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Comparison of ascorbate metabolism in fruits of two citrus species with obvious difference in ascorbate content in pulp

Xiao-Yan Yang[a,b], Jin-Xia Xie[a,b], Fang-Fang Wang[a,b], Jing Zhong[a,b], Yong-Zhong Liu[a,b,*], Guo-Huai Li[a,b], Shu-Ang Peng[a,b]

[a] Key Laboratory of Horticultural Plant Biology (Ministry of Education), Huazhong Agricultural University, Wuhan 430070, People's Republic of China
[b] College of Horticulture and Forestry Science, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

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ABSTRACT

Citrus fruit is widely consumed and provides ascorbate for human health. The ascorbate content in pulp is generally higher in orange (*Citrus sinensis* Osb.) than in Satsuma mandarin (*Citrus unshiu* Marc.). However, what contributes to such difference is still unknown. In the present study, ascorbate accumulation, expression profiles of genes involved in L-galactose pathway and activity changes of enzymes related with L-ascorbic acid (AA) oxidation and recycling were investigated during fruit development and ripening in fruit pulp of Satsuma mandarin and orange. As fruit ripens, total ascorbate (T-ASC) or AA content increased in mandarin whereas fluctuated on a relatively high level in orange. Concentrations of T-ASC or AA in pulp of orange were over 1.5-fold higher than that in pulp of Satsuma mandarin during fruit ripening. Further analysis showed that each transcript of four genes (encoding GDP-D-mannose-3′,5′-epimerase, GDP-L-galactose-pyrophosphatase, L-galactose dehydrogenase and L-galactono-1,4-lactone dehydrogenase respectively) in orange was almost on a higher level and the activities of oxidation enzymes (ascorbate oxidase and ascorbate peroxidase) were lower during fruit ripening as compared with Satsuma mandarin. As ascorbate pool size is decided by the combination of biosynthesis, oxidation and recycling, therefore, higher expression of four genes along with lower activity of oxidation enzymes should contribute at least partially to the higher ASC accumulation in orange pulp.

Introduction

Ascorbate (ASC), also called vitamin C, includes two bioactive forms: the reduced form (L-ascorbic acid, AA) and the oxidized form (dehydroascorbate, DHA). It is widely known that ASC plays crucial roles not only in different plant development processes mainly for its functions as antioxidant and enzymatic cofactors (Olmos et al., 2006; Kotchoni et al., 2009), but also in maintaining human health such as reducing risk of chronic diseases (for example cancer, cardiovascular disease and cataract), collagen formation, normal bone development and application in cancer treatment (Sarkar et al., 2009). However, humans cannot synthesize their own AA due to the absence of L-gulonolactone oxidase and ASC cannot be stored in the body either. Thus, humans have to obtain such important vitamins regularly from their dietary food, mainly from vegetables and fruits (Naidu, 2003).

Although ASC roles have been well documented, the mechanism controlling ASC level is still poorly understood. ASC component and level are attributable to the balance of its biosynthesis, catabolism and recycling whereas AA biosynthesis was considered as the main source of ASC accumulation in plant cells (Ishikawa et al., 2006). AA biosynthetic pathways in plants are more complicated than in animals because at least four distinct pathways for AA biosynthesis have been described until now, including L-galactose or Smirnoff–Wheeler pathway, L-glucose pathway, galacturonic acid pathway, and *myo*-inositol pathway (Linster and Clarke, 2008). Among them, L-galactose pathway was evidenced as a major biosynthetic pathway in high plants (Linster and Clarke, 2008). This pathway involves six enzymatic steps from GDP-D-mannose-1-p to L-ascorbate via the intermediate formation of GDP-D-mannose and L-galactose (Fig. 1). The first conversion of GDP-D-mannose-1-p to GDP-D-mannose is catalyzed by GDP-D-mannose pyrophosphorylase

Abbreviations: AA, L-ascorbic acid; AO, ascorbate oxidase; APX, ascorbate peroxidase; ASC, ascorbate; CES, crude enzyme solution; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GDH, L-galactose dehydrogenase; GGP, GDP-L-galactose-pyrophosphatase; GLDH, L-galactono-1,4-lactone dehydrogenase; GME, GDP-D-mannose-3′,5′-epimerase; GMP, GDP-D-mannose pyrophosphorylase; GPP, L-galactose-1-P phosphatase; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; OD, optical density; TA, titratable acidity; T-ASC, total ASC; TSS, total soluble solid.

* Corresponding author at: College of Horticulture and Forestry Science, Huazhong Agricultural University, Shizishan Street 1#, Hongshan District, Wuhan 430070, People's Republic of China. Tel.: +86 27 62415825; fax: +86 27 87282010.
E-mail addresses: liuyongzhong@mail.hzau.edu.cn, liuyz05@126.com (Y.-Z. Liu).

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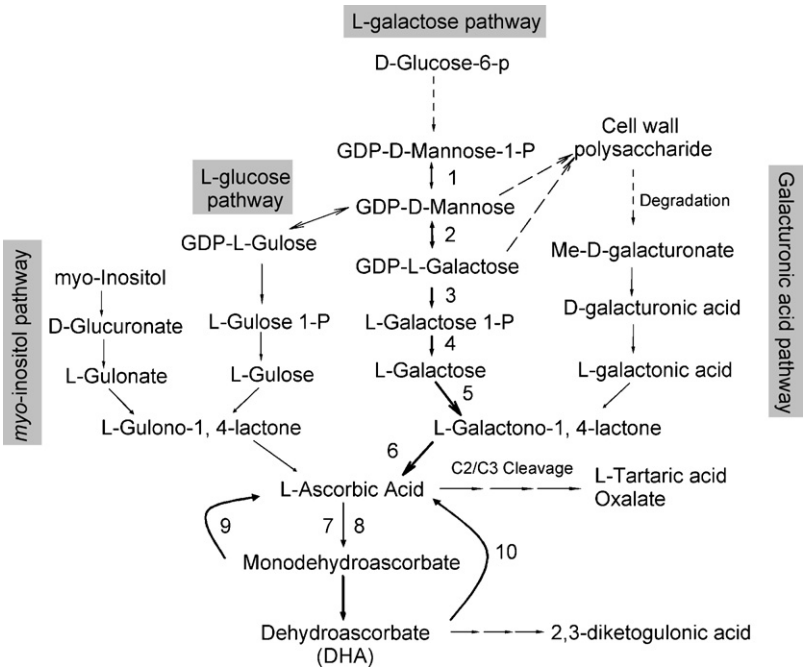

Fig. 1. Simple scheme of ascorbate biosynthetic, catabolic and recycling pathways in plants. The gray background indicated the four possible biosynthetic pathways in plants (Linster and Clarke, 2008). Enzymes catalyzing the numbered reactions are: 1, GDP-D-mannose pyrophosphorylase; 2, GDP-Man-3′,5′-epimerase; 3, GDP-L-galactose phosphorylase; 4, L-galactose-1-P phosphatase; 5, L-galactose dehydrogenase; 6, L-galactono-1,4-lactone dehydrogenase; 7, ascorbate peroxidase; 8, ascorbate oxidase; 9, monodehydroascorbate reductase; 10, dehydroascorbate reductase.

(GMP). Subsequently, GDP-D-mannose undergoes double epimerization catalyzed by GDP-D-mannose-3′,5′-epimerase (GME) to form GDP-L-galactose. Under the catalysis of GDP-L-galactose-pyrophosphatase (GGP) and L-galactose-1-P phosphatase (GPP), GDP-D-galactose is converted into L-galactose. The resulting L-galactose is then oxidized into L-galactono-1,4-lactone by the catalysis of L-galactose dehydrogenase (GDH). The final step in the biosynthesis of AA is catalyzed by L-galactono-1,4-lactone dehydrogenase (GLDH), which is located at the mitochondrial inner membrane (Fig. 1).

As for AA catabolism, it includes oxidation and cleavage (Fig. 1). Its oxidation is catalyzed by ascorbate oxidase (AO), and ascorbate peroxidase (APX). AO catalyzes AA in the presence of oxygen, while APX catalyzes the reduction of hydrogen peroxide to water with the simultaneous oxidation of AA. Both ways of AA oxidation produce monodehydroascorbate (MDHA) which, if not rapidly reduced by MDHA reductase (MDHAR), will subsequently be disproportionate and form AA and DHA (Ishikawa et al., 2006). In the end, if DHA could not be completely reduced back to AA in time, it would easily undergo irreversible hydrolysis to 2,3-diketogulonic acid (Washko et al., 1992) or it can be a biosynthetic precursor in the formation of oxalic acid, L-threonic acid and L-tartaric acid by carbon cleavage (Debolt et al., 2007).

Besides the affection of biosynthesis and catabolism, the AA recycling process also plays a very important role in ASC accumulation in plant tissues (Chen et al., 2003). This process involves two enzymes: an NAD+-dependent MDHAR and a reduced glutathione-dependent dehydroascorbate reductase (DHAR). When MDHA is produced by AA oxidation, it will be spontaneously converted to DHA or be enzymatically reduced to AA by MDHAR, while DHA can be also reduced to AA by DHAR using glutathione as the reducing substrate (Noctor and Foyer, 1998).

Fruit is an important source of vitamin C for humans (Naidu, 2003). However, their vitamin C contents vary greatly. For example, high ASC-accumulating fruit such as kiwifruit (*Actinidia deliciosa* cv. Hayward) contained over 200 μmol vitamin C per gram of fresh weight (Bulley et al., 2009), while in low ASC-accumulating fruit such as apple (*Malus domestica* cv. Gala), flesh only contained about 0.4 μmol vitamin C per gram of fresh weight (Li et al., 2009). Owing to its important functions for plant normal development and for human health, to study the mechanism of AA control in plant edible parts seems very important (Hancock and Viola, 2005). To date, significant efforts have been made to investigate ASC metabolism in many fruit species, such as kiwifruit (Bulley et al., 2009), apple (Li et al., 2008), peach (Imai et al., 2009), tomato (Alhagdow et al., 2007; Ioannidi et al., 2009), strawberry (Do Nascimento et al., 2005) and so on, implying that ASC contents in fruit can be modulated during development (Li et al., 2008; Bulley et al., 2009; Imai et al., 2009; Ioannidi et al., 2009).

Compared with fruits mentioned above, very limited information is currently available on the mechanism controlling ASC levels in citrus fruit, which is one of the most economically important fruits in the world. Citrus fruit and related products are rich in ASC (Nagy, 1980). They are widely consumed as a functional food in the world and provide an important source of ASC for human health (Martí et al., 2009). The ASC content in citrus fruit juice sacs is 20–100 mg per 100 mL juice, which varies greatly depending on species or variety (Nagy, 1980; Lee and Kader, 2000). For instance, orange (*Citrus sinensis* Osb.) and Satsuma mandarin (*Citrus unshiu* Marc.) are two commercial species with significant difference in ASC levels (Nagy, 1980). Our analysis in 2001 and 2002 showed that the average AA contents in fruit juice sacs of orange and mandarin at the maturing period are 48.9 ± 8.4 and 27.3 ± 7.1 mg per 100 mL juice, respectively (data unpublished). In the present study, to gain insight into the potential mechanism regulating ASC accumulation in citrus fruit, we systematically investigated the expression profiles of six L-galactose pathway-related genes as well as ASC contents between fruit pulps of *C. sinensis* cv. Newhall and *C. unshiu* cv. Guoqing No.1 during fruit development and ripening. The genes measured herein were *GMP*, *GME*, *GGP*, *GPP*, *GDH* and *GLDH*. On