Effects of exogenous indole-3-acetic acid on the growth and cadmium accumulation of lettuce under cadmium stress

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Abstract. Indole-3-acetic acid (IAA) plays crucial roles in plant growth and stress tolerance. In present study, the effects of spraying different concentrations (0, 25, 50, 100 and 200 $\mu\text{mol/L}$) of IAA on the growth and cadmium (Cd) accumulation in lettuce (*Lactuca sativa*) were investigated. The lettuce exposed to Cd exhibited a substantial decline in growth, and the Cd content of them significantly increased. Spraying exogenous IAA resulted in alleviating the inhibitory of Cd toxicity to lettuce. The dry weight in shoots of lettuce increased by spraying with IAA compared with the Cd treatment alone, but the dry weight of roots had no significant differences. Although exogenous IAA increased the root Cd content, it significantly reduced shoot Cd content, indicating its role in Cd transport. Therefore, spraying IAA effectively alleviated Cd toxicity and reduced Cd uptake in the edible parts of lettuce, and the 100 $\mu\text{mol/L}$ IAA was the optimal dose.

1 Introduction

Lettuce (*Lactuca sativa*) is a common type of leafy vegetable that is widely cultivated worldwide. Due to limited availability of arable land and the high market demand for vegetables around the world, lettuce is frequently cultivated under unfavourable conditions [1]. Currently, heavy metal contamination of soil is a significant worldwide environmental problem, and the cadmium (Cd) contamination levels are higher than the other metals [2]. The Cd is toxic trace pollutant for both plants and humans, which enters the soil mainly from human activities and then is transferred to the food chain [3]. Lettuce is a plant species that shows high accumulation of Cd [4], and has been considered as a Cd bio-indicator. However, there are no symptoms of dehydration, chlorosis, or necrosis are found on the Cd-contaminated lettuce plants [5].

In recent years, plant growth regulators are gaining increased attention because of its involvement in regulation of abiotic stresses. Indole-3-acetic acid (IAA), as a signal hormone in higher plants, regulates the growth and development of plants, and has a crucial function in stress resistance [6-7]. At present, some studies have demonstrated IAA alleviated Cd-induced toxicity on plants. When 10 $\mu\text{mol/L}$ IAA is sprayed on eggplant seedlings grown in 3 mg/kg and 9 mg/kg Cd contaminated soil, it increases biomass of root and shoot, and decrease the Cd content of root and shoot [8]. IAA at low doses alleviate the Cd toxicity by regulating the ascorbate-glutathione cycle as well as activity of other antioxidants in *Trigonella* seedlings [9]. Agami and Mohamed investigate the resistance mechanism of IAA

to cadmium tolerance in wheat seedlings, pre-treatment with IAA enhance the antioxidant defense activities in Cd stressed wheat, and enhance Cd tolerance [10].

In the present investigation, the effect of different concentrations of IAA on the growth and Cd accumulation of lettuce under Cd stress were studied. The objective was to determine whether exogenous IAA could mitigate the Cd toxicity in lettuce by improving the growth and alleviating Cd accumulation of lettuce.

2 Materials and methods

2.1. Materials

Seeds of “Glass lettuce” were purchased from the Chengdu seed station. This cultivar shows high purity, good quality, good resistance and suitable growing period (50 d from sowing to harvest), IAA was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Experimental design

The pot experiment was conducted at the Institute of Pomology and Olericulture of Sichuan Agricultural University from February 2017 to June 2017. In February, the uniform size of lettuce seeds were sterilized in 10% H_2O_2 (v/v) for 10 min, and then rinsed with ultrapure water. The sterilized seeds were placed evenly in culture dishes lined with moist filter paper and germinated in an artificial incubator at 20 °C. When the radicle length was 5 mm, the germinated seeds were sown in an enamel tray containing clean sand and grown in an incubator

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maintained at 23 °C/18 °C (light/dark) under light intensity of 200 $\mu\text{mol}/(\text{m}^{-2}\cdot\text{s}^{-1})$ and photoperiod of 14 h/10 h (light/dark). The 1/2 Hoagland nutrient solution was applied every day.

After 15 d of culturing, the lettuce plants with four completely unfolded leaves were transplanted into plastic pots (10 cm in diameter and height) filled with clean sand, two seedlings per pot. Every three pots were placed in a plastic tray (8 cm in height) filled with Hoagland's nutrient solution, and the nutrient solution was replaced at 2 d intervals. 3 d after transplanted, the cultivation conditions were changed to the Hoagland's nutrient solution with 10 mg/L $\text{CdCl}_2\cdot 2.5\text{H}_2\text{O}$. Lettuce seedlings were cultivated in nutrient solution without Cd as the control. At the same time, the leaves of the Cd-treated lettuce seedlings were sprayed with different concentrations of IAA solution (0, 25, 50, 100, or 200 $\mu\text{mol}/\text{L}$) at 09:00. The control and Cd treatment alone were sprayed with distilled water. The IAA solutions were applied three times at 2 d intervals. Each treatment consisted of three pots (on plastic trays) and which was replicated three times. The pots were kept under ambient environmental conditions with natural sunlight and temperature (from March to April). Air temperature ranged between 23 ± 2.5 °C (day) and 16 ± 2.5 °C (night). Relative humidity varied from 65 to 84%. The lettuce was harvested during the period of organ formation (40 days after transplanted).

2.3. Determination of Growth parameters and Cd Content

The whole plant samples ($n = 3$) were rinsed with tap water and washed again with deionized water repeatedly. After drying with absorbent paper, the plant height and root length were measured with a ruler, and stem diameter was measured with vernier caliper. Then, the plants were divided into shoots and roots. The shoots and roots of each treatment were packed separately and dried at 80 °C to a constant weight. And the dry weight was weighed with an electronic balance. The root/shoot ratio was calculated as root dry weight divided by shoot dry weight. The dried samples were milled and then 0.5 g

powder was digested with $\text{HNO}_3\text{-HClO}_4$ (4:1 v/v). The Cd content was determined using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA) [11]. The translocation factor was calculated as shoot Cd content divided by root Cd content.

2.4. Statistical Analyses

All data were processed with Excel 2010 software and analysed by means of one-way ANOVA in combination with the least significant difference (LSD) test using SPSS 20.0 statistical software (IBM Corporation, Armonk, NY, USA). Significance was assessed at the 5% level of probability.

3 Results

3.1. Growth parameters of lettuce

Cd stress significantly inhibited the growth of lettuce plants. Compared with the control, plant height, stem diameter, and root length were reduced by 7.03% ($P < 0.05$), 28.67% ($P < 0.05$), and 28.32% ($P < 0.05$), respectively (Table 1). Under Cd stress, the inhibition of growth was alleviated by spraying IAA solution, which increased plant height and stem diameter compared with Cd treatment alone. Plant height and stem diameter were highest after spraying 100 $\mu\text{mol}/\text{L}$ IAA, increased by 10.34% ($P < 0.05$) and 22.85% ($P < 0.05$) respectively, compared with Cd treatment alone. No significant difference in root length and root dry weight was observed between the Cd + IAA treatments and Cd treatment alone. Under Cd stress, the maximum dry weight of shoot was attained when the IAA concentration of 100 $\mu\text{mol}/\text{L}$, which was 19.36% ($P < 0.05$) higher than Cd treatment alone. With increased in the IAA concentration, the root/shoot ratio of lettuce decreased slightly. The lowest root/shoot ratio was observed after treatment with 100 $\mu\text{mol}/\text{L}$ IAA, which was 8.51% lower than Cd treatment alone.

Table 1. Effects of exogenous IAA on growth parameters of lettuce plants under Cd stress.

Treatment Cd + IAA	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Shoot biomass (g/plant)	Root biomass (g/plant)	Root/shoot ratio
Control	15.08 $\pm$ 0.36 ab	9.14 $\pm$ 0.21 a	13.42 $\pm$ 0.43 a	1.80 $\pm$ 0.07 a	0.49 $\pm$ 0.02 a	0.27 $\pm$ 0.00 a
1	14.02 $\pm$ 0.47 cd	6.52 $\pm$ 0.28 d	9.62 $\pm$ 0.31 b	0.70 $\pm$ 0.03 d	0.16 $\pm$ 0.01 b	0.24 $\pm$ 0.00 b
2	14.48 $\pm$ 0.44 c	6.72 $\pm$ 0.26 d	9.46 $\pm$ 0.29 b	0.73 $\pm$ 0.02 cd	0.17 $\pm$ 0.01 b	0.23 $\pm$ 0.00 b
3	14.97 $\pm$ 0.40 b	7.10 $\pm$ 0.30 c	10.00 $\pm$ 0.29 b	0.76 $\pm$ 0.04 bc	0.17 $\pm$ 0.01 b	0.22 $\pm$ 0.00 b
4	15.47 $\pm$ 0.39 a	8.01 $\pm$ 0.22 b	9.30 $\pm$ 0.14 b	0.78 $\pm$ 0.03 b	0.17 $\pm$ 0.01 b	0.22 $\pm$ 0.00 b
5	13.94 $\pm$ 0.48 d	7.21 $\pm$ 0.33 c	9.26 $\pm$ 0.36 b	0.73 $\pm$ 0.05 cd	0.16 $\pm$ 0.02 b	0.23 $\pm$ 0.00 b

Data represent the mean value $\pm$ standard deviation from three replicates. Different letters within same column indicate significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test. The root/shoot ratio was calculated as the biomass in roots divided by the biomass

in shoots. The number 1 represents Cd+0 $\mu\text{mol}/\text{L}$ IAA; The number 2 represents Cd+25 $\mu\text{mol}/\text{L}$ IAA; The number 3 represents Cd+50 $\mu\text{mol}/\text{L}$ IAA; The number 4 represents Cd+100 $\mu\text{mol}/\text{L}$ IAA; The number 5 represents Cd+200 $\mu\text{mol}/\text{L}$ IAA.

3.2. Shoot and Root Cd Contents

The Cd content of shoots and roots in Cd-treated lettuce significantly increased compared with the control (Table 2). Under Cd stress, application of IAA significantly reduced the Cd content of shoot in lettuce plants. The lowest Cd content of shoot was attained in treatment with 100 $\mu\text{mol/L}$ IAA, which was 17.59% ($P < 0.05$) lower than Cd treatment alone. The trend for Cd content of the root was opposite to that for the shoot. The root Cd

content was highest in the presence of 50 $\mu\text{mol/L}$ IAA, which was significantly higher than that of all other treatments. At IAA concentration of 25 and 200 $\mu\text{mol/L}$, no significant difference in root Cd content was observed between the Cd + IAA treatments and Cd treatment alone. The Cd translocation factor of the lettuce plants significantly decreased after treatments with IAA compared with the Cd treatment alone. The minimum Cd translocation factor was 0.346 in treatment with 50 $\mu\text{mol/L}$ IAA.

Table 2 Effects of exogenous IAA on Cd content of lettuce under Cd stress.

Treatment	Shoot Cd content (mg/kg)	Root Cd content (mg/kg)	TF
Control	3.63 $\pm$ 0.03 f	6.69 $\pm$ 0.054 e	0.54 $\pm$ 0.00 a
Cd+0 $\mu\text{mol/L}$ IAA	68.29 $\pm$ 0.28 a	147.34 $\pm$ 4.80 c	0.46 $\pm$ 0.00 b
Cd+25 $\mu\text{mol/L}$ IAA	62.16 $\pm$ 0.12 c	144.10 $\pm$ 1.90 c	0.43 $\pm$ 0.00 c
Cd+50 $\mu\text{mol/L}$ IAA	61.35 $\pm$ 0.58 d	177.37 $\pm$ 0.71 a	0.35 $\pm$ 0.00 d
Cd+100 $\mu\text{mol/L}$ IAA	56.28 $\pm$ 0.10 e	160.62 $\pm$ 1.95 b	0.35 $\pm$ 0.00 d
Cd+200 $\mu\text{mol/L}$ IAA	63.50 $\pm$ 0.32 b	150.06 $\pm$ 0.96 c	0.42 $\pm$ 0.00 c

Data represent the mean value $\pm$ standard deviation from three replicates. Different letters within same column indicate significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test. The translocation factor (TF) was calculated as the Cd content in shoots divided by the Cd content in roots.

4 Conclusion

Cd stress suppressed the growth and increased the Cd content of lettuce plants. Exogenous IAA decreased the Cd content in shoots of lettuce, alleviated the toxic effect of Cd on growth. The greatest biomass and the lowest shoot Cd content, were found in lettuce after foliar application of 100 $\mu\text{mol/L}$ IAA. In summary, IAA alleviated the negative effects of Cd toxicity on growth, Cd content in lettuce plants, which aids in a safe lettuce production in areas with Cd-contaminated soil.

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