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Research article

Low temperature storage affects the ascorbic acid metabolism of cherry tomato fruits

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ABSTRACT

Tomato fruits are an important source of L-Ascorbic acid, which is an essential compound of human diet. The effect of the widespread practice of cold storing (5–10 °C) tomato fruits was monitored to determine its impact on the concentration and redox status of L-Ascorbic acid. Total L-Ascorbic acid levels were well maintained in both attached fruits and cold treated fruits, while in other treatments its levels were considerably reduced. However, low temperature storage conditions enhanced the expression of most genes coding for enzymes involved in L-Ascorbic acid biosynthesis and redox reactions. The findings suggest that the transcriptional up-regulation under chilling stress conditions of most genes coding for L-Ascorbic acid biosynthetic genes galactono-1,4-lactone dehydrogenase, GDP-D-mannose 3,5-epimerase but also for the isoenzymes of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase enzyme, glutathione reductase that are strongly correlated to the L-Ascorbic redox status. Moreover, fruits stored at 10 °C exhibited higher levels of transcript accumulation of *MDHAR2*, *DHAR1*, *DHAR2*, *GR1* and *GR2* genes, pointing to a better ability to manage chilling stress in comparison to fruits stored at 5 °C.

1. Introduction

In plants, L-ascorbic acid (Shigeoka et al., 2002) metabolism is correlated with oxidative stress defense, while AsA accumulation in plant tissues and organs is altered by physiological phenomena such as senescence, cell expansion development and, various biotic and abiotic stimulations (Davey et al., 2006, 2000). Most of the biological roles of AsA derive from its great capacity to act as a reducing agent. Indeed, the primary function of AsA is to detoxify hydrogen peroxide H_2O_2 produced by metabolism during photosynthesis and especially in stress conditions (Smirnoff, 2011).

In plant cells, AsA contributes to Reactive Oxygen Species (ROS) detoxification via a four-step biochemical pathway, known as the L-ascorbate–glutathione cycle or the Foyer, Halliwell, Ashada cycle (Foyer and Noctor, 2011). In AsA metabolism several enzymes are involved, ascorbate peroxidase oxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase enzyme (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.8.1.7) (Noctor and Foyer, 1998). The function of this peroxidation-recycling system is believed to be more or less constant in plant tissues, but is enhanced under stress conditions resulting in the maintenance of AsA pool (Alhagdow et al., 2007).

Unlike plants, which biosynthesize AsA, also known as vitamin C, in relatively high concentrations (Grantz et al., 1995), humans are unable to biosynthesize AsA due to the lack of the L-glucono-1,4-lactone oxidase enzyme (Smirnoff, 2011). As a consequence, around 90% of vitamin C in the human diet is derived from fresh vegetables and fruit (Jacob and Sotoudeh, 2002). Due to its high volume of per capita consumption, tomato fruit constitutes a significant source of AsA intake for humans (Stevens et al., 2008).

Abbreviations: AsA, L-Ascorbic acid; ROS, Reactive Oxygen Species; APX, Ascorbate peroxidase oxidase; MDHAR, Monodehydroascorbate reductase; DHAR, Dehydroascorbate reductase enzyme; GR, Glutathione reductase.

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Cherry tomatoes are enjoying a continuously increasing commercial demand because of several important quality traits such as higher dry matter and higher levels of soluble solids in comparison to normal-sized tomato fruits. Moreover, due to their higher levels of sugars and organic acids, cherry tomatoes exhibit a sweeter taste and richer aroma (Raffo et al., 2002).

Tomato ripening is a complicated and extensively studied physiological process, which continues during the post-harvest life of fruits (Adams-Phillips et al., 2004). As a result, post-harvest treatments and storage conditions could have a dramatic effect on phytonutrient content and nutritional value of tomatoes (Javanmardi and Kubota, 2006). During tomato post-harvest life, AsA levels among other nutrients are susceptible to heavy reduction resulting in significant loss of nutrient value. Research data strongly suggest that actions should be taken during the post-harvest life of tomato fruits in order to preserve AsA levels until consumption (Oms-Oliu et al., 2011).

Common post-harvest treatments of tomato fruits involve the storage of fruits for some days near the plantation, the transportation in refrigerator trucks at near 10 °C, and the storage in domestic fridges until consumption at approximately 5 °C (Kirkland et al., 2009). While low temperature storage can preserve the nutrient quality of tomato fruit, it is well established that it can trigger stress responses. Such storage can cause chilling injury in fruits associated with oxidative stress which, in turn, is correlated to membrane phase separation of membrane lipids (Gharezi et al., 2012; Malacrida et al., 2006; Vega-García et al., 2010). Cold or freezing stress is also linked to ROS production, while plant cold acclimation is associated with ROS-scavenging enzymatic activities of several enzymes including APX (Suzuki and Mittler, 2006). In addition, it has been found that low temperatures also stimulate AsA oxidation through the activity of APX (Hodges et al., 2004; Ioannidi et al., 2009; Malacrida et al., 2006). Moreover, enzymes involved in ROS scavenging mechanisms, including glutathione reductase (GR, EC 1.8.1.7) activity, en enzyme which participates in AsA recycling (Malacrida et al., 2006) was higher after storage of tomatoes at 4 °C.

The aim of this study is to investigate the effects of some common postharvest treatments on AsA metabolism during the main stages of postharvest life of tomato fruits. The results suggest that the accumulation of transcripts of most genes involved in AsA biosynthesis and metabolism was significantly higher after cold storage. AsA levels are similar to attached fruits as well as to fruits stored at 5 °C and 10 °C for the same period. The data suggest that AsA is recycled under low temperature conditions, which is attributed to chilling-produced ROS scavenging by AsA catabolism. Thus, AsA levels are maintained in both attached fruits and cold treated fruits, while in low temperatures AsA concentration is retained through the transcriptional stimulation of AsA metabolism.

2. Materials and methods

2.1. Plant material and growth conditions

Plants of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme* cv. Conchita F1; de Ruiter seeds, Melbourne Australia), a productive hybrid with a long shelf-life, were cultivated in a glasshouse at the Agricultural University of Athens, Greece between December and May 2012. Mean minimum and maximum temperatures in the greenhouse were respectively 15.7 ± 2.0 and 26.6 ± 4.3 °C in spring (March–May) and 12.9 ± 1.9 and 23.9 ± 4.4 °C in winter (October–February). Solar radiation varied between 700 and 1400 μmol m^{-2} s^{-1} PAR. During postharvest storage, the fruits were stored in darkness inside clear PET cases appropriate for food that allowed gas exchange. The dimensions of the cases were 13.1 cm × 11.3 cm × 7 cm. The cases were filled with fruits in the commercial maturity stage. Inside storage units the relative humidity was 75% the temperature was adjusted according to the treatments simulating common postharvest conditions. List of all treatments and plant material is shown in Table 1.

Each harvest was carried out at 11:00 am and replicated three times creating three lots. Samples were immediately frozen in liquid nitrogen, homogenized using a pestle and mortar and then stored at −80 °C.

2.2. Real time PCR expression analysis

Real-time PCR reactions were conducted as previously described (Tsaniklidis et al., 2012, 2014). Samples for qPCR analysis were prepared mixing 1 gr from each lot (described above). Total RNA was isolated from each sample using RNeasy extraction Kit (Qiagen, Hilden Germany). Quantity and quality of total RNA were assessed by spectrophotometric and electrophoretic analysis, measuring the absorbance at 260 nm and the absorbance ratio of 260/280 nm in Nanodrop (Thermo, Wilmington USA) and by 1.5% w/v agarose-gel electrophoresis. To eliminate total DNA, samples were treated with RNAse free DNaseI (Takara, Otsu Shiga Japan) according to the manufacturer's instructions. The complete DNA removal was tested with primers designed against the ubiquitous expressed gene of *UBIQUITIN* (*UBQ*), while *S. lycopersicum* genomic DNA was used as a positive control.

First strand cDNA was reverse transcribed by the Affinity ScriptTM Multi Temperature reverse transcriptase using oligo (Aradhya et al., 2010) primers according to the manufacturer's instructions (Stratagene, Santa Clara USA). The resulting first-strand cDNA was then normalized for the expression of the housekeeping gene of *UBQ*. Gene specific primers were designed by Beacon designer v 7.01 software (Premier biosoft, Palo Alto USA) against *GalLDG* (Aoki et al., 2010), *APX* (Aoki et al., 2010; Najami et al., 2008), MDHAR (Grantz et al., 1995; Li et al., 2010), DHAR (Chen and Gallie, 2006; Zou et al., 2006), GDP-D-mannose 3,5-epimerase (GME, EC 5.3.1.18) (Gilbert et al., 2009) and UBQ genes (Table 2).

Quantitative real time PCR reactions were performed on MX-3005P system (Stratagene, Santa Clara USA) with Kapa Fast Universal 2X qPCR Master Mix (Kapa, Woburn USA) and gene-specific primers. Annealing was set at 60 °C (45 s), elongation at 72 °C (11 s) and denaturation at 95 °C (3 s) for 40 circles, final extension followed at 72 °C for 5 min. The primer specificity and the formation of primer-dimers were monitored by dissociation curve analysis and agarose gel electrophoresis on a 2% (w/v) gel. In all samples examined, a single amplicon was detected.

The expression levels of *S. lycopersicum UBQ* gene were used as internal standards to normalize concentrations of the cDNA templates. For the relative quantification of gene expression, a

Table 1
Plant materials.

Treatment	Abbreviation
Fruits at commercial maturity (47 days post flowering attached to plants)	CM
CM fruits left for 5 more days on plants to mature attached to plants	RR
Detached CM fruits left in transportation boxes near the plantation to mature	NTP
CM fruits stored at 5 °C for 5 days	5d5 °C
CM fruits stored at 5 °C for 5 days and then for 2 more days at RT	5d5 °C+2dRT
CM fruits stored at 10 °C for 5 days in transportation boxes	5d10 °C
CM fruits stored at 10 °C for 10 days in transportation boxes	10d10 °C