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Effects of Exogenous Melatonin on Growth and Physiological Characteristics of Chinese cabbage seedlings under Salt Stress

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Abstract. A pot experiment was conducted to study the effects of spraying exogenous melatonin (MT) on growth and physiological characteristics of Chinese cabbage seedling under 50 mmol·L^{-1} NaCl salt stress. The results showed that exogenous MT could promote the growth of Chinese cabbage seedlings under salt stress and increase water content, chlorophyll content, soluble protein content. Besides, the MT not only strengthened the activity of antioxidant enzymes system, but also restrained MDA accumulation. Therefore, exogenous MT can enhance the resistance of Chinese cabbage seedlings to salt stress, and the concentration of 200 μmol·L^{-1}MT was the best.

Introduction

Chinese cabbage originating in China, has a long history of cultivation, rich variety of resources, is China's largest cultivated area of vegetables [1]. In recent years, the land salinization has become one of the main factors restricting the development of China's vegetable industry, there are about 100 million hectares of land salinity problems on the land [2], and vegetables situation in our country today [3], Salinization will affect the growth of vegetables, resulting in a serious decline in yield and quality [4]. Melatonin (MT) is an indole derivative of tryptophan. It has been found that melatonin, as an endogenous free radical scavenger with strong antioxidant activity, can increase the seed germination rate [5], effective mitigation of heavy metal stress [6], high temperature stress [7], low temperature stress [8], UV radiation [9] and other abiotic stress on plant growth damage. In addition, by reducing lipid peroxidation and improve antioxidant enzyme activity to promote salt stress pennisetum seed germination [10]. These studies suggest that melatonin can modulate the physiological properties of plants under salt stress. Therefore, in this experiment, the effects of melatonin on the physiological regulation of Chinese Cabbage under salt stress were studied in order to provide some reference for the subsequent research and the production of salted cabbage.

Materials and Methods

Materials. The seeds of 'Kuaike 35F' precocious cabbage were purchased from Sichuan Agricultural University (Chengdu Campus). Melatonin (MT) was purchased from SIGMA-ALDRICH Company.

Experimental Design. Select the seeds of Chinese cabbage, sterilization soaking germination. When the seeds were white seeded in nursery pots (10 cm × 10 cm) with perlite and vermiculite (perlite: vermiculite = 1: 1). Watering once a day until the cotyledons unearthed, then watering Hoagland nutrient solution (water volume of 20 ml/pot) every 2 d. After the two leaves of the Chinese cabbage were fully expanded, the seedlings were transplanted to pots filled with medium. The size of the pots was 21 cm × 20 cm (diameter × height). The pots were placed in plastic greenhouses and grown under natural conditions, and once every 2 d watering 50 mmol·L^{-1} NaCl Hoagland nutrient solution for salt treatment until the end of the trial, in order to prevent water shortages, according to weather conditions appropriate to add water. After the 4 leaves of the Chinese cabbage were fully expanded, sprayed with MT solution of 50, 100, 200 and 400 μmol·L^{-1} at 9:00 once every 2 d, sprayed three times, and each treatment was repeated three times. After 30 d, the indicators were determined.

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

Growth and biomass. As shown in Table 1 and Table 2, the plant height, stem diameter and root length of cabbage seedlings treated with exogenous MT were all higher than CK;Under the concentration of 0 ~ 200 μmol·L^{-1}, the plant height, stem diameter, root length, root fresh weight, aerial parts dry weight and aboveground water content were positively correlated with spraying MT concentration, However, when the concentration reached 400 μmol·L^{-1}, the indicators have decreased. The root water content increased with the increase of MT concentration in the range of 0 ~ 100 μmol·L^{-1}, and reached the maximum at 100 μmol·L^{-1}, but not significantly with 200 μmol·L^{-1}.While under 400 μmol·L^{-1} treatment, the underground water content was significantly lower than the maximum. The results showed that spraying melatonin promoted the growth of salt-stressed cabbage seedlings in a certain range, and enhanced its resistance to salt stress.

Table 1 Effects of exogenous melatonin on the growth of Chinese cabbage seedlings under salt stress

MT concentration (μmol·L^{-1})	Plant height (cm)	Stem diameter (cm)	Root length (cm)	Shoot fresh weight (g·$plant^{-1}$)	Root fresh weight (g·$plant^{-1}$)
0	8.66±0.230 c	0.32±0.007 c	21.98±0.455c	4.23±0.057 e	1.08±0.030 d
50	8.80±0.255bc	0.33±0.003 c	22.60±0.791bc	4.73±0.051 c	1.43±0.060 b
100	8.97±0.234b	0.36±0.007 b	23.50±0.510 b	5.18±0.057 b	1.48±0.037ab
200	9.50±0.122a	0.38±0.010 a	26.37±0.914 a	5.52±0.050 a	1.49±0.027 a
400	9.32±0.164a	0.35±0.006b	22.72±0.766bc	4.62±0.044 d	1.23±0.017 c

Note: there is a significant difference between 0.05 levels of different letters, the same as following tables.

Table 2 Effects of melatonin on dry weight and water content of salt-stressed Chinese cabbage seedlings

MT concentration (μmol·L^{-1})	Shoot dry weight (g·plant^{-1})	Rootdry weight(g·plant^{-1})	Shoot water content (%)	Root water content (%)
0	0.70±0.016 c	0.19±0.006 a	83.45	82.41
50	0.78±0.022 b	0.19±0.006 a	83.51	86.71
100	0.85±0.016 a	0.20±0.007 a	83.59	86.81
200	0.87±0.028 a	0.20±0.008 a	84.15	86.71
400	0.76±0.019 b	0.19±0.005 a	83.48	84.55

Photosynthetic pigment content. As shown in Table 3, the contents of chlorophyll a, chlorophyll b and carotenoid increased first and then decreased with the increase of MT concentration, and the total chlorophyll content was the highest at the concentration of 200 μmol·L^{-1}. When the concentration of MT reached 400 μmol·L^{-1}, carotenoids, chlorophyll a and b decreased, but still higher than the control. The chlorophyll a content increased by 8.62%, 9.59%, 11.54% and 4.55%, the chlorophyll b increased by 15.07%, 15.87%, 23.02% and 12.70%, and the carotenoids increased by 5.00 %, 7.27%, 8.64%, 1.36%.

Table 3 Effect of exogenous MT on chlorophyll content of Chinese cabbage seedlings under salt stress

MT concentration (μmol·L^{-1})	Chlorophyll a content (mg·g^{-1}FW)	Chlorophyll b content (mg·g^{-1} FW)	Chlorophyll content (mg·g^{-1} FW)	Carotenoid content (mg·g^{-1} FW)
0	0.615±0.001 b	0.126±0.008 a	0.741±0.007 c	0.220±0.004 b
50	0.668±0.029 a	0.145±0.000 a	0.813±0.029ab	0.231±0.006ab
100	0.674±0.022 a	0.146±0.028 a	0.820±0.006 a	0.236±0.001 a
200	0.686±0.016 a	0.155±0.013 a	0.841±0.004 a	0.239±0.004 a
400	0.643±0.022 a	0.142±0.017 a	0.785±0.006 b	0.223±0.008 b

The enzymatic antioxidant system. The results showed that the activities of SOD, POD and CAT in leaves of cabbage seedlings treated with exogenous MT were significantly changed. In the range of 0 ~ 200 μmol·L^{-1}, with the increase of MT concentration, SOD, POD and CAT activities were higher than CK and reached the maximum at the peak of 200 μmol·L^{-1}, but the activities of SOD, POD and CAT were lower than CK when the concentration reached 400 μmol·L^{-1}.

Table 4 Effect of exogenous MT on antioxidant enzyme activities of Chinese cabbage seedlings under salt stress

MT concentration (μmol·L^{-1})	SOD activity (U·g^{-1}FW)	POD activity (U·g^{-1}FW)	CAT activity (U·g^{-1}FW)
0	171.51±3.13c	2380±57 b	18.30±0.42 b
50	172.54±2.67c	2414±88 b	19.50±0.42 b
100	179.73±1.59b	2514±184 b	25.50±0.42 a
200	187.29±2.43 a	3998±257 a	26.70±0.42 a
400	170.80±1.17 c	1614±122 c	14.40±0.85 c

MDA and soluble protein. As shown in Fig.1, the soluble protein content increased first and then decreased With the increase of concentration of spraying exogenous MT. MT concentration of 50 μmol·L^{-1} is not significant difference with control, but at the concentration of 200 μmol·L^{-1} are

extremely significant difference with control and soluble protein content increased by 9.35 %. The Fig.2 shows that spraying exogenous MT could significantly decrease MDA content in shepherd's-purse leaves under salt stress. Therefor low concentration of MT could increase the content of soluble protein in plant cells and decrease the content of MDA in plant cells, but high concentrations of MT led to the decrease of soluble protein content and the increase of MDA content. So high concentrations of MT have a certain toxic effect to plants.

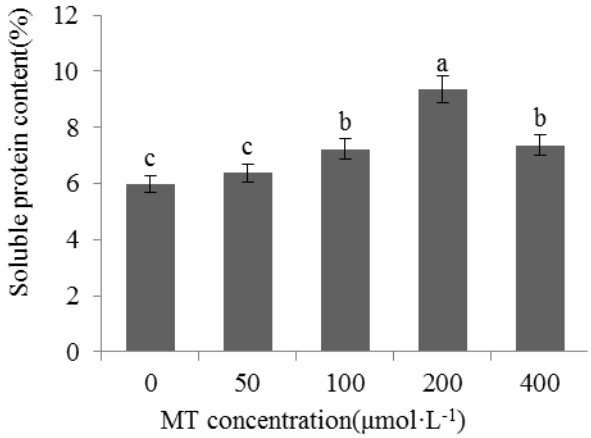

Fig. 1 The influence of soluble protein

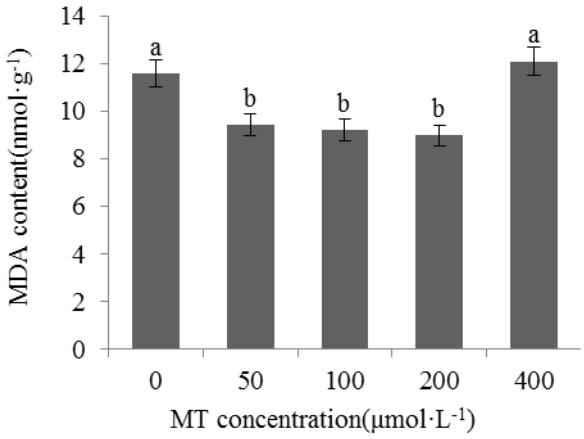

Fig. 2 The influence of MDA content

Discussion

Salt stress seriously restricted the growth and development of vegetables, which not only inhibited the growth and differentiation of plant organs, resulting in delayed plant development; lead to abnormal chloroplast structure, plant chlorophyll content and carotenoid content decreased, old leaves withered withered [11]. Studies have shown that exogenous melatonin can increase the chlorophyll content of plants under salt stress [12]. The results showed that exogenous MT promoted the growth of cabbage seedlings and increased the content of photosynthetic pigments. in the range of 0 ~ 200 μmol·L^{-1} the photosynthetic pigment content was increased. However, when the concentration reached 400 μmol·L^{-1}, the content of photosynthetic pigment was higher than CK but lower than 200 μmol·L^{-1}.

Under salt stress plants produce large amounts of reactive oxygen species. Active oxygen can not be promptly removed, the integrity of the cell will be destroyed, resulting in a large number of intracellular accumulation of MDA. MDA is the final decomposition product of membrane lipid peroxidation, MDA level can reflect the degree of plant stress damage [13]. The activities of SOD, POD and CAT in the range of 0 ~ 200 μmol·L^{-1} were increased with the increase of MT concentration, and reached the maximum at 200 μmol·L^{-1}, Which could reduce the membrane lipid peroxidation level and reduce the MDA content in the leaves of Chinese cabbage seedlings. When the concentration of MT was 400 μmol·L^{-1}, it was probably due to the high concentration of MT, the activity of SOD, POD and CAT was lower than that of CK, which aggravated the effects of salt stress. The MDA content of cabbage seedlings was significantly higher than that of CK. In addition, MT promoted the accumulation of soluble protein, decreased cell osmotic potential and increased osmotic adjustment ability, which may reduce the loss of water content in cabbage seedlings, and the water content of cabbage seedlings was significantly increased, Thus enhancing the resistance of cabbage seedlings.

In summary: Exogenous MT in a certain concentration range could effectively alleviate the damage of cabbage seedlings to salt stress. In the range of 0~200 μmol·L^{-1}, the growth of cabbage seedlings was enhanced, the water content increased, the activity of antioxidant enzymes in seedlings was increased, Which decreased the MDA content in seedling, effectively alleviated the damage of photosynthetic system of Chinese cabbage seedlings under salt stress, and enhanced the adaptability of Chinese cabbage seedlings under salt stress.The treatment with 200 μmol·L^{-1} MT was the best.

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