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Effects of exogenous melatonin on growth and cadmium content of *Zizyphus acidojujuba* seedlings

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Abstract. To study the effects of exogenous melatonin on the repair of fruit tree seedlings under heavy metal stress, *Zizyphus acidojujuba* seedlings were treated with different concentrations of exogenous melatonin (0, 50, 100, 150, and 200 μmol L⁻¹) by root-irrigation under cadmium (Cd) stress (5 mg kg⁻¹) by a pot experiment. The results showed that compared with the control (0 μmol L⁻¹), root-irrigation with exogenous melatonin reduced the leaves biomass and photosynthetic pigment content of the *Z. acidojujuba* seedlings, and also reduced the SOD, POD activity and soluble protein content. In addition to 50 μmol L⁻¹ exogenous melatonin reduced the Cd content in roots of *Z. acidojujuba* seedlings, the other treatments increased the Cd content in the roots and leaves of *Z. acidojujuba* seedlings under irrigated exogenous melatonin, and exogenous melatonin also promoted the transport of Cd from the roots of *Z. acidojujuba* seedlings to the shoots, with all the maximum appeared at 200 μmol L⁻¹. Therefore, under the conditions of Cd pollution, exogenous melatonin inhibited the growth and increased the Cd content of *Z. acidojujuba* seedlings. As the concentration of exogenous melatonin increased, the inhibitory intensity and increased amount was also increased.

1. Introduction
In recent years, the damage caused by soil cadmium (Cd) pollution has attracted more and more attention. Cd gets into the soil through natural factors and human factors[1], which not only damages the growth and development of plants[2], but also can get into the human body through food, and seriously endangers human health[3]. Fruit tree is an important economic crop in agricultural production. But at present, the orchard soil of China is polluted by Cd in many areas[4-5], which led the Cd content in fruits increased[4, 6-7]. Therefore, it is very necessary to study how to reduce the Cd content in fruits in the Cd-contaminated areas.

Melatonin (N-acetyl-5-methoxy-monochromoamine), a hormone that plants can synthesize themselves, is not only involved in the regulation of plant growth and development, but also related to the effects of abiotic stress and biotic stress[8]. Furthermore, the application of exogenous melatonin increases the tolerance of plants to abiotic stresses such as drought, radiation, extreme temperatures, and toxic elements[9]. Previous studies on cucumber and tomato have shown that exogenous melatonin can inhibit the accumulation of Cd in plants under Cd stress and relieve the damage of Cd stress to plants[10-11]. The experiments of Liu et al.[12] also showed that exogenous melatonin can effectively alleviate the toxic effects of Cd stress on rice. But on the seedlings of rice, the content of Cd increases at lower concentrations of exogenous melatonin, while the content of Cd decreases at...
higher concentrations of exogenous melatonin[13]. The experimental results of Huang et al.[14] on radish showed that melatonin at a suitable concentration reduces the Cd content in radish under Cd stress, while the lower concentrations and higher concentrations of exogenous melatonin increases the Cd content. Posmyk et al.[15] found that higher concentrations of exogenous melatonin reduces the germination rate of cabbage seeds under copper stress. It can be seen that different concentrations of exogenous melatonin may lead to different absorption of heavy metals by plants.

As a common wild fruit tree, *Zizyphus acidojujuba* not only can be used as the rootstock of cultivated jujube, but also has high nutritional and medicinal value. However, soil Cd pollution has brought damage to the growth of *Z. acidojujuba*[16], and it is urgent to solve this problem. The research on the exogenous melatonin has been widely studied in crops and vegetables for its resistance to Cd pollution, but the study of Cd accumulation in fruit trees is still lacking. Therefore, in this experiment, the effects of exogenous melatonin on the growth and Cd content of jujube seedlings was studied by using different concentrations of exogenous melatonin to irrigate *Z. acidojujuba* seedlings. In order to screen out the concentration of exogenous melatonin which can significantly reduce the Cd content and promote growth of *Z. acidojujuba* seedlings, also provides a reference for jujube production in Cd-contaminated areas.

2. Materials and Methods

2.1 Materials

Seeds of *Z. acidojujuba* were purchased in Xingtai County, Hebei Province. Melatonin is produced by Sigma-aldrich company with a specification of 1 g. The soil used for the experiment was sandy loam and was taken from polluted-free farmland near the Chengdu campus of Sichuan Agricultural University.

2.2 Experiment method

The sandy loam was air-dried and sieved through a 5 mm sieve. Then added CdCl$_2$·2.5H$_2$O analytical pure solution to it and mixed thoroughly to make the soil Cd content 5 mg kg$^{-1}$. Stored in a ventilated and sheltered place for one month and then mixed again, afterwards, 2.5 kg of soil was weighed into a pot (18 cm diameter, 21 cm high) for a total of 30 pots. Selected plump *Z. acidojujuba* seeds, then soaked the seeds after disinfection to promote its germination, until 70 %-75 % of the seeds were germinated and then putted them in a tray with clean river sand for breeding. When the seedlings grew to the height of 10 cm, the seedlings with the same growth tendency were transplanted into pots, and 4 seedlings were randomly planted in each pot.

After transplanted for 15 days, *Z. acidojujuba* seedlings were treated with exogenous melatonin by root-irrigation. A total of 5 treatments were performed at concentrations of 0 (clean water, CK), 50, 100, 150, and 200 μmol L$^{-1}$. Each pot was irrigated with 100 mL of exogenous melatonin every 7 days, irrigated at 19:00 on the day, and each treatment concentration was repeated 6 times. The potted seedlings were placed in a transparent canopy, irregularly irrigate according to the actual situation of soil moisture, to ensure that the soil moisture is maintained at about 80% of field capacity.

2.3 Sample collection and analysis

The whole plant was harvested after the last root-irrigation of exogenous melatonin for 6 days. A portion of *Z. acidojujuba* seedlings were used to take fresh leaves of their 6 th to 10 th positions (middle and upper parts of the plant). The chlorophyll $a$, chlorophyll $b$ and carotenoid contents were determined by Spectrophotometry. Superoxide dismutase (SOD) activity was measured by NBT Reductive method, catalase (CAT) activity was measured by Potassium Permanganate Titration method, peroxidase (POD) activity was measured by Guaiacol method, and soluble protein content was measured by Coomassie Brilliant Blue G-250 method[17]. Another part of *Z. acidojujuba* seedlings were used to measure the plant height with a ruler, then washed with distilled water, cut
roots, stems, and leaves of the plants, put them in an oven at 115 °C for 20 min and dried at 75 °C until constant weight, then used an electronic balance to measure the biomass index (dry weight of roots, stems, and leaves). Weighed 1.000 g of the plant sample, added nitric acid-perchloric acid (4:1 by volume) and let stand for 12 h until solution is transparent, then filter and dilute to 50 mL, used the iCAP 6300 ICP spectrometer (Thermo Scientific, USA) to determination the content of Cd[18].

2.4 Statistical analyses
SPSS Statistics 17.0 software (IBM, Chicago, USA) were used for analysis of variance ($p < 0.05$). Root/shoot ratio = root biomass/shoot biomass, transport factor (TF) = Cd content in shoot/ Cd content in root[19-20].

3. Results and analysis

3.1 Biomass and plant height of Z. acidojujuba seedlings
Compared with the control, root-irrigation with exogenous melatonin had no significant effect on the roots biomass, stems biomass, and plant height of Z. acidojujuba seedlings under Cd pollution (Table 1). The leaves biomass and shoots biomass of Z. acidojujuba seedlings were decreased significantly compared with the control, however the ratio of root/shoot significantly increased. Compared with the control, after irrigated 100 μmol L –1 exogenous melatonin, the leaves biomass of Z. acidojujuba seedlings decreased by 17.35% ($p < 0.05$), and after irrigated 50, 150, and 200 μmol L –1 exogenous melatonin, the leaves biomass of Z. acidojujuba seedlings decreased by 31.63% ($p < 0.05$), 36.73% ($p < 0.05$), and 50.31% ($p < 0.05$), respectively. Compared with the control, after irrigated 200 μmol L –1 exogenous melatonin, the shoots biomass of Z. acidojujuba seedlings reduced by 23.43% ($p < 0.05$), and the root/shoot ratio of Z. acidojujuba increased by 27.16% ($p < 0.05$).

| Treatments | Roots (g plant –1) | Stems (g plant –1) | Leaves (g plant –1) | Shoots (g plant –1) | Root/shoot ratio | Plant height (cm) |
|------------|--------------------|--------------------|--------------------|--------------------|------------------|------------------|
| CK         | 0.39±0.03a         | 0.071±0.01a        | 0.098±0.01a        | 0.17±0.01a         | 2.32±0.11b       | 10.88±0.06a      |
| 50 μmol L –1| 0.38±0.00a         | 0.071±0.00a        | 0.067±0.00c        | 0.14±0.00c         | 2.74±0.02a       | 10.50±0.13a      |
| 100 μmol L –1| 0.42±0.02a        | 0.070±0.00a        | 0.081±0.02b        | 0.15±0.00b         | 2.77±0.15a       | 10.38±0.29a      |
| 150 μmol L –1| 0.38±0.01a        | 0.077±0.00a        | 0.062±0.00c        | 0.14±0.00c         | 2.77±0.17a       | 10.40±0.19a      |
| 200 μmol L –1| 0.38±0.01a        | 0.080±0.00a        | 0.049±0.00d        | 0.13±0.00c         | 2.95±0.11a       | 10.34±0.24a      |

Note: The data are mean±standard error. The lowercase letters in the same column of data indicate that the differences between different treatments have significant differences ($p<0.05$). Same as below.

3.2 Photosynthetic pigment of Z. acidojujuba seedings
Compared with the control, root-irrigation with exogenous melatonin significantly reduced the photosynthetic pigment content of Z. acidojujuba seedlings (Table 2). The contents of chlorophyll a of Z. acidojujuba seedlings significantly decreased compared with the control, with the minimum of decreased range appeared at 100 μmol L –1, and the maximum of decreased range appeared at 200 μmol L –1, which reached 32.66% ($p < 0.05$) and 50.98% ($p < 0.05$), respectively. Compared with the control, chlorophyll b content of Z. acidojujuba seedlings did not change significantly after irrigated 100 μmol L –1 exogenous melatonin, while treated by 50, 150, and 200 μmol L –1 exogenous melatonin significantly decreased by 22.73% ($p < 0.05$), 29.75% ($p < 0.05$), and 29.34% ($p < 0.05$), respectively. Compared with the control, the content of total chlorophyll of Z. acidojujuba seedlings did not change significantly after treated by 50 and 100 μmol L –1 exogenous melatonin, while irrigated 150 and 200 μmol L –1 exogenous melatonin, significantly decreased by 29.75% ($p < 0.05$), and 29.34% ($p < 0.05$), respectively. The content of carotenoids in Z. acidojujuba seedlings respectively decreased by 27.45% ($p < 0.05$), 25.49% ($p < 0.05$), 29.41% ($p < 0.05$), and 50.98% ($p < 0.05$) after treated by 50, 100, 150, and 200 μmol L –1 exogenous melatonin compared with the control.
Table 2. Photosynthetic pigment of Z. acidojujuba seedings

| Treatments | Chlorophyll \(a\) (mg g\(^{-1}\)) | Chlorophyll \(b\) (mg g\(^{-1}\)) | Total chlorophyll (mg g\(^{-1}\)) | Chlorophyl \(a/b\) (mg g\(^{-1}\)) | Carotenoids (mg g\(^{-1}\)) |
|------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CK         | 1.88±0.00a      | 0.54±0.01a      | 2.42±0.01a      | 3.51±0.04a      | 0.51±0.04a      |
| 50 μmol L\(^{-1}\) | 1.40±0.09bc | 0.47±0.01b      | 1.87±0.08ab     | 2.95±0.24b      | 0.37±0.01b      |
| 100 μmol L\(^{-1}\) | 1.46±0.08b | 0.54±0.01a      | 2.00±0.08a      | 2.70±0.15b      | 0.38±0.04b      |
| 150 μmol L\(^{-1}\) | 1.33±0.01bc | 0.37±0.01c      | 1.70±0.01b      | 3.55±0.06a      | 0.36±0.04b      |
| 200 μmol L\(^{-1}\) | 1.27±0.02c | 0.45±0.01b      | 1.71±0.13b      | 2.84±0.06b      | 0.25±0.01c      |

3.3 Antioxidant enzyme activity and soluble protein content of Z. acidojujuba seedings

SOD and POD activities of Z. acidojujuba seedings significantly decreased after treated with exogenous melatonin by root-irrigation compared with the control, and the CAT activity did not change significantly (Table 3). There was no significant difference in soluble protein content compared with the control after irrigated 50 and 100 μmol L\(^{-1}\) exogenous melatonin, while it is significantly reduced after irrigated 150 and 200 μmol L\(^{-1}\) exogenous melatonin. Compared with the control, after irrigated 50, 100, 150, and 200 μmol L\(^{-1}\) exogenous melatonin, the SOD activity of Z. acidojujuba seedlings respectively decreased by 5.43% \((p < 0.05)\), 3.58% \((p < 0.05)\), 6.77% \((p < 0.05)\), and 7.40% \((p < 0.05)\), and the POD activity of Z. acidojujuba seedlings respectively decreased by 14.47% \((p < 0.05)\), 18.65% \((p < 0.05)\), 26.65% \((p < 0.05)\), and 22.81% \((p < 0.05)\). After irrigated exogenous melatonin was applied at 150 μmol L\(^{-1}\) and 200 μmol L\(^{-1}\), soluble protein content was respectively decreased by 11.76% \((p < 0.05)\) and 10.59% \((p < 0.05)\).

Table 3. Antioxidant enzyme activity and soluble protein content of Z. acidojujuba seedings

| Treatments | SOD activity (U g\(^{-1}\)) | POD activity (U g\(^{-1}\) min\(^{-1}\)) | CAT activity (mg g\(^{-1}\) min\(^{-1}\)) | soluble protein content (mg g\(^{-1}\)) |
|------------|-----------------|-----------------|-----------------|-----------------|
| CK         | 178.6±1.43a     | 811.2±9.70a     | 9.08±0.44a      | 0.85±0.03a      |
| 50 μmol L\(^{-1}\) | 168.9±1.05c | 693.8±2.22b     | 9.09±0.29a      | 0.78±0.02ab     |
| 100 μmol L\(^{-1}\) | 172.2±0.44b | 659.9±12.44bc   | 10.98±1.30a     | 0.81±0.03ab     |
| 150 μmol L\(^{-1}\) | 166.5±0.53cd | 595.0±3.84d     | 10.33±0.90a     | 0.75±0.03b      |
| 200 μmol L\(^{-1}\) | 165.3±1.69d | 626.2±28.48cd   | 9.41±0.20a      | 0.76±0.02b      |

3.4 Cd content of Z. acidojujuba seedings

compared with the control, 50 μmol L\(^{-1}\) exogenous melatonin significantly reduced the Cd content in roots of Z. acidojujuba seedings, while the other treatments significantly increased the Cd content in roots (Table 4). After irrigated exogenous melatonin, the Cd content in stems did not change significantly compared with the control, but the leaves Cd content, shoots Cd content, and the TF increased significantly. After irrigated 50 μmol L\(^{-1}\) exogenous melatonin, the Cd content in roots of Z. acidojujuba seedings decreased by 9.11% \((p < 0.05)\) compared with the control, while the other three treatments respectively increased by 27.18% \((p < 0.05)\), 29.26% \((p < 0.05)\), and 36.58% \((p < 0.05)\). The increased range of Cd content in leaves of Z. acidojujuba seedings enhanced with the increased of exogenous melatonin concentration, the Cd content in leaves of Z. acidojujuba seedings respectively increased by 131.66% \((p < 0.05)\) after irrigated 200 μmol L\(^{-1}\) exogenous melatonin, compared with the control. The Cd content in shoots of Z. acidojujuba seedings increased by 66.60% \((p < 0.05)\), 45.11% \((p < 0.05)\), 63.19% \((p < 0.05)\), and 112.55% \((p < 0.05)\) after irrigated 50, 100, 150, and 200 μmol L\(^{-1}\) exogenous melatonin, compared with the control.
Table 4. Cd content of *Z. acidojujuba* seedlings

| Treatments          | Roots (mg kg⁻¹) | Stems (mg kg⁻¹) | Leaves (mg kg⁻¹) | Shoots (mg kg⁻¹) | TF     |
|---------------------|-----------------|-----------------|------------------|------------------|--------|
| CK                  | 11.92±0.01c     | 7.54±0.01a      | 3.79±0.05e       | 4.70±0.19c       | 0.39±0.02d |
| 50 μmol L⁻¹         | 10.63±0.20d     | 7.81±0.03a      | 6.58±0.04d       | 7.83±0.19b       | 0.74±0.01a |
| 100 μmol L⁻¹        | 15.16±0.12b     | 7.93±0.02a      | 6.85±0.08c       | 6.82±0.19b       | 0.45±0.01cd |
| 150 μmol L⁻¹        | 15.17±0.28b     | 7.60±0.10a      | 7.31±0.05b       | 7.67±0.09b       | 0.51±0.01c |
| 200 μmol L⁻¹        | 16.28±0.02a     | 7.70±0.25a      | 8.78±0.03a       | 9.99±1.00a       | 0.61±0.06b |

4. Discussion

The effect of melatonin on plants is similar to that of indole acetic acid (IAA), which promotes plant growth[21], and melatonin can induce indole acetic acid accumulation[22]. In this experiment, exogenous melatonin reduced the leaves biomass of *Z. acidojujuba* seedlings, Zhang et al.[23] studied on Polygonum cuspidatum showed that lower concentrations of exogenous melatonin have a significant effect on the growth of roots and leaves, while higher concentrations have inhibitory effects on growth. This result may be due to the characteristic that lower concentrations of melatonin promoted growth and higher concentrations of melatonin inhibit growth.

Cd stress significantly reduces chlorophyll content in jujube leaves[24]. However, melatonin pretreatment alleviates the decrease of photosynthetic rate of watermelon under abiotic stress, and it was dose-dependent[25]. In this experiment, the content of photosynthetic pigment decreased significantly after treated with exogenous melatonin by root-irrigation, and the decreased range of higher concentrations exogenous melatonin more than that of lower concentrations, which was consistent with the results of the study on cherries that higher concentrations of melatonin significantly decrease the chlorophyll content[26]. This may be due to the higher concentrations of melatonin inhibit the loading of sucrose in the leaves, and excessive accumulation of sucrose feedback inhibits photosynthesis[27].

Cd stress induces plants to produce large amounts of reactive oxygen species and inhibit plant growth[28-29]. In this experiment, SOD activity and POD activity of *Z. acidojujuba* seedlings were significantly decreased after irrigated exogenous melatonin, which was consistent with the changes of the biomass of *Z. acidojujuba* seedings and this may be due to poor growth vigor of *Z. acidojujuba* seedings resulting in reduced antioxidant capacity. In this experiment, there was no significant difference in the CAT activity of *Z. acidojujuba* seedlings compared with the control, which is inconsistent with the results of previous studies on the treatment of melatonin[9, 11, 13]. However, studies by Hegedüs et al.[30] showed that there is no significant change in CAT activity of wheat under Cd stress. Therefore, it may be due to the difference in experimental materials, CAT does not participate in the regulation under Cd stress in *Z. acidojujuba* seedlings, so there was no significant difference of CAT activity after irrigated exogenous melatonin. The soluble protein content was significantly reduced in this experiment, which is consistent with previous study on the drought stress of Perilla frutescens seedlings[31].

The results of this experiment showed that after irrigated exogenous melatonin, except for irrigated 50 μmol L⁻¹ exogenous melatonin reduced Cd content in roots and leaves of *Z. acidojujuba* seedings, other treatments increased these content compared to the control. The Cd content in roots of *Z. acidojujuba* seedlings reached the maximum after irrigated 200 μmol L⁻¹ exogenous melatonin. Previous studies on rice and radish showed that lower concentrations and higher concentrations of exogenous melatonin increase the ability of plants to absorb Cd under Cd stress[13–14]. Posmyk et al.[15] found that higher concentrations of exogenous melatonin reduces the germination rate of red cabbage seeds compared with the control under copper stress, while lower concentration and appropriate concentrations of exogenous melatonin enhances the germination rate of cabbage. The results showed that different concentrations of exogenous melatonin have different effects on plant under heavy metal stress, and there are significant differences in the sensitivity to exogenous
melatonin of different species. In this study, exogenous melatonin at four concentrations enhanced the Cd accumulation ability of *Z. acidojujuba* seedlings, the higher the concentration of exogenous melatonin is, the more the increased range of Cd content is. It is inferred that *Z. acidojujuba* seedlings are highly sensitive to exogenous melatonin. *Z. acidojujuba* is often used as rootstock in cultivation, there is no research show that after grafting jujube cultivar, the Cd content in fruit of cultivars will increase, so under Cd pollution, root irrigation with exogenous melatonin will reduce the Cd content of jujube fruit or not is yet to be studied.

5. Conclusions
Under the condition of Cd pollution, the four concentrations of exogenous melatonin used by root-irrigation in this experiment reduced the biomass, photosynthetic pigment and SOD activity of the leaves of *Z. acidojujuba* seedlings, indicating that exogenous melatonin inhibited the growth of *Z. acidojujuba* seedlings. Under the condition of Cd pollution, exogenous melatonin increased the Cd content in the root and leaves of *Z. acidojujuba* seedlings, and enhanced the ability of Cd in *Z. acidojujuba* seedlings to migrate from the roots to the aerial parts. Therefore, the four concentrations of exogenous melatonin used in this experiment are not suitable for the repair of *Z. acidojujuba* seedlings under the conditions of Cd pollution.

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