Chemosphere 117 (2014) 567–574

Contents lists available at ScienceDirect

Chemosphere

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

Eucalyptus tolerance mechanisms to lanthanum and cerium: Subcellular distribution, antioxidant system and thiol pools

Yichang Shen [a,b], Shirong Zhang [b,c,*], Sen Li [a,b], Xiaoxun Xu [b], Yongxia Jia [b], Guoshu Gong [d]

[a] Key Laboratory of Soil Environment Protection of Sichuan Province, Wenjiang 611130, PR China
[b] College of Resources and Environment, Sichuan Agricultural University, Wenjiang 611130, PR China
[c] Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Wenjiang 611130, PR China
[d] Agricultural College, Sichuan Agricultural University, Wenjiang 611130, PR China

HIGHLIGHTS

- La and Ce accumulation in eucalyptus were 696.5 and 493.7 mg plant^{-1}, respectively.
- Cell walls stored 45.40–63.44% of the metals under La or Ce stress.
- POD and CAT activity enhanced at low La or Ce; but no change in GSH or AsA.
- NPT concentration increased at low La or Ce, PCs concentration continued to increase under La or Ce stress.

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 14 August 2014
Accepted 7 September 2014
Available online 7 October 2014

Handling Editor: Shane Snyder

Keywords:
Eucalyptus
Lanthanum
Cerium
Subcellular distribution
Antioxidant system
Thiol pools

ABSTRACT

Guanglin 9 (*Eucalyptus grandis* × *Eucalyptus urophlla*) and *Eucalyptus grandis* 5 are two eucalyptus species which have been found to grow normally in soils contaminated with lanthanum and cerium, but the tolerance mechanisms are not clear yet. In this study, a pot experiment was conducted to investigate the tolerance mechanisms of the eucalyptus to lanthanum and cerium. Cell walls stored 45.40–63.44% of the metals under lanthanum or cerium stress. Peroxidase and catalase activities enhanced with increasing soil La or Ce concentrations up to 200 mg kg^{-1}, while there were no obvious changes in glutathione and ascorbate concentrations. Non-protein thiols concentrations increased with increasing treatment levels up to 200 mg kg^{-1}, and then decreased. Phytochelatins concentrations continued to increase under La or Ce stress. Therefore, the two eucalyptus species are La and Ce tolerant plants, and the tolerance mechanisms include cell wall deposition, antioxidant system response, and thiol compound synthesis.

1. Introduction

In recent years, high demand for rare earth elements (REEs) in industry and agriculture has led to the accumulation of REEs in soils (Li et al., 2010; Wang et al., 2011a). However, REEs are heavy metals with low to moderate toxicity (Jiang et al., 2012). Low concentrations of REEs can promote plant growth, but excessive REEs can inhibit plant growth (Kobayashi et al., 2007; Wen et al., 2011; Wang et al., 2011a) and impact human health (Rapôso et al., 2007; Damment et al., 2009; Oral et al., 2010). Lanthanum (La) and cerium (Ce) are representatives of the REEs, and different from Cd, Pb, and Cu. La and Ce are widely used in new materials and agricultural rare-earth micronutrient fertilizers (Babula et al., 2008); therefore, these two elements are common soil pollutants and in this study are used as representatives of various REEs that occur as soil contaminants (Nicodemus et al., 2009).

Conventional methods can be effective in removing toxic metals from contaminated soils; these include adsorption, land filling, and selective leaching (Aparajith et al., 2010; Bayat and Sari, 2010; Boparai et al., 2011; Vasudevan et al., 2011). However, these methods are usually expensive, and they may destroy soil properties (Usman and Mohamed, 2009). Phytoremediation is an alternative method that has been widely considered because of its advantages: it is easily employed, its effect on the soil is permanent, and it is inexpensive (Yamato et al., 2008).

* Corresponding author at: College of Resources and Environment, Sichuan Agricultural University, Wenjiang 611130, PR China. Tel.: +86 028 86290995; fax: +86 028 86290983.
E-mail address: rsz01@163.com (S. Zhang).

http://dx.doi.org/10.1016/j.chemosphere.2014.09.015
0045-6535/

Phytoremediation is a green remediation approach that uses green plants to decontaminate or detoxify pollutants (Chehregani et al., 2009; Suchkova et al., 2010). When applied to soil polluted with La and Ce, the two elements can be recycled after accumulator or hyperaccumulator plants are ashed. In recent years, research has been conducted on the phytoremediation of REEs contaminated soils, but there are very few reports containing information about REE tolerance mechanisms of plants (Shan et al., 2003; Lai et al., 2006). Research has been conducted on the accumulation and tolerance mechanisms for toxic metals in plants. Several hypotheses, including compartmentalization of the cell (Weng et al., 2012), the response of the antioxidant system, and the chelation of metals by thiol compounds and other low molecular weight thiols (Kupper et al., 2004; Hernandez-Allica et al., 2006), have been proposed to explain cell detoxification mechanisms during phytoremediation.

Subcellular distribution of metals may be associated with metal tolerance and detoxification in plants; this includes such processes as cell wall deposition and vacuolar compartmentation. Research has indicated that a large amount of metals stored in the cell wall fraction could prevent metal contaminants from entering the cell interior and participating in cell physiological activities (Wójcik et al., 2005; Wang et al., 2008). Ramos et al. (2002) observed that most of the Cd present in a lettuce plant occurred in the cell wall. However, most Cd in *Phytolacca americana* was present in the soluble fraction of the cell (Fu et al., 2011). Therefore, compartmentalization of metals in plants varies between species.

Plant cells will produce large amounts of reactive oxygen species when under metal stress. Free radicals can damage intracellular macromolecules, resulting in membrane lipid peroxidation (Srivastava et al., 2007; Douchiche et al. 2010; Zhang et al., 2013). Antioxidant systems can help plants withstand harmful effects of environmental stress (Formigari et al., 2007). The effectiveness of oxidative defense system in plants can be measured by the activities of antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) and by the concentrations of non-enzymatic antioxidants including glutathione (GSH) and ascorbic acid (AsA) (Ahmad et al., 2012). High concentrations of REEs have been observed to cause an increase in intracellular reactive oxygen species (Wang et al., 2011a). Activities of some antioxidant enzymes enhanced in wheat seedlings (Zeng et al., 1999) and spinach seedlings (Liu et al., 2004) under REE stress. Lanthanum nitrate treatments also induced changes in activities of antioxidant enzymes and glutathione contents in *Triticum durum* (Aquino et al., 2009). Therefore, CAT, POD, GSH, and AsA may be important compounds for intracellular free radical scavenging.

Plants may synthesize mercapto group-containing compounds when they are exposed to metals stress (Cobbett, 2000; Najmanova et al., 2012). These thiol compounds include non-protein thiols (NPT), glutathione (GSH), and phytochelatins (PCs). PCs sequester heavy metals through thiolate coordination (Najmanova et al., 2012). Cys, GSH, and PCs play pivotal roles in plant metal tolerance and detoxification (Dominguez-Solis et al., 2004; Lima et al., 2006; Vázquez et al., 2009; Weng et al., 2012). Nevertheless, some studies have demonstrated that the detoxification of lead (Pb) and cadmium (Cd) in *Sedum alfredii* is not related to PCs but to GSH (Sun et al., 2007; Gupta et al., 2010).

In general, the roles of the various detoxification processes may vary between plants, and it is necessary to conduct research targeted on different plants to elucidate detoxification mechanisms (Weng et al., 2012).

Eucalyptus is grown widely throughout the world because of its adaptability and high productivity (Forrest and Moore, 2008), and mainly distributed in Australia, the Philippines and other western Pacific islands. China is now the second largest producer of eucalyptus in the world (Huang et al., 2007). In China, the plant species mainly distributed in Guangxi, Guangdong, Hainan, Sichuan, and Fujian. In preliminary experiments, the accumulations of La and Ce in eucalyptus in 30 months were 696.50 and 493.68 mg plant^{-1}, respectively. The two eucalyptus species, Gn9 (Guanglin9: *Eucalyptus grandis* × *Eucalyptus urophlla*) and Eg5 (*Eucalyptus grandis* 5), grew normally in soil containing high concentrations of La and Ce, but their tolerance mechanisms are unclear.

The objectives of this study were to determine the concentrations and subcellular distributions of La and Ce in roots and leaves in Gn9 and Eg5, to explore the response of the antioxidant system induced by the presence of La and Ce, and to evaluate the roles of thiol pools in alleviating La and Ce toxicity.

2. Materials and methods

2.1. Plant and soil description

The Gn9 and Eg5 were used in our study. Soil samples used in the pot experiments contained 426, 158, and 416 g kg^{-1} of clay, silt, and sand, respectively. The soil organic matter and total N were 32.42 and 1.89 g kg^{-1}, respectively; available N, Olsen P and available K were 102.7, 22.27, and 152.9 mg kg^{-1}, respectively; and the pH was 6.98.

2.2. Experimental design

From August 2012 to January 2013, the experiment was conducted in Wenjiang, Sichuan, which has a humid monsoon subtropical climate with an average annual temperature of 15.9 °C and average annual precipitation of 972 mm. Annual sunshine time is 1168 h with an average relative humidity of 84%.

The pot experiment was conducted in a net house with transparent polythene sheets to protect them from rainwater leaching. Each plastic pot (35 cm in diameter, 40 cm in height) was filled with 8.0 kg of ground soil (the soil was ground and passed through a 4-mm mesh) that was mixed with three levels of La and Ce in solution (prepared by dissolving analytical grade $LaCl_3{\cdot}7H_2O$ and $CeCl_3{\cdot}6H_2O$). The levels of treatment were control (0), 30, 200, and 500 mg kg^{-1}. The soil was then incubated for 4 weeks, after which two uniform eucalyptus seedlings were transplanted into each pot. Four replicates were run for each treatment. During the experiment, the pots were watered with tap water (containing no detectable La and Ce) based on the amounts of water lost from the plants. The plant samples were harvested 150 d after transplanting.

2.3. Sampling and pretreatment

Plant samples were collected and washed thoroughly with running tap water followed by deionized water. Plants were divided into roots, stems, and leaves, and the fresh weight (FW) was determined immediately. Some fresh samples were used for the determination of physical signs and subcellular distribution, and the rest were dried to a constant weight at 75 °C. The dried plant samples were finely ground and sieved through a 1-mm nylon sieve for chemical analysis. Soil samples were taken immediately adjacent to the plants in the pot. All the soil samples were air-dried at room temperature for 15 d then ground and passed through a 2-mm nylon sieve.

2.4. Determination of enzyme activity

Plant material (0.5 g) was homogenized on ice with 5 mL of ice-cooled 50 mM Na-phosphate buffer (pH 7.8, containing 0.1 mM EDTA) and polyvinylpolypyrrolidone. The mixture was then