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Oxalic acid pretreatment reduces chilling injury in Hami melons (*Cucumis melo* var. *reticulatus* Naud.) by regulating enzymes involved in antioxidative pathways

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ABSTRACT

The Hami melon (*Cucumis melo* var. *reticulatus* Naud.) is a tropical fruit with a short storage period. To prolong freshness, the fruit is stored at low temperatures (3 °C), although this can lead to chilling injury (CI) involving skin lesions, numerous tiny brown spots on the rind of the fruit, susceptibility to decay, especially upon re-warming, the formation of small pits, and browning of the surface associated with a loss of sensory quality. Our preliminary research revealed that pretreatment with 15 mmol L^{-1} oxalic acid (OA) for 10 min at 25 °C effectively reduced CI in Hami melons stored at 3 °C. The current work was conducted to confirm the above results and determine whether enzymes involved in antioxidative pathways play a role in the decreased CI. The activities of enzymes related to antioxidative pathways and expression levels of the corresponding genes were measured. OA pretreatment prior to storage at 3 °C for 42 d increased the activities of glutathione reductase (GR), ascorbate peroxidase (APX), and peroxidase (POD), and led to higher expression levels of the Cm-GR, Cm-APX, and Cm-POD genes relative to the control group. The OA-treated group also maintained higher contents of ascorbic acid (AsA) and glutathione(GSH) compared with the control group. OA treatment inhibited H_2O_2 and O_2^- accumulation, delayed membrane lipid oxidation, and led to a lower CI index compared with the control fruit. These results indicate that enzymes involved in antioxidative pathways play a role in the alleviation of CI upon OA treatment.

1. Introduction

The Hami melon (*Cucumis melo* L. var. *inodorus* Jacq.) is an economically important crop in Xinjiang province in northwestern China with a delicious flavor and crispy and juicy flesh (Bi and Zhang, 1991). However, it is sensitive to low temperatures and therefore susceptible to chilling injury (CI). Typical symptoms of chilling injury in Hami melons include skin lesions, numerous tiny brown spots on the rind of the fruit, and susceptibility to decay (Edna et al., 2011). In particular, upon re-warming, the formation of tiny pits and browning of the surface are related to a decrease in sensory quality (Benamor et al., 1999). Previous studies have suggested that low antioxidant activity produced unbalanced reactive oxygen species (ROS),which damaged cell membrane structure, moreover abnormal respiration rate, ethylene production are the main factors responsible for CI in melon (García et al., 2005; Fogelman et al., 2011). Different cultivars of Hami melons have been reported to possess different levels of cold tolerance (Zhang et al., 2017). Some methods for alleviating CI in cold-stored Hami melons have been developed based on hot-water treatment, innovative packaging, nitric oxide (NO) treatment, and the expression of antisense aminocyclopropanecarboxylate oxidase (ACO) gene (Fogelman et al., 2011; Rij and Ross, 1988; Zhang et al., 2017; Benamor et al., 1999).

Several abiotic methods to enhance resistance to chilling stress can be used prior to storage to delay the deterioration of low-temperature-sensitive commodities, such as cold shock, salinity, and heat shock (Erkan et al., 2005). Oxalic acid (OA) is an organic acid that occurs naturally in plants and plays several distinct roles in biological organisms (Shimada et al., 1997). Recently, OA has received considerable attention as a post-harvest treatment to delay senescence and preserve vegetable and fruit quality in mangoes (Kashif et al., 2015; Zheng et al.,

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2007; Zheng et al., 2012) and sweet cherries (Daniel et al., 2011) and improve the resistance of fruits toward various diseases (Deng et al., 2015; Jiang et al., 2013; Tian et al., 2006; Wang et al., 2009). OA treatment has also been reported to mitigate chilling injury and preserve the quality of mangoes (Ding et al., 2007; Li et al., 2014), peaches (Jin et al., 2014), and "Taify" pomegranates (Awad et al., 2013; Sayyari et al., 2010). The underlying mechanisms have also been investigated, revealing that OA reduces ROS accumulation, preserves cell membrane integrity (Ding et al., 2007), increases proline content by increasing Δ1-pyrroline-5-carboxylate synthetase (P5CS) activity and decreasing proline dehydrogenase (PrDH) activity, maintains a high ATP level and energy charge (Jin et al., 2014; Li et al., 2014), increases the ratio of unsaturated/saturated fatty acids (Jin et al., 2014), elevates lycopene accumulation by upregulating transcription factors PSY1 and ZDS expression (Li et al., 2014), and regulates sugar metabolism (Wang et al., 2016). These findings demonstrate that OA treatment induces the resistance systems and affords protection against CI in fruit exposed to chilling stress.

The chilling temperature influences the plant cell membrane structural integrity (Rui et al., 2010). Electrolyte leakage and malondialdehyde (MDA) content can be used to evaluate the level of membrane lipid peroxidation (Lin et al., 2014). The appearance of chilling damage in fruits is related to oxidative stress from excess ROS (Hodges et al., 2004) and the effective elimination of ROS and the mitigation of CI is associated with high activities of antioxidant enzymes (Gualanduzzi et al., 2009; Imahori et al., 2008; Sala, 1998) including peroxidase (POD), ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) (Xu et al., 2012; Yang et al., 2011). The combined role of antioxidant enzymes and non-enzymatic antioxidants has been reported to increase antioxidant ability in plants (Ahmad et al., 2010; Foyer and Noctor, 2005). Previous studies have shown that a higher content of reducing substances such as AsA and GSH, which mediated the ascorbate–glutathione cycle, increased the ROS elimination capacity in longan fruit due to decreasing ROS accumulation and helped maintain cellular compartmentalization (Lin et al., 2015).

In our preliminary investigation of Hami melons, we observed remarkable alleviation of CI in fruit treated with 15 mmol L^{-1} OA compared with the control. To the best of our knowledge, no information regarding the inhibition of CI in harvested Hami melons by OA treatment or the underlying physiological and molecular mechanisms has been previously reported.

In this study, the effects of exogenous OA treatment on CI and the antioxidative capacity of Hami melons during storage at 3 °C were evaluated by measuring the following indicators: CI, electrolyte leakage, MDA content, H_2O_2, O_2^-, AsA, oxidation of ascorbic acid (DHA), GSH, oxidized glutathione (GSSG), AsA/DHA and GSH/GSSG ratios, and activities and gene expression patterns of APX, POD, and GR. We sought to provide empirical evidence for the mechanistic involvement of the antioxidant system in the effects of the OA treatment of Hami melons exposed to chilling stress.

2. Materials and methods

2.1. Melon harvesting and OA treatment

Hami melons (var. *inodorus* Jacq.) cv. "Xizhoumi 25 hao" at the commercially mature stage (sugar content at center 17%) were harvested in Turpan Shan Shan, Xinjiang province, China, on July 25, 2016. The fruits were individually packed in foam plastic net bags to protect them and minimize vibration during transportation and then loaded into standard melon shipping cartons (40 cm × 35 cm × 28 cm) with four melons per carton. The average weight was 2.7 ± 0.1 kg. Upon arrival at the laboratory, the melons were sorted to obtain those without defects and with a uniform gray color.

In our preliminary study, harvested Hami melons were treated by dipping the fruits in various concentrations of OA (0, 10, 15, and 20 mmol L^{-1}, containing 0.5% v/v Tween 20 as a surfactant) at 25 °C for 10 min followed by storage at 3 °C for 42 days. The 15 mmol L^{-1} OA treatment was found to be the most effective for decreasing the CI index and maintaining the quality of the fruit. Therefore, an OA concentration of 15 mmol L^{-1} was used in this study.

For the main study, 420 melons were randomly divided into two groups each containing 210 individual fruits, which represented the control and 15 mmol L^{-1} OA treatment groups. The melons in the treatment and control groups were dipped in 15 mmol L^{-1} OA solution or water, respectively, at 25 °C for 10 min. After allowing the fruits to dry in air, the control and OA-treated fruits were repacked in standard melon shipping cartons (40 cm × 35 cm × 28 cm) with four melons per carton and stored at 3 °C and 90% relative humidity (RH) for 42 days. Three replicates of each group were performed, five fruits (a total of 15 fruits) were randomly removed from each of the control and treatment on 0, 7, 14, 21, 28, 35, 42 day and maintained at 25 °C for two days, and then evaluated for pericarp chilling injury. Furthermore, 105 fruits samples of each group were analyzed the other parameters during low temperature storage (five fruits in triplicate for each date). Pericarp tissue was excised to a depth of 2 mm around the equator of each fruit. Each sample was packed individually in aluminum foil, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

2.2. CI index

The CI index was evaluated using a subjective scale of visual percentage based on the brown spots according to the method described by Bi et al. (2003) with some modifications. The rinds of the fruits were assessed on a chilling scale from 0 to 4, as follows: 0, no signs of brown spots on the rind; 1, slight symptoms < 25%; 2, symptoms from 25% to < 50%; 3, symptoms from 51% to < 75% ; 4, symptoms ≥ 75%. CI index = (chilling scale × number of fruit in each class)/(number of total fruit × highest chilling scale).

2.3. Electrolyte leakage and MDA content

Cylinders of the peel tissues of five Hami melon fruits from each group were halved at the equatorial region, 20 discs (5 mm × 10 mm) was evaluated according to the method described by Wang et al. (2013) with minor modifications. The electrolyte leakage is expressed as a percentage.

The MDA concentration was measured according to the method described by Chen et al. (2008) with minor modifications. Frozen melon pulp tissues (1.0 g) were homogenized in 8 ml of phosphate buffer (pH 7.8, 50 mmol L^{-1}). The MDA concentration is expressed in nmol g^{-1} fresh weight (FW).

2.4. H_2O_2 content and O_2^- production rate

The H_2O_2 content was measured based on the method described by Ferguson et al. (1983) with some modifications. Frozen pulp tissue (5.0 g) was homogenized in cold acetone (5.0 mL) and then centrifuged at 12,000 × *g* and 4 °C for 20 min. Titanium reagent (0.1 mL, 10% $TiCl_4$ in concentrated HCl) was added to 1 ml of the extract supernatant, and the precipitate was washed at least five times with acetone, separated, and dissolved in 3 ml of 1 mmol L^{-1} H_2SO_4, the absorbance was measured at 415 nm, and the content of H_2O_2 in the samples was calculated according to the standard curve. The H_2O_2 content is expressed in μmol g^{-1} fresh weight (FW).

The O_2^- production rate was determined according to the method described by Xu et al. (2012) with minor modifications. Frozen pulp tissue (5.0 g) was homogenized in sodium phosphate buffer (5.0 mL, pH 7.8, 50 mmol L^{-1}) containing 1.0 mmol L^{-1} EDTA, 0.3% Triton X-100, and 2% polyvinylpyrrolidone (PVP) and then centrifuged at 12,000 × *g* for 20 min A portion of the supernatant (1.0 mL) was mixed with