JOURNAL OF ENVIRONMENTAL SCIENCES 26 (2014) 2048–2055

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Cadmium accumulation and tolerance of two castor cultivars in relation to antioxidant systems

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ARTICLE INFO

Article history:
Received 6 November 2013
Revised 18 December 2013
Accepted 19 February 2014
Available online 10 August 2014

Keywords:
Cadmium
Castor
Antioxidant activity
Tolerance
Phytoremediation

ABSTRACT

To investigate the effects of Cd on tolerance and antioxidant activities of castor, two different castor (*Ricinus communis* L.) cultivars (Zibo No. 5 and Zibo No. 8) were used for a hydroponic experiment (0, 1 and 2 mg/L Cd) and a pot experiment using Cd-contaminated soil (34 mg/kg) with the addition of ethylenedinitrilotetraacetic acid (EDTA). The results indicated that there were significant differences between the two cultivars with respect to Cd uptake in shoots (113–248 mg/kg for Zibo No. 5 and 130–288 mg/kg Zibo No. 8), biomass tolerance indexes (64.9%–74.6% for Zibo No. 5 and 80.1%–90.9% for Zibo No. 8) in the hydroponic experiment and survival rates (0% for Zibo No. 5 and 100% for Zibo No. 8) determined by the addition of EDTA in the pot experiment, suggesting that Zibo No. 8 has higher tolerance than Zibo No. 5. Moreover, the castor cultivars have low bioconcentration factors (4.80% for Zibo No. 5 and 5.43% for Zibo No. 8) and low translocation factors (<1%). Consequently, Zibo No. 8 can participate in Cd phytostabilization in highly Cd-polluted areas. The results indicated that glutathione (GSH) as a non-enzymatic antioxidant, and antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX), were cultivar- and dose-dependent. The higher tolerance of Zibo No. 8 compared with Zibo No. 5 can be attributed to the higher GSH levels in the root and higher GPX activity in the leaf.

Introduction

Cadmium (Cd) pollution has caused worldwide concern because of its high toxicity to plants, animals and humans. Furthermore, it is one of the most ubiquitous pollutants in soil (Huang et al., 2011). When Cd-contaminated land is used for crop planting, Cd is easily transferred from the soil to human body *via* the food chain, endangering human health (Jarup, 2003). In China, at least 13,330 ha farmland is contaminated by Cd, according to a recent soil survey from 11 provinces (Biao and Nan, 2000). Therefore, many Chinese people are confronted with potentially serious

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http://dx.doi.org/10.1016/j.jes.2014.08.005

health risks due to Cd pollution. Hence, it is of utmost importance to remediate Cd-contaminated soil.

Phytoremediation can be applied for the reduction of heavy metals from polluted soil using green plants (Shi and Cai, 2009). It is generally accepted that phytoremediation technology is cheap, convenient, and not harmful to the environment (McGrath and Zhao, 2003). However, the key point of phytoremediation is to search for the most efficient plant to treat, for example, Cd-polluted soil. Castor (*Ricinus communis* L.) is a C_3 plant of the Euphorbiaceae family from tropical Africa. It develops large biomass and a strong root system, and can be planted in a wide range of geographical environments in China. Some evidence has been provided that castor could phytoremediate soil polluted by Cd (Lu and He, 2008; Shi and Cai, 2009; Huang et al., 2011). This plant is characterized by a high tolerance to Cd concentrations exceeding 200 mg/kg (Shi and Cai, 2009), revealing a higher remediation efficiency compared to Indian mustard (*Brassica juncea* L.), which is considered to be a potential phytoremediator (Bauddh and Singh, 2012a,b). In addition, castor is an important oil crop for industry, but not edible for humans or animals (Olivares et al., 2013), and grown on marginal lands that are usually unsuitable for food crops (Berman et al., 2011). In addition, castor is a perennial plant, which can constantly remove Cd from contaminated soil (Bauddh and Singh, 2012a). It is also an excellent rotation and companion crop (Olivares et al., 2013), which is able to phytoremediate Cd-polluted soil in cooperation with other plants such as Indian mustard (Bauddh and Singh, 2012a). Consequently, castor can be cultivated for phytoremediation and for bioenergy production, which simultaneously addresses two critical global problems — increasing energy demands and remediation of Cd-polluted soil. Thus, it is a highly valuable renewable resource.

Cadmium, a non-essential toxic heavy metal, leads to alterations of the morphology and physiology of plants, caused by oxygen free-radical-mediated oxidative stress and peroxidation of membrane lipids (Tappel, 1973; Foyer et al., 1994; Chaoui et al., 1997; Szollosi et al., 2009). For plants' self-protection, plant cells induce the activity of oxygen radical detoxifying enzymes, such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX), and non-enzymatic antioxidants such as glutathione (GSH), in order to resist to oxidative stress caused by toxic metal concentrations (Vanassche and Clijsters, 1990; Chaoui et al., 1997). Antioxidants play an important role in the defensive mechanism of plants against Cd, as found in the citrus rootstock of citrumelo (Podazza et al., 2012), safflower (Shi et al., 2010) or Indian mustard (Liu et al., 2011). Glutathione is also active in many plants, for instance in garden cress (Gill et al., 2012) and wheat (Sun et al., 2005). However, reports about the Cd tolerance mechanisms of differently tolerant castor cultivars are few.

Therefore, the goals of the present study were (1) to compare the Cd tolerance, uptake and accumulation in two different castor cultivars; (2) to investigate the tolerance mechanisms of the castor cultivars exposed to Cd stress with the focus on antioxidant enzymes (GPX, CAT, and SOD) and a non-enzymatic antioxidant (GSH); and (3) to verify whether the castor cultivars can grow in a highly Cd-polluted soil (34 mg/kg) for phytoremediation.

1. Materials and methods

1.1. Selection and preparation of plant cultivars

Seeds of castor Zibo No. 5 and Zibo No. 8 cultivars, with high cadmium-phytoextraction ability and adaptability to the conditions in many parts of China, were obtained from Zibo Academy of Agricultural Sciences, Zibo City, Shandong Province, China. Castor seeds were initially grown on artificial non-polluted soil for 2 to 3 weeks until the seedlings developed two healthy tender leaves. These uniform seedlings were used for hydroponic experiments and pot experiments in the greenhouse located at the Center for Environmental Remediation, Institute of Geographical Sciences and Natural Resources, Chinese Academy of Sciences, Beijing, China.

1.2. Hydroponic experiments

1.2.1. Plant culture

The uniform seedlings of the castor plants were transplanted to 1 L pots containing 400 mL of half-strength Hoagland's solution with the following composition: 2.5 mmol/L $Ca(NO_3)_2$, 2.5 μmol/L KNO_3, 0.5 mmol/L KH_2PO_4, 0.5 mmol/L $MgSO_4$, 25 μmol/L H_3BO_3, 2.25 μmol/L $MnCl_2$, 1.9 μmol/L $ZnSO_4$, 0.15 μmol/L $CuSO_4$, 0.05 mmol/L $(NH_4)_6Mo_7O_{24}$ and 5 μmol/L Fe-EDTA. $CdCl_2{\cdot}2.5H_2O$ (guaranteed reagent) was used for providing Cd pollution. The Cd salt was added to the hydroponic culture. The pH of the nutrient solution was maintained at (6.0 ± 0.1) by the addition of 0.1 mol/L NaOH. Eight replicates were each treated with 0, 1 or 2 mg/L Cd: three replicates for the determination of biomass and Cd concentration, and the others for analysis of physiological indexes. Plants were kept in a greenhouse at temperatures of 25/15 °C during the day/night and a 16 hr photoperiod of about 300 mE/(m^2·sec) intensity, as well as 60% average relative humidity. The nutrient culture was replaced every 3 days, and the seedlings of castor grew for 3 weeks in the nutrient culture.

1.2.2. Growth parameters

At the end of the experiments, the plants for determination of biomass and Cd concentration were harvested and washed by distilled water, and then divided into two parts: root and shoot. All plant parts were dried in an oven at 70 °C for 48 hr to constant weight. The dry weights were measured by electronic balance.

Roots and shoots were ground in a mill, digested in flasks on an electric heating plate at 60 °C and treated with concentrated HNO_3 (guaranteed reagent). The temperature was then increased to 110 °C and kept stable until the sample solution became clear (Alexander et al., 2006). The sample volume was adjusted to 25 mL with ultrapure water. The Cd concentration of the sample was measured by flame atomic absorption spectroscopy (ContraAA 700, Analytikjena, Germany). A reference material GBW07603 (GSV-2) was used to monitor the Cd recovery of the plant samples (recovery: 90% ± 10%).

1.2.3. Measurement of antioxidant enzymes

After washing with distilled water, the plants were divided into three parts for analysis of physiological indexes: root, stem and leaf. These parts were stored in liquid nitrogen to maintain the activity of their enzymes. The leaves and roots were used for the analysis of antioxidants. Fresh samples were homogenized with the extracting solutions and ground with a chilled mortar and pestle, and then centrifuged at 10,000 × *g* for 25 min at 4 °C. The supernatants were stored at 4 °C and used for the analysis of antioxidants.

Glutathione was extracted using a 5 mmol/L EDTA-TCA solution and analyzed according to the method developed by Eyer and Podhradsky (1986).