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Variation in Antioxidant Enzyme Activities of Two Strawberry Cultivars with Short-term Low Temperature Stress

Zhang Yong, Tang Hao-Ru and Luo Ya

Forestry and Horticultural College, Sichuan Agricultural University,
Ya'an, 625014, People's Republic of China

Abstract: Changes of superoxide(O_2) and hydrogen peroxide(H_2O_2), malondialdehyde(MDA) contents and activities of enzymes involving cell defense in leaves of strawberry (*Fragaria×ananassa* Duch) plantlets under different time of low temperature stress were studied. With the increase of stress times, the rates of O_2 generation and contents of H_2O_2 increased to a certain degree and then decreased. The MDA contents and the relative conductivity fluctuated were increased during the treatment. The activities of antioxidant enzymes, such as superoxide dismutase(SOD), catalase(CAT), peroxidase(POD) and ascorbate peroxidase(APX) were gradually increased to a certain degree and then decreased. The results clearly suggested that low temperature stress triggered an increase of reactive oxygen species (ROS) and the early accumulation of ROS in plants might lead to the production of antioxidant defense system. If the stress were too strong, the defense system of plants could not remove the more production of ROS effectively and result in severely damage to plants or even death.

Key words: Strawberry · Low temperature stress · Reactive oxygen species · Antioxidant enzyme · Lipid peroxidation

INTRODUCTION

Low temperature is one of the most important environmental factors that regulate plant growth and development and limit plant production [1]. Plants can respond and adapt to low temperature stress by several physiological, biochemical and molecular responses [2]. There is increasing evidence that low temperature stress induced the increase generation of reactive oxygen species (ROS) such as superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxide radicals (OH-) and singlet oxygen ($^1O_2$), which have greater toxicity potentials on biomolecules and membranes in plants [3]. Plants have antioxidant enzymes to keep the increase generation of ROS which includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) [4].

Strawberry (*Fragaria×ananassa* Duch) is one of nutritious fruits in the world. Injury of low temperature on strawberry is a serious problem in strawberry cultivation. Though there are many reports in the literatures detailing changes in the activities of enzymes involved in antioxidant metabolism in response to low temperature stress [5, 6], which clearly suggests that plants grown under low temperatures could trigger the changes of defense enzymes. To our knowledge, very little is known about the antioxidant defense mechanism in strawberry against stressful environments. We have carried out a study on the relationship between different times of low temperature stress and the antioxidant responses in two strawberry cultivars. Changes of the activities of the antioxidant enzymes (SOD, CAT, POD and APX) were monitored to evaluate the degree of tolerance in the two different strawberry cultivars subjected to low temperature stress.

MATERIALS AND METHODS

Plant Materials and Treatments: The leaves of two strawberry cultivars (*Fragaria×ananassa* cv. 'Toyonaka' and 'Zoji') were obtained from 4-week-old in vitro cultures. The culture was maintained on MS medium supplied with 0.5mg/L BA and 0.1mg/ L IBA with a 4-week interval in a growth chamber at 25±1°C and a light intensity of 4800lx provided by cool-white fluorescent lamps. For low temperature treatment (0°C), the cultures were transferred to the artificial intelligent

Corresponding Author: Dr. Tang Hao-Ru, Forestry and Horticultural College, Sichuan Agricultural University, Ya'an, 625014, People's Republic of China

growth chamber. The leaves were harvested at regular intervals of 0, 12, 24, 48 and 72 hours after low temperature treatment.

Determination of ROS: O_2 production was measured as described by determination kit (Nanjing Jiancheng Bioengineering Institute NJBI). O_2 production rates were demonstrated with □OD_{550} . min^{-1} . mg^{+} proteins.

H_2O_2 was extracted and its content was measured by monitoring the absorbance of the titanium-peroxide complex at 405 nm according to the method of Nanjing Jiancheng Bioengineering Institute (NJBI) determination kit. The contents of H_2O_2 was demonstrated with mmol•L^{-1}.

Enzyme Activity Assay: Total SOD (EC 1.15.1.1) activity was assayed by determination kit (Nanjing Jiancheng Bioengineering Institute NJBI). One unit of SOD activity was defined as the amount of enzyme required for 1mg tissue proteins in 1ml of a reaction mixture SOD inhibition rates to 50% as monitored at 550 nm. The activities of SOD were demonstrated with U mg^{-1} proteins.

CAT (EC 1.11.1.6) activity was assayed by determination kit (Nanjing Jiancheng Bioengineering Institute NJBI). One unit of CAT activity was defined as 1mg tissue proteins consumed 1μmol H_2O_2 at 405 nm for 1 second. The activities o f CAT were demonstrated with mg^{-1} proteins.

POD (EC 1.11.1.7) activity was determined specifically with guaiacol at 470 nm following the method of Amako *et al*. [7]. One ml of the enzyme extract was added to the reaction mixture containing 0.855 μl guaiacol solution and 1.355 μl hydrogen peroxide solution in 3 ml phosphate buffer (pH7.0). One unit of POD activity was defined as 1mg tissue proteins catalysised 0.01 μmol H_2O_2 for 1 min. The activities of POD were demonstrated with U mg^{-1} proteins.

APX (EC 1.11.1.11) activity was determined by following the method of Nakano and Asada [8]. Two ml of a reaction mixture containing 1.66 ml of 0.5 mmol/L ascorbate in phosphate buffer (pH 7.0), 0.26 ml of 2 mmol/L H_2O_2, both of which were freshly prepared and 0.08 ml of enzyme extract. One unit of APX activity was defined as 1mg tissue proteins catalysised 1 μmol ascorbate at 290 nm for 1 min. The activities of APX were demonstrated with U mg^{-1} proteins.

Lipid Peroxidation: Relative conductivity was determined according to the method of Blum and Ebercon [9]. Samples of leaves were rinsed with distilled water for 2 h. The conductivity of the solution (R1) was measured with a conductivity meter (Model DDS-11A, Shanghai Dazhong Instrument Inc., Shanghai, China). Samples were then heated in boiling water for 5 min and then cooled to room temperature. The conductivity of killed tissue (R2) was again measured. Relative conductivity was calculated as the ratios of R1 to R2.

Lipid peroxidation was determined as the concentration of TBA-reactive products, equated with malondialdehyde (MDA), as originally described by Hendry *et al.* [10]. Four ml of a reaction mixture containing 3 ml of 0.5% TBA and 1 ml of extract were boiled for 10 min and then quickly cooled to room temperature. The contents of TBA reactive products (MDA) were expressed as μmol•g^{-1}FW.

Statistical Analysis: The experiments were repeated at least 3 times with 3 replicates for each. All the data in this study were expressed as means±SD. The data were analyzed using one-way analysis of variance and Duncan's multiple range test at the 5% level of significance from the DPS 7.05 package for windows.

RESULTS

Effects of Low Temperature Stress on Ros Contents in Strawberry Leaves: The effects of low temperature stress on the production of O_2 and the contents of H_2O_2 in leaves of the two cultivars 'Toyonaka' and 'Zoji' were shown in Fig. 1. Low temperature stress led to an increase in the generation of $O_2^{£-}$ and H_2O_2, but a sharp increase in the production of O_2 in both strawberry cultivars occurred within the first 12 h after treatment and the production of O_2 reached the maximum at 24 h or 48 h after treatment (Fig. 1A). While the contents of H_2O_2 reached the highest at 12 h and 48 h after treatment respectively for 'Toyonaka' and 'Zoji' (Fig. 1B). After that, although the production of O_2 and H_2O_2 slowly decreased but it was significantly higher than that of control.

Effects of Low Temperature Stress on Antioxidant Enzymes Activities in Strawberry Leaves: The strawberry plantlets treated with 0°C showed a significant enhancement in the activities of SOD, CAT and POD during all the period of the experiment (Fig. 2A, B, C). The activity of SOD increased rapidly at the beginning of the treatment and reached the highest for 'Toyonaka' and 'Zoji'. After that, the activity of SOD showed a little decrease, but kept at a high level (Fig. 2A). The activity of CAT showed a rapid increase and a fast decrease at the

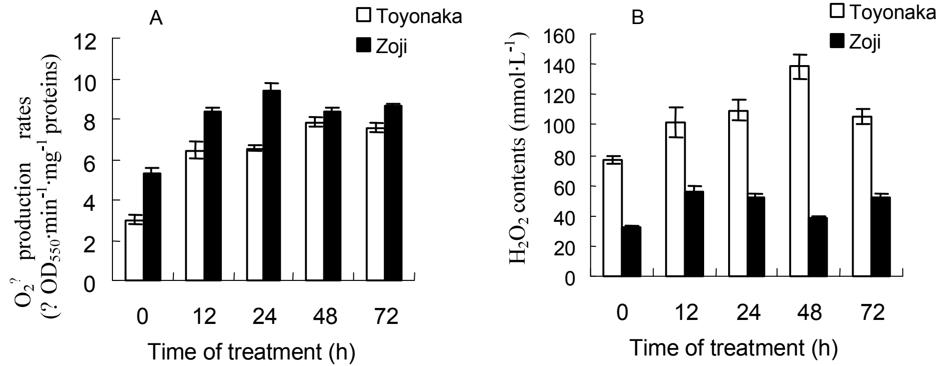

Fig. 1: Changes in $O_2^{£-}$ production rates (A) and contents of H_2O_2 (B) in leaves of strawberry treated with 0°C for different times. All values are means ± SD of three replicates

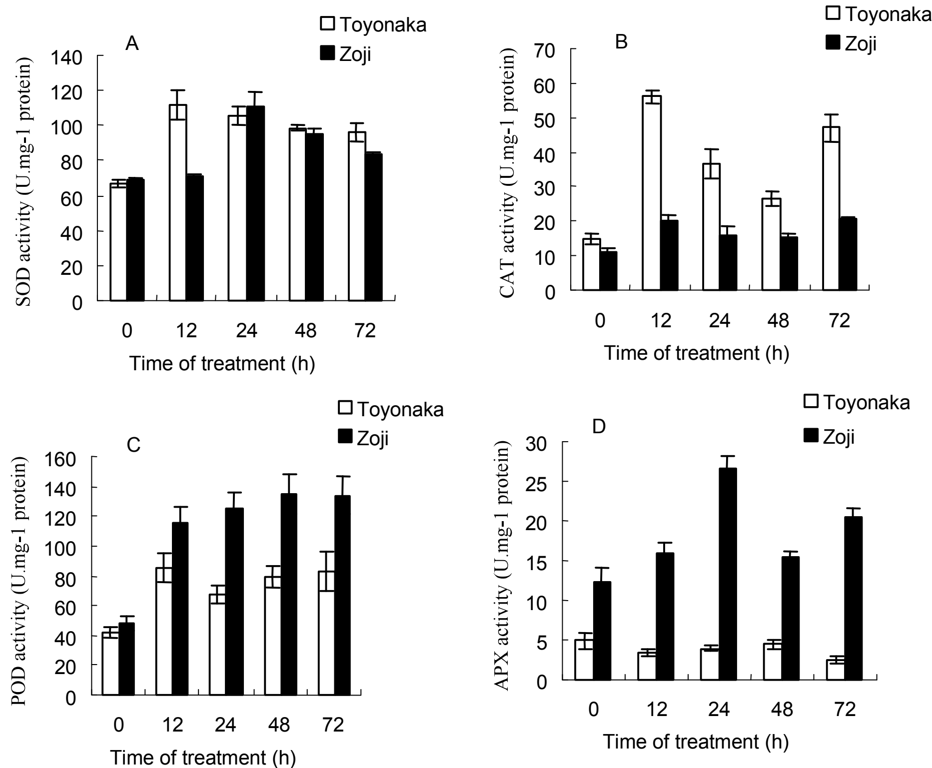

Fig. 2: Changes of the activities of antioxidant enzymes SOD (A), CAT (B), POD (C) and APX (D) in leaves of strawberry plantlets treated with 0°C for different times. All values are means ± *SD* of three replicates

initial phase of cold stress and then increased and kept in a high level (Fig. 2B). The activity of POD showed a sharp increase at the beginning of the treatment and a stable increase during the experimental period in both strawberry cultivars except the 24 h treatment (Fig. 2C). The activities of APX in 'Zoji' and 'Toyonaka' showed a different variation. In 'Zoji' leaves, the activities of APX increased rapidly and reached the maximum firstly, which was 3.7 times higher compared to the normal temperature leaves. After that, it decreased at the end of the treatment, but it was obvious higher than that of control. In 'Toyonaka' leaves, the activities of APX showed little changes and always kept at a very low level during all the period of the treatment (Fig. 2D).

Effects of Low Temperature Stress on Lipid Peroxidation of Strawberry Leaves: Low temperature stress led to an increase in the generation of MDA in both strawberry

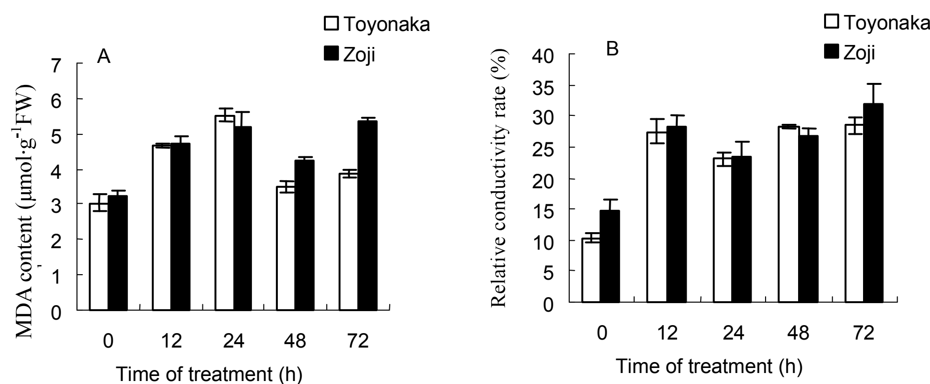

Fig. 3: Changes in the contents of MDA (A) and the relative conductivity (B) in leaves of strawberry treated with 0°C for different times. All values are means ± *SD* of three replicates

leaves. The production of MDA increased rapidly and reached the highest quickly and kept at the high level during the experimental periods except the 48 h treatment (Fig. 3A). As the contents increasing of MDA, low temperature stress increased the relative conductivity of both strawberry cultivars were fluctuated increased by 55~141% and 37~114%, respectively during the time of low temperature treatment (Fig. 3B).

DISCUSSION

The imposition of both abiotic and biotic stresses causes overproduction of ROS, which ultimately imposes a secondary oxidative stress in plant cells [11]. Overproduction of ROS might have a detrimental effect on the metabolism, growth and development of cells through their ability to initiate reaction cascades that resulted in the production of toxic chemical species, such as hydroxyl radicals and lipid peroxides, ending in cell dysfunction and death [12, 13]. In parallel, however, ROS might also have a positive role in plant growth and development. In this study, the fast increasing and keeping at a high level of production of O_2^- and the contents of H_2O_2 in both strawberry leaves treated with low temperature suggested that O_2^- and H_2O_2 might serve as signal molecules. The early accumulation of ROS in *Arabidopsis* and maize under chilling was found to signal productions of antioxidant enzymes [14, 15]. A dual role for H_2O_2 in the regeneration of protoplasts had also been demonstrated [16, 17].

The effects of antioxidant enzymes in low temperature stress have been demonstrated in many experiments [5, 6, 18]. SOD converts O_2^- radicals into H_2O_2 and O_2. CAT dismutases H_2O_2 into water and O_2, whereas POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds or antioxidants. APX utilises AsA as an electron donor in the neutralization of H_2O_2[19, 20].

CAT, POD and APX appeared to play an essential protective role in the scavenging processes when coordinated with SOD activity [21]. They are chloroplastic or cytosolic enzymes which scavenged H_2O_2 generated primarily through SOD action. An increase in the activity of CAT and POD were observed in both strawberry leaves on low temperature treatment (Fig. 2B, C). The activity of APX increased almost coordinately with SOD activity in both strawberry leaves (Fig. 2D), although the activity of APX in the two cultivars showed a different changement under low temperature stress. This might demonstrate gene-dependence in changes of antioxidant enzymes.

Under the consecutive low temperature stress, the relative conductivity of strawberry leaves and the contents of MDA were increasing. Peroxidation of lipids (primarily the phospholipids of cell membranes) was mechanistically important from free-radical production perspective as it was one of the few examples of carbon centred radical production in plant cells [13]. Low temperature stress directly leads to accumulation of ROS and lipids peroxidation in plant cells appears to be initiated by a number of ROS [4].

In conclusion, our results clearly suggested that low temperature stress triggered an increase of ROS and the early accumulation of ROS in plants might lead to the production of antioxidant defense system. The overproduction of ROS might cause membrane lipid peroxidation, which was injurious for plant cells. The cell defense enzyme could increase its activities to prevent the plants not to be damaged and to keep their homeostasis. If the stress were too strong, the defense system of plants could not remove the more production of ROS effectively and the antioxidant enzymes activities decreased, resulting in severely damage to plants or even death.

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