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Metabolite content of harvested Micro-Tom tomato (*Solanum lycopersicum* L.) fruit is altered by chilling and protective heat-shock treatments as shown by GC–MS metabolic profiling

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ABSTRACT

The primary aim of this study was to identify metabolites associated with chilling tolerance that was engendered by a heat-shock treatment of tomato fruit pericarp (*Solanum lycopersicum* L. cv. Micro-Tom). Harvested mature-green fruit were immersed in 20 or 40 °C water for 7 min ('Heat-Shock') and then stored at 2.5 °C for 0 or 14 d ('Chilled'). A reduction in chilling injury symptoms (i.e., slow or abnormal ripening, increased ion leakage, and increased respiration following chilling) was used to select this heat-shock treatment as optimal. Using GC–MS (Gas Chromatography–Mass Spectrometry) metabolite profiling, 363 analytes were detected in fruit pericarp of which 65 are identified metabolites. Principal Component Analysis of these data led to distinct groups among the samples based on their treatments; 'Chilled' and 'Chilled + Heat-Shocked' fruit were markedly different from each other, while the 'Non-Chilled Control' and 'Heat-Shocked' fruit were similar and grouped closer to the 'Chilled + Heat-Shocked' fruit. These results indicate that the heat treatment provided protection from chilling in part by altering levels of fruit metabolites. The levels of arabinose, fructose-6-phosphate, valine and shikimic acid appear to be associated with this heat-shock induced chilling tolerance since their levels were altered in the 'Chilled' samples ($p < 0.05$), relative to the control and the heat-shocked protected fruit. We also describe the metabolites we identified that could be further studied as being indicative of incipient chilling injury in mature-green tomato fruit.

1. Introduction

Chilling injury (CI) is a complex disorder that is detrimental to the quality of tropical and subtropical fruit and often leads to severe economic loss (Sevillano et al., 2009). Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops produced globally (Luengwilai and Beckles, 2009) and like most subtropical fruit is susceptible to CI. When fruit are exposed to low, non-freezing temperatures, a number of physiological and biochemical changes can occur, including a failure to ripen normally, surface indentations, discoloration, and increased rates of respiration and water loss (Morris, 1982; Saltveit and Morris, 1990). An early event of CI is the marked increase in membrane permeability, with increased leakage of ions from within cells (i.e., symplast) to intercellular (apoplastic) spaces within the tissue (Saltveit, 2005). While symptoms can develop during chilling, most become pronounced after the chilled tissue is held at a non-chilling temperature (ca. 20 °C) for a few days (Saltveit and Morris, 1990).

Chilling injury in plants has been studied for over a century, but little is known about its molecular basis. Pre-conditioning at temperatures of ~40–50 °C before chilling mitigates the development of CI symptoms and that mitigation may be mediated by altered protein synthesis including the synthesis and accumulation of heat shock proteins (HSPs) (Ding et al., 2001; Lurie and Klein, 1991; Lurie et al., 1997; Saltveit, 2005). Low temperature stress causes changes in the accumulation of distinct metabolites in many plant species (Bohnert et al., 1995; Gusta et al., 1996; Guy, 1990; Hannah et al., 2006; Kaplan et al., 2004; Kishitani et al., 1994) and changes in the levels of a subset in response to pre-treatments may also contribute to induced chilling tolerance (Sevillano et al., 2009).

We do not know the metabolites that are altered in the early phases of CI in mature-green tomato fruit. Most CI research on mature-green fruit focuses on secondary events, i.e. when visual symptoms are manifest making it difficult to separate the initial 'cause' from the subsequent 'effect'. Some attempts to study the physiological and transcriptional processes initiated early in chilling stress in breaker and ripened tomato fruit have been made

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(Gomez et al., 2009; Weiss and Egea-Cortines, 2009). For example Gomez et al. (2009) took a targeted approach to examine the biochemical changes associated with postharvest chilling of tomato fruit cv. Micro-Tom. They assayed sugars (glucose, fructose and sucrose), organic acids (malic, citric, succinic and tartaric acid) and some carotenoids of breaker and red-ripe fruit and found changes in response to increasing incubation (after as little as 4 d) at low temperature (6 °C) mainly in breaker fruit (Gomez et al., 2009). This work was important in showing that Micro-Tom is a good model for CI studies and in pinpointing how specific compound were altered.

In contrast to targeted measurement of compounds, GC–MS and other hyphenated mass spectrometry technologies are powerful as they provide a view of a larger set of metabolites (Roessner and Beckles, 2009). They are proving to be an invaluable tool for understanding metabolic and physiological processes in tomatoes (Carrari et al., 2006; Moco et al., 2006, 2007; Schauer et al., 2006), and are also useful in identifying metabolite fingerprint associated with various postharvest disorders in a range of tissues (Nicolai et al., 2010; Pico et al., 2010; Rudell et al., 2008, 2009; Vikram et al., 2004, 2006). Adoption of these technologies coupled with a good description of fruit physiology may become indispensible for understanding postharvest biology and for developing diagnostic tests for fruit quality.

The aim of this work was to better understand metabolic alterations associated with early events in chilling injury in mature green tomato fruit. We had two specific goals. First, we wished to determine if it is possible to distinguish, based on GC–MS metabolite profiling, harvested tomato fruit that were subjected to chilling stress from those with a prior heat-shock treatments that increase chilling tolerance before symptoms manifest. Second we wished to identify the specific metabolites, which changed in response to these treatments. Examining the response of mature-green tomato fruit rather than in breaker fruit may offer further insight since (i) they are more susceptible to chilling injury and (ii) the complex metabolic changes associated with ripening at breaker would not confound the analysis. GC–MS analysis was done in the same pericarp fruit tissue samples that were used to characterize the effects of heat-shock treatments that induced chilling tolerance and reduced the rate of chilling-induced ion leakage (Luengwilai et al., 2011). There are clear benefits to monitoring changes in physiological and metabolic processes simultaneously during chilling rather than relying on visual symptoms that develop during subsequent storage at non-chilling temperatures. Metabolites associated with the earliest response of sensitive tissue to chilling injury may be identified. Such metabolites may be investigated as a marker(s) for breeding programs and in ascertaining whether sensitive crops have been exposed to injurious chilling temperatures on their way to market.

2. Materials and methods

2.1. Plant growth, fruit sampling and postharvest treatments

Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) plants were grown as previously described (Luengwilai et al., 2011). Five to ten mature-green fruit (i.e., fruit with softened locular tissue and seeds that could not be cut with a knife; Saltveit, 1991) were hand harvested from each plant. Uniform (4–6 g, fresh weight), non-damaged fruit were washed in commercial bleach (1:20 dilution of 5% (v/v) sodium hypochlorite) and air-dried under a laminar flow hood.

The experimental design consisted of six replicates of four treatments: a control treatment (*Control*: non-heat-shock, non-chilled), a heat-shock treatment (*HS*: heat-shocked, non-chilled), a chilled treatment (*Chilled*: non-heat-shock, chilled), and a heat-shock/chilled treatment (*HS + Chilled*: heat-shocked, chilled). Fruit were immersed in water at 20 °C (control) or at 40 °C (heat-shock) for 7 min. The fruit were then air-dried for 30 min before being chilled at 2.5 °C for 14 d as previously described (Luengwilai et al., 2011).

2.2. Metabolite sample extraction and derivatisation

Frozen pericarp disks from the base of each fruit were ground in liquid nitrogen and 50 mg were extracted in 1500 μL of 100% (v/v) methanol. The extract was vortexed for 10 s, shaken for 15 min at 70 °C, centrifuged for 15 min at 3200 × *g*, and the supernatant was then transferred to new tube. The pellet was mixed vigorously with 500 μL of DI water, centrifuged for 15 min at 3200 × *g* and the supernatant was combined with the previous extract. Aliquots of 50 μL of the supernatants were dried in a Speed Vac concentrator (SVC 100, Savant instrument, Famingdale, NY). The derivatisation and GC–MS system was done as previously described (Fiehn et al., 2008). Analytes (363) were automatically detected using the BinBase algorithm (Fiehn et al., 2008), and 65 were unambiguously identified by comparing their retention times to that derived from mass spectral libraries. The identities of the remaining compounds (298) are unknown, but they appear consistently between samples and therefore may have a biological origin.

2.3. Data analyses

A one-way ANOVA and The Student's *t*-test at $p < 0.05$ was used to detect significant changes due to chilling and heat-induced chilling tolerance. False discovery rates were calculated using Metaboanalyst (Xia et al., 2009). For all multivariate analyses the data were $\log_{10}$ transformed prior to analysis. Log_{10} transformation provided the closest approximation to a normal distribution compared to other methods such as Pareto, Autoscaling and Range Scaling (van den Berg et al., 2006) contained within the Metaboanalyst program. Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) were performed using Statistica Dataminer (Statsoft, 2003). Both methods are designed to provide a general overview of the relationships among different samples (Stamova et al., 2009).

3. Results and discussion

3.1. Physiological response to chilling

Fruit-specific changes associated with the duration of chilling and chilling temperature are known to vary significantly from harvest to harvest and within and between experiments (Saltveit and Morris, 1990). Therefore we performed extensive physiological characterization of the chilling response (Luengwilai et al., 2011) and coupled that with a metabolomic study. As a result, molecular responses could be directly related to underlying biological processes in our experiments.

The rate of respiration of whole mature-green tomato fruit after chilling and the rate of chilling-induced ion leakage from excised mature-green pericarp tissue into an isotonic mannitol solution were both significantly reduced by heating the fruit to 40 °C for 7 min prior to chilling at 2.5 °C for 14 d. For 'Control' and 'HS' fruit – before chilling, the rate of ion leakage was 3.3 ± 1.1% total conductivity per hour. After 14 d at 2.5 °C the rate of ion leakage from control fruit increased 3.3-fold to 14 ± 0.1% total conductivity per hour, while the rate from 'HS' fruit only increased 2.4-fold to 8.0 ± 1.2% total conductivity per hour (Luengwilai et al., 2011). As we wanted to relate the metabolic changes to physiological changes, GC–MS was performed on polar extracts of tomato fruit that were used for respiration and ion leakage measurement.