Differences of Ascorbic Acid Synthesis in Different Genotype of Red-fleshed Kiwifruits

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Abstract: To investigate the ascorbic acid (AsA) content of different varieties of red-fleshed kiwifruit, four kinds of red flesh kiwifruit were used as materials to determine the content of AsA and glutathione (GSH). Results showed that there were significant differences not only in the contents of AsA and GSH but also the ratio of AsA/DHA and GSH/GSSG in flesh of 4 kiwifruit genotypes. T-AsA and AsA levels of 'Hongshi 2' were higher than those of other red-fleshed kiwifruits, as well as its ratio of AsA/DHA was the highest. And ‘808’ had the highest ratio of GSH/GSSG which was 24 times of the ‘Hongshi 2’. Besides, ‘Hongshi 2’ contained the highest contents of T-GSH in flesh whereas the lowest values were measured from ‘Tetra-Chine’. ‘808’ contained the highest GSH content which was 3.29 times of ‘Hongshi 2’. All of these results indicated that higher efficiency AsA-GSH cycle was likely to be responsible for the higher AsA accumulation in flesh of kiwifruit.

1. Introduction
Ascorbic acid (AsA), also named vitamin C (Vc), is an important antioxidant in plants. Fruit and vegetables are a primary source of dietary intake of vitamin C for humans. Ascorbic acid have been reported to have clear health benefits after human consumption[1]. AsA accumulation is controlled by biosynthesis and recycling along with plant growth process. Up to now, the L-galactose pathway, a series of successive reactions, has been deemed to be the main route for AsA accumulation in different species [2,3]. The ascorbate-glutathione (AsA-GSH) cycle in plants is an efficient balance among biosynthesis, oxidation and recycling[4].

Kiwifruit (Actinidia) is one of the most popular fruits in the world, especially in rich sources of biologically active compounds for example AsA[5]. China is the most kiwifruit producer worldwide and possesses abundant Actinidia germplasm resources and cultivars. Its AsA content is higher than that of most fruits and it has the reputation of "King of Vc". The kiwifruit with red flesh is favored by consumers because of its relatively rare and showy appeal[6]. To gain insight into the mechanisms responsible for controlling AsA levels in kiwifruit, we investigated the AsA and GSH levels in 4 red-fleshed kiwifruit genotypes which grown in China. Results from the present study would provide useful information for utilizing kiwifruit cultivars and further understanding about controlling mechanisms of AsA in plants.
2. Materials and Methods

2.1 Plant Material
All kiwifruit genotypes used in this study were harvested from a 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 sample. 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 AsA, DHA, GSH, GSSG, T-AsA and T-GSH content
AsA and DHA content were determined by the method of Kampfenkel et al. [7], GSH and GSSG content was determined by the method of Griffith[8], T-AsA and T-GSH content was determined by the slightly improved method of Yuan Yulin[9].

2.3 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 ± SD.

3. Results and Discussion

3.1 T-AsA and AsA levels in flesh of 4 kiwifruit genotypes
As shown in Figure.1, the T-AsA content in flesh of ‘Hongshi 2’ and ‘Tetra-Chine’ have no obvious differences, as well as ‘808’ and ‘Hongyang’. However, the T-AsA content in flesh of ‘Hongshi 2’ and ‘Tetra-Chine’ were significantly higher than those of ‘808’ and ‘Hongyang’. In our findings, AsA levels showed great differences in four red-fleshed kiwifruit genotypes. The AsA content of ‘Hongshi 2’ was significant higher than that of other genotypes, while the lowest values for this parameter was found in ‘808’. Values for AsA ranged from 3.94 (808) to 10.54μmol/g FW (Hongshi 2), the highest content was 2.68 times of the lowest.

![Fig.1 Contents of T-AsA and AsA in four red-fleshed kiwifruit genotypes.](image)

Note: Data with the different letters indicate the difference is significant (P<0.05).The same is as below.

3.2 DHA, GSH, GSSG, T-AsA and T-GSH content
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3.2 T-GSH and GSH levels in flesh of 4 kiwifruit genotypes
GSH, another important antioxidant, was connected with AsA accumulation by AsA-GSH recycle [10]. In the present study, T-GSH and GSH levels also showed great variations in different kiwifruit genotypes. Values for T-GSH differed significantly among kiwifruit genotypes, ranging from 0.57 to 0.68 μmol/g FW in flesh (Figure 2). ‘Hongshi 2’ contained the highest contents of T-GSH in flesh whereas the lowest values was measured from ‘Tetra-Chine’. For all genotypes, the GSH ranged from 0.07 to 0.23 μmol/g FW in flesh (Figure 2). ‘808’ contained the highest GSH content while the lowest was found in ‘Hongshi 2’.

![Fig.2 Contents of T-GSH and GSH in four red-fleshed kiwifruit genotypes.](image)

3.3 The ratio of AsA/DHA and GSH/GSSG in flesh of 4 kiwifruit genotypes
Ascorbic acid is divided into reduced AsA and oxidized DHA, and the composition ratio of the two reflects the redox state of ascorbic acid in the plant. The higher the AsA/DHA ratio, the better the regulation of the plant's own redox state. The oxidation reduction status of glutathione is measured by the GSH/GSSG ratio. As shown in Figure 3, the ratio of AsA/DHA and GSH/GSSG had great differences in 4 red-fleshed kiwifruit genotypes. The ratio of AsA/DHA in ‘Hongshi 2’ was the highest, while ‘Hongyang’ was the lowest. The highest content was 2.88 times of the lowest. In addition, the ratio of AsA / DHA in ‘808’ and ‘Tetra-Chine’ had no significant differences. Different from the results of AsA / DHA, the results of GSH / GSSG in four red-fleshed kiwifruit genotypes differ significantly. Interestingly, ‘808’ had the highest ratio of GSH/GSSG which was 24 times of the lowest (Hongshi 2).
4. Conclusions
This study found that there were significant differences in the levels of ascorbic acid in different genotypes of red-fleshed kiwifruit. The differences in ascorbic acid levels of different genotypes of kiwifruit were determined by many factors such as AsA synthesis ability, AsA–GSH cycle regenerative capacity, degradation level and reduction state (AsA/DHA ratio)\(^{[11-13]}\). Results indicated that ascorbic acid levels of ‘Hongshi 2’ was the highest, because it had the highest T-AsA content, AsA content and AsA/DHA ratio. Besides, glutathione is coupled with ascorbic acid to form the AsA-GSH cycle. GSH acts as an electron acceptor of the reduced DHAR and is regenerated into AsA by reduction with dehydroascorbate reductase, and coupled with AsA to form the AsA-GSH cycle. The GSH level of kiwifruit had obvious diversity. GSH content and GSH / GSSG in ‘Hongshi 2’ were the lowest in this study. The difference in T-GSH of four red-fleshed kiwifruit genotypes was smaller than the difference in T-AsA of four red-fleshed kiwifruit genotypes. In summary, there were significant differences in AsA levels in different genotypes of red-fleshed kiwifruit.

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