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EFFECTS OF LOW TEMPERATURE ON MEMBRANE LIPID PEROXIDATION AND ACTIVITIES OF PROTECTIVE ENZYMES IN PERSIMMON (*DIOSPYROS* SPP.) ROOTSTOCKS

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Abstract

The relative electric conductivity, MDA content and activities of protective enzymes (SOD、POD and CAT) in isolated branches of *Diospyros lotus* L., *Diospyros D. oleifera* W.C. Cheng and *Diospyros D. glaucifolia* Metcalf were determined, and the effects of low temperature on the membrane lipid peroxidation and activities of protective enzymes of persimmon rootstocks branches were analyzed. The results indicated that, with the decrease of temperature, the relative electric conductivity and MDA content presented an upward trend, but their increment in *D. lotus* was lower than that in *D. oleifera* and *D. glaucifolia*. The activities of SOD, POD and CAT increased at first and then decreased in all the three species, but the increment in *D. lotus* was higher than that of *D. oleifera* and *D. glaucifolia*. It may be concluded that *D. lotus* could effectively enhance the activities of protective enzymes，further inhibit the accumulation of free radicals during low temperature，and thus reduce the damage of free radicals to *D. lotus*. This might be one of important reasons for higher cold-resistance of *D. lotus*.

Introduction

Temperature is an important factor which limits the geographical distribution of plants and affects their biological production. By freeze injury, the production of fruit trees was found to reduce, and even frozen to die in many areas. Under low temperature, a series of changes in plant morphology and physiological characteristics happen to increase its cold resistance. Under artificially simulated low temperature, cold resistance of fruit trees was appraised and evaluated by measuring the changes of physiological and biochemical indexes. At present, the cold resistance of apricot plum (Liu and Huo 2008), pomegranate (Song *et al*. 2012), walnut (Xiang *et al*. 2011, Xiang *et al*. 2014), peach (Yang *et al*. 2005), strawberry (Luo *et al*. 2007), grape (Li *et al*. 2017), pear (Zhang *et al*. 2004), apple (Gao *et al*. 2000) and other fruit trees were reported, and the study of the cold resistance of persimmon and its rootstock was few. Among 3 species and 17 cultivated species of persimmon plants, the difference of cold resistance among them was higher than that of their cultivated varieties (Leng and Zhi 2001). In terms of frost symptoms, tree types of freezing injury, frost damage and variety types, the cold tolerance of persimmon trees was investigated and analyzed (Yang *et al*. 2010). By using principal component analysis and membership function method, the cold resistance of 39 persimmon varieties was comprehensively evaluated (Zhao *et al*. 2010).

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In the present experiment, the relative electrical conductivity, elaborate MDA content, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity of 3 kinds of persimmon rootstock were measured, and the relationship between the indecies and the cold resistance in them was discussed. This provided the theoretical basis for selection of cold resistant rootstocks and for the physiological study of cold resistance of persimmon.

Materials and Methods

Three different cold-resistant rootstocks of persimmon, such as *D. lotus*, *D. oleifera* and *D. glaucifolia,* were used. In early November, 2015 the branches were collected from the national Persimmon Germplasm garden of Northwest Agriculture and Forestry University. Three big trees of each species with strong growth and free of disease and insect pests were selected. At the periphery of the crown, 40 healthy young shoots with medium growth and no pests and diseases were cut. The collected branches were cut into about 20cm, rinsed with tap water and then with distilled water for 3 times, the water was drained with absorbent paper, wrapped in a damp gauze bag and placed in the refrigerator (4°C) for further research. The treated branches were wrapped in damp gauze bag and placed into temperature-controlled refrigerator for low temperature treatment. The temperature gradient was as follows: normal temperature (4°C), −5, −10, −15, −20, −25, −30 and −35°C. The cooling rate was 4°C/hr, and the temperature was maintained 12 hrs after reaching the target temperature, then gradually warmed up, the heating rate was 4°C/hrs. The branches at 4°C centigrade were compared with the control (CK). The treated branches were grinded into small slices of 0.1 - 0.2 cm and 0.5 g was used for the determination of various physiological indecies. Each treatment was set 3 times.

According to Li Hesheng's method (Li 2000), the relative conductivity was measured by DDS-720 type conductivity meter; the content of malondialdehyde (MDA) was determined by thiobarbituric acid method; the activity of superoxide dismutase (SOD) was determined by NBT reduction method; the activity of peroxisase (POD) was determined by guaiacol method; and the activity of catalase (CAT) was determined by UV spectrophotometry.

The experimental data were analyzed by using SPSS 13, and Microsoft Excel 2003 for plotting.

Results and Discussion

The relative electrical conductivity of 3 persimmon rootstocks under low temperature is shown in Fig. 1. With the decrease of treatment temperature, the relative electrical conductivity of 3 species of persimmon rootstocks showed an upward trend. Taking the case of *D. lotus*, the relative electrical conductivity of the branches changed little, when the temperature was reduced from 4 to −5°C. When the temperature dropped from −5 to −10°C, the relative conductivity of the branches increased sharply, and the increase was 22.1%.When the temperature dropped from −10 to −20, the relative conductivity of the branches increased slowly; when the temperature dropped from −20 to −25°C, the relative electrical conductivity of branches increased again and increased by 31.1%. When the temperature dropped from −25 to −35°C, the relative electrical conductivity of branches increased slowly. The similar change was observed in *D. oleifera* and *D. glaucifolia*. Comparing the relative conductivity of different varieties, it was found that when the temperature dropped from 4 to −35°C, the relative conductivity of the 3 persimmon rootstock increased 2.9 (*D. lotus*), 3.1 (*D. oleifera*) and 4.1 times (*D. glaucifolia*), which indicated that the low temperature resistance of *D. lotus* was stronger than that of *D. oleifera* and *D. glaucifolia*.

The malondialdehyde content of 3 persimmon rootstocks under low temperature is presented in Fig. 2. With the decrease of treatment temperature, the malondialdehyde content of 3 species of persimmon rootstocks was found to increase. Taking the case of *D. lotus*, the malondialdehyde content in the branches increased slowly when the temperature dropped from 4 to −20°C. When the temperature dropped from −20 to −35°C, the malondialdehyde content increased sharply, and the increase was 0.87, 1.15 and 1.59 times, respectively. The similar change was observed in *D. oleifera* and *D. glaucifolia*. When the temperature dropped from 4 to −35°C, the increase of malondialdehyde content (3.95 times) of *D. lotus* was similar to that of *D. oleifera* (3.96 times), both of which were lower than that of *D.glaucifolia* (4.39 times). This finding indicates that the 3 kinds of persimmon rootstocks showed different ability to resist the low temperature.

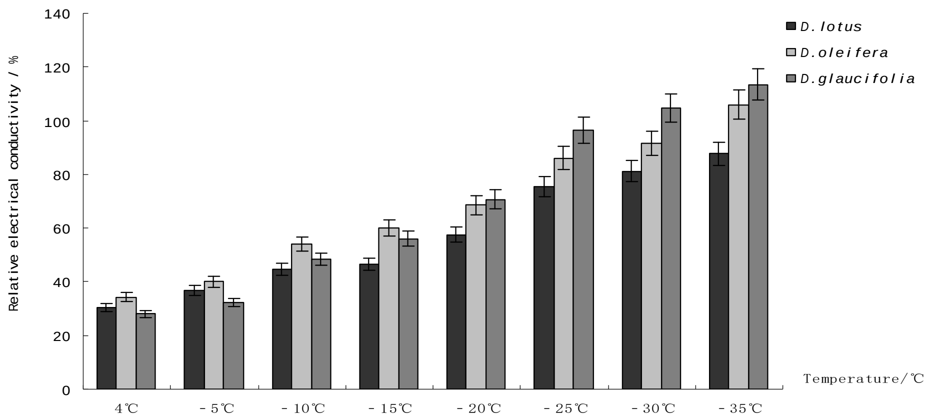

Fig. 1. Effect of low temperature on relative electrical conductivity of persimmon rootstocks.

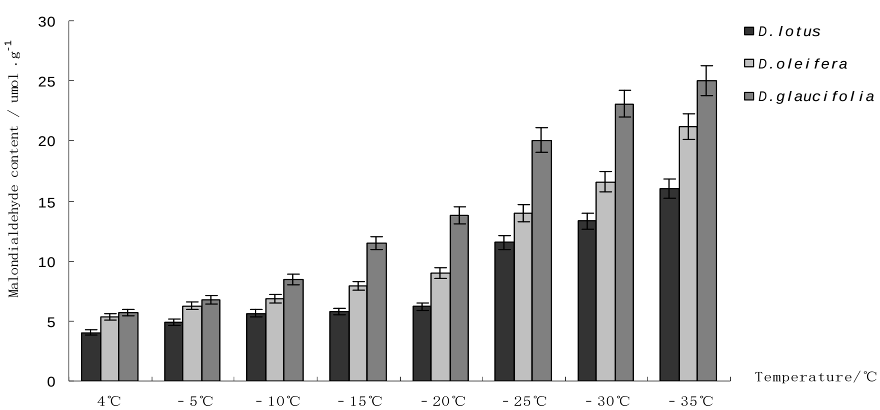

Fig. 2. Effect of low temperature on malondialdehyde content of persimmon rootstocks.

Under low temperature, the changes of SOD activity of 3 persimmon rootstocks are shown in Fig. 3. With the decrease of treatment temperature, the SOD activity of 3 persimmon rootstocks increased first and then decreased. Taking *D. lotus* as an example, the activity of SOD was 2.12 u• g^{-1}• min^{-1} at 4°C and increased rapidly at the temperature from 4 to –20ºC. The highest value of SOD activity was 15.26 u•g^{-1}• min^{-1}at –20ºC, the increase was 7.2 times. Then the SOD activity decreased with the reduction of treatment temperature, and the activity of SOD was only 6.43 u• g^{-1}• min^{-1} at −35°C. The similar change was observed in *D. oleifera* and *D. glaucifolia*. The SOD activity of the persimmon rootstocks reached the highest value at −20°C, which was 15.26, 12 and 13.79 u•g^{-1}•min^{-1}, respectively, and the increase was 7.2, 2.89 and 4.81 times, respectively. Under low temperature, *D. lotus* showed a strong ability to scavenge free radicals.

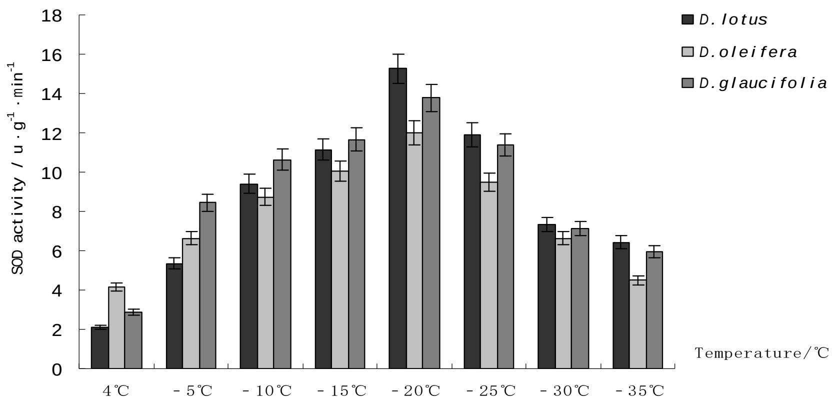

Fig. 3. Effects of low temperature on SOD activity of persimmon rootstocks.

The changes of POD activity in the branches of 3 persimmon rootstocks under low temperature are presented in Fig. 4. With the reduction of treatment temperature, the POD activity of the 3 persimmon rootstock branches rose first and then declined, which was similar to the SOD activity. In case of *D. lotus*, the POD activity was 1.34 u•g^{-1}•min^{-1} at 4°C. When the temperature was reduced from 4 to –20°C, the POD activity increased rapidly, and reached the highest value 10.72 u• g^{-1}• min^{-1} at –20°C, and the increase was 8 times. Then the POD activity decreased with the reduction of the temperature. The activity of POD was only 4.07 u•g^{-1}•min^{-1} at –35°C. The similar change was observed in *D. oleifera* and *D. glaucifolia*. The POD activity in the branches of 3 persimmon rootstocks reached the highest value at -20ºC,which were 10.72, 8.44 and 9.55 u•g^{-1}• min^{-1}, respectively, and the increase was 8, 3.44 and 4.53 times, respectively.

Under low temperature, the changes of CAT activity of 3 persimmon rootstocks were observed with the reduction of treatment temperature (Fig. 5). The CAT activity of 3 persimmon rootstocks rose first and then declined. , This result is similar to that of SOD and POD. Taking *D. lotus* as an example, the CAT activity was 5.42 u• g^{-1} • min^{-1} at 4ºC and increased rapidly at the temperature from 4 to –20ºC, reached the highest value at 22.66 u• g^{-1} • min^{-1}, and the increase was 4.18 times. Then the CAT activity decreased with the reduction of treatment temperature, and the CAT activity was only 4.03 u•g^{-1}•min^{-1} at –35ºC. The similar change was observed in *D. oleifera* and *D. glaucifolia*. The CAT activity of 3 persimmon rootstocks reached the highest value 22.66, 23.21 and 18.69 u•g^{-1}•min^{-1}, respectively at –2ºC, and the increase was 4.18, 3.77 and 3.18 times, respectively.

Under normal conditions, free radical scavenging systems were composed of SOD, POD and CAT in plants. The intracellular free radicals were at a low level, which was not enough to cause destruction to them. When plants were under stress, the protection system was destroyed, and the free radicals would accumulate in large quantities. Too many free radicals would injure the cells, causing the increase of cell membrane permeability and producing a large number of MDA

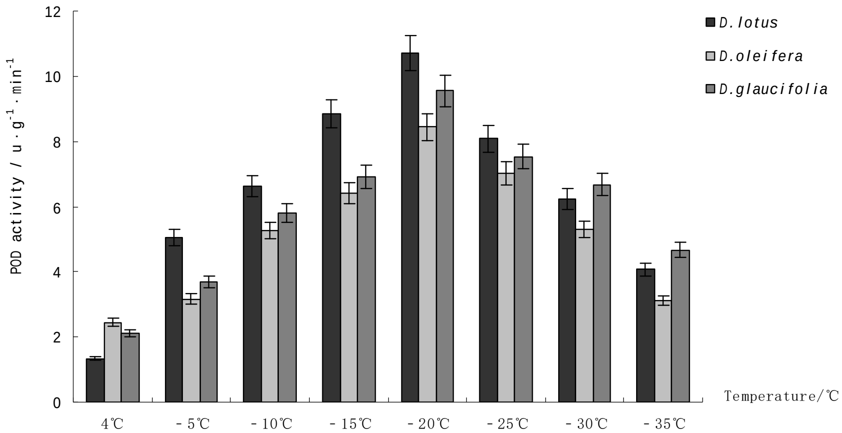

Fig. 4.Effects of low temperature on POD activity of persimmon rootstocks.

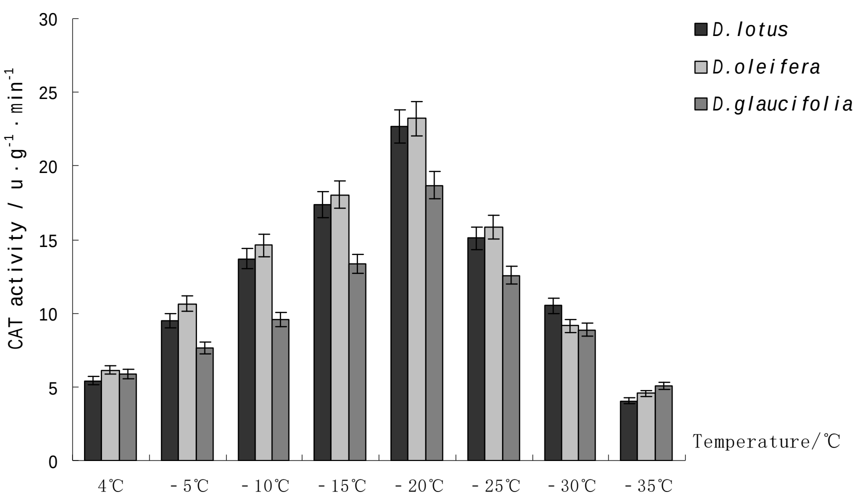

Fig. 5. Effect of low temperature on CAT activity of persimmon rootstocks.

(Liu and Huo 2008, Song *et al*. 2012). In the present study, it was found that with the decrease of temperature, the relative conductivity and malondialdehyde content of 3 persimmon rootstocks showed a rising trend. But the increase of *D. lotus* was lower than that of *D. oleifera* and *D. glaucifolia*, which indicated that the degree of damage in *D. lotus* was lower than that of *D. oleifera* and *D. glaucifolia*. These observations are in agreement with the results of the study on the walnut (Xiang *et al*. 2011, Xiang *et al*. 2014). At the same time, the activity of SOD, POD and CAT in the branches of 3 persimmon rootstocks increased rapidly when the temperature was reduced from 4 to −20°C and reached the highest value at −20°C. At lower temperatures, the SOD, POD and CAT activities were increased to remove the excessive free radicals in the cells, and to protect the cell membrane, which enhanced the cold resistance of 3 persimmon rootstocks. When the temperature dropped from −20 to −35°C, the activity of SOD, POD and CAT in the branches of 3 persimmon rootstocks declined rapidly. It might be due to the decrease of the temperature in the plant, the damage caused by the low temperature eventually broke the metabolic balance in the plant, and the activity of the enzyme decreased, and the ability to remove the free radical decreased simultaneously, causing the plant to decrease. The imbalance of the production and the elimination of free radicals in the body eventually lead to the occurrence of low temperature injury symptoms in plants (Luo *et al.* 2007). By comparing the increase of the enzyme activity in the branches of 3 persimmon rootstocks, it was found that the increase of the enzyme activity of *D. lotus* was higher than that of *D. oleifera* and *D. glaucifolia*, which further indicated that the ability of *D. lotus* to resist the low temperature was higher than that of *D. oleifera* and *D. glaucifolia*.

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