Effects of Spraying Abscisic Acid on Growth and Antioxidant Enzyme of Lettuce Seedlings under Salt Stress

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Abstract. The glass lettuce was made as material, the effect of different concentrations (0, 1, 5, 10, 20 μmol·L⁻¹) of exogenous abscisic acid on growth, antioxidase activity and osmotica of lettuce seedlings under salt stress was studied. The results showed that the root length, stem diameter, and dry fresh weight of lettuce sprayed with abscisic acid were higher than those of the control. Within a certain concentration range, the activities of antioxidant enzymes POD, SOD and CAT increased with the increase of abscisic acid concentration. The soluble protein content increased but the MDA content decreased, which maintained the balance of intracellular reactive oxygen metabolism and prevented membrane lipid peroxidation. Therefore, spraying the exogenous abscisic acid could increase the resistance of lettuce to salt stress, and the best effect was obtained when the concentration was 10μmol·L⁻¹.

1. Introduction
Lettuce (Lactuca sativa L.) has wide adaptability to the soil, and it is not strict with the light requirement during the growing period. It has become one of the important leafy vegetables in greenhouse [1].

Under the condition of facility mulching, the cultivation and management measures of the irrational fertilization and irrigation of the soil lead to the accumulation of salt on the ground surface, which leads to secondary salinization of soil [2]. Under the condition of secondary salinization of plant soil, obvious physiological drought and growth adverse reaction can be found in crops, leading to dehydration, wilting and death of plants [3-5].

Abscisic acid (ABA) is currently recognized as one of the five major traditional plant hormones [6]. ABA can not only regulate the growth and development of plants, but also regulate the adaptability of plants to the environment [7-8]. So it is considered to be the stress hormone of plants [9]. Therefore, this study explored the changes of growth and physiological characteristics of lettuce under salt stress by spraying ABA, trying to find the optimum ABA concentration which can alleviate the harm of salt stress, enhance salt tolerance, and provide reference for salted vegetable cultivation.

2. Materials and Methods

2.1 Materials.
The seeds of 'Glass lettuce' were purchased from Sichuan Agricultural University (Chengdu Campus). Melatonin was purchased from Sigma-Aldrich (St. Louis, MO, USA).
2.2 Experimental Design.

The plump lettuce seeds were chosen and sterilized with 1.2% sodium hypochlorite solution for 10 min, then rinsed with deionized water three times, placed in a petri dish covered with wet filter paper, and placed in a constant temperature incubator at 22 °C for germination. When the seeds piped, the germinated seeds were selected and sown them into the pots filled with the substrate. The size of the pots was 21 cm × 20 cm (diameter × height). The ratio of the substrate was vermiculite: perlite = 1:1, the pots were placed in a plastic greenhouse, and 1/2 Hoagland nutrient solution was poured every 2 days. After the second true leaf of lettuce was fully developed, the lettuce seedlings with the same growth vigour were treated with salt stress with Hoagland nutrient solution containing 50 mmol·L⁻¹ NaCl, and were poured every 2 days, 20 ml each time, until the end of the experiment and daily replenished water depend on the weather. When three true leaves of lettuce are fully spread, the four seedlings with the same growth were retained in per pot. Different concentration of abscisic acid solution with 0 (control, fresh water treatment), 1, 5, 10, 20 μmol·L⁻¹ was sprayed on the leaves at 18:00 until leaf dripping, once every two days and for a total of 3 times. Each treatment was repeated 6 times for a total of 30 pots. The growth indexes, antioxidant activity and content of osmotica of lettuce were determined 30 days after sprayed melatonin.

2.3 Statistic analyses.

Statistical analyses were performed using SPSS 20.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way ANOVA with least significant difference (LSD) at a 5% confidence level.

3. Results

3.1 Growth and biomass.

As shown in Table 1, spraying ABA greatly increased the growth of lettuce under salt stress. During the concentration range of 0~10 μmol·L⁻¹, with the increasing of exogenous ABA concentration, the root length, stem diameter and fresh weight of the lettuce also showed an upward trend, but when the concentration of exogenous ABA concentration reached 20 μmol·L⁻¹, the indexes of lettuce showed a downward trend, and the growth of lettuce was significantly higher than that of control when 10 μmol·L⁻¹ ABA was applied. The above results indicate that spraying a certain concentration of ABA can significantly promote the normal growth of plants.

### Table 1. The growth and biomass of lettuce seedlings.

| ABA concentration (μmol·L⁻¹) | Root length (cm) | Stem diameter (cm) | Fresh weight of shoot (g) | Fresh weight of root (g) | Dry weight of shoot (g) | Dry weight of root (g) |
|------------------------------|------------------|--------------------|---------------------------|-------------------------|------------------------|------------------------|
| 0                            | 20.09±0.186a     | 0.26±0.024b        | 2.44±0.114b               | 0.78±0.033b             | 0.45±0.006c            | 0.63±0.009c            |
| 1                            | 22.78±1.51ab     | 0.32±0.016ab       | 2.47±0.108ab              | 1.10±0.109ab            | 0.48±0.008b            | 0.72±0.007b            |
| 5                            | 24.08±0.65ab     | 0.35±0.023a        | 2.70±0.150ab              | 1.13±0.066ab            | 0.48±0.007b            | 0.78±0.005b            |
| 10                           | 27.03±2.78a      | 0.37±0.022a        | 3.10±0.261a               | 1.23±0.232a             | 0.49±0.005a            | 0.82±0.007a            |
| 20                           | 25.32±1.81ab     | 0.36±0.012a        | 2.87±0.235ab              | 1.20±0.104ab            | 0.48±0.004b            | 0.79±0.004b            |

Note: There is a significant difference between 0.05 levels of different letters, the same below.

3.2 Antioxidant enzyme activities.

It can be seen from Figure 1 that the activity of SOD in lettuce treated with ABA was significantly higher than that of control, and the activity of SOD respectively increased by 38.16%, 50.03%, 69.75%, 6.51% than control, and the differences were significant. In the range of 0~20 μmol·L⁻¹, POD activity and CAT activity also increased to some extent after applying ABA, and reached a significant level at 10 μmol·L⁻¹.

3.3 MDA and soluble protein content.

It can be seen from Figure 2 that under salt stress, the MDA content in control is significantly higher than that in other treatments, which indicated that salt stress caused a large amount of active oxygen
accumulated in lettuce leaves, accelerated cell membrane lipid peroxidation, and continuously increases lettuce. After spraying ABA, the damage of lettuce was alleviated. In the range of 0~20 μmol·L⁻¹, the MDA content decreased first and then increased, the MDA content was lowest at 10 μmol·L⁻¹, and decreased by 25.45% compared with control \((P<0.05)\).

Figure 1. The antioxidant enzyme activities of lettuce seedlings.

When the lettuce was exposed to different concentrations of ABA under salt stress, which showed a trend of increasing and then decreasing, and the soluble protein content increased more than control. After spraying 1, 5, 10, 20 μmol·L⁻¹ ABA, the soluble protein content in lettuce leaves increased by 11.63%, 25.50%, 34.90% and 25.25% than control respectively. Low concentration of ABA has a higher promoting effect on salt-induced lettuce, while the promotion effect is lower at high concentration. Among them, when the concentration of ABA was 10 μmol·L⁻¹, the soluble protein content of lettuce was highest.
4. Conclusions
Under salt stress, the plant showed growth inhibition, small leaves, and fresh weight and biomass reduction. Under salt stress, plant cells undergo osmotic stress, which balances the external osmotic potential of plant cells. Plant cells actively accumulate some soluble substances to reduce intracellular osmotic potential and ensure the normal supply of water. When accumulated soluble substances cannot be reduced in the intracellular osmotic potential, it is necessary to provide exogenous substances to enhance the osmotic adjustment ability of plant cells and reduce salt damage. Studies have shown that exogenous ABA can alleviate the inhibition of salt stress on plants, and can improve the osmotic adjustment ability of plant cells under salt stress. The results of this study showed that foliar application of ABA can effectively alleviate the inhibition of lettuce growth by salt stress, and make lettuce grow stronger in secondary salinized soil. Exogenous ABA can increase the soluble protein content, help to alleviate the permeation damage caused by salt stress, enhance the SOD and POD activity in lettuce leaves, reduce the accumulation of MDA, and effectively prevent the generation of excess reactive oxygen species caused by stress. The peroxidation damage increased the growth of lettuce, especially with 10 μmol·L⁻¹ABA.

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