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Effects of exogenous melatonin on growth and physiological characteristics of Chinese Cabbage Seedlings under Aluminum Stress

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Abstract: The effects of exogenous melatonin on growth and physiological characteristics of Chinese cabbage seedlings under 50μmol/L aluminum stress were studied by spraying 5 different concentrations of MT（0、50、100、200、400）$\mu mol \cdot L^{-1}$solution on leaves in Chinese cabbage seedlings. The result showed that exogenous melatonin could promote the growth of Chinese cabbage seedlings under aluminum stress, increase the biomass, water content, light and pigment content and soluble protein content; Improve the activity of SOD, POD and CAT; Reduce the MDA content. Therefore, exogenous MT could improve the stress tolerance of Chinese cabbage seedlings to aluminum stress and alleviate the damage caused by aluminum stress on Chinese cabbage seedlings, and the 200 μmol/L was the best concentration.

Introduction

At present, the acid soil accounts for 21% of the total area of our country, and the aluminum poisoning is an important barrier factor for vegetable production in acidic soil[1]. The results showed that the photosynthesis of soybean, longan, rape and other crops was obviously inhibited under aluminum stress, and Al^{3+} can be combined with proteins, lipids, nucleic acids, interfering with plant cell ion metabolism, affecting the normal physiological and biochemical processes, inhibiting plant growth[2].

Melatonin (melatonin, MT) was first found in the pineal gland of animals, which is a type of indole[3]. It has been found that MT is widely existed in plants, which is an endogenous free radical scavenger with strong antioxidant[4], and it plays an important role in plant stress defence[5]. Researches show that, MT can effectively alleviate the damage of high temperature[6], low temperature[7], salt stress[8], UV radiation[9] and other non-biological factors on plant, prevent chlorophyll degradation, maintain cell membrane integrity. MT can also protect plants against chemical and heavy metal ions damage[10]. Zhang and other studies found that the beans can resist heavy metal copper ions damage by adding MT in the soil, and greatly improving the survival rate of plants[11].

Chinese cabbage (*Brassica rapa ssp. pekinensis*) is rich in nutrients and has certain medicinal value for preventing stomach illness and scurvy, which is one of the most popular vegetables. Its cultivated area and consumption is the top of all kinds of vegetables in China[12]. In recent years, MT has been used more and more widely in alleviating plant stress, but the research on the physiological and biochemical effects of cabbage under stress has not yet been reported. Therefore, the experiment is based on the seedling of cabbages as materials, studied the effects of exogenous melatonin on the growth and physiological characteristics of Chinese cabbage seedlings.

Materials and Methods

Materials. 'Crack 35F' early-maturing cabbage materials; MT is purchased from SIGMA ALDRICH company, a small amount of anhydrous ethanol dissolves with distilled water as 500μmol·L^{-1} mother liquor, 4 ºC preservation, according to the design concentration dilution.

Experimental Design. Select full Chinese cabbage seeds, lukewarm water soaked after 15 minutes (constantly stirring, natural cooling) to take out the seeds with gauze wrapped and place in 28 ºC incubator, humidity 80%. After the seeds were exposed, sown in 20 cm × 21cm (diameter × height) nutrition bowl (vermiculite: perlite = 1: 1,V/V), 3 strains per bowl, each processing 2, repeat 3 times, then put the pots into plastic greenhouses, growing under natural conditions and watering 20 ml 1/2Hoaglandnutrient solution every other day. After 2 true leaves fully expanded, water Hoagland nutrient solution which containing 50μmol·L^{-1} aluminum until the end of test, Once in 2d and 20 ml per bowl. When cabbage seedlings 3 true leaves fully expanded, spraying 5 different concentrations of MT（0、50、100、150、200μmol·L^{-1}）on leaves at 17:00 every other day, a total of 3 times. After 30 days, measure growth index and physiological index.

Statistic analyses. Excel2010 data preprocessing. 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 analyses

Growth and biomass. Table 1, with the increase of MT concentration, the plant height, stem diameter, root length and fresh weight of root of cabbage seedling increased first and then decreased, and were higher than CK. In the concentration range of 0 ~ 200 μmol·L^{-1}, the growth index increased with the increase of MT concentration, and when the MT concentration reached 200 μmol·L^{-1}the indexes reached the maximum, and the difference was significant. However, when the concentration reached 400 μmol·L^{-1}, the indexes decreased, but still higher than CK. From table 2, it was found that the effects of different MT concentrations on the dry weight of Chinese cabbage seedlings under aluminum stress were significantly different. With the increase of MT concentration, the dry weight of ground part (root) and the water content of aboveground (root) increased first and then decreased, and reached the maximum at 200μmol·L^{-1}, but when the concentration reached 400μmol·L^{-1}, the indicators have decreased, but still higher than CK.

Photosynthetic pigment content. The effects of different concentrations of exogenous melatonin on the photosynthetic pigment content of Chinese cabbage seedlings under aluminum stress were significantly different. With the increase of MT concentration, the photosynthetic pigment content of Chinese cabbage seedlings under the aluminum stress showed the trend of increasing first and then descending, and all higher than CK. The chlorophyll a content, chlorophyll b content, total chlorophyll content and carotenoid content of Chinese cabbage seedlings were significantly higher than those of CK. At the concentration of 200 μmol·L^{-1}, which compared with the CK improved 30.26%, 29.41 %, 30.26% and 21.19%.

Table 1 Effects of exogenous melatonin on the growth of Chinese cabbage seedlings aluminum 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	8.51±0.10d	0.270±0.014 d	19.10±0.21d	3.33±0.25 c	0.90±0.00 d
50	9.17±0.08c	0.329±0.005 bc	22.28±0.35c	4.54±0.38 ab	1.21±0.02 c
100	9.80±0.15 b	0.351±0.005 b	25.62±0.64 b	4.94±0.42 a	1.37±0.06 b
200	10.47±0.21 a	0.389±0.001 a	28.78±0.98a	5.59±0.45 a	1.69±0.05 a
400	9.34±0.06bc	0.305±0.007 c	23.97±0.39bc	3.68±0.33bc	1.29±0.10 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 aluminum -stressed Chinese cabbage seedlings

MT concentration (μmol·L^{-1})	Shoot dry weight (g·plant^{-1})	Root dry weight(g·plant^{-1})	Shoot water content (%)	Root water content （%）
0	0.363±0.001 b	0.093±0.004 c	89.11	89.77
50	0.378±0.000 b	0.106±0.007 bc	91.69	91.11
100	0.389±0.003 ab	0.122±0.004 ab	92.14	91.30
200	0.418±0.019 a	0.134±0.002 a	92.53	92.11
400	0.379±0.003 b	0.119±0.001 ab	89.73	90.80

Table 3 Effect of exogenous MT on chlorophyll content of Chinese cabbage seedlings under aluminum 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	0.532±0.015 d	0.119±0.005 c	0.651±0.010 d	0.151±0.004 c
50	0.593±0.012 c	0.131±0.002 bc	0.724±0.010 c	0.163±0.001 b
100	0.635±0.004 b	0.135±0.004 b	0.770±0.008 b	0.169±0.005 b
200	0.693±0.005 a	0.154±0.002 a	0.848±0.008 a	0.183±0.003 a
400	0.600±0.003 c	0.133±0.005 bc	0.733±0.002 c	0.162±0.003 b

The enzymatic antioxidant system、MDA and soluble protein. It was found that the activities of

SOD, POD and CAT in Chinese cabbage seedlings increased first and then decreased with the increase of MT concentration, and were higher than CK. When the concentration of MT was 200 μmol·L^{-1}, the activities of SOD and CAT were significantly higher than CK, which were 4.65% and 30.26% higher than CK respectively. The POD activity of Chinese cabbage leaves reached the maximum at MT concentration of 200 μmol·L^{-1}, which was 28.35% higher than that of CK. When the MT concentration was 50, 100 and 400 μmol·L^{-1}, the POD activity of Chinese cabbage seedlings was not significantly different from CK. For the soluble protein content, MT treatment at a concentration of 400 μmol·L^{-1} was significantly higher than that of CK and 50 μmol·L^{-1}. And for the MDA content, with the increase of MT concentration, it showed a tendency to decrease first and then decrease, and all lower than CK. MT content of 100 μmol·L^{-1} and 200 μmol·L^{-1}under aluminum stress was significantly lower than that of CK, which was 26.88% and 25.34% lower than that of CK.

Table 4Effect of exogenous MT on antioxidant enzyme activities, MDA and soluble protein content of Chinese cabbage seedlings under Aluminum stress

MTconcentration (μmol·L^{-1})	SOD activity (U·g^{-1}FW)	POD activity (U·g^{-1}FW)	CAT activity (U·g^{-1}FW)	MDA content (nmol·g^{-1}FW)	Soluble protein (mg·g^{-1}FW)
0	752.04±2.41 c	3163.33±130.00 b	26.17±0.39 d	11.72±1.07 a	0.272±0.001 c
50	761.24±3.57 bc	3380.00±220.00 b	28.76±0.12 c	10.35±1.16 ab	0.274±0.012 c
100	766.46±3.69 b	3726.66±426.66 ab	30.17±0.19 b	8.75±0.504 b	0.298±0.007 bc
200	787.4±2.91 a	4060.00±43.33 a	34.09±0.44 a	8.57±0.153 b	0.311±0.004 b
400	758.63±1.68 bc	3710.00±53.33 ab	30.19±0.26 b	9.94±0.325 ab	0.360±0.010 a

Discussion

Many studies have shown that aluminium can be toxic to plants. The research on rice indicate that aluminum stress reduces root and aboveground dry weights as well as root length and plant height[13].Researchers in the study of wheat, rice, and Pinusmassoniana found that aluminum stress caused plant membrane lipid peroxidation, membrane structure and function were destroyed[14].The results of this test show that exogenous MT promoted the growth of Chinese cabbage seedlings under aluminum stress and increased the biomass, at the same time the content of MDA decreased, at the concentration of 200 μmol·L^{-1} meet minimum, but it was not significant when compared with 100 μmol·L^{-1}

MT as an antioxidant, can protect the chlorophyll, thereby delaying leaf senescence, improve photosynthetic efficiency. Studies have shown that MT can effectively inhibit the reduction of chlorophyll a in cucumber seedlings under high temperature stress, increase the content of carotenoids and improve the photosynthetic efficiency[15]. In this study, the chlorophyll content and carotenoid content of cabbage were studied under different concentrations of MT, and the effect was the most significant when the concentration was 200 μmol·L^{-1}.

Under the aluminum stress, the plant produces a large amount of reactive oxygen species, the structure and function of the cell membrane is destroyed, membrane lipid peroxidation increased, resulting in a large number of MDA accumulation. In this experiment, the activity of SOD, POD and CAT increased with the increase of MT concentration in the range of 0~200 $\mu mol \cdot L^{-1}$, which indicated that MT could effectively alleviate the destruction of cell membrane of Chinese cabbage seedlings under aluminum stress, thereby reducing the membrane lipid peroxidation level. In addition, MT promoted the accumulation of soluble protein in cabbage seedlings and increased the ability of osmotic adjustment. Therefore, MT could reduce the water loss of Chinese cabbage seedlings, and the water content of aboveground (root) of cabbage seedlings increased significantly, which enhanced the resistance of Chinese cabbage seedlings sex.

In conclusion, suitable concentration of exogenous MT can effectively alleviate the damage of Chinese cabbage seedlings under aluminum stress, significantly promoted the growth, increased the photosynthetic pigment content, enhanced the activity of antioxidant enzymes in cabbage seedlings. The content of osmotic regulation was improve, the content of MDA was decreased, and effectively relieved the damage of photosynthetic system and enhanced the resistance of Chinese cabbage seedlings under aluminum stress. Among them, the concentration of 200 $\mu mol \cdot L^{-1}$ was the best.

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