Vitamin C in fleshy fruits: biosynthesis, recycling, genes, and enzymes

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L-ascorbic acid (vitamin C) is a plant secondary metabolite that has a variety of functions both in plant tissues and in the human body. Plants are the main source of vitamin C in human nutrition, especially citrus, rose hip, tomato, strawberry, pepper, papaya, kiwi, and currant fruits. However, in spite of the biological significance of L-ascorbic acid, the pathways of its biosynthesis in plants were fully understood only in 2007 by the example of a model plant Arabidopsis thaliana. In the present review, the main biosynthetic pathways of vitamin C are described: the L-galactose pathway, L-gulose pathway, galacturonic and myo-inositol pathway. To date, the best studied is the L-galactose pathway (Smyrnoff–Wheeler pathway). Only for this pathway all the enzymes and the entire cascade of reactions have been described. For other pathways, only hypothetical metabolites are proposed and not all the catalyzing enzymes have been identified. The key genes participating in ascorbic acid biosynthesis and accumulation in fleshy fruits are highlighted. Among them are L-galactose pathway proteins (GDP-mannose phosphorylase (GMP, VTC1), GDP-D-mannose epimerase (GME), GDP-L-galactose phosphorylase (GGP, VTC2/VTC5), L-galactose-1-phosphate phosphatase (GPP/VTC4), L-galactose-1-dehydrogenase (GalDH), and L-galactono-1,4-lactone dehydrogenase (GalLDH)); D-galacturonic pathway enzymes (NADPH-dependent D-galacturonate reductase (GalUR)); and proteins, controlling the recycling of ascorbic acid (dehydroascorbate reductase (DHAR1) and monodehydroascorbate reductase (MDHAR)). Until now, there is no clear and unequivocal evidence for the existence of one predominant pathway of vitamin C biosynthesis in fleshy fruits. For example, the L-galactose pathway is predominant in peach and kiwi fruits, whereas the D-galacturonic pathway seems to be the most essential in grape and strawberry fruits. However, in some plants, such as citrus and tomato fruits, there is a switch between different pathways during ripening. It is noted that the final ascorbic acid content in fruits depends not only on biosynthesis but also on the rate of its oxidation and recirculation.

Key words: L-ascorbic acid; vitamin C; fruits; metabolism; the key genes of ascorbic acid biosynthesis.


Накопление витамина С в сочных плодах: биосинтез и рециркуляция, гены и ферменты

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L-аскорбиновая кислота (витамин C) – вторичный метаболит растений, выполняющий множество разнообразных функций как в растительных тканях, так и в организме человека. Основным источником витамина C в питании человека служат растения, и прежде всего плоды цитрусовых, шиповника, перца, смородины, томата, клубники, папайи, киви. Однако, несмотря на то что L-аскорбиновая кислота – важное биологически активное вещество, путь ее биосинтеза в растительной клетке был описан лишь в 2007 г. на примере модельного растения Arabidopsis thaliana. В настоящем обзоре рассмотрены известные на сегодняшний день пути биосинтеза L-аскорбиновой кислоты в тканях растений. Это L-галактовый, L-гулозный, галактурановый и мио-инозитоловый пути. Наиболее изучен из них L-галактовый путь (путь Смирнова–Уилера), для которого определены все ферменты, катализирующие последовательную цепь реакций. Для других путей известна лишь предположительная последовательность метаболитов, при этом многие ферменты, катализирующие их превращение, еще не выявлены. Выделены ключевые гены, которые участвуют в биосинтезе и накоплении аскорбиновой кислоты в сочных плодах. Среди них ферменты L-галактового пути (ГДФ-маннозоfosфорилаза (GMP, VTC1), ГДФ-D-маннозо-3’5’-эпимераза (GME), ГДФ-L-галактофосфорилаза (GGP, VTC2/VTC5), L-галактофосфатдегидрогеназа (GalDH) и L-галактоно-L,4-лактондегидрогеназа (GalLDH)); ферменты D-галактуронового пути (NADPH-зависимая D-галактуронатредуктаза (DHAR1) и monodehydroascorbate reductase (MDHAR)).

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**Introduction**

L-Ascorbic acid (vitamin C) is a secondary plant metabolite involved in manifold functions in the cell (Davey et al., 2000; Iqbal et al., 2009; Smirnoff, 2018). L-Ascorbic acid (L-AsA) acts as an expression regulator for many genes, influences plant growth and development via phytohormones, and, which is no less important, is involved in the plant cell response to the impact of biotic and abiotic stress factors (Pastori et al., 2003; Gest et al., 2013; Li et al., 2013). Some plant species utilize ascorbates as a substrate for biosynthesis of other important metabolites, for example, oxalates and tartrates (Loewus F.A., Loewus M.W., 1987; Loewus F.A., 1999; Cruz-Rus et al., 2010).

Vitamin C is especially valuable in human diet since humans and other higher primates had lost the ability to produce L-AsA because of a mutation in one of the enzymes involved in its biosynthesis (Nishikimi, Yagi, 1996). Vitamin C plays an important role in the normal functioning of the body, being a coenzyme in several metabolic processes. It also possesses antioxidant properties and eliminates free radicals, which contribute to carcinogenesis and body aging (Figueroa-Méndez, Rivas-Arancibia, 2015). In addition, vitamin C improves human immunity by activating phagocytes, prevents the cardiovascular diseases associated with atherosclerosis, and enhances collagen formation and development of the cartilage tissue ( Diplock et al., 1998). The main source of this vitamin in human diet is plants; fruits of citruses, sweet-brier, actinidia (kiwi), sand thorn, papaya, strawberry, mountain ash, sweet pepper, and tomato display the highest content of vitamin C (Iqbal et al., 2009; Streltsova et al., 2010).

**Biosynthesis of ascorbic acid in plant cell**

Strange it may seem but the biosynthesis of L-AsA in plant cell was finally described in a model plant, Arabidopsis thaliana, only as late as 2007 (Linster et al., 2007) despite its evident importance in the life of plants and human health. Unlike animals, which synthesize L-AsA from glucuronic acid, the plant cell has at least four alternative pathways of its biosynthesis, namely, L-galactose, L-gulose, galacturonic, and myo-inositol pathways (see the Figure) (Li et al., 2010; Yang et al., 2011).

**L-Galactose pathway**

The L-galactose pathway, or Smirnoff–Wheeler pathway (Wheeler et al., 1998), is currently regarded as the main pathway of L-AsA biosynthesis in plants. The initial substrate for this pathway is a glucose molecule. The Smirnoff–Wheeler pathway comprises 10 successive stages. Interestingly, the first eight stages transform D-glucose into L-galactose, which differs from D-glucose only by the spatial positions of hydrogen and hydroxyl groups at the fourth carbon atom (Linster et al., 2007).

GDP-D-mannose and GDP-L-galactose are important metabolites of this pathway. The enzyme GDP-D-mannose-3′,5′-epimerase controls their mutual transformation. It is important here that the main part of the products in this reaction is spent for the primary metabolic reactions, namely, the biosynthesis of cell wall polysaccharides (Roberts, 1971; Baydoun, Fry, 1988), which is maximally active in growing organs and tissues. This suggests that the initial stages of this metabolic pathway are mainly involved in the growth of organs (leaves, fruits, and so on). In the formed (mature) organs, the secondary metabolic reactions are switched on and provide further transformation of GDP-L-galactose to L-AsA. Therefore, the limiting stage in this metabolic pathway of vitamin C synthesis is the reaction that directly generates L-AsA and is controlled by the enzyme GDP-L-galactose phosphorylase, VTC2 (see the Figure). It is believed that VTC2 is of a key importance for production of vitamin C and that its activity depends on the need of a cell in synthesizing cell wall polysaccharides ( Bulley et al., 2012; Wang et al., 2014).

**L-Gulose pathway**

The case study of arabidopsis has shown that one of the enzymes of the above detailed pathway, GDP-D-mannose-3′,5′-epimerase (GME), putatively possesses 5′-isomerase activity and is able to catalyze GDP-D-mannose transformation to GDP-L-gulose (Wolucka, Van Montagu, 2003) along with the 3′,5′-isomerase activity, converting GDP-D-mannose to GDP-L-galactose. The next assumption was that GDP-L-gulose in the course of subsequent transformation into L-gulose-1-phosphate, L-gulose, and L-gulono-1,4-lactone could be also converted to L-AsA (see the Figure). However, the corresponding catalytic enzymes of the L-gulose pathway of vitamin C synthesis in plants have not been yet discovered except for L-gulono-1,4-lactone oxidase of arabidopsis (Maruta et al., 2010). Interestingly, the overexpression of rat L-gulono-1,4-lactone oxidase (ALO) elevated the L-ascorbic acid content in tobacco and lettuce (Jain, Nessler, 2000). An overexpression of rat ALO in the arabidopsis plants carrying a mutation in VTC gene (deficient in vitamin C) completely restored the L-AsA level (Radzio et al., 2003). This suggests that the L-gulose pathway may be regarded as one of the alternative pathways in L-AsA biosynthesis in plants.
**Myo-inositol pathway**

Myo-inositol is a carbohydrate metabolite synthesized by most cells and necessary for a normal plant growth and development. In a form of inositol phosphates and phosphatidylinositol lipids, myo-inositol is involved in intracellular signal transduction in various cascades (Michell, 2007).

The myo-inositol pathway of L-AsA biosynthesis in plants comprises four stages (see the Figure). Myo-inositol is oxidized by myo-inositol oxygenase to D-glucuronic acid, which is further converted by glucuronate reductase to L-gulonic acid with subsequent transformation to L-gulono-1,4-lactone, which is catalyzed by aldonolactonase. The last reaction in this line is the transformation of L-gulono-1,4-lactone to L-AsA, catalyzed by L-gulonolactone dehydrogenase (Lorence et al., 2004).

Myo-inositol oxygenase (MIOX) is the key enzyme in this pathway: it is shown by the case study of arabidopsis that an overexpression of MIOX doubles the vitamin C content in flowers and leaves (Lorence et al., 2004). The remaining enzymes involved in the subsequent reactions in plants are still not determined.

**D-Galacturonic pathway**

As was shown in the early 1960s, galacturonic acid methyl ester is convertible to L-AsA. The existence of this metabolic pathway was for the first time demonstrated in the case study of a protist, *Euglena gracilis* (Shigeoka et al., 1979). In plants, exogenous application of D-galacturonic acid methyl ester elevates the ascorbic acid content in different tissues and arabidopsis cell culture (Loewus, Kelly, 1961; Davey et al., 1999), thereby demonstrating the presence of this pathway of ascorbic acid biosynthesis.

Note that the initial substrates for the D-galacturonic pathway are the degradation products of cell wall polysaccharides.
AsA recycling (ascorbate–glutathione cycle)
The current data volume accumulated while studying the AsA metabolism in plant cells suggests that the AsA levels depend not only on its biosynthesis, but also on it oxidation and further recycling (Li et al., 2013). The L-AsA formed in one of the above described biosynthesis cycles acts in the plant cell as an antioxidant, protecting from oxidative stress (Akram et al., 2017). In these processes, oxidized forms (monodehydroascorbic and dehydroascorbic acids) are formed. During the recycling, the oxidized forms are again reduced to AsA with the help of two reductases – mono-dehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR). This is known as the ascorbate–glutathione cycle (see the Figure). The biological function of this cycle is determined by that the cell contains reactive oxygen species, on the one hand, and AsA has antioxidant properties, on the other.

The first stage in this pathway is detoxification of reactive oxygen species with the help of ascorbate peroxidase (APX) or ascorbate oxidase (AO). This gives monodehydroascorbic acid, which is either again reduced to AsA by MDHAR, or dehydroascorbic acid, which is reduced by DHAR.

Thus, the AsA final content in plant organs and tissues depends on both its biosynthesis and recycling.

Specific features of AsA biosynthesis and accumulation in fruits
L-AsA is involved in manifold functions in the life of a plant organism. The AsA biosynthesis is triggered in response to various endogenous and exogenous impacts and takes place in almost all plant tissues and organs. Therefore, the presence of at least four pathways for AsA synthesis is not surprising as well as switching between these pathways depending on the particular demands and conditions in the cell. However, our interest is naturally focused on how vitamin C is accumulated in edible parts of the plants; correspondingly, it is necessary to clearly understand which metabolic pathway of AsA biosynthesis is major in the fruits of different plant species. Although this issue is of considerable importance, the studies on the specific features of the vitamin C accumulation in plant fruits are few. Currently, the pathways of AsA biosynthesis and accumulation in fruits have been examined in sufficient detail in some cultivated plants, such as the strawberry, tomato, grape, kiwi, apple, pear, sweet cherry, and citrus (Agius et al., 2003; Hancock et al., 2007; Bulley et al., 2009; Cruz-Rus et al., 2010; Di Matteo et al., 2010; Li et al., 2010; Walker et al., 2010; Badejo et al., 2012; Alós et al., 2014).

As a rule, AsA is gradually accumulated with an increase in the mass of the developing fruit with the highest rate on days 75–100 after anthesis. This pattern of AsA accumulation has been observed, for example, for the tomato (Ioannidi et al., 2009). However, AsA more rapidly accumulates at early stages of fruit development in kiwi (Bulley et al., 2009) and black currant (Hancock et al., 2007) fruits, when the cell biosynthetic activity is maximal (Li et al., 2011).

A significant role of the major (L-galactose) pathway has been shown for several species. In particular, it has been shown that the content of vitamin C in the black currant (Ribes nigrum) fruits considerably varies depending on both climatic conditions and genotype; note that the correlation of expression with vitamin C accumulation is shown for only one gene, the gene coding for GDP-D-mannose-3′5′-epimerase (Walker et al., 2010).

The maximum content of vitamin C in kiwi (Actinidia delicosa variety Qinmei) fruits is observable on day 30 after anthesis with a gradual decrease by day 60. Expression patterns are similar for most of the studied key genes except for the genes encoding L-galactono-1,4-lactone dehydrogenase (GalLDH) and L-galactose-1-phosphate phosphatase (GPP/VTC4). Note that only GPP expression correlates with AsA accumulation (Li et al., 2010).

An important role of the enzymes involved in the D-galacturonic pathway has been also demonstrated for the content of vitamin C in tomato (Solanum lycopersicum) fruits, typically reaching 35 mg per 100 g. Moreover, an increase in AsA with tomato (cultivar Micro-Tom) fruit ripening is inversely correlated with the expression of the genes involved in the major (L-galactose) pathway (Badejo et al., 2012). The L-galactose and D-galactouronate treatment of tomato plants increased the vitamin C content in the mature fruits; however, this result was unachievable by treating the plants with L-gulono-1,4-lactone, formed in the L-gulose and myo-inositol pathways (see the Figure) (Badejo et al., 2012). This suggests that the AsA synthesis during tomato fruit ripening may start with the Smirnoff–Wheeler pathway to further switch to the D-galacturonic pathway, the enzymes of which work at the late stage of fruit ripening. D-Galacturonic acid is produced via the cleavage of cell wall pectin; this suggests that the pathway in question is activated during the maceration of tomato fruits (Badejo et al., 2012). As has been earlier demonstrated, expression of the genes coding for pectinesterases and polygalacturonases (the enzymes involved in pectin degradation) is very high in an introgression line, IL 12-4 (S. pennellii and S. lycopersicum), differing from the parental line by a considerably higher vitamin C content. This is another evidence for that the D-galacturonic pathway is directly involved in the accumulation of vitamin C in tomato fruits (Di Matteo et al., 2010).

AsA is a most valuable component in the grapes (Vitis vinifera), acting as the substrate for synthesis of tartaric acid (Cholet et al., 2016). Similar to the strawberry and tomato fruits, the AsA content increases with grape ripening reaching
its maximum in fully mature fruits. Expression analysis of the genes controlling different AsA biosynthesis pathways in grape fruits demonstrates a strong correlation of the transcription of D-galactouronate reductase gene (GalUR) and the quantitative content of vitamin C (Cruz-Rus et al., 2010). Thus, the D-galacturonic pathway is also regarded as a major pathway for vitamin C biosynthesis during grape fruit development and ripening (Cruz-Rus et al., 2010).

It is known that the mature strawberries (Fragaria sp.) are rich in vitamin C, containing on the average 60 mg AsA per 100 g fresh tissue (Agius et al., 2003). As is shown, the D-galacturonic pathway is significant for the AsA accumulation in strawberry fruits. Expression of one of the enzymes of this pathway, NADPH-dependent D-galacturonate reductase (GalUR), in strawberry fruits increases proportionally to the accumulation of vitamin C there. Overexpression of the strawberry GalUR gene in arabidopsis leaves gives a twofold increase in vitamin C content there, suggesting an important role of this particular enzyme in AsA biosynthesis (Agius et al., 2003).

Note that the final vitamin C content in fruits depends not only on the rate of AsA biosynthesis, but also on its recycling (see the Figure). In particular, it has been shown that the expression of MDHAR gene (ascorbate–glutathione pathway) in strawberries positively correlates with the accumulation of vitamin C in developing fruits (Cruz-Rus et al., 2011).

The maximum AsA content in sweet cherry (Prunus avium cultivar Hongdeng) fruits is observed during their setting followed by a gradual decrease during fruit development and a moderate increase in the mature fruit (Liang et al., 2017). Nonetheless, AsA content continues to increase with the weight of fresh fruits. Full-sized cDNAs of 10 genes involved in the L-galactose pathway of AsA biosynthesis have been described as well as of 10 genes involved in AsA recycling. The expression levels of the genes encoding GDP-L-galactose phosphorylase (GGP2), L-galactono-1,4-lactone dehydrogenase (GalLDH), ascorbate peroxidase (AO2), glutathione reductase (GR1), and dehydroascorbate reductase (DHAR1) correlate with the quantitative content of vitamin C during fruit development, which suggests that the joint work of all these genes involved in AsA biosynthesis, degradation, and recycling together regulates the AsA accumulation in sweet cherry fruits (Liang et al., 2017).

The enzymatic activities involved in the AsA biosynthesis via the Smirnoff–Wheeler pathway and its recycling in different fruit tissues have been comprehensively studied in pear (Pyrus pyrifolia cultivar Aikansui) fruits (Huang et al., 2013). Biochemical analysis demonstrates that the AsA content increases with fruit development to reach the maximum 30 days after anthesis and then decreases and is maintained at the same level. The highest AsA concentration is observed in the pear peel, which is a result of a high L-galactose dehydrogenase and L-galactono-1,4-lactone dehydrogenase activities, on the one hand, and dehydroascorbate reductase and monodehydroascorbate reductase (involved in AsA recycling), on the other. An exogenous application of the precursors of AsA biosynthesis has shown that the peel displays a higher ability to synthesize the target substance via the Smirnoff–Wheeler and D-galacturonic pathways, whereas the pulp and core were less capable of synthesizing AsA (Huang et al., 2013).

According to a study of apple (Malus domestica) fruits, AsA can be synthesized via the L-galactose pathway (Li et al., 2008). Further studies of the dynamics of vitamin C accumulation during fruit (cultivar Gala) ripening have shown that the transcription levels of the genes coding for GDP-L-galactose phosphorylase, GDP-L-mannose pyrophosphorylase, D-galacturonate reductase, and L-galactono-1,4-lactone dehydrogenase, regulated on a posttranscriptional level, do not correlate with vitamin C accumulation. On the other hand, the expression patterns of L-galactose dehydrogenase, L-galactose-1-phosphate phosphatase, and GDP-D-mannose-3′S-epimerase are in general similar to the AsA accumulation pattern. Interestingly, the expression and activity of the genes of monodehydroascorbate reductase and dehydroascorbate reductase, which degrade AsA in fruits, do not correlate with the AsA accumulation during fruit development (Li et al., 2011).

In addition, the search for the quantitative trait loci (QTL) responsible for the vitamin C accumulation in the M. domestica fruits (Mellidou et al., 2012) has succeeded in finding a linkage group that comprises the genes of GDP-L-galactose phosphorylase (GGP) and dehydroascorbate reductase (DHAR). Of special interest are three GGP paralogous genes, all residing within the AsA-QTL cluster. The association between some allelic variants of the GGP gene and increased vitamin C content has been observed. Comparison of the GGP expression patterns in the specimens displaying high and low vitamin C contents suggests a key role of GGP in the accumulation of vitamin C. Molecular markers (SNPs) have been found; these markers are helpful when breeding new cultivars with an increased vitamin C content in apple fruits (Mellidou et al., 2012). In addition, a correlation between DHAR and a QTL associated with the resistance to flesh browning has been shown (Mellidou et al., 2012).

Citrus are known as an important source of vitamin C. Expression patterns of 13 genes involved in vitamin C metabolism (including its synthesis, degradation, and recycling) in two citrus fruits – orange (Citrus sinensis) and tangerine (C. unshiu) – have been studied. The L-galactose pathway is shown to be major for the synthesis of vitamin C. Note that AsA accumulation is maximum in the peel and pulp, correlating with the expression profiles of the genes involved in the L-galactose pathway, whereas the myo-inositol pathway is prevalent for the AsA synthesis in immature fruit peel. GGP and GPP are regarded as the key genes controlling the AsA synthesis in the pulp; as for the peel, the function of GMP and MIOX is also important (Alós et al., 2014). In addition, a relative expression of the MDHAR and DHAR genes (involved in recycling) correlates with the AsA accumulation during fruit ripening and the cultivars with an elevated AsA content displayed an upregulation of these genes (Alós et al., 2014).

Thus, it is currently impossible to unambiguously state which of the four known pathways of AsA biosynthesis is prevalent in plant fruits. In particular, the L-galactose pathway is prevalent in peach (Imai et al., 2009) and kiwi (Bulley et al., 2009) versus grapes (Cruz-Rus et al., 2010) and strawberries (Agius et al., 2003), where the L-galacturonic pathway is
likely to be prevalent. On the other hand, the prevalence of AsA pathways changes during fruit ripening in several plants, for example, citruses, or tomatoes (Bajero et al., 2012; Alós et al., 2013).

Key enzymes determining L-AsA biosynthesis and accumulation in berries, vegetables, and fruits

Enzymes of L-galactose pathway
GDP-mannose phosphorylase (GMP or VTC1), EC 2.7.7.22, displays a mannose-1-phosphate guanylyltransferase activity. The gene coding for this enzyme was for the first time detected in and cloned from a mutant arabidopsis plant with a decreased AsA content (Conklin et al., 1999). As has been later shown, an inhibition of GMP in the tomato decreases the AsA content in fruits (Keller et al., 1999).

The NCBI database contains VTC1 gene sequences of the arabidopsis (A. thaliana), tomato (S. lycopersicum), turnip (Brassica rapa), cabbage (B. oleracea), potato (Solanum tuberosum), papaya (Carica papaya), sweet cherry (P. avium), rice (Oryza sativa), tobacco (Nicotiana tabacum), jujube (Ziziphus jujuba), and pepper (Capsicum annuum). The gene length of approximately 2500 bp is rather conserved in its structure, comprising four exons and four introns.

GDP-D-mannose-3′,5′-epimerase (GME), EC 5.1.3.18, catalyzes the reversible epimerization of GDP-D-mannose, which is among the main reactions in the L-galactose pathway of AsA biosynthesis. In this reaction, a high-energy glycosyl-pyrophosphoryl linkage is hydrolyzed. GME is able to catalyze two different reactions with formation of either GDP-L-galactose or GDP-L-gulose from GDP-D-mannose. GDP-L-gulose is the initial substrate for the alternative gulose pathway of AsA biosynthesis. The other reaction product, GDP-L-galactose, can be further utilized not only for synthesizing vitamin C, but also in biosynthesis of the cell wall and glycoproteins, which is primarily necessary for the development of vegetative organs (Lukowitz et al., 2001; Gilbert et al., 2009; Mounet-Gilbert et al., 2016). As has been shown, this enzyme activity can also influence pollen development and seed formation (Mounet-Gilbert et al., 2016).

Currently, GME is regarded as one of the most important enzymes in the L-AsA biosynthesis in plants. The genes coding for this enzyme have been isolated and characterized in the arabidopsis (Wolucka et al., 2001; Wolucka, Van Montagu, 2003), rice (Watanabe et al., 2006), and tomato (Zhang C.J. et al., 2011; Zhang Y.Y. et al., 2011). Interestingly, the rice and tomato genomes carry two paralogous genes each (Watanabe et al., 2006; Zhang C.J. et al., 2011; Zhang Y.Y. et al., 2011). A positive correlation between expression of this gene and AsA content is observed in the apple (Li et al., 2010) and blueberry (Liu et al., 2015). However, this pattern is unobservable in the kiwi (Bulley et al., 2009), peach (Imai et al., 2009), and tomato (Ioannidi et al., 2009; Mellidou et al., 2012). Taking into account the need in maintenance of the metabolic balance between the syntheses of AsA and cell wall, competing for the common substrate (GDP-L-galactose), it is shown that an overexpression of the GME gene does not lead to an increase in the AsA content in arabidopsis (Yoshimura et al., 2014). On the other hand, a joint overexpression of GME and the gene coding for the enzyme catalyzing the limiting stage of the L-galactose pathway, GPP, results in a considerably increased AsA accumulation as compared with the overexpression of GPP alone (Bulley et al., 2009). Analogous data have been also obtained for kiwi fruits (Bulley et al., 2009). This demonstrates a higher importance of the GME and GPP genes and their joint control in the L-galactose pathway.

In plants, two paralogous genes are known, GME1 and GME2. These genes have a length of approximately 1500 bp, display a high homology, and consist of six exons and five introns. Among the horticultural crops, these genes are known in the grape vine (V. vinifera), tomato (S. lycopersicum), pineapple (Ananas comosus), melon (Cucumis melo), and mulberry (Morus notabilis).

GDP-L-galactose phosphorylase (GGP or VTC2/VTC5), EC 2.7.7.69, catalyzes the GDP-L-galactose phosphorylation to L-galactose-1-phosphate. An important role of the VTC2 gene was demonstrated in the arabidopsis (Dowdle et al., 2007). The recombinant VTC2 alleles of the wild-type arabidopsis and two mutants were expressed in Escherichia coli. The product of one mutant allele did not lead to vitamin C synthesis and the product of the other mutant allele displayed only 2 % of the total wild-type VTC2 activity. However, a comprehensive analysis of the plants has shown that the vtc2 mutants of arabidopsis still accumulated AsA at the level of 10–20 % of its content in wild-type plants. This suggested existence of certain other pathways of AsA biosynthesis (Dowdle et al., 2007; Laing et al., 2007; Linster et al., 2007, 2008).

In addition, the changes in AsA level in response to the VTC2 overexpression in arabidopsis, tomato, strawberry, potato, and rice have been experimentally studied (Bulley et al., 2012; Wang et al., 2014). The plants transformed with the constructs carrying VTC2 gene displayed a considerable increase in the AsA content (Bulley et al., 2012; Wang et al., 2014).

Certain data demonstrate that not only the coding sequence of VTC2 gene, but also a region in its promoter sequence influences the AsA accumulation in fruits. As has been shown, the AsA biosynthesis may be controlled via a posttranscriptional repression of GDP-L-galactose phosphorylase. This regulation involves an additional reading frame (uORF). Its “switch-off” leads to the synthesis of GDP-L-phosphorylase and, as a consequence, to an increase in the AsA concentration. This posttranslational AsA regulation is most likely a rather ancient control mechanism, since the uORF is retained in GDP genes from the mosses to angiosperms (Laing et al., 2015).

All this suggests that GGP/VTC2 in some plants is the key regulator of AsA biosynthesis; many biotechnological studies aimed at an increase in the vitamin C content have been performed utilizing this particular gene (Zhou et al., 2012).

So far, the NCBI database contains the sequence of several GGP paralogs from the arabidopsis (A. thaliana), turnip (B. rapa), field mustard (Brassica arvensis), cabbage (B. oleracea), maize (Zea mays), sunflower (Helianthus annuus), potato (S. tuberosum), and tobacco (N. attenuata). The lengths of these DNAs vary around 2740 bp and they comprise seven exons and six introns.

L-galactose-1-phosphate phosphatase (GPP/VTC4), EC 3.1.3.89, catalyzes dephosphorylation with formation of L-galactose. GPP is also regarded as an efficient enzyme for
regulation of AsA synthesis. This was first assumed for kiwi fruits (Laing et al., 2004; Bulley et al., 2009), apple (Mellidou et al., 2012), and tomato (Ioannidi et al., 2009). However, note that AsA is synthesized even in the case of GPP gene knockout although in a smaller quantity, which suggests that either there are other functional phosphatases involved in AsA biosynthesis or AsA is synthesized via other pathways (Conklin et al., 2006; Torabinejad et al., 2009).

It has been shown that GPP is a bifunctional enzyme able to catalyze the biosynthesis not only of AsA, but also of myo-inositol. Thus, GPP activity may represent the junction point of the L-galactose and myo-inositol pathways of AsA biosynthesis (Torabinejad et al., 2009).

Homologous GPP genes comprising 12 exons and 11 introns in the arabidopsis (length, 2413 bp) and nine exons and nine introns in turnip (length, 7196 bp) are known in the kingdom of plants.

L-galactose-1-dehydrogenase (GalDH), EC 1.1.1.316, and L-galactono-1,4-lactone dehydrogenase (GalLDH), EC 1.3.2.3. GalDH oxidizes L-galactose to L-galacto-1,4-lactone using NAD⁺ as electron acceptor. GalDH is the final enzyme of the L-galactose pathway and directly leads to AsA synthesis. So far, the GalLDH gene has been isolated from the arabidopsis, pea, kiwi, and apple (Gatzek et al., 2002; Laing et al., 2004; Mieda et al., 2004). As has been shown for pear fruits, a high AsA concentration in the peel is in part determined by high activities of GalDH and the next enzyme in the biosynthesis pathway, GalLDH (Huang et al., 2013). GalLDH has been characterized for several plant species, including the sweet potato (Imai et al., 1998), cauliflower (Oesterhelt et al., 1997), spinach (Mutsuda et al., 1995), tobacco (Yabuta et al., 2000), strawberry (do Nascimento et al., 2008), melon (Pateraki et al., 2004), tomato (Zhang et al., 2005), strawberry (Agius et al., 2003) as well as the key role of this enzyme in the AsA biosynthesis. Note that the reactions of the D-galacturonic pathway in plants are yet insufficiently studied and require further biochemical, physiological, and genetic studies. Homologs of the GalUR gene have been annotated in the genomes of the sunflower (H. annuus) and tobacco (N. attenuata); their lengths are 2671 and 12 453 bp, respectively; and they comprise four exons and three introns.

Enzymes involved in AsA recycling

Dehydroascorbate reductase (DHAR1) and monodehydroascorbate reductase (MDHAR). MDHAR enzyme activity is tightly correlated with the AsA accumulation in tomatoes at a decreased temperature, suggesting its significant role in the regulation of AsA synthesis under stress.

Identification of the MDHAR and DHAR genes and their further functional analysis have shown that an overexpression of the DHAR gene provides a 1.6-fold increase in the AsA content in the tomato fruits grown at a relatively low illumination. A study of expression levels of two MDHAR isoforms demonstrates that an increase in the transcription of this gene is negatively correlated with an increase in the AsA level during tomato fruit ripening (Li et al., 2013). However, it is assumed that MDHAR is an important determinant of the changes in AsA level under stress (Ioannidi et al., 2009). In particular, the MDHAR activity considerably elevates the AsA content in fruits in the case of cold (Li et al., 2013) and oxidative (Gest et al., 2013) stresses.

The role of MDHAR in the increase of AsA content has been unambiguously demonstrated for tomatoes by QTL mapping (Sauvage et al., 2014) and by expression and enzyme activity profiling during fruit ripening (Mellidou et al., 2012). Expression of this gene also correlates with the vitamin C accumulation in blueberries (Liu et al., 2015). The suppression of MDHAR in tomato fruits decreases the AsA content, thereby suggesting that the recycling control via an increase in MDHAR activity may be an efficient way for increasing the vitamin C content (Truffault et al., 2017). Moreover, it has been shown using siRNA that a decrease in this enzyme activity makes tomato plants unable to resist cold stress (El Airaj et al., 2013).

Expression of the DHAR gene correlates with the AsA accumulation in the chestnut rose (Huang et al., 2014) and blueberry (Liu et al., 2015) fruits.

Three paralogous genes – DHAR1, DHAR2, and DHAR3 – are known in plants. The paralogous genes display a low degree of homology. The DHAR1 gene has a length of approximately 6000 bp and comprises six exons and five introns. Among the horticultural crops, DHAR genes are known in the grape vine (V. vinifera), tomato (S. lycopersicum), sweet cherry (P. avium), pepper (C. annuum), and apple (M. domestica).

The MDHAR paralogs also display a low degree of homology. The number of exons in these genes varies from nine to 17. These genes have been found in the tomato (S. lycopersicum), arabisidopsis (A. thaliana), and pepper (C. annuum).

Thus, the activities of the enzymes involved in AsA recycling needs further studies, the more so since the enzymes of this pathway may be directly associated with the resistance
to cold and oxidative stress, which has an important applied value.

**Conclusion**

This paper briefly the main pathways of L-AsA biosynthesis and recycling in plant tissues. The key genes involved in the AsA biosynthesis and accumulation in fruits are considered. A huge volume of data on this issue demonstrates that the most significant role in the AsA biosynthesis, accumulation, and recycling is played by a synergistic interaction of all these components. Most likely, the insight into these interactions will form the background for the research into the vitamin C metabolism in plants during the next decade.

**References**


Vitamin C in fleshy fruits: biosynthesis, recycling, genes, and enzymes


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