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Tomato cv. 'Micro-Tom' as a model system to study postharvest chilling tolerance

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ABSTRACT

Storage at low temperature is a common practice to extend the market life of many vegetables. Among other horticultural crops, tomato fruit suffers chilling injury when it is storage under refrigerated conditions. Much effort has been made to understand the mechanisms of generation of this physiologic disturbance, but many aspects need to be clarified yet. Tomato (*Solanum lycopersicum*) cv. "Micro-Tom" is a miniature tomato plant with various properties that make it useful as a model system in plant biology. In this work, the potential of tomato cv. "Micro-Tom" fruit as a model to study chilling injury was investigated. The effect of postharvest chilling was compared on cvs. "Micro-Tom" and "Minitomato", another variety with fruit of similar size. Green mature fruits cvs. "Micro-Tom" and "Minitomato" were harvested and stored during 4 weeks at 4 °C. It was observed that tomato cv. "Micro-Tom" fruit was clearly tolerant to chilling while tomato cv. "Minitomato" fruit developed severe chilling injury symptoms and avoided ripening. Harvest and chilling altered the length of time between the different ripening stages, the development of red full color and smell. Also, it was shown that harvesting fruits by visual appreciation is a rapid and useful method for distinguishing the different ripening stages during tomato cv. "Micro-Tom" fruit ripening. These results showed that tomato cv. "Micro-Tom" fruit was able to counteract the strain resulting from the imposed chilling stress. Altogether these data indicate that tomato cv. "Micro-Tom" fruit is a good model to study the mechanism of postharvest chilling response and tolerance in tomato.

1. Introduction

The market life of fruits and vegetables can be extended through storage at low temperature (Kader, 2003). This practice decreases metabolic and pathogenic activities and facilitates exportation by allowing long distance shipment and more regulated supply of fruit in the market. However, storing these products below critical temperatures may result in a physiological disorder known as chilling injury that generates high economic losses and stresses applied to fruit production. While physiological and biochemical events involved in chilling injury have been extensively described, much remains to be understood about the precise molecular mechanisms of its generation and tolerance mechanisms.

Tomato (*Solanum lycopersicum*) fruit is chilling sensitive at temperatures below 10 °C if held for longer than 2 weeks or at 5 °C for longer than 6–8 days affecting the final fruit quality (http://postharvest.ucdavis.edu/pfvegetable/Tomato/). Symptoms of chilling injury are failure to ripen and to develop full color and flavor, premature softening, skin depressions (pitting), browning of seeds, and increased decay. Development of chilling injury symptoms depends upon several factors: preharvest conditions, maturity at harvest, postharvest handling including storage time and temperature and cultivar sensitivity. It has been reported than green mature fruit cv. "Micro-Tom", a dwarf cultivar bred for home gardening purpose (Scott and Harbaugh, 1989), stored at 4 °C during a month is capable of ripening to the red stage inducing its antioxidant system (Malacrida et al., 2006). Tomato cv. "Micro-Tom" small size, rapid growth, short life cycle for the fruit harvest after sowing and easy transformation (Sun et al., 2006), have made tomato cv. "Micro-Tom" plants a convenient model system for research on different fields. Several studies have been conducted on tomato genetics (Meissner et al., 1997), carbohydrate metabolism

Abbreviations: G_{on}, Y_{on}, O_{on} and R_{on} green, yellow, orange and red fruit ripened on the vine; Y_{off}, O_{off} and R_{off} yellow, orange and red fruit ripened off the vine; G_{ch}, green fruit chilled; Y_{ch}, O_{ch} and R_{ch} yellow, orange and red fruit chilled and ripened off the vine.

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(Obiadalla-Ali et al., 2004), hormonal functions and interactions (Campos et al., 2010), microbial plant interaction (Park et al., 2007), arbuscular mycorrhizal symbiosis (Salvioli et al., 2012), *Solanaceae* genomics (Aoki et al., 2010), amino acids metabolism (Ferraro et al., 2012; Scarpeci et al., 2007; Sorrequieta et al., 2010), and in molecular breeding of tomato fruit shelf-life (Okabe et al., 2012). The "Micro-Tom" phenotype is due to at least three mutations: *self-pruning* (producing a determinate phenotype), *dwarf* (reducing internode length and producing smaller, rugose, and dark-green leaves) and *miniature* (likely to be associated with gibberellin signaling) (Marti et al., 2006; Meissner et al., 1997). An important characteristic of tomato cv. "Micro-Tom" fruit is that it can ripen after postharvest chilling at 4 °C. Additionally, this fruit showed a high antioxidant response (Malacrida et al., 2006). These data led us to evaluate deeply the behavior of tomato cv. "Micro-Tom" fruit after postharvest chilling.

To reduce the degree of variability in ripening experiments, assessment of ripening stages should be as accurate and rapid as possible. The harvest of tomato fruit is usually based on a comparison of color fruit to color charts, time from anthesis (Zhang et al., 2009), time from breaker stage or green mature stage, determination of the color parameters or the firmness, or both, and Raman spectroscopy (Qin et al., 2012). Several of these techniques are time-consuming and not adequate when immediate fruit processing is required. On the other hand, when tomato cv. "Micro-Tom" fruits were harvested employing periods' based methods and visually inspected (externally and internally), they do not show developmental synchrony. In this work, it is shown that external visual appreciation of color corresponds with color parameters. This criterion helps to make a quick harvesting of fruits and fulfills the requisite of uniformity in fruit developmental stages. Additionally this approach was used to evaluate the chilling effect on the final quality of tomato cv. "Micro-Tom" fruit.

2. Materials and methods

2.1. Plant material

Tomato (*S. lycopersicum*) plants (cvs. "Micro-Tom" and "Minitomato") (Kisaka and Kida, 2003) were grown in a controlled environment cabinet under a light intensity of 400 μmol s^{-1} m^{-2} at the top of the plants containing the fruit. The temperature ranged from 25 °C during the light period (14 h) to 18 °C in the dark, and the relative humidity was 70%. Plants were grown in soil, continuously maintained under optimal irrigation, and supplied weekly with half strength Hoagland solution (Malacrida et al., 2006).

Fruit ripening was tested under three different conditions (Fig. S1): on the vine (fruits were allowed to ripen naturally on the plant), off the vine (fruits were picked at the mature green stage and directly placed on a shelf in the growing cabinet), and chilled (fruits were harvested at the mature green stage, stored for four weeks at 4 °C, and then transferred back to the growing cabinet). Fruits were collected by visual appreciation at the mature green (*G*), yellow (*Y*), orange (*O*), and red (*R*) stages. *G*, *Y*, *O* and *R* are accompanied by a subscript referring to the ripening conditions: on—ripening on the vine, off—ripening off the vine, ch—4 weeks postharvest storage at 4 °C and ripening on the shelf. Mature green fruit was collected when fruit stopped growing and had a whitish coloration at the blossom end.

2.2. Chilling injury evaluation

Chilling injury severity was assessed by determining the capacity of the fruits to reach the red stage under the different ripening conditions. Their maturation was checked visually during 15 days. Also, the fruits were inspected to determine deterioration, infections and pitting (decay). Fifty fruits for each variety and treatment were tested.

2.3. Determination of the duration of transition periods between different ripening stages

Flowers were tagged at anthesis and the development and ripening of fruits on the vine, off the vine and off the vine with previous chilling were registered and monitored daily. The ripening stage (*G*, *Y*, *O* and *R*) was defined by visual appreciation.

2.4. Color determination

Fruits were cleaned, dried, cut transversely through the center and placed on the scanner (Hewllet Packard) with the cut side down and a black background. The images obtained were analyzed employing "Tomato Analyzer Color Test" (Rodriguez et al., 2010) designed to quantify the color parameters *R* (red), *G* (green), and *B* (blue) of the RGB color space. The average RGB values were employed to calculate L^*, a^*, b^* of the CIELAB color space and Hue and Chroma color descriptors. The scanner color calibration was achieved employing Color checker Munsell Color X-write.

2.5. Assessment of total soluble solids and firmness

The soluble solids content was determined employing a hand SO-RH digital refractometer, previously calibrated with distilled water. Values were expressed as a percentage of glucose and fructose in the juice from ripe fruits (1 °Bx). Firmness was determined on two points of each fruit using a Fruit Pressure Tester-12.5-N type shore with a tip of 0.1 in 0–100 scale. Analyses were carried out in forty fruits.

2.6. Sensory analyses

Ten tomatoes from each treatment were washed, dried and placed on white trays labeled with three-digit numbers. The same ten tomatoes were evaluated by all panelists for skin color and appearance. Another ten tomatoes from each treatment were washed with tap water, dried and cut in half in order to evaluate odor and sweetness, acidity and juiciness-by-mouth. Two tomatoes were evaluated by each panelist. Ten panelists (5 men, 5 women) who responded affirmatively to liking and frequently consuming tomatoes were recruited from students or staff at IBR. Sensory attributes and their definitions were discussed. All panelists were given the opportunity to practice the procedure in advance. Panelists evaluated the attributes of tomato samples on four-point scales.

3. Results

3.1. Chilling injury of tomato cvs. "Micro-Tom" and "Minitomato" fruits

Tomato cvs. "Micro-Tom" and "Minitomato" fruits were harvested at mature green stage and evaluated for chilling injury symptoms after storage during four weeks at 4 °C. These varieties were chosen for comparison because of their fruit similar size, and to avoid the effect of surface to area volume ratio. Green fruits of the two varieties (Fig. 1a and f) were also picked from the plant and ripened off the vine. These fruits evolved on the shelf to a red-ripe stage (Fig. 1c and h) visually similar to tomatoes fully ripened on the vine (Fig. 1b and g). When tomato cvs. "Micro-Tom" and "Minitomato" fruits were taken off the camera after four weeks at 4 °C storage, they were still green and were mostly free of chilling