

Effect of Astaxanthin on the Growth and Resistance of Strawberry Seedling under Cadmium Stress

Yu'ting Jiang ^{1,a}, Ya Luo^{1,b}, Fan Mo^{1,c}, Ya'jie Ling^{1,d}, Qin Mo^{1,e}, Shu Luo^{1,f} and Hao'ru Tang^{1,g*}

¹College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

^a2863170989@qq.com, ^b173698873@qq.com, ^c853861848@qq.com, ^d2208312818@qq.com, ^e943156105@qq.com, ^f626567947@qq.com and ^g1048019614@qq.com

*Corresponding author. Yu'ting Jiang, Ya Luo and Fan Mo contributed equally to this work.

Keywords: Astaxanthin; Strawberry Tissue culture seedlings; Cadmium stress; Resistant

Abstract: In this experiment, we studied the effects of astaxanthin on the growth and resistance of strawberry tissue culture seedlings under the stress of heavy metal cadmium. Strawberry seedlings were planted in MS medium supplemented with different concentrations of astaxanthin and with 25 mg/L GdCl₂. The results showed that the content of chlorophyll increased and the content of MDA decreased in the strawberry plantlets treated with astaxanthin compared with the control. The activity of SOD, POD and CAT increased in astaxanthin treated plantlets with low concentration (5 μmol/L~100 μmol/L), but decreased with high concentration. The content of vitamin C increased with astaxanthin treatment of 5~50 μmol/L, and decreased with the treatment of 100~200 μmol/L of astaxanthin. Meanwhile, astaxanthin treatment at 5 μmol/L showed the best antioxidant activity. Thus, low concentrations of astaxanthin could alleviate the damage in strawberry tissue culture seedlings under the stress of heavy metal cadmium, but the high concentration of astaxanthin could not relieve the damage. According to the indexes, the optimal concentration of astaxanthin to alleviate cadmium stress is between 10~100 μmol/L.

Introduction

Strawberry (*Fragaria ananassa* Duch.) is Rosaceae strawberry genus perennial herb evergreen plants.

It is an adaptable strawberry with early results, short growth cycle, rich nutrient and high economic value, loved by the people of fruit [1]. In the world of small berries production, strawberry is first, and it is also known as the yacon and cranberry; native to Europe, introduced to our country in the early 20th century, classified as berry in horticulture. Strawberry berry is full of bright red, juicy, sweet, sour, delicious and nutritious, deeply loved by consumers, thus as the fruit treasures [2].

Astaxanthin, referred to as ASTA, is a purple crystalline powder that is insoluble in water and soluble in solvents such as acetone and chloroform. The chemical name of astaxanthin is 3,3'-dihydroxy-4,4'-dione-β, β'-carotene, which molecular formula is C₄₀H₅₂O₄, and molecular mass is 596.86 [3]. Due to the longer conjugate double bond [4] in astaxanthin molecules, the hydroxyl group and the unsaturated ketone at the end of the conjugated double bond, in which the hydroxyl group and the carbonyl group, in turn, constitute an α-hydroxyketone such that the structure has a more energetic electronic effect that provides electrons to free radicals or absorbs unpaired electrons in free radicals, easy to react with free radicals so as to eliminate it play a role in

scavenging free radicals.

Many studies have shown that the astaxanthin antioxidant activity is rather than other carotenoids. Astaxanthin also has the ability to inhibit lipid peroxidation, which is 100 times more potent than alpha-tocopherol in inhibiting lipid peroxidation and protects phosphatidylcholine lipids against oxidation [5].

Heavy metals continue to accumulate in the soil, resulting in deteriorating soil quality, decreasing the quality of agricultural products, and also entering the human body through the food chain and endangering human health. The application of biogas fertilizer on the contents of heavy metals in strawberry fruits has been studied [6], and the results show that the application of biogas slurry can effectively reduce the content of strawberry fruit mercury, arsenic and cadmium. Studies have shown that selenium can protect the integrity of cell membranes and reduce the absorption of heavy metals cadmium, lead by removing malondialdehyde (MDA), a membrane lipid peroxidation product [7]. That astaxanthin scavenging free radicals and inhibiting lipid peroxidation, astaxanthin can effectively improve plant stress resistance in heavy metal environment has not been reported. In this experiment, the effects of different concentrations of astaxanthin on the growth and resistance of strawberry tissue culture seedlings under heavy metal stress were studied. In order to clarify the role of astaxanthin on the resistance of strawberry seedlings in order to provide relevant theoretical basis for astaxanthin strawberries under heavy metal stress.

Materials and Methods

Materials collection. 'Benihoppe' strawberry (*Fragaria ananassa* Duch.) plantlets, taken from Sichuan Agricultural University of Horticulture biotechnology laboratory.

Experimental Design. Using MS as the basic medium, CdCl₂ was first added at a concentration of 25 mg/L and astaxanthin at 0, 5, 10, 50, 100 and 200 µmol/L respectively. Including sucrose 30g/L, agar 7g/L, culture room temperature was 25°C, and light hours was 16h/d. Strawberry seedlings were inoculated into the above medium. Five strawberry seedlings were inoculated into each bottle, and there were 12 bottles. After 20 days, strawberry growth and related physiological indexes were measured and repeated 3 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

Chlorophyll content. Different concentrations of astaxanthin have a significant effect on the content of chlorophyll of strawberry tissue culture seedlings under the stress of heavy metal cadmium (Figure 1). Under the concentration of 5 µmol/L~10 µmol/L astaxanthin, the chlorophyll content of strawberry tissue culture seedlings increased and the content of chlorophyll increased with the increase of astaxanthin concentration. The chlorophyll content of strawberry tissue culture seedlings decreased with astaxanthin concentration from 50 µmol/L to 200 µmol/L, and the content of chlorophyll decreased with the increase of astaxanthin concentration; The chlorophyll content in 100µmol/L and 200µmol/L astaxanthin treatments was significantly lower than that of the control. Thus, astaxanthin treatment at low concentration (5 µmol/L~50 µmol/L) could increase the chlorophyll content of strawberry tissue culture seedlings under Cd stress. However, treatment with

astaxanthin at high concentration (100 $\mu\text{mol/L}$ ~200 $\mu\text{mol/L}$) reduced the chlorophyll content of strawberry tissue culture seedlings and did not alleviate the stress of heavy metal cadmium.

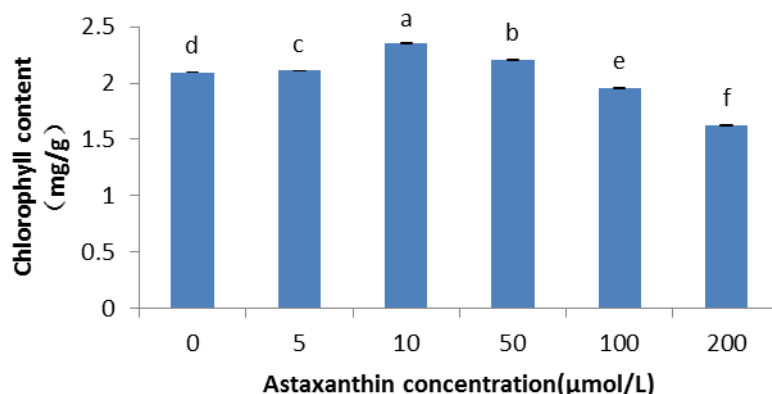


Fig.1 Effects of astaxanthin on chlorophyll content of strawberry groups under the stress of heavy metal cadmium.

MDA content. Astaxanthin at different concentrations had a significant effect on the content of MDA in strawberry seedlings under Cd stress (Figure 2). Under the treatment of astaxanthin at the concentration of 10 $\mu\text{mol/L}$, the content of MDA in strawberry tissue culture seedlings was the lowest (26.85 $\text{nmol}\cdot\text{g}^{-1}\text{FW}$). The concentration of 5 $\mu\text{mol/L}$, 50 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$ of astaxanthin did not show any significant difference compared with the control. Under the treatment of 200 $\mu\text{mol/L}$ astaxanthin, the content of MDA in the tissue culture seedlings of strawberry was significantly higher than that of the control. Thus, a certain concentration (10 $\mu\text{mol/L}$) of astaxanthin treatment can ease the stress of strawberry tissue culture seedlings of cadmium, and astaxanthin at a high concentration (200 $\mu\text{mol/L}$) increased the stress of strawberry seedlings.

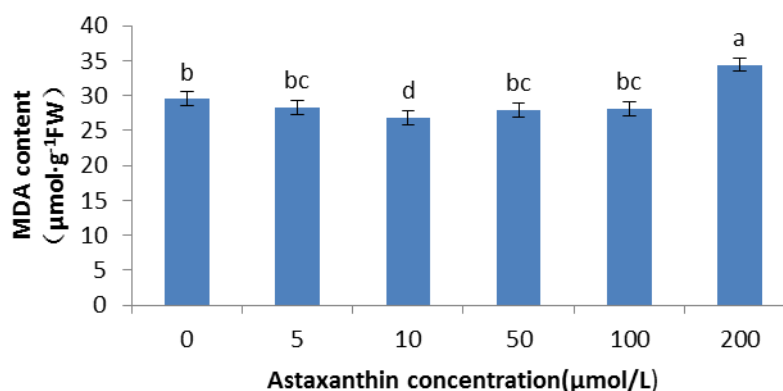


Fig.2 Effects of astaxanthin on MDA content of strawberry group seedling under the stress of heavy metal cadmium.

SOD content. The effects of different concentrations of astaxanthin on the activity of SOD in strawberry tissue culture seedlings under the stress of heavy metal cadmium are shown (Fig. 3). Under the treatment of astaxanthin at various concentrations, the SOD activity of strawberry tissue culture seedlings were higher than the control. Under low concentration of astaxanthin (5 $\mu\text{mol/L}$ ~100 $\mu\text{mol/L}$), SOD activity increased with the increase of astaxanthin concentration. However, SOD activity decreased with high concentration of astaxanthin (200 $\mu\text{mol/L}$). The activity of SOD (167.09 U/g FW) was significantly higher than that of the control (86.80 U/g FW) under

astaxanthin concentration of 100 μ mol/L. Thus, astaxanthin treatment can alleviate the effect of heavy metal cadmium on the SOD activity of strawberry tissue culture seedlings, and the optimum concentration is between with 50 μ mol/L and 100 μ mol/L.

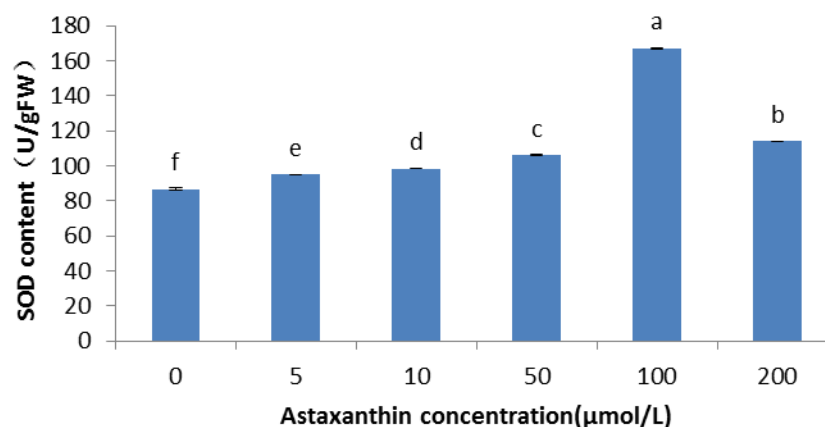


Fig.3 Effect of astaxanthin on the SOD activity of strawberry tissue culture seedlings under the stress of heavy metal cadmium

CAT content. The astaxanthin treatment at various concentrations had significant effects on the CAT content of strawberry tissue culture seedlings compared with the control (Fig. 4). Each concentration of astaxanthin treatment compared with the control, CAT content increased significantly. The content of CAT increased with the increase of astaxanthin concentration from 5 μ mol/L to 50 μ mol/L, and then it decreased with the increasing of concentration of astaxanthin from 100 μ mol/L to 200 μ mol/L. The CAT content (30.50 U/gFW) was significantly higher than that of the control (23.32 U/g FW) when the astaxanthin concentration was 50 μ mol/L. Thus, a certain concentration of astaxanthin treatment increased the content of CAT in strawberry tissue culture seedlings under heavy metal cadmium stress, which could alleviate the stress of strawberry seedlings in the presence of cadmium, the optimal concentration was between 10 μ mol/L and 50 μ mol/L

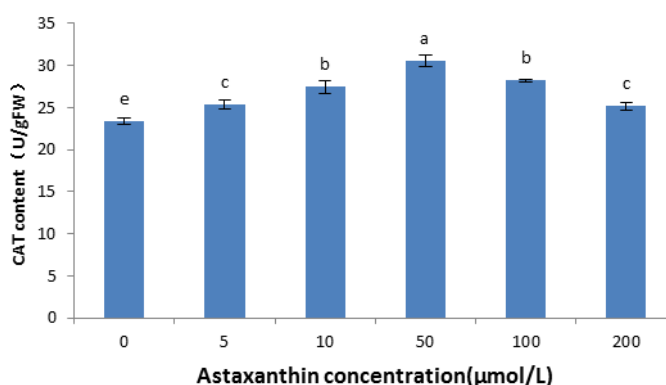


Fig.4 Effects of astaxanthin on CAT activity of strawberry group seedling under the stress of heavy metal cadmium.

POD content. Compared with the control, astaxanthin at various concentrations had significant effects on the activity of POD in strawberry tissue culture seedlings (Fig.5). All showed an increasing trend compared with the control. The POD activity of strawberry tissue culture seedlings

treated with 5 $\mu\text{mol/L}$ ~100 $\mu\text{mol/L}$ astaxanthin increased with the increase of astaxanthin concentration, and then decreased with increasing concentration of astaxanthin. POD activity (41.37 U/gFW) was significantly higher than that of control (32.74 U/gFW) when it was treated with 100 $\mu\text{mol/L}$ astaxanthin. Thus astaxanthin treatment can alleviate the impact of heavy metal cadmium on the POD activity of strawberry tissue culture seedlings, the optimum concentration is between with 50 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$.

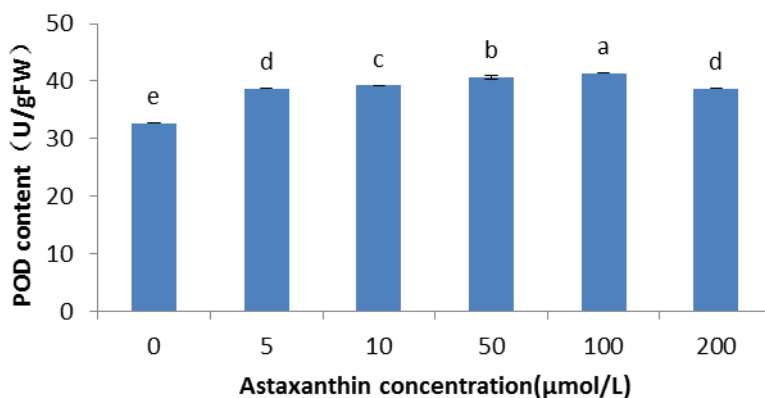


Fig.5 Effect of astaxanthin on POD activity of strawberry tissue culture seedlings under heavy metal cadmium stress

Ascorbic acid content. Astaxanthin treatment at various concentrations compared with the control had significant effects on the Vc content of strawberry tissue culture seedlings (Figure. 6). Compared with control, the content of Vc increased significantly under astaxanthin treatment. Vc content increased with the increase of astaxanthin concentration from 5 $\mu\text{mol/L}$ to 100 $\mu\text{mol/L}$. The Vc content (0.44 mg/g) of strawberries was obviously higher than that of the control (0.08 mg/g) under astaxanthin treatment of 100 $\mu\text{mol/L}$. Thus, a certain concentration of astaxanthin treatment under Cd stress in strawberry tissue culture increased Vc content, and astaxanthin treatment can alleviate the stress of heavy metal cadmium strawberry tissue culture seedlings. Appropriate concentration is between with 50 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$.

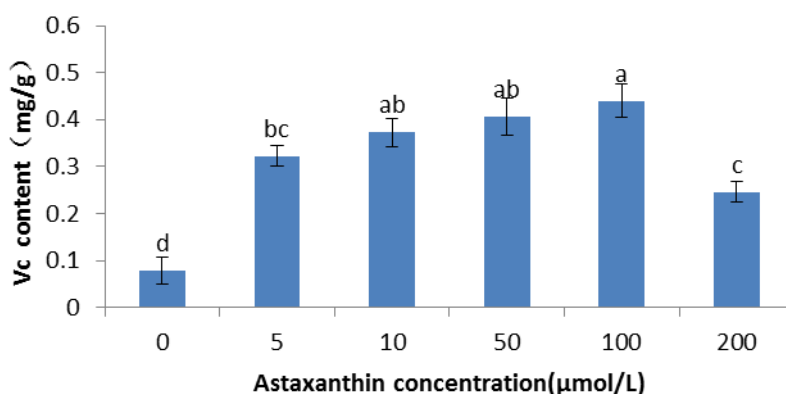


Fig.6 Effects of astaxanthin on ascorbic acid content of strawberry group seedling under the stress of heavy metal cadmium.

Conclusions

Heavy metal ions may compete with other nutrient elements for the absorption point of plant roots, or affect the physiological and biochemical processes of plants, thereby changing the plant's

absorption and transport of various nutrients [8]. Under the condition of cadmium stress, the balance of strawberry body is damaged, which affects all kinds of metabolic activities of strawberries and ultimately affects the growth of strawberries. Astaxanthin has a special molecular structure, with the function of scavenging oxygen free radicals, is a very effective antioxidant.

Leaf is the most important organ of plants for photosynthesis [9]. Chlorophyll in leaves is one of the main photosynthetic pigments that absorb light energy during photosynthesis [10]. The content of chlorophyll could be used as a factor to reflect the change of photosynthetic efficiency under heavy metal cadmium stress. Under heavy metal cadmium stress, the content of chlorophyll of strawberry tissue culture decreased and the photosynthesis was inhibited. The astaxanthin concentration of $5 \mu\text{mol} / \text{L} \sim 50 \mu\text{mol} / \text{L}$ could increase the chlorophyll content of strawberry tissue culture seedlings under Cd stress in the present study. However, the treatment with astaxanthin at a concentration of $100 \mu\text{mol} / \text{L} \sim 200 \mu\text{mol} / \text{L}$ reduced the chlorophyll content of strawberry tissue culture seedlings and did not alleviate the stress of heavy metal cadmium. Therefore, the impact of astaxanthin on the chlorophyll content changes with the different concentration.

Malondialdehyde (MDA) is the final product of membrane lipid peroxidation [11], and its content reflects the degree of membrane lipid peroxidation damage. The results showed that astaxanthin at the concentration of $10 \mu\text{mol/L}$ could alleviate the stress of strawberry seedlings by heavy metal cadmium. This indicated that astaxanthin treatment at a certain concentration could alleviate the accumulation of MDA in strawberry, but the high concentration of astaxanthin would increase MDA content. This experiment shows that astaxanthin can increase the activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) to different extents. However, the best activity for different enzymes corresponds to different optimum concentrations. Vitamin C, also known as L-ascorbic acid, is an antioxidant [12]. Vitamin C was obviously increased under certain astaxanthin concentration, while vitamin C content was highest under astaxanthin concentration of $100 \mu\text{mol/L}$.

Acknowledgements

Supported by the Scientific Research Foundation for Returned Overseas Chinese Scholars, State Education Ministry and Key projects of Sichuan Provincial Education Department(172A0319)

References

- [1] S. D. Cao. Study on tissue culture and rapid propagation of strawberry detoxification seedlings [J]. Journal of Shandong Forestry Science and Technology, 2002, 142(5): 19-21.
- [2] M. Z. Zhao. Pocket fruit and vegetable cultivation of high quality books [M]. Beijing: Science and Technology Literature Publishing House, 2005. 08.
- [3] C. P. Sun, W. Y. Tian. Astaxanthin[M]. Beijing: China Medical Science and Technology Press, 2016.

- [4] Dufosse L. Microbial production of food grade pigments [J]. Food Biotechnology, 2006, 44 (3): 313-321.
- [5] J. Huang. Antioxidant functional food ingredients - Natural astaxanthin [C]. China International Food Additives and Ingredients Exhibition. 2010.
- [6] T. Ai, J. X. Li, Q. Y .Liu. Effect of Biogas manure on heavy metal content in strawberry [J]. Northern gardening, 2010, (3) : 38-40.
- [7] H. Y. Zhang, Q. Han, L. Tian, et al. Effect of leaf selenium on strawberry cadmium and lead accumulation in strawberry leaves [J]. Horticulture Journal, 2011, 38(3): 409-416.
- [8] Y. S. Li., T. H. Sun . Research advances on the mechanism of plant hyperaccumulation by heavy metals [C] .National Circular Economy and Ecological Industry Academic Seminar. 2009.
- [9] M. U. Hongping, Y. E. Wanhui, Y. Z. Chen, et al. Photosynthesis and Growth of and under Different Phosphorus Nutrition Levels [J]. Botany in Wuhan, 2008, 26(5):514-519.
- [10] F. Qi. Effect of spiraea anthocyanins on pigment PS \square photochemical activity and differences among varieties [D]. Northeast Forestry University, 2014.
- [11] S. Q. Zhu. Traits Related to Chilling-Induced Photoinhibition in Leaves of indica and japonica Rice (*Oryza sativa*) [J]. Rice Science), 2004, 11(4):205-213.
- [12] H. J. Wang. Study on the stability of vitamin C and determination of content in fruits and vegetables by ultraviolet spectrophotometry [D].Shanxi Medical University, 2015.