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Effect of astaxanthin on Strawberry Seedlings under low temperature stress on active oxygen metabolism

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Keywords: Astaxanthin; Strawberry; Low temperature stress; Active oxygen

Abstract: In order to solve the problem of low temperature damage in strawberry production, 'Benihoppe' strawberry (*Fragaria ananassa* Duch) was used as a material, so the effects of astaxanthin treatment on strawberry seedlings under low temperature stress was studies. The results showed that compared with the control, the antioxidant enzyme (SOD, POD, CAT, APX) activity and FRAP value of strawberry leaves increased; meanwhile the production rate of O_2^-, H_2O_2 and MDA were decreased in all astaxanthin treated strawberry seedlings under low temperature stress. Especially the treatment of 50 μmol/L astaxanthin had the best effect, indicating that the astaxanthin treatment in suitable concentration can effectively reduce the injuries in strawberry seedlings under low temperature.

Introduction

Low temperature is one of the main limiting factors affecting the growth, development and distribution of plants, and low temperature adversity leads to excessive production and accumulation of reactive oxygen species (O_2^- and H_2O_2, OH^- and et al), so as to aggravate the hyperoxidation of cell membrane lipids, denaturation of protein and nucleotide's injury, even leading to the death of cell [1]. When plant is under low temperature stress, its own will produce a series of defense mechanisms, such as anti-oxidation enzymes, anti-stress proteins and other substances to combat free radicals,so it can protect plants from reactive oxygen [2] ,and enhance the resistance of plants to cold. Therefore, antioxidant metabolism plays an important role in protecting plants under low temperature stress. Astaxanthin is a carotenoid that is not a source of vitamin A [3], and the important property is that it is extremely strong resistance to oxidation, many previous studies have shown that it is a good antioxidant, playing an important role in quenching free radicals [4, 5]. Manys studies have been conducted on the use of astaxanthin for human medicine and livestock feed, and the use of biotechnology for the production of astaxanthina yet at present [6-8]. However there was little research about the effect of astaxanthin on the antioxidant activity and the metabolism of plant active oxygen.

Strawberry (*Fragaria* × *ananassa* Duch), is a perennial fruit tree crop, the fruit is soft, and juicy has rich nutrition. Its yield and cultivation area have always been the first in the production of small berries [9]. Because the protection facilities are poor, the short-term low temperature injury occasionally would occured in the strawberry cultivation process in winter, thus causing great loss to strawberry production. Based on the strong antioxidant properities of astaxanthin, this study was to explore the effecs of astaxanthin on active oxygen metabolism of strawberry seedlings under low temperature.

Materials and Methods

Materials collection. In this experiment, the test-tube seedling leaves of 'Benihoppe ' were used as experimental materials. The test-tube seedlings were established and preserved by biotechnology of college of horticulture, Sichuan Agricultural Uiniversity in September 2016. The MS medium includes 1 mg/L BA, 30 g/L sucrose and 7 g/L agar. The culture condition is 25±1℃, 2500 Lx and daylight for 16 hours a day.

Experimental Design. Through preliminary pilot study, screening and determining the proper astaxanthin concentration in this study. First, the strawberry tube-seedlings were cultivated for four weeks, and then 0, 5, 10, 50 and 100 μmol/L astaxanthin were respectively added to the medium. After half a month of cultivating, the test-tube seedlings were put in incubator at 4℃. After 0, 3, 6, 12, 24, 48 hours in low temperature, the leaves were respectively cut from the seedlings,which were used to measure the relevant indicators,such as the superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) and ascorbic acid peroxidase (APX), FRAP and the production rate of superoxide anion (O_2^-), hydrogen peroxide(H_2O_2) and malondialdehyde(MDA) content. The experiment was repeated three times.

Statistical Analyses Statistical analyses were conducted using statistical software of SPSS 17.0. Data were analyzed by one-way ANOVA with least significant difference at 5% confidence level.

Results and Discussion

The superoxide anion production rate(O_2^-). As showed in Fig.1, compared with the control, at low temperature stress, the 5，10，50 and 100 μmol/L astaxanthin all can reduce the production rate of superoxide significantly. To some extent, as the treatment concentration increases, the rate of the production is also rising, but the effect of 50 μmol/L is better than 100 μmol/L. And at 12 hours of cold treatment, the rate of production is maximized. The results showed that the proper concentration of astaxanthin could effectively reduce the production rate of superoxide anion under cold stress.

Hydrogen peroxide (H_2O_2). As showed in Fig.2, compared with the control, at low temperature stress, the 5，10，50 and 100 μmol/L astaxanthin can reduce the amount of hydrogen peroxide. And with the cold treatment time increases, H_2O_2 content also increases. Similar to the superoxide anions, with the increase of astaxanthin content, the effect of removing hydrogen peroxide is better.

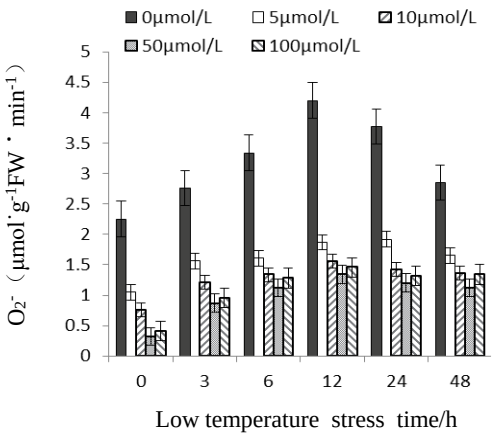

Fig.1 Effect of astaxanthin on the production rate of the oxygenatede in the leaves of strawberry seedlings at low temperature stress

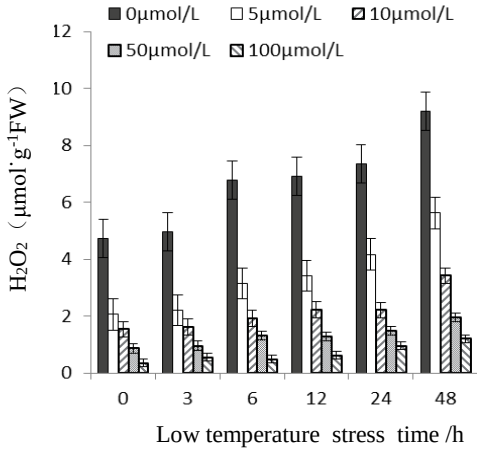

Fig.2 Effect of astaxanthin on the concent of hydrogen peroxide in the leaves of strawberry seedling at low temperature stress

Activity of protective enzyme activity (SOD 、CAT、POD、APX). As showed in Fig.3, under cold stress, the activity of four antioxidant enzymes SOD, CAT, POD and APX were significantly increased under different concentrations of astaxanthin. However, cold treatment at different times

did not significantly affect the antioxidant enzyme activities, and there is no obvious change in its laws. Among them, 50 μmol/L treatment had the best effect on the enhancement of SOD and POD activity; however, the optimal concentrations for enhancing CAT and APX activities were 10 μmol/L and 100 μmol/L, respectively.

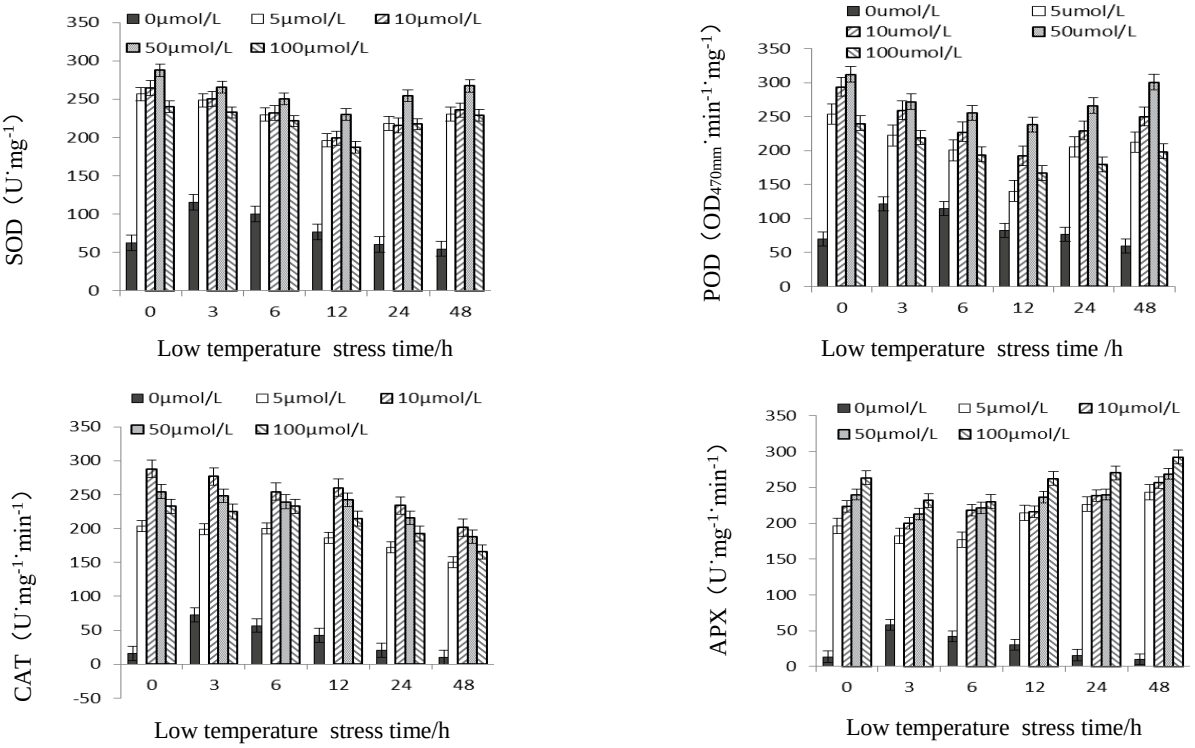

Fig.3 Effects of astaxanthin on the activity of SOD、POD、CAT and APX in strawberry seedling leaves under low temperature stress

Conclusions

The damage to plants caused by low temperature stress is derived from membrane system [10]. It decreased the activity of the enzymes in plants, and the enzymes can scaveng free radicals; therefore, the accumulation of free radicals is one of the important reasons to damage plants. So, removing radicals such as O_2^- and H_2O_2 in time, and avoiding excessive oxidation of planst cell membrane lipids is crucial. The research data showed that SOD was the enzyme to remove the O_2^-,while CAT, APX and POD were the key enzymes to remove H_2O_2[11,12].In this experiment, under low temperature stress, compared with the control, the four concentrations of astaxanthin can improve the activity of protective enzymes (SOD、POD、CAT、APX).

Recent research shows that low temperature adversity can promote the accumulation of reactive oxygen (O_2^-, H_2O_2, etc.) and harm plant cells. Therefore, the production rate of O_2^- and the accumulation of H_2O_2 in plants reflect the degree of damage to plants[13-14]. In this experiment, under low temperature stress, compared with the control, the four concentrations of astaxanthin can reduce the production rate of O_2^- and amount of H_2O_2.

The results of this study showed that the treatment of astaxanthin can increase the antioxidant capacity of strawberry seedling, and the astaxanthin treatment in suitable concentration can effectively reduce the damage of temperature stress in strawberry seedlings under low temperature stress. Especially, the concentration of 50μmol/L had the best effect.

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