Journal of Plant Physiology 171 (2014) 1205–1216

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Molecular Biology

L-Ascorbic acid metabolism during fruit development in an ascorbate-rich fruit crop chestnut rose (*Rosa roxburghii* Tratt)

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 28 February 2014
Accepted 3 March 2014
Available online 15 April 2014

Keywords:
Accumulation
Chestnut rose
Dehydroascorbate reductase
Fruit development
L-Ascorbic acid (Vitamin C)

SUMMARY

Chestnut rose (*Rosa roxburghii* Tratt) is a fruit crop that contains unusually high levels of L-ascorbic acid (AsA; ~1300 mg 100 g^{-1} FW). To explore the mechanisms underlying AsA metabolism, we investigated the distribution and abundance of AsA during fruit development. We also analyzed gene expression patterns, enzyme activities, and content of metabolites related to AsA biosynthesis and recycling. AsA first accumulated during late fruit development and continued to accumulate during ripening, with the highest accumulation rate near fruit maturity. The redox state of AsA in fruit was also enhanced during late fruit development, while leaf and other tissues had much lower levels of AsA and the redox state of AsA was lower. In mature fruit, AsA was mainly distributed in the cytoplasm of the mesocarp. Correlation analysis suggested that the gene expression patterns, enzyme activities, and related metabolite concentrations involved in the L-galactose pathway showed relatively high correlations with the accumulation rate of AsA. The gene expression pattern and activity of dehydroascorbate reductase (DHAR, EC 1.8.5.1) correlated strongly with AsA concentration, possibly indicating the crucial role of DHAR in the accumulation of high levels of AsA in chestnut rose fruit. Over expression of *DHAR* in *Arabidopsis* significantly increased the reduced AsA content and redox state. This was more effective than over expression of the L-galactose pathway gene GDP-D-mannose-3,5-epimerase (EC 5.1.3.18). These findings will enhance understanding of the molecular mechanisms regulating accumulation of AsA in chestnut rose.

Introduction

L-Ascorbic acid (AsA), commonly called vitamin C, is a highly abundant and essential metabolite for plants and animals. AsA is an important dietary supplement for some animals, such as primates and humans, which lack the capacity to synthesize AsA. Fruit and vegetables contain relatively high concentrations of AsA, but content varies widely among plant species and plant tissues. The level of AsA accumulation depends on a balance between biosynthesis and degradation, and the balance between reduction and oxidation.

Four biosynthetic pathways have been proposed for AsA biosynthesis in plants, with the pathway that occurs *via* L-galactose the best established (Fig. 1). The L-galactose pathway contains ten steps, converting D-glucose to AsA *via* GDP-D-mannose. The corresponding ten enzymes have been recently identified, and the last five, which catalyze the conversion of GDP-D-mannose to AsA, are specific to AsA biosynthesis (Laing et al., 2007; Linster et al., 2007). The L-galactose pathway is thought to be the dominant route for AsA biosynthesis in many plants (Imai et al., 2009; Linster et al., 2007; Linster and Clarke, 2008) and promises to be the most effective target for improving AsA content in plants *via* genetic modification (Cronje et al., 2012). The other three proposed biosynthetic routes for AsA synthesis include the D-galacturonate pathway (Agius et al., 2003), the L-gulose pathway (Wolucka and Van Montagu, 2003), and the myo-inositol pathway (Lorence et al., 2004); however, some of the specific enzymes and genes involved

Abbreviations: ALase, aldono-lactonase; AO, ascorbate oxidase; APX, ascorbate peroxidase; AsA, L-ascorbic acid (vitamin C); DAA, days after anthesis; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GalUR, D-galacturonic acid reductase; GalDH, L-galactose dehydrogenase; GER, GDP-4-keto-6-deoxy-D-mannose 3,5-epimerase-4-reductase; GGP, GDP-L-galactose phosphorylase; GalLDH, L-galactono-1,4-lactone dehydrogenase; GMD, GDP-D-mannose-4,6-dehydratase; GME, GDP-D-mannose-3,5-epimerase; GMP, GDP-D-mannose pyrophosphorylase; GPP, L-galactose-1-phosphate phosphatase; GulDH, L-gulose dehydrogenase; GulLO, L-gulono-1,4-lactone oxidase; IPS, L-myo-inositol 1-phosphate synthase; MDHAR, monodehydroascorbate reductase; MDHA, monodehydroascorbate; MIOX, myo-inositol oxygenase; PGI, glucose-6-phosphate isomerase; PMI, mannose-6-phosphate isomerase; PMM, phosphomannomutase.

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http://dx.doi.org/10.1016/j.jplph.2014.03.010
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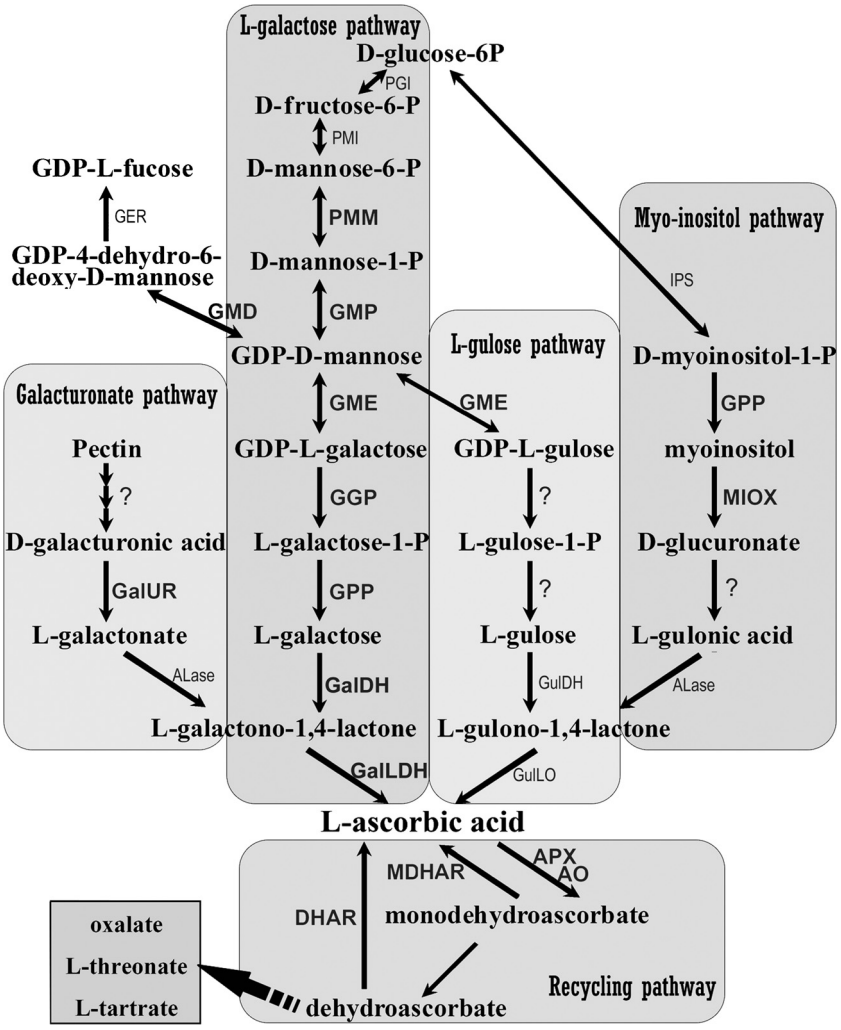

Fig. 1. Proposed pathways for AsA biosynthesis and recycling in plants. Differentially regions represent different pathways for AsA biosynthesis and recycling. Arrows connect metabolites of substrates to products, with the corresponding enzymes indicated. Question marks indicate unconfirmed reactions. Enzymes investigated in the current study are highlighted in larger bold font.

in these pathways have not been identified. The D-galacturonate pathway synthesizes AsA by breaking down cell walls, and is thought to be important for AsA biosynthesis in some plants (Badejo et al., 2012; Cruz-Rus et al., 2010, 2011; Di Matteo et al., 2010; Oller et al., 2009). The L-gulose pathway is a branch of the L-galactose pathway, beginning with GDP-D-mannose and synthesizing AsA *via* L-gulose, while the myo-inositol pathway utilizes myo-inositol as its precursor, which can also be produced from glucose. But at present, whether the two routes contribute to AsA biosynthesis is uncertain (Endres and Tenhaken, 2009; Maruta et al., 2010).

In addition to the four AsA biosynthetic pathways, AsA can also be produced through ascorbate–glutathione cycle, in which AsA is produced by reducing previously oxidized AsA (Foyer and Halliwell, 1976). AsA can be continuously oxidized as a result of enzymatic or non-enzymatic reactions and can be reduced back to AsA by recycling reductases. Additionally, part of oxidized AsA can be further catabolized into smaller molecules such as L-tartaric acid, L-threonic acid, L-glyceric acid, and oxalic acid (Cruz-Rus et al., 2012; Ishikawa et al., 2006). Recycling of AsA is important in the maintenance of the AsA level in plants (Cruz-Rus et al., 2011; Stevens et al., 2008), and genetic enhancement of AsA recycling could also increase AsA content (Chen et al., 2003; Haroldsen et al., 2011; Naqvi et al., 2009; Qin et al., 2011).

Chestnut rose (*Rosa roxburghii* Tratt) is a rare fruit crop in Southwest China whose fruits contain a variety of phytochemicals beneficial for human health. Particularly, fruit of this crop is abundant in AsA (1000–2000 mg $100\,g^{-1}$ FW), that is higher than most common fruit crops, *e.g.* tomato (~20 mg), strawberry (~50 mg), and kiwifruit (*Actinidia deliciosa*, ~100 mg); it is even close to those fruits with top AsA concentration, *e.g.* kiwifruit (*Actinidia eriantha*, ~1500 mg), acerola (*Malpighia glabra*, ~1700 mg), rosehip (*Rosa* sp. cv. 1000–2500 mg). In recent decades, intensive efforts have focused on understanding how AsA metabolism is controlled in several model plants such as *Arabidopsis* and tomato, as well as in fruit crops such as kiwifruit. The mechanisms of AsA production in chestnut rose are not well understood, despite the high AsA content of its fruit. To increase understanding of AsA production in chestnut rose during fruit development, we systematically analyzed changes in the abundance of AsA and related metabolites, the cellular distribution of AsA, gene expression patterns, and the activities of enzymes involved in AsA biosynthesis and recycling. Results not only revealed a special pattern of AsA accumulation, but also indicated that biosynthesis is just one of the ways leading to AsA accumulation in the fruit and that AsA recycling by dehydroascorbate reductase (DHAR) may play a more important role in AsA accumulation. These results will be beneficial in developing