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EFFECTS OF SALT STRESS ON NUTRITIONAL QUALITY OF ORANGE-HEADING CHINESE CABBAGE SEEDLINGS

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Abstract

To study the effects of NaCl on nutritional quality in Chinese cabbage is of great significance to clarify the mechanism of salt stress on the formation of fruit and vegetable quality, orange-heading Chinese cabbage was treated with 0, 50, 100, 150 and 200 mmol/L NaCl, the effects of NaCl on the carotenoids, vitamin C, soluble sugar and crude fiber in leaves were studied, to study the gene expressions of key enzymes in carotenoid metabolism pathway, total RNA was extracted and the first strand of cDNA was synthesized by reverse transcription. The results showed carotenoids in orange-heading Chinese cabbage treated with 100 mmol/L NaCl was higher than that of the control, and the difference was significant ($p<0.05$). The vitamin C increased first and then decreased, vitamin C in orange-heading Chinese cabbage treated with 150 mmol/L NaCl was higher than that of the control. The soluble sugar increased with the NaCl increase, and the difference was significant between the control and NaCl treatments. The crude fiber decreased first and then increased, and there was not much differences among different NaCl treatments. The genes expression of carotenoids synthesis enzymes PDS, ZDS and LYCE increased, and reached the highest under 100 mmol/L NaCl, then decreased under 150 mmol/L NaCl, while increased under 200 mmol/L NaCl. The results indicated NaCl stress might have effects on the carotenoid, vitamin C, soluble sugar and crude fiber in orange-heading Chinese cabbage. Especially the changes between the carotenoid contents and the genes expression of carotenoids synthesis enzymes were similar, therefore salt stress had a positive effect on the production of orange-heading Chinese cabbage carotenoids and it is likely influencing the quality of orange-heading Chinese cabbage.

Key words: Salinity stress, Orange-heading Chinese cabbage, Carotenoids, Quality, Gene expression.

Introduction

Salinization is a serious environmental problem, and one-fifth of irrigated agricultural areas are adversely affected by soil salinity. Approximately 831 million hm^2 of soil in worldwide is threatened by salinization. Secondary salinized soils make up approximately 77 million hm^2 of land area, 58% of which is distributed in irrigated agricultural areas, accounting for 20% of the total irrigated soil all over the world, and the proportion is still increasing (Wang *et al*., 2019). Therefore, developing salt-resistant crops is essential for maintaining food production (Chinnusamy *et al*., 2005). The total area of saline soil is approximately 3.6×10^7 hm^2, accounting for 4.9% of the available land area (Yang, 2008), and it has become an important land resource in China (Wang *et al*., 2009). Soil salinization refers to the process by which salt in soil or in groundwater rises to the soil surface through capillary water and accumulates on the soil surface after water evaporation (Fan *et al*., 2021). Salinization leads to poor soil permeability, increased osmotic pressure of solutions, physiological drought in plants, ion toxicity and the destruction of normal metabolism. Carotenoids are important markers used to evaluate the quality of fruits and vegetables. In plants, carotenoids have various functions related to photosynthesis and photo-protection, and they are precursors of phytohormones. Carotenoids act as powerful antioxidants in many biological systems due to their ability to react with free radicals. They also have powerful activity against singlet oxygen generated from lipid peroxidation or radiation (Kazimierczak *et al*., 2020). Carotenoids are also essential metabolic precursors and antioxidants for animal growth and development. They are a diverse group of pigments that are widely distributed in nature that produce distinct colors in fruits and flowers and play critical roles in plant growth and development (Ma *et al*., 2016).

High salinity is an abiotic stress that not only causes physiological drought and ionic toxicity but also reduces photosynthesis, therefore salt stress has adverse effects on plant growth. Reactive oxygen species (ROS) are a byproduct of the electron transport chain in photosynthesis of plant. High salinity leads to produce many ROS, and can induce the accumulation of secondary metabolites to eliminate ROS. Carotenoids are one of important secondary metabolites, and can effectively eliminate ROS, thus play a very important role in the process of plant salt tolerance. Chinese cabbage is an annual or biennial vegetable in Cruciferae, and it is also a popular cold-resistant vegetable in China. The inner leaves in orange-heading Chinese cabbage are bright orange, are different from those of common Chinese cabbage. Orange-heading Chinese cabbage also is rich in carotenoids such as carotene, lycopene and lutein. There are few studies on the effects of salt stress on seed germination and seedling growth in Chinese cabbage (Gao, 2018). There is little knowledge about the expression of carotenoid biosynthetic pathway genes and changes of other quality substances in orange-heading Chinese cabbage under salinity condition. In this study, we investigated the effect of NaCl on the contents of vitamin C, soluble sugar, coarse fiber and the expression of key genes involved in the carotenoid biosynthesis in orange-heading Chinese cabbage. Therefore, we aimed to determine the effect of salinity stress on the levels of carotenoids, vitamin C, soluble sugar, coarse fiber and the expression of carotenoid biosynthetic pathway genes in orange-heading Chinese cabbage by using various concentrations of NaCl.

Materials and Methods

Experimental material and planting: Orange-heading Chinese cabbage bred at Northwest A & F University in China was used as the research material. After soaking the seeds for 24 hours, they were evenly spread on wet filter paper, and were placed in a constant temperature incubator for 12 hours. When the radicle length reached more than 1/2 of the seed length, the germinated seeds were planted in a pot containing mixed soil (Cultivated soil: nutrient soil: organic fertilizer = 6:2:1), and were watered with tap water.

Salt stress treatment: After reaching the four-true-leaf stage, orange-heading Chinese cabbage was irrigated using Hoagland nutrient solution with 0, 50, 100, 150, and 200 mmol/L NaCl. Each orange-heading Chinese cabbage was irrigated with 50 mL of Hoagland-NaCl solution each time, and was stressed by NaCl once every 7 days. After 21 days, the tender leaves with similar size and stage were collected, and were stored at -80°C to determine physiological indicators and genes expression.

Determination of nutritional indices in leaves of orange-heading Chinese cabbage carotenoid contents: To measure the carotenoid contents, 2 g of fresh leaves samples were placed in a mortar, then an appropriate amount of quartzite, calcium carbonate and 0.1% volume fraction of BHT (2,6-di-tert-butyl-p-cresol) solution were added, and an appropriate amount of organic solvent were added for grinding in the dark. Carotenoids were extracted, and their absorbance values were measured at 440, 645 and 663 nm, and carotenoid contents were calculated according to the formula:

$$\mathbf{C_K = 4.7A_{440} - 5.454A_{645} - 2.165A_{663}}.$$

Determination of vitamin C: Vitamin C was determined by 2,6-dichlorophen indigo titration. Two grams of fresh orange-heading Chinese cabbage leaves were ground with 2% oxalic acid and the abrasive fluid were adjusted to 100 mL to make the extract. The standard curve of ascorbic acid was prepared with 2,6-dichlorophen indigo titration. The vitamin C was calculated as follows:

$$\text{Vitamin C contents (mg/100 g)} = \frac{(V1 - V2 \times K \times V)}{W \times V3}$$

W: Sample weight (g);
V1: Volume of dye used in the titration (mL);
V2: Volume of dye used in the blank (mL);
V3: Volume of extract used in the sample titration (mL);
K: Amount of vitamin C oxidized by 1 mL of 2,6-dichlorophenol indophenol (mg).

Determination of soluble sugar: The soluble sugar contents was determined using the anthracenone method. The soluble sugars were removed from orange-heading Chinese cabbage leaves with 80% ethanol solution for 2 times. Then, the clear liquid was mixed, decolored and adjusted to the correct volume. 5 mL anthracenone reagent was added to 1 mL of extract, and the absorption value at 625 nm was measured. A standard curve was made using a glucose solution. The soluble sugar was calculated as follows:

$$\text{Soluble sugar contents} = \frac{C \times V}{M \times 10^3}$$

C: Concentration found in the standard curve (μg/mL);
V: Constant volume of extract (mL);
M: Sample weight (g).

Determination of crude fiber: The crude fiber was determined by acid-base hydrolysis. Approximately 1–3 g of fresh leaves from orange-heading Chinese cabbage were boiled with 200 mL of hot sulfuric acid for 30 min. The samples were then boiled with 200 mL of hot 12.5 g/L NaOH for 30 min to digest the residue, and ethanol was used to remove the soluble matter. Finally, the filter paper and residue were weighed. The weight of crude fiber was calculated as follows:

$$\text{Crude fiber weight} = m_1 - m_2$$

m_1: Weight of the filter paper and residue;
m_2: Mass of the filter paper.

The expression of key enzyme genes involved in carotenoid synthesis: Total RNA was isolated from orange-heading Chinese cabbage subjected to 0, 50, 100, 150, or 200 mmol/L NaCl for 72 h, and was used to synthesize first-strand cDNA with an M-MLV reverse transcriptase kit (Promega, America). The 28S rRNA gene were used as reference to detect the expression of carotenoid synthetase genes by semiquantitative RT–PCR. The amplification conditions were as follows: 94°C for 5 min; 94°C for 30 s, 50°C for 30 s and 72°C for 1 min (27 cycles for 28S rRNA gene, and 35 or 40 cycles for the carotenoid synthetase genes); and a final extension at 72°C for 10 min. For the target gene, PCR was carried out in three biological repeats. Photoshop software was used to analyze the brightness of electrophoresis bands, and to quantify relatively.

Statistical analysis

The determination of physiological indices was repeated three times. Excel was used for statistical analysis, SPSS 17.0 was used to analyze the significance of the differences, a statistical significance threshold of $p<0.05$ was used to evaluate one-way ANOVA results, and Origin 7.5 was used for drawing.

Results

Effect of NaCl stress on carotenoid contents in orange-heading Chinese cabbage: To further study the effects of salt stress on carotenoids in the leaves of orange-heading Chinese cabbage, carotenoid contents were analyzed in seedlings treated with Hoagland solutions containing different NaCl concentrations. The results showed that 50~100 mmol/L NaCl significantly increased the carotenoid contents in the leaves of orange-heading Chinese cabbage, but the carotenoid contents in those leaves decreased under 150 mmol/L NaCl (Fig. 1).

Effect of NaCl stress on vitamin C in orange-heading Chinese cabbage: Fig. 2 shows that the vitamin C contents in orange-heading Chinese cabbage first increased and then decreased with NaCl increase. The vitamin C contents were the lowest under 0 mmol/L NaCl. Under 150 mmol/L NaCl, the vitamin C contents increased significantly, and reached the highest value. When the concentration of NaCl was 200 mmol/L, the vitamin C contents decreased significantly. The results showed that when NaCl was less than 150 mmol/L, the accumulation of vitamin C in orange-heading Chinese cabbage was promoted. However, when NaCl was more than 150 mmol/L, the accumulation of vitamin C was inhibited.

Effect of NaCl stress on soluble sugar in orange-heading Chinese cabbage: With NaCl increase, the soluble sugar contents in orange-heading Chinese cabbage leaves increased. The soluble sugar contents were the lowest in control, and then the soluble sugar contents increased significantly under 50~150 mmol/L NaCl, and when NaCl was 150~200 mmol/L, the soluble sugar contents increased slightly, the difference was not significant. The results showed that salt stress could promote the accumulation of soluble sugar in orange-heading Chinese cabbage (Fig. 3).

Effect of NaCl stress on crude fiber in orange-heading Chinese cabbage: Fig. 4 shows that the crude fiber contents in orange-heading Chinese cabbage first decreased, and then increased along with NaCl increased, but the difference was not significant. The crude fiber contents decreased under 0~100 mmol/L NaCl. When NaCl was 100 mmol/L, the crude fiber contents were the lowest. Under 100~200 mmol/L NaCl, the crude fiber contents increased. The results showed that the effect of NaCl on crude fiber might be not obvious in orange-heading Chinese cabbage.

Carotenoid synthase gene expression under salt stress in orange-heading Chinese cabbage: The expression of PDS increased significantly under 50~100 mmol/L NaCl (Fig. 5). At 150 mmol/L NaCl, the expression of ZDS and LYCE decreased sharply. Compared with the control, the expression of ZDS (Fig. 6) and LYCE (Fig. 7) increased under 100 mmol/L NaCl, while the expression of all three genes decreased at 150 mmol/L NaCl.

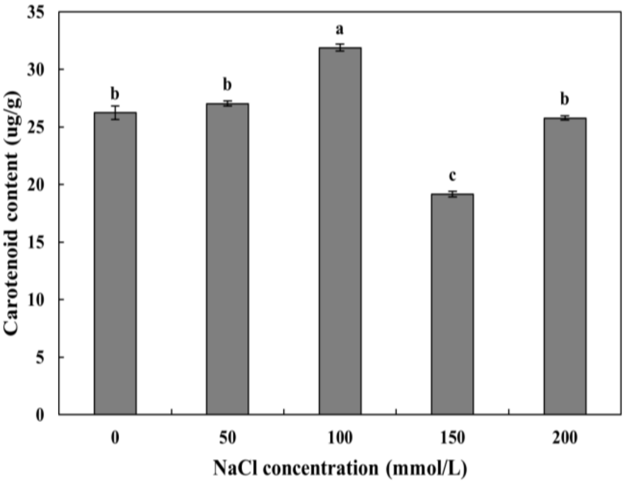

Fig. 1. The change of carotenoid in orange-heading Chinese cabbage leaves under NaCl stress.

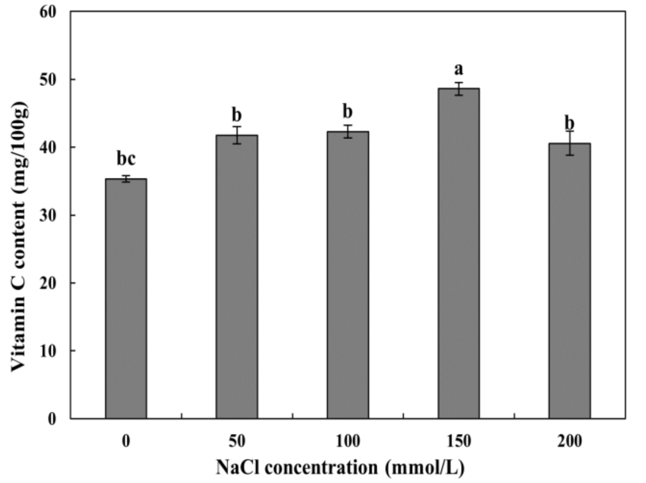

Fig. 2. Change of vitamin C contents in orange-heading Chinese cabbage under NaCl stress.

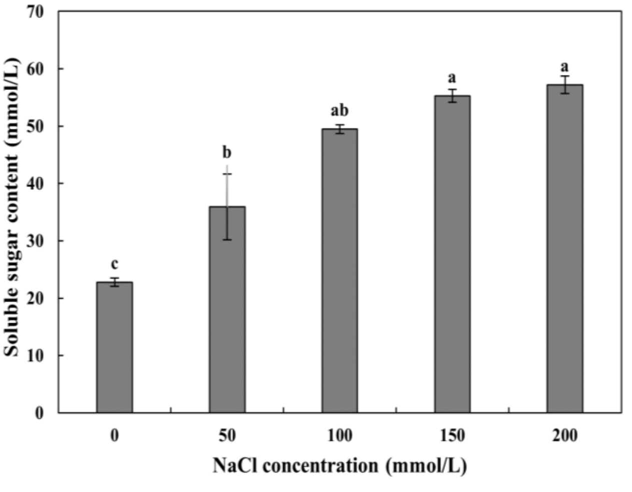

Fig. 3. Change of soluble sugar contents in orange-heading Chinese cabbage under NaCl stress.

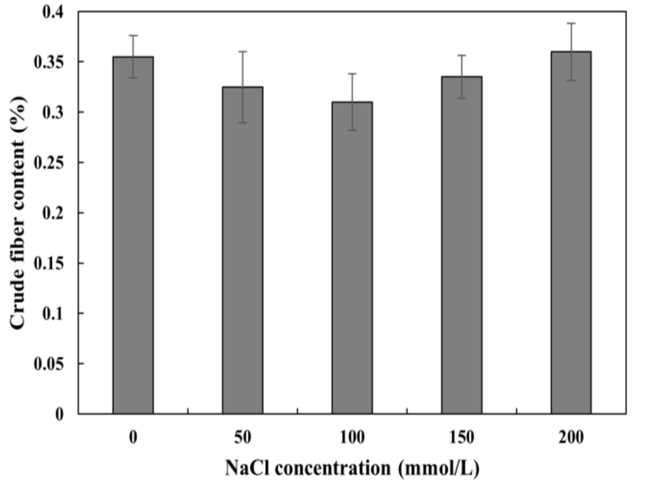

Fig. 4. Change of crude fiber contents of orange-heading Chinese cabbage under different NaCl stress.

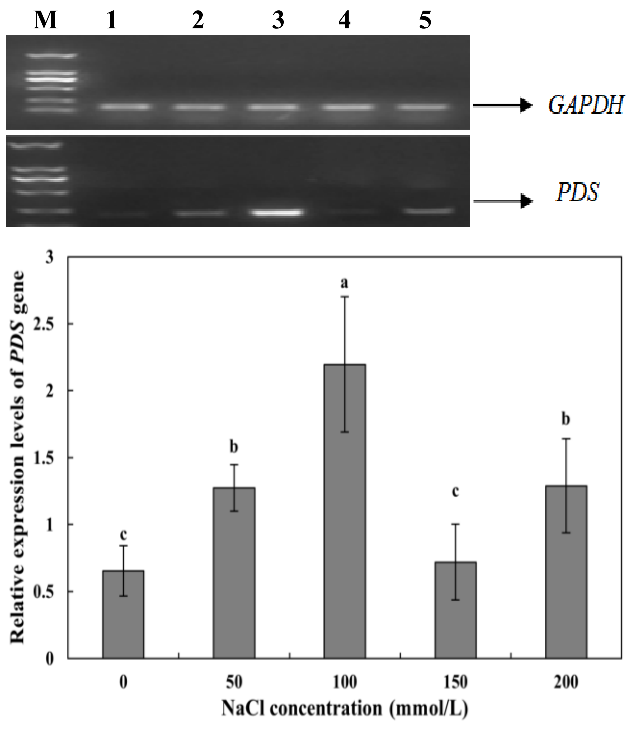

Fig. 5. Expression patterns of the *PDS* gene under NaCl stress. Semi-quantitative RT-PCR analysis was carried out with specific primers using the RNA isolated from orange-heading Chinese cabbage leaves subjected to NaCl stress. *GAPDH* was used as the internal control.

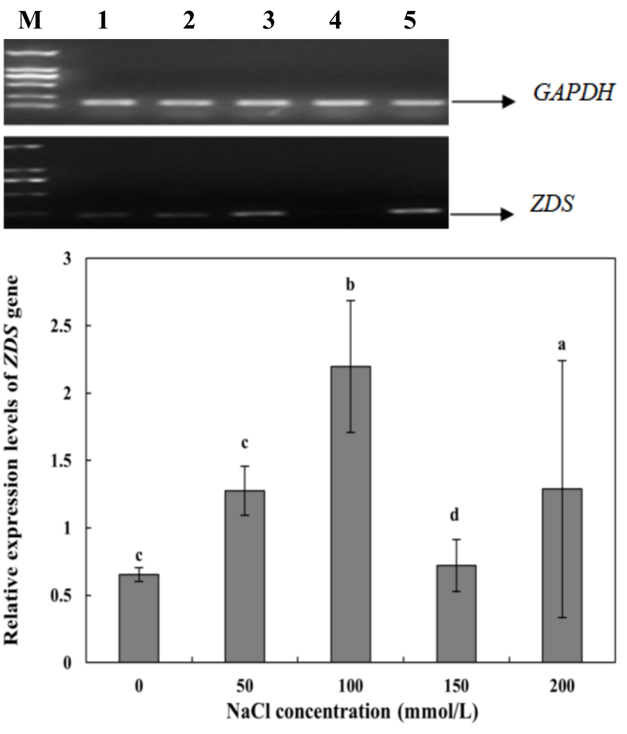

Fig. 6. Expression patterns of the *ZDS* gene under NaCl stress. Semi-quantitative RT-PCR analysis was carried out with specific primers using the RNA isolated from orange-heading Chinese cabbage leaves subjected to NaCl stress. *GAPDH* was used as the internal control.

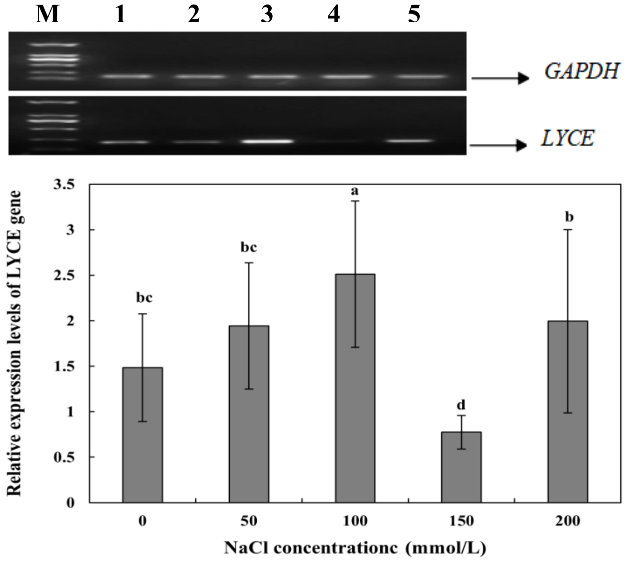

Fig. 7. Expression patterns of the *LYCE* gene under NaCl stress. Semi-quantitative RT-PCR analysis was carried out with specific primers using the RNA isolated from orange-heading Chinese cabbage leaves subjected to NaCl stress. *GAPDH* was used as the internal control.

Discussion

With the decrease in freshwater resources and poor watering and fertilizer management, land salinization is particularly serious, and salt-resistance research in plants and the breeding of salt-resistant plants is becoming important. Salinity has a serious influence on plant growth and productivity. Salt stress affects the quality and yield of rice (Razzaq *et al*., 2020). Chinese cabbage is a better commercial vegetable variety, and salt stress affects its growth and development processes and photosynthesis (Qiu *et al*., 2017), as well as its nutritional quality. Li *et al*., (2015) studied the effects of NaCl stress on Chinese cabbage seedlings, and found that 100 mmol/L NaCl was beneficial to Chinese cabbage; these results are consistent with our results.

Vitamin C is an important antioxidant in the human body, and eating vegetables and fruits is the main way to obtain vitamin C, therefore, vitamin C is an important factor which is used to evaluate the nutritional quality of vegetables. Previous studies have shown increases in vitamin C of Amaranthus leafy vegetables under 50 mmol/L and 100 mmol/L NaCl (Sarker & Oba, 2019). Similarly, Liu *et al*., (2016) found that vitamin C in lettuce showed an increasing trend at low NaCl and a decreasing trend at high NaCl. Our experiments showed that vitamin C in orange-heading cabbage increased at low NaCl and decreased at high NaCl, which was consistent with previous studies, indicating that a certain NaCl will promote the accumulation of vitamin C in orange-heading Chinese cabbage.

Soluble sugar acts as a permeable regulatory material to help plants adapt to abiotic stress. The increase in the soluble sugar in vegetables can improve their taste. Bai (2019) showed that the soluble sugar in soybean leaves increases under salt stress. Our results showed that the soluble sugar increased with NaCl increase, which is consistent with previous studies, and indicated that soluble sugar accumulated in orange-heading Chinese cabbage under salt stress. This increase may be to regulate the cell water potential, thereby improving the plant's salt tolerance.

Crude fiber is also beneficial to the human body. Reducing the crude fiber improves the taste and the quality of Chinese cabbage. Our experiments showed that with the increase in NaCl, the crude fiber first decreased and then increased, but the difference was not significant, indicating that salt stress had little impact on the crude fiber contents in orange-heading Chinese cabbage. Wu (2021) found the crude fiber sharply dropped in *Medicago sativa* L. when subjected to salinity. The crude fiber contents are differences in different study materials under salt stress, this may be due to the salt tolerance of plants is different, and then the crude fiber in the plants differ in their sensitivity to salt induction.

Wang *et al*., (2019) found that the gene expression levels of PSY, PDS and ZDS in germinated maize treated with 300 mmol/L NaCl were 1.63, 1.53 and 1.31 times higher than those in the control, respectively, which accelerated the synthesis of carotenoids. Under 450 and 600 mmol/L NaCl treatment, the relative expression levels of the three genes decreased, and the carotenoid synthesis rate slowed down. Li *et al*., (2020) found that the overexpression of *PDS* and *ZDS* significantly increased the accumulation of carotenoids and enhanced the salt tolerance of transgenic tobacco. These results are consistent with the results in orange-heading Chinese cabbage, our study found that the carotenoid contents first increased and then decreased, finally increased again under salt stress, which was similar to the expression trend of its synthetase genes PDS, ZDS, LYCE in orange-heading Chinese cabbage. The possible reason is that salt stress affects the contents of carotenoid by affecting the expression of carotenoid synthase genes. It also shows that suitable salt can improve the quality of carotenoids in orange-heading Chinese cabbage, which is suitable for the future use of saline soil. To improve the quality of orange-heading Chinese cabbage to provide a theoretical basis, the mechanism that affects its carotenoid synthesis under salt stress still needs more in-depth research.

Orange-heading Chinese cabbage is a vegetable with high nutrition and good quality, abiotic stress such as salt stress is an important factor affecting the growth and quality of plants, and there are few reports on the effect of salt stress on the quality of orange-heading Chinese cabbage. Therefore, to research the effect of NaCl stress on the nutritional quality of the plant is needed. This study showed that NaCl stress had different influences on the nutritional quality of orange-heading Chinese cabbage. Appropriate NaCl can promote carotenoids, vitamin C and soluble sugar contents in orange-heading Chinese cabbage, can affect barely the crude fiber contents. The results of this study can lay the foundation for the response of orange-heading Chinese cabbage to salt stress and salt resistance.

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