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Transcriptional changes associated with chilling tolerance and susceptibility in 'Micro-Tom' tomato fruit using RNA-Seq

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ABSTRACT

Tomato fruit are horticultural products of great economic and nutritional value, whose transportation and marketing at low temperature are limited due to their susceptibility to develop chilling injury (CI). Hot water (HW) pre-treatments have been shown to reduce the CI symptoms in tomato fruit, but the molecular mechanisms involved in the acquisition of CI tolerance remain unclear. In the present work, a comparative transcriptomic analysis between HW treated and non-treated fruit before and after cold storage was carried out. RNA-Seq analysis detected a large number of differentially expressed genes that ranged from 2235 (heat shock) to 5433 (cold storage). Three clusters of genes were identified after 2 weeks of cold storage: the chilling-response included the down-regulation of genes involved in photosynthesis, metabolism of cell wall, lipid and ethylene, as well as the up-regulation of genes for trehalose synthesis and transcription factors (DOF and MYB); the chilling-susceptibility was associated with the down-regulation of genes involved in carotenoid biosynthesis, which correlates with the main CI symptom of uneven ripening; meanwhile, the chilling-tolerance was related to the up-regulation of genes for heat stress (heat shock proteins and heat shock transcription factors) and detoxification (glutathione *S*-transferases). The induced tolerance to CI in tomato fruit seems to be related first with the protection of cell wall and membranes integrity, and second with the restoration of ethylene biosynthesis and signaling.

1. Introduction

Low-temperature storage is the most important method of reducing postharvest decay and maintaining the organoleptic and nutritional quality of fruits and vegetables; however, exposing tropical and subtropical crops to temperatures below 12 °C induces the development of a physiological disorder known as chilling injury (CI), which leads to a decrease in the postharvest quality and thus to important economic losses (Polenta et al., 2007; Aghdam et al., 2013). Remarkably, CI disorders usually become visible when fruits reach the consumers, turning fruits unpalatable and leading to consumer rejection (Lauxmann et al., 2012).

Physiological responses to CI have been comprehensively reviewed (Sevillano et al., 2009); the first response is related to alterations in cell membrane conformation and structure, caused by changes in the lipid composition of the membrane that results in a decrease of its fluidity and permeability; these changes affect the functionality of mitochondria and chloroplast, causing an overproduction of reactive oxygen species (ROS) and thus an increase in the oxidative stress, which is considered a secondary response to CI.

Tomato is the second most important horticultural crop worldwide; nevertheless, its transportation and marketing are limited by its susceptibility to develop CI, being 12 °C the minimum safe temperature for storage. The main CI symptoms in tomato fruit include uneven ripening, pitting and decay, and they usually appear when fruit are transferred to a ripening temperature (20–22 °C) after being stored at low temperature (2–6 °C) more than 2 weeks (Vega-García et al., 2010).

Among the existing tomato genotypes, the cultivar Micro-Tom is considered a model system because of its unique characteristics,

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such as small size, ability to grow in high densities, and short life cycle (Meissner et al., 1997). 'Micro-Tom' presents a very low frequency (0.06%) of nucleotide mismatch in exons with respect to the genome-sequenced cultivar Heinz 1706 (Aoki et al., 2010), and efficient genetic transformation protocols (Dan et al., 2006; Cruz-Mendívil et al., 2011) have been developed for this model system, making it a valuable tool for functional genomics. Furthermore, some studies have shown that 'Micro-Tom' fruit are susceptible to developing CI when exposed to low temperatures (Malacrida et al., 2006; Gómez et al., 2009; Luengwilai et al., 2012a, 2012b; Müller et al., 2013), but to a lower extent when compared to commercial tomato cultivars (Vega-García et al., 2010).

Omics-based approaches have allowed us to address the complex global biological systems that underlie various plant functions and responses (Mochida and Shinozaki, 2011). In this sense, proteomic studies in cold-stored tomato fruit have revealed that tolerance or defense mechanisms are related to the accumulation of heat shock proteins (HSPs), late embryogenesis abundant (LEA) proteins, molecular chaperones (GR-RBP) and antioxidant enzymes (TPxI); meanwhile, the susceptibility mechanisms include the uncoupling of photosynthetic processes (ATP synthase) and protein degradation machinery (26S proteosome) (Page et al., 2010; Vega-García et al., 2010; Sanchez-Bel et al., 2012).

Whereas proteomics enables the identification of posttranscriptional and posttranslational modifications, transcriptomic analysis allows the identification of genes coding for proteins that are difficult to determine by 2D gel electrophoresis (Seliger et al., 2009); in addition to stress-responsive transcription factors (TFs) which may be potential targets for crop breeding (Calderon-Vazquez et al., 2008). A transcriptomic analysis of 'Micro-Tom' fruit after a short cold storage (48 h at 6 °C) showed that 38 genes were up-regulated by cold, but only one coding for a dehydrin was related to previously identified cold-stress genes (Weiss and Egea-Cortines, 2009). Dehydrins belong to the group II of LEA family and protect proteins and membranes against unfavorable structural changes (Kosová et al., 2007). On the other hand, an expression analysis of tomato fruit after a long period of cold storage (4 weeks at 3 °C) showed that the CI visual symptom of uneven ripening was the outcome of alterations in genes from carotenoid biosynthesis, cell wall modification, ethylene biosynthesis and signaling (Rugkong et al., 2011). These transcriptomic approaches were focused on chilling response and susceptibility; therefore, studies focusing on CI tolerance mechanisms are still required.

Heat treatments (air, steam or water) have been previously used to alleviate the CI symptoms in tomato fruit, where hot water (HW) treatments have been the most efficient in terms of heat transfer and CI symptoms reduction (Lurie et al., 1997; Yang et al., 2009; Luengwilai et al., 2012a). These treatments are applied prior to cold storage, and consist in the immersion of fruit in HW (38–54 °C) for short times (1–15 min). It is thought that exposure to a high temperature triggers physiological responses that allow the tissue to cope in a better way with subsequent stress conditions (Lara et al., 2009); there is evidence that induced tolerance to CI in tomato fruit after a heat treatment is related to the accumulation of HSPs (Polenta et al., 2007), metabolites such as arachidic acid and 2-ketoisocaproic acid (Luengwilai et al., 2012b), and enhanced arginase activity (Zhang et al., 2013). However, the global transcriptomic changes in tomato fruit with induced tolerance to CI have not been analyzed yet, which may be useful in the understanding of the molecular network induced by a postharvest heat treatment.

Considering the above mentioned, in the present work a transcriptomic approach using RNA-Seq was undertaken to identify differentially expressed (DE) genes in tomato fruit after the heat shock, cold storage and subsequent ripening. DE genes were grouped in chilling-responsive regulons to identify common and unique responses to chilling, followed by a functional categorization to obtain a biological context of the chilling tolerance and susceptibility mechanisms in tomato fruit.

2. Materials and methods

2.1. Plant material and postharvest treatments

Tomato fruit (*Solanum lycopersicum* cv. Micro-Tom) were harvested at mature-green stage, and selected based on epidermis color (a^* = −13 to −10) and weight (2–3 g). The mature-green stage was selected due to their higher susceptibility to develop CI, and also to avoid the complex transcriptomic changes associated with ripening at breaker and later stages. After washing, tomato fruit were immersed for 7 min in water at 40 °C (HW) or at 20 °C (control), air-dried for 30 min at room temperature, and then stored for 14 days at 5 °C to induce chilling stress, followed by 14 days at 20 °C to allow ripening and symptoms development (Luengwilai et al., 2012a). Relative humidity was maintained above 90% during the storage.

2.2. CI index, physical and physiological parameters

CI index (Vega-García et al., 2010) and physical parameters of fruit quality (weight loss, color and firmness) were determined after 14 days at 5 °C + 14 days at 20 °C. The severity of the symptoms (U = uneven ripening, and W = wilting) was assessed visually as injury level (IL) using a five-point scale based on the percentage of tissue affected for each criterion (0 = no injury, 1 ≤10%, 2 = 11–25%, 3 = 26–40%, 4 ≥40%). CI index was calculated with the formula: (ILU + ILW)/2. The weight loss (%) was calculated using the values of initial weight (W_0) and final weight (W_f) with the following expression: $[(W_0 - W_f)/W_0] \times 100$. Color changes on the surface of tomato fruit were quantified by the values of L^*, a^*, b^* and Hue° using a colorimeter CR-200 (Konica Minolta Inc., Tokyo, Japan), and the total difference of color (ΔE) was calculated with the expression $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$. The pericarp firmness was evaluated with a digital force gauge Chatillon E-DFE-100 (Ametek, Berwyn, PA). Each fruit was cut into four segments and the locular tissue was removed; these segments were placed with the pericarp upward and penetrated in the equatorial region to a depth of 1 mm, using a 1 mm diameter tip and a constant speed of 0.83 mm s^{-1}. The maximum compression force was expressed in Newtons (N).

Ion leakage was measured in pericarp discs (1 cm diameter) after 0, 7 and 14 days of storage at 5 °C, and expressed as % (Zhao et al., 2009a). Respiration rate was assessed in chilled fruit (5 °C for 14 days) during reconditioning at 20 °C (0, 4, 8, 12, 16 and 24 h), and the production of CO_2 was expressed in μg kg^{-1} s^{-1} (López-Valenzuela et al., 2011).

For all postharvest assays, the experimental unit consisted in 10 fruit, and three replicates per treatment were used in each experiment. Data were subjected to analysis of variance and comparison of means with the Fisher's least significant difference test (LSD, $P \leq 0.05$), using the STATGRAPHICS Plus 5.1 software.

2.3. RNA isolation and high-throughput sequencing

Tomato fruit from both control and HW treatments were sampled after 0 h, 14 days at 5 °C, and 14 days at 5 °C + 14 days at 20 °C, and named C-0h, C-14d, C-14+14d, HW-0h, HW-14d and HW-14+14d, respectively. Five fruits were pooled for each condition, pericarp tissues were ground in liquid nitrogen and stored at −70 °C until use. Total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) and purified using RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. RNA quality was monitored by gel electrophoresis, A_{260}/A_{280} ratio, and RNA integrity number (RIN). High quality RNA samples