Effects of exogenous indole-3-acetic acid on antioxidant enzyme activities and osmotic substance contents of lettuce under cadmium stress

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Abstract. In plant growth and stress tolerance, indole-3-acetic acid (IAA) plays an important role. In this research, the effects of spraying different concentration (0, 25, 50, 100 and 200 μmol/L) of IAA on the antioxidant system in lettuce were investigated. The lettuce (Lactuca sativa) exposed to cadmium (Cd) exhibited the SOD, POD activities and MDA content obviously increased. Spraying exogenous IAA resulted that the CAT activity, soluble protein and proline content in shoots of lettuce increased compared with the Cd treatment alone, and MDA content with 50 μmol/L IAA was lowest. Therefore, spraying IAA effectively alleviated Cd toxicity and enhanced antioxidant system of lettuce, and the 100 μmol/L IAA was the optimal dose.

1 Introduction

Accumulation of contaminants such as cadmium (Cd) in soil involves natural or anthropogenic processes it is a toxic metal that causes deleterious effect on both plants and humans [1]. Currently, heavy metal contamination of soil is a significant worldwide environmental problem. Lettuce (Lactuca sativa) is a common type of leafy vegetable that is widely cultivated worldwide. Lettuce is a plant species that shows high accumulation of Cd [2], and has been considered as a Cd bio-indicator [3]. Lettuce plants grow in Cd-contaminated soil, however, there are no symptoms of dehydration and chlorosis are found on the Cd-contaminated lettuce plants [4].

In recent years, plant growth regulators are gaining increased attention because of its involvement in regulation of abiotic stresses. Indole-3-acetic acid (IAA) regulates the growth and development of plants, and has a crucial function in stress resistance [5]. The Trigonella seedlings are alleviated with IAA at low doses by regulating activity of some antioxidants under the Cd stress [6]. But, there is no research to investigate the effect that exogenous IAA on oxidative stress in lettuce under Cd stress.

In the present research, the effect of different concentrations of IAA on oxidative stress of lettuce under Cd stress was studied. The objective was to determine whether exogenous IAA could mitigate the Cd toxicity in lettuce by alleviating the oxidative stress caused by Cd.

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), and 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, Sichuan Agricultural University from February 2017 to June 2017. In February, the uniform size of lettuce seeds were sterilized in 10% H2O2 (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 maintained at 23 °C/18 °C (light/dark) under light intensity of 200 μmol/(m²·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 CdCl₂·2.5H₂O. 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 μmol/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 Antioxidant Enzyme Activity

Tender leaves (0.5 g; n = 6) were homogenized with a pestle and mortar in 6 mL of 0.05 mol/L ice-cold potassium phosphate buffer (pH 7.8). The homogenate was transferred to a plastic centrifuge tube and centrifuged at 10,000 × g for 15 min, and the supernatants were used for analysis of antioxidant enzyme activity. All procedures were performed at 4 °C. The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were measured in accordance with the method of Zhang, Huang and Chen [7].

2.4. Determination of malondialdehyde and Osmotic Adjustment Substances

The concentration of malondialdehyde (MDA), which is an indicator of the degree of membrane lipid peroxidation, was assayed using the thiobarbituric acid test. The soluble protein content was evaluated by anthranone-ethyl acetate method. The proline content was assayed using the sulfo-salicileic acid method. They were measured in accordance with the method of Zhang, Huang and Chen [7].

2.5. 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. Antioxidant Enzyme Activities

The activities of POD and SOD induced by Cd stress were significantly increased by 71.69% (P < 0.05) and 151.23% (P < 0.05), respectively, compared with the control (Table 1). After treatments with IAA, the activities of POD and SOD decreased compared with Cd treatment alone. When the IAA concentration was 100 μmol/L, the activities of POD and SOD were lowest, being 32.54% (P < 0.05) and 31.16% (P < 0.05) less than Cd treatment alone, respectively. In contrast, Cd treatment alone resulted in a decrease in CAT activity by 35.85% (P < 0.05) compared with the control. The CAT activity of lettuce increased with elevation in IAA concentration under Cd stress. The highest activity of CAT was observed when spraying 100 μmol/L IAA, which was 52.58% (P < 0.05) higher than Cd treatment alone.

Table 1. Effects of exogenous IAA on antioxidant enzyme activities of lettuce under Cd stress.

| Treatment | POD activity (U/g) | SOD activity (U/g) | CAT activity (U/g) |
|-----------|--------------------|--------------------|--------------------|
| Control   | 455.56±13.12 c     | 57.23±2.84 e       | 7.56±0.34 a        |
| Cd+0 μmol/L IAA | 782.15±17.29 a | 143.78±5.86 a     | 4.85±0.21 e        |
| Cd+25 μmol/L IAA | 731.10±19.37 b | 134.86±2.96 b     | 6.05±0.19 d        |
| Cd+50 μmol/L IAA | 669.82±13.58 c | 107.37±0.65 c     | 6.63±0.37 c        |
| Cd+100 μmol/L IAA | 527.63±13.75 d | 98.92±2.73 d      | 7.40±0.21 b        |
| Cd+200 μmol/L IAA | 696.31±16.55 c | 107.83±2.47 c     | 5.73±0.09 d        |

Data represent the mean value ± 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.

3.2. The contents of MDA and Osmotic Adjustment Substances

The MDA content of leaves in lettuce treated with Cd alone increased by 57.38% (P < 0.05) compared with the control (Table 2). Under Cd stress, treatments with IAA decreased the MDA content in leaves compared with Cd treatment alone. The MDA content of Cd-stress lettuce treatment with 50 μmol/L IAA was lowest, being 19.54% (P < 0.05) lower than Cd treatment alone. Compared with the control, Cd stress caused a 52.89% (P < 0.05) reduction in the soluble protein content of the leaves (Table 2). Spraying IAA significantly increased the soluble protein content in the
leaves under Cd stress, and the highest soluble protein content was observed at the IAA concentration of 100 μmol/L. The opposite result was found in proline content compared with the control, Cd stress significantly increased the proline content. Cd-stress lettuce treated with 25 and 200 μmol/L IAA, the proline content decreased compared with Cd treatment alone. But the proline content significantly increased when 50 and 100 μmol/L IAA were sprayed, being \( P < 0.05 \) 9.36% and 40.04% \( P < 0.05 \) higher than Cd treatment alone, respectively.

| Treatment               | MDA content (μmol/g) | Soluble protein content (mg/g) | Proline content (μg/g) |
|-------------------------|----------------------|-------------------------------|------------------------|
| Control                 | 6.64±0.17 d          | 1.142±0.007 a                 | 24.42±0.68 e           |
| Cd+0 μmol/L IAA         | 10.45±0.64 a         | 0.538±0.013 d                 | 35.24±1.34 c           |
| Cd+25 μmol/L IAA        | 10.01±0.40 b         | 0.602±0.004 c                 | 35.12±0.82 c           |
| Cd+50 μmol/L IAA        | 8.46±0.22 c          | 0.618±0.026 bc                | 38.54±1.87 b           |
| Cd+100 μmol/L IAA       | 10.22±0.32 ab        | 0.650±0.021 b                 | 49.53±1.86 a           |
| Cd+200 μmol/L IAA       | 10.29±0.32 ab        | 0.563±0.011 d                 | 28.23±0.67 d           |

Data represent the mean value ± 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.

4 Conclusion

The growth of lettuce plants by inducing oxidative stress despite of enhanced antioxidant system under Cd stress suppressed. Exogenous IAA improved osmotic adjustment and CAT content, decreased the SOD and POD content, hence, alleviated the toxic effect of Cd on growth. The greatest soluble protein and proline content, as well as the lowest SOD and POD activities, were found in lettuce after leaf application of 100 μmol/L IAA. In summary, IAA alleviated the negative effects of Cd toxicity on oxidative damages in lettuce plants, which aids in a safe lettuce production in areas with Cd-contaminated soil.

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