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Effects of Melatonin-Soaked Seeds on the Cadmium Accumulation in Broadbean (*Vicia faba*) Seedlings

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Abstract: In order to discuss the melatonin (MT) on the growth and cadmium (Cd) accumulation in vegetables, broadbean (*Vicia faba*) seeds were soaked in five doses (0, 50, 100, 150 and 200 µmol/L) of MT and then planted in pots containing Cd-contaminated soil. Different doses of MT increased the biomasses of broadbean seedlings' roots and shoots compared with the control, with 200 µmol/L of MT producing the maximum values. MT effectively decreased the Cd contents in roots and shoots of broadbean seedlings compared with the control, and the maximum reduction rate of 29.29% occurred at the 200-µmol/L MT dose. Thus, MT could promote broadbean growth and reduce Cd accumulation in broadbean, with 200 µmol/L being the optimum dose in this experiment.

Introduction

Melatonin (MT) is an indole amine hormone that can regulate plant photoperiods and improve the plants' responses to adverse environmental conditions, such as heavy metals, ultraviolet radiation, temperature changes and other functions [1]. Under heavy metal stress, exogenous MT can chelate a high concentration of metal ions, preventing them from damaging biological macromolecules, and it also produces antioxidant protection against seeds and seedlings [2]. The application of an appropriate exogenous MT concentration can promote the growth of young rice buds under nickel (Ni) stress and alleviate the toxicity of Ni to rice seedlings [3]. Under high-temperature stress, MT inhibits reactive oxygen species (ROS) production in cucumber seedlings, improves antioxidant enzyme activities, inhibits the reduction of chlorophyll a, and increases the chlorophyll a/b ratio and carotenoid content, which reduces the damage caused by high-temperature stress on cucumber seedlings and maintains the photosynthetic rate at a relatively high level [4-5]. MT can also enhance the resistance of tomato to cadmium (Cd) by improving antioxidant enzyme activities and promoting the integration and isolation of Cd in plants [6]. Thus, MT can alleviate the damage to plants caused by heavy metal stress. In this research, the effects of MT on growth and Cd-related characteristics of broadbean seedlings were studied to determine whether MT can reduce Cd accumulation in broadbean seedlings.

Materials and Methods

Materials. Local varieties of broadbean grown in Chengdu, Sichuan Province, China were used in this study. The soil samples were fluvo-aquic soils, which were collected from the Chengdu Campus Farm of Sichuan Agricultural University (30°42′ N, 103°51′ E) in October 2015. The soil basic properties and its Cd content were described in Liu et al. (2015) [7]. Then, 0.5 kg of air-dried soil was placed into each polyethylene pot (10-cm high, 8-cm diameter). Cd ($CdCl_2 \cdot 2.5\ H_2O$) was added to the soil samples at a dose of 10 mg/kg according to [8]. The soil moisture was maintained at 80% of field capacity for one month.

Experimental Design. In November 2015, the seeds of broadbean were soaked in different doses (0, 50, 100, 150 and 200 µmol/L) of MT for 48 h. A 50-mL solution soaked approximately 15 seeds.

Then, the seeds were placed in an incubator to germinate at 26°C for 48 h, and four uniform seeds with buds were transplanted into the prepared soil of each pot in the greenhouse of the Chengdu Campus Farm. Every treatment was replicated four times (four pots). We watered every day to keep the soil moist. When the broadbean seedlings had grown for one month (December 2015), the whole broadbean seedlings were harvested and washed with tap water and deionized water. The fresh weights of the roots, stems and leaves were weighted. After that, the fresh organ of plant samples (5.000 g) were ground into homogenates, digested in HNO_3:$HClO_4$, and determined the Cd concentration using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA) [9]. The measured values of the Cd contents were checked according to Zhang *et al.* (2011) [10].

Statistical Analyses. Data were analyzed using SPSS 13.0 statistical software (IBM, Chicago, IL, USA).

Results and Discussion

Biomasses of Broadbean Seedlings. Compared with the control, soaking seeds in different MT concentrations increased the biomasses of the root, leaf and shoot of broadbean seedlings (Table 1). The 50-μmol/L MT dose had no significant effects on the stem biomass of broadbean seedlings compared with the control, but 100, 150 and 200-μmol/L MT doses increased that the stem biomass. When the MT doses were 100, 150 and 200 μmol/L, the shoot biomasses of broadbean seedlings significantly increased by 9.47%, 13.49% and 19.20%, respectively, compared with the control. Thus, the 200-μmol/L MT dose produced the optimum results in this experiment.

Table 1 Biomasses of broadbean seedlings

Melatonin concentrations (μmol/L)	Roots (g/plant fresh wt)	Stems (g/plant fresh wt)	Leaves (g/plant fresh wt)	Shoots (g/plant fresh wt)
0	5.68±0.03d	3.91±0.02b	4.31±0.05e	8.23±0.05e
50	5.95±0.04c	3.95±0.03b	4.58±0.06d	8.53±0.06d
100	6.05±0.05c	4.10±0.04a	4.91±0.07c	9.01±0.07c
150	6.25±0.02b	4.15±0.01a	5.19±0.08b	9.34±0.06b
200	6.58±0.03a	4.23±0.02a	5.59±0.09a	9.81±0.06a

Values are means (± SE) of four replicate pots. Significant differences (indicated by different lowercase letters) within a column are based on a one-way analysis of variance with the least significant difference test ($p < 0.05$), and followed by the post-hoc test.

Cd Content in Broadbean Seedlings. Compared with the control, MT decreased the Cd content in the roots of broadbean seedlings, but there were no significant differences among the 50-, 100-, 150- and 200-μmol/L MT doses (Table 2). MT had no significant effects on the Cd contents in the stems of broadbean seedlings compared with the control. When the doses of MT were 50, 100, 150 and 200 μmol/L, compared with the control, the Cd contents in the leaves decreased by 11.29%, 20.16%, 34.27% and 40.32%, respectively. For the Cd content in shoots, the 50-μmol/L MT dose had no significant effects on the Cd contents in the shoots compared with the control, and 100-, 150- and 200-μmol/L MT doses decreased the Cd contents in the shoots. When the doses of MT were 100, 150 and 200 μmol/L, the Cd contents in the shoots decreased by 13.64%, 24.75% and 29.29%, respectively, compared with the control. Thus, MT could effectively decrease the Cd content in broadbean seedlings.

Discussion

Under stress conditions, MT plays an important role in promoting plant growth [11-13]. Other studies indicated that only low MT levels promote plant growth, while high MT levels inhibit growth [14-15]. In this study, 100-, 150- and 200-μmol/L MT doses increased the biomass of broadbean seedlings, while the 50-μmol/L MT dose had no significant effect. This was not completely consistent with previous studies [11-15]. This might be related to the different sensitivities of plants to MT and requires further study. In this experiment, MT effectively decreased the Cd contents in roots, leaves, and shoots of broadbean seedlings, but not in stems, which might be related to the distribution of Cd in different broadbean organs [6], and the integration and isolation of Cd in plants [6]. Thus, MT can reduce Cd accumulation in broadbean and should be used in broadbean production areas having Cd-contaminated soil.

Table 2 Cadmium contents in broadbean seedlings

Melatonin concentrations (μmol/L)	Roots (mg/kg fresh wt)	Stems (mg/kg fresh wt)	Leaves (mg/kg fresh wt)	Shoots (mg/kg fresh wt)
0	3.56±0.04a	0.143±0.006a	0.248±0.004a	0.198±0.002a
50	3.05±0.05b	0.142±0.005a	0.220±0.006b	0.184±0.004ab
100	2.92±0.05b	0.140±0.003a	0.198±0.008c	0.171±0.004b
150	2.91±0.08b	0.131±0.002a	0.163±0.009d	0.149±0.005c
200	2.82±0.01b	0.129±0.005a	0.148±0.004d	0.140±0.003c

Values are means (± SE) of four replicate pots. Significant differences (indicated by different lowercase letters) within a column are based on a one-way analysis of variance with the least significant difference test ($p < 0.05$), and followed by the post-hoc test.

Conclusions

Different doses of MT increased the biomasses of broadbean seedlings' roots and shoots compared with the control, with 200 μmol/L of MT producing the maximum values. MT effectively decreased the Cd contents in roots and shoots of broadbean seedlings compared with the control, and the maximum reduction rate of 29.29% occurred at the 200-μmol/L MT dose. Thus, MT could promote broadbean growth and reduce Cd accumulation in broadbean, with 200 μmol/L being the optimum dose in this experiment.

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