Effect of Exogenous Melatonin on Antioxidant Enzyme Activity in Kiwifruit *Hayward* under Salt Stress

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**Abstract.** In order to explore the effect of exogenous melatonin (MT) on antioxidant enzyme activity in kiwi under the salt stress, *Hayward* seedlings primed with 0.1μmol•L⁻¹ MT were used as the experimental materials and treated with 100 mmol•L⁻¹ NaCl in hydroponic condition. The results showed that exogenous MT had certain effect on the activity of SOD, POD during the early stage of salt stress, but the effect was not significant.

1. **Introduction**

Soil salinization is a global problem. Up to now, about 80% of China's salinized soil has not been developed and utilized, which has become one of the biggest constraints on China's agricultural development [1]. Studies have shown that melatonin (MT) has the function for improving the resistance to biological stimuli, and improving the toleration to several biological and abiotic stresses [2-5]. The *Actinidia* plant is an important fruit tree resource originated on China [6]. "Hayward" is the earliest kiwi variety, belonging to the series of *A. deliciosa*, which is the main variety cultivated in China. Its fruit has rich aroma, delicate and sweet taste, rich nutrition value and good medicinal value, and is known as the "King of Fruits".

In this study, the "Hayward" seedlings were used as the material to determine physiological indexes such as membrane lipid peroxidation, osmotic regulatory substances and antioxidant enzyme activity in kiwi leaves, so as to explore the effects of exogenous MT on plant resistance to sodium salt stress and provide theoretical basis for the application of exogenous MT in production.

2. **Materials and methods**

2.1. **Material preparation**

After stored at 4℃ for 60 days to break dormancy, seeds of *Hayward* were treated with variable temperature to accelerate germination. Germinated seeds were sown into a hole tray containing mixed matrix (nutritive soil: vermiculite: perlite =3:1:1), placed in the artificial climate chamber (25±1 ℃, 12 h/12 h). When cultivated to 3 true leaf, seedlings were transferred to pots (23 cm×18 cm), with 3 seedlings every pot.

2.2. **Material Treatments**

When grew to 9 true leaves, the seedlings with consistent growth were divided into 3 groups, cultured...
in 20×35 cm plastic box filled with 1500mL 1/2 Hoagland's nutrition. Firstly, seedlings were treated with 1/2 Hoagland's nutrition or plus 0.1μmol·L⁻¹MT (50 mL) for 5 consecutive days, then treated with plus 100 mmol·L⁻¹ NaCl. 3 treatments were set as: (1) CK (1/2 Hoagland's nutrition); (2) ST (1/2 Hoagland's nutrition +100 mmol·L⁻¹ NaCl); (3) ST+MT (100 mmol·L⁻¹ NaCl +0.1μmol·L⁻¹ MT). Each treatment was repeated 4 times, and each repeat used one box with 9 seedlings. 3-5 leaves were taken as samples at 0, 36, 48, 60 h, and immediately frozen and stored at -80 ℃ for following determination of some physiological indexes.

2.3. Measurement Items and Methods
H₂O₂ content was determined by the method of Hao JJ et al. [7]. Superoxide dismutase (SOD) activity was determined by nitrogen blue tetrazole method [8]. The POD activity was determined by guaiacol colorimetry [8]. Catalase (CAT) activity was determined by ultraviolet spectrophotometry [9].

2.4. Data Analysis
SPSS 20.0 was used for significance analysis, and Excel 2010 software was used for data processing and chart drawing.

3. Results and Analysis

3.1. Effect of exogenous MT on H₂O₂ content
In this study, the content of H₂O₂ in the leaves of CK group showed a trend of increasing first and then decreasing, while that in ST and ST+MT group went up continuously (Figure 1). The differences of H₂O₂ content among three groups were basically insignificant before 60h. At 60h, H₂O₂ content in ST was significant high than that in CK, and much higher than ST+MT (insignificant), indicating MT alleviating the salt stress at a certain degree.

![Figure 1. Changes of hydrogen peroxide (H₂O₂) content in leaves of hydroponic "Hayward" seedlings. Note: Different lowercase letters in the figure indicate a significant difference at the 5% level, the same as below.](image-url)

3.2. Effect of exogenous MT on antioxidant enzyme activity
The main functions of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are to remove reactive oxygen radicals and prevent their excessive accumulation [10]. In the study (Figure 2), SOD activity in three groups showed basically no significant difference during the whole salt stress procession except at the 0h. After primed with MT (at 0h), SOD activity in ST+MT group was significant lower than CK and ST, indicating that MT made the seedlings adapt to the hydroponics environment to some extent.
Figure 2. Changes of SOD activity in leaves of hydroponic "Hayward" seedlings.

In this study (Figure 3), POD activity in the ST+MT group showed the same change trend as that in the CK group, which showed a trend of increasing and decreasing later, but the change range was larger than that in the CK group. While the activity of ST group increased first, then decreased, and then recovered slightly. During the whole stress process, POD activity in the three treatments showed no significant difference except 0 and 60 h, indicating MT made the seedlings adapt to the hydroponics environment and alleviating the salt stress to a certain degree.

Figure 3. Changes of POD activity in leaves of hydroponic "Hayward" seedlings.

In this study (Figure 4), CAT activity in the CK group showed a upward trend followed by a downward trend, while the ST group showed a consistent change trend, however, the turning point was delayed, and the ST+MT group first rose and then fell, and recovered slightly after the turning point in the ST group. During the whole stress process, there was no significant difference in CAT activity among the leaves of kiwifruit seedlings treated by the three groups, indicating MT had no significant effect on CAT activity.

Figure 4. Changes of CAT activity in leaves of hydroponic "Hayward" seedlings.
4. Conclusion
SOD can catalyze disproportionation under water and salt stress to convert superoxide radicals into \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \), and then decompose \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \) into non-toxic \( \text{H}_2\text{O} \) and \( \text{O}_2 \) through CAT and POD to prevent the interaction between \( \text{H}_2\text{O}_2 \) and reactive oxygen species, maintain the balance of reactive oxygen metabolism and protect aerobic organisms from damage \([11-15]\). In comparison with the changes of antioxidant enzyme activity in the three treatment groups in the experiment, it is believed that melatonin (MT) might play a certain role in helping hydroponic seedlings to adapt to the hydroponics environment and to resist salt stress, and its effect on SOD and POD was obvious, but the effect is not significant. It may be related to the kiwi seedlings which are not suitable for hydroponic culture.

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