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Update

L-Ascorbic acid metabolism and regulation in fruit crops

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Abstract

L-Ascorbic acid (AsA) is more commonly known as vitamin C and is an indispensable compound for human health. As a major antioxidant, AsA not only maintains redox balance and resists biological and abiotic stress but also regulates plant growth, induces flowering, and delays senescence through complex signal transduction networks. However, AsA content varies greatly in horticultural crops, especially in fruit crops. The AsA content of the highest species is approximately 1,800 times higher than that of the lowest species. There have been significant advancements in the understanding of AsA accumulation in the past 20 years. The most noteworthy accomplishment was the identification of the critical rate-limiting genes for the 2 major AsA synthesis pathways (L-galactose pathway and D-galacturonic acid pathway) in fruit crops. The rate-limiting genes of the former are *GMP*, *GME*, *GGP*, and *GPP*, and the rate-limiting gene of the latter is *GalUR*. Moreover, *APX*, *MDHAR*, and *DHAR* are also regarded as key genes in degradation and regeneration pathways. Interestingly, some of these key genes are sensitive to environmental factors, such as *GGP* being induced by light. The efficiency of enhancing AsA content is high by editing upstream open reading frames (uORF) of the key genes and constructing multi-gene expression vectors. In summary, the AsA metabolism has been well understood in fruit crops, but the transport mechanism of AsA and the synergistic improvement of AsA and other traits is less known, which will be the focus of AsA research in fruit crops.

Introduction

L-Ascorbic acid (AsA), namely vitamin C, has important functions and antioxidant effects in organisms. It is an indispensable nutrient for human health. However, primates have lost the ability to synthesize AsA due to the mutation of the enzyme in the last step of AsA synthesis. Therefore, AsA must be part of the diet from AsA-rich vegetables and fruits. For plants, AsA not only plays important roles as an antioxidant and quenching free radical, in particular during photosynthesis and photoprotection (Smirnoff 2011), but also is involved in cell growth and division and plant hormone biosynthesis (Lisko et al. 2014). Significant progress has been made in our understanding of AsA metabolism in plants. Many of the major advances in AsA research have been achieved by studying AsA-enriched fruit crops. Although AsA can be detected in all plants, AsA levels show wide variation in fruit crops, and within the same plant there are also significant differences between tissues and organs (Davey et al. 2000). Therefore, various and multifaceted regulatory mechanisms are expected to exist in fruit crops that control AsA metabolism (Liu et al. 2022a).

Wide variation of AsA levels in fruit crops

The AsA content of different species of fruit varies greatly, and the AsA content of the same fruit will also have

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significant differences under different growth conditions or maturities (Valente et al. 2011). The AsA content of the main horticultural crops is listed in Fig. 1A and Supplemental Table S1; all the data were collected from related studies and reports. In fruit, the highest AsA content is the Kakadu plum and the lowest is pomegranate, with the former approximately 1,800 times that of the latter (Miller et al. 1993; Valente et al. 2011). The content of AsA in kiwifruit also varies greatly, the highest being in *Actinidia eriantha*, which can reach 2,127 mg/100 g fresh weight (FW) (Liao et al. 2021a). In vegetables, the highest AsA content is in sweet pepper and the lowest is in eggplant, which can be 159 mg/100 and 4 mg/100 g, respectively (Ye 2011). In flowers, the highest is in jasmine with 210.63 mg/100 g, and the lowest is in tulip with 1.58 mg/100 g (Wang 2003; Xing 2004).

The AsA content also shows different accumulation patterns among fruit crops. Generally, fruit crops can be classified into 3 types according to the periods of AsA peak accumulation appears during fruit growth and development. The first type is AsA peak accumulation at the young fruit period, such as kiwifruit (Liao et al. 2021b) and apple (Li 2009) (Fig. 1B). The second type is AsA peak accumulation at the fruit expansion period, such as jujube (Chen 2015) (Fig. 1C). The third type is fruit AsA peak accumulation at the maturity period, such as chestnut rose (Huang 2013), strawberry (Luo et al. 2019), and tomato (Ioannidi et al. 2009) (Fig. 1D).

Tissue and subcellular distribution of AsA

Different plant tissues have different AsA levels, with photosynthetic and storage tissues generally having higher AsA content, and younger tissue having higher AsA content than aged tissue. In *Actinidia chinensis* cv. "Jinyan" and apple cv. "Gala," mature leaves were found to have higher AsA content than young leaves, and the pericarp had higher AsA content than the pulp (Li et al. 2008; Liao et al. 2022). In addition, AsA content in different tissues are cultivar specific among different cultivars of the same species. For example, in *A. chinensis* cv. "Hongyang," the pulp has higher AsA content than the pericarp (Liao et al. 2022). There is a developing consensus that AsA synthesis can occur within the phloem (Hancock et al. 2003). There were reports on AsA biosynthesis in sink organs (e.g. fruit), including tomato, kiwifruit, chestnut rose, and apple, with higher AsA levels found in the vascular tissues (Li et al. 2008; Huang 2013). Studies on apple and chestnut rose fruit suggest that the accumulation of AsA in fruit may utilize AsA synthesized in other tissues, transported in the long-distance transport tissue in the form of oxidized DHA, which together with in situ synthesis leads to the accumulation of AsA (Li et al. 2008; Huang 2013) (Fig. 2). However, this research cannot fully answer the question of whether AsA accumulation in sink organs occurs as a result of biosynthesis in situ or import from the leaves. Some studies have demonstrated that in long-distance AsA transport from source to sink in model plants, AsA was transported to root tips, shoots, and floral organs but not to mature leaves (Franceschi and Tarlyn 2002; Tedone et al. 2004) (Fig. 2).

AsA content also differs among organelles. Usually, the cytoplasm and peroxisomes contain higher levels of AsA, approximately 20 to 40 mM and 10 to 23 mM, respectively (Zechmann et al. 2011). This is followed by the nucleus and chloroplast, approximately 6 to 30 mM and 10 to 20 mM, respectively, with mitochondria having approximately 9 to 12 mM. The lowest levels are in the vacuole, with approximately 2 to 4 mM (Bartoli et al. 2000). Amyloplasts are essentially free of AsA. In addition, the AsA content in chloroplasts was significantly increased under photo-oxidative stress (Zechmann et al. 2011). Due to the last enzymatic step of the main AsA synthesis pathway being located in the mitochondria, much of the synthesized AsA is derived within the mitochondria. However, the concentration of AsA in the mitochondria is lower than that in the cytoplasm, which indicates that AsA leaves the mitochondria via transporters after being synthesized (Horemans et al. 2000) (Fig. 2). It has been found that transmembrane transport of AsA occurs in the chloroplast, vacuole, and plasma membrane, and the transport of AsA on the chloroplast and plasma membrane is an active transport process mediated by a protein carrier (Horemans et al. 2000; Szarka et al. 2004, 2007). The transmembrane transport of AsA into or out of the mitochondria and peroxisomes is unclear, with the possibility that AsA diffuses from mitochondria to the cytoplasm. It is worth mentioning that the apoplast lacks NADPH, GSH, and the corresponding reaction enzymes, resulting in a lack of AsA recycling in the apoplast (Horemans et al. 2008). Therefore, the transporters of AsA and DHA must be present between symplastically isolated cells and tissues. Except for Cytb, which was shown to indirectly cause the transfer of AsA, other putative transporters have not been validated at the molecular level (Rivas et al. 2008; Kosti et al. 2012).

Recognized pathways and genes contributing AsA accumulation in fruit crops

The metabolic pathway of AsA in plants is more complicated than in animals. At present, AsA metabolic pathways are mainly divided into biosynthetic, degradation, and cyclic regeneration pathways (Fig. 3), and it is recognized that there are 4 major biosynthetic pathways: L-galactose, D-galacturonic acid, inositol, and L-gulose. These pathways have been well studied in fruits, such as strawberry and kiwifruit (Foyer et al. 2020; Liao et al. 2021c; Liu et al. 2022a).

L-galactose pathway

The L-galactose pathway was the first discovered and is recognized as the main synthetic pathway in fruit crops, including apple (Li 2009), kiwifruit (Wei et al. 2021; Liao et al.

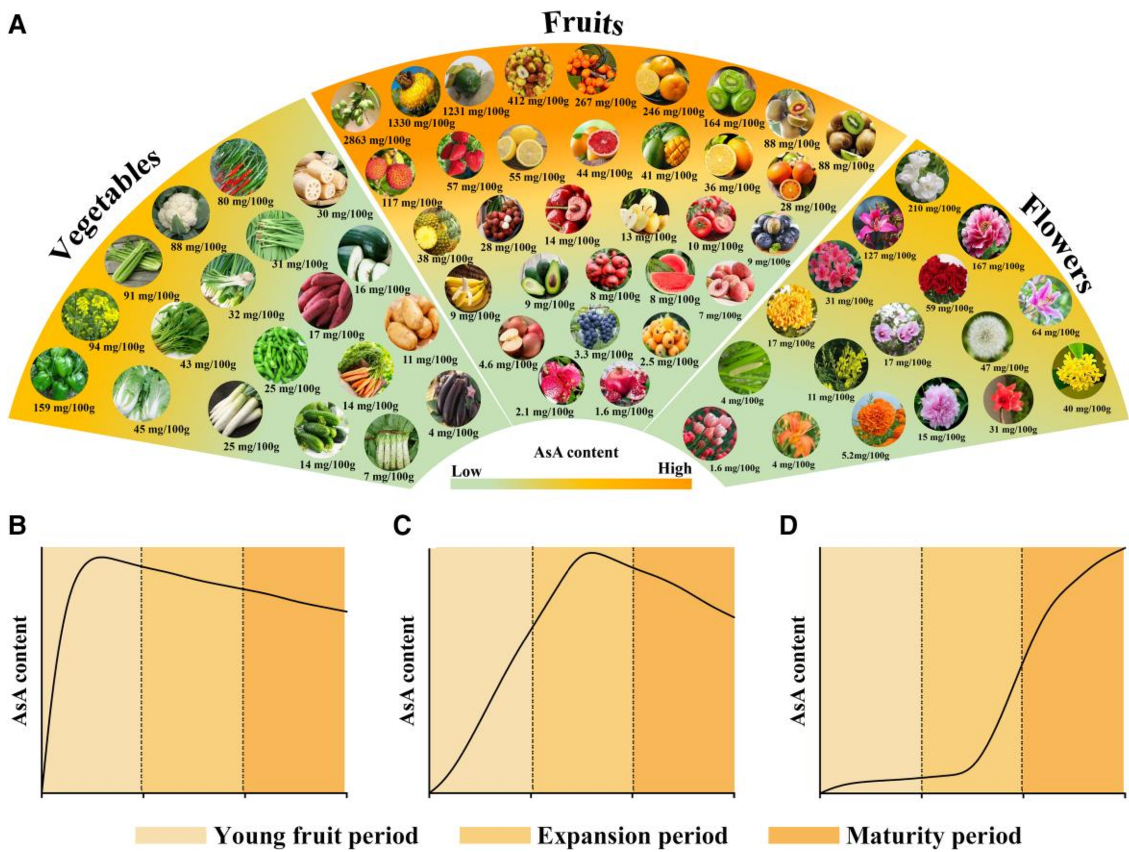

Figure 1. AsA content in various horticultural crops (A) and accumulation patterns (B–D). All the data and figures were collected from related studies (Ye 2011; Huang 2013) and reports as well as the Web of Science, China National Knowledge Infrastructure, and public websites. Panels B–D represent different AsA accumulation patterns. The data used to draw schematic diagram were obtained from kiwifruit (*A. eriantha*), Chinese jujube (cv "Mazao"), and chestnut rose (cv "Guinong 5") (Huang 2013; Lu et al. 2022; Liu et al. 2022b).

2021d), and jujube (Lu et al. 2022). The key enzymes involved in the L-galactose pathway have been largely characterized. Among these, there are 4 recognized key rate-limiting enzyme genes in this pathway: *GMP*, *GME*, *GGP*, and *GPP*.

GMP (EC 2.7.7.22, GDP-D-mannose pyrophosphorylase) is considered to be the first rate-limiting enzyme of the L-galactose pathway. There was a high correlation between *GMP* expression and AsA content; AsA content of the plant decreases, and the plant dies rapidly after silencing *GMP* (Zou et al. 2006; Badejo et al. 2008; Lin et al. 2021). The *GMP* gene promoter of acerola (*Malpighia glabra*) had higher activity than the cauliflower mosaic virus *35S* and *Arabidopsis GMP* promoters (Badejo et al. 2008). GMP gene family members also had tissue expression specificity; for example, *SlGMP3* and *AeGMP2* were confirmed to be involved in AsA synthesis in leaves of tomato (Zhang et al. 2013) and kiwifruit (Liao et al. 2021a), respectively.

GME (EC 5.1.3.18, GDP-D-mannose-3′,5′-epimerase) not only catalyzes GDP-mannose to GDP-L-galactose but also to GDP-L-gulose, considered key evidence for the existence of the L-gulose pathway. In kiwifruit (Bulley et al. 2009) and blueberry (Liu et al. 2015), there was a significant correlation between *GME* expression level and AsA content during fruit development. A quantitative trait locus (QTL) study of tomato found that *GME* and AsA contents were closely related (Zou et al. 2006; Stevens et al. 2007). After inhibition of the *GME* genes, AsA content of the plant was significantly reduced, reactive oxygen species accumulated, and leaves were bleached (Gilbert et al. 2009). Conversely, overexpression of the *GME* gene can significantly increase AsA content and enhance plant resistance to stress (Imai et al. 2012; Ma et al. 2014). However, some studies have suggested that *GME* does not limit AsA content. For example, there was no significant correlation between the expression of the *GME* gene and AsA in tomato (Ioannidi et al. 2009), and overexpression of *GME* of kiwifruit in *Arabidopsis* did not significantly change the AsA level (Bulley et al. 2009).

GGP (EC 2.7.7.69, GDP-L-galactose phosphorylase) is considered to be the core gene that regulates AsA (Alegre et al. 2020; Anisimova et al. 2021). The expression of *GGP* during fruit development in kiwifruit with differing AsA levels was consistent with the changes in the accumulation rate of AsA (Bulley et al. 2009). Recent research on high AsA (*A. eriantha*) and low AsA (*Actinidia rufa*) of kiwifruit found that *AceGGP3* was highly expressed and positively correlated with high AsA content in the fruit. Furthermore, *GGP3*

expression also was correlated to AsA concentration in the *A. eriantha* × *A. rufa* hybrid, and the expression of the *AceGGP3* allele derived from *A. eriantha* was significantly higher than that of the *A. rufa*–derived allele *AcrGGP3* (Liu et al. 2022a). When the *GGP* gene of kiwifruit was overexpressed in tobacco, the AsA content in tobacco leaves increased 3-fold, and the AsA content in *Arabidopsis* was increased by approximately 4-fold. If *GGP* and *GME* of kiwifruit were co-transformed into *Arabidopsis*, the AsA content can be increased by approximately 7-fold (Bulley et al. 2009). In addition, the expression of *GGP* was also regulated by light (Dowdle et al. 2007) and an upstream open reading frame (Laing et al. 2015; Zhang et al. 2018) to regulate AsA metabolism. Using research on kiwifruit variation in the *GGP1* promoter region appears to be key to differences in *GGP* expression and AsA content in *A. eriantha* and *A. rufa* (Wei et al. 2021).

GPP (L-galactose-1-phosphate) was first isolated from kiwifruit, and GPP protein exists as a dimer in kiwifruit with a high AsA content (Laing et al. 2004a) but exists in monomer form in apple with low content (Guo et al., 2011). However, when the *GPP* gene was suppressed, the AsA content and GPP activity could be detected, indicating that there were other AsA synthesis pathways or other enzymes with GPP-like catalytic activity (Lorence et al. 2004; Conklin et al. 2006; Zhang et al. 2008). In the studies of tomato (Ioannidi et al. 2009), apple leaves (Li et al. 2009), and AsA overaccumulation mutant lines of *Arabidopsis* (Matteo et al. 2003), *GPP* was the only gene whose expression was consistent with changes in AsA content. Therefore, the transcriptional regulation of *GPP* plays an important role in regulating the synthesis and accumulation of AsA (Li et al. 2013a).

The D-galacturonic acid pathway

The D-galacturonic acid pathway is regarded as the main biosynthetic pathway in chestnut rose (An 2004; Huang 2013), sweet orange (Xu et al. 2013), and grape (Cruz-Rus et al. 2010) and is also used as a secondary biosynthetic pathway in kiwifruit (Li et al. 2011). Moreover, this pathway is considered to play a greater role in some tissues at certain stages of plant development. For example, AsA in chestnut rose leaves is mainly synthesized through the D-galacturonic acid pathway (An 2004).

GalUR (EC 1.1.1.19, D-galacturonic acid reductase) was first cloned from strawberry, and overexpression of strawberry *GalUR* caused a 10-fold to 50-fold increase in *GalUR* activity and a 2-fold to 3-fold increase in AsA content (Agius et al. 2003). The expression of *GalUR* in different tissues and development of kiwifruit has shown that *GalUR* was highly correlated with AsA content. However, the homology of GalUR among different kiwifruit species was low, of approximately 50% to 60% (Li et al. 2011; Wu 2015). Although *GalUR* gene expression was unrelated to AsA content in chestnut rose, *GalUR* gene expression was significantly upregulated during the rapid increases in AsA, and the L-galactose pathway was not active at this time (Huang 2013). Studies have

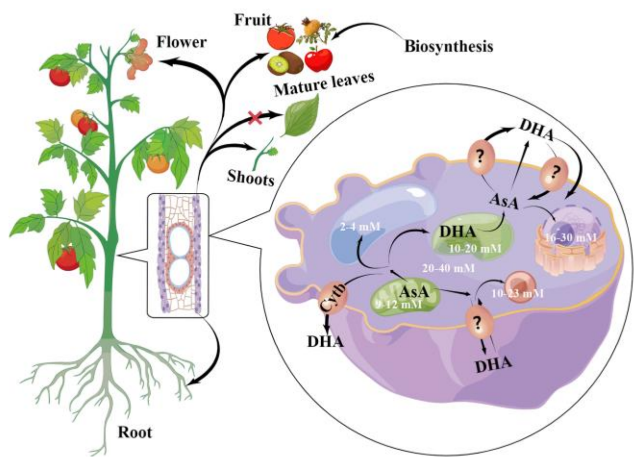

Figure 2. Long-distance transport and metabolism of AsA in plant organelles. AsA was accumulated into phloem and transported to root tips, shoots, and floral organs, but generally not to mature leaves. At the cytological level, AsA is synthesized in mitochondria and then enters the cytoplasm for transport to various organelles. In addition, AsA and DHA can also be transported outside the cell membrane by simple diffusion or transport proteins, such as the Cytb. After AsA functions in the chloroplast, DHA is produced and transported into the cytoplasm. Elements were modified from FigDraw (https://www.figdraw.com/static/index.html).

also reported that iron deficiency stress can induce the expression of the *GalUR* gene in apple leaves (Tian 2007). However, it was shown that *GalUR* on kiwifruit and apples does not participate in the biosynthesis of AsA in leaves (Li 2009). The functional characterization of *GalUR*-encoded protein has not yet been well characterized, and its selectivity and specificity for D-galacturonic acid has not been determined.

The inositol pathway and L-gulose pathway

Research on the inositol pathway and the L-gulose pathway has mainly occurred in model plants, and the understanding of these pathways in fruit crops is still relatively limited. So far, the gene for the L-gulose pathway in plants has not been identified. Although key genes on the inositol pathway were identified on the chestnut rose, there was no significant correlation between their expression and AsA (Huang 2013).

MIOX (EC 1.13.99.1, Myo-inositol oxygenase) was involved in not only AsA synthesis but also in cell wall formation (Arner et al. 2002). Studies have reported that AsA content increases when *MIOX* was overexpressed (Lorence et al. 2004; Endres and Tenhaken 2009), but other studies show the opposite results (Kanter et al. 2005; Siddique et al. 2013). Findings in chestnut rose were consistent with the latter, suggesting no significant correlation between *MIOX* and AsA content (Huang 2013). In *A. eriantha*, *MIOX* was found to be involved in the accumulation of AsA during fruit development and was also closely related to *A. eriantha* fruit ripening (Liao et al. 2021a). MIOX has also been found to play an important role in other metabolism (Siddique et al.

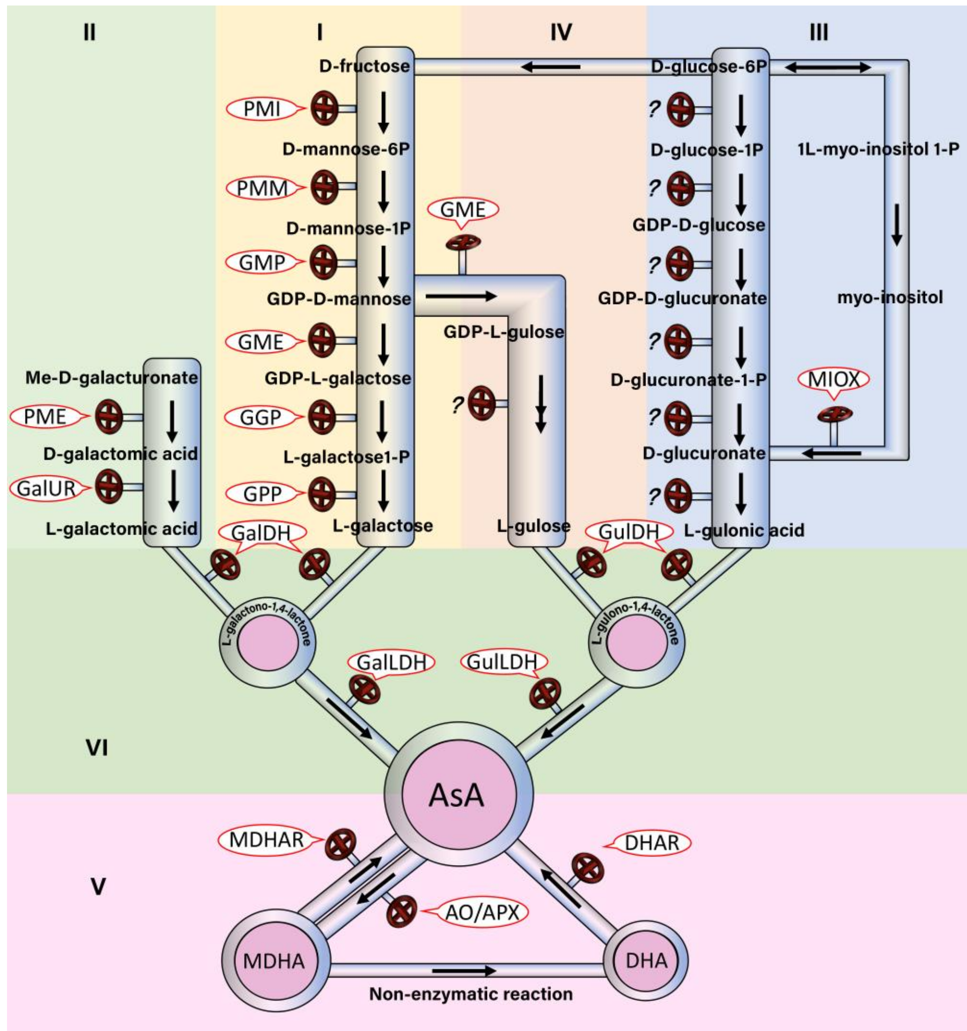

Figure 3. Proposed pathways for AsA metabolism. L-galactose pathway (I), D-galacturonic acid pathway (II), inositol pathway (III), L-gulose pathway (IV), degradation and cyclic regeneration pathway (V) of AsA metabolism, common in the synthetic pathway (VI). PME: Methylesterase; PMI: Mannose-6-phosphate isomerase; PMM: Phosphomannose mutase; GulDH: L-gulose-1,4-lactyl dehydrogenase; GR: Glutathione reductase.

2009; Eckardt 2010; Pieslinger et al. 2010), suggesting the inositol pathway in plants is secondary to AsA synthesis. In addition, SlIMP3 demonstrated high affinity with the L-galactose 1-phosphat and D-myoinositol 3-phosphate and acted as a bifunctional enzyme in the biosynthesis of AsA and myoinositol. Overexpression of *SlIMP3* not only improved AsA and myoinositol content but also increased cell wall thickness, improved fruit firmness, delayed fruit softening, decreased water loss, and extended shelf-life (Zheng et al. 2022a).

Common genes that function in several synthetic pathways

Among the many enzymes involved in AsA synthesis, 2 enzymes are shared by the 4 AsA biosynthetic pathways, namely GalDH and GalLDH. These 2 enzymes are also considered to be necessary for AsA biosynthesis and have been studied in several fruit crops.

Among all AsA synthesis–related enzymes, GalDH (EC 1.1.1.117, L-galactose dehydrogenase) is the only one that participates in AsA synthesis but not in any other biochemical reactions. Although the *GalDH* gene has been cloned from fruit crops such as kiwifruit (Laing et al. 2004b) and apple (Xiao et al. 2007), the relationship between GalDH activity and AsA accumulation has not been clearly reported. Overexpression of *GalDH* can increase the activity of GalDH but not AsA content (Gatzek et al. 2002), perhaps due to the feedback inhibition of GalDH enzyme by high AsA. This feedback mechanism has been confirmed in spinach, where 1 mM AsA can reduce GalDH enzyme activity by 41% (Pallanca and Smirnoff 2000; Mieda et al. 2004). In addition, due to the very high conversion efficiency of GalDH enzyme toward L-galactose, exogenous L-galactose can be converted into AsA (Smirnoff and Wheeler 2000), resulting in a low L-galactose content.

GalLDH (EC 1.1.1.117, L-galactose-1,4-lactone dehydrogenase) is the last enzyme in the synthesis of AsA. The *GalLDH*

gene has been identified in many plants, such as kiwifruit (Wu 2015) and chestnut rose (An et al. 2005a). GalLDH was highly specific for L-galactose-1,4-lactone, and the enzymatic activity was inhibited by high concentrations of L-galactose-1,4-lactone. A large number of studies have confirmed that the *GalLDH* gene and AsA content were highly correlated (Pateraki et al. 2004; Liao et al. 2021a). Antisense suppression of *GalLDH* mRNA led to a significant decline in the GalLDH activity (Tabata et al. 2001). However, attempts to increase AsA via GalLDH failed, mainly because this protein was located in the inner mitochondria membrane (Alhagdow et al. 2007). It has been reported that this gene may be induced by light to regulate AsA content (Smirnoff and Wheeler 2000; Dowdle et al. 2007; Liao et al. 2019), perhaps via light induction of expression (Tamaoki et al. 2003) or photorespiration-dependent changes directly affecting GalLDH enzyme activity (Millar et al. 2003; Bartoli et al. 2006).

Degradation and cyclic regeneration pathway

In plants, the degradation and cyclic regeneration pathways are coupled together to maintain the balance of AsA. Due to AsA's role in removing reactive oxygen species, these pathways are integral to plant resistance to biotic and abiotic stresses (Chen et al. 2003; Singh et al. 2014). Plants mainly degrade AsA through AO (EC 1.10.3.3, ascorbate oxidase) and APX (EC:1.11.1.11, ascorbate peroxidase) and regenerate AsA through MDHAR (EC 1.6.5.4, monodehydroascorbate reductase) and DHAR (EC 1.8.5.1, dehydroascorbate reductase).

AO and APX both would use AsA as an electron donor, and it reduces H_2O_2 to H_2O. The inhibition of *AO* gene expression can increase the AsA content by 40% and improve the salt tolerance of the plant (Sanmartin et al. 2003; Yamamoto et al. 2005; Zhang et al. 2011a). Silencing of *AO* results in inhibition growth and altered AsA levels and ripening patterns in melon fruit (Chatzopoulou et al. 2020). Unlike AO, many studies have shown that the activity of APX enzyme has a strong correlation with AsA content (Singh et al. 2014; Chiang et al. 2015). It appears that fruit crops have more members of the APX gene family than herbaceous crops. Research has focused on the ability of APX to improve resistance to external stress (Wang et al. 2005) and not the role of *APX* gene in regulating AsA content. The *APX* gene that plays a key role in fruit development has been identified in *A. eriantha* (Liao et al. 2020), which also responds to light stress.

In plants, MDHAR is widely distributed and found in chloroplast, cytoplasm, mitochondria, and peroxisome (Lunde et al. 2006; Li et al. 2010). Studies on persimmon show that *MDHAR* was closely correlated to the metabolism of AsA content in the leaves and fruit (Pu 2008). Overexpression of the *MDHAR* gene not only increased the AsA content but also enhanced the resistance of transgenic lines to ozone, salt damage, and drought stress (Eltayeb et al. 2007; Stevens et al. 2008). However, overexpressed *MDHAR* gene from fruit crops showed that the AsA content of transgenic plants was significantly downregulated (Haroldsen et al. 2011; Gest et al. 2013). The results with the latter were also obtained in kiwifruit (*A. eriantha*); this study speculated that increased MDHAR enzyme activity promotes APX enzyme activity, which leads to the decrease of AsA content (He 2022).

DHAR combines with GSH to catalyze DHA and reduce it into AsA. *DHAR* genes have been cloned from spinach, chestnut rose, kiwifruit, and other fruit crops (Chen et al. 2003; Niu et al. 2007) and have shown that *DHAR* had a significant positive regulatory effect on AsA content. However, *DHAR* was not been considered as the key gene for the accumulation of high AsA levels in chestnut rose (An et al. 2005b). Overexpression of *DHAR* in model plants has shown that the AsA content increased by 2 to 4 times, and the content of glutathione also significantly increased (Chen et al. 2003; Chen and Gallie 2005). In contrast, inhibition of *DHAR* gene expression led to inhibition of plant growth (Chen and Gallie 2006), and resistance to O_3 decreased (Chen and Gallie 2005), resulting in obvious photoinhibition (Chen and Gallie 2008).

Transcriptional regulators of AsA levels and response to environmental factors

Although many studies on the functional genes related to AsA metabolism have been reported, the abundant variation in AsA content among different species in plants suggests there was complex interaction between genes and transcription factors (TFs). There were several reports on the regulation of AsA content by TFs in fruit crops (Zheng et al. 2022a) (Fig. 4). For example, MdERF98, an apple ethylene response factor, can directly bind to the promoter of *MdGMP1* to activate transcription (Zhang et al. 2012; Ma et al. 2022). Studies in pears have shown that PbrMYB5 can bind to the promoter of *PbrDHAR2* to regulate AsA content as well as affect the cold tolerance of plants (Xing et al. 2018). In cabbage, BcERF70 acts on the DRE (dehydration responsive element) motif in 7 target gene promoters to regulate AsA content (Yuan et al. 2020). In addition, a recent study showed that AcERF91 (Chen et al. 2021), AceMYBS1 (Liu et al. 2022b), and AcMYBR (Liu et al. 2021) could affect AsA content in kiwifruit fruit by regulating the expression of *AcGGP3*.

The biosynthesis of AsA is extremely sensitive to light. Studies have shown that AsA content significantly decreases after bagging treatments in many fruits, such as kiwifruit (Liao et al. 2019), pear (Xing et al. 2018), and apple (Ma et al. 2022). The transcript abundance of genes encoding enzymes involved in AsA biosynthesis shows diurnal fluctuations influenced by light. This presumably reflects a need for antioxidants to detoxify reactive oxygen species produced during photosynthesis. Studies in apples show that the F-box protein MdAMR1L1 interacts with *MdGMP1* and promotes its degradation through the ubiquitination pathway, thereby inhibiting AsA synthesis (Ma et al. 2022). Light negatively

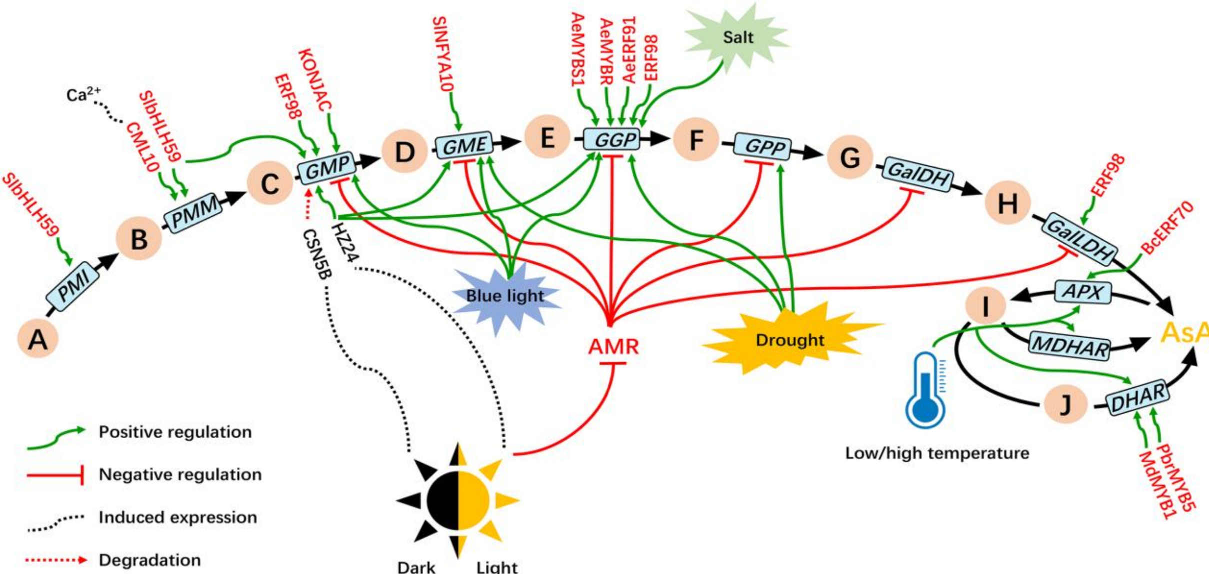

Figure 4. Reported TFs and environmental regulatory networks for AsA accumulation. Green arrows indicate promotion of expression or AsA accumulation, red arrows indicate inhibition of expression or AsA accumulation. A) D-fructose-6P, (B) D-mannose-6P, (C) D-mannose-1P, (D) GDP-D-mannose, (E) GDP-L-galactose, (F) L-galactose1-P, (G) L-galactose, (H) L-galactono-1,4-lactone, (I) monodehydroascorbate, (J) dehydroascorbate. AMR: AsA mannose pathway regulator.

regulates *AMR* gene expression, which then regulates expression of other key genes, affecting AsA levels (Zhang et al. 2009). CSN5B, part of the COP9 signalosome complex, promotes *GMP* expression (Wang et al. 2013). Similar studies have reported that *AtAMR1* can negatively regulate the genes in the L-galactose pathway, including *GMP*, *GME*, *GGP*, *GPP*, *GalDH*, and *GalLDH* (Zhang et al. 2009) (Fig. 4). At the same time, the *GGP* gene is induced not only by blue light but also by drought and salt stress (Wang et al. 2022). Also light can directly affect the expression of other AsA metabolism-related genes, including *GalLDH*, *MDHAR* (Liao et al. 2019), and *PMI* (Majed and Karim 2017).

The regulation of AsA content by transcriptional activation or repression is also affected by variations in the promoter regions of functional genes. One study of different kiwifruit species showed that there was a 183-bp deletion in the GGP promoter of *A. eriantha*, resulting in different *GGP* expression and AsA content in *A. eriantha* (high AsA content) and *A. rufa* (low AsA content) (Wei et al. 2021). Sequence analysis of the deleted fragment found that there were some negatively regulated cis-acting elements in the *GGP* promoter of *A. rufa*, which reduced the transcription level of *GGP* (Li et al. 2014). Cis-acting elements such as G-Box and ABRE motifs in the promoter of the *GGP* gene of kiwifruit can regulate *GGP* expression under different light conditions (Li et al. 2013a, 2013b).

Challenges and promising ways to enhance AsA content in fruit crops

Enhancement of AsA content in fruit crops attracts considerable attention, not only to strengthen its nutritional value but also to improve stress tolerance. At present, studies on AsA in fruit crops have turned to elaborate regulatory networks, and functional characterization of the key structural genes and TFs has been ongoing. The next primary focus is to use these genes and TFs for genetic improvement in crops. In molecular breeding, genetic engineering is one of the preferred technologies for scientists and breeders. Among the studies of improving the AsA content by using genetic engineering technology, *GGP*, *GME*, *GMP*, and *DHAR* have a large number of research reports, which can respectively increase the AsA content of tomato fruit by 6.2 times, 1.6 times, 1.5 times, and 1.6 times, respectively (Bulley et al. 2011; Haroldsen et al. 2011; Zhang et al. 2011b; Cronje et al. 2012). However, overexpression of these genes was also accompanied by some morphological fruit alterations, such as seedlessness. It is highly likely that the dynamic balance of reactive oxygen species in plants was disrupted. Using multiple expression vectors to simultaneously overexpress the genes related to AsA synthesis, degradation and regeneration will be an alternative strategy to enhance the AsA content in fruit crops. The disadvantage of this method is the need for the acquisition of numerous transgenic lines to seek the best overexpression combination. In addition, the key regulatory TFs that regulate AsA through population genetic mapping can also be used to construct multiple expression vectors. It must be mentioned that editing the uORF of key genes of AsA provides a generalizable, efficient method for manipulating translation of mRNA that could be applied to enhance crop vitamin C, especially for *GGP* (Laing et al. 2015; Li et al. 2018; Zhang et al. 2018).

Using physical or chemical methods to induce the accumulation of AsA content is another simple strategy in fruit

crops. This needs to be based on the studies of effects of treatments on key genes related to AsA metabolism. At present, we know that AsA content would be affected by some abiotic factors, including light, abscisic acid (ABA), and methyl jasmonate (Liao et al. 2019; Liu et al. 2022a). This knowledge will be useful for the applications of AsA enhancements in fruit crops.

Conclusions

The content of AsA in plants is affected by the biosynthesis, degradation, regeneration, and transport of AsA. There has been intensive study of these pathways with the key genes in the L-galactose pathway being identified, with a large number of studies confirming the function of various key genes. The genes related to AsA degradation and regeneration are less studied. The degradation and regeneration of AsA play important roles in both biotic and abiotic stresses. Genes that encode proteins that transport AsA or transcriptionally regulate AsA metabolism have recently been identified. The study of the regulation of AsA levels is becoming clear, especially in response to changes in the environment.

Competing interests

The authors declare no conflict of interest.

Author contributions

GLL: Writing original draft. QX and ACA: Reviewing and editing. XBX: Supervision, conceptualization. All the authors read and approved the final manuscript.

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Data availability

The datasets supporting the conclusions of this article are included.

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