

Comparison of Enzyme Activities Involved in AsA-GSH Cycle in Red-flesh Kiwifruit Varieties

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Abstract. Kiwifruit is rich in ascorbic acid. In this study, activities five enzymes involved in ascorbate-glutathione cycle (AsA-GSH cycle) were determined in four red-flesh kiwifruit varieties. Results showed that ‘Tetra-Chine’ had higher activities in APX (ascorbate peroxidase) and AO, while ‘Hongshi 2’ had higher activities of dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR). In addition, ‘Hongyang’ contained the highest content of H₂O₂, ‘808’ contained the highest activity of glutathione reductase (GR). All of these results indicated that there were significant differences in the activities of enzymes in different genotypes.

1. Introduction

Ascorbic acid (AsA, vitamin C) is one of the most abundant low molecular weight antioxidants in plant tissues, which is involved in antioxidant in plant cell, the protection of photosynthesis, cell division, growth and signal transduction and so on[1-3]. AsA accumulation is controlled by biosynthesis and recycling along with plant growth process. The most important regeneration mechanism of AsA in plant cells is by AsA-GSH cycle system. GSH, AsA and MDHAR, DHAR, APX and GR, constitute the antioxidant defense system in the AsA-GSH cycle in plants and play an important role in scavenging reactive oxygen species [4].

Most plants in genus *Actinidia* are native to China, and fruits are rich in vitamin C, known as "the king of fruit"[5]. To gain insight into the mechanisms responsible for controlling AsA levels in kiwifruits, we investigated the enzyme activities in AsA-GSH cycle in 4 red-flesh kiwifruit genotypes which were planted in China. Results from the present study will help us to reveal physiological mechanism of AsA accumulation in kiwifruit.

2. Materials and Methods

2.1 Plant Material

All kiwifruit genotypes used in this study were harvested from the Kiwifruit Resource Orchard in Shifang (104° 16'N, 31° 13'E), Chengdu, China. Fruits were selected according to the uniformity of the shape when samples have reached physiological maturity (total soluble solid content was 7-8%). At least 10 fruits were harvested for every varietie. Prior to preparation of the test samples, the fruit



samples were exposed to room temperature to reach eating maturity (total soluble solid content was 10-11%). These fruits were chopped and homogenised under liquid nitrogen in a high-speed blender for 1 min, then immediately frozen in liquid nitrogen and stored at -72°C until use.

2.2 Assays of AO, APX, GR, DHAR and MDHAR activities

GR, DHAR and MDHAR activities were assayed using the method of Ma and Cheng [6]. APX activity was assayed using the method of Nakano and Asada [7]. AO activity was assayed using the method of Pignochi and Foyer[8].

2.3 Assays of H_2O_2 Content

H_2O_2 content was determined by the method of Hao et al [9].

2.4 Data Analysis

All data was processed using Excel 2010 software and analysis of variance (ANOVA) was performed by the SPSS 20.0 and significant differences ($P < 0.05$) between treatments were determined using Duncan's test. Data were expressed as mean $\pm$ SD.

3. Results and Discussion

3.1 Activities of APX and AO and H_2O_2 content in flesh of 4 kiwifruit genotypes

APX catalyzes the conversion of H_2O_2 to H_2O and O_2 using AsA as specific electron donor. As shown in Figure 1A, the activity of APX ranged from 0.14 (808) to 0.32 U/g FW (Tetra-Chine). The activity of APX in 'Tetra-Chine' was the highest, and there was no significant difference of APX activity in other three kiwifruit genotypes. Besides, a significant difference of H_2O_2 content was found in flesh of 4 kiwifruit genotypes. The H_2O_2 content in 'Hongyang' was the highest, by 4.63 times of 'Hongshi 2' (Figure 1B). The higher H_2O_2 content may be due to lower APX activity in red-flesh kiwifruit, because APX can prevent the accumulation of toxic levels of H_2O_2 in the cell[10].

The AO catalyzes a complex reaction, which reduces safely the molecular oxygen into water without the release of ROS. A significant difference of AO activity was found in flesh of 4 genotypes. 'Tetra-Chine' contained the highest activity of AO in flesh, it was 2.3 times of '808', 3.29 times of 'Hongshi 2' and 7.67 times of 'Hongyang' (Figure 1C). AO not only apparently works to decrease oxygen content, thus limiting the formation of reactive oxygen species (ROS), but also oxidizes AsA to DHA. These strongly suggest that AO has an actual role in regulating AsA content[11].

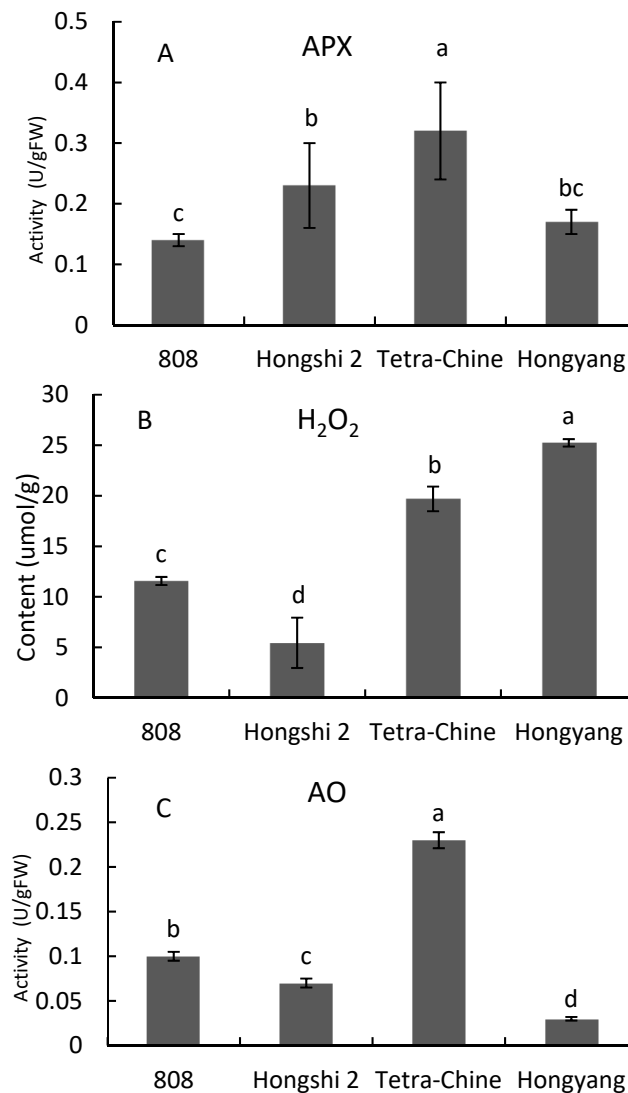


Fig.1 Activities of APX and AO and H₂O₂ content in four red-flesh kiwifruit genotypes.

3.2 Activities of DHAR, MDHAR and GR in flesh of 4 kiwifruit genotypes

DHAR, MDHAR and GR were key enzymes involved in AsA-GSH cycle to regulate AsA accumulate in plant cell. As a major anti-oxidant in plants, AsA is oxidized to DHA via successive reversible electron transfers with MDHA as a free radical intermediate. MDHAR can recycle MDHA molecules into AsA and DHAR reduce DHA to AsA by with GSH as an electron donor. GR regenerate the reduced form of GSH to maintain the cellular redox state.

As indicated in Figure 2A, there was no significant difference in DHAR activities among 'Hongshi 2', 'Tetra-Chine' and 'Hongyang', but their DHAR activities was significantly higher than that of '808', which was 3 times of '808'. The MDHAR activities in 'Hongshi 2' and 'Hongyang' were higher than that in 'Tetra-Chine' and '808'(Figure 2B). As for GR activity, the highest activity was measured in '808' and the lowest was in 'Hongshi 2' and 'Hongyang'. The highest one was the 7 times of the lowest one (Figure 2C).

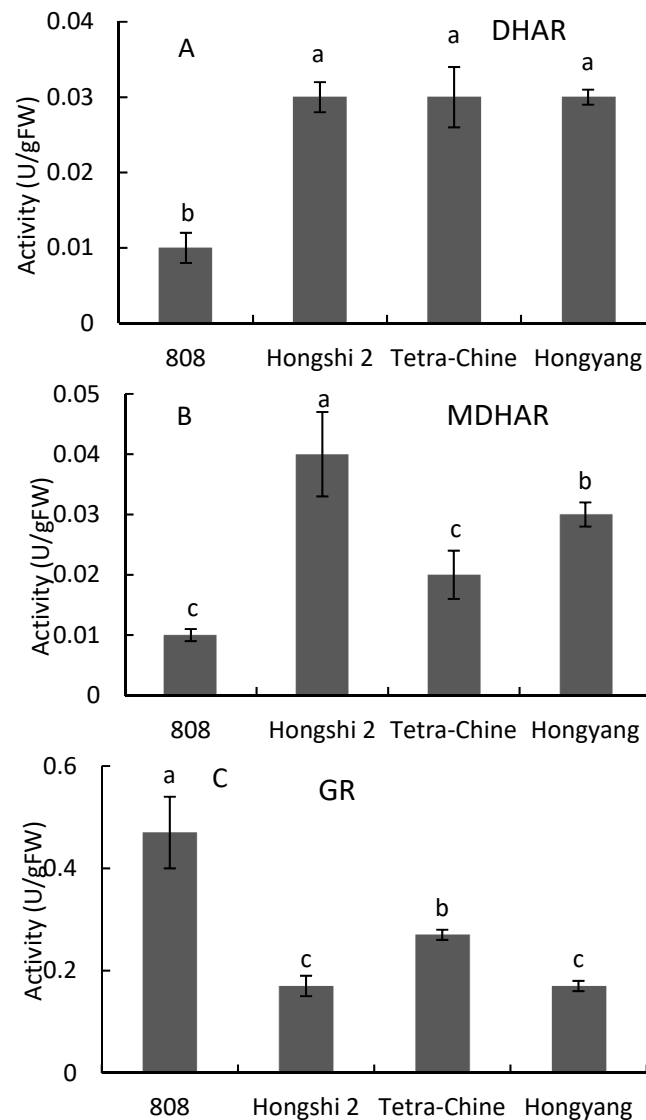


Fig.2 Activities of DHAR, MDHAR and GR in four red-flesh kiwifruit genotypes.

4. Conclusions

Several previous studies had reported that there was obvious correlation between contents of T-AsA, AsA and GSH and activities of MDHAR, DHAR and GR, which indicated that those three enzymes also played an important role in maintaining AsA redox state [12, 13]. In plants, AsA content is also highly regulated by the regeneration system, and the AsA–GSH circulatory system is an important pathway for oxidative ascorbate regeneration in plants [14]. In this study, we analyzed the activities of the important oxidoreductases such as APX, DHAR, MDHAR, and GR which are involved in AsA–GSH cycle. We found that ‘Tetra-Chine’ performed higher activities of APX and AO, ‘Hongshi 2’ performed higher activities of DHAR and MDHAR. It showed that they had high antioxidant activity which could eliminate toxic H_2O_2 in plants. Therefore, it is presumed that their AsA content is also high. In a whole, there were still many differences in the enzyme activity of red-fleshed kiwifruits with different genotypes.

References

- [1] Smirnoff N, Wheeler GL (2000). Ascorbic acid in plants: biosynthesis and function. *Crit Rev Biochem Mol Biol*, 35(35): 291-314.

- [2] Wheeler GL, Jones MA, Smirnoff N (1998). The biosynthetic pathway of vitamin C in higher plants. *Nature*, 393 (6683): 365–369
- [3] De Tullio MC, Arrigoni O (2004). Hopes disillusions and more hopes from vitamin C. *Cell Mol Life Sci*, 61(2): 209-219.
- [4] Asada K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50: 601-639.
- [5] Huang H W, Gong J J, Wang S M, et al. Genetic diversity in the genus *Actinidia*[J]. *Chinese Biodiversity*, 2000.
- [6] Ma, F. W. and Cheng, L. L. 2003. The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shade peel. *Plant Sci*. 165:819-827.
- [7] Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 22, 867-880.
- [8] Pignocchi, C. and Foyer, C. H. 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Curr Opin Plant Biol*. 6:379-389.
- [9] J.J. Hao, Z.L. Kang and Y. Yu: *Experimental techniques of Plant Physiology References* (First Edition) (Trans Chemical Industry Press, China 2006).
- [10] Pandey, P.; Singh, J.; Achary, V. M. M.; Reddy, M. K. 2015. Redox homeostasis via gene families of ascorbate-glutathione pathway. *Front. Environ. Sci*. 3:25.
- [11] De Tullio, M.; Guether, M.; Balestrini, R. 2013. Ascorbate oxidase is the potential conductor of a symphony of signaling pathways. *Plant Signal. Behav*. 8(3): e23213
- [12] Li, M. J.; Liang, D.; Pu, F.; Ma, F. W.; Hou, C. M.; Lu, T. 2009. Ascorbate levels and the activity of key enzymes in ascorbate biosynthesis and recycling in the leaves of 22 chinese persimmon cultivars. *Sci. Hort*. 120:250-256.
- [13] Pu, F. and Ren, X. L. Ascorbate levels and activities of enzymes related to the glutathione-ascorbate cycle in fruits of chinese persimmon cultivars. 2014. *Hort. Environ. Biotechnol*. 55(4):315-321.
- [14] Foyer, C. H. and Noctor, G. 2003. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plantarum*. 119:355-364.