

# Effects of Exogenous Melatonin on Growth and Physiological Characteristics of Leaves in Shepherd's-purse Seeding under NaCl Stress

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**Abstract:** The effects of exogenous melatonin on growth and physiological characteristics of Shepherd's-purse seedlings under salt Stress were studied by spraying 5 different concentrations of MT (0, 50, 100, 150, 200  $\mu\text{mol}\cdot\text{L}^{-1}$ ) solution on leaves in Shepherd's-purse seedlings. The result showed that spraying MT on leaves in Shepherd's-purse seedlings which plant height, root length, fresh and dry weight were higher than control. With the increase of concentration of MT, the general tends of chlorophyll and carotenoid content on leaves in Shepherd's-purse seedlings were all first increased then decreased together. Besides, spraying MT not only strengthened the activity of anti-oxygenic enzyme and enhanced anti-oxygenic ability, but also restrained MDA accumulation and increased soluble protein content. Therefore, exogenous MT could efficiently enhance resistance to salt stress, and the 150  $\mu\text{mol}\cdot\text{L}^{-1}$  was the best concentration.

## Introduction

In recent years soil salinization has become one of the main problems faced by modern agriculture. Large areas of soil salinization not only cause a waste of resources, but also limit plant growth, which leading to crop failures and lower quality, a serious threat to the sustainable development of agriculture in China [1-2]

Melatonin (N- acetyl-5-methoxy tryptamine, Melatonin, MT) is a strong antioxidant endogenous free radical scavenger [3]. Melatonin affect some oxidation and antioxidant enzymes in cells and tissues through its receptor, such as to enhance catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-P) and other activities to achieve the free radical scavenging effect [4]. Zhang et al. have shown that melatonin can improve Pennisetum resistance to salt stress [5]. Li et al. also showed that administration of 1  $\mu\text{mol}\cdot\text{L}^{-1}$  MT can effectively alleviate the inhibitory effects of salt stress on the growth of Hubei Begonia, reduce the degradation of chlorophyll and oxidative damage, and enhance the photosynthetic rate [6]. Zhang et al. studies have also found that exogenous melatonin reduced oxidative damage caused by salt stress by enhancing antioxidant gene expression, which significantly increased cucumber seeds SOD, POD, CAT activity [7].

Shepherd's-purse has high nutritional and medicinal value, which in tenderstem leaf for food [8]. With the increase of soil secondary salinization area, shepherd's growth is affected and it's yield and quality reducing. In this experiment, with shepherd's-purse seedlings as materials, we study effects of exogenous melatonin on salt stress on growth and physiological characteristics of shepherd's

purse.

## Materials and Methods

**Materials.** Shepherd's-purse seedlings were collected from chengdu, Sichuan Agricultural University campus surrounding farmland. MT is purchased from SIGMA ALDRICH company, a small amount of anhydrous ethanol dissolves with distilled water as  $500 \mu\text{mol}\cdot\text{L}^{-1}$  mother liquor,  $4^{\circ}\text{C}$  preservation, according to the design concentration dilution. All chemicals used in experiments were of analytical grade.

**Experimental Design.** Clean the shepherd's-purse seedlings, select Growing evenly seedlings and transplant to pots which filled with substrate and the size is  $21 \text{ cm} \times 20 \text{ cm}$  (diameter  $\times$  height), then put the pots into plastic greenhouses, growing under natural conditions and watering 20 mL 1/2 Hoagland nutrient solution every other day. After seedling transplanting survival, water Hoagland nutrient solution which containing  $50 \text{ mmol}\cdot\text{L}^{-1}$  NaCl until the end of the trial in order to prevent water shortages, according to the weather conditions add moisture properly. When shepherd's-purse 4 true leaves fully expanded, choose the same size of shepherd's-purse seedlings to spray 5 different concentrations of MT ( $0, 50, 100, 150, 200 \mu\text{mol}\cdot\text{L}^{-1}$ ) on leaves at 17:00 every other day, a total of 3 times. Every pot 3 strains and each processing. after 30 days, measure growth index and physiological index.

**Statistic analyses.** Statistical analyses were performed using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way ANOVA with least significant difference (LSD) at a 5% confidence level.

## Results and Discussion

**Growth and biomass.** We can see from Table 1, shepherd's-purse spray melatonin whose height, root length, fresh and dry weight were higher than no spraying, and with the increasing concentration the general tends were all first increased then decreased together. When the concentration of  $150 \text{ mg}\cdot\text{L}^{-1}$  reaches maximum value and concentration of  $200 \text{ mg}\cdot\text{L}^{-1}$  compared with  $150 \text{ mg}\cdot\text{L}^{-1}$  has decreased, but it's still higher than the control. This shows that spraying melatonin within a certain range can promote the growth of salt stress shepherd's-purse, improve its resistance to salt stress.

Table 1 Effects of MT on the growth and biomass in Shepherd's purse under salt stress

MT concentration $/(\mu\text{mol}\cdot\text{L}^{-1})$	Plant height $/(\text{cm})$	Root length $/(\text{cm})$	Shoot fresh mass $/(\text{g})$	Shoot fresh mass $/(\text{g})$	Root dry mass $/(\text{g})$	Shoot dry mass $/(\text{g})$
0	$18.13 \pm 0.06\text{d}$	$14.67 \pm 0.32\text{e}$	$0.189 \pm 0.01\text{e}$	$2.14 \pm 0.01\text{e}$	$0.097 \pm 0.01\text{c}$	$0.540 \pm 0.01\text{d}$
50	$19.87 \pm 0.72\text{c}$	$16.03 \pm 0.31\text{d}$	$0.248 \pm 0.02\text{d}$	$2.25 \pm 0.01\text{d}$	$0.104 \pm 0.01\text{c}$	$0.575 \pm 0.01\text{c}$
100	$21.07 \pm 0.31\text{b}$	$17.57 \pm 0.35\text{b}$	$0.281 \pm 0.02\text{c}$	$2.33 \pm 0.01\text{c}$	$0.123 \pm 0.01\text{b}$	$0.599 \pm 0.01\text{b}$
150	$22.03 \pm 0.31\text{a}$	$18.53 \pm 0.25\text{a}$	$0.366 \pm 0.01\text{a}$	$2.50 \pm 0.02\text{a}$	$0.128 \pm 0.01\text{ab}$	$0.615 \pm 0.01\text{a}$
200	$20.03 \pm 0.51\text{c}$	$16.67 \pm 0.25\text{c}$	$0.321 \pm 0.01\text{b}$	$2.35 \pm 0.01\text{b}$	$0.114 \pm 0.01\text{b}$	$0.596 \pm 0.01\text{b}$

Note: Data followed different letters within column indicate significant difference of 0.05 level.

**Photosynthetic pigment content.** We can see from Table 2, chlorophyll a, chlorophyll b and carotenoid content were increased firstly and then decreased with increasing concentration of MT, and it is the most effective when the concentration of  $150 \mu\text{mol}\cdot\text{L}^{-1}$ , compared with the control improved 13.79%, 13.64%, 12.5%. Chlorophyll a/b showed the extent of the thylakoid membrane crib fold, shepherd's-purse seedlings spray melatonin that chlorophyll a/b were higher than control, at the concentration of  $150 \mu\text{mol}\cdot\text{L}^{-1}$ , the peak, and then reduced, but the difference was not

significant.

Table 2 Effects of MT on the contents of photosynthetic pigments in Shepherd' s purse under salt stress

MT concentration /( $\mu\text{mol}\cdot\text{L}^{-1}$ )	Chlorophyll a /( $\text{mg}\cdot\text{g}^{-1}$ )	Chlorophyll b /( $\text{mg}\cdot\text{g}^{-1}$ )	Chlorophyll a+b /( $\text{mg}\cdot\text{g}^{-1}$ )	Chlorophyll a/b /( $\text{mg}\cdot\text{g}^{-1}$ )	Carotenoid /( $\text{mg}\cdot\text{g}^{-1}$ )
0	1.45±0.01e	0.44±0.01 d	1.89±0.01 e	3.27±0.04 a	0.32±0.01b
50	1.50±0.01d	0.46±0.01 c	1.96±0.01 d	3.30±0.03 a	0.33±0.02 b
100	1.55±0.01c	0.47±0.01 b	2.02±0.01 c	3.31±0.01 a	0.33±0.01 b
150	1.65±0.01 a	0.50±0.01 a	2.15±0.01 a	3.33±0.04 a	0.36±0.01 a
200	1.57±0.01 b	0.47±0.01 b	2.04±0.01 b	3.31±0.04 a	0.34±0.02 b

**The enzymatic antioxidant system.** The Fig. 2 shows that the activity of Shepherd's-purse leaf of SOD, POD, CAT had same change trend, when the concentration was between 0~150  $\mu\text{mol}\cdot\text{L}^{-1}$  MT significantly increased with the increase of the concentration of melatonin, and it reached the maximum at the peak of 150  $\mu\text{mol}\cdot\text{L}^{-1}$ , and SOD, POD, CAT activity increased by 150.38% , 36.35%

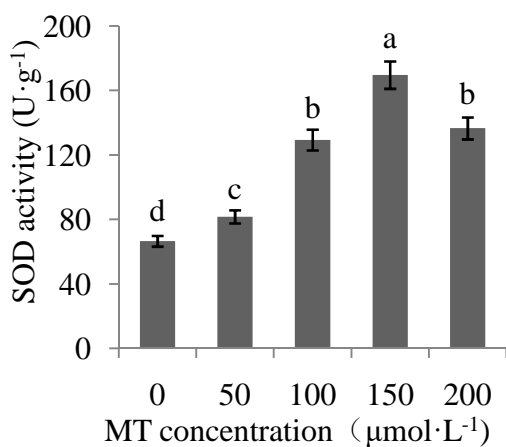


Fig.1 Effects of MT on SOD activity in Shepherd' s purse under salt stress

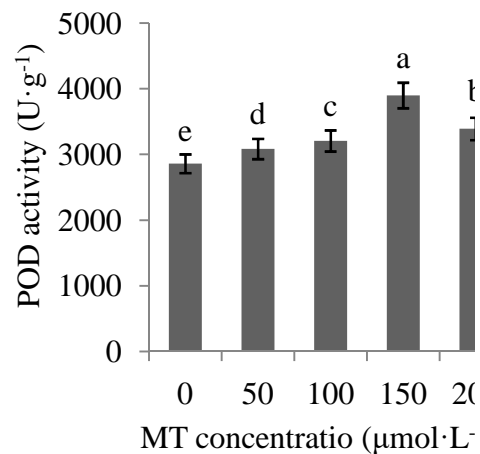


Fig.2 Effects of MT on SOD activity in Shepherd' s purse under salt stress

and 64.53% compared with the control. At a concentration of 200  $\mu\text{mol}\cdot\text{L}^{-1}$  the activity of SOD, POD and CAT has declined compared to 150  $\mu\text{mol}\cdot\text{L}^{-1}$ , but it is still significantly higher than the control.

**MDA and soluble protein.** MDA is the production of cell membrane lipid peroxidation, The higher MDA content in plant the greater damage of cell membranes. The figure shows that spraying exogenous MT could significantly decrease MDA content in shepherd's-purse leaves under salt stress. When the concentration was 50, 100, 150 and 200  $\mu\text{mol}\cdot\text{L}^{-1}$  MDA content decreased by 11.13%, 25.30%, 37.32%, 30.28%. Protein is the control and executor of plant traits and life activities, Figure 3 shows that soluble protein content increased first and then decreased With the increase of concentration of spraying exogenous MT. MT concentration of 50  $\mu\text{mol}\cdot\text{L}^{-1}$  is not significant difference with control, but at the concentration of 150  $\mu\text{mol}\cdot\text{L}^{-1}$  are extremely significant difference with control and soluble protein content increased by 87.18%.

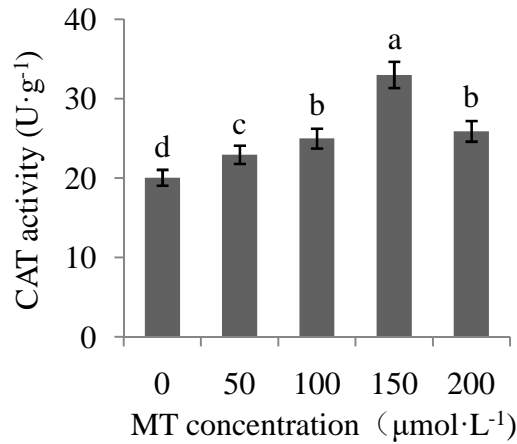


Fig. 3 Effects of MT on CAT activity in Shepherd' s purse under salt stress

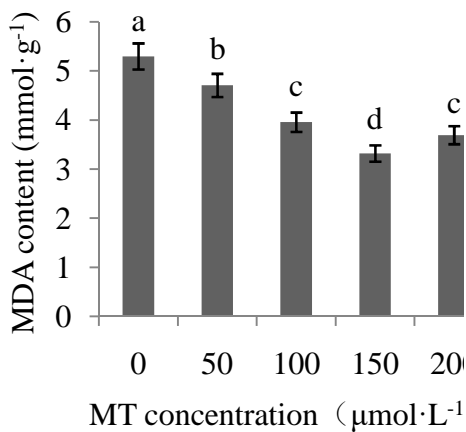


Fig. 4 Effects of MDA on CAT activity in Shepherd' s purse under salt stress

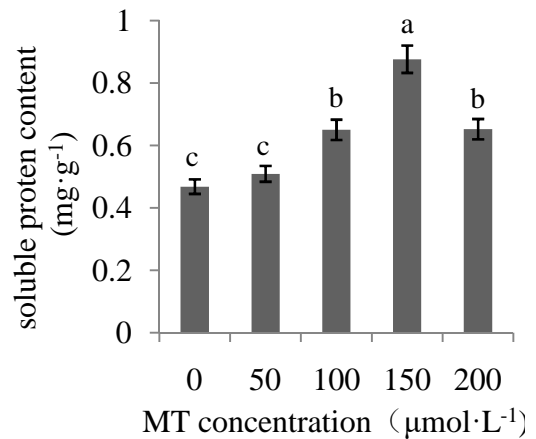


Fig. 5 Effects of MT on soluble protein content activity in Shepherd' s purse under salt stress

## Conclusions

Melatonin treatment improved the photosynthetic pigment content of shepherd's-purse seedlings which under salt stress, and enhanced photosynthesis, Promoted the growth of the shepherd's-purse, increased dry matter accumulation. Analysis it's resilience indicators proved that Melatonin treatment can increase antioxidant enzyme POD, CAT, SOD activity, maintain the balance of active oxygen metabolism, reduce MDA content and Improve the resistance ability of shepherd's-purse seedlings that under salt stress. And the best concentration was  $150 \mu\text{mol}\cdot\text{L}^{-1}$ .

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