

## Effects of Exogenous Chitosan on Physiological Characteristics in Waxy Maize Seedlings under Salt Stress

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**Abstract:** Salinized soil is widely distributed in Tianjin. Waxy corn has high nutritional value and plays an important role in urban agriculture. It is very important to study the salt-tolerant characteristics of waxy corn for the utilization of salinized soil and using salinized soil to plant waxy corn. The effects of exogenous chitosan on the physiological characteristics of waxy corn seedlings under different salt stress were analyzed using jinnuo 72 as test material. The results showed that the content of soluble protein and proline in waxy corn seedlings treated with exogenous chitosan were significantly increased compared with the control group in the range of 0.04% to 0.52% soil salt content. The content of soluble sugar, catalase activity and superoxide dismutase activity in waxy corn seedlings treated with exogenous chitosan were significantly increased compared with the control group in the range of 0.20% to 0.52% soil salt content. The content of malondialdehyde in waxy corn seedlings treated with chitosan was significantly reduced compared with the control group under salt stress. Under certain salt stress, exogenous chitosan treatment could alleviate the damage of salt stress to waxy corn seedlings by increasing the content of osmotic substances, enhancing the activity of antioxidant enzymes and decreasing the content of malondialdehyde.

### 1. Introduction

Irrigated land affected by different degrees of salinization exists widely all over the world, and the problem of soil salinization in Tianjin is very prominent. Crop growth was seriously compromised by soil salinization, and soil salinization is one of the main stress factors affecting agricultural production [1]. Waxy corn has high nutritional and commercial value, which is widely loved by people. In addition to fresh food, waxy corn is also widely used in livestock feed. Waxy corn plays an important role in the development of urban agriculture. One of the effective methods to improve the salt tolerance of maize is to use exogenous growth regulation substances [2-3]. The chitosan (CTS) is a kind of high molecular substance, which exists in arthropod insects, crustaceans and fungi. It has biodegradability, biocompatibility, non-toxic and bacteriostasis and other physiological functions [4]. Studies have shown that CTS can regulate plant growth and development, and can be used as an inducer of abiotic stress resistance to enhance crop resistance. The activity of superoxide dismutase (SOD) of soybean under salt stress was increased by CTS[5],the activities of SOD, peroxidase(POD) and catalase(CAT) of Chinese cabbage under salt stress were increased by CTS[6],the  $\beta$ -amylase activity of rice under salt stress was increased by CTS[7],the germination and seedling growth of wheat under salt stress were promoted by CTS[8], and CTS alleviate salt stress damage. The effect of exogenous gibberellin on maize seedling physiology under salt stress has been reported [9], There are few studies on the effects of exogenous CTS on the physiological characteristics of waxy corn seedlings under different soil salt content. In

order to investigate the alleviating effect of exogenous CTS on the salt damage of waxy corn, the effects of CTS on the related enzymes and osmotic substances of waxy corn seedlings under different soil salt content were studied. It is expected to provide a theoretical reference for the application of CTS as an exogenous salt resistance agent and the planting of waxy corn on salinized soil, which is of great significance for the full utilization of salinized soil.

## **2. Materials and Methods**

### **2.1 Experimental Materials**

The experimental material was Jinnuo 72 of waxy corn variety, which was provided by Tianjin zhongtian runnong technology Co., Ltd.

### **2.2 Experimental Method**

Seeds were randomly selected to be uniform, Soak the seeds in 1% sodium hypochlorite solution for 10 minutes and rinse with water. The experiment was divided into control group (ck) and treatment group (CTS treatment). The disinfected seeds were immersed in water for 12 h in ck group, and were immersed in 0.3% CTS for 12 h in the CTS treatment group. Two kinds of treated seeds were planted separately in five types of salt content soil, ten holes was evenly sown each basin with two seeds in each hole. The 5 kinds of soils were adjusted with 0, 40, 80, 120 and 160 mmol/L NaCl solution, respectively, and the soil salt content was adjusted according to the method [10]. After adjustment, the measured salt content was 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, respectively. Each treatment was repeated for 3 times. The seeding experiment was carried out on May 10, 2020. When the seedling height reached 3 cm, each basin of the CTS treatment group was sprayed with 3 mL 0.3% CTS. The contents of proline, soluble sugar, soluble protein and malondialdehyde (MDA) in the leaves were measured when the seedlings grew to 12 cm, and the activities of CAT and SOD in the leaves were measured.

### **2.3 Determine Items and Measurement Methods**

The content of soluble protein was determined by coomassie bright blue method [11], the content of soluble sugar was determined by anthrone colorimetric method [11], the content of MDA was determined by thiobarbituric acid (TBA) method [11], the content of proline was determined by colorimetric method [11], the activity of CAT was determined by ultraviolet absorption method [11] and the activity of SOD was determined by nitro-blue tetrazolium (NBT) method [11]. (Kit: Nanjing Jiancheng Reagent Company)

### **2.4 Data Processing**

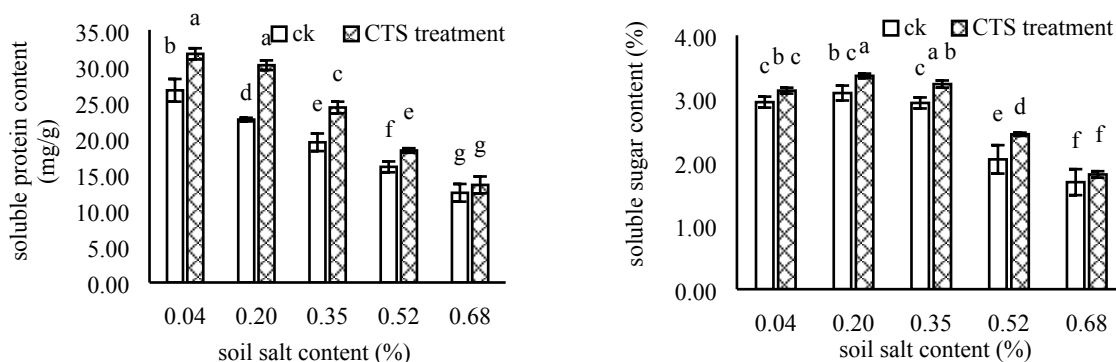
Data processing and draw graphs were performed by using Excel 2013, and data statistical analysis was used by SPSS 18.0 software.

## **3. Results and Analysis**

### **3.1 Effects of Exogenous CTS on Soluble Protein and Soluble Sugar Contents in Waxy Maize Seedlings under Salt Stress**

As can be seen from figure 1, the soluble protein content of waxy corn seedlings decreased significantly with the increase of soil salt content. In the range of 0.04% to 0.52% soil salt content, the soluble protein content of waxy corn seedlings was significantly increased by exogenous CTS. When the soil salt content was 0.68%, the soluble protein content of the treatment group was 8.8% higher than that of the control group, but not significant. The soluble sugar content of waxy corn seedlings was decreased with the increase of soil salt content under low salt stress, but not significantly. The soluble sugar content was significantly decreased under high salt stress. The soluble sugar content of waxy corn seedlings significantly increased by exogenous CTS in the range of 0.20% to 0.52% soil salt content. Although the soluble sugar content of waxy corn seedlings was

increased respectively by 6.1% and 7.2% with exogenous CTS treatment at 0.20% and 0.52% soil salt content, compared with the control waxy corn seedlings, but not significantly. It was showed that exogenous CTS could improve the water retention ability of cells and enhance the salt tolerance of maize seedlings by increasing the content of soluble protein and soluble sugar, which physiological osmotic regulating substance.

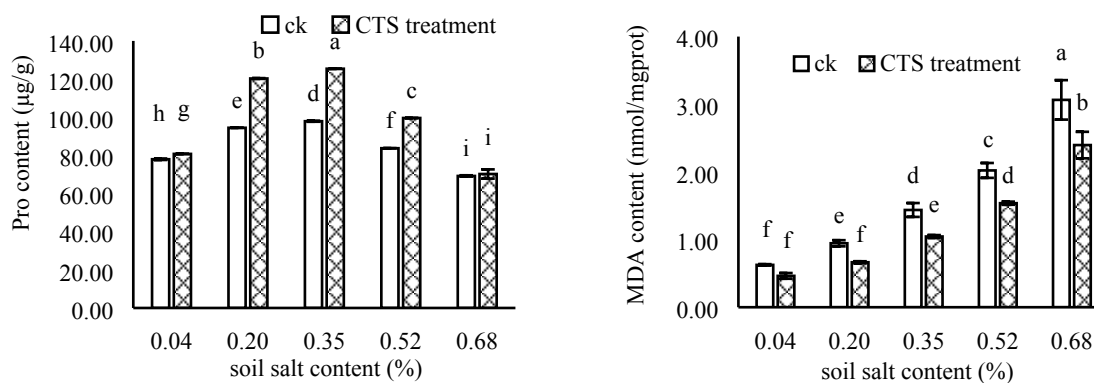


**Fig 1.** Changes of soluble protein content and soluble sugar content in waxy corn seedlings treated with CTS treatment under different salt stress

Note: Different lower-case letters above the bar mean significant difference at the 0.05 probability level, the same below.

### 3.2 Effects of Exogenous CTS on Proline and MDA Contents in Waxy Maize Seedlings under Salt Stress

The proline content of waxy corn seedlings showed a trend of significant increase with the increase of soil salt content at less than 0.35%, and a trend of significant decrease with the further increase of soil salt content. The proline content of waxy corn seedlings was significantly increased by exogenous CTS in the range of 0.04% to 0.52% soil salt content. The proline content of the treatment group was 1.5% higher than that of the control group at 0.68% soil salt content, but not significant (figure 2). It can be seen that CTS can improve the salt tolerance of waxy corn seedlings by increasing the content of proline. MDA content can reflect the damage degree caused by salt stress. As can be seen from the change of MDA content in Figure 2, the MDA content of waxy corn seedlings showed a significant increase trend with the increase of soil salt content. The content of MDA in waxy corn seedlings could be significantly reduced by exogenous CTS under salt stress. The content of MDA in plant tissues was increased under the influence of salt stress, indicating that the tissues were damaged and their salt resistance decreased. The content of MDA in waxy corn seedlings could be reduced by CTS treatment under salt stress, and the salt tolerance of waxy corn seedlings could be increased.

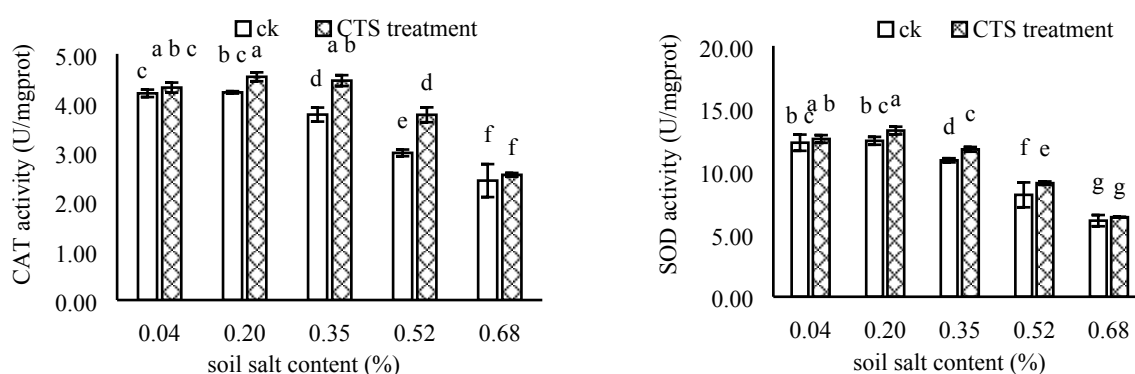


**Fig 2.** Changes of proline content and MDA content in waxy corn seedlings treated with CTS treatment under different salt stress

### 3.3 Effects of Exogenous CTS on Activities of CAT and SOD in Waxy Maize Seedlings under Salt Stress

CAT can remove harmful metabolites in plants and prevent peroxidation damage to plants. As can be seen from figure 3, the CAT activity of waxy corn seedlings showed a trend of significant decrease with the increase of soil salt content, when the salt content was above 0.20%. In the range of soil salt content from 0.20% to 0.52%, the CAT activity of waxy corn seedlings was significantly increased by exogenous CTS treatment. Compared with the control waxy corn seedlings, the CAT activity of waxy corn seedlings was increased by 2.8% and 4.8% by exogenous CTS treatment, respectively, under 0.04% and 0.68% salt content, but not significant. CTS can reduce salt injury of waxy maize seedlings by increasing CAT activity under low salt stress.

SOD activity also showed a similar trend, the SOD activity of waxy corn seedlings showed a trend of significant decrease with the increase of soil salt content, when the salt content was above 0.20%. In the range of soil salt content from 0.20% to 0.52%, the SOD activity of waxy corn seedlings was significantly increased by exogenous CTS treatment. Compared with the control waxy corn seedlings, the SOD activity of waxy corn seedlings was increased by 2.4% and 4.5% by exogenous CTS treatment, respectively, under 0.04% and 0.68% salt content, but not significant. CTS can reduce salt damage of waxy maize seedlings by increasing SOD activity under low salt stress.



**Fig 3.** Changes of activities of CAT and SOD in waxy corn seedlings treated with CTS treatment under different salt stress

#### 4. Discussion and Conclusion

In this experiment, the content of proline in waxy corn seedlings was significantly increased by CTS treatment, which was consistent with the research results on the effect of CTS on physiological and biochemical indexes of tomato seedlings under salt stress [12]. The content of proline, soluble protein and soluble sugar in waxy maize seedlings was significantly increased by CTS treatment. The trend was consistent with the research results on the effect of H<sub>2</sub>S on physiological indexes and antioxidant activity of melilotus seedlings under NaCl stress [13]. And the trend was also consistent with the results of the study on the effect of salicylic acid on the physiological characteristics of pepper seedlings under salt stress [14]. The contents of soluble sugar and proline in rice could be increased by CTS treatment under salt stress [7], and proline content of wheat seedlings could be increased by CTS treatment [8,16]. These results are consistent with the results of this experiment. These results indicate that CTS treatment can promote the accumulation of soluble sugar, soluble protein and proline in plants under salt stress. The synthesis and accumulation of these osmotic regulatory substances can reduce the osmotic potential of plant cells, thus improving the water absorption capacity of plant cells and alleviating the damage caused by stress [15]. MDA content is one of the physiological indexes of salt stress resistance [17]. Salt stress can stimulate the increase of reactive oxygen species in plant cells and further degrade biological molecules such as proteins in plant tissues. Plant tissues and cells are subjected to oxidative damage under salt stress [18]. The oxidation of cell membrane lipids will increase the content of MDA in cell membrane and further inhibit the synthesis of protein [19]. Reducing MDA content can alleviate the damage of salt stress to plants. The results of this study showed that the content of MDA in waxy maize seedlings was significantly reduced by CTS treatment under salt stress. The salt tolerance of waxy maize seedling

was increased. Which was basically consistent with the research results of Chinese cabbage [6], soybean [5], wheat [8] and cowpea [20] under salt stress.

The activity of antioxidant enzymes in plants could be induced by CTS under salt stress to alleviate the damage caused by salt stress. The activity of CAT and SOD of waxy maize seedling was significantly increased by exogenous CTS treatment under certain salt stress degree in this experiment. This result is consistent with the results that CTS treatment can increase the CAT and SOD activities in cowpea [20], wheat [8], Chinese cabbage [6], lentil [21] and soybean seedlings [5] under salt stress. A certain concentration of CTS can enhance the activities of various enzymes in plants, keep the nutrients in plants under environmental stress stable, reduce the degree of membrane peroxidation caused by salt stress, so as to improve the salt tolerance of waxy maize seedlings.

The results showed that the content of soluble protein and proline in waxy maize seedlings was significantly increased by exogenous CTS in the range of 0.04% to 0.52% soil salt content. The content of soluble sugar, activity of CAT and SOD in waxy maize seedlings were significantly increased by exogenous CTS from 0.20% to 0.52% soil salt content. The content of MDA in waxy maize seedlings could be significantly reduced by exogenous CTS under salt stress. Exogenous CTS treatment could alleviate the damage of salt stress to waxy maize seedlings by increasing the content of osmotic substances, enhancing the activity of antioxidant enzymes and reducing the content of MDA under salt stress. Exogenous CTS treatment is one of the effective methods to improve salt tolerance of waxy maize seedlings.

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