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Light avoidance reduces ascorbic acid accumulation in the peel of *Citrus* fruit

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ABSTRACT

Citrus fruits are highly consumed worldwide and represent one of the most important sources of ascorbic acid (AsA). However, information about the molecular mechanisms regulating AsA accumulation in *Citrus* fruit and the effects of environmental factors is scarce. In this study we have investigated the effect of fruit shading on AsA content and the expression of AsA biosynthetic, degrading and recycling genes in fruits of different *Citrus* species. Immature-green fruits were covered at the end of the cell enlargement phase and AsA concentration in the flavedo declined and remained at low levels as compared with light-exposed fruits. Fruit shading marginally altered the expression of genes from the L-galactose pathway and this effect was variable in the four *Citrus* species. However, specific isoforms (*GalUR8* or *GalUR12*) from the L-galacturonic acid pathway were significantly repressed paralleling the reduction in AsA concentration. No significant effect of shading was detected in transcription of genes of the *myo*-inositol and L-gulose pathways as well as recycling and degradation. Collectively, results indicate that light avoidance inhibited accumulation of AsA in the flavedo of *Citrus* fruits and suggest that the L-galacturonic acid pathway has a relevant contribution to AsA content in this tissue.

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1. Introduction

Ascorbic acid (AsA; or vitamin C) is the most abundant antioxidant in the plant cells and plays important roles in a wide range of processes such as photosynthesis, growth regulation and senescence. Additionally, AsA is a cofactor for enzymes and affects the expression of genes involved in defense and hormone signaling pathways [reviewed in 1]. Interestingly, the enhancement of AsA concentrations in crops is expected to extend shelf-life, and increases stress tolerance to soil salinity, drought and pollution [2]. Furthermore, AsA is essential for human survival and must be obtained mainly through the consumption of fruits and vegetables since humans have lost the capability to synthesize it [3].

In plants, four AsA biosynthetic pathways have been proposed: the L-galactose, the L-gulose, the *myo*-inositol and the D-galacturonic acid pathways. The L-galactose pathway has been suggested to be a major biosynthetic route especially active during the development of several fleshy fruits like apple, kiwi, tomato and *Citrus* fruits [4–10], whereas in strawberry fruit the D-galacturonic acid and the *myo*-inositol pathways seemed to prevail [11,12]. Moreover, feeding experiments with AsA precursors demonstrated that the peel of apple fruits is capable of synthesizing *de novo* AsA via the L-galactose and the D-galacturonic acid pathways, while the flesh and seeds are only able to synthesize AsA through the L-galactose pathway [13]. Hence, the predominating AsA biosynthetic pathways seem to be specie-specific and can change depending on the developmental stage and the fruit tissue.

In addition to the *de novo* biosynthesis of AsA, other mechanisms such as degradation and recycling can considerably contribute to the regulation of AsA pool in plants (Fig. 1). Thus, AsA can be transformed into monodehydroascorbate (MDHA) by the enzymes ascorbate oxidase (AO) and ascorbate peroxidase (APX) [14]. Then, the MDHA radical can either be recycled into AsA by monodehydroascorbate reductase (MDHAR) or undergo disproportionation into dehydroascorbate (DHA) and AsA [15,16]. Moreover, DHA can

Abbreviations: AsA, ascorbic acid; qRT-PCR, quantitative reverse transcription polymerase chain reaction; HPLC, high performance liquid chromatography; PDA, photodiode array detector; DTT, dithiothreitol; cDNA, complementary DNA; DNase, desoxyribonuclease I; NC, non-covered; C, covered.

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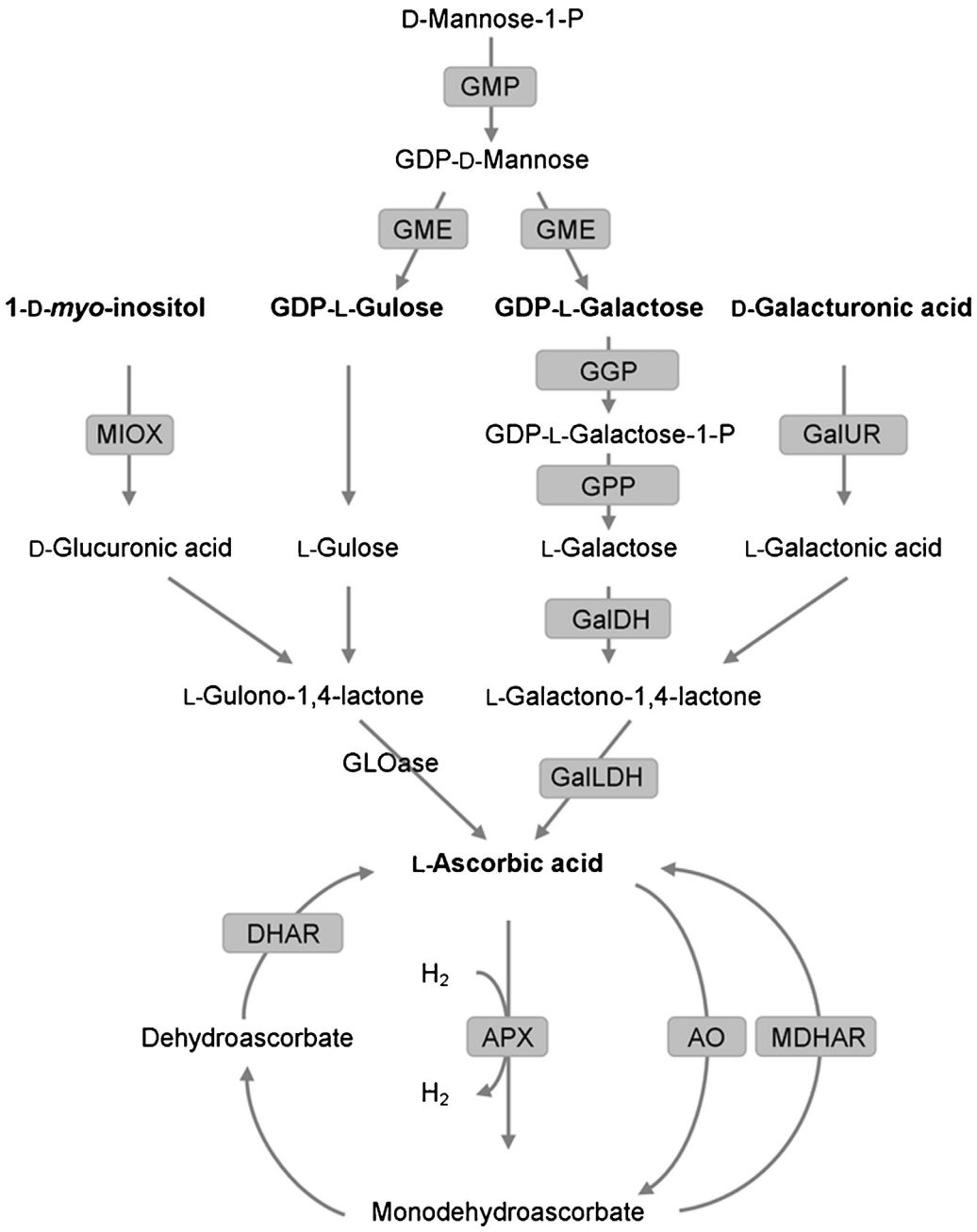

Fig. 1. Schematic representation of the biosynthetic, degrading and recycling pathways of AsA in plants. The enzymes catalyzing the reactions are: GDP-mannose pyrophosphorylase (GMP), GDP-mannose-3′–5′-epimerase (GME), GDP-L-galactose transferase (GGP), L-galactose-1-phosphate phosphatase (GPP), L-galactose dehydrogenase (GalDH), L-galactono-1,4-lactone dehydrogenase (GalLDH), D-galacturonic acid reductase (GalUR), myo-inositol oxygenase (MIOX), L-gulono-1,4-lactone oxidase (GLOase), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), ascorbate oxidase (AO), ascorbate peroxidase (APX). Adapted from [1].

be recycled into AsA by dehydroascorbate reductase (DHAR) before being irrevocably hydrolyzed (Fig. 1) [reviewed in 17].

The overexpression of genes encoding AsA biosynthetic [18–20] and recycling enzymes [13,21,22] or the suppression of degrading enzymes [23] in transgenic plants have been addressed to increase AsA concentrations in several plant species and have highlighted the importance of the balance between AsA biosynthesis and degradation/recycling in AsA homeostasis.

Among fruits and vegetables, *Citrus* fruit are highly consumed worldwide and are ranked among the top ten relevant sources of AsA [24]. A wide variation in AsA content has been described between *Citrus* varieties and tissues, and also notable changes occur at different stages of fruit development. AsA concentration in the flavedo (the outer colored part of the peel) oscillates between 75 and 374 mg/100 g FW while the albedo (the white internal part of the peel) accounts for 11–190 mg/100 g FW and the content in the pulp ranges between 20 and 70 mg/100 g FW [9,25,26]. Oranges, in general, contain the highest AsA levels (29 and 82 mg/100 mL of juice), followed by lemons (30–50 mg/100 mL), grapefruits (30–60 mg/100 mL) and mandarins (20–60 mg/100 mL) [25,26]. Recent studies on AsA metabolism in *Citrus* fruits have revealed that during natural fruit ripening AsA content increased in the flavedo while the opposite occurred in the pulp [9]. In addition, the differences in AsA concentration between oranges and mandarins have been suggested to be associated with differences in the expression of genes from the L-galactose pathway during ripening as well as with the differential activity of enzymes involved in AsA recycling and degradation [8,9]. Moreover, the high levels of gene