African Journal of Biotechnology Vol. 12(16), pp. 1952-1961, 17 April, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4048
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Distribution and metabolism of ascorbic acid in pear fruits (*Pyrus pyrifolia* Nakai cv. Aikansui)

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Accepted 15 June, 2012

Ascorbate accumulation levels, distribution and key enzyme activities involved in synthesizing via Smirnoff-Wheeler pathway and recycling in different pear fruit tissues during development were studied. Results show that the ascorbate contents increased with the fruit development, and reached the highest titers in 30 days after anthesis (DAA), then decreased and maintained a level. The higher contents of ascorbate in the peel of pear fruit were observed, which results from a combination of higher activities of L-galactose dehydrogenase (GalDH) and L-galactono-1,4-lactone (GalLDH) involving ascorbate biosynthesis and higher dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) activities used to recycle ascorbate. Exogenous feeding of ascorbate synthesis precursors demonstrated that the peel had stronger capability of *de novo* ascorbate biosynthesis *via* Smirnoff-Wheeler pathway and uronic acid pathway whereas the flesh and core had lower capability for ascorbate synthesis. These results suggest that the pear fruit is able to cause *de novo* ascorbate biosynthesis and the peel had higher capability for ascorbate biosynthesis than the flesh and core.

Key words: *Pyrus pyrifolia*, ascorbate, biosynthesis.

INTRODUCTION

L-Ascorbic acid (AsA), also called vitamin C (Vc) or ascorbate (ASC), is a vital antioxidant compound that plays a critical role in the cellular metabolism of plants and animals (Banhegyi et al., 1997; Noctor and Foyer, 1998). Plants and most animals can synthesize AsA, however a few mammalian species including primates, humans and guinea pigs have lost this capability (Nishikimi et al., 1994), for lacking L-gulono-1,4-lactone oxidase, an enzyme essential for its synthesis.

Consequently, humans are unable to synthesize AsA in their bodies and deficiency state occurs with a wide spectrum of clinical manifestations. Scurvy is one of the clinical expressions of ascorbate deficiency, which is a lethal condition unless appropriately treated (Diplock et al., 1998). Previous studies have also showed that AsA is involved in preventing humans from various oxidative stress-related diseases such as cancers and cardiovascular and aging (Davey et al., 2000). Thus, in order to survive humans must ingest vitamin C, mainly from fresh fruits, vegetables and other crop produce (Li and Schellhorn, 2007).

No plant mutant completely devoid of AsA has ever been described which clearly indicate that plant which is unable to synthesize is lethal, implicating a role of this compound in a wide range of physiological phenomena (Ishikawa et al., 2008). In general, AsA is known to operate as an antioxidant either by direct chemical interaction with reactive oxygen species, or in the reaction catalyzed by AsA peroxidase during photosynthesis, environment-induced oxidative stress, such as ozone (Chen and Gallie, 2005), UV (Chen et al., 2008), high light (Li et al., 2009), SO_2 (Kubo et al., 1995) and wound- and pathogen-induced oxidative processes

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Abbreviations: DAA, Days after anthesis; **GalDH,** L-galactose dehydrogenase; **GalDH,** L-galactose dehydrogenase; **DHAR,** dehydroascorbate reductase; **MDHAR,** monodehydroascorbate reductase; **AsA,** ascorbic acid.

(Foyer and Noctor, 2009; Pavet et al., 2005). Additionally, ascorbate is a cofactor for enzymes, and affects the expression of genes involved in defense and hormone signaling pathways (Pastori et al., 2003). AsA is also crucially involved in cell division (De Pinto et al., 1999), cell wall metabolism (Fry, 1998), and plant pathogen interactions (El-Zahaby et al., 1995).

The ascorbate biosynthetic pathway in mammals has been known since the 1950s. Uracil-diphosphate (UDP)-D-glucose derived from glycogen is considered to be the main substrate for the *de novo* synthesis of ascorbate, and intermediates include D-glucuronate, L-gulonate and L-gulono-1,4-lactone as the last component which is oxidized by a L-gulono-1,4-lactone oxidase to produce AsA (Smirnoff, 2001). In plant, AsA biosynthetic pathway was different from that in animals (Loewus, 1999); it was proposed that ascorbate is synthesized in plants by oxidation of L-galactose (L-Gal), namely Smirnoff-wheeler's pathway, in which guanosine 5'-diphospho (GDP)-D-mannose, generated from D-mannose-L-phosphate, is continuously converted to GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-1,4-lactone, and finally to L-ascorbate (Linster and Clarke, 2008; Wheeler et al., 1998). The roles of many of the genes and enzymes have been confirmed in this process (Laing et al., 2007; Linster et al., 2007). In addition, recent studies showed that in the existence of alternative pathway of AsA biosynthesis in plants, L-gulose and myo-inositol have been proposed as intermediates in AsA biosynthesis, indicating that part of the animal pathway could also operate in plant. An alternative pathway with L-galactonic acid intermediate has also been reported in fruit crop, e.g. in strawberry and grape fruit (Ishikawa et al., 2006; Zhang et al., 2008).

Pear (*Pyrus pyrifolia* Nakai) is an important economic fruit crop in temperate regions. Although L-ascorbate (Asc) is also an important nutritional constituent, up to now little attention has been paid on AsA content in pear fruit. There is no report regarding the changes in AsA content during fruit development as our knowledge. Researches on distribution and metabolism of ascorbic acid in pear fruits is of great importance for further understanding to gain novel insights into the regulatory mechanisms of AsA accumulation in pear. The purpose of this study was to investigate AsA content, AsA biosynthesis related enzyme activities, as well as recycling of AsA in fruits of pear cv. Ankansui during its development and ripening. The results should provide useful information on regulatory mechanisms and breeding programs aiming at the improvement of AsA levels in pear fruits.

MATERIALS AND METHODS

Plant materials

Six-year-old pear trees (*P. pyrifolia* Nakai cv. Aikansui) were used in the present study. All the trees were grown at a density of 2 × 4 m in the Nanjing Agricultural University Experimental Orchard located in Nanjing (32°24′N, 118°46′E), China. Defect-free fruits were harvested at 10 days intervals following anthesis (days after anthesis, DAA), and continuing 110 days after attainment of physiological maturity. Fruits were harvested from the top part of the canopy receiving full light and used for all experiments. For determining differences in AsA content and recycle system in different fruit tissues, fruits were divided into peel, fresh and core. Samples were weighed and immediately frozen in liquid nitrogen, followed by storage at -72°C until use for analysis of contents of AsA and enzyme activities.

Assays for AsA

AsA and dehydroascorbate (DHA) contents were assayed according to the method of Kampfenkel et al. (1995), with some modifications. Samples (0.5 g) were homogenized in 4 ml 6% (w/v) metaphosphoric acid containing 1 mM ethylenediamine tetraacetic acid (EDTA) on ice and centrifuged at 12000 g for 20 min. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid in acidic solution. Fe^{2+} forms complexes with bipyridyl, yielding a pink color with the absorbance peak at 525 nm. Total ascorbate (AsA + DHA, T-AsA) was determined with 200 µl extracted solution by initially incubating for 20 min in 200 mM phosphate buffer solution (pH 7.4) and 1.5 mM dithiothreitol (DTT) to reduce all DHA to AsA. After incubation, 200 µl of 0.5% (w/v) N-ethylmaleimide (NEM) was added to remove excess DTT. Reduced ascorbate was analyzed in a similar manner except that 400 µl deionized H_2O was substituted for DTT and NEM. Color was developed in both series of reaction mixtures (total and reduced ascorbate) with the addition of 1000 µl 10% trichloroacetic acid, 800 µl 42% phosphoric acid, 800 µl 65 mM 2,2'-dipyridyl in 70% (v/v) ethanol, and 400 µl 3% (w/v) $FeCl_3$. The reaction mixtures were then incubated at 42°C for 1 h and quantified at 525 nm. A standard curve of AsA was established and used for quantification, DHA concentration was calculated from differences between total ascorbate and reduced ascorbate.

Assays of L-galactono-1,4-lactone (GalLDH) and L-galactose dehydrogenase (GalDH) activities

Crude GalLDH enzyme extract was prepared using the method of Do Nascimento et al. (2005), with some modifications. Samples (3 g) were homogenized in 10 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 0.4 M sucrose, 0.3% (v/v) mercaptoethanol and 1 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 500 g for 10 min at 2°C, and the supernatant was centrifuged at 16,000 g for 40 min at 2°C. The pellet was suspended in 2 ml of 0.1 M phosphate buffer (pH 8.0) that contained 5 mM glutathione, 1 mM EDTA, and 10% (v/v) glycerol. This suspended solution was again centrifuged at 2000 g for 10 min at 2°C, and the supernatant was utilized to determine the activity of GalLDH. A reaction mixture (1.5 ml), containing 60 mM cytochrome c, 1 mM sodium azide, 2.5 mM L- galactose, 0.1% (v/v) Triton X-100, and 0.1 ml of the enzyme extract in 50 mM Tris-HCl (pH 8.5), was pre-incubated at 27°C for 5 min. Subsequently, reduction of cytochrome C was monitored by the increase in absorption at 550 nm. One unit of activity was defined as the reduction of 1 mmol of cytochrome C per minute.

GalDH activity was assayed according to a method described by Hancock et al. (2003), with some modifications. Samples were extracted on ice cold 50 mM Hepes buffer (pH 8.0) containing 5 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, and 1% (w/v) PVP-4000. The homogenates were centrifuged at 16,000 g for 20 min at 2°C and the supernatants were collected as enzyme extracts. A reaction mixture (1.5 ml),

consisting of enzyme extract, 50 mM Hepes buffer (pH 8.0), 5 mM $MgCl_2$, 0.5 mM NAD^+ was started by the addition of 2 mM L-galactose and then pre-incubated at 27°C for 2 min; reduction of NAD^+ was monitored by the increase in absorption at 340 nm. Activity was calculated in mmol terms of NAD^+ reduced per minute.

Assays of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) activities

DHAR and MDHAR were assayed using the method described by Ma and Cheng (2004) with some modifications. Samples (2 g) were homogenized with 8 mL of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM DTT, 0.1% (v/v) Triton X-100, and 2% (w/v) mercaptoethanol with 0.5 g PVP. The homogenates were centrifuged at 16,000 g for 20 min at 4°C and the supernatants were collected for enzyme assays. MDHAR activity was assayed at 340 nm in 3 ml reaction mixture containing 50 mM Hepes–KOH (pH 7.6), 0.1 mM NADH, 0.25 mM AsA, 0.25 units AsA oxidase, and 0.1 ml of the supernatants. The reaction was initiated by adding AsA oxidase. DHAR activity was measured at 265 nm in 3 ml of assay solution containing 100 mM Hepes–KOH (pH 7.0), 1 mM EDTA, 2.5 mM GSH, 0.2 mM DHA, and 0.1 ml of the supernatants. The reaction was initiated by adding DHA. MDHAR activity was calculated in mmol terms of NADH oxidized per minute, while that of DHAR was expressed as mmol of AsA produced per minute.

AsA biosynthesis by feeding with candidate precursors

Flesh, peel and core from young (DAA, 30 days) and mature (DAA, 100 days) fruit were used in this experiment, respectively. The peel tissues were taken using a 2 mm thick peeler and cut into discs in 1.0 × 1.0 cm. The flesh and core tissue were taken using a 1.0 cm-diameter cork-borer and cut into discs in 0.3 cm thickness. As described by Hancock et al. (2003), the fresh samples were pre-incubated in 25 ml buffer containing 20 mM 2-(N-morpholino)ethanesulfonic acid (MES, pH 5.5), 300 mM manitol, 5 mM $MgCl_2$, 2 mM KCl, 1 mM $CaCl_2$ and 1 mM $CaSO_4$ with a rotary shaker at 100 rpm at 25°C for 1 h. then each of precursors, including D-glucose (D-Glu), L-galactose (L-Gal), L-galactono-1,4-lactone (L-GalL), L-gulono-1,4-lactone (L-GulL), D-glucuronic acid (D-GluA), D-galacturonic acid (D-GalUA) and myoinositol (MI) was added, with a final concentration of each treatment solution reaching 10 mM. Following incubation for 24 h at a rotary shaker (100 rpm) at 25°C, incubated samples were washed with the sterile water and surface-dried on filter paper, followed by immersion in liquid nitrogen. Total AsA concentration was determined by a spectrophotometer as described above.

RESULTS

Distribution of AsA during fruit development and ripening

The changes in AsA contents in different pear fruit tissues during fruit development and ripening are illustrated in Figure 1.

AsA, DHA and T-AsA were measured and their content was calculated on fresh weight basis. T-AsA and AsA levels of the different young fruit tissues were much higher than those of the mature fruits. The highest T-AsA contents were in peel, with the concentration of 1.8 μmol g^{-1} FW, at the early developmental stage at 30 DAA (Figure 1A). After then, it decreased continuously until 90 DAA. During the maturation and ripening stage (90 to 110 DAA), it remained at range of 0.15 to 0.20 μmol g^{-1}FW (Figure 1A). Distribution pattern of T-AsA, AsA and DHA in different fruit tissues was similar during development and ripening. The peel had the highest contents of T-AsA, AsA and DHA while the flesh had the lowest.

Changes of GalLDH and GalDH activity during fruit development and ripening

The changes of GalDH and GalLDH activities in different fruit tissue during fruit development and ripening are illustrated in Figure 2. GalDH activities in the core did not change significantly during pear development and ripening and the significantly lowest activity was maintained compared with other part of fruits. While the activity of GalDH in the peel changed significantly during fruit development and ripening. With fruit development, the activity of GalDH increased until 30 days after anthesis, and then the activity slowly declined with fruit growth. The most rapid drop was observed from 90 to 110 days (Figure 2A).

The GalLDH activity showed similar patterns as GalDH during fruits growth and ripening. The activity of GalLDH in fruit peel increased significantly at early stages of the development, and the highest GalLDH activity at 30 was observed, and then the activity of GalLDH significantly decreased from 50 to 70 DAA, followed by a slowly decline from 70 to 110 days (Figure 2B). The GalLDH activity in flesh showed similar developmental patterns as peel. However, the activity of GalLDH was higher in the peel than flesh and core. The activities of GalLDH in core were maintained lower levels during fruit development.

Changes of MADHAR and DHAR activities during fruit development and ripening

MDHAR activities in all tissues during the fruit development showed a similar change pattern (Figure 3A). Activities of MDHAR in all tissues increased steadily with the fruit development, and it reached the highest levels at 90 DAA, after that the activity decreased.

The significant difference of MDHAR activities were observed in different fruit tissues. The peel had the highest MDHAR activity, but had no differences between the flesh and core.

DHAR activities showed a different change trends during fruit development compared to MDHAR activities (Figure 3B). There were two spouts in the peel at 30 and 90 DAA, respectively. The significantly highest DHAR activity in the peel samples was observed at 90 DAA. The activities of DHAR in flesh and core were maintained at lower levels during fruit development.

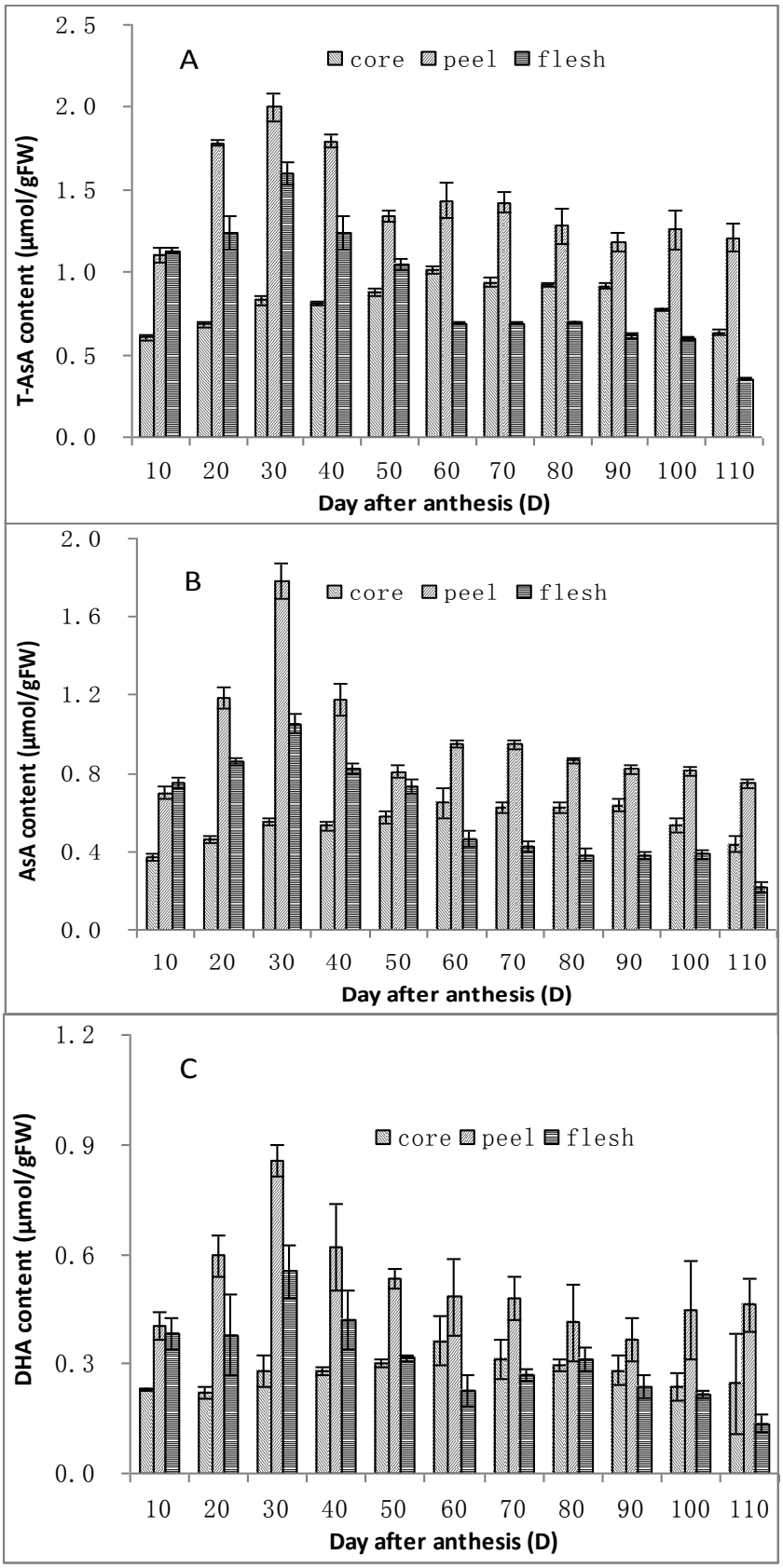

Figure 1. Changes of T-AsA **(A)**, AsA **(B)** and DHA **(C)** content in different pear fruit tissue and development stage. Values are means of three replicates±SD.

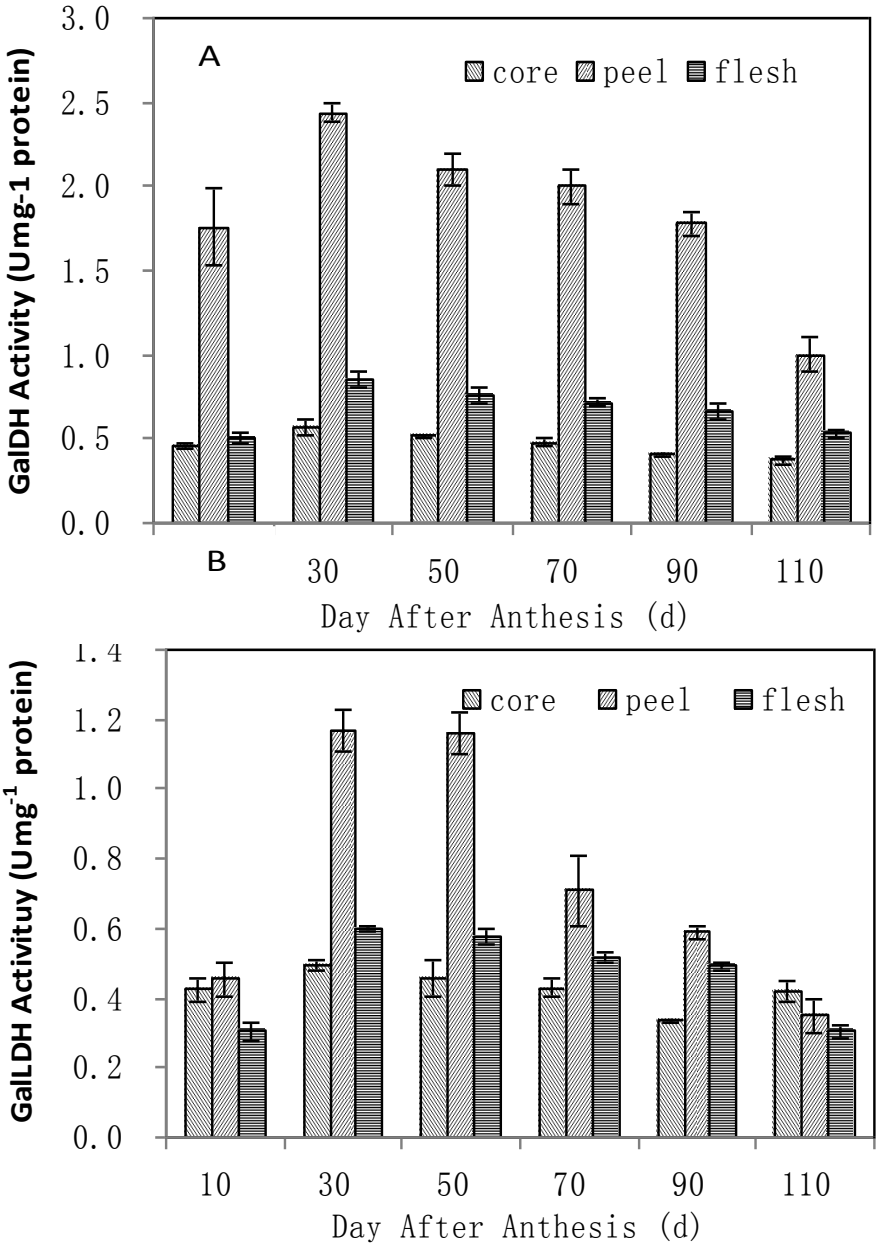

Figure 2. Changes of enzyme activity of GalDH **(A)** and GalLDH **(B)** in different tissues and development stage of pear fruit. Values are means of three replicates ±SD.

AsA biosynthesis in fruit of different stages

To understand the difference in AsA biosynthesis in the young (30 DAA) and mature (100 DAA) fruit tissue, different samples were treated *in vitro* with potential precursors of AsA biosynthesis. Results indicate that T-AsA content in the different fruit tissue of different development stages responded to different precursor candidates, differently (Figure 4). Treatment with L-Gal and L-GalL resulted in clearly increased T-AsA content in all samples compared with the control which was treated with sucrose. Compared with mature fruit tissue, tissues of young fruit showed the higher increase in T-AsA content with L-Gal and L-GalL. In young fruit core, the maximum AsA increase was obtained from treatment with L-Gal or L-GalL. On the other hand, in mature fruit core, the T-AsA levels were increased about 23 and 33% with the L-Gal and L-GalL treatment, respectively. Regarding the different fruit tissues, the fruit core had the maximum AsA increment, but the AsA contents in flesh and peel increments were lower. Additionally, increased T-AsA contents were also found in the mature fruit tissue in the

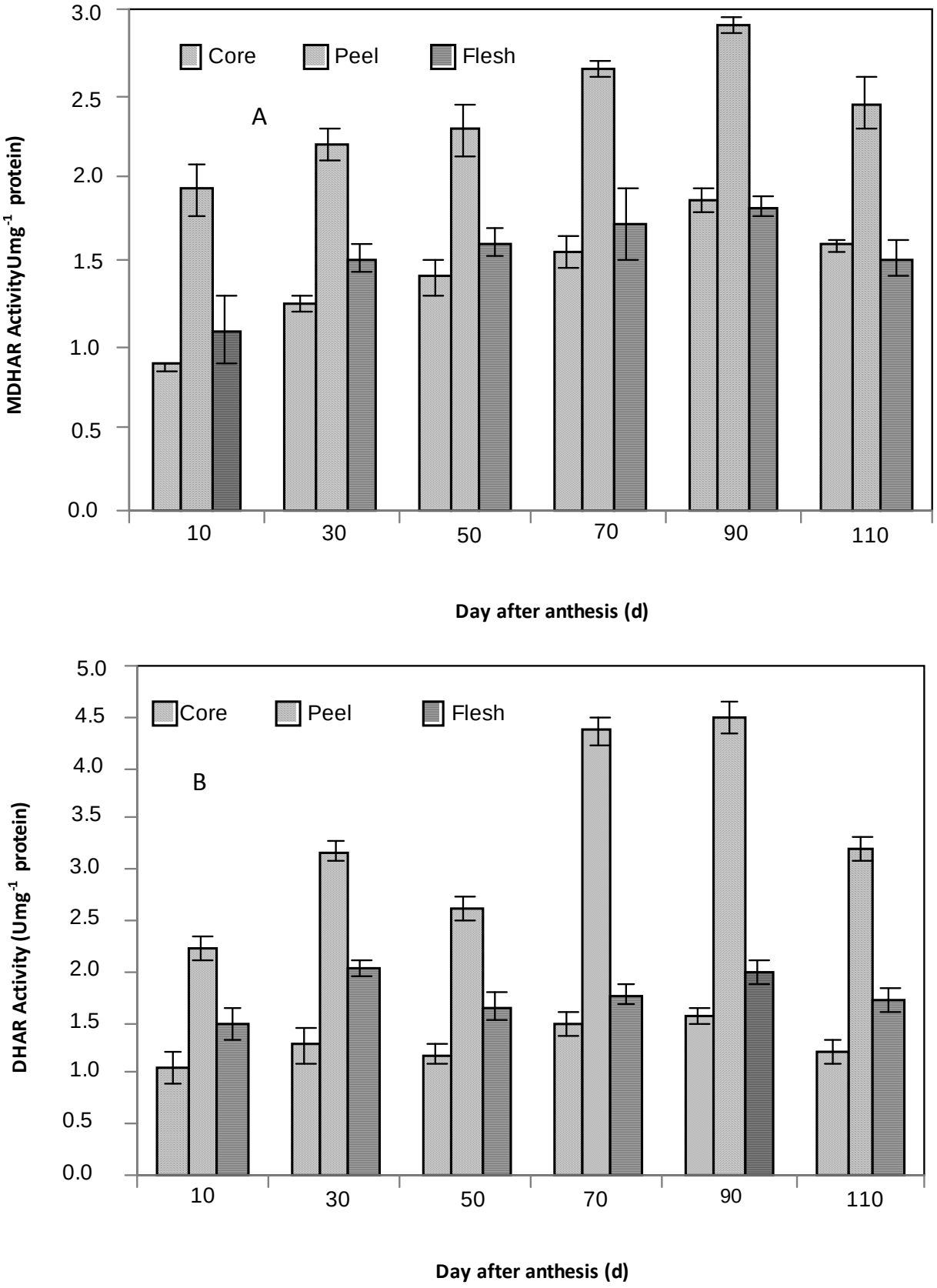

Figure 3. Changes of enzyme activity of MDHAR **(A)** and DHAR **(B)** in different tissues and stage of pear fruit. Values are means of three replicates ±SD.

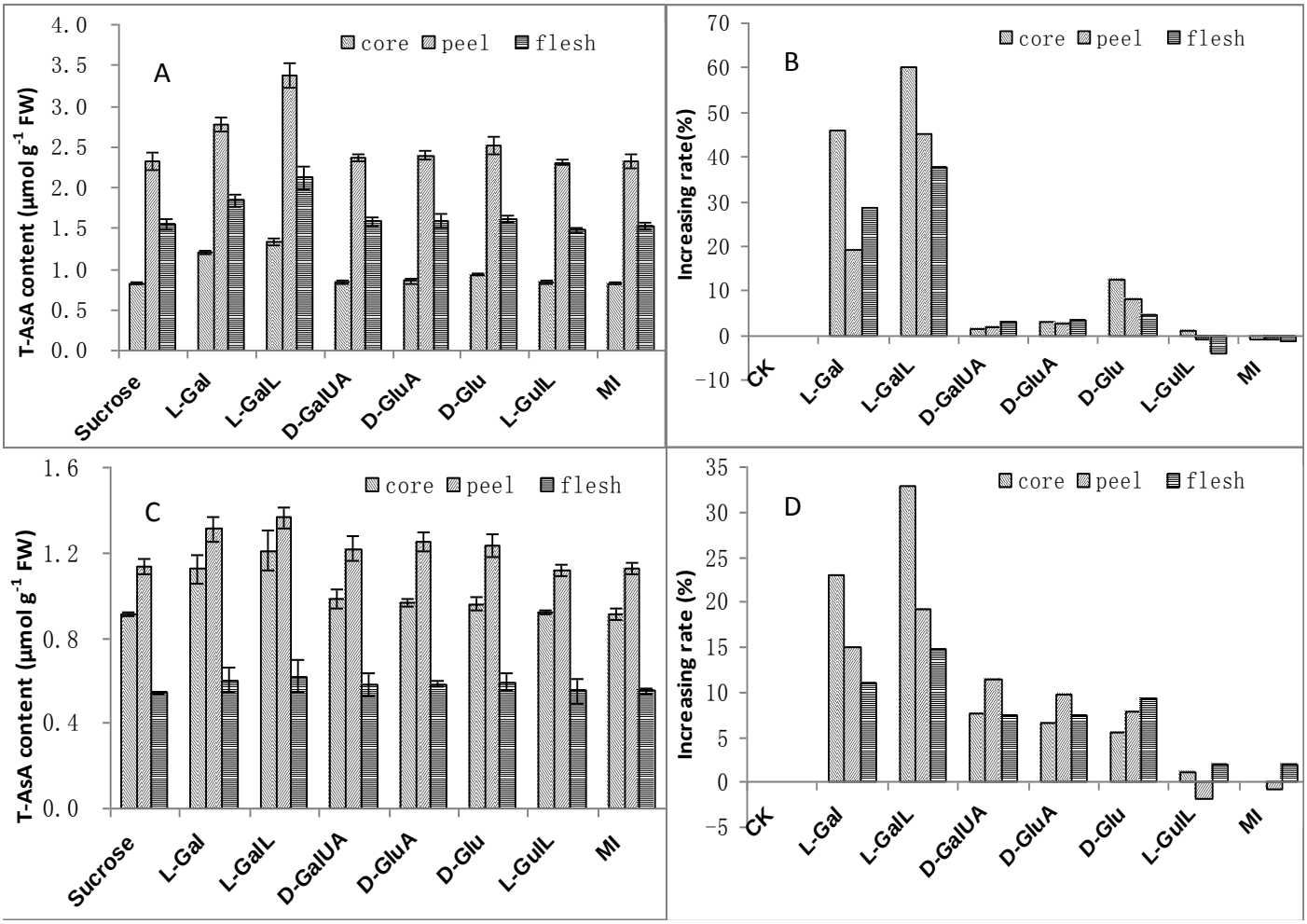

Figure 4. Effect of different precursor candidates for AsA biosynthesis on total AsA content in the young (A, 30 days) and mature (C, 100 days) fruits of pear. **A** and **C.** T-AsA content after feeding. **B** and **D.** Increasing rate of T-AsA content after feeding compared to control. Sucrose was used as control. L-Gal, L-galactose; L-GalL, L-galactono-1,4-lactone; D-GalUA, D-galacturonic acid; D-GluA, D-glucuronic acid; D-Glc, D-glucose; L-GulL, L-gluono-1,4-lactone; MI, myoinositol. Values are means of three replicates±SD.

treatment of D-GluA and D-GalUA, but contents in the young fruit tissues were not increased.

DISCUSSION

AsA contents and accumulation capability in fruits of different species have significant difference. For instance, Davey et al. (2000) reported that blackcurrant (11.2 to 11.8 μmol/g FW), strawberry (3.37 μmol/g FW) and kiwifruit (3.41 μmol/g FW) are fruits particularly rich in AsA. The results of this work demonstrate that accumulated AsA contents in pear (*P. pyrifolia* Nakai) of cultivar Aikansui were similar to those reported in cranberry (0.67 μmol/g FW), apple (0.11 to 0.56 μmol/g FW) and apricots (0.39 to 0.56 μmol/g FW) (Davey et al., 2000).

AsA levels were varied between different tissues and the same tissue at different developmental stages. The peel of fruit is the interface between fruit and environment and frequently experiences biotic and abiotic stress (Ma et al., 2004). The phytocompounds including triterpenoids (He and Liu, 2007), phenols (McGhie et al., 2005), AsA and GSH (Davey et al., 2004; Guo et al., 2003) levels in the peel of fruit were much higher than that in the flesh. Results of the present study also show that AsA levels in the peel of fruits of cv. Aikansui pear were much higher than that in the flesh and core.

Previous reports have shown two types of ascorbate accumulation pattern in fruit: i) the ascorbate levels are basically unchanged or increased slightly during fruit growth and ripening. The crops in this category include melon, tomato, strawberry, kiwifruit and blackcurrant (Agius et al., 2003; Pateraki et al., 2004; Bulley et al., 2009). ii) The concentration of ascorbate declined with fruit development, and finally reached a stable level

during fruit maturation. The crops in this category include apple, orange (Davey et al., 2004; Li et al., 2008) and acerola (Badejo et al., 2007; Okuse and Ryugo, 1981). Our research shows that pear of Aikansui cultivar belongs to the second type. It means that ascorbate contents (with $\mu molg^{-1}$ FW) exhibited decrements during its development. The T-AsA levels in mature pear fruit was approximately 0.18 $\mu molg^{-1}$ FW (Figure 1), which is lower compared with early stages of fruits development.

The *de novo* biosynthesis is a main reason of AsA accumulation in plant cells. The majority of plant L-ascorbates seems to be produced by the Smirnoff-Wheeler's pathway (Conklin et al., 2006). In this pathway, L-galctose is reduced to L-galactono-1,4-lactone by GalDH and then L-galactono-1,4-lactone is oxidized to L-ascorbic acid by the highly specific mitochondrial inner membrane-associated GalLDH (Wheeler et al., 1998). Therefore, the activities of these enzymes are essential to the metabolism of plant cell. The positive correlation between the activities of GalDH or GalLDH and ascorbic acid levels in plant tissues were reported by several authors (Ba et al., 1994; Oba, 1994; Pateraki et al., 2004; Tabata et al., 2001, 2002; Tamaoki et al., 2003). In the present study, changes in activities of GalDH and GalLDH during fruit growth and ripening showed positively consistent with ascorbate content in the development of fruit. The results are well agreed with that in apple which was reported by Li et al. (2008). Though, it still have other opinions that GalLDH and GalDH are not related to the ascorbate contents and does not determine the ascorbate content in special tissue (Tamaoki et al., 2003). Over-expression of *Arabidopsis GalDH* has no effect on ascorbate contents in tobacco under natural conditions (Gatzek et al., 2002). Thus, further investigation is necessary to understand the roles of GalDH and GalLDH in controlling ascorbate synthesis *via* Smirnoff-Wheeler pathway.

AsA is not a stable metabolic product though, it is utilized both as a cellular reductant and substrate for the synthesis of particular organic acids (Melino et al., 2009), therefore capability of ascorbate recycle play a important role in the determination of AsA concentration in plant tissues (Chen et al., 2003). In ascorbate recycle pathway, the reduced ascorbate is oxidized into a MDHA radical, which can be converted back to reduced ascorbate via MDHAR. MDHA is further rapidly converted to DHA, which is converted back to reduced ascorbate by the action of DHAR (Asada, 2006; Mittler, 2002; Mittler et al., 2004; Smirnoff and Wheeler, 2000). The importance of ascorbate recycling in cell are confirmed in studies of trangenetic plants, the over-expression of a DHAR enzyme in tobacco increased the ascorbic acid levels from two to four-fold (Chen et al., 2003) and over-expression of cytosolic DHAR significantly increased DHAR activities and ascorbate contents in potato leaves (Qin et al., 2011). These results indicate that ascorbate contents in the cell can be elevated by enhancing recycling ascorbate *via* DHAR or MDHAR over-expression. We investigated the changes of MDHAR and DHAR activities and the relationships between these enzymes activities and ascorbate contents in pear fruit tissues. Results indicate that the peel with higher activities of MDHAR and DHAR compared with that of the flesh and core was consistent with the ascorbate concentrations in different tissues. The results also indicate that the peel of pear fruit have stronger synthesis and recycling capability of AsA.

Feeding experiments as a main method were used to study the metabolic pathways in plants. It is recently used in vitamin metabolism research in many plants. In this study, samples representing different developmental stages were fed with non-labeled putative substrates to synthesize ascorbate involved in L-galactose pathway, including D-Glu, L-Gal and L-GalL and in uronic acid pathway of D-glucuronic acid (D-GluA) and D-galacturonic acid (D-GalUA). In L-gulose pathway, L-gulono-1,4-lactone. L-Gal and L-GalL significantly increased ascorbate levels in the core, flesh and peel of 30 and 100 DAA 'Aikansui' fruit. The results indicate that these tissues of pear fruit are capable of ascorbic acid biosynthesis *via* the Smirnoff-Wheeler pathways, and that the capacity to produce ascorbate was greater at 30 DAA than 100 DAA or later, which is in agreement with patterns of AsA biosynthesis in apple (Li et al., 2008) and kiwi (Li et al., 2010). Furthermore, when feeding with L-Gal and L-GalL, the capacity of synthesis AsA in core was stronger than that in the flesh and peel. D-GaluA and D-GluA also stimulated ascorbate accumulation indicating that fruits tissues utilize uronic acid for ascorbate synthesis (Agius et al., 2003; Chen et al., 2003). D-GaluA and D-GluA appear as products of the turnover of cell wall pectin. Thus there may be a certain relationship between ascorbate synthesis and turnover of cell wall carbohydrates (Davey et al., 2000). All results suggest that the two proposed alternative pathways for AsA biosynthesis, the Smirnoff-Wheeler pathway (L-Galactose Pathway) and the D-galacturonic acid pathway, are possibly presented in pear fruit, but these results need to be confirmed in future studies by feeding radiolabelled precursors and genetic experiments.

Taken together, each tissue in pear fruit seems to have its own characteristic pattern of ascorbate synthesis and recycling. All these results suggest that the peel of pear fruit has the highest titers of ascorbate and enzyme activities which are used to synthesis, and to recycle ascorbate. Ascorbate concentrations in each tissue of pear fruit were correlated with biosynthetic capacity by the Smirnoff-Wheeler pathway. Moreover, feeding experiments indicated that the peel of 'Aikansui' pear was able to *de novo* synthesis of ascorbate *via* Smirnoff-Wheeler pathway and uronic acid pathway together. However, D-GalUA and D-GluA were not precursors of ascorbate biosynthesis in the flesh and core. Finally, an understanding of the mechanism(s) of ascorbate genera-

tion and accumulation in pear fruit will help us to breed pear cultivars with high concentration of AsA in the fruit.

ACKNOWLEDGEMENT

This work was supported by the Nature Science Fund of china (31071759).

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