Chemosphere 86 (2012) 530–537

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Biphasic effects of lanthanum on *Vicia faba* L. seedlings under cadmium stress, implicating finite antioxidation and potential ecological risk

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ARTICLE INFO

Article history:
Received 25 June 2011
Received in revised form 14 October 2011
Accepted 20 October 2011
Available online 14 November 2011

Keywords:
Rare earth elements (REEs)
Cadmium
Antioxidation
Prooxidation
Hormetic effects
Ecological risk

ABSTRACT

In the present study, lanthanum (La) as a representative REE was used to explore the mechanisms for alleviation of Cd-induced oxidative damage by extraneous La at appropriate concentrations, and to assess ecological risk of combination of Cd and La at higher concentrations in roots of *Vicia faba* L. seedlings. The seedlings were hydroponically cultured for 15 d under nutrient solution, 6 μmol L^{-1} $CdCl_2$, and combination of 6 μmol L^{-1} $CdCl_2$ and increasing concentrations of La, respectively. The results showed that the supplementation with low concentrations of exogenous La (<120 μmol L^{-1}) led to reduced contents of Cd, Ca, Cu, Zn, Mn or Fe element and increased activities of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) isozymes as well as heat shock protein 70 (HSP 70) production in the roots. However, the supplementation with higher La (>120 μmol L^{-1}) showed the adverse effects. The contents of Cd elevated above the single Cd treatment in the roots, accompanying with the decline of antioxidant isozyme's activities and HSP 70, and increment of carbonylated proteins and endoprotease isozyme's activities. The results also showed that the root growth was not only related to carbonylated proteins, but also to indole acetic acid oxidase activities. Therefore, the supplemented extraneous La contributed to biphasic effects: stimulated antioxidation at lower concentrations and pro-oxidation at higher concentrations against Cd-induced oxidative stress in the roots.

1. Introduction

Heavy-metal contamination has been greatly accelerated since the beginning of industrial revolution, which has become an increasing worldwide ecological and environmental problem. Cadmium (Cd), as one of the most toxic heavy metals, mainly derives from mining, smelting and manufacturing and enters agricultural and ecological systems via sludge utilization, sewage irrigation and Cd-containing phosphate fertilizers (Zhao et al., 2011). Previous studies revealed that after uptaken by plant roots, Cd could disturb plant growth, photosynthesis, mineral nutrition, and membrane structure, etc. (Pietrini et al., 2003; Scebba et al., 2006). Cd not only brings detrimental impacts to terrestrial and aquatic ecosystems (Sánchez, 2008), but also human health through bioaccumulation (Duruibe et al., 2007; Thijssen et al., 2007). It is thus highly important to reduce toxic effects of Cd on crops.

To mitigate the oxidative damage initiated by Cd, plants usually adopt antioxidative defense and cellular metabolism to keep cellular redox homeostasis (Zeng et al., 2011). Antioxidative enzymes (e.g. superoxide dismutase (SOD), catalase (CAT), and peroxidase (GPX)) are involved in the detoxification of $O_2^{\cdot-}$ and H_2O_2 and inhibition of the formation of $^{\cdot}OH$ radicals. Ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione and ascorbate acid are important components of the ascorbate–glutathione cycle and responsible for removal of H_2O_2 in different cellular compartments (Rodríguez-Serrano et al., 2009).

Rare earth elements (REEs) have been proved to have extensively biological effects on plants, animals, and human beings (Qiu et al., 2005). In China, REE-based microfertilizers have been widely applied for over 30 years to enhance yield and improve quality of crops and vegetables owing to their specific properties (Liu et al., 2006; Wang et al., 2007). REEs have been also proved to have resistance to salt, flood, aridity, acid rain, and heavy metal stress in plants (Ni, 1995; He et al., 2005).

Lanthanum (La) as a representative REE was reported to improve the resistance to heavy metals and alleviate the phytotoxicity in some plants (Choi et al., 2001; Pang et al., 2002). Inconsistent reports showed that the contents of heavy metals elevated with the increasing concentrations of extraneous REE(s), and that

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doi:10.1016/j.chemosphere.2011.10.030

mineral elements altered inversely in plants (Ding et al., 2004; Wang et al., 2008b). Related studies were very limited to date (Pang et al., 2002). Therefore, the potential mechanisms for relief of heavy metal stress by REEs need to be further investigated.

In the present study, 6 μmol L^{-1} Cd was used as the tested Cd-contaminated solution, and *Vicia faba* L. seedlings were employed as the tested plants. The concentration was chosen because it represents the contamination level of Cd in many polluted regions, which is several hundred times higher than fifth standard (equal to 0.009 μmol L^{-1}) for surface water Cd in China (GB3838-2002). The objective is (1) to explore the mechanisms for alleviation of Cd-induced oxidative damage by extraneous La at appropriate concentrations; (2) to assess ecological risk of combination of Cd and La at higher concentrations in roots of the seedlings.

2. Material and methods

2.1. Plant materials and lanthanum treatment

Seeds of *V. faba* L. were surface sterilized with 0.1% (m/v) sodium hypochlorite solution for 10 min and rinsed thoroughly in distilled water. Subsequently, the seeds were sown in moist fine gravel in plastic cylinders with perforated bottom at 20–23 °C. Six germinated seeds with nearly 2-centimeter length of primary roots were selected and transplanted into 1.2-L containers filled with equal Hoagland solution prepared according to methods of Lucretti et al. (1999). After a day of culture in the nutrient solution, cadmium chloride ($CdCl_2$) (Sigma–Aldrich) or/and lanthanum nitrate ($La(NO_3)_3$) were added to the containers and final treatments were control (Hoagland solution only), 6 μmol L^{-1} Cd, 6 μmol L^{-1} Cd + 2 μmol L^{-1} La, 6 μmol L^{-1} Cd + 8 μmol L^{-1} La, 6 μmol L^{-1} Cd + 30 μmol L^{-1} La, 6 μmol L^{-1} Cd + 60 μmol L^{-1} La, 6 μmol L^{-1} Cd + 120 μmol L^{-1} La, 6 μmol L^{-1} Cd + 240 μmol L^{-1} La, and 6 μmol L^{-1} Cd + 480 μmol L^{-1} La, respectively. The seedlings were incubated in the aerated Hoagland solution, which were replaced with freshly prepared ones every 3 d and pH values were adjusted within the range of 5.5–5.8. The containers were placed in a growth chamber with 15-h photoperiod (light at 230 μmol m^{-2} s^{-1}) at 23 °C, 6-h nights at 20 °C and 75% relative humidity. Three containers were prepared in each treatment in an independent experiment. Roots were harvested for chemical analysis and biological measurements immediately after 15 d of the treatments.

2.2. Measurement of root lengths

Lengths between stem base and root tips were measured to be represented as root lengths of the seedlings. Six seedlings were measured in each container, and three containers were included in each treatment.

2.3. Measurement of metal contents by ICP–OES

Fresh roots were consecutively rinsed with 1 mol L^{-1} HCl and distilled water. Digestion of samples was performed according to the previous protocol (Wang et al., 2010). Contents of La, calcium (Ca), ferrum (Fe), potassium (K), cadmium (Cd), zinc (Zn), cuprum (Cu), magnesium (Mg) and manganum (Mn) elements were detected by inductively coupled plasma optical emission spectrometer (ICP–OES, Perkin-Elmer, Optima 5300 DV, USA) and expressed as μg g^{-1} dry weight (DW). Certified standard samples (GBW07429) and triplicates of all samples were used to ensure accuracy and precision. The detection limit of 0.02 μg L^{-1} for this method was applied for all results.

2.4. Enzymes extraction and activity assays

Crude enzyme extract was prepared according to method described by Wang et al. (2010). 1 g of fresh roots was ground immediately to be fine powder under liquid nitrogen and homogenized in extraction buffer (0.1 mol L^{-1} Tris-HCl, pH 8.0, 10% (v/v) glycerol, 0.1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 0.2% (v/v) Triton X-100, 5% (w/v) PVPP, and 1 mmol L^{-1} phenylmethylsulphonyl fluoride (PMSF), 1 mmol L^{-1} benzamidine, 1 μg mL^{-1} leupeptin and 2 μg mL^{-1} apratinin). The extract was centrifuged for 20 min at 15 000 g, and supernatant was recovered and used for determination of all isozymes and activities listed below. Soluble protein content was determined by Bradford (1976) with BSA as standard. All operations were performed at 4 °C.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was determined according to method described by García-Limones et al. (2002) and Wang et al. (2008a) with slight modification. 3 mL of assayed mixture contained 0.1 mol L^{-1} Tris-HCl, pH 8.0, 50 μmol L^{-1} NBT, and 0.03% Triton X-100, 0.1 mmol L^{-1} EDTA, 14.5 mmol L^{-1} methionine, 1.05 μmol L^{-1} riboflavin and 100 μL crude enzyme extract. The reaction was started by adding riboflavin and A_{560} was measured immediately after 15 min illumination with light intensity of 2000 Lux. Control assays with and without the enzyme were also run. SOD activity is expressed as units min^{-1} mg^{-1} protein. One unit of enzyme activity is defined as the amount of crude enzyme extract required to inhibit the NBT reduction by 50%.

Guaiacol peroxidase (GPX) (EC 1.11.1.7), Catalase (CAT) (EC 1.11.1.6) and ascorbate peroxidase (APX) (EC 1.11.1.11) activities were also measured according to methods of García-Limones et al. (2002) with minor modifications, respectively. For GPX assay, 3 mL of assayed mixture contained 0.1 mol L^{-1} Tris-HCl, pH 7.0, 15 mmol L^{-1} guaiacol, 0.06% (v/v) H_2O_2 and 100 μL crude enzyme extract. The reaction was started by adding H_2O_2 and the oxidation of guaiacol was determined by the increase of A_{470} ($\varepsilon = 26.6$ $mmol^{-1}$ cm^{-1}). One GPX unit is defined as the amount of enzyme that produces 1 μmol min^{-1} oxidized guaiacol in the mixture.

For CAT assay, 3 mL of assayed mixture contained 0.1 mol L^{-1} Tris-HCl, pH 7.0, 20 mmol L^{-1} H_2O_2 and 100 μL crude enzyme extract. The reaction was started by adding H_2O_2. One unit of CAT is defined as the amount of crude enzyme extract necessary to decompose 1 μmol min^{-1} H_2O_2 in the mixture. For APX assay, 3 mL of assayed mixture contained 0.1 mol L^{-1} Tris-HCl, pH 7.0, 0.3 mmol L^{-1} sodium ascorbate, 5 mmol L^{-1} H_2O_2 and 100 μL crude enzyme extract. One unit of APX is defined as the amount of enzyme that oxidizes 1 μmol min^{-1} ascorbate in the mixture.

2.5. Determination of antioxidant isozymes

Isozyme's patterns were determined by native polyacrylamide gel electrophoresis (PAGE) using High-throughput Mini-PROTEIN 3 Electrophoresis System (Bio-Rad, USA). Crude enzyme extracts corresponding to 78.0 μg of total soluble protein, mixed with glycerin and bromophenol blue, were loaded into each lane for separation of antioxidant isozymes. Electrophoresis was subjected to constant voltage of 70 V reaching separating gel, and resumed to end by 120 V in ice bath using a 25 mmol L^{-1} Tris, 192 mmol L^{-1} glycin solution (pH 8.3) as running buffer.

SOD, GPX and APX isozymes were visualized by the methods of García-Limones et al. (2002) and CAT isozymes by the methods of Verma and Dubey (2003). Experiments were conducted in triplicate for each treatment.

2.6. Determination of patterns of indole acetic acid (IAA) oxidase

IAA oxidase isozymes were detected according to the methods of de Forchetti and Tigier (1983) with minor modification. Crude