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Antioxidative responses of ripe tomato fruit to postharvest chilling and heating treatments

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ABSTRACT

The objective of this research was to determine the antioxidative responses to chilling and heating stresses in ripe tomatoes cv. 'Sanibel'. Full ripe fruit were treated with either chilling (5 °C for 5 days), hot water (52 °C for 15 min, then cooled to 25 °C with tap water), or left untreated as the control. Fruit samples were taken directly after treatment or after 4 days storage at 20 °C. Directly after treatments, heating remarkably increased the activity of lipoxygenase (LOX), and chilling increased malondialdehyde (MDA) content. LOX catalyzes the oxidation of lipids, and MDA is a compound produced from lipids under oxidative stress. The increase of LOX activity and MDA content indicates escalated reactive oxygen species (ROS) in the tissues. The heat treatment also increased ascorbate levels, and induced the activities of catalase (CAT) and peroxidase (POD) as well. After 4 days storage at 20 °C, antioxidant reductive capacity (ARC) and the activity of ascorbate peroxidase (APX) was significantly higher in the chilling treated fruit than in the control. Chilling directly increased MDA and ascorbate levels, and led to a remarkable increase in H_2O_2 on day 4 after treatment. The activities of CAT and POD were lower in chilled fruit than in the control on day 4 after treatment. These results indicate that oxidative stress may be induced by chilling treatment even without visual injury on the fruit surface. Heat treatment enhanced the antioxidant enzyme system to potentially protect fruit from environment stress.

1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are often harvested at mature green stage commercially and such fruits are susceptible to chilling injury during storage and transportation at refrigerate temperatures (Biswas et al., 2015; Paull, 1990). Chilling injury causes improper ripening, sunken spots, and increased susceptibility to *Alternaria* rot (Ding et al., 2002), and often associated with increased oxidative stresses (Stevens et al., 2008). However, like many other chilling sensitive crops such as avocado, peach and mango, the ripe tomato fruits are more tolerant to chilling injury than the unripe ones (Paull, 1990). Previous research showed that even without visual disorder in the ripe tomatoes, flavor loss was observed (Bai et al., 2011; Wang et al., 2015) and the influences were evidenced in gene express and enzyme activity levels when exposure fruits to chilling temperatures (Bai et al., 2011).

Heat treatments have been determined to alleviate chilling injury for unripe tomatoes (Biswas et al., 2015; Ding et al., 2002; Wang et al., 2015) for the visual quality, decay protection and maintenance of a better flavor quality. There is little information available for the oxidative stress in ripe tomatoes under such chilling or heating exposure.

Changes in the environmental conditions can cause stress to plants. The stress causes a variety of biochemical, physiological and metabolic changes. When exposed to abiotic stress conditions, such as a low temperature, high temperature, water deficit stress, and ozone pollution, production of reactive oxygen species (ROS) increases and causes oxidative stress (Imahori et al., 2008). Oxidative stress occurs when the generation of ROS exceeds the capacity of plant to maintain cellular redox homeostasis or to scavenge ROS (Paull and Chen, 2000; Hodges et al., 2004; Vicente et al., 2006). ROS accumulation may cause oxidative damage to lipids, forming toxic products such as malondialdehyde (MDA) (Imahori et al., 2008). Lipoxygenase (LOX) can promote oxidation of polyunsaturated fatty acids by catalyzing the conjugation and hydroperoxidation of structures within fatty acids (Parkhey et al., 2012). ROS accumulation may cause oxidative damage to lipids, forming toxic products

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such as MDA. Decrease in antioxidant activity in stressed tissues results in higher levels of ROS that may contribute to cellular injury.

Scavenging of ROS is important for maintenance of normal plant growth. Plants have evolved an efficient antioxidant defense system that would prevent the accumulation of ROS and repair oxidative damage. This system involves lipid-soluble antioxidants (e.g. α-tocopherol and carotene), water-soluble reductants (e.g. ascorbate and glutathione), and antioxidative enzymes (Imahori et al., 2008). Superoxide dismutase (SOD) dismutases superoxide radical to H_2O_2 and O_2, thus protecting the cells from damage by superoxide radical reaction products. The product, which is a potentially toxic compound, is then reduced to water by a number of enzymes such as catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) (Kochhar et al., 2003). In addition to reacting non-enzymatically with ROS, those reducing substances are known to form an ascorbate-glutathione cycle. Hence, this cycle could play an important role to scavenge H_2O_2 in the plant (Foyer et al., 1997; del Río et al., 1998). Thus, plants have developed a series of both enzymatic and non-enzymatic detoxification systems to scavenge ROS, so that protecting plant cells from oxidative stress (Wahid et al., 2007).

Moderate stress not only induces the resistance to this kind of severe stress, but also can improve tolerance to other stresses. It has been shown that a number of abiotic stresses such as a low temperature, high temperature, and ultraviolet light, applied before storage increase tolerance to stresses (Gonzalez-Aguilar et al., 2010; Pesis, 2005). Most postharvest treatments involve the alteration of the natural conditions of the horticultural commodities in order to prolong its postharvest life (Dumas et al., 2003; Gonzalez-Aguilar et al., 2010). Some postharvest treatments could induce some mechanisms that affect the metabolic activity of the treated produce, such as the triggering of the antioxidant mechanism of horticultural crops. The activation of the antioxidant system as a response to postharvest stress can result in improving the antioxidant status of horticulture crops (Gonzalez-Aguilar et al., 2010). Hence, activation of various pathways leading to the accumulation of antioxidants has been associated with responses of ripe tomato fruits to several abiotic stresses such as chilling and heating.

The purpose of this research was to determine how chilling and heating affected the antioxidant status in full ripe tomato fruit, and to discuss the possible mechanisms.

2. Materials and methods

2.1. Plant materials and treatments

Tomato (*S. lycopersicum* L. cv. Sanibel) was grown in outdoor field plot at the University of Florida Gulf Coast Research and Education Center, Wimauma, FL, and harvested at the full red stage (surface color a* value reached a plateau (>20)). After washing and air drying, fruit were then treated with either chilling (5 °C refrigerator for 5 days), hot water (heating, 52 °C water bath for 15 min, then cooled with tap water to 25 °C), or not treated as the control. Fruit samples were taken directly after treatment or after 4 days storage at 20 °C to determine the hysteresis effects and injury recovery. There were three replicates of three fruit each per treatment per sampling day. For sampling, pericarp tissue from three fruit per replicate were quickly collected with a sharp stainless steel knife and pulverized in liquid nitrogen, then stored at −80 °C until analysis.

2.2. Extraction and measurement of redox products and intermadiets

Samples were extracted and analyzed as described by Stevens et al. (2006), except stated otherwise. Briefly, tomato tissue was ground in liquid nitrogen, and 5 g of powder was homogenized with 3 mL of ice-cold 6% trichloroacetic acid. Samples were centrifuged for 15 min at 25 000 × *g* at 4 °C. The supernatant was used for following assays. A 96-well microplate reader (Model SynergHT, BioTek, Winooski, VT, USA) was used for measurement of absorbance.

MDA content was determined by the thiobarbituric acid (TBA) reaction with malondialdehyde assay kit (NWK-MDA01, Vancouver, WA, USA) according to the protocol of manufacturer. The absorbance of the samples at 532 nm was used for determination of MDA content.

H_2O_2 content was determined with H_2O_2 assay kit (NWK-HYP01, Vancouver, WA, USA) according to the protocol of manufacturer. The absorbance of the samples at 560 and 595 nm was used for determination of H_2O_2 content.

For ascorbate (AsA) assay, the AsA standards were prepared freshly. Two assays were carried out on each sample, one to measure the total AsA (including the addition of 5 mM dithiothreitol (DTT)) and another to quantify the reduced AsA content (omission of DTT from the assay). The absorbance was read at 550 nm was used for determination of AsA content.

Glutathione (GSH) content was determined with glutathione assay kit (NWK-GSH01, Vancouver, WA, USA) according to the protocol of manufacturer. The absorbance of the sample at 405 nm was used for determination of GSH content.

Antioxidant reductive capacity (ARC) was determined with antioxidant reductive capacity assay kit (NWK-ARC01, Vancouver, WA, USA) according to the protocol of manufacturer. The absorbance of the sample at 490 nm was used for determination of ARC.

2.3. Extraction and assay of lipoxygenase (LOX), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD)

Extraction of enzymes were carried out according to Yilmaz et al. (2001) with some modification. The composition of homogenization buffer was 150 mM Tris–HCl (pH 8.0), including 250 mM sorbitol, 10 mM $MgCl_2$, 1% glycerol (v/v), 0.2% PVP (w/v), 5 mM DTT, and the following protease inhibitors: 0.1 mM PMFS, 0.1 mM benzamidine, and 5 mM aminocaproic acid. Frozen pericarp tissue 10 g with 10 mL homogenization buffer were thawed and blended at high speed for 30 s in an ice bath. After filtration through two layers of Miracloth (Calbiochem, La Jolla, CA), the homogenate was centrifuged for 20 min at 12,000 × *g* at 4 °C, and the supernatant was collected as the crude enzyme source and assayed immediately or flash-frozen in liquid nitrogen and stored at −80 °C until analysis. A 96-well microplate reader (Model SynergHT, BioTek, Winooski, VT, USA) was used for kinetic property and measurement of enzyme reaction.

The assay of lipoxygenase (LOX) was adapted from Riley et al. (1996) with the linoleic acid substrate prepared according to Surrey (1964). An increase in absorbance at 234 nm was followed for 20 min at 20 °C after addition of 10 μL substrate solution into a well containing 10 μL of the enzyme extract, 10 μL of assay buffer (150 mM Tris–HCl buffer, pH 8.0, containing all compounds present in the homogenization buffer except glycerol and protease inhibitors), and diluted by adding 200 μL water. LOX activity was calculated by monitoring the formation of conjugated diene at 234 nm using an extinction coefficient of 25 mM^{-1} cm^{-1} (Axelrod et al., 1981).