Effects of Exogenous Melatonin on Growth and Antioxidant System of Lettuce Seedlings under Salt Stress

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Abstract. The glass lettuce was made as material, the effect of different concentrations (0, 50, 100, 150, 200 μmol·L^{-1}) of exogenous melatonin on growth, antioxidant enzyme 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 melatonin 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 melatonin concentration. The soluble protein content increased but the MDA content decreased, which maintaining the balance of intracellular reactive oxygen metabolism and preventing membrane lipid peroxidation. Therefore, spraying the exogenous melatonin could increase the resistance of lettuce to salt stress, and the best effect was obtained when the concentration was 100 μmol·L^{-1}.

1. Introduction

Facilities cultivation has become an important industry for promoting agricultural modernization. Long-term cover cultivation will lead to problems such as salt accumulation on the surface and secondary salinization of soil, which has restricted the development of vegetable industry [1]. Soil salinization is one of the major limiting factors in agricultural production and seriously affects the growth and survival of crops. Under the condition of salt stress, the balance between the generation and elimination of reactive oxygen species in the cell is destroyed and membrane lipid peroxidation is enhanced, resulting in increased plasma membrane permeability, imbalance of ion balance, and metabolic disorders [2, 3]. Melatonin is a tryptophan derivative and widely found in plants. Its physiological function is similar to that of IAA. It not only promotes adventitious roots, but also regulates photoperiod [4]; it can also eliminate freedom radical and protect the integrity of the membrane and prevent the degradation of chlorophyll [5]. As an antioxidant, it can effectively inhibit the damage to plant cell caused by abiotic stresses such as high temperature [6], low temperature [7], drought [8] and salt stress [9]. With the increase of soil secondary salinization in facilities, the growth and development of lettuce has been greatly affected. Therefore, this study explored the effects of exogenous melatonin on growth and antioxidant system of lettuce under salt stress.

2. Materials and Methods

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

Experimental Design. The plump lettuce seeds were chose 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 the three true leaves of lettuce are fully spread, the four seedlings with the same growth were retaining in per pot. Different concentration of melatonin solution with 0 (CK, fresh water treatment), 50, 100, 150, 200 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.

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

Growth and Biomass of Lettuce Seedlings under Salt Stress. As shown in Table 1, the root length, Stem diameter, fresh weight and dry weight of lettuce seedlings sprayed with different concentrations of melatonin the leaves were higher than those of the control, and they were increased first and then decreased with increasing treatment concentration. The peak was reached at the concentration was 100 μmol·L^{-1} and thereafter decreased. Although the values of treatments of 150 μmol·L^{-1} and 200 μmol·L^{-1} were lower than those treated with 100 μmol·L^{-1}, they were still significantly higher than that of control. It indicated that exogenous spraying of melatonin at a certain concentration can promote the normal growth of lettuce seedlings under salt stress, and the effect is most significant when melatonin concentration is 100 μmol·L^{-1}.

Table 1 Growth and biomass of lettuce seedlings under salt stress

Melatonin 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 shoot /g
0	20.94±0.93b	0.287±0.02b	2.188±0.05c	0.838±0.03c	0.155±0.03d	0.215±0.01d
50	21.44±0.77b	0.315±0.03b	2.573±0.19b	1.018±0.04b	0.166±0.05bc	0.238±0.04c
100	26.00±0.89a	0.386±0.01a	3.288±0.13a	1.345±0.02a	0.182±0.02a	0.283±0.05a
150	23.22±1.07a	0.334±0.02b	2..879±0.84b	1.238±0.05a	0.167±0.03b	0.248±0.05b
200	22.60±1.22b	0.325±0.01b	2..668±0.38b	1.099±0.05b	0.164±0.01c	0.243±0.03bc

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

Antioxidant enzyme activity of Lettuce Seedlings under Salt Stress. Fig. a showed that the activity of SOD, POD and CAT enzymes in the salt-stressed lettuce seedlings were significantly changed after spraying melatonin, and the change trends were similar, that is, as the melatonin concentration increased, the indicators first increased and then decreased, and when melatonin concentration was 100 μmol·L^{-1}, they were increased by 129.6%, 114.1%, and 85.5% respectively compared to the control. Although the activities of SOD, POD and CAT decreased at concentrations of 150 μmol·L^{-1} and 200 μmol·L^{-1}, they were still significantly higher than the control. From the above results, we can see that in a certain concentration range, low concentration of melatonin treatment can enhance SOD, POD, CAT enzyme activity of lettuce seedlings, but the high concentration of melatonin leaded to SOD, POD, CAT enzyme activity decreased, and the spraying melatonin concentration was 100 μmol·L^{-1} worked best.

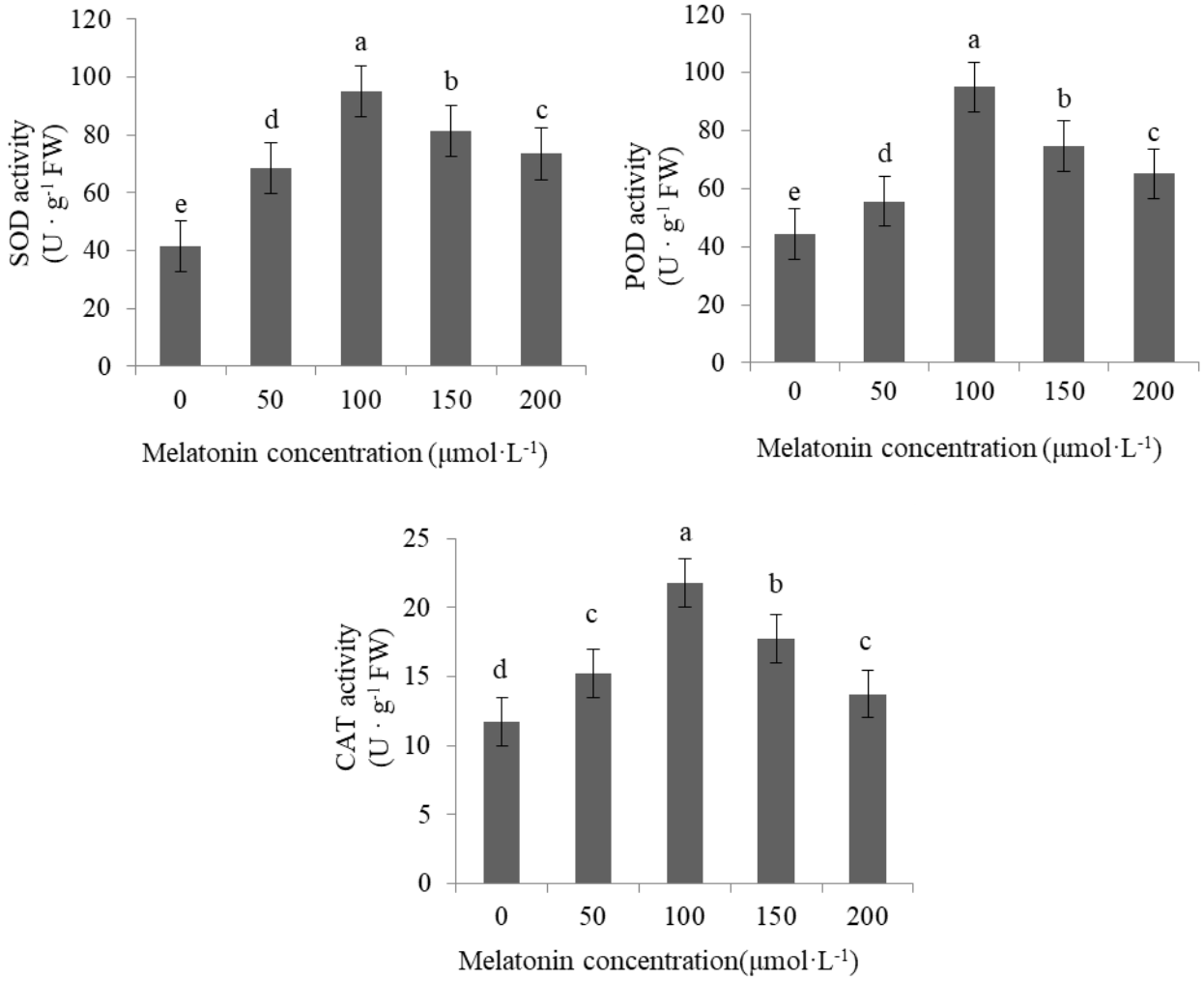

Fig. 1 Antioxidant enzyme activity of lettuce seedlings under salt stress

MDA and Soluble Protein Content in Lettuce Seedlings under Salt Stress. As shown in Fig. 2, the soluble protein content of lettuce seedlings increased first and then decreased with the increasing melatonin concentration under salt stress, and peaked at melatonin concentration was 100 μmol·L^{-1}, an increase of 40.7% compared with the control, the value of each concentration treatment was higher than that of the control. In general, the content of MDA was positively correlated to the degree of damage to the cell membrane. In Fig. 2, the trend of MDA content in each melatonin treatment was opposite to that of soluble protein. When melatonin was 100 μmol·L^{-1}, it was 37.8% lower than that of the control.

4. Conclusions

Under salt stress conditions, large amounts of reactive oxygen species (ROS) are produced in plants. SOD, POD, CAT and other protective enzymes act synergistically effect in plants to remove excess ROS during stress, which maintain the metabolic balance of ROS, and protect the membrane structure, so that plants tolerate, mitigate or resist adverse stress injury to a certain extent. Exogenous spraying of melatonin under salt stress enhanced the activities of three important protective enzymes, thus reducing the damage of salt stress on lettuce seedlings. MDA is one of the most important products of membrane lipid peroxidation. The extent of membrane lipid peroxidation can be understood by MDA to indirectly determine the extent of membrane system damage and the plant's resistance to stress. Soluble protein is an important osmotica in plants, which can increase the infiltration concentration of cells and the number of functional proteins and help maintain the normal metabolism of cells. The results showed that exogenous application of melatonin significantly decreased the content of MDA in leaves of lettuce seedlings and reduced the

damage caused by salt stress. In addition, soluble protein content in leaves of lettuce seedlings can also be increased to ensure normal seedling growth. In brief, the melatonin concentration of 100 μmol·L^{-1} put up the most significant effect.

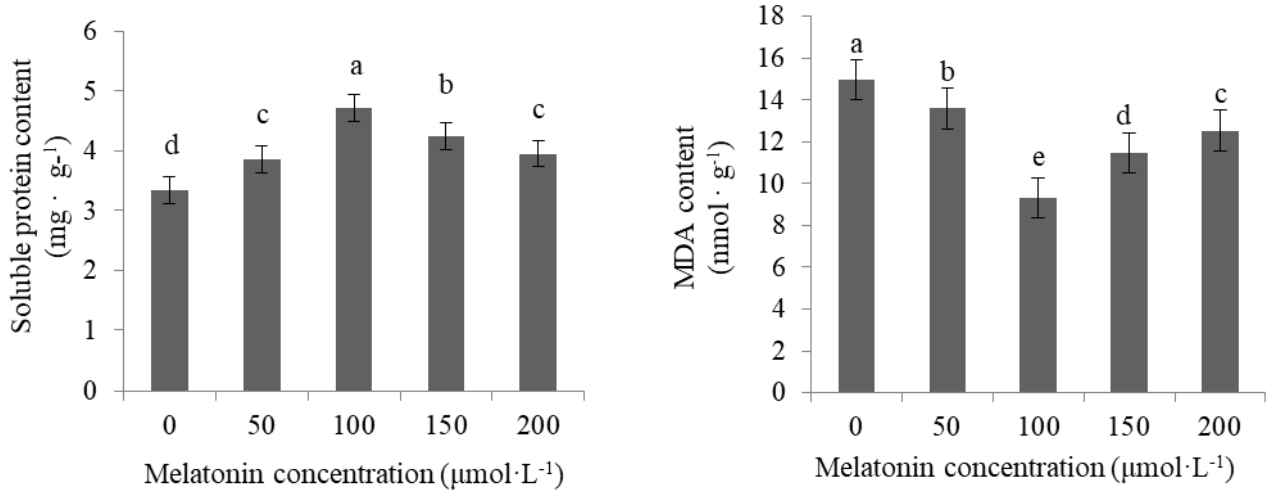

Fig. 2 Soluble protein and MDA content of lettuce seedlings under salt stress

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