990). Moreover, Our resent research has revealed that blue light-emitting diode (LED) light was effective for stimulating anthocyanin accumulation in postharvest strawberry fruit, which was resulted from the activation of key enzymes in the pentose phosphate, shikimate, phenylpropanoid, and flavonoid pathways (Xu et al., 2014).

To date, many studies have been performed to prolong the storage life of Chinese bayberry fruit (Wang et al., 2009; Yang et al., 2009; Jin et al., 2012), however, information on the effect of blue light on the accumulation anthocyanin in bayberry is still limited. In the present study, to enhance the content of anthoycanin in bayberries, the effects of blue (470 nm, 40 μmol m^{-2} s^{-1}) LED light on anthocyanin accumulation and the expression of related genes was investigated.

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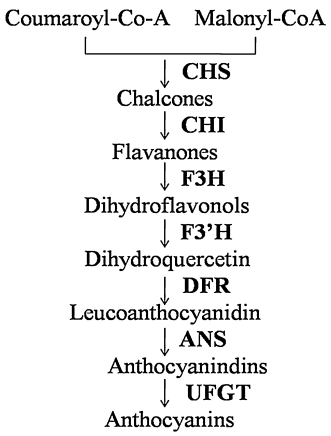

Fig. 1. Diagrammatic representation of the flavonoid pathway. Enzymes for each step are shown in bold, and include: chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-β-hydroxylase (F3H), flavonoid 3′-hydroxylase, dihydroflavonol-4-reductase (DFR); anthocyanidin synthase (ANS), and UDP-glucose: flavonoid-3-O-glycosyltranferase (UFGT).

2. Materials and methods

2.1. Plant materials

Bayberries (*Myrica rubra* Sieb. and Zucc. cv. Biqi) used in this study were hand-harvested at 99 days after flowering when nearly 3/4 of the fruit turned red (CIRG = 1.74) from six adult trees grown in a homogeneous orchard located at Cixi county (Zhejiang, China) under normal culture practice. All fruit were transported to the laboratory within one hour and selected for uniformity without any damage.

2.2. LED blue light irradiation

Bayberries were irradiated with blue (470 nm) light at an intensity of 40 μmol m^{-2} s^{-1} for 8 days at 10 °C. Fruit stored at 10 °C in the dark were used as the control. There were three replicates of five kilograms of fruit each per treatment, and the experiment was conducted twice. Fruit samples were taken at 2-day intervals during storage and immediately frozen in liquid nitrogen and kept at −80 °C until required.

2.3. Color index determination

Fruit surface color was measured with a Minolta CR-410 colorimeter, using the CIELAB color system. The color index of bayberries was calculated as CIRG = $(180 - h)/(C + L)$ (Carreñom et al., 1995), where L is the lightness and corresponds to a black-white scale, h is the hue angle on the color wheel, and C is the chroma, a measure of the intensity of color. Three equidistant color measurements were made around the equator of each berry, and the mean values for twenty fruit from each treatment were subjected to statistical analyses.

2.4. Total anthocyanin content determination

To prepare the fruit extract, 1 g samples from each replicate were homogenized with 5 mL of pre-cooled acidified water (3% formic acid), and after centrifugation at 10,000 g for 15 min (4 °C), then another 5 mL of pre-cooled acidified water was used to extract the residue again. The supernatant was combined to make the final volume of 25 mL for analysis. Total anthocyanin content of bayberry extract was measured using the pH differential method (Cheng and Breen, 1991). Absorbance was measured at 510 and 700 nm, respectively, in different buffers at 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-glucoside (Cy-3-Glu) of 29,600. Results were expressed as milligrams of Cy-3-Glu equivalents per gram of fresh weight.

2.5. Total RNA extraction and cDNA synthesis

Total RNA used for q-PCR analysis was extracted from 10 fruits of each treatment using the Plant Total RNA Extraction Kit (Genotheramics, Suzhou, China) according to the manufacturer's instructions. Extracted RNA was treated with amplification grade RNase-free DNase1 (Omega, Norcross, GA, USA) to remove any DNA contamination prior to cDNA synthesis. Reverse transcription (RT) was carried out using 2 μg of total RNA and the SuperRT First Strand cDNA Synthesis Kit (CWBIO, Beijing, China), following the manufacturer's instructions.

2.6. Quantitative real-time PCR (q-PCR)

The sequences of the primers used for q-PCR are shown in Table 1. Q-PCR analysis was performed using the Mx3000P q-PCR System (Agilent Stratagene, Santa Clara, CA, USA). Two-step q-PCR analysis was performed in a total volume of 12.5 μl, containing 0.5 μl of the synthesized cDNA, 0.25 μl of 10 μM each forward and reverse primers (Table 1), 6.5 μl of the SYBR Green PCR master mix (Thermo Scientific, Pittsburgh, PA, United States) and 5 μl RNase-free water. The thermal cycling conditions consisted of an initial denaturation step of 95 °C for 7 min, followed by 40 cycles of 95 °C for 15 s combined with each primer specific annealing temperature for 30 s ranged from 50 °C to 60 °C, then completed with a melting curve analysis program. *MrACT* was used to normalize as endogenous reference. Three measurements for each biological replicate sample were performed.

2.7. Statistical analysis

All values are shown as the mean ± SD. Student's unpaired T test was used to compare the means at $P < 0.05$.

3. Results

3.1. Effect of blue light treatment on color development, and total anthocyanin content

CIRG value was used to express as fruit color of Chinese bayberries in our study. The values in the control bayberries did not change significantly during the whole storage, however, a significant increase was observed in blue light-exposed bayberries (Fig. 2A). The levels of anthocyanin accumulated gradually in bayberries during storage, which was increased by the blue light treatment. At the end of storage, the levels were about 1.8-fold of the dark control fruit (Fig. 2B).

3.2. Effect of blue light treatment on expression of MrMYB1 gene

The expression of *MrMYB1* gene increased in bayberries during storage, which was induced by blue light treatment. In blue light exposed-bayberries, the transcript levels were increased by approximately 3.3-fold on day 8 (Fig. 3).

3.3. Effect of blue light treatment on flavonoid biosynthesis pathway genes

As shown in Fig. 4, there was a general increase in expression of *MrCHS* and *MrF3H* with storage time in dark control bayberries,