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Involvement of abscisic acid in postharvest water-deficit stress associated with the accumulation of anthocyanins in strawberry fruit

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ABSTRACT

Fruits are prone to suffer water-deficit stress and accelerated senescence after harvest. To understand the effect of postharvest water-deficit on the senescence progress of strawberry fruit, fruit peduncles were dipped into water or abscisic acid solution and stored at 20 °C with 50% relative humidity in the dark. The results showed that detached fruit without water supply through the peduncles suffered serious water-deficit stress with great weight loss and low moisture content. However, fruit absorbing water through their peduncles successfully avoided the water-deficit stress with a slight change of weight loss and moisture content. Water-deficit significantly promoted anthocyanin levels with increased PAL, C4H and DFR activities. The expressions of transcriptional factors that regulate anthocyanin biosynthesis (*FaMYB1*, *FabHLH3* and *FaTTG1*) were also up-regulated under water-deficit stress. Meanwhile, stress elevated the abscisic acid level as well as *FaNCED1* and *FaASR* expression in strawberry fruit. Furthermore, exogenous ABA application exhibited the similar features with water-deficit stress. These results suggested that postharvest water-deficit would accelerate anthocyanin accumulation in strawberry fruit and that abscisic acid may play a role in the response to water-deficit stress.

1. Introduction

Harvested fruit experience a range of biotic and abiotic stresses that lead to premature senescence and eventual decline in quality and commercial value (Rizzini et al., 2009; Romero et al., 2013a). Water-deficit occurs in plants when the rate of transpiration exceeds water uptake, and is an aspect of various abiotic stresses on higher plant, such as drought, salinity, low temperature (Bray, 1997). For postharvest fruit, once detached from the plant, the fruit has no renewable source of water to compensate for that lost through transpiration (Nakano et al., 2003). Thus, harvested fruits are vulnerable to be impacted by water-deficit stress, which could be primarily responsible for remarkable metabolic disorders involved in the senescence of fruit during postharvest handling and storage.

Several studies in grapes suggested that when undergoing postharvest water-deficit, there were increases in soluble solids, hormones (ethylene and abscisic acid), anthocyanins, ethanol, and enhancements of respiration rate, and lipoxygenase and alcohol dehydrogenase activities (Bellincontro et al., 2004; Costantini et al., 2006). Transcript profiling also indicated that postharvest water-deficit stress in grape berry was associated with modifications on the expressions of genes involved in transcriptional regulation factors, hormone and sugar metabolism, polyphenol biosynthesis and water transport, such as *MybA*, *MybB*, *ACO*, *PAL*, *CHS*, *F3H*, *LDOX*, *PIP2*, *POD* and *PPO* (Bonghi et al., 2012; Rizzini et al., 2009). In response to postharvest dehydration in citrus fruit, $PLA_{2\alpha}$, $PLA_{2\beta}$, $PLD\alpha$, and $PLD\beta$ were also activated and triggered lipid catabolism in cell plasma membrane (Romero et al., 2013a).

Abscisic acid (ABA) plays a crucial role during the ripening and senescence of fruit, especially in non-climacteric fruit (Jia et al., 2011). Moreover, ABA acts as a long-distance signal between roots and shoots for plant under water-deficit stress (Osakabe et al., 2014). Seasonal water-deficit in the field could markedly increase ABA concentration in Cabernet Sauvignon at maturity and one week following maturity (Deluc et al., 2009). Under water-deficit, strawberry fruit connected to the plant also showed high level in ABA (Terry et al., 2007). In citrus fruit, PYP/PYL/RCAR-inactivated clade A, protein phosphatases 2C (*PP2CA*) mediated by ABA is a key regulator in the response to postharvest dehydration (Romero

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et al., 2013b). Downstream of ABA signaling, postharvest water-deficit could activate phospholipase activities and trigger lipid catabolism in cell plasma membrane in citrus fruit. Thus, ABA may be a key regulator of the response to postharvest water-deficit in citrus fruit (Romero et al., 2013a).

A previous study from this laboratory found that detachment (harvesting) accelerated ripening and senescence progress of strawberry fruit accompanying water loss (Chen et al., 2014). So far, little information about the involvement of water-deficit in the senescence of postharvest fruit is available, which may cover up novel opportunities to extend the postharvest life. In this paper, the evidentiary involvement of water-deficit and ABA in the accelerated ripening of postharvest strawberry fruit was investigated with an additional imposed supply of water and ABA. A range of physical, physiological and biochemical changes were analyzed to assess the postharvest ripening features in fruit pigmentation. ABA content and expressions of ABA-related genes, such as *FaNCED1* and *FaASR*, in the fruit were also determined.

2. Materials and methods

2.1. Plant material and treatments

Strawberry fruits (*Fragaria* × *ananassa*) at 23 d after anthesis were harvested from a plastic greenhouse located in the suburb of Hangzhou, China. Three-hundred fruits were detached from the branch with their peduncles (about 30 mm length). The fruits were of similar size, with no diseases and insect pests. The harvested fruits were transported to the laboratory within 90 min. Fruits were surface sterilized by immersion in 0.1% sodium hypchlorite solution for 2 min, followed by triply rinsing in sterile deionized water and drying naturally on a clean bench at room temperature. Samples without mechanical damage were randomly divided into three groups, consisting of eighty fruit in each group. Fruits of group one were put on the mouth (20 mm diameter) of 25 mL vials (water-deficit group, WD). Fruits of group two were put on the mouth of vials with 10 mm of fruit peduncles inserted in sterile deionized water (water supply group, WS). Fruits of group three were put on the mouth of vials with 10 mm of fruit peduncles inserted in 100 μmol L^{-1} ABA solution (Water plus ABA group, WA). A schematic plot of sample treatment is shown in Fig. 1. All fruits were stored at (20 ± 2) °C with (50 ± 5)% relative humidity in the dark. Twenty fresh fruits were used as the sample at day 0. Twenty fruits per treatment were randomly sampled for analysis on days 1–4. The whole experiment was repeated with three replications.

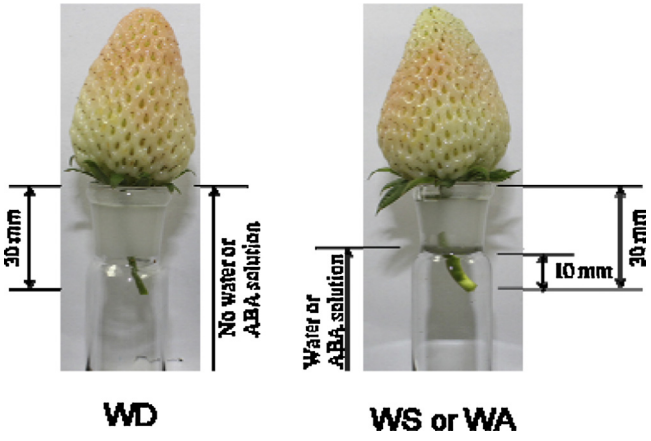

Fig. 1. Diagram of sample treatment. WD represents water-deficit; WS represents water supply; WA represents water plus ABA.

2.2. Determination of weight loss and moisture content

Weight loss was determined for ten fruits from each group. The results were expressed as the percentage loss of initial weight as described below: Weight loss (%) = (IW − FW)/IW × 100, where IW was initial weight (g) and FW was final weight (g).

To determine moisture content, ten strawberries were longitudinally cut into four identical portions, which were randomly distributed in four sub-samples. These sub-samples were individually crushed into a puree. The puree was used for moisture content determination by drying at 100 °C. The% of moisture was determined gravimetrically.

2.3. Determination of tristimulus color and total anthocyanin

Tristimulus color was measured on two opposite sides (equatorial area) of each fruit using Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Japan) and a^* was recorded to represent red/green.

Total anthocyanin content (TAC) was estimated by the pH differential method according to Ornelas-Paz et al. (2013). The frozen sample (10 g) was ground to a fine powder in liquid nitrogen using a mortar and pestle. About 300 mg sample powder was weighed in a 5 mL centrifuge tube with 3 mL of 1% HCl–methanol solution. The mixture was homogenized and kept on ice for 10 min. Then it was centrifuged at 1500 × *g* for 10 min at 4 °C and the supernatant was left for measurement. TAC was expressed on a fresh weight basis as milligrams of pelargonidin-3-glucoside per kilogram, with the molar extinction coefficient of 22,400 L mol^{-1} cm^{-1} (Cheng and Breen, 1991).

2.4. Analysis of abscisic acid (ABA)

ABA content was determined by ultra-performance liquid chromatography–mass spectrometry (UPLC–MS). The method of milling the fruit samples was the same as that of TAC. The extraction procedure was according to the method described by Symons et al. (2012). One gram of powder was mixed with ten milliliters of 80% methanol that contained one milligram of 2,6-di-*tert*-butyl-4-methylphenol (BHT, >99.0%, Aladdin Industrial Inc., China) and then soaked overnight at 4 °C.

On the second day, the mixture was filtered through a Whatman No. 1 filter paper and concentrated to less than 1 mL under vacuum at 35 °C and then taken up in 3 × 3 mL of 10% (v/v) methanol-0.4% (v/v) acetic acid in double distilled water and loaded onto a Sep-Pak C_{18} (12 cm kg^{-1}, Waters Corporation, USA) cartridge, preconditioned with 15 mL 100% methanol followed by 15 mL 0.4% (v/v) acetic acid in double distilled water. The hormones were eluted from the Sep-Pak with 0.4% (v/v) acetic acid–methanol solution after a rinse of 10% (v/v) methanol-0.4% (v/v) acetic acid in double distilled water. Then the eluates were dried under vacuum at 35 °C and the residue was re-suspended in 20% (v/v) methanol-0.4% (v/v) acetic acid in double distilled water, centrifuged at 12,000 × *g* for 3 min.

Analysis of ABA was performed with an Agilent 6460 triple quadruple LC/MS system (Agilent Technologies Inc., USA). (±) 2-*cis*-4-*trans*-abscisic acid (ABA, 98%, Aladdin Industrial Inc., China) was used as a standard. ZORBAX Eclipse XDB-C18 column (2.1 × 150 mm, 3.5 μm, Agilent Technologies Inc., USA) was used with mobile phases A = methanol and B = 0.1% methanoic acid in water. The flow rate was 5 μL s^{-1} and the column was held at 35 °C. The program was 40% A to 60% B for 1.5 min, then a linear gradient to 100% A over 6.5 min and followed by a linear gradient to 60% B over the next 2 min, and 40% A to 60% B for the final 7 min.

MS was conducted in the electrospray and multiple reaction monitoring (MRM) modes, monitoring negative ions for ABA. The