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Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit

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ABSTRACT

Peach fruit deteriorates and senesces quickly during storage at ambient temperature. Research confirms that melatonin (MT) is involved in plant senescence. However, little information is available on the effects of MT on postharvest senescence. In this study, two cultivars of peach fruit 'Shahong' and 'Qinmi' were treated with MT at 0.1 mmol L^{-1} and then stored at ambient temperature (25–28 °C)for 7 days. The results showed that MT treatment effectively slowed process of senescence in both peach cultivars, as indicated by reduced weight loss, decay incidence and respiration rate as well as maintained firmness, total soluble solids and ascorbic acid contents. MT treatment significantly enhanced the activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase in both cultivars, but decreased the activity of lipoxygenase, levels of superoxide anion and hydrogen peroxide, and malondialdehyde content. These results indicate that the activation of antioxidant enzymes to scavenge superoxide anion and hydrogen peroxide by MT treatment was associated with the maintenance of membrane integrity, which might be a part of the mechanism implicated in delay of senescence in peach fruit.

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1. Introduction

Peach is one of the most ancient fruit originated from China and it is increasingly becoming world-popular because of its high marketing value with bright color, favorable flavor and abundant phytonutrients (Lurie and Crisosto, 2005). However, the fruit inevitably undergoes a rapid senescence after harvest, leading to a severe quality reduction, as manifested by dehydration, shriveling, softening, decrease in ascorbic acid content and decay. Therefore, quality deterioration of peach fruit greatly restricts its edibility and commodity value (Bonghi et al., 1999). Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical have been implicated as the cause of oxidative damage to membrane lipids, one of the most convincing mechanisms of apoptosis and natural senescence in plants (Mittler, 2002). To date, various postharvest treatments including salicylic acid (Wang et al., 2006), oxalic acid (Zheng et al., 2007), nitric oxide (Flores et al., 2008), active packaging (Montero-Prado et al., 2011), UV-C (Yang et al., 2014) and hypobaric treatment (Wang et al., 2015) have been tested to effectively maintain the redox equilibrium and prevent the lipid peroxidation by mediating ROS production, contributing to maintenance of high quality and extension of storage life in peach fruit.

Melatonin (MT), a naturally occurring indoleamine once thought to be unique to animals, is also widely distributed in the plant kingdom. The presence of MT has been identified in different organs of plants, including root, stem, leaf, flower, fruit and seed (Jemima et al., 2011; Reiter et al., 2015). MT performs diverse physiological functions in plants. In addition to serve as darkness signaling and plant growth-promoting regulators, another noticeable role is its antioxidant activity associated with the protection of plants against internal and environmental oxidative stresses (Reiter et al., 2015; Tan, 2015; Zhang et al., 2015). Antioxidant activity of MT can be implemented through several patterns: neutralize free radicals, and enhance enzymatic or non-enzymatic antioxidant activites; prevent intracellular antioxidant enzymes inactivation as well as inhibit free radicals production (Rodríguez et al., 2004; Leon et al., 2005; Galano et al., 2011). Based on the above-mentioned mechanisms, the possible role of MT in relation to leaf senescence was investigated by Arnao and Hernández-Ruiz (2009) who found that treatment of barley leaves with MT resulted in a delay of dark-induced senescence and

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decrease of chlorophyll degradation. Also, works by Wang et al. (2013) and Shi et al. (2015) have proved that MT-mediated chlorophyll preservation involves in controlling senescence in *Malus hupehensis* and rosette leaves. In consideration of ROS damage to chloroplasts (Apel and Hirt, 2004), the authors speculated that MT might quench the over-production of ROS, and consequently reduced the chlorophyll loss and delayed the senescence process (Arnao and Hernández-Ruiz, 2009). Wang et al. (2012) noted that application of 10 mmol L^{-1} MT up-regulated the expression of genes encoding these enzymes, as well as promoted the cycle of ascorbic acid and glutathione, contributing to a reduction of ROS level and delay of the senescence in detached apple leaves. Additionally, results from metabonomics-based investigations and proteomic analysis also revealed the ability of MT to delay leaf senescence (Wang et al., 2013, 2014). These findings provide valuable evidence that MT might participate in regulation of plant senescence. However, there is little information available on the effect of MT as a postharvest factor on storage life and quality of horticultural crops.

The objective of this study was to investigate the effects of MT application on senescence of peach fruit during storage at ambient conditions, using two cultivars. Included were analyses of quality parameters and ROS metabolism. The study was helpful to further understand physiological functions of MT in harvested fruit.

2. Material and methods

2.1. Plant material and treatments

'Shahong' (SH) and 'Qinmi' (QM) peach (*Prunus persica* Batsch) fruit were harvested at the commercial maturity stage (ripe but preclimacteric) from a commercial orchard in Xi'an, Shaanxi province of China. Fruit were chosen on the basis of uniform shape and appearance and without visible defects. The selected fruit were randomly divided into 45 lots of 30 fruits at random for each cultivar. Three lots were used to evaluate the fruit characteristics at harvest and the rest of 42 lots were divided into 2 groups for the following treatments in triplicate: distilled water containing 1% absolute ethanol (control) and MT (Yuanye Biotechnology Co., Ltd., Shanghai, China) at 0.1 mmol L^{-1} for 10 min. To obtain MT solution, 23.23 mg of MT was dissolved in 10 mL absolute ethanol, and then the mixture was diluted to 0.1 mmol L^{-1} using distilled water. Fruit were then air-dried and stored at 25–28 °C and 60–70% relative humidity for up to 7 days. The samples were daily taken for evaluating decay incidence, weight loss, respiration rate, firmness and total soluble solids content. Meanwhile, samples of flesh tissue derived from 10 fruit, with 3 replicates for each treatment, were collected and stored at −80 °C for analysis of levels of ascorbic acid, malondialdehyde (MDA) and $O_2^{\bullet-}$ and H_2O_2, and activities of lipoxygenase (LOX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and APX.

2.2. Decay incidence and weight loss

Peach fruit with rot, visible fungal growth or bacterial lesions were considered decay. Decay incidence was the number of fruits showing decay symptoms relative to the total number of fruit in each treatment expressed in %. Weight loss was evaluated by weighing the peach fruit before and after the storage period and presented as % of weight loss compared to initial weight.

2.3. Respiration rate, firmness, total soluble solids and ascorbic acid contents

Respiration rate was estimated using a modified closed method (Jiang et al., 2012). In each treatment, five peach fruit was randomly sampled and sealed in a glass container at 25–28 °C for 1 h, which contained 10 mL 0.4 M NaOH. Then, 5 mL saturated $BaCl_2$ and 2 drops of phenolphthalein were added, and titrated with 0.1 mol L^{-1} oxalic acid until the end point. The respiration rate of samples was expressed as mg CO_2 kg^{-1} h^{-1}.

Firmness was determined using a pressure tester (GY-3, Aidebao Instrument Co., Ltd., Leqing, China) equipped with an 8 mm diameter probe and expressed as N. For SSC, the flesh tissue (2 g) were ground in a mortar and centrifuged at 8000*g* for 15 min, the supernatant was dropped to a WYT-4 hand-held refractometer (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) and the value was read. SSC content was expressed as °Brix.

Ascorbic acid content was measured using a modified method of Bessey and King (1933). Peach flesh tissue (5 g) was homogenized in 25 mL of 2% oxalic acid solution and centrifuged at 8000*g* and 4 °C for 15 min. After centrifugation, 10 mL of the supernatant was transferred into a 50 mL triangle bottle and titrated with a calibrated 2,6-dichlorpphenolindophenol solution until a permanent pink color. Ascorbic acid content was expressed on a fresh weight basis as mg 100 g^{-1}.

2.4. MDA content

MDA content was determined using a method of Dhindsa et al. (1981) with some modification. Peach flesh tissue (2 g) was homogenized with 10 mL of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was then heated at 100 °C for 10 min. After rapid cooling, the mixture was centrifuged at 5000*g* for 15 min. The absorbance of the supernatant was measured at 450, 532, and 600 nm. The MDA content was expressed on a fresh weight basis as μmol g^{-1}.

2.5. $O_2^{\bullet-}$ production rate and H_2O_2 content

$O_2^{\bullet-}$ production rate was determined in 3 g of peach flesh tissue following a modified method (Wang and Luo, 1990). $O_2^{\bullet-}$production rate was calculated using $NaNO_2$ as a standard and expressed on a fresh weight basis as nmol g^{-1} min^{-1}. H_2O_2 content was performed according to Patterson et al. (1984) with some modifications. Peach flesh tissue (5 g) were homogenized in 5 mL of cold acetone and centrifuged at 5000*g* and 4 °C for 15 min. The supernatant (1 mL) was mixed with 0.1 mL of 22 mmol L^{-1} titanium sulphate and 0.2 mL ammonia, and again centrifuged at 5000 g and 4 °C for 10 min. Then, the pellets were dissolved in 3 mL of 1 mol L^{-1} sulfuric acid and centrifuged for 10 min at 5000*g*. H_2O_2 content was calculated using H_2O_2 as a standard and expressed on a fresh weight basis as μmol g^{-1}.

2.6. Enzyme assays

Peach flesh tissue (2 g) was homogenized in various precooled buffers (4 °C) to prepare extracts for assay of the following enzymes: 6 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7.8) containing polyvinylpyrrolidone for SOD; 8 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 6.8) containing polyvinylpyrrolidone for POD, CAT and LOX; 8 mL of 100 mmol L^{-1} potassium phosphate buffer (pH 7.5) containing 0.1 mmol L^{-1} ethylene diamine tetra-acetic acid, 1 mmol L^{-1} ascorbic acid and polyvinylpyrrolidone for APX. The tissue homogenates were then centrifuged at 12,000*g* and 4 °C for 15 min. The supernatants were used for the enzyme assays.

LOX activity was measured according to Surrey (1963). LOX activity was estimated by the increase in absorbance at 234 nm, and expressed on a fresh weight basis as U g^{-1}, where U = 0.01 ΔA_{234nm} per min.

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