Determination of Ascorbic Acid of Five Green Flesh Kiwifruit Genotypes by High Performance Liquid Chromatography

Dong Liang[1,2,a], Zhiyou Ni[1,b], Fan Gao[1,c], Hui Xia[1,2,d*]

[1] College of Horticulture, Sichuan Agricultural University, 611130, Chengdu, China
[2] Institute of Pomology and Olericulture, Sichuan Agricultural University, 611130 Chengdu, China

[a]liangeast@sina.com, [b]1606748902@qq.com, [c]272650516@qq.com, [d]susanxia_2001@163.com

*Corresponding author.

Abstract. This study measured the content of ascorbic acid (AsA) in five green-flesh kiwifruit genotypes by high performance liquid chromatography (HPLC). The content of AsA and GSH were compared in different green flesh kiwifruit genotypes. The results indicated that the AsA levels changed vary remarkably in different green flesh kiwifruit genotypes, and the AsA level of 'Wuzhi 3' kiwifruit were higher.

1. Introduction

Kiwifruit originated in China, which riches in Vc and has high nutritional value, known as "the king of fruits" [1]. Ascorbic acid (AsA) is a high-abundance small molecule antioxidant that is commonly found in plant tissues [2-4]. AsA is widely found in plants and exists in almost all plant organelles. AsA not only participates in antioxidant defense as the most important antioxidant in plant antioxidant system, but also regulates cell division and elongation, regulates transcription and translation of certain genes, and maintains cell redox balance [3]. The lack of plant AsA causes a weakening of resistance to adversity [5-6], and inhibits the growth [7-8]. At present, there are few studies on the determination and comparison of AsA content in many varieties of kiwifruit. In this study, we determined the AsA content of five green flesh kiwifruit genotypes using high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Plant material

Five green kiwifruit genotypes used in this study were harvested from a kiwifruit resource orchard in Shifang (104°16′N, 31°13′E), Chengdu, China (Table 1). 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 easting 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 -80°C until use.

2.2. Assays for AsA

Frozen tissue (0.5g) was added to 3ml of 0.2% metaphosphoric acid and ground. The homogenate was centrifuged and the supernatant was diluted with 0.2% metaphosphoric acid to 10ml and used for AsA determination. To determine the total AsA (T-AsA) level, as the method described by Li et al [9-10]. Thus, a 1000μl aliquot of supernatant was incubated for 4h in the dark with10μl of 200mM dithiothreitol (DTT). AsA was determined as described by Huang et al [11] and Zhang et al [12] via a high performance liquid chromatography (HPLC) with system with a photodiode array detector, Chromeleon software (Dinex), and a reverse C18 column. The mobile phase was composed of 15% methanol and 85% metaphosphoric acid aqueous solution, pH2.5. The column temperature was set at 35℃. Spectra were acquired at wavelengths between 200 and 400nm and AsA quantification was performed at 243nm.

Table 1 Description of 5 green flesh kiwifruit genotypes examined in this study.

Genotypes	Flesh color	Species	Application
Hayward	Green	*A. deliciosa*	Cultivar
Cuiyu	Green	*A.chinensis*	Cultivar
Wuzhi No.3	Green	*A.chinensis*	Cultivar
Kuimi	Green	*A.chinensis*	Cultivar
Miliang No.1	Green	*A. deliciosa*	Cultivar

3. Results and discussion

3.1. T-AsA and AsA levels

As is shown in Figure 1, T-AsA and AsA levels showed great differences in 5 green flesh kiwifruit genotypes. The contents of T-AsA and AsA ranged from 11.15 (Miliang 1) to 30.39 μmol/g FW (Wuzhi 3) and 5.23 (Miliang 1) to 18.65 μmol/g FW (Wuzhi 3) (Figure 1 A and B). There was no significant difference in AsA content between 'Wuzhi 3' and 'Hayward' kiwifruit, but it was significantly higher than other genotypes (Figure 1 A). The AsA content of 'Wuzhi 3' was significant higher than that of other genotypes (Figure 1 B). While the lowest values for T-AsA and AsA contents were found in 'Miliang 1' (Figure 1 A and B). Meanwhile, the results indicated that T-AsA and AsA levels of 'Wuzhi 3' kiwifruit were higher than 'Miliang 1' kiwifruit (Figure 1 A and B). The ratios of AsA/DHA in 'Wuzhi 3' and 'Kuimi' kiwifruit were both more than one, and the values were 1.58 and 1.13, respectively (Figure 1 C). While that in 'Miliang 1' and 'Cuiyu' kiwifruit were all less than one. The ratio of AsA/DHA in 'Hayward' kiwifruit was 1.03. The ratio of AsA/DHA results showed that the AsA in 'Wuzhi 3' and 'Kuimi' kiwifruit flesh was mainly in the reduced state, while that in 'Miliang 1' and 'Cuiyu' kiwifruit genotypes was chiefly in the oxidation state (Figure 1 C).

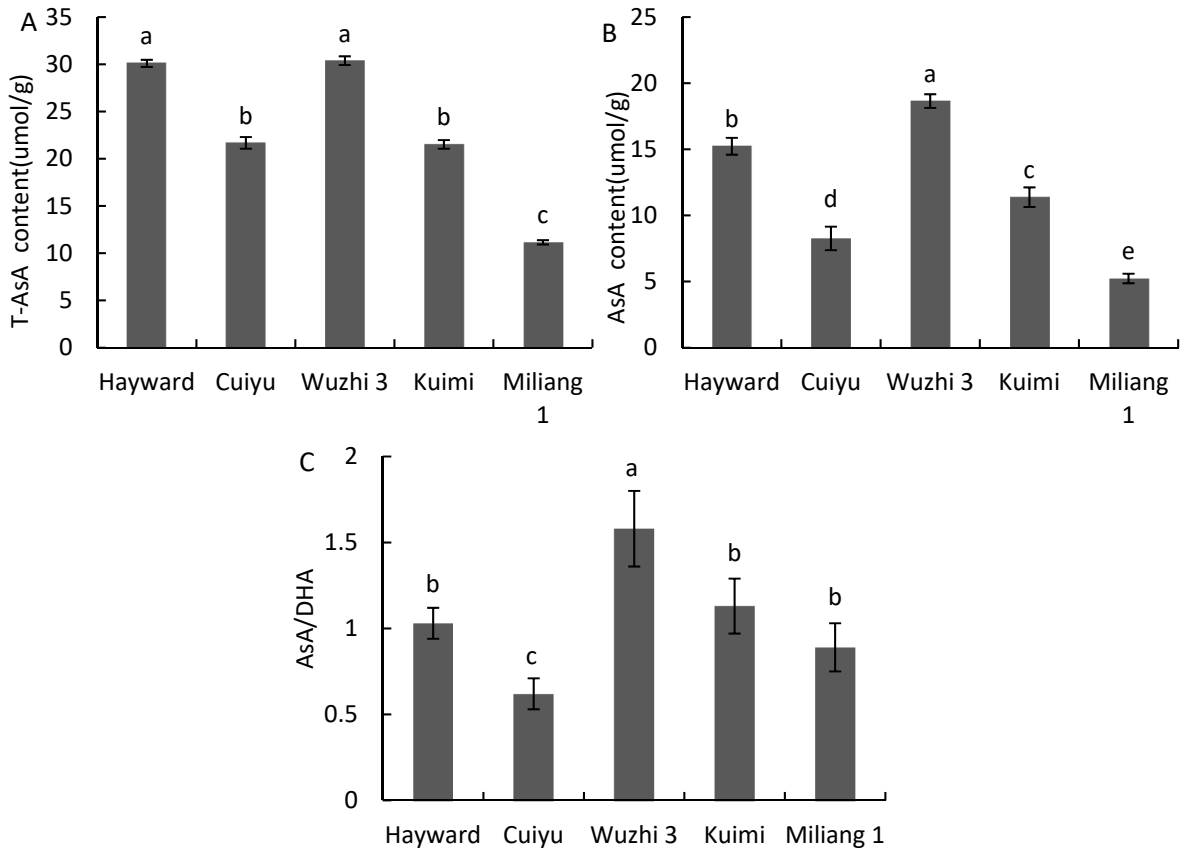

Figure 1 The contents of T-AsA (A), AsA (B) and ratio of AsA/DHA (C) in flesh of 5 green flesh kiwifruit genotypes.

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

3.2. T-GSH and GSH levels

The content of T-GSH and GSH differed significantly among 5 green flesh kiwifruit genotypes, ranging from 0.43 (Cuiyu) to 0.60 μmol/g FW (Kuimi) and 0.11 (Hayward) to 0.30 μmol/g FW (Miliang 1) in flesh (Figure 2 A and B). The T-GSH content of 'Kuimi' kiwifruit was significant higher than 'Cuiyu' kiwifruit, and did not differ significantly from other genotypes (Figure 2 A). The GSH content of 'Miliang 1' kiwifruit was significant higher than 'Hayward' kiwifruit, and had no difference compared to other genotypes (Figure 2 B). The ratio of GSH/GSSG of 'Miliang 1' kiwifruit was more than one, while that of 'Kuimi', 'Wuzhi 3' and 'Hayward' kiwifruit were less than one, and that of 'Cuiyu' kiwifruit was 1 (Figure 2 C). The ratio of GSH/GSSG results showed that the GSH in 'Miliang 1' kiwifruit was mainly in the reduced state, while that in 'Kuimi', 'Wuzhi 3' and 'Hayward' kiwifruit were chiefly in the oxidation state (Figure 2 C). The results indicated that GSH content would not be a key factor of controlling AsA content, which was consistent with the results of previous studies [13-15].

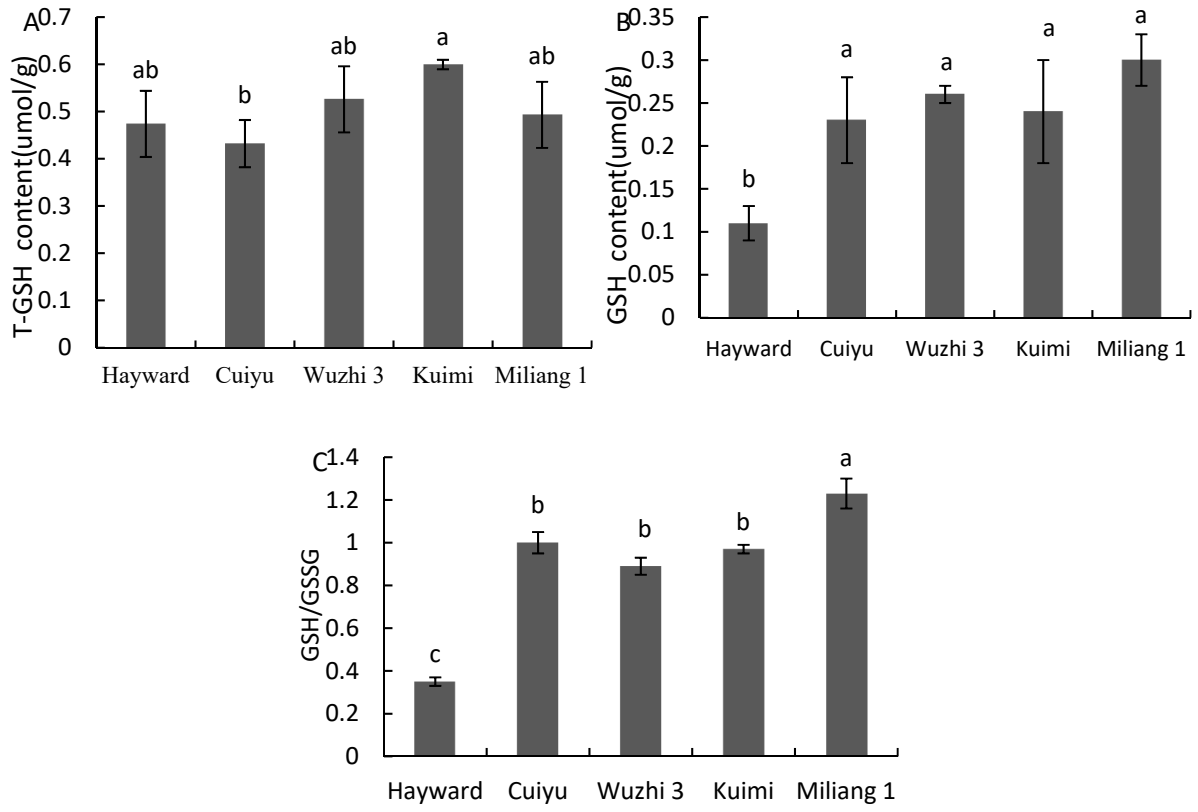

Figure 2 Contents of T-GSH (A), GSH (B) and ratio of GSH/GSSG (C) in flesh of 5 green flesh kiwifruit genotypes.

4. Conclusion

AsA is not only an essential substance for maintaining human health, but also has important physiological functions for the plant itself. Ascorbic acid is an important antioxidant and a cofactor of many enzymes in organisms and plays an important role in plant growth and development and its resistance to stress. The results indicated that the AsA levels changed vary obviously in different green flesh kiwifruit genotypes, and the AsA level of 'Wuzhi 3' kiwifruit was higher. And there is no correlation between the contents of T-AsA, AsA, T-GSH and GSH.

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