Study on Physiological Changes of Seed *Broccoli* under Salt Stress

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Abstract. In this paper, the typical neutral salt NaCl in salt soil was used to simulate salt stress. The changes of CAT, H\(_2\)O\(_2\), POD, proline, total SOD and leaf thickness of H17, a seed broccoli variety, under the concentration of 100, 200 and 300 mmol/L NaCl were detected at water sensitive stage of seed broccoli. The results showed that the total SOD activity of seed broccoli was not significantly affected, and the activity of POD was more sensitive to low salt stress (100 mmol/L). The activity of POD remained basically stable when the salt concentration continued to increase. The activity of CAT showed a tendency to decrease first and then rise. The H\(_2\)O\(_2\) and proline exhibited more sensitive to low and moderate salt concentration with a trend of rise first and fall then. The content of H\(_2\)O\(_2\) and proline reached the peak at middle salt concentration (200 mmol/L). Additionally, the leaf thickness was positively correlated with salt stress concentration.

1. Introduction

Plant growth and yield will be affected by low temperature, drought, salinity and other environmental stresses [1]. Soil salinization is one of the global agricultural environmental and ecological problems. According to the statistics of FAO and UNESCO, the saline soil in the world is 9.5 × 10^8 hm\(^2\), which is about 10% of the land area. China's salinized soil area is about 1.0 × 10^8 hm\(^2\) [2], accounting for one third of Asia. With the development and deterioration of the following factors, such as the aggravation of industrial pollution, the development and utilization of wastewater and seawater, the input of chemical fertilizer and the development of agricultural production, the secondary salinization of soil tends to increase. Soil secondary salinization has seriously affected the yield and quality of agricultural products [3-4]. Therefore, the cultivation of salt tolerant varieties and the development of salt land planting can maintain water pressure and salt and improve soil structure and microenvironment, which is of great significance to the development of salt land and characteristic planting industry [5].

Brassica oleracea var. Italica, known as green cauliflower, broccoli and green cauliflower, originated from Europe, belongs to Cruciferae Brassica herb. It has important edible and medicinal value. The vitamin a content of white vegetables is 2 times that of tomatoes, and the vitamin a content of white vegetables is 6 times that of tomatoes. Its protein content is 2 times that of broccoli. Its V\(_a\) content is 240 times and 6 times that of broccoli and tomato, and its calcium content is 2 times that of tomato. More importantly, Brassica oleracea var. Italica has anti-cancer effect. It is regarded as a good health vegetable and "spring preserving vegetable" by European and American nutritionists, and is an...
indispensable vegetable variety in people's life [6]. In salt stress, plants will produce a large number of free radicals to increase the permeability of cell plasma membrane and disorder the mineral metabolism and water metabolism [7]. Meanwhile, the morphological characteristics of leaves and other organs changed. Up to now, few studies have been conducted to reveal the salt tolerance of crops, such as cowpea [8], wheat [9] and elaeagnus angustifolia [10]. However, there is no study on salt tolerance of seed broccoli. Therefore, in the present study, pot culture method was used to detect the physiological response of seed broccoli under salt stress. The purpose of this study is to provide reference for the cultivation of salt tolerant seed broccoli and salt field planting.

2. Materials and Methods

2.1. Materials

The test materials were self-bred broccoli line H17. The NaCl (analytically pure) used in this study is produced by Boao Weixin Co.

2.2. Plant Cultivation

In this study, broccoli seeds with uniform size, plumpness and health were selected. Broccoli seeds were soaked in 70% ethanol for 1 min, washed with water, and then treated with 1% NaHCO$_3$ for 10 min. After washing with water, it was placed in the tray and cultured at 25 °C. When three true leaves grew, they were transplanted into a 25cm×16.5cm plastic pot for soil culture, and irrigated with 1/2 Hoagland nutrient solution regularly. After the seedlings grew to 10-15 leaves, 20 pots with the same growth and normal growth were selected and treated with salt stress in the greenhouse.

2.3 Salt Stress Treatment

The pot seedlings were randomly divided into 4 groups with 5 pots in each group. In the first group, normal 1/2 Hoagland nutrient solution was applied, 500ml/pot each time, and then applied after the soil became white. The second group was treated with 1/2 Hoagland solution mixed with 100 mmol/L NaCl. The third group was treated with 200 mmol/L NaCl mixed solution prepared with 1/2 Hoagland solution. The fourth group was treated with 1/2 Hoagland solution mixed with 300 mmol/L NaCl. The physiological and morphological indexes were detected 15 days later.

2.4 Physiological and Morphological Detections under Salt Tolerance

2.4.1. Sampling and Morphological Detection. Take 3-5 mature leaves from the middle and upper parts of each pot and number them in groups. The spiral micrometer was used for measurement. The mesophyll thickness near the vein in the middle of the leaf was measured at three places randomly, and the average value was calculated to estimate the leaf thickness.

2.4.2. Physiological Indexes under Salt Tolerance. 0.5g and 4ml of 0.05mol/l phosphate buffer (pH 7.8) were accurately weighed from the leaves of the plants sampled above, and a little quartz sand was added and placed in the mortar. The homogenate was grinded in ice bath and centrifuged at 4000R/min for 15min. Proline was determined by ninhydrin method [11]. The activity of catalase was determined by ammonium molybdate complexation method [12]. The activity of superoxide dismutase (SOD), peroxidase (POD) and the content of hydrogen peroxide (H$_2$O$_2$) were tested by the test kit of Nanjing Jiancheng Co.

2.5 Statistical analysis

All data management and analysis were performed using Microsoft Office Excel 2007 and DPS 2000. DMRT method was used for multiple comparisons.
3. Results and Analysis

3.1. Effect of Salt Stress on SOD Activity

The activity of SOD was not significantly affected by salt concentration in seed broccoli leaves ($P=0.3848$), which indicated that the total SOD activity of seed broccoli was not sensitive to salt stress (Table 1).

| NaCl (mmol·L⁻¹) | CAT (U·ml⁻¹) | H₂O₂ (mmol·L⁻¹) | POD (U·mgprot⁻¹) | Proline (µg·g⁻¹) | SOD (U·g⁻¹) | Leaf thickness (mm) |
|----------------|--------------|-----------------|------------------|------------------|-------------|-------------------|
| 0              | 11.56ᵇᴬ      | 61.38ᵇᴮ       | 0.0145ᵇᴬ         | 39.81ᶜᶜ       | 5.36ᵃᴬ      | 0.2433ᵈᶜ         |
| 100            | 10.61ᵇᴬ      | 64.28ᵇᴮ       | 0.0221ᵃᴬ         | 53.31ᵇᴮᶜ      | 5.36ᵃᴬ      | 0.4333ᶜᴮ         |
| 200            | 10.36ᵇᴬ      | 78.32ᵃᴬ       | 0.0173ᵇᴬ         | 73.95ᵃᴬ       | 5.30ᵃᴬ      | 0.5467ᵇᴬ         |
| 300            | 11.01ᵇᴬ      | 64.56ᵇᴮ       | 0.0185ᵇᴬ         | 61.22ᵇᴮᶜ      | 5.35ᵃᴬ      | 0.6233ᵃᴬ         |

3.2. Effects on POD Activity

The results showed that different salt concentrations had significant effects on the activity of POD ($P=0.0335$). At low salt concentration, the activity of POD exhibited an upward trend, and reached the highest value of 0.0221U/mgprot at 100 mmol/L salt concentration, which was significantly higher than that in the blank control (0.0145 U/mgprot). The content of POD exhibited an upward trend with the increase of salt concentration.

There was no significant difference in POD content between the salt concentrations 200 mmol/L and 300 mmol/L, which indicated that the content of POD was more sensitive to low salt stress (100 mmol/L) in seed broccoli. When the salt concentration increased to 200 mmol/L and 300 mmol/L, the POD content remained basically stable.

3.3. Effects on CAT Activity

The activity of CAT became weak under low and medium salt concentration. With the increase of salt concentration, the CAT activity of seed broccoli began to increase.

Table 1 showed that the CAT activity of seed broccoli decreased under the treatment of low and medium salt concentration. At 200 mmol/L salt concentration, CAT activity reached the lowest value (10.36 U/ml), which was 10.38% less than that of the blank control group ($P=0.0922$). With the increase of salt concentration, the CAT activity of seed broccoli increased to 11.01 U/ml at 300 mmol/L salt concentration, which was 4.76% less than that in the control group. The overall trend of CAT activity of seed broccoli was first decreased and then increased.

3.4. Effects on H₂O₂ Content

Under the treatment of low salt concentration, the content of H₂O₂ in the seed broccoli leaves continued to rise. When the salt concentration exceeded the tolerance level of 200 mmol/L, the H₂O₂ content begins to decrease. Table 1 showed that under the medium and low salt concentration, the H₂O₂ content of seed broccoli showed a trend of continuous increase. The H₂O₂ content of seed broccoli reached the maximum value of 78.32mmol/L when the salt concentration was 200mmol/L, which was 127.66% of the blank group and had a very significant difference from the control group ($P=0.0027$).

The content of H₂O₂ in seed broccoli decreases with the increase of salt concentration. When the salt concentration reached 300mmol/L, the H₂O₂ content of seeded broccoli was 64.56 mmol/L, which was only 105.18% of the control group.

3.5. Effects on Pro Content

The results showed that salt concentration had a significant effect on Pro level ($P=0.0017$). Table 1
showed that the content of Pro first increased and then decreased. With the increase of salt concentration, the content of Pro increased continuously. When the salt concentration was 200mmol/L, the Pro content reached the maximum of 73.95 g/g, which was extremely significantly higher than that of the control group. After 200mmol/L concentration, the content of Pro began to decrease. The results indicated that content of Pro under salt stress reached its tolerance peak under medium and high salt concentration.

3.6. Effects on Leaf Thickness

The data showed that the leaf thickness of seed broccoli increased with the increase of salt concentration. When the salt concentration was 300mmol/L, the maximum leaf thickness reached 0.623mm, which was significantly higher than that of the control group (P=0.0001).

4. Discussion

A large number of studies have been conducted to explore the physiological changes of plants under stress, indicating that reactive oxygen species are closely related to the peroxidation of membrane lipids [10, 13-16]. Reactive oxygen species (ROS) include hydrogen peroxide and three types of free radicals (superoxide, hydroxyl and peroxide radicals). These radicals lead to the peroxidation of membrane lipids after accumulating in large quantities in cells. In general, an antioxidant system can remove these free radicals. For example, SOD, CAT and POD can scavenge superoxide radicals, hydrogen peroxide radicals, and peroxide radicals, respectively. However, under adverse conditions, the activity of antioxidant enzymes is inhibited, which leads to the accumulation of free radicals and adversely affects cell membranes [17]. In the antioxidant system, the decrease of SOD may be related to cell senescence [18]. CAT and POD can remove the oxidative effect of H$_2$O$_2$ on cells in plants. Therefore, CAT is a key factor for plants to against the OH-toxicity. Under salt stress, Pro can prevent dehydration and maintain the osmotic potential of cells at a normal level [5].

In this study, the Pro content of seed broccoli leaves increased with the increase of salt concentration. Pro ensures water absorption in high salt environments by reducing osmotic potential. However, there is a limit to this regulatory capacity. With the significant increase of Pro, the plant will consume too much energy, resulting in malnutrition. In the present study, when the salt concentration reached 200mmol/L, the Pro content reached its maximum value and exceed the maximum tolerance of seed broccoli. After the concentration of 200mmol/L, the content Pro decreased.

The detection of enzyme activity showed that when the POD content in the seed broccoli leaves was low in salt concentration, it would increase with the increase of salt concentration. The activity of CAT and total SOD showed a steady decrease trend. Dong proposed that POD activity of low-resistant wheat strains decreased while that of high-resistant wheat strains increased under the same salt stress, and the SOD activity of both strains remained at a high level [14]. However, in this study, POD activity reached its peak value when the salt concentration was 100mmol/L. There was no significant change in CAT and SOD activity. This indicated that Broccoli was sensitive to salt stress when the salt concentration was low, and a certain amount of POD was produced to protect the membrane structure, reflecting the low NaCl tolerance potential.

In the present study, the physiological indexes of seed broccoli showed different changes under salt stress. With the increase of salt concentration, the leaf thickness of seed broccoli increased, which was consistent with the change of leaf morphology of soybean plants in saline alkali environment. The reason for this phenomenon may be that the excessive salt content made the leaf tissue increased and arranged closely, which eventually leaded to the increase of leaf thickness. The activities of SOD and CAT in seed broccoli showed a gentle change trend, which was different from the results in previous studies [19-20], and this may be due to the strong salt tolerance of seed broccoli varieties. The change trend of proline and hydrogen peroxide was same as basically, which was consistent with that of common broccoli under salt stress, exhibiting the trend of first rising and then declining. Under salt stress, the POD activity of seed broccoli was significantly different from that of common broccoli, indicating that the maximum tolerance of POD activity of seed broccoli was lower than that of
common broccoli. In conclusion, the present study showed that H17 had a certain salt tolerance, and H17 showed strong salt tolerance when the concentration of NaCl reached the strongest salt stress at 100 mmol/L.

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