

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

Yue Zhong<sup>1,a</sup>, Ya Luo<sup>1,b</sup>, Cong Ge<sup>1,c</sup>, Qin Mo<sup>1,d</sup>, Ya'jie Lin<sup>1,e</sup>, Shu Luo<sup>1,f</sup> and Hao'ru Tang<sup>1,g\*</sup>

<sup>1</sup>College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

<sup>a</sup>519116070@qq.com, <sup>b</sup>173698873@qq.com, <sup>c</sup>442477804@qq.com, <sup>d</sup>943156105@qq.com, <sup>e</sup>2208312818@qq.com, <sup>f</sup>626567947@qq.com and <sup>g</sup>1048019614@qq.com

\*Corresponding author. Yue Zhong, Ya Luo and Cong Ge contributed equally to this work.

**Keywords:** Astaxanthin; Strawberry tissue culture seedlings; Salt stress; Resistance

**Abstract:** In this experiment, we studied the effects of astaxanthin on the growth and resistance of strawberry tissue culture seedlings under salt stress. The samples were treated with astaxanthin concentrations of 0, 5, 10, 50, 100, 200 µmol/L. The results show that there is a rise in content of chlorophyll, ascorbic acid and the antioxidant capacity of CAT in strawberry seedlings which were treated with astaxanthin of 5, 10, 50 and 100 µmol/L, compared with the controls; meanwhile at the same time MDA showed a downward trend under the treatment. And the effect of 10 and 50 µmol/L astaxanthin treatments is the best. Astaxanthin of 200 µmol/L contributed to the damage to the strawberry seedlings under salt stress. The proper concentration of astaxanthin can improve the physiological activity of strawberry seedlings and enhance the salt tolerance of strawberry seedlings.

## Introduction

Strawberry (*Fragaria ananassa* Duch.) is a perennial herb of roseberry, which is a kind of bright red, soft and juicy fruit. Strawberries are rich in nutrients and have many active ingredients, and they can slow ageing and prevent various diseases. Therefore, its yield and cultivation area have been in the forefront of small berry planting <sup>[1]</sup>. But because the strawberry is extremely sensitive to salt, the growth of strawberries will be affected when the soil salt content is higher than 0.05%, which can lead to the occurrence of abnormal fruit <sup>[2]</sup>, resulting in the decline in production. The salinized soil has greatly restricted the production and development of strawberries, so it is of great significance for strawberry to carry out salt-resistant research.

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 scientific name of astaxanthin is 3,3'-dihydroxy-4,4'-dione-β, β'-carotene, which molecular formula is C<sub>40</sub>H<sub>52</sub>O<sub>4</sub>, a natural pigment that is widely found in crustaceans, green algae and red yeast, and it's a natural pigment, belongs to a kind of carotenoids <sup>[3]</sup>. In recent years, there has been a great deal of research on astaxanthin in China and abroad. The results show that the natural astaxanthin has a strong antioxidant activity and is known as "super antioxidant". Recent experiments in vitro show that it can quench single-line oxygen and have the powerful function of eliminating oxygen free radicals effectively, which is a very effective antioxidant reagent <sup>[5]</sup>. In *Haematococcus pluvialis* living cells, it acts as an antioxidant and photoprotective agent <sup>[6]</sup>, which can effectively quench a variety of reactive oxygen

species and prevent reactive oxygen from causing damage to cells.

In recent years, astaxanthin is often packaged as a healthy food ingredient in the market, and there are many patents around astaxanthin. The function of its product mainly focused on the prevention of disease of heart head blood-vessel, anticancer, anti-aging, improving vision, enhancing immunity, etc <sup>[7]</sup>. However, astaxanthin has not been widely used in plants so far, and it has not been reported in the study of resistance of strawberry. Therefore, this experiment was conducted to study the effects of different concentrations of astaxanthin on the growth and resistance of strawberry tissue culture seedlings under the salt stress, to clarify the influence on the chlorophyll and the resistance of strawberry plants under the salt stress, and to provide the theoretical basis for the study on the anti-salinity of strawberry plants.

## Materials and Methods

### Test materials and reagents

**Test material.** The ‘Benihoppe’ strawberry tissue culture seedlings, from the biotechnology laboratory of the horticulture college of Sichuan agricultural university.

**Reagents and drugs.** Distilled water, aseptic water, mercury, MS medium, 150 mmol/LNaCl, astaxanthin, ethanol, sucrose, agar, etc.

**Instruments and equipment.** Ultra-clean working table, high pressure steam sterilization pan, electric furnace, low temperature high speed centrifuge, spectrophotometer, thermostatic water bath pan, mortar, balance, beaker, measuring cylinder, volumetric bottle, liquid gun, etc.

### Experimental method

**Treat with astaxanthin.** Take the grown strawberry stolons longing 3 ~ 5 cm from the garden and flush them under the running water for around 1 h. Put the materials in 70% alcohol for half a minute on ultra-clean working table in bioclean room, and then immersed them in 0.1% mercuric chloride water for 15 min disinfection. Rinse them 3-5 times with sterile water and dry the surface of the material with sterile paper <sup>[8]</sup>. Finally, place the sterilized material in a petri dish (which has been sterilized by high pressure sterilization) containing the filter paper with tweezers. Peel off their stem point meristems and inoculate them in the culture medium.Using MS as the basic medium, NaCl was first added at a concentration of 150 mmol/L and astaxanthin at 0, 5, 10, 50, 100 and 200  $\mu\text{mol/L}$  respectively. Including 30 g/L sucrose,7 g/L agar , 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.

## Results and Discussion

**Chlorophyll content.** Fig.1 shows that the chlorophyll content of strawberry seedlings treated by astaxanthin concentration of 10, 50  $\mu\text{mol/L}$  was significantly higher than that in control under salt stress and the chlorophyll content of strawberry seedlings treated by astaxanthin concentration of 100, 200  $\mu\text{mol/L}$  was lower than that in control under salt stress.It can be seen that the chlorophyll content in strawberry seedlings can be improved by the proper concentration of astaxanthin.

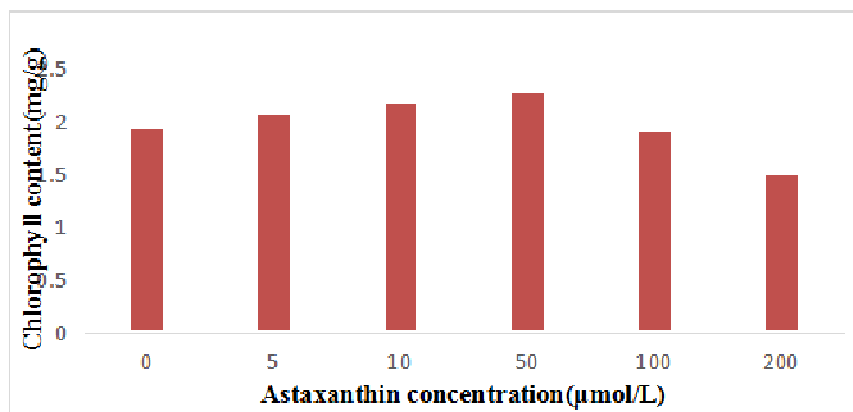


Fig.1 Effects of astaxanthin on chlorophyll content of strawberry tissue culture seedlings under salt stress

**MDA content.** Fig.2 shows that the MDA content of strawberry seedlings treated by astaxanthin concentration of 5 ,10 ,50 ,100 μmol/L was lower than that in control under salt stress. It can be seen that the MDA content in strawberry seedlings can be suppressed by the proper concentration of astaxanthin.

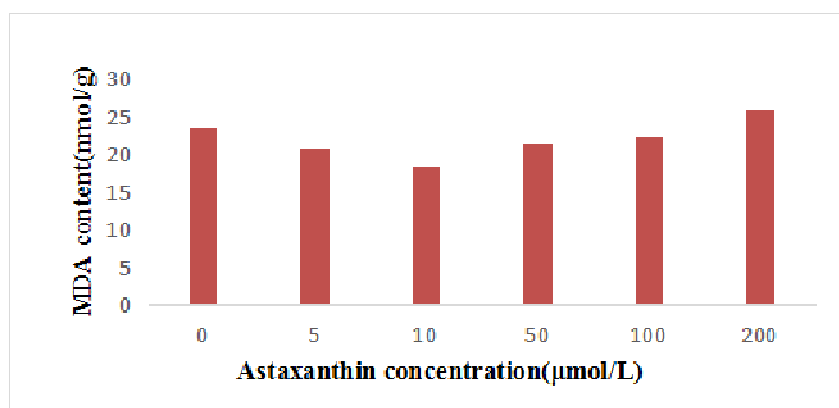


Fig.2 Effects of astaxanthin on MDA content of strawberry tissue culture seedlings under salt stress

**CAT content.** Fig.3 shows that the CAT content of strawberry seedlings treated by astaxanthin concentration of 5 ,10 ,50 ,100 ,200 μmol/L was significantly higher than that in control under salt stress. It can be seen that the CAT content in strawberry seedlings can be improved by the proper concentration of astaxanthin and the best effect of strawberry CAT activity is on the concentration of 10-100 μmol/L.

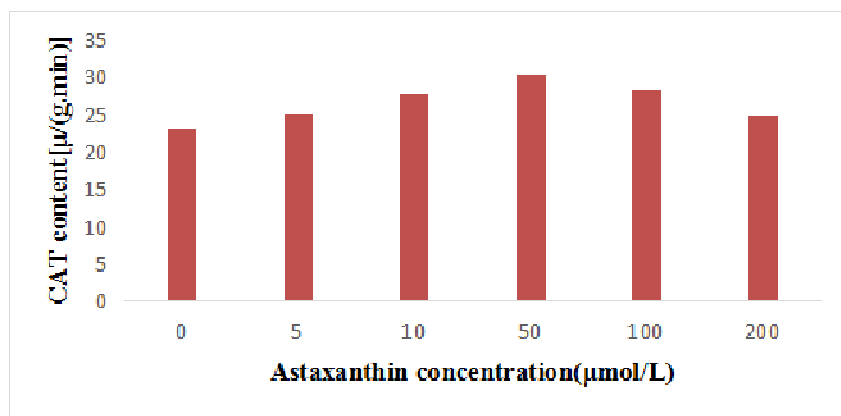


Fig.3 Effects of astaxanthin on CAT activity of strawberry tissue culture seedlings under salt stress

**Ascorbic acid content.** Fig.4 shows that the ascorbic acid content of strawberry seedlings treated by astaxanthin concentration of 5 , 10 , 50 , 100 μmol/L was significantly higher than that in control under salt stress. It can be seen that the ascorbic acid content in strawberry seedlings can be improved by the proper concentration of astaxanthin.

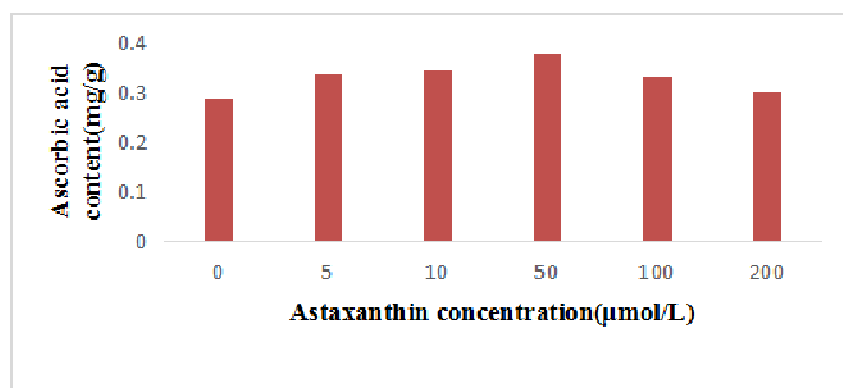


Fig.4 Effects of astaxanthin on ascorbic acid content of strawberry tissue culture seedlings under salt stress

## Conclusions

In order to determine the effect of astaxanthin on the resistance of strawberry plants under salt stress, this experiment used six different concentrations of astaxanthin to treat strawberry tissue culture seedlings under salt stress. Chlorophyll in leaves is one of the main photosynthetic pigments that absorb light energy during photosynthesis <sup>[8]</sup>. Malondialdehyde (MDA) is the final product of membrane lipid peroxidation <sup>[9]</sup>, and its content reflects the degree of membrane lipid peroxidation damage. The results show that the concentration of 5, 10, 50, 100 μmol/L was effective in improving the salt tolerance of strawberry seedlings. Among them, the concentration of 5, 100 μmol/L doesn't work very well in improving the salinity effect of strawberry seedling. However the concentration of 200 μmol/L of astaxanthin increased the damage to strawberry seedlings. It can be seen that the concentration of 10, 50 μmol/L can improve the physiological activity of strawberry seedlings to a certain extent, and enhance the salt tolerance of strawberry seedlings. Ascorbic acid and CAT are important indicators of antioxidant capacity in plants, the generation of reactive oxygen species is the main hazard brought by salt stress <sup>[10-11]</sup>. In summary, we can say that the appropriate

concentration of astaxanthin can reduce the damage of strawberry seedlings under salt stress.

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