Postharvest Biology and Technology 97 (2014) 62–67

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Magnetic resonance imaging provides spatial resolution of Chilling Injury in Micro-Tom tomato (*Solanum lycopersicum* L.) fruit

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ARTICLE INFO

Article history:
Received 20 February 2014
Accepted 4 June 2014

Keywords:
Chilling injury
Tomato fruit
Magnetic resonance imaging (MRI)
Apparent diffusion coefficient (ADC)

ABSTRACT

Magnetic resonance imaging (MRI) was used to monitor internal changes in harvested tomato (*Solanum lycopersicum* L. cv. Micro-Tom) fruit. Measurements of ethylene evolution, respiration, and ion leakage indicated that the fruit developed chilling injury (CI) after storage at 0 °C. Unlike these measurements, MRI provided spatially resolved data. The apparent diffusion coefficient (ADC), which is an indication of water mobility in tissues, was calculated from MRIs of the different parts of the fruit. Storage for 1 or 2 weeks at 0 °C caused no difference in the ADCs (*D*-values) in the pericarp, but it did lead to higher values in the inner tissues i.e., the columella and locular region compared to non-chilled fruit ($P < 0.05$). Changes in inner fruit *D*-values after 1 and 2 weeks of chilling at 0 °C were similar to changes in respiration, ethylene production and ion leakage which increased ($P < 0.05$) compared to the non-chilled controls. Most CI studies of tomato fruit used pericarp tissue. Our data indicate that columella tissue changes occur in response to chilling injury in tomato fruit and suggest that more caution is needed when interpreting data from experiments commonly used to study this phenomenon.

1. Introduction

Exposure of susceptible plant tissues to non-freezing temperatures below 10–12 °C induces a physiological disorder called Chilling Injury (CI) (Saltveit, 2000, 2005). There appears to be two phases in the development of CI. The first phase is initiated in the cold (Lyons, 1973) and could involve a change in membrane fluidity or enzyme activity (Saltveit, 2000). Overt symptoms develop after prolonged chilling or upon warming to non-chilling temperatures (e.g., 20 °C) (Saltveit, 2000, 2003). These secondary symptoms are predicated by primordial events initiated in the cold, and include a host of metabolic and physiological changes that include increased membrane permeability (Saltveit, 2005), increased respiration and ethylene production (Saltveit, 2003), uneven ripening, disease susceptibility, water soaking and surface pitting (Luengwilai et al., 2012a; Morris, 1982; Sharom et al., 1994). A technique that could detect the earliest physiological changes associated with CI would foster a better understanding of the initial events leading to this disorder, and point to more effective ameliorative action.

Magnetic resonance imaging (MRI) is a nondestructive imaging technique, which is increasingly used to visualize and quantify fruit physiological response to endogenous or exogenous stimuli (Abbott, 1999; Defraeye et al., 2013). MRI uses the magnetic properties of nuclei and their interactions with radio frequency and applied magnetic fields to produce an image (Clark et al., 1997). Variations in the chemical composition and integrity of cellular structures can change the movement of water within and among tissues. These changes can be detected as modifications in the relaxation times of the protons in water, which in turn alters the signals used to construct MR images (Zhang and McCarthy, 2012). Diffusion-weighted MRI of tissues provides a quantitative measure (*D*-values) of the apparent diffusion coefficient of water, instead of estimations of water mobility from relaxation measurement that include the influence of translational mobility, composition and other factors (Zhang and McCarthy, 2012). In addition, a spatially resolved map of the apparent diffusion coefficient (ADC) of water can be obtained, which could help to understand and quantify the development of disorders such as CI within the tissue.

MRI has been used to gain insight into early phases of different postharvest physiological disorders before the manifestation

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http://dx.doi.org/10.1016/j.postharvbio.2014.06.005
0925-5214/

of external symptoms (Nicolai et al., 2014). These include core breakdown in pear (Lammertyn et al., 2003) watercore disorder (Herremans et al., 2014), internal browning (Gonzalez et al., 2001) and mealiness in apple (Barreiro et al., 1999; Letal et al., 2003). There are few reports where MRI was used to detect the early stages of CI in sensitive produce. In persimmon, MR images of cold-stored fruit were distinct from those stored at ambient temperature (Clark and Forbes, 1994). In zucchini squash (Wang and Wang, 1992), MRI provided enough data to act as a predictor of where water soaking would occur in the epidermis after the cold-storage. These studies both indicated that MRI has great potential for studying CI in fruit tissues.

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops both economically (Beckles et al., 2012) and as a genomics, molecular, biochemical, and physiological model for biological processes occurring in fleshy fruits (Seymour et al., 2013). Like most subtropical fruit, tomato is susceptible to CI. Studies with tomato fruit could leverage existing functional genomics resources to pinpoint the molecular basis of this trait. To our knowledge, MRI has not been used to study CI in this species. We used the dwarf cultivar 'Micro-Tom' because it is the functional genomics model for tomato (Meissner et al., 1997). Its high-density growth, short life cycle and concentrated fruit-set (i.e., many fruit of a similar age) makes it possible to obtain harvests of 500 fruit or more per square meter per year (Meissner et al., 1997). Because tomato postharvest studies can be hampered by biological variability (Hertog et al., 2004), the availability of numerous, similarly aged fruit makes Micro-Tom a convenient experimental model for postharvest studies (Gomez et al., 2009; Luengwilai et al., 2012a,b; Malacrida et al., 2006; Re et al., 2012; Sorrequieta et al., 2013; Vega-Garcia et al., 2010; Weiss and Egea-Cortines, 2009). Furthermore, we have previously characterized Micro-Tom fruit physiological response to a range of postharvest chilling temperature-time combinations (Luengwilai et al., 2012a), and used this information to design a metabolomics investigation of CI (Luengwilai et al., 2012b). This has established a baseline with this cultivar for the further CI studies we exploit here.

The specific objective of this study was to determine if MRI could detect some of the earliest physiological changes that accompany CI in tomato fruit. Current methods of assessing the occurrence and severity of CI are: (1) time consuming (e.g., enzyme assays, carbon dioxide and ethylene production), (2) destructive (e.g., measurement of ion leakage from excised tissue, firmness tests), or (3) occur only after the activation of secondary, downstream events (e.g., the CI index). These methods are time-proven and are indispensable, but there is a need for non-destructive methods with equivalent or better sensitivity to those currently used. MRI potentially offers such advantages and could be an important complementary tool for studying incipient CI. We show that MRI can provide spatio-temporal resolution of chilling induced changes in Micro-Tom tomato fruit prior to development of downstream symptoms.

2. Materials and methods

2.1. Plant growth conditions

Tomato (*S. 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 August 2012 in greenhouses located in Davis, CA as previously described (Luengwilai et al., 2012a).

2.2. Fruit sampling and postharvest treatments

Mature green fruit were hand-harvested between 7 and 8 am (Saltveit, 1991). Unblemished fruit that were both uniform in size and external color were washed in commercial bleach (1:20 dilution of 5% (v/v) sodium hypochlorite) and allowed to dry in a transfer hood. A total of 12 fruit were used in each of the four treatments. Control fruit were held at 20 °C, while the remaining treatments were as follows: (1) 0 °C for 1 week, (2) 0 °C for two weeks, and (3) 0 °C for 2 weeks followed by storage at 20 °C for an additional week.

2.3. Respiration rate and ethylene production

Chilled fruit were removed from 0 °C and allowed to slowly (2–3 h) warm to 20 °C before CO_2 and ethylene production were measured. Twelve fruit were evaluated per treatment by placing four fruit in each of three 500 mL glass containers. These jars were sealed for 1 h and a 1-mL sample of the head space was withdrawn using a syringe and its CO_2 concentration was measured with an infrared gas analyzer as previously described (Saltveit and Strike, 1989). Ethylene production was measured from a 2.5 mL sample of the head-space using a Gas Chromatograph (Model Carle 211, Hach Carle, Loveland, CO) equipped with a flame ionization detector. These two samples were taken within 30 s of each other from the same jar.

2.4. Ion leakage measurement

Each fruit was cut into four radial segments, cleaned of adhering locular tissue, washed for 5 s in running tap water, blotted dry, and one segment was placed in each sector of a 4-sectored Petri dish under aseptic conditions. The dishes were placed in 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 allow the tissue to overcome the wound-induced alterations in membrane permeability (Saltveit, 2005). After transferring to room temperature (~18 °C) for 1 h, the four aged segments from each Petri dish were put into a 50 mL plastic centrifuge tube containing 20 mL of an aqueous solution of 0.2 M mannitol. Preliminary experiments determined that 0.2 M was isotonic for these excised radial segments (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. Ion leakage was expressed as percent of total and plotted over time. The linear increase in ion leakage from 0.5 to 2.0 h was used to calculate the rate of ion leakage (Saltveit, 2002, 2005).

2.5. MR imaging and data acquisition

MRI data were acquired on a 1 T permanent magnet NMR spectrometer (Aspect Imaging, Industrial Area Hevel Modi'in, Shoham, Israel) with a 60 mm inside diameter (ID) coil. Each tomato fruit was positioned at the center of the coil during imaging. A diffusion weighted spin echo (SE-DW) imaging sequence was used to obtain the image of the equatorial slice of the sample. A set of 12 images for each sample was acquired with a series of motion-encoding gradients composed of 12 steps (b in Eq. (1)), applied in the spin echo sequence with a repetition time (TR) of 1000 ms, an echo time (TE) of 30 ms, field of view (FOV) of 64 mm × 64 mm, and slick thickness of 2 mm. In the SE-DW images, the signal intensity (S) of any voxel is given as:

$$S = S_0 e^{-bD} \quad (1)$$