Scientia Horticulturae 197 (2015) 657–664

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Effect of blue light on ethylene biosynthesis, signalling and fruit ripening in postharvest peaches

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ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 12 October 2015
Accepted 19 October 2015
Available online 4 November 2015

Keywords:
Blue light
Ethylene biosynthesis
Ethylene perception and signalling
Ripening
Peach
Quality attributes

ABSTRACT

The involvement of ethylene gas in the ripening of peach fruit is a symbolic phenomenon that has a place in the history of research on plant hormones. It is well documented that fruit ripening is developmentally regulated and affected by environmental factors such as temperature and light. Blue light has been shown to induce pigment synthesis in postharvest fruit. However, information on its effect on fruit ripening is still limited. This study aimed to investigate the effect of light-emitting diode blue light treatment at 40 $\mu mol\ m^{-2}\ s^{-1}$ on fruit ripening, ethylene biosynthesis and signalling in postharvest peaches during storage at 10 °C. Blue light exposure induced fruit ripening as expressed by the enhanced decline of fruit firmness and increases in skin colour parameters, which was associated with the increase in ethylene production because of the up-regulated expression of *PpLOXs* and ethylene biosynthetic genes such as *PpACO1* and *PpACS3*. Meanwhile, six genes in relation to ethylene perception and signalling were also investigated using real-time PCR. Among them, blue light treatment significantly inhibited expression of *PpERS1* and enhanced transcript levels of *PpEIN2*, *PpEIL1*, *PpEIL2* and *PpERF2*, which led to an induction ethylene signalling pathway and resulted in turn into the promotion of ethylene production and fruit softening in postharvest peaches. Compared to control fruit stored at darkness, higher expression level of ethylene biosynthetic and signalling genes in blue light exposed peaches could be beneficial for its ethylene production and consequently fruit ripening.

1. Introduction

Fruit ripening is a complex and genetically programmed process that results in numerous physiological, biochemical, and structural changes in colour, flavour, aroma, texture, and nutritional value of the flesh (Giovannoni, 2004; Klee and Giovannoni, 2011). These changes are the result of the coordinated activation of multiple transcription regulatory and biochemical pathways, which are influenced by endogenous and environmental factors (Martel et al., 2011). Ethylene, a simple gaseous plant hormone is considered as a trigger of the ripening process in climacteric fruits (Guo and Ecker, 2004). In climacteric fruit, an increase in ethylene production is observed before the initiation of ripening and increasing evidence shows that the control of climacteric fruit ripening relies largely on the modulation of ethylene production and/or action, and auto-regulation of ethylene biosynthesis at the transcriptional level (Inaba et al., 2007; Zhang et al., 2009).

Peach, a typical climacteric fruit, is characterized by a peak in ethylene production that coordinates ripening-associated processes (Tonutti et al., 1991). Fruit softening of peaches is partially controlled by ethylene (Hayama et al., 2006). Application of exogenous ethylene to fruit caused faster softening while treatment with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, maintained fruit firmness and delayed ripening process, which was associated with an altered gene expression pattern as observed for endopolygalacturonase (Dal Cin et al., 2006; Zhang et al., 2012). So far, some genes related to ethylene biosynthesis and signalling pathways have been isolated from peach fruit, including ACC syn-

Abbreviations: 1-MCP, 1-methylcyclopropene; ACC, 1-aminocyclopropane-1-carboxylic acid; ACS, ACC synthase; ACO, ACC oxidase; EIL, ethylene insensitive 3-like genes; EIN2, ethylene insensitive 2; ERF, ethylene response factor; ERS1, ethylene sensor 1; ETR, ethylene receptor; LED, light-emitting diode; LOX, lipoxygenases; TF, transcription factors.

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http://dx.doi.org/10.1016/j.scienta.2015.10.034
0304-4238/

thase (ACS), ACC oxidase (ACO), ethylene receptor (ETR), a CTR1 orthologue, and ethylene insensitive 3-like genes (EIL) and ethylene response factor (ERF) (Hayama et al., 2006; Bassett et al., 2002; Rasori et al., 2002; Begheldo et al., 2008; Sherif et al., 2012).

Blue light is a vital environmental factor that affects many aspects of plant growth and development (Lin, 2000). It has also been reported that blue light plays important roles in pigment biosynthesis and disease resistance in postharvest fruit. For example, exposure of tangerines to blue light reduced infection by *Penicillium digitatum* and induced phospholipase A2 gene expression, which strongly suggested that induction of lipid signalling cascade by blue light inhibited fungal colonization by *P. digitatum* in citrus fruit. (Alferez et al., 2013). Moreover, blue light could regulate flavonoid biosynthetic pathway in strawberries and Chinese bayberries after harvest, which resulted in increased concentration of anthocyanins during storage (Shi et al., 2014; Xu et al., 2014). However, to the best of our knowledge, little information is available on the effect of blue light treatment on ripening process in postharvest fruit. The objective of this study was to investigate the effect of light-emitting diode (LED) blue light treatment on fruit ripening, ethylene biosynthesis and signalling in ripening peaches during postharvest storage.

2. Materials and methods

2.1. Plant material and treatment

Peach fruit (*Prunus persica* cv. Jinli) were harvest at commercial maturity stage from a commercial plantation at Ningbo, Zhejiang province and transported within 2 h to the laboratory. Fruit were selected for uniform size and colour, and then divided into two groups randomly. The first group of peaches were irradiated with blue (470 nm) light at an intensity of 40 μmol m^{-2} s^{-1} for 15 days at 10 °C (90% relative humidity). This dose was chosen in our present study based on our preliminary trials (data not shown). GreenPower LED Research Module Blue (Koninklijke Philips Electronics N.V., Holland) were used and the flux intensity of the LEDs was measured with a digital visible light meter (Model 9.4 Blue Light, Solartech Inc, USA). The second group of fruit was stored at 10 °C in the dark (90% relative humidity) and considered as the control (CK). There were three replicates of 80 fruit each per treatment, and samples were taken initially and at 3-day intervals during storage. Fruit quality and ethylene production was determined on whole fruit, while, for molecular analyses, fruit exocarp and mesocarp tissues were immediately frozen in liquid nitrogen separately and stored at −80 °C until used.

2.2. Determination of fruit colour and firmness, total soluble solid and titratable acidity contents, and ethylene production

Fruit surface colour was measured with a CR-410 chroma meter (Konica Minolta Sensing, Inc., Japan), using the CIELAB colour system. Three equidistant colour measurements were made around the equator of each fruit, and the mean values for five fruit from each replicate were subjected to statistical analyses.

Total soluble solid (TSS) contents were determined by using an Abb refractometer (AR12, Schmidt + Haensch GmbH & Co., Germany). Titratable acidity (TA) was determined by titrating 5 ml juice with 0.1 mol/L NaOH to pH 8.2 and was expressed as% citric acid.

Three replicates of five fruit each were held in an air-tight chamber at 10 °C for 2 h prior to gas sampling. One ml of headspace gas was withdrawn from the chamber for each measurement and injected into a gas chromatograph. Ethylene production was monitored using a Shimadzu 14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an activated alumina column and flame ionization detectors, and the results were expressed as micro liter kg^{-1} h^{-1}. Fruit firmness was measured on two pared sides of five fruit from each replicate (skin removed) using a TA-XT2i texture analyzer (Stable Micro System Ltd., UK) with a 5 mm diameter probe.

2.3. Total RNA extraction and cDNA synthesis

Fruit exocarp and mesocarp was carefully grounded in liquid nitrogen. Total RNA was extracted using Plant RNA Kit (Omega Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions. RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The A260/280 ratios of RNA samples were in the range of 1.8–2.0. The RNA was treated with amplification grade RNase-free DNase1 (Omega Bio-Tek Inc., 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) as recommended by the manufacturer. The cDNA was then used as a template for PCR amplification.

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

Q-PCR reactions were performed with an Mx3000P q-PCR System (Agilent Stratagene, Santa Clara, CA, USA) in triplicates using gene specific primers (Table 1). 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 100 μmol/l each forward and reverse primers, 6.5 μl of the SYBR Green PCR master mix (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) and 5 μl RNase-free water. The thermal cycling conditions consisted of an initial denaturation at 95 °C for 7 min, and then for 40 cycles as follows: denaturation at 95 °C for 15 s combined with each primer specific annealing temperature ranged from 50 °C to 60 °C for 30 s, then completed with a melting curve analysis program. Real-time PCR data was calibrated relative to *PpTEF2* (JQ732180) expression level at zero time for each treatment, following the 2^{-DDCt} method for relative quantification. Three independent biological replicates were analysed for each sample and data were indicated as mean ± SE ($n = 3$).

2.5. Statistical analysis

All values are shown as the mean ± SE. Statistical analysis was performed using the SPSS package program version 16.0 (SPSS Inc., Chicago, IL, US) by one-way analysis of variance (ANOVA) and Student's t test to compare means at $P < 0.05$.

3. Results

3.1. Effect of blue light treatment on fruit firmness, ethylene production, TSS, TA and fruit colour in peach fruit

Softening as expressed by firmness declination is a major symptom of ripening in peach fruit after harvest. Fruit firmness decreased gradually with storage time in both samples, peaches exposed to darkness and peaches exposed to blue light. However, the trend was significantly enhanced by blue light (Fig. 1A). A climacteric rise in ethylene production was observed in both control and blue light treated peaches. In fruit stored at darkness, ethylene production increased gradually and reached the highest level on day 6th of storage. However, blue light irradiation delayed the peak time to day 9th and promoted higher ethylene production significantly during the whole storage (Fig. 1B).