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Alleviation of chilling injury in tomato fruit by exogenous application of oxalic acid

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ABSTRACT

The effects of oxalic acid on the development of chilling injury (CI), energy metabolism and lycopene metabolism in tomato fruit (*Solanum lycopersicum* L.) were investigated. Mature green tomatoes were dipped in 10 mmol l^{-1} oxalic acid (OA) solution for 10 min at 25 °C. Tomatoes were subsequently stored at 4 ± 0.5 °C for 20 days before being transferred to 25 °C for 12 days. Oxalic acid treatment apparently alleviated CI development and membrane damage; maintained higher levels of ATP and ADP; increased activities of succinic dehydrogenase (SDH), Ca^{2+}-adenosine triphosphatase (Ca^{2+}-ATPase) and H^{+}-adenosine triphosphatase (H^{+}-ATPase); and elevated lycopene accumulation associated with the upregulation of *PSY1* and *ZDS* expression in tomatoes during a period at room temperature following exposure to chilling stress. Thus, oxalic acid treatment benefited the control of CI and the maintenance of fruit quality in tomatoes stored for long periods (approximately 32 days).

1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are usually stored at low temperature to delay ripening and extend shelf life, but the fruit is susceptible to chilling injury (CI) when continuously exposed to temperatures below 12 °C but above freezing (Wang, 1993; Zhang et al., 2010). Although incipient CI in tomatoes is not generally apparent during storage at low temperatures, visible symptoms of CI, such as failure to ripen normally, surface lesion or indentations, discolouration, and increased water loss and decay, develop following transfer to warmer temperatures. These changes result in decreased fruit quality, consumer acceptability and shelf life, resulting in substantial economic losses (Luengwilai, Beckles, & Saltveit, 2012; Sharom, Willemot, & Thompson, 1994; Wang, 1993; Zhao et al., 2009). Recently, hot water treatment (Luengwilai et al., 2012) and application of exogenous chemicals, such as brassinosteroids (Aghdam, Asghari, Farmani, Mohayeji, & Moradbeygi, 2012), arginine (Zhang, Shen, Li, Meng, & Sheng, 2013), salicylic acid (Aghdam, Asghari, Khorsandi, & Mohayeji, 2014), and gibberellins (Ding et al., 2015), have shown to be effective in reducing CI in harvested tomatoes.

Oxalic acid is ubiquitous in plant species and plays important roles in living organisms, such as regulation to calcium concentration in plant tissues, and involvement in plant defense against herbivores and in heavy metal tolerance (Franceschi & Nakata, 2005). Recently, studies have revealed that pre-storage application of oxalic acid alleviates CI in harvested mangoes (Ding, Tian, Zheng, Zhou, & Xu, 2007; Li, Zheng, Liu, & Zhu, 2014), pomegranates (Sayyari et al., 2010) and peaches (Jin, Zhu, Wang, Shan, & Zheng, 2014). These effects of oxalic acid have been attributed to enhanced membrane integrity, increased antioxidant capacity and increased energy status. However, no information is available concerning the role of oxalic acid on the response to CI in postharvest tomatoes. Thus, the goal of this study was to examine the influence of oxalic acid on CI, lycopene accumulation, and energy metabolism in tomatoes.

2. Materials and methods

2.1. Fruit material and experiments

"Oumeiyuan" tomatoes were hand-harvested from a commercial orchard in Hangzhou city (China) at the mature-green stage when fruits showed incipient yellow colouration at the end of blossom. Harvested fruit that were uniform in shape and color and without mechanical damage were selected and randomly assigned

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to two lots of 350 fruits. One lot was dipped in 10 mmol l^{-1} oxalic acid solution (the most effective concentration for tomatoes according to our preliminary experiments within a range of concentrations from 5–15 mmol l^{-1} oxalic acid) at 25 °C for 10 min, and the other was dipped in water (as a control). Control and oxalic acid-treated fruit were air dried for approximately 3 h. Fifteen fruits for each treatment were placed inside a clean polypropylene plastic box with the fruit touching, and each box was wrapped in a 0.02 mm thick low-density polyethylene (LDPE) bag. The bags were then placed in low temperature humidity chambers (Sanyo, MIR-554) at 4 ± 0.5 °C for 20 days and were subsequently transferred to 25 °C for 12 days to simulate shelf conditions. Ninety fruits for each treatment were used to evaluate CI during storage. Analysis of ten fruits each in triplicate was conducted at 5-day and 3-day intervals during storage at 4 °C and 25 °C, respectively.

2.2. Determination of the a^* and b^* values

Surface color was measured with a minoltachromameter CR400 (Minolta, Osaka, Japan) on the surface of each fruit at three locations around the equator of the fruit.

2.3. Determination of CI index

There were five classes used for rating the surface area affected by external CI symptoms, such as pitting and uneven ripening. 0: no visible symptoms; 1: trace, symptoms <10%; 2: slight, symptoms from 10% to <20%; 3: moderate, symptoms from 20% to <30%; 4: severe, symptoms ⩾30%. CI index was calculated using the formula: CI (%) = $\sum$ (chilling scale × number of fruit in each class)/(number of total fruit × highest chilling scale) × 100%.

2.4. Determination of MDA and relative leakage rate

MDA contents were assayed according to the method of Jin et al. (2013) with some modifications. Briefly, tomato flesh (1 g) was homogenized with 5 ml of 100 g · l^{-1} trichloroacetic acid and then centrifuged at 10 000×g for 20 min at 4 °C. MDA content was expressed as nmol · g^{-1}FW.

Relative leakage rate was determined according to the method of Zheng, Tian, Meng, and Li (2007). Ten mesocarp discs (3–4 mm thickness × 10 mm diameter) from 10 fruits at the equatorial region were rinsed and immersed in 30 ml distilled water for 4 h. Initial conductivity was determined with a conductivity meter (DDS-11A, Shanghai, China). Final conductivity was also measured while each sample continued to be rinsed for 4 h after being boiled for 15 min. Relative leakage rate was defined as relative conductivity: (initial conductivity/final conductivity × 100%).

2.5. Determination of ATP, ADP and AMP content

The contents of ATP, ADP and AMP were determined using the method of Li et al. (2014). Two grams of flesh from the mesocarp of 10 fruits around the equatorial region were ground with 6 ml of 0.6 mol l^{-1} perchloric acid and then centrifuged at 16 000×g for 15 min at 4 °C. The supernatant was diluted to 5 ml after the solution was neutralized to pH 6.5–6.8 with 1 M KOH. The solution was then passed through a 0.45 μm filter. Contents of ATP, ADP and AMP were measured using Agilent 1100 high-performance liquid chromatography equipped with a reverse Spherisorb C-18 analytical column and an UV detector at 254 nm. Mobile phase A contained 0.06 M dipotassium hydrogen phosphate and 0.04 mol l^{-1} potassium dihydrogen phosphate and was adjusted to pH 7.0 with 0.1 mol l^{-1} KOH. Mobile phase B consisted of pure methyl alcohol. Elution was conducted by a continuous gradient elution with 100–75% for A and 0–25% for B for 14 min. Flow rate of the mobile phase was 0.9 ml min^{-1}. Samples of 20 μl were injected into the HPLC system for analysis of ATP, ADP and AMP contents. Energy charge (EC) was calculated by the following formula: (ATP + 1/2 ADP)/(ATP + ADP + AMP).

2.6. Determination of related enzymes in energy metabolism

Mitochondria of tomatoes were isolated using the protocol of Jin et al. (2013). For preparation of mitochondria, 20 g of flesh from the mesocarp of 10 fruits around the equatorial region were homogenized in an ice bath with 30 ml of grinding medium, composing of 50 mmol l^{-1} Tris–HCl buffer (pH 7.5), 0.25 M sucrose, 0.3 mol l^{-1} mannite, 1 mmol l^{-1} EDTA, 0.5% (w/v) polyvinyl pyrrolidone (PVP), 0.1% (w/v) bovine serum albumin (BSA) and 0.1% (v/v) beta-mercaptoethanol. The homogenate was squeezed through 6 layers of cotton gauze and centrifuged at 5 000×g for 20 min, and the supernatant was centrifuged at 20 000×g for 30 min. The precipitate was then washed twice with 10 ml washing medium, composed of 10 mol l^{-1} Tris–HCl buffer (pH 7.5), 0.25 mol l^{-1} sucrose, 0.3 mol l^{-1} mannite and 1 mmol l^{-1} EDTA. The precipitate was then centrifuged again. The final precipitate was suspended in 3 ml of wash medium for the enzyme assays. All steps were performed at low temperatures of approximately 4 °C.

Succinic dehydrogenase (SDH) activity was determined using the method of Ackrell, Keamery, and Singer (1978) with slight modifications. The reaction was conducted at 30 °C for 5 min in a reaction mixture containing 3 ml of 0.2 m mol l^{-1} phosphate buffer (pH 7.4), 1 ml of 0.2 mmol l^{-1} sodium succinate (pH 7.4), 0.1 ml of 0.9 mmol l^{-1} 2,6-dichlorophenol indophenol (DCPIP), 0.1 ml of 0.33% 5-methylphenazinium methosulfate (PMS) and 0.3 ml crude mitochondrial extract. One unit of SDH activity was defined as a change of 0.01 in absorbance per minute at 600 nm under the assay conditions.

Cytochrome C oxidase (CCO) activity was measured using the method of Jin et al. (2013). The assay medium consisted of 0.2 ml of 0.3 mmol l^{-1} cytochrome C solution, 0.5 ml 20 mol l^{-1} dimethyl phenylenediamine, and 0.2 ml crude mitochondria extract. The assay was performed at 37 °C for 3 min. The oxidizing velocity of reduced cytochrome C at 510 nm was determined. One unit of CCO activity was defined as a change of 0.01 in absorbance per minute at 510 nm under the assay conditions.

The activity of Ca^{2+}-ATPase and H^+-ATPase was measured using the method of Jin et al. (2013). For Ca^{2+}-ATPase, 3 ml of total reaction mixture consisted of 30 mmol l^{-1} pH 8.0 Tris–HCl, 3 mmol l^{-1} Mg_2SO_4, 0.1 mmol l^{-1} Na_3VO_4, 50 mmol l^{-1} $NaNO_3$, 3 mmol l^{-1} $Ca(NO_3)_2$, 0.1 mmol l^{-1} ammonium molybdate, and 0.3 ml mitochondrial extract. For H^+-ATPase, 3 ml assay medium consisted of 30 mM pH 8.0 Tris–HCl, 3 mM Mg_2SO_4, 0.1 mmol l^{-1} mM Na_3VO_4, 50 mmol l^{-1} $NaNO_3$, 0.1 mmol l^{-1} ammonium molybdate, and 0.3 ml mitochondrial extract. 100 μl of 30 mmol l^{-1} ATP-Tris (pH 8.0) was added to initiate the reaction. After 30 min of incubation at 37 °C, the reaction was stopped by 55% trichloroacetic acid. The quantity of inorganic phosphorus was determined using the "Inorganic Phosphorus Detection Kit" per the manufacturer's protocol (NJBI, Nanjing, China). One unit of Ca^{2+}-ATPase and H^+-ATPase activities was defined as the release of 1 μmol l^{-1} of phosphorus in absorbance per minute at 660 nm under the assay conditions.

The activities of SDH, CCO, Ca^{2+}-ATPase and H^+-ATPase were expressed in U · mg^{-1}protein units. Protein contents were measured using the method of Bradford (1976). Bovine serum albumin (BSA) was used as a standard.

2.7. Determination of lycopene

Lycopene content in tomato mesocarp was determined using the method of Toor and Savage (2006). Lycopene from mesocarp