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Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism

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ABSTRACT

Effects of 0.1 mM melatonin (MT) on chilling injury (CI), membrane fatty acid content and phenolic metabolism in peach fruit were studied during storage at 1°C for 28 days. MT treatment delayed the development of CI in peach fruit, as was illustrated by MT-treated fruit showing lower CI incidence, CI index and firmness loss than the control. MT treatment prevented membrane lipid peroxidation and contributed to maintaining a higher ratio of unsaturated to saturated fatty acids in peach fruit. MT treatment also stimulated the activities of glucose-6-phosphate dehydrogenase, shikimate dehydrogenase and phenylalanine ammonia lyase, but inhibited the activities of polyphenol oxidase and peroxidase. This would help in activating the accumulation of total phenolic and endogenous salicylic acid that might have a direct function in alleviation of CI. These results indicate that MT treatment can be an effective technique to reduce postharvest CI during low temperature storage of peach fruit.

1. Introduction

Chilling injury (CI) is a physiological disorder commonly occurring in a large collection of peach cultivars during low temperature storage, especially when storing at a potential risk zone of temperature between 2.2 and 7.6 °C (Lurie & Crisosto, 2005). The disorder is characterized mainly by flesh browning and/or mealiness, abnormal ripening and higher sensitivity to decay (Lurie & Crisosto, 2005), and is considered as a primary limitation of application of low temperature to preserve peach fruit. Many hypotheses have been put forward for a CI mechanism in plants, such as involvement of imbalances in metabolism, accumulation of toxic compounds, decreased water dynamic state and increased permeability (Lyons, 1973; Naruke et al., 2003; Purwanto et al., 2013). To deal with this problem, researchers have been devoting a great deal of attention to finding economical, convenient and highly effective techniques to reduce CI in peach fruit.

Melatonin (MT) is an indoleamine hormone ubiquitous in nature. In plants, MT has been identified in nearly all organs and tissues, and is shown to be a signaling molecule involved in numerous physiological processes such as differentiation, growth, ripening and senescence of plant and the protective effect against various forms of environmental stress (Reiter et al., 2015; Zhang Huber et al., 2015; Zhang Sun et al., 2015). Exogenous MT has been demonstrated to be effective in alleviating chilling stress-induced damage in plants through different mechanisms. For example, Posmyk, Balabusta, Wieczorek, Sliwinska, and Janas (2009) revealed that MT treatment significantly counteracts the adverse effect of chilling stress on cucumber seeds by influencing the structure and function of cellular membrane. Likewise, in another study, researchers found that application of MT contributes to reducing the risk of ultrastructural damage in meristematic cells of *Vigna radiata* roots due to chilling exposure (Szafrańska, Glińska, & Janas, 2013). Their data also documented that MT-medicated *Vigna radiata* root acclimation to chilling stress is associated with its ability to activate the pathway of phenolic (Szafrańska, Szewczyk, & Janas, 2014). It was also shown that MT has a positive regulation in the expression of C-repeat-binding factors and reactive oxygen species (ROS)-related antioxidant genes, which helps Arabidopsis thaliana induce resistance to chilling stress (Bajwa, Shukla, Sherif, Murch, & Saxena, 2014). However, more recently, attention has been partially directed toward the effect of exogenous MT application on oxidative stress-induced senescence and CI in postharvest fruit. Sun et al. (2016) found that MT plays a crucial role in the regulation of senescence in tomato fruit. Gao, Zhang, & Lv et al. (2016) putted forward a similar result that MT at a concentration of 0.1 mM leads to a clear delay of senescence in peach fruit during ambient storage, through antioxidative mechanism. Aghdam and Fard (2017) reported that MT is conductive to attenuating postharvest decay in strawberry fruit by mechanisms that trigger the accumulation of hydrogen peroxide and phenolic compounds and induces the activity of

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γ-aminobutyric acid shunt. Cao et al. (2016) proved that MT ensures better prevention of CI in peach fruit during low temperature storage, and such effect has been attributed in part to MT-induced promotion of polyamine, γ-aminobutyric acid and proline. These findings undoubtedly reveal new insights into the physiological roles of MT in plants. However, further work along these lines would be valuable, since there is only a limited understanding at present.

The purpose of this paper is to advance our understanding of the possible underlying mechanisms of how MT induces a defence response of peach fruit to avoid CI. In peach fruit, previous studies have reported that both biosynthesis of phenolic compounds (Gao, Zhang, & Lv et al., 2016; Gao, Zhang, & Chai et al., 2016; Jin, Zheng, Tang, Rui, & Wang, 2009) and maintenance of a higher ratio of unsaturated to saturated fatty acids (Jin, Zhu, Wang, Shan, & Zheng, 2014) may account for the enhancement of chilling tolerance. Therefore, the focus of the present study was on whether the MT-induced changes in membrane fatty acid contents and phenolic metabolism are linked to the enhanced tolerance to CI in peach fruit during low temperature storage.

2. Material and methods

2.1. Plant material and treatments

Mature pre-climacteric fruit of peach (*Prunus persica* Batsch cv 'Chuanzhongdao') were hand-collected from a well-managed commercial orchard in Xi'an, China. Fruit were chosen for uniformity without any defects, and then separated into 2 lots at random. Fruit of the first lot were dipped in a solution of 0.1 mM MT for 10 min in the low light to prevent light-induced MT degradation (Gao, Zhang, & Lv et al., 2016; Gao, Zhang, & Chai et al., 2016). Under the same condition, fruit of the second lot were soaked in distilled water and designed as the control. Afterwards, all fruit were dried in the air and stored at 1 °C with a relative humidity of 85–90% for 28 days. Fruit were removed from each lot after 0, 7, 14, 21 and 28 d of low temperature storage to evaluate CI incidence, CI index and firmness loss. Meanwhile, flesh tissue samples derived from 10 fruit were collected and stored at −80 °C for subsequent measurements. For each lot, three replicates were performed.

2.2. CI incidence, CI index and firmness

CI incidence was defined as the ratio of CI-fruit to total fruit and expressed as%.

CI index was assessed visually based on the percentage of the cut surface of peach slices that exhibited browning with a scale from 0 to 4: 0 (none), 1 (< 25%), 2 (25–50%), 3 (50–75%), 4 (> 75%), according to Wang, Chen, Kong, Li, and Archbold (2006). CI index was obtained from the formula: CI index = Σ (CI scale × number of fruit in each scale)/(4 × total number of fruit).

Firmness was determined with a fruit firmness tester (GY-3, Aidebao Instrument Co., Ltd, Leqing, China) fitted with an 8 mm diameter probe and expressed as N.

2.3. Malondialdehyde content

Malondialdehyde (MDA) content was tested using a modified method described by Dhindsa, Pulmb-Dhindsa, and Thorpe (1981). Flesh tissue (2 g) was homogenized with 10 ml of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was boiled at 100 °C for 10 min, cooled quickly thereafter, and centrifuged at 5000*g* for 15 min. Absorbance of the supernatant was measured at 450, 532 and 600 nm. MDA content was expressed on a fresh weight basis as μmol g^{-1}.

2.4. Measurement of composition of fatty acid

The extraction and quantification of fatty acids was performed according to the method described by Jin et al. (2014). Flesh tissue (20 g) was homogenized with chloroform/methanol (2:1) solution and the mixture was acidified with 0.1 N HCl. After centrifugation at 4000*g* for 10 min, the organic layer was collected and taken to dryness. Total fatty acids were methylated by adding trifluoride 14% methanolic solution and methylated fatty acids were extracted with hexane. After that, the solvent was dried and the methylated fatty acids were redissolved in 0.2 ml chloroform and analyzed for fatty acid composition by a Hitachi 663–30 Gas Chromatograph with a flame ionization detector. Fatty acids were identified based on the retention time of the internal standard free fatty acid C17:0. The ratio of unsaturated to saturated fatty acid was calculated from the formula: [oleic acid (18:1) + linoleic acid (18:2) + linolenic acid (18:3)]/[palmitic acid (16:0) + stearic acid (18:0)].

2.5. Total phenolic and endogenous salicylic acid contents

Flesh tissue (2 g) was homogenized in 5 ml of methanol. After centrifugation at 12,000 × *g* for 15 min, the supernatant was collected and used to measure the content of total phenolic. In brief, 0.5 ml of supernatant was gently mixed with 1.0 ml of Folin-Ciocalteu reagent and 3 ml of 1 M sodium carbonate and the total volume of the mixture were adjusted to 10 ml with distilled water. After the mixture had been kept at room temperature for 1 h, the absorbance was read at 760 nm (Hinneburg, Dorman, & Hiltunen, 2006). Results were expressed as the mass of gallic acid equivalents on a fresh weight basis in mg g^{-1}.

Endogenous salicylic acid content was measured by an enzyme-linked immunosorbent assay (ELISA). A plant salicylic acid ELISA kit was used following the instructions of the manufacturer (Nanjing Senbeijia Biological Technology Co., Ltd, Nanjing, China). In brief, flesh tissue (1 g) was homogenized in 5 ml of methanol and the supernatant collected. To test the supernatant 10 μl was diluted with 50 μl sample dilution in a 96-well microplate. The mixtures were incubated in the water bath at 37 °C for 30 min. After washing 5 repeats, HRP-Conjugate reagent and chromogen solution were added and incubated for 15 min at 37 °C in the dark. The reaction was stopped by sulfuric acid and the changes in colour were noted at 450 nm. The content of endogenous salicylic acid was calculated following the standard curve.

2.6. Measurement of enzymes activities associated with phenolic metabolism

For determination of lipoxygenase (LOX), shikimate dehydrogenase (SKDH), polyphenol oxidase (PPO) and peroxidase (POD), flesh tissue (2 g) was homogenized in 5 ml of 50 mM potassium-phosphate buffer, pH 6.8. And then sample was centrifuged at 12,000*g* for 15 min at 4 °C. For determination of glucose-6-phosphate dehydrogenase (G6PDH), flesh tissue (2*g*) was homogenized in 10 ml of potassium-phosphate buffer, pH 7, containing 1 mM EDTA, 3 mM of magnesium sulfate and 1 mM polyvinylpyrrolidone. The sample was then centrifuged at 12,000*g* for 15 min at 4 °C. For determination of phenylalanine ammonia-lyase (PAL), flesh tissue (2*g*) was homogenized with 5 ml of 0.2 M borate buffer, pH 8.8, containing 6*g* of polyvinylpyrrolidone. The sample was then centrifuged at 12,000*g* for 15 min at 4 °C. The supernatants were then used for the enzyme activity.

LOX activity was assayed according to the method of Surrey (1963). Reaction mixture consisted 2.775 ml 100 mM sodium acetate buffer, pH 5.5, 25 μl linoleic acid and 0.2 ml of supernatant. The absorbance was measured by an increase in absorbance at 234 nm. LOX activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{234\ nm}$ per min.

G6PDH activity was determined using the method described in Debnam and Emes (1999). 0.2 ml of supernatant was mixed with 1.8 ml of 50 mM Hepes-NaOH phosphate buffer, pH 8, 5 mM glucose-6-phosphate disodium salt, 0.8 mM $NADP^+$ and 2 mM magnesium chloride. The absorbance was measured by an increase in absorbance at 340 nm.

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