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Effect of Cd on growth, physiological response, Cd subcellular distribution and chemical forms of *Koelreuteria paniculata*

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ABSTRACT

Koelreuteria paniculata were cultivated in nutrient solution with different concentrations of Cd (0, 50, 150, 250 and 500 μM) and sampled after 90 days. The resistance, translocation, accumulation and stress responses in *Koelreuteria paniculata* were investigated by hydroponic experiments. The results showed that *Koelreuteria paniculata* is an efficient Cd excluder that can tolerate high concentrations of Cd (up to 150–250 μM of Cd). The concentration of Cd never exceeds 5 ppm in leaves and 10 ppm in roots. The high concentration of Cd (≥ 250 μM) had a toxic effect on *K. paniculata* and significantly restricted the plant growth. The accumulation ability of Cd by different plant tissues followed the sequence of roots > leaves > stems. The bioconcentration factors and translocation factors both were less than 1. Cd has the highest content in the cell wall and is migrated to soluble fractions and organelles at high concentrations. Undissolved Cd phosphate, pectates and protein-bound Cd were the predominant forms. The low concentration of Cd (≤150 μM) promoted the synthesis of soluble proteins, AsA and GSH, while high concentration of Cd clearly inhibited the physiological and biochemical process, caused membrane lipid peroxidation and severe membrane damages, and increased MDA and H_2O_2 contents. POD, CAT and SOD exhibited positive and effective responses to low concentration Cd stress, but could not remove the toxicity caused by high concentration Cd stress. The content of IAA, GA and ZT decreased and ABA content was significantly increased under high-concentration Cd stress.

1. Introduction

The heavy use of cadmium (Cd) containing fertilizers, and mining exploitations has led to a gradual concentrations of cadmium in the soil environment. Cd cannot be microbially degrade in soil, with the property of persistence and bioaccumulation (Azevedo et al., 2012; Dhir et al., 2009; Xin et al., 2013). Cd is not an essential element for biological growth. It is highly toxic and poses a threat to living organisms, from microorganisms to animals, affecting its normal growth and causing death (Dalcorso et al., 2010; Alessandro et al., 2012). For the heavy metal pollution in soil, phytoremediation is safe and reliable, with good economic and ecological benefits (Xue et al., 2014; He et al., 2014; Bjelková et al., 2011). In addition, phytoremediation can prevent soil erosion and water loss, improve the condition of landscape. On this foundation, phytoremediation is considered a sustainable way for the wide application in environment remediation of heavy metal contaminants (Cluis, 2004; Ghosh and Singh, 2005). Many herbaceous species including *Typha latifoia*, *Thlaspi caerulescens*, *Arabidopsis halleri*, *Miscanthus floridulus* (Labill) Warb, *Eremochloa ciliaris* L., *Sedum alfredii* H. and *Solanum nigrum* L. have been confirmed to be able to accumulate and transfer heavy metals such as Pb, Cd and Mn in mine areas. Herbaceous plants are widely used in the soil restoration of heavy metal contaminated areas. However, each species produces a low biomass. Additionally, shallow root systems for these plants limit the removal of heavy metal from deep soil (Keller et al., 2003). There are also some non-hyper-accumulator plants, such as willows, eucalypts, poplars, *Brassica napus* and sunflower (Iori et al., 2017; Guo et al., 2015; Ehsan et al., 2014), can be used for phytoremediation because they have large biomass, rapid growth and deep root system (He et al., 2013). However, these plants are generally less resistant to Cd, which limits their repair efficiency. Woody plants with great biomass and high tolerance to heavy metals may be an alternative. (Pulford and Watson, 2003; Mukhopadhyay and Maiti, 2010).

Koelreuteria paniculata is a deciduous species of the sapindaceae family native to China, Japan, and Korea. It can resist salinity, drought and short-term flooding. This species have a deep root system, and is strongly adaptable to the environment (Zhang et al., 2017). These characteristics make it a good tree species for phytoremediation in

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heavy metal contaminated areas. In 2004, a three-square-kilometer base with *K. paniculata* ecological restoration was established in Xiangtan Manganese Mine, Hunan, China. It is a successful case of using *K. paniculata* to repair waste mining area (Tian et al., 2009). It tentatively confirmed that *K. paniculata* is highly resistant to various heavy metals and has certain potential of phytoremediation for heavy metal contaminated soils. So far, the reports of *K. paniculata* mainly focus on biological characteristics, pharmacology and chemical composition (Huang et al., 2015; Luo et al., 2015; Pipinis et al., 2015; Mostafa et al., 2014). The mechanism of plant tolerance to Cd has been reported quite a lot, but relatively few reports on woody plants and even more rarely reported on *K. paniculata. Koelreuteria paniculata* is known to grow on metal contaminated sites and thus might be involved in phytoremediation process. But its response to metal exposure (notably Cd exposure) has never been described so far. So, the resistance, translocation, accumulation and stress responses of *K. paniculata* to different concentrations Cd has yet to be studied thoroughly. Therefore, the present research is intended to: (1) investigate the effects of Cd on the growth of *K. paniculata*; (2) explore the subcellular distribution and chemical forms of Cd in *K. paniculata*; (3) examine the effects of Cd on physiological and biochemical indexes of *K. paniculata*, including chlorophyll, malondialdehyde, soluble protein, hormone contents, and antioxidant enzymes in order to uncover the potential mechanisms with regard to the uptake, accumulation, translocation and tolerance of Cd in *K. paniculata*. The results of this study are expected to provide the theoretical basis for the application and improvement of phytostabilization of *K. paniculata* in heavy metal contaminated soil.

2. Materials and methods

2.1. Plant cultivation

K. paniculata seedlings were obtained from Central South University of Forestry and Technology, Changsha, PR China. All seedlings were 1-year-old and of similar size: 50 cm high, with a diameter of 0.5 cm. They were grown in 20% Hoagland-Arnon nutrient solution and cut off all branches. The seedlings of *K. paniculata* were precultured for 4 weeks for the initiation of the new roots before they were exposed to Cd stress. Then they were used for pot experiment. Pot experiment was conducted by means of hydroponics in intelligent incubator at 25/20 ℃, a 16/8 h photoperiod, 65 ± 5% relative humidity, and irradiation of 350 μmol m^{-2} s^{-1}. The selected plants were cultivated in Cd treatment solutions (0, 50, 150, 250, 500 μM), supplied as $CdCl_2{\cdot}2H_2O$, with four replicates for each treatment. Plants were cultivated with deionized water daily to maintain volume of culture medium, and supplied 20% Hoagland-Arnon nutrient solution every week. After a growth period of 90 days all plants were thoroughly washed with deionized water to eliminate any particles attached to roots surface and used for next experimental treatment. Initial individual explant dry weight (grams), branch numbers, maximum root lengths (cm) and plant height (cm) were recorded. Each plant is labeled and the percent growth rate of entire plant (%GR) was calculated as change in fresh weight at 90 d. Tolerance Index (TI) were calculated as 100[root $length_{Cd\text{-}exposed}$/root $length_{control}$] (Clabeaux et al., 2011).

2.2. Plant Cd uptake

The plant roots were washed in 20 mM Na^2-EDTA for 20 min to eliminate Cd adsorbing to the roots surfaces. The whole plants were rinsed with deionized water and separated into roots, stems and leaves. All plant samples were frozen immediately in liquid nitrogen and divided into three sections for analysis of total Cd concentrations, subcellular distribution and chemical forms. The bioconcentration factor (BCF) was equal to the ratio of Cd concentrations in plant to soil Cd concentrations. The translocation factor (TF) was calculated as the Cd concentrations in the shoot divided by that in the root.

2.3. Subcellular fractions of Cd in leaves, stems and roots

The plant cells in fresh tissues of leaves, stems and roots were separated into three parts (cell wall fraction, organelle fraction and soluble fraction) on the basis of the method depicted by Y. Wang et al. (2012) and C. Wang et al. (2012). Plant tissues were homogenized in cooled extraction buffer [250 mM sucrose, 1.0 mM DTT ($C_4H_{10}O_2S_2$) and 50 mM Tris-HCl, pH 7.5] with a cooled mortar. The homogenate was sifted through nylon cloth (80 μM). As residue included mainly cell walls and cell wall debris, it was named the 'cell wall fraction'. The filter liquor was centrifuged at 12,000 × *g* for 40 min. The subsidence was designated as the 'organelle fraction' and the supernatant was the 'soluble fraction'. All procedures were carried out at 4 ℃.

2.4. Chemical forms of Cd in leaves, stems and roots

Cd chemical forms were extracted from the following steps: (1) 80% ethanol, which extracts inorganic Cd giving priority to nitrate/nitrite, chloride, and aminophenol Cd; (2) deionized water, which extracts Cd-organic acid complexes; (3) 1 M NaCl, which extracts pectate and protein-integrated Cd; (4) 2% acetic acid (HAc), which extracts undissolved Cd phosphate; (5) 0.6 M HCl, which extracts Cd oxalate; and (6) the residual Cd form (Fu et al., 2011). The frozen plant tissues were homogenized in an extraction solution with a mortar, then diluted in a rate of 1:100 (W/V), shaken for 22 h at 25 ℃. The homogenate was then centrifuged at 5000 × *g* for 10 min to obtain the first supernatant in a centrifuge tube. The precipitate was suspended twice in the extractant, shaken for 2 h and centrifuged at 5000 × *g* for 10 min at 25 ℃. The supernatants of the three suspensions were then combined. Using the above procedure, the residue was extracted sequentially with the next extractant. Each of the solution was evaporated to constant weight on an electroplated plate at 70 ℃.

2.5. Analysis of chlorophyll, malondialdehyde, soluble protein, H_2O_2, glutathione, ascorbic acid and antioxidant enzyme

The method of Porra (2002), was used to determine total chlorophyll, chlorophyll a and b. The degree of lipid peroxidation was estimated as malondialdehyde (MDA) content in leaves, stems and roots following the method of Esposito and Domingos (2014). Soluble protein content in leaves, stems and roots was following the method of Vassilev and Lidon (2011). Hydrogen peroxide (H_2O_2) in leaves, stems and roots was measured spectrophotometrically according to Alexieva et al. (2001). Determination of glutathione (GSH) content in leaves, stems and roots follows the method of Aravind and Prasad (2005). Ascorbic acid (AsA) content in leaves, stems and roots was measured according to the method of Hodges and Forney (2000). Superoxide dismutase activity (SOD) in leaves was estimated as 50% reduction of nitroblue tetrazolium (NBT) was determined by the method of Fatima and Ahmad (2005). Peroxidase activity (POD) in leaves was measured by the method of Wu et al. (2016). Catalase activity (CAT) in leaves was measured by the method of Apodaca et al. (2017).

2.6. Analysis of hormone

Approximately 2 g of the freeze-dried leaf sample was weighed and then milled in an ice bath with the pre-cooled methanol solution (80%). The homogenate was immersed in a freezer (4 ℃) for 15 h, then centrifuged at 5000 × *g* for 10 min. The supernatant was concentrated to half with a rotary evaporator at 40 ℃ and transferred to a separatory funnel. 10 mL petroleum ether was added to the funnel, separated the layers after the shock, then discard the upper liquid (repeat 3 times). The remaining liquid was concentrated to 5 mL with a rotary evaporator, and the pH of liquid was adjusted to 2.8 with 0.1 mM HCl. The remaining liquid was extracted twice with an equal volume of ethyl acetate in a separatory funnel, and the ethyl acetate phase was merged.