Ascorbate metabolism in vegetative and reproductive organs of “cherry” tomato

G. Tsaniklidis[1], N. Nikoloudakis[2], C. Delis[3], G. Aivalakis[1]

[1]Laboratory of Plant Physiology and Morphology, Agricultural University of Athens, Athens, Greece
[2]Vegetative Propagation Material Control Station, Hellenic Ministry of Rural Development and Food, Athens, Greece
[3]Department of Agricultural Technology, Technological Institute of Kalamata, Kalamata, Greece

Abstract

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Ascorbate metabolism is an essential procedure for all plant cells that plays important roles in several physiological processes such as plant development and reactive oxygen species detoxification. To shed more light on ascorbate metabolism in certain organs of tomato plants, we performed a detailed compartmentalized analysis of ascorbate concentration, ascorbate peroxidase/dehydroascorbate reductase enzyme activities and transcript accumulation of genes related to ascorbate metabolism. Our results showed higher level of ascorbate concentration and ascorbate peroxidase and dehydroascorbate reductase activities in young leaves and shoot tips, while min. ascorbate concentration was recorded in root tips. The study of the expression of the genes involved in ascorbate metabolism revealed that several genes followed similar patterns. However, *APX*3 gene expression was considerably higher in reproductive organs, while plastidial *APX6* and *DHAR2* genes transcripts were barely detectable in root tips. Organ-specific expression of genes involved in ascorbate metabolism suggests that different isoenzymes have a specific role in regulation of the redox status of some of the organs in tomato plants.

Keywords: ascorbic acid; ascorbate peroxidase; dehydroascorbate reductase; monodehydroascorbate reductase; glutathione reductase

As a highly active, low-molecular-weight reducing agent, ascorbate (AsA) is involved in various processes of cell metabolism (Smirnoff 2011). It is well established that AsA protects cells against oxidative damage caused by reactive oxygen species (ROS) including the extremely active hydrogen peroxide (H_2O_2), which is a by-product of aerobic metabolism (Gest et al. 2013). AsA redox reactions are often referred to in literature as ascorbate–glutathione circle, which plays an important role in plants in redox status regulation through the activity of four enzymes; ascorbate Peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (*GR*), encoded by multigene families (Mittler 2002). AsA is mostly associated to various biotic and abiotic stresses including pathogens, drought and oxidizing agents and also to the regulation of chloroplast-mitochondria interactions in photosynthetic cells (Conklin, Barth 2004; Fotopoulos et al. 2008; Smirnoff 2011; Talla et al. 2011). AsA also acts as a co-factor and protects sev-

eral enzymes with active sites susceptible to oxidation (Prescott, John 1996). Additional roles were also proposed for AsA in plants; thus several studies suggest that AsA is an essential element that participates in a number of growth and developmental processes like cell division, cell expansion and root growth (Davey 2000; Cordoba-Pedregosa et al. 2003). In tomato plants, high accumulation of transcripts of the genes involved in AsA biosynthesis were correlated to fruit growth and maturation suggesting an important role of AsA in certain developmental stages (Ioannidi et al. 2009). Considering the differences in the physiological status and functionality of the plant organs we undertook the task to elucidate the distinguishing roles of certain isoenzymes involved in AsA metabolism among the vegetative and reproductive cherry tomato organs. Moreover, total AsA content was determined in parallel with the activity of key enzymes of AsA metabolism.

MATERIALS AND METHODS

Plant material and growth conditions. Plants of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme* cv. Conchita F_1; de Ruiter seeds, Melbourne Australia) were cultivated in a glasshouse of the Agricultural University of Athens, Greece between December and May. Mean min. and max. temperatures in the greenhouse were 15.7 ± 2.0°C and 26.6 ± 4.3°C, respectively, (Spring: March–May) and 12.9 ± 1.9°C and 23.9 ± 4.4°C (Winter: Oct–Feb). Solar radiation varied between 700–1,400 μmol/m·s PAR. Mature and young leaves (from sixth and third knot, respectively), shoot tips (top 2 cm), shoot (with diameter 1,5 cm), root tips (2 cm), open flowers, pericarp and central region of red ripe fruits (pulp) (52 days after flowering) were collected and prepared simultaneously. Each harvest was carried out at 11 a.m. during springtime with similar conditions 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.

qPCR analysis. Real-time polymerase chain reaction (PCR) experiments were conducted with the gene-specific primers sequences as previously recorded (Tsaniklidis et al. 2014).

Ascorbic acid determination. Total AsA contents of tissues were determined as previously described (Tsaniklidis et al. 2014).

APX and DHAR enzyme assay. The activity of APX and DHAR was assessed as previously described (Tsaniklidis et al. 2014).

Statistical analysis. Statistics were performed using the Statgraphics Centurion (Statpoint Technologies, Warrenton, USA). Significant differences between treatments were determined by two-way ANOVA and post-hoc comparisons by least significant difference ($P < 5\%$).

RESULTS AND DISCUSSION

AsA metabolism in shoot tips and shoot

The fast growing shoot tips were among the most metabolically active plant tissues in terms of enzyme activity (APX and DHAR) and transcript accumulation levels (APX, MDHAR, DHAR and GR) (Figs 1–4). With the exceptions of *APX*3 and of both GR isoenzymes, all genes exhibited the highest accumulation of transcripts in shoot tips. Moreover, AsA was accumulated in high levels in growing shoot tips. Zhang et al. (2011) reported that AsA is important for controlling the redox status during cell division and expansion. AsA possibly moderates plant growth by regulating numerous basic biological processes, such as (i) the biosynthesis of hydroxyproline-rich proteins which are essential for the advancement of the cell cycle (ii) the bonding of cell wall glycoproteins and other polymers, and (iii) redox reactions at the plasmalemma involved in elongation mechanisms. The AsA free radical also

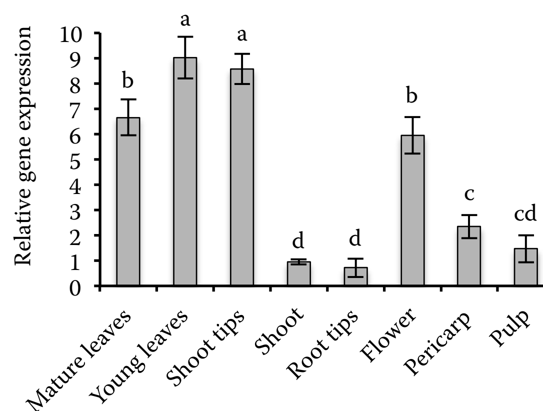

Fig. 1. Total AsA (tAsA) (reduced + oxidized AsA) content of the organs of cv. Conchita tomato plants

bars represent means (+ standard error) of three biological replications; indicator letters in common denote a lack of a significant difference

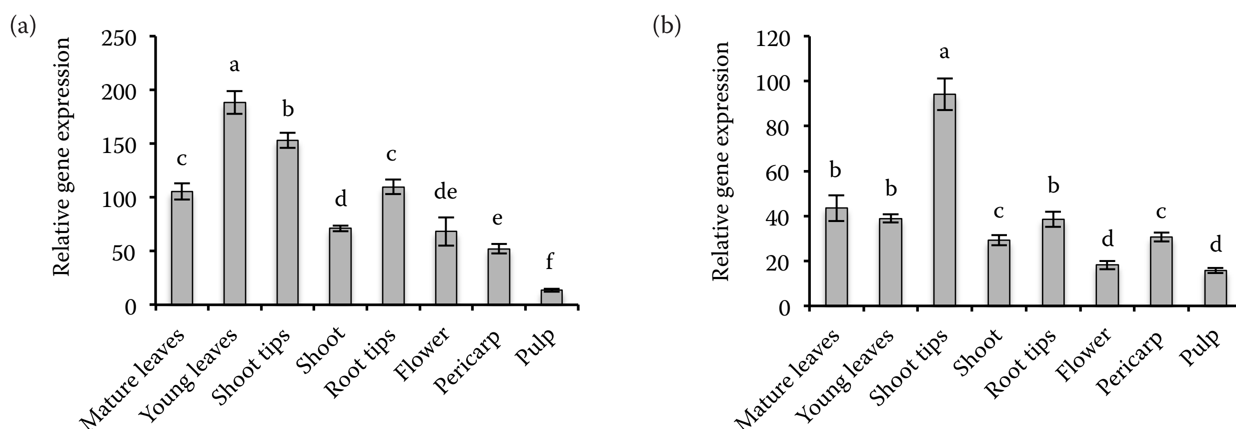

Fig. 2. APX (a) and DHAR (b) enzyme activity in the organs of cv. Conchita tomato plants
bars represent means (+ standard error) of three biological replications; indicator letters in common denote a lack of a significant difference

stimulates a high vacuolization accountable for cell elongation (Smirnoff 2011). The combination of cell division and expansion with photosynthesis is believed to have led to the observed up-regulation of the transcription of AsA metabolism-related genes. Concerning AsA metabolism in shoot, Antonova et al. (2009) showed a sound escalation of AsA concentration in cambium, conductive phloem and the cell enlargement zone, with a following sharp decrease during subsequent cell maturation and lignification. AsA eliminates free radicals involved in xylogen synthesis and controls the lignification of cell wall (Takahama 1993). The balance amongst AsA and hydrogen peroxide regulates the polymerization of xylogen monomers, modulating the cell wall lignification (Davey et al 2000). Another possible explanation of the comparably low levels of AsA in mature shoot is that the slow metabolism and actual photosynthesis of this organ probably do not require high levels of AsA for antioxidant protection (Smirnoff 2000). The combination of cell division and expansion with photosynthesis is believed to lead to an up-regulation of the transcription of AsA metabolism-related genes. The findings of this study reinforce the hypothesis that AsA is crucial for cell expansion and morphogenesis.

Antonova et al. (2009) showed a sound escalation of AsA concentration in cambium, conductive phloem and the cell enlargement zone, with a following sharp decrease during subsequent cell maturation and lignification. AsA eliminates free radicals involved in xylogen synthesis, controls the polymerization of xylogen monomers and also adjusts the lignification of cell wall by regulating the free radicals into the apoplast (Takahama 1993; Davey et al. 2000). Another possible explanation of the comparably low levels of AsA in mature shoot is that the slow metabolism and actual photosynthesis of this organ probably do not require high levels of AsA for antioxidant protection (Smirnoff 2000).

AsA metabolism in young and mature leaves

Total AsA levels in young tomato leaves were higher comparing to mature leaves and ranged between 6.5 to 9 μM/g fresh weight (FW) (Fig. 1). Bartoli et al. (2000) also reported higher tAsA contents in young potato leaves with comparable concentrations. Moreover Li et al. (2010b) found that tASA concentration in apple leaves increased rapidly with their development and reached the highest level in 20-day-old leaves remaining nearly constant until senescence. The lower tASA concentration in mature leaves could be attributed to both lower AsA biosynthesis and reduction capacity (Bartoli et al. 2000). Zhang (2013) reported that low accumulation of AsA accelerates senescence while its high content delays the process.

Moreover, our results showed higher APX activity in young leaves than in mature ones. This finding indicates that a higher antioxidant requirement probably exists in developing young leaves (Fig. 2). In contrast, as in the case of apple leaves, DHAR activity was rather constant in both mature and young leaves, indicating a different metabolic function (Li et al. 2010b).

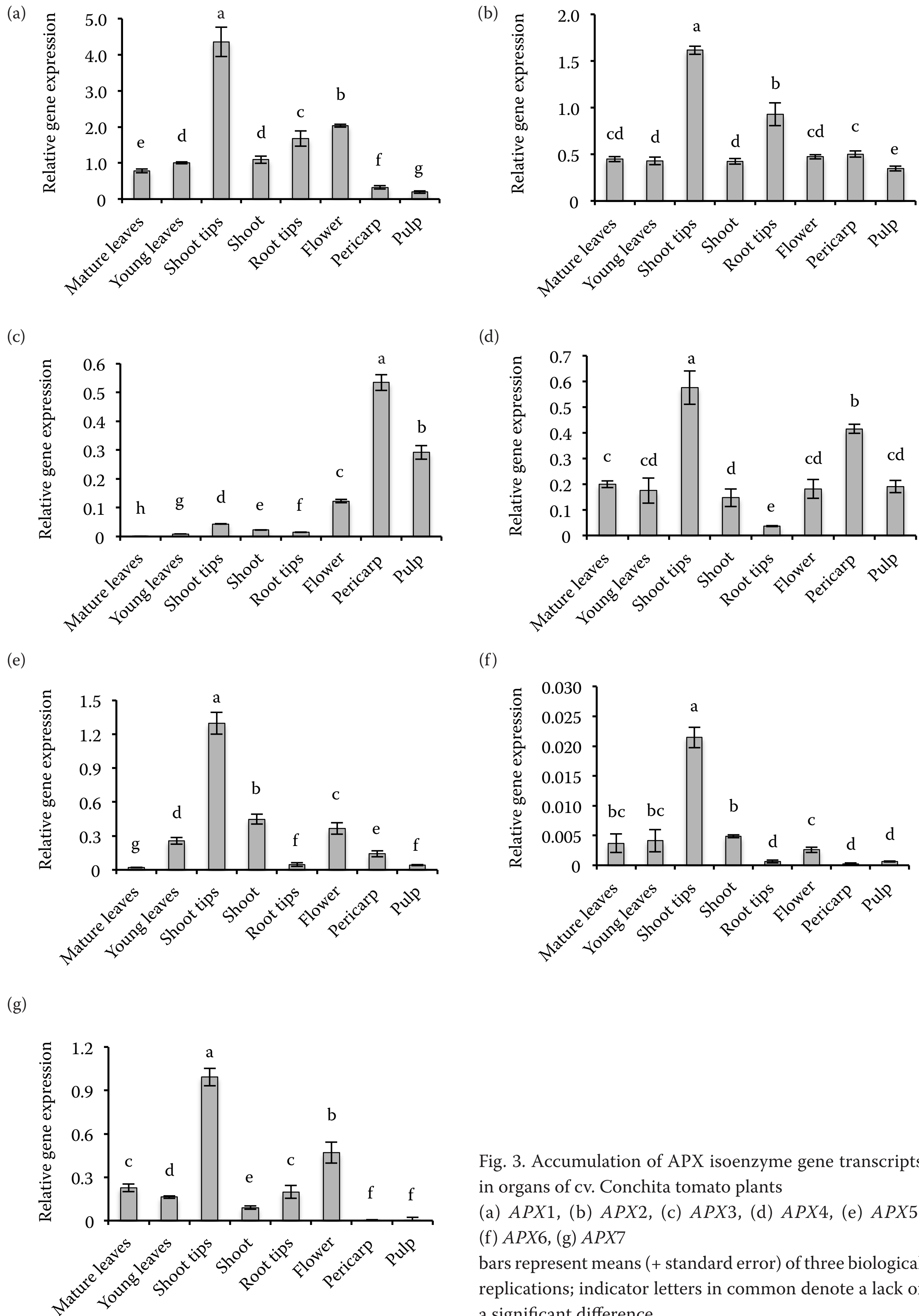

Fig. 3. Accumulation of APX isoenzyme gene transcripts in organs of cv. Conchita tomato plants
(a) *APX*1, (b) *APX*2, (c) *APX*3, (d) *APX*4, (e) *APX*5, (f) *APX*6, (g) *APX*7
bars represent means (+ standard error) of three biological replications; indicator letters in common denote a lack of a significant difference

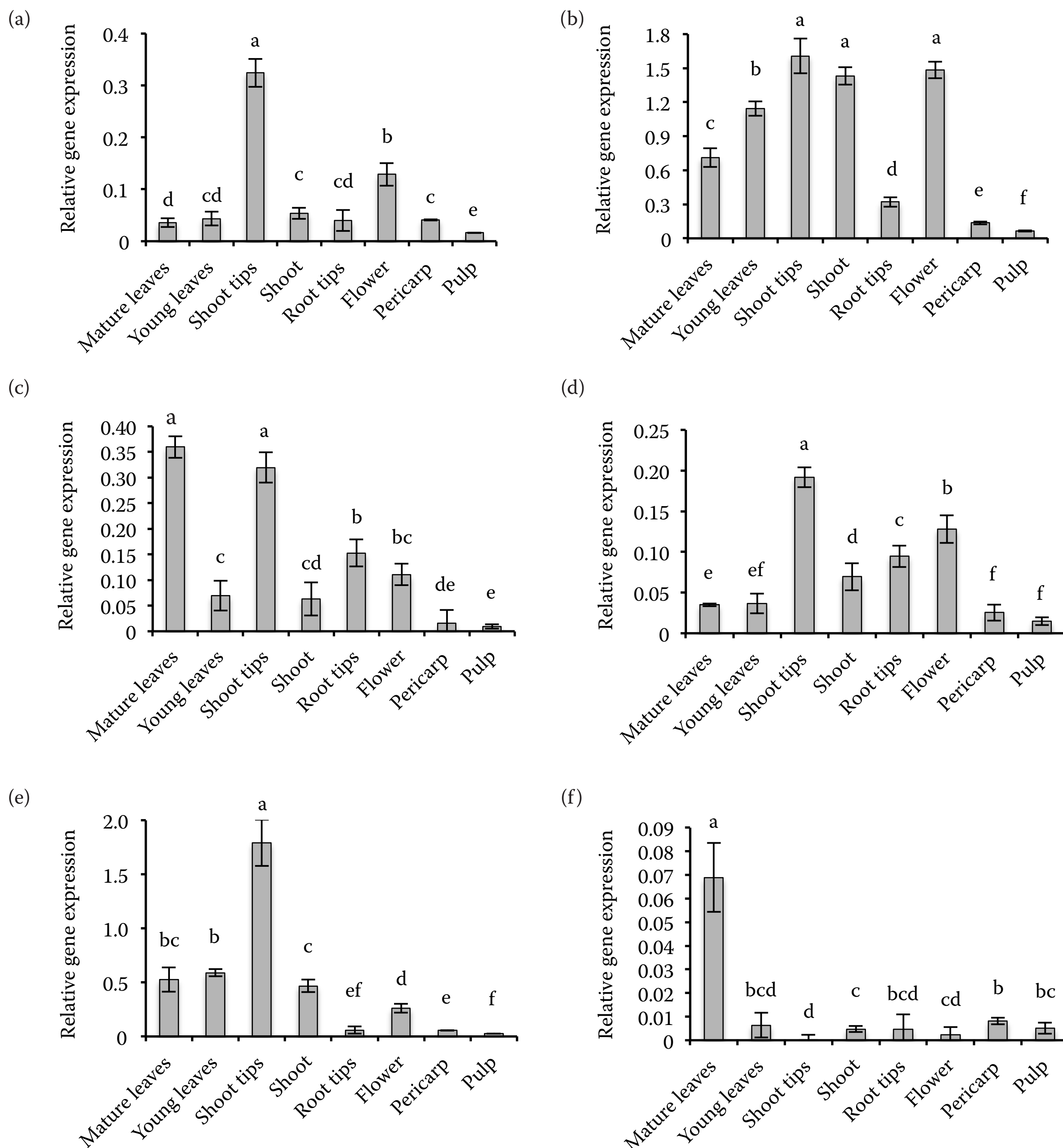

Fig. 4. Accumulation of transcripts of AsA recycle enzymes in organs of cv. Conchita tomato plants
(a) *MDHAR*1, (b) *DHAR*1, (c) *GR*1, (d) *MDHAR*2, (e) *DHAR*2, (f) *GR*2
bars represent means (+ standard error) of three biological replications; indicator letters in common denote a lack of a significant difference

Interestingly, differences of transcript accumulation for most genes among young and mature leaves were minor. Similar results for APX transcription were obtained by Panchuk et al. (2005). In *Arabidopsis,* Li et al. (2010a) also found minor differences in the transcripts levels of MDAHR and DAHR between young and mature leaves, as recorded in the current study as well. Thus, it could be said that different molecular mechanisms participate in controlling the transcriptional regula-

tion of ASA metabolism genes in young and mature leaves. However, both cytosolic and plastidial GR isoenzymes followed completely different patterns of transcription among mature and young leaves and also in other tissues (Fig. 4). It is established that glutathione and by extension GR, are involved in numerous physiological processes comparing to other enzymes of AsA metabolism with more specific roles and probably have different specificity/function (Nagalakshmi, Prasad 2001). Young and mature leaves as well as shoots exhibited similar DHAR activity, while in shoot tips the activity was considerable higher and in flowers it was fairly lower (Fig. 2). On the other hand, APX activity was higher in young leaves and shoot tips following similar pattern to tAsA levels. These results suggest that in cherry tomatoes, although APX activity is related to tAPX levels, the DHAR activity follows a different pattern possibly related to the redox status of APX.

AsA metabolism in root tips

AsA is considered to be actively involved with photoprotection of photosynthesis and in reactions to biotic and abiotic stress conditions (Smirnoff 2011). The fact that in root tips photosynthesis is absent makes the study of AsA metabolism appealing.

Root tips showed the lowest tASA levels among all other plant tissues examined. (Fig. 1). Lin et al. (2004) reported that tAsA levels in tomato roots were around 0.45 μM/g FW, which is comparable to the tASA concentration of the present study (0.7 μM/g FW), while in onion roots up to 0.19 μM/g FW were found (Cordoba-Pedregosa et al. 2003). Interestingly, despite low tASA levels, APX and DHAR activities in root tips were relatively high (Fig. 2), which could point to a higher turnover between oxidized and reduced forms of AsA. Comparable results were obtained by Cordoba-Pedregosa et al. (2003) and Lin et al. (2004) for tomato and onion roots respectively. AsA level is also related to growth of root architecture and root reaction to gravity. Transcript accumulation in root tips revealed a complicated picture; transcripts levels of *APX*1, *APX*2, *MDHAR*1, *MDHAR*2 and *GR*1 were comparable to the other vegetative organs, while *APX4*, *APX5*, *APX6* and *DHAR2* were considerably lower (Figs 3 and 4). The corresponding thylacoid bound APX isoenzyme of rice was also not detected in roots. However, a plastidial APX isoenzyme of rice showed high levels of transcript accumulation as was the case of plastidial *APX*7 (Teixeira et al. 2006).

AsA metabolism in flower and fruit compartments

It is widely known form other studies that AsA is involved in anthesis (Attolico, de Tullio 2006). Indeed, our results showed high transcript accumulation levels for most studied genes comparable or, in some cases, even higher than leaves (Figs 3 and 4). The fact that in flowers both photosynthesis and cell division and extension take place possibly explains this finding (Muller et al. 2010). To better understand AsA metabolism in fruits, red ripe cherry tomato fruits were divided in two parts, pericarp and pulp (containing seeds, locular parenchyma and placenta). tAsA levels in fruits were lower than in leaves, shoot tips and flowers, while they were higher than in shoot and root tips (Fig. 1). In accordance to our results, Massot et al. (2012) reported almost two-fold difference in concentration of tAsA in leaves than in fruits of tomato. Our results revealed that the most active fruit tissue is the pericarp, where transcripts were, in most cases, almost twofold higher compared to the pulp. Zushi and Matsuzoe (2012) also reported similar results in tomato. These findings, along with the higher tAsA levels and enzyme activities, probably indicate higher AsA metabolism in the pericarp. Li et al. (2009) showed a positive correlation between light intensity and AsA metabolism in apple fruits; this finding could be one of the factors that explain the difference in AsA metabolism between pericarp and the pulp. Interestingly, *APX*3 was strongly expressed only in reproductive organs suggesting a specific role for this gene in their physiological processes (Fig. 3). Moreover, different progression of transcript accumulation was found in developing cherry tomato fruits concerning *APX*3 in comparison to other cytosolic APX isoenzymes (Tsaniklidis et al. 2014). It is possible that *APX*3 along with *APX*4 are important for controlling AsA redox status in ripe fruits. In contrast, transcripts of most of the genes exhibited lower accumulation in fruits than in tissues with high photosynthetic potential as well as in roots. These results can be attributed into two factors; the differences in light effect in fruit and in organs with high photosynthetic potential (Massot et al. 2012) and the different respira-

tion rate between mature fruits and fast growing organs as flowers and root tips. Indeed, both factors are reported to have an effect on AsA metabolism (Bartoli et al. 2006).

CONCLUSION

Our analysis confirmed the presence of AsA metabolism in all organs studied. Moreover, it was shown that both AsA metabolite concentration and transcripts accumulation depend on the functionality and the physiological role of the organ examined. The specificity of the accumulation of transcripts of some genes could suggest that several isoenzymes have differential importance in AsA redox status in each organ.

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Corresponding author:

Dr. Georgios Tsaniklidis, Agricultural University of Athens, Laboratory of Plant Physiology and Morphology, Iera Odos 75, 11855 Botanikos, Athens, Greece
phone: + 30 210 529 4224; fax: + 30 210 529 4286, e-mail: giorgos.tsaniklidis@gmail.com