Postharvest Biology and Technology 63 (2012) 123–128

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Chilling-injury of harvested tomato (*Solanum lycopersicum* L.) cv. Micro-Tom fruit is reduced by temperature pre-treatments

Kietsuda Luengwilai[1], Diane M. Beckles, Mikal E. Saltveit*

Department of Plant Sciences, University of California-Davis, One Peter Shields Avenue, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 31 August 2010
Accepted 26 June 2011

Keywords:
Tomato fruit
Chilling injury
Electrolyte leakage

ABSTRACT

Heat-shocks were used to reduce the development of chilling injury symptoms during ripening of tomato fruit (*Solanum lycopersicum L.* cv. Micro-Tom). Mature green tomatoes were immersed in 30–50 °C water for 3–9 min before being chilled at 2.5 °C for 0, 0.5, 1, 2, 3, or 14 days, and then held at 20 °C for an additional 7–14 days. The affect of both heat-shock and chilling treatments were independent of fruit weight. Measured at 20 °C after 14 days of chilling, fruit exposed to 40 °C for 7 min exhibited reduced chilling injury symptoms, as measured by their advanced ripening score and decreased rate of ion leakage into an isotonic 0.2 M mannitol solution. Reduced rates of leakage from the symplastic compartment probably contributed to the 2-fold decrease in the amount of ions in the apoplastic space, when compared to the control. A subsequent paper will report the results of metabolic profiling of Micro-Tom tomato fruit subjected to treatments that significantly decreased their development of chilling injury symptoms.

1. Introduction

Chilling injury (CI) is a physiological disorder of plants and plant organs caused by exposure to low, but non-freezing temperatures (ca. >10 °C) (Raison and Lyons, 1986). Chilling-sensitive agriculturally important crops are often of tropical and subtropical origin and include avocados, bananas, soybeans, and tomatoes. The severity of injury depends on numerous intrinsic (e.g., cultivar, prior growing conditions, and prior exposure to stress) and extrinsic (e.g., temperature, duration of exposure, surrounding relative humidity, sanitation, and level of mechanical injury) factors. CI can occur before or after harvest, i.e., in the field, and during transport, storage and marketing (Morris, 1982). Symptoms are manifold, and include tissue browning, pitting and discoloration of the skin, uneven ripening, and increased disease susceptibility. These detrimental changes reduce quality and consumer acceptability leading to substantial economic loss.

Chilling can adversely affect harvested crops in two ways. First, exposure to low temperatures for certain durations can result in the development of chilling injury symptoms as described above. Second, since sensitive crops must be stored at temperatures above their chilling threshold, they cannot take full advantage of low temperature storage to slow metabolic activity and preserve quality. Storage at these elevated temperatures shortens their market-life compared to crops that can be stored just above their freezing point. CI has been recognized, described, and studied for over 100 years (Saltveit and Morris, 1990), yet the primary cause and subsequent development of chilling injury symptoms remains unresolved. There is therefore both a practical need and an intellectual curiosity to understand the basis of this physiological disorder.

Conceptually, CI can be subdivided into two events; a primary event that is temperature-dependent and is initiated when the temperature falls below a threshold temperature for a specified duration, and causes some metabolic dysfunction. The secondary event is time-dependent and includes a multitude of metabolic processes that can be adversely affected as a consequence of the primary event, and lead to the development of measurable symptoms characteristic of chilling injury (Orr and Raison, 1990). Separating CI into these two stages helps to delineate the fundamental molecular mechanisms underlying this phenomenon, which is enormously complex. Moreover, it becomes possible to differentiate the primary 'cause' (i.e., the initial event happening upon chilling) from the secondary 'effect' (i.e., the subsequent events that produce physiological and visual signs of chilling injury) (Orr and Raison, 1990).

Heat-shock treatments reduce the development of chilling injury symptoms in tomato fruit (Saltveit, 2001). Exposure to temperatures at about 10–20 °C above the normal growing temperature induces the production of a unique set of heat shock proteins (HSPs) that may function as molecular chaperones. These HSPs bind to unfolded or denatured proteins and prevent cell damage at low, chilling temperatures (Saltveit, 2002). In the experiments reported,

* Corresponding author.
E-mail address: mesaltveit@ucdavis.edu (M.E. Saltveit).
[1] Current address: Horticulture Department, Kasetsart University, Kampangsaen campus, Kampangsaen, Nakorn Pathom 73140, Thailand.

0925-5214/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.postharvbio.2011.06.017

a heat-shock protocol is identified that reduces the development of chilling injury symptoms (i.e., preserves normal ripening, and decreases chilling-induced increases in ion leakage) in harvested mature green Micro-Tom tomato fruit.

2. Materials and methods

2.1. Plant growth conditions

Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) seeds were a gift from Dr. David Weiss (The Hebrew University of Jerusalem, Israel). Tomato plants were grown from May to October 2009 in the Environmental Horticultural greenhouse, UC Davis, CA. Plants were grown in 15-cm diameter pots in standard soil media. Liquid fertilization with 100 mg/L nitrogen, 26 mg/L phosphorous, and 124 mg/L potassium was applied following standard cultural practices. Average daily PAR light in the greenhouses was 25.25 ± 2.62 mole m^{-2} s^{-1}. Temperatures averaged 20 °C at night and 25 °C during the day. Humidity was approx. 50% during the day and 90% at night.

2.2. Fruit sampling and postharvest treatments

Mature green fruit (i.e., liquefying locular tissue, seeds not cut with a knife) (Saltveit, 1991) were hand-harvested between 7 and 8 am. Uniform and non-damaged fruit were washed in commercial bleach (1:20 dilution of 5% (v/v) sodium hypochlorite), and air-dried under a transfer hood. Three replicates of 4 fruit each were used for each of the 10 time points studied. Control fruit were held at 20 °C and heat-shocked fruit were immersed in a water bath at 30–50 °C for 3–9 min. All fruit were air-dried before being chilled at 2.5 °C for 0, 0.5, 1, 2, 3, or 14 days. Fruit were then ripened at 20 °C for an additional 0, 0.5, 1, 2, 7, 14 or 21 days. These experiments were repeated four times with slight variations in the duration of chilling exposure.

2.3. Ripening score

The degree of ripening was expressed as an average ripening score for each replicate. Ripening was scored as a visual determination of the surface color, where 1 = the surface completely green, 2 = less than 25% of the surface pink or red, 3 = more than 25% but not more than 60% of the surface pink or red, and 4 = more than 60% of the surface pink or red.

2.4. Respiration rate

The chilled fruit were removed from 2.5 °C and held at 20 °C for 1, 4, 8 or 24 h before respiration rate was measured. Four fruit per replicate were placed into a sealed 500 mL glass container for 1 h. A 1 mL sample of the head space was withdrawn using a syringe and its CO_2 concentration measured with an infrared gas analyzer as previously described (Saltveit and Strike, 1989). Fruit were then dissected for subsequent study.

2.5. Dissection of fruit

A 1 cm diameter stainless steel cork borer was used to remove the central core from each fruit. The bottom pericarp disk thus excised was rinsed in deionized (DI) water to remove adhering tissue and immediately frozen in liquid nitrogen and kept at −80 °C until used for metabolic profiling (to be reported in a subsequent paper). The remaining locular tissue was cut into four radial segments and washed of adhering tissue with DI water. One radial segment from each of four fruit (one replicate) was placed into each section of a 4-sector 25 × 100-mm diameter plastic Petri dish. Each of the four sections thereby contained one radial segment from each of the four fruit per replicate, and each dish contained all the radial segments from the four fruit in a replicate.

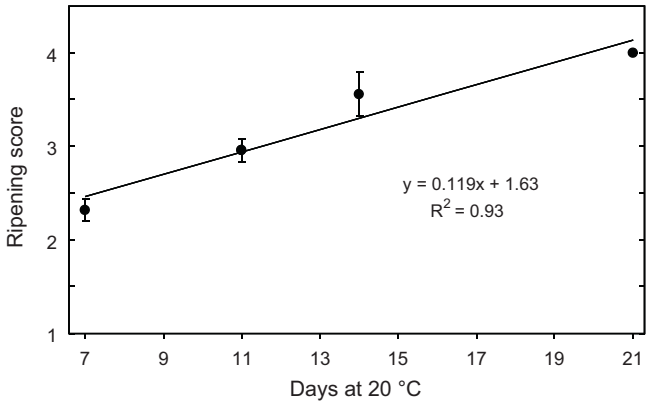

Fig. 1. Ripening scores of harvested Micro-Tom fruit. The fruit were harvested at the mature green stage (soften locular tissue, seeds not cut with a knife) and stored at 20 °C. The ripening score was calculated as described in the text. Values are mean ± S.D. of 10–40 fruit.

2.6. Ion leakage measurement

The dishes with the excised radial segments were placed into plastic tubs lined with wet paper towels and loosely covered with aluminum foil. The tubs were held at 12.5 °C for 18 h to produce 'aged' tissue, i.e., to overcome the wound-induced alterations in membrane permeability (Saltveit, 2005).

After transferring to room temperature (approx. 18 °C), the four aged segments from each sector of the Petri dish were put into a 50 mL plastic centrifuge tube containing 20 mL of an aqueous solution of 0.2 M mannitol. It had previously been determined that 0.2 M was isotonic for this tissue (Saltveit, 2005). The conductivity of the bathing solution was measured with an Extech Model 480 digital conductivity meter (Waltham, MA) every 5 min for 30 min and then less frequently for 180 min with gently shaken between readings. After 3 h the tubes were capped, frozen at −20 °C and warmed to room temperature and frozen and thawed twice before the total conductivity of the solution was measured at room temperature after 1 h of shaking.

3. Results and discussion

3.1. Response of harvested mature green fruit to chilling

Micro-Tom tomato fruit have been used as a model system to study chilling injury (Neta-Sharir et al., 2005; Malacrida et al., 2006; Gomez et al., 2009; Weiss and Egea-Cortines, 2009). The ripening score of mature green fruit held at 20 °C increased linearly ($R^2 > 0.93$) from 7 to 21 days after harvest (Fig. 1). The fruit were harvested at the mature green stage and developed ripening scores of 2.3, 3.6 and 4.0 after 7, 14 and 21 days, respectively. In contrast, chilling the mature green fruit at 2.5 °C for 0 to 3 weeks produced a linear decrease ($R^2 > 0.99$) in their ripening score measured after holding the chilled fruit at 20 °C for an additional 14 days (Fig. 2). The ripening score decreased 15% (from 3.3 to 2.8) after one week of chilling, but it was not significantly different from that of the non-chilled control ($P = 0.05$). Extending chilling for 2–3 weeks produced a significant 35% and 2-fold decrease in the ripening score, respectively. Two weeks was selected as the duration of chilling because fruit could recover from this modest level of injury and treatments that modulated the fruit's chilling tolerance would produce a greater change in the response (i.e., changes in the ripening