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Antioxidative systems, metal ion homeostasis and cadmium distribution in *Iris lactea* exposed to cadmium stress

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ABSTRACT

Iris lactea is a perennial halophyte and is tolerant to Cd. However, the mechanisms underlying this Cd tolerance are still poorly understood. In this study, morphological, physiological and biochemical responses of *I. lactea* to a 21 d exposure to different concentrations of Cd (0–150 mg L^{-1}) were investigated. *I. lactea* plants showed no toxicity symptoms except for a small reduction in growth at 100 and 150 mg L^{-1} Cd, along with the enhancement of H_2O_2 and MDA content in comparison to the control. The activities of SOD and POD were significantly enhanced and Ca accumulated with increasing Cd concentrations. Moreover, most Cd was retained in roots and only a small amount was transported to the shoots with increasing external Cd concentrations. Cd content had a negative correlation with content of K, Fe, Zn, and Mn and a positive correlation with Mg content in shoots and roots, which had no influence on these contents of mineral nutrients in shoots and chlorophyll levels with the increase of Cd concentrations. The Cd translocation factors were always less than 1 and bioaccumulation factors ranged from 3.43 to 15.6 across all treatments, suggesting that *I. lactea* might be effectively used in phytostabilization of Cd contaminated soils. Overall, the findings suggest that *I. lactea* could reduce photoinhibition and oxidative damage and maintain metal ion homeostasis in plant tissue by limiting translocation of Cd from roots to shoots and enhancing induction of antioxidant enzyme activities, thereby improving its Cd tolerance.

1. Introduction

Cadmium (Cd) is a non-essential heavy metal with high toxicity to plants, animals and humans due to its large solubility in water (Guo et al., 2014). It is estimated that annually around 30,000 t of Cd are released into the environment, of which 13,000 t result from human activity (Gallego et al., 2012). Consequently, Cd contamination of soil has become a serious environmental concern and also a threat to human health via accumulation of Cd in the food chain (Satoh-Nagasawa et al., 2012). Once excess Cd^{2+} enters into the cell, it may result in alterations to numerous physiological processes caused at a cellular/molecular level by inactivating enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for mineral nutrients and disrupting membrane integrity, finally leading to cell death (Rascio and Navari-Izzo, 2011). To cope with Cd stress, plants have evolved multifarious adaptation strategies to maintaining cellular metabolism, including exclusion at the surface of plasmalemma, sequestration in vacuoles, complexation via metal-chelating molecules in cytosol, as well as induction of antioxidant machinery and stress proteins (Clemens et al., 2002; Vázquez et al., 2006). The use of plants to cleanup soils and water contaminated with pollutants, a technique known as phytoremediation, is emerging as a new tool for in situ remediation (Yang et al., 2005). In Cd phytoremediation, plants are used either to absorb Cd from the soil or translocate it to harvestable shoots (phytoextraction) or to stabilize metal contaminants in soils through accumulation by precipitation within root zones (phytostabilization) (Zhang et al., 2010).

Iris lactea var. *chinensis* (Fisch.) Koidz. is a perennial monocotyledonous halophyte found in saline meadow and desert steppe in north China (Bai and Li, 2005). The plant has attractive leaves and flowers, a wide range abundant seeds, salt and drought tolerance, pest and disease resistance and easy cultivation. So they are widely planted in gardens, streets and along highways or salt affected areas as ornamental plants in China (Huang et al., 2003). It deposits Cd in cell walls, in the cytoplasm and on the inner-surface of xylem vessels in the root tip, so might have potential for the phytoremediation of Cd contaminated soils (Han et al., 2007). These authors suggested that studies on Cd tolerance mechanisms of *I. lactea* had focused only on the sub-cellular localization of Cd in the tissues and cells in the root tip. Cd can lead to oxidative stress by the production of reactive oxygen species (ROS) in plant cells that

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damage biomolecules and biomembranes through cell membrane lipid peroxidation and protein carbonylation (Gill et al., 2012; Nahar et al., 2016). Plants have evolved scavenging systems to control effectively ROS using non-enzymatic and enzymatic antioxidants. It has been documented that antioxidative enzymes, such as SOD (superoxide dismutase) and POD (peroxidases), could be involved in the control of ROS accumulation (Qiu et al., 2008). However, little is known about the extent to which responses in antioxidative systems and metal ion homeostasis contributes to the mitigation of Cd toxicity in *I. lactea* plants subjected to external Cd stress.

Therefore, to further understand the mechanisms of Cd tolerance in *I. lactea*, the present study aimed to: (1) evaluate the growth response by analyzing shoot and root length, and relative growth rates; (2) investigate biochemical and physiological responses, such as the lipid peroxidation, content of chlorophyll and H_2O_2, and anti-oxidative defense activity (SOD and POD); (3) examine the accumulation and distribution of Cd and mineral nutrients including potassium (K), iron (Fe), zinc (Zn), manganese (Mn), calcium (Ca), and magnesium (Mg) when *I. lactea* plants was exposed to different concentrations of Cd. Results obtained from this study would further understanding of the mechanisms of Cd tolerance in *I. lactea*.

2. Materials and methods

2.1. Plant growth conditions and treatments in the greenhouse

Seeds of *Iris lactea* were collected from the experimental site at Xiaotangshan in the Beijing Academy of Agriculture and Forestry Sciences. The seeds were sterilized with 5% sodium hypochlorite solution (v/v) for 5 min and rinsed thoroughly with distilled water, soaked in water for 56 h at 40 °C, then sown in plastic culture pots (8 cm in diameter, 12 cm in height) containing peat and sand (v/v, 2:1) under a 16hphotoperiod (light intensity was 600 μmol m^{-2} s^{-1}) at 28 ± 2 °C, and relative humidity (RH) between 50% and 60%. Pots watered every 3 days for 6 weeks. Once plants had three leaves, seedlings were selected for uniformity, then transferred into plastic containers (length 19 cm, width 14 cm and height 8 cm) filled with 0.6 L modified Hoagland solution (2 mM KNO_3, 1 mM $NH_4H_2PO_4$, 0.5 mM Ca $(NO_3)_2{\cdot}4H_2O$, 0.5 mM $MgSO_4{\cdot}7H_2O$, 60 μM Fe-citrate, 92 μM H_3BO_3, 18 μM $MnCl_2{\cdot}4H_2O$, 1.6 μM $ZnSO_4{\cdot}7H_2O$, 0.6 μM $CuSO_4{\cdot}5H_2O$ and 0.7 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$) for three weeks. All the seedlings were grown in the same chamber with a 16 h photoperiod at 28 ± 2 °C, and RH between 50% and 60%, irrigated every three days with Hoagland nutrient solution. The seedlings were treated with the 0.6 L Hoagland nutrient solution supplemented with 0, 5, 10, 25, 50, 100 and 150 mg L^{-1} of Cd as $CdCl_2$ for 21 days. The hydroponic solution was renewed every three days to keep relatively stable concentrations of Cd treatment. The plastic containers were arranged in a completely randomized block design, and each treatment was repeated eight times independently with three seedlings in each replicate.

2.2. Calculation of the relative growth rate

The relative growth rate (RGR) of whole plants was calculated using the formula RGR = (lnWj- lnWi)/Δt, where Wj and Wi are final (after 21 d of treatments) and initial(before treatments) dry weights (DW), respectively, and Δt is the time elapsed (days) between the two measurements, initial dry weight was determined before treatments (Martínez et al., 2005).

2.3. Determination of chlorophyll content

Chlorophyll (Chl) contents were estimated using a method modified from Ma et al. (2012). Fresh leaf samples was ground using fine quartz sand with 80% acetone and centrifuged at 12,000 × *g* for 10 min at 4 °C. The optical density of the solution was recorded at 645 nm and 663 nm using a UV-T6 spectrophotometer (Persee, Beijing, China), and Chl content was expressed as mg g^{-1} fresh weight (FW).

2.4. Lipid peroxidation

Lipid peroxidation in terms of malondialdehyde (MDA) formation was measured by the method of Dhindsa et al. (1981) with slight modifications. Supernatant (1 cm^3) was mixed with 4 cm^3 of 20% (m/v) trichloroacetic acid containing 0.5% (m/v) thiobarbituric acid, and centrifuged at 15,000 × *g* and 4 °C for 30 min. Test-tubes were heated at 95 °C for 30 min, then quickly cooled in an ice bath, and the precipitate was removed by centrifugation. The absorbance at 450, 532, and 600 nm were determined using aUV-T6 spectrophotometer (Persee, Beijing, China). Contents were calculated as μmol g^{-1} FW.

2.5. Determination of H_2O_2 content

Hydrogen peroxide (H_2O_2) was measured according to the method of Guo et al. (2013) with minor modifications. 500 mg of fresh leaves were frozen in liquid N_2 and homogenized in an ice bath with 10 ml of 0.1% trichloroacetic acid. After centrifugation for 20 min (12,000 × *g*, 4 °C), 1 ml of the supernatant was mixed with 1 ml of 10 mM sodium phosphate buffer (pH7.0) plus 2 ml of 1 M KI. The photometric absorption of the reaction solution supernatant was read at 390 nm. Contents were calculated as nmol g^{-1} FW.

2.6. Assay of antioxidant enzymes

For the analysis of antioxidant enzyme activities, 500 mg of fresh leaves was homogenized in 4 cm^3 of phosphate buffer (50 mM, pH 7.0) containing 1% (m/v) soluble polyvinylpyrrolidone and 0.2 mM ascorbic acid.

The homogenate was centrifuged at 15,000 × *g* and 4 °C for 10 min and the supernatant was then used for enzyme assays. Protein content was determined according to the method of Bradford (1976) with bovine serum albumin as a standard. The activity of SOD (EC 1.15.1.1) was determined according to the method described previously by Jiang and Huang (2001). The POD (EC 1.11.1.7) activity was measured at 470 nm as guaiacol oxidation by H_2O_2 (coefficient of absorbance 26.6 mM cm^{-1}) according to Chance and Maehly (1955).

2.7. Trace element analysis of plant samples

Harvested plants were washed thoroughly with deionized water, divided into shoots and roots, and oven dried at 80 °C for 3 d to constant weight. Dried plant tissues were ground and digested in a $HNO_3/HClO_4$ mixture (v/v, 4/1) for 24 h, and then heated at 150–200 °C until near dryness. The cooled residue was dissolved in ddH_2O up to 20 mL of total volume. The content of mineral nutrients (K, Ca, Mg, Fe, Mn, Zn and Cd) was measured using an atomic absorption spectrophotometer (AA-6300C, Shimadza, Kyoto, Japan).

Translocation factor (TF) and bioaccumulation factor (BCF) were calculated as follows: TF = (Cd in shoots)/(Cd in roots) indicates a plant's ability to translocate Cd from roots to shoots (Guo et al., 2014); BCF = (Cd in shoots)/(Cd in solution) reflects the ability of plants to accumulate Cd (Chen et al., 2011).

2.8. Statistical analysis

All the data are presented as means with standard deviation (SD). Statistical analyses including one-way ANOVA, Duncan's multiple range tests were performed by statistical software (Ver.13.0, SPSS Inc, Chicago, IL, USA).

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