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Research article

Comparison of ascorbic acid biosynthesis in different tissues of three non-heading Chinese cabbage cultivars

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ABSTRACT

Ascorbic acid (L-AsA) is an important antioxidant in plants and humans. Vegetables are one of the main sources of ascorbic acid for humans. For instance, non-heading Chinese cabbage (*Brassica campestris* ssp. *chinensis* Makino) is considered as one of the most important vegetables in south China. To elucidate the mechanism by which AsA accumulates, we systematically investigated the expression profiles of D-mannose/L-galactose pathway-related genes. We also investigated the recycling-related genes and AsA contents in different tissues of three non-heading Chinese cabbage cultivars, 'Suzhouqing', 'Wutacai' and 'Erqing' containing different amounts of AsA. Our results showed that six genes [D-mannose-6-phosphate isomerase 1 (*PMI1*), GDP-L-galactose phosphorylase 1 (*GGP1*), *GGP2*, *GGP4*, GDP-mannose-3′, 5′-epimerase1 (*GME1*), and *GME2*] were expressed at high level and ascorbate oxidase (*AAO*) was expressed at low level. This expression pattern contributes, at least partially, to higher AsA accumulation in the leaves and petioles than in the roots. Eight genes (*PMI1*, *GME*, *GGP*, L-galactose-1-phosphate phosphatase, L-galactose dehydrogenase, L-galactono-1, 4-lactone dehydrogenase, monodehydroascorbate reductase 1, and glutathione reductase1) were also expressed at high level; *AAO* and ascorbate peroxidase (APX) were expressed at low level. This expression pattern may similarly contribute to higher AsA accumulation in 'Wutacai' and 'Suzhouqing' than in 'Erqing'. Therefore, the high expression levels of *PMI*, *GME*, and *GGP* and the low expression level of *AAO* contributed to the high AsA accumulation in non-heading Chinese cabbage.

1. Introduction

L-Ascorbic acid (AsA, vitamin C) is an abundant antioxidant that functions in stress responses and plant defense; AsA is also a co-factor of many dioxygenases of reactive oxygen species in plants [1]. Furthermore, AsA is involved in diverse physiological functions, such as cell cycle regulation, cell wall synthesis, cell expansion, electron transfer, plant morphology modulation, flowering time, and onset of senescence in plants [2–4]. AsA also helps prevent various oxidative stress-related diseases, such as cancers and cardiovascular diseases, and aging in humans who have lost the ability to synthesize AsA, thereby providing human health benefits [5]. The main AsA sources for humans are fruits and vegetables. Ascorbate levels in plants are influenced by three genetically controlled pathways, including synthesis, degradation, and recycling [6].

In plants, the AsA synthesis pathway involves a complex network of D-mannose/L-galactose (D-Man/L-Gal) [7], L-gulose [8], D-galacturonate (D-GalA) [9], and *myo*-inositol (MI) [10] as entry points into the network (Fig. 1) [3]. The D-Man/L-Gal pathway, commonly called the Smirnoff-Wheeler pathway, is considered as the main route of AsA biosynthesis [7,11,12]. All of the genes encoding the enzymes involved in this pathway are fully

Abbreviations: AAO, ascorbate oxidase; APX, ascorbate peroxidase; AsA, ascorbic acid (vitamin C); DHAR, dehydroascorbate reductase; D-GalUA, D-galacturonic acid; D-Glu, D-glucose; D-GluA, D-glucuronic acid; L-Gal, L-galactose; L-GalL, L-galactono-1,4-lactone; L-GulL, L-gulono-1,4-lactone; MI, *myo*-inositol; GDH, L-galactose dehydrogenase; GGP, GDP-L-galactose phosphorylase; GLDH, L-galactono-1,4-lactone dehydrogenase; GME, GDP-mannose-3′, 5′-epimerase; GMP, GDP-mannose pyrophosphorylase (mannose-1-phosphateguanyltransferase); GPP, L-galactose-1-phosphate phosphatase; GR, glutathione reductase; PMI, D-mannose-6-phosphate isomerase; PMM, phosphomannomutase; MDHAR, monodehydroascorbate reductase; qRT-PCR, quantitative real-time PCR; T-AsA, total AsA.

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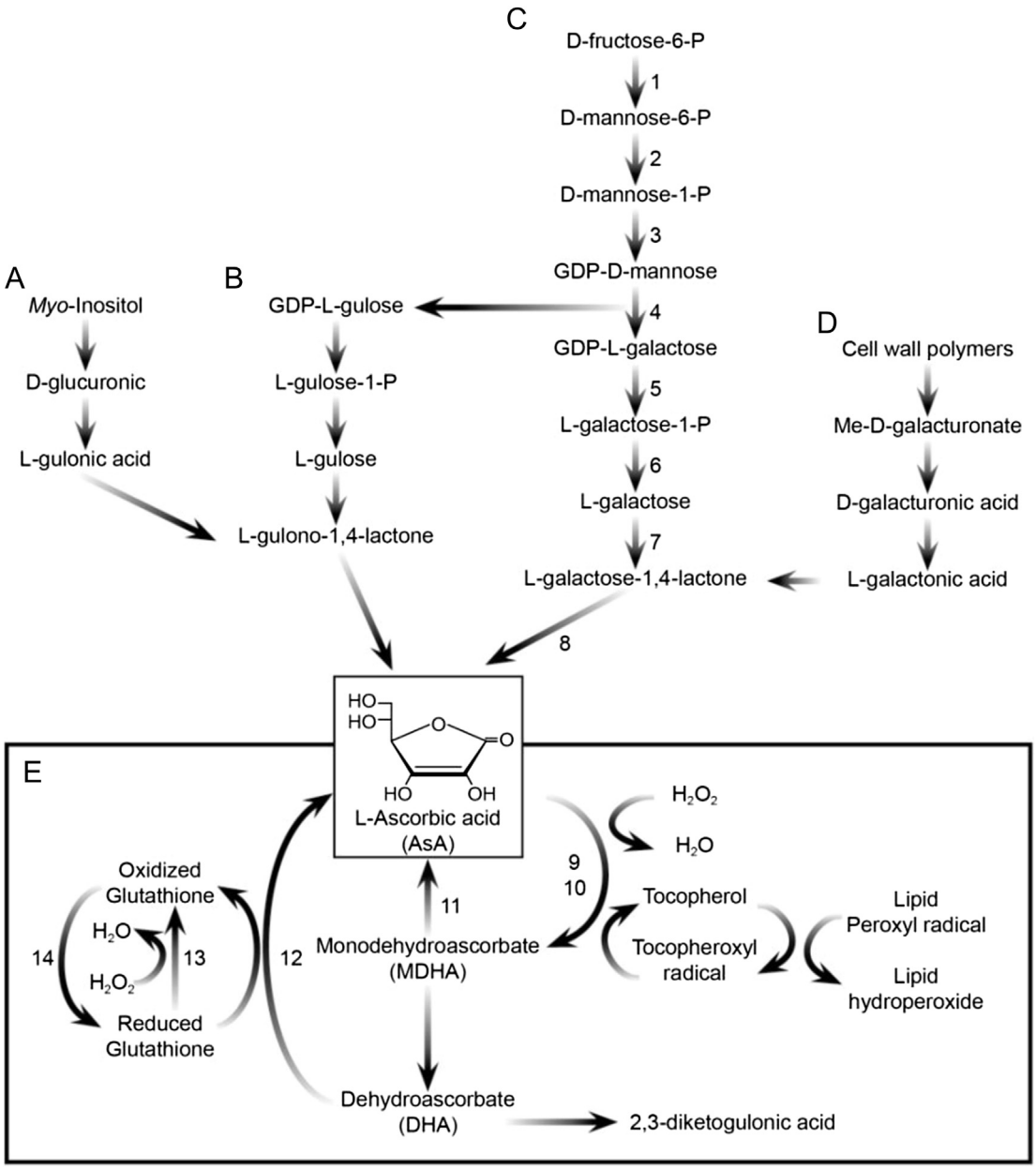

Fig. 1. Proposed the four biosynthetic pathways of AsA in plants ((A) *myo*-inositol, (B) L-gulose, (C) D-mannose/L-galactose (D-Man/L-Gal), (D) D-galacturonate (D-GalA)) and recycling pathway (E) [42]. The enzymes catalyzing these reactions are listed as follows: 1, D-mannose-6-phosphate isomerase (PMI); 2, phosphomannomutase (PMM); 3, GDP-mannose pyrophosphorylase (mannose-1-phosphateguanyltransferase) (GMP); 4, GDP-mannose-3′,5′-epimerase (GME); 5, GDP-L-galactose phosphorylase (GGP); 6, L-galactose-1-phosphate phosphatase (GPP); 7, L-galactose dehydrogenase (GDH); 8, L-galactono-1,4-lactone dehydrogenase (GLDH); 9, ascorbate peroxidase (APX); 10, ascorbate oxidase (AAO); 11, monodehydroascorbate reductase (MDHAR); 12, dehydroascorbate reductase (DHAR); 13, glutathione peroxidase (GRX); and 14, glutathione reductase (GR) [42].

characterized in the model plant *Arabidopsis thaliana* [7,11,13]. AsA is an unstable metabolic product and can be oxidized to a monodehydroascorbate (MDHA) radical while ascorbate peroxidase (APX; EC 1.11.1.11) uses AsA as an electron donor to scavenge hydrogen peroxide (H_2O_2) [14] or ascorbate oxidase (AAO, EC 1.10.3.3) catalyzes AsA in the presence of oxygen. MDHA is subsequently disproportionated and oxidized to DHA if not rapidly reduced by MDHA reductase (MDHAR, EC 1.6.5.4) [15]. DHA is then irreversibly hydrolyzed to 2, 3-diketogulonic acid or is reduced to AsA by dehydroascorbate reductase (DHAR, EC 1.8.5.1), which uses glutathione (GSH) as a reductant [16]. The resulting oxidized glutathione is then reduced back to GSH by an NADPH-dependent glutathione reductase (GR; EC1.6.4.2) [16].

AsA biosynthesis and metabolism have been well studied in plants; for example, reduction/overexpression of genes encoding various enzymes in the AsA biosynthesis and metabolic network leads to a decrease/increase in AsA content; such genes may regulate AsA content [17–19]. However, the mechanisms controlling AsA levels in plants remain unexplored. The molecular regulation of AsA accumulation in tissues (e.g., petioles and roots) also remains unknown. Previous biochemical analyses confirmed that heterotrophic tissues can undergo AsA biosynthesis [20,21]. AsA translocation from source to sink tissues has also been demonstrated [20,22], but the relative contributions of biosynthesis and transport of AsA concentration in sink tissues have not been quantified. This phenomenon may influence AsA levels.