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Analysis of Antioxidant Enzyme Activity Involved in Ascorbate-glutathione Cycle in Green-flesh Kiwifruits

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Abstract: To compare the activities of enzymes related to ascorbate-glutathione cycle (AsA-GSH cycle) in different varieties, the fruits of five green-fleshed kiwifruit varieties were used as materials and activities of ascorbate peroxidase (APX), monodehydroascorbic acid (MDHA), dehydroascorbate reductase (DHAR) were measured by ultraviolet spectrophotometry. Results showed that 'Cuiyu' had higher MDHAR activities. The higher enzyme activity is responsible for the higher accumulation of AsA in kiwifruit. All of these results indicated that there were significant differences in the activities of enzymes in 5 kiwifruit genotypes.

1. Introduction

Kiwifruit is an ancient plant in China. The fruit is a typical berry, rich in trace elements, amino acids and rich in vitamin C. Kiwifruit has higher nutritional value and economic value than ordinary fruits. Therefore, the kiwifruit industry is increasingly concerned by the world [1]. Ascorbic acid (AsA), vitamin C (Vc), is an important antioxidant in plants and plays an important role in human health. AsA accumulation is controlled by biosynthesis and recycling along with plant growth process. The most important regeneration mechanism of AsA in plant cells is the AsA-GSH cycle system (Figure 1). In this pathway, ascorbate peroxidase (APX) oxidizes AsA to monodehydroascorbic acid (MDHA), while H_2O_2 is scavenged with AsA as the electron donor. A portion of MDHA can be reduced to AsA by the catalysis of MDHAR, a portion of which can be generated by non-enzymatic disproportionation reactions to AsA and dehydroascorbic acid (DHA), while DHA can be reduced to AsA with the participation of DHAR and GSH. If DHA cannot be reduced in time, it will be further oxidatively degraded to oxalic acid (OA) and tartaric acid (TA) and lost [2]. To gain insight into the mechanisms responsible for controlling AsA levels in kiwifruit, we investigated the activities of enzymes related to AsA-GSH cycle system in 5 green-fleshed kiwifruit genotypes which grown in China.

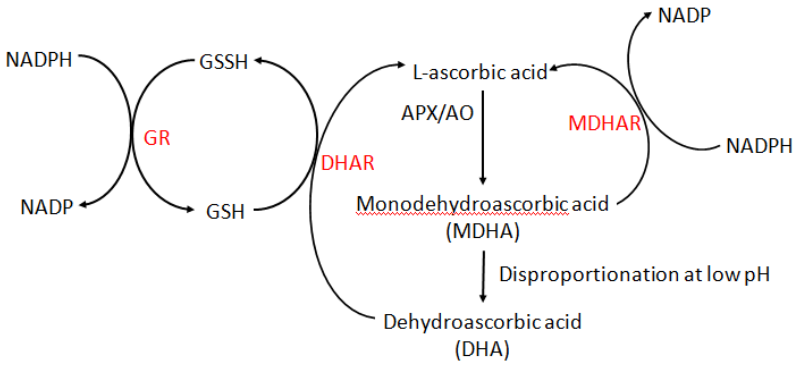

Fig.1 AsA-GSH cycle in plants

2. Materials and Methods

2.1 Plant Material

All kiwifruit genotypes used in this study were harvested from 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 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 -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 [3]. APX activity was assayed using the method of Nakano and Asada [4]. AO activity was assayed using the method of Pignochi and Foyer[5].

2.3 Assays of H_2O_2 Content

H_2O_2 content was determined by the method of Hao et al [6].

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 ± SD.

3. Results and Discussion

3.1 Activities of APX and AO and H_2O_2 content in flesh of 5 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 2A, a significant difference of APX activity was found in flesh of 5 green kiwifruit genotypes, the activity of APX ranged from 0.53 (Cuiyu) to 0.97U/g FW (Hayward). And there was no significant difference of APX activity in other three kiwifruit genotypes. As displayed in Figure 2B, the H_2O_2 content of 'Hayward' was the lowest, and there was no significant difference of H_2O_2 content in other four kiwifruit genotypes. The lower H_2O_2 content may be due to higher APX activity in green-fleshed kiwifruit, because APX can prevent the accumulation of toxic levels of H_2O_2 in the cell [7].

As shown in Figure 2C, AO activity ranged from 0.02 (Cuiyu) to 0.06 U/gFW (Kuimi). AO activity of 'Kuimi' kiwifruit was significantly higher than other kiwifruit genotypes, which was 3

times high than that in 'Cuiyu'. The AO catalyzes a complex reaction, which reduces safely the molecular oxygen into water without the release of ROS. 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 [8].

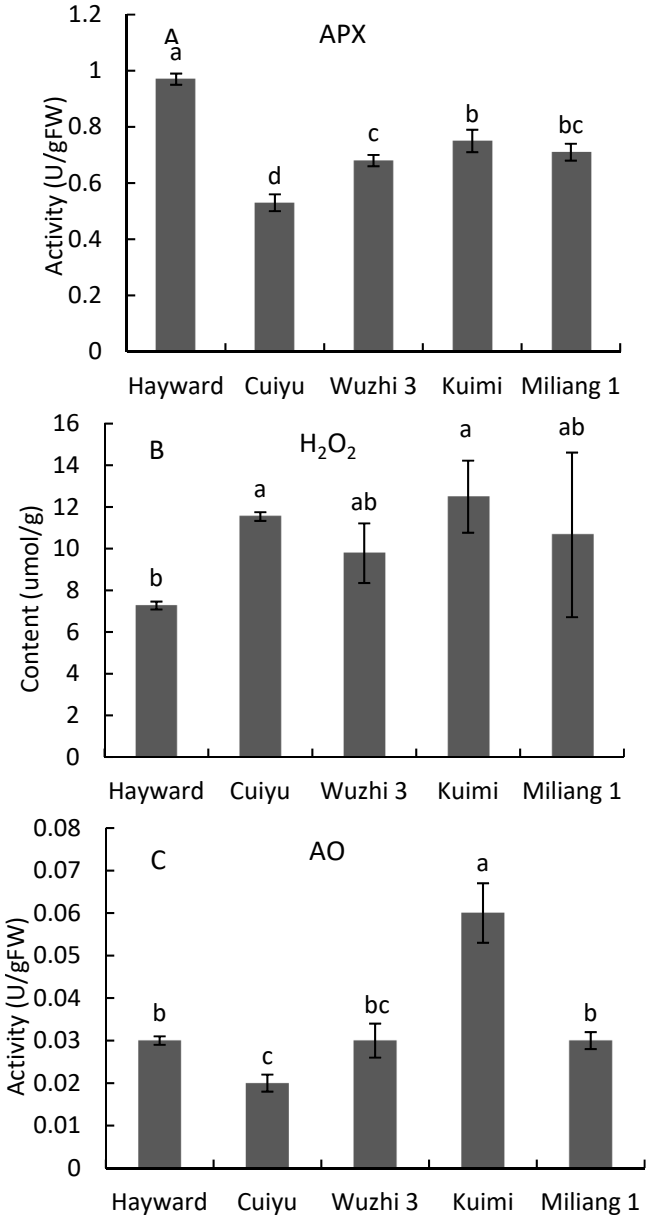

Fig.2 Activities of APX and AO and H_2O_2 content in five green-fleshed kiwifruit genotypes.
Note: Data with the different letters indicate the difference is significant ($P<0.05$). The same is as below.

3.2 Activities of DHAR, MDHAR and GR in flesh of 5 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[9]. As indicated in Figure 3A and B, the DHAR and MDHAR activities in 'Cuiyu' were the highest in five green-fleshed kiwifruit genotypes.

They were significant higher than those in other four genotypes. The DHAR activity in 'Cuiyu'is 1.87 times higher than that of 'Kuimi', and the activity of MDHAR is 4.67 times that of 'Kuimi'. As shown in Figure 3C, the highest activity of GR was measured in 'Wuzhi 3' and the lowest was in 'Hayward'. The highest one was the 2.5 times of the lowest.

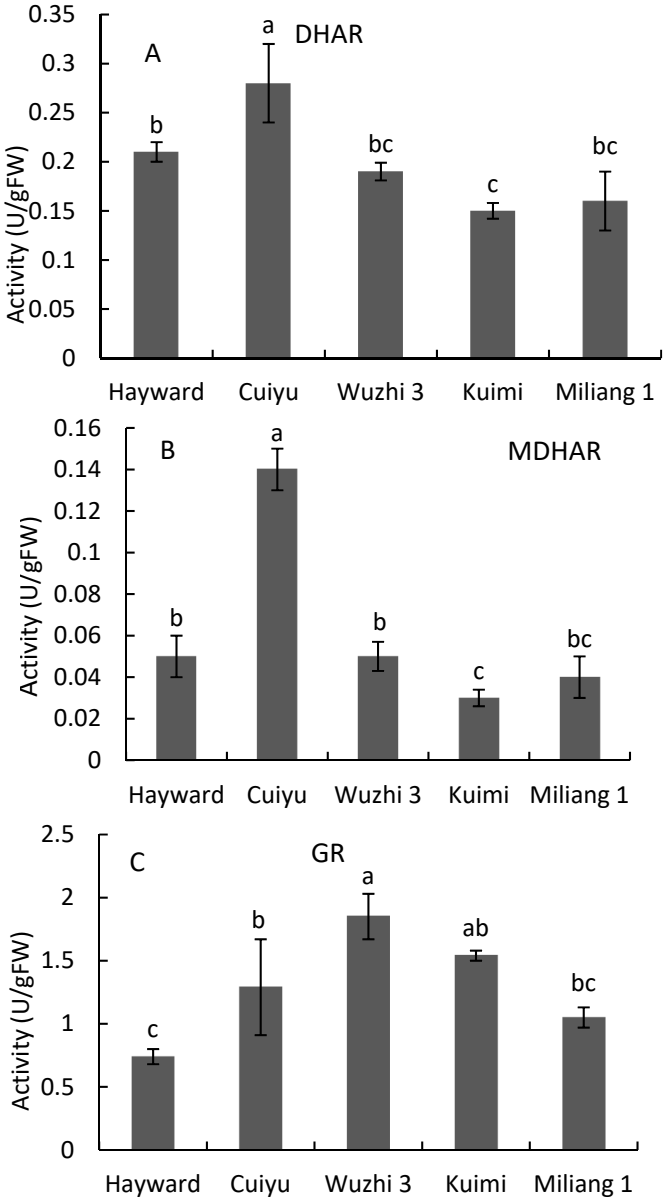

Fig.3 Activities of DHAR, MDHAR and GR in five green-fleshed kiwifruit genotypes.

4. Conclusions

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 [10]. H_2O_2 is active oxygen that is toxic to plants. The AsA-GSH cycle can effectively remove it under the action of APX and reduce its damage to plants cell. The activity of MDHAR, DHAR and GR played an important role in AsA-GSH cycle. In this study, we analyzed the activities of the important enzyme activity such as APX, DHAR, MDHAR, and GR which are involved in AsA–GSH cycle. We found that 'Hayward' performed highest activity of APX, 'Kuimi' performed highest activity of AO, 'Cuiyu' performed highest activities of DHAR and MDHAR, and 'Wuzhi 3' performed highest activity of GR. It showed

that they had high antioxidant activity. Overall, we found that there were still some differences in the enzyme activity of green-fleshed kiwifruits with different genotypes.

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