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Short communication

Lanthanum tolerance and accumulation characteristics of two *Eucalyptus* species

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A B S T R A C T

Because of extensive mining of rare earth elements (REEs) and overuse of REE-containing fertilizers, the remediation of soils polluted with REEs such as lanthanum (La) has become an international concern. In this study, pot and plot experiments were conducted to investigate the La tolerance and accumulation of a *Eucalyptus grandis* × *Eucalyptus urophylla* hybrid, Guanglin 9 (Gn9) and *E. grandis* 5 (Eg5). In the pot experiment, the two species grew normally in soil added La concentrations below 500 mg kg^{-1}, and shoot biomass showed no significant decrease compared with the control. Peroxidase and catalase activities and glutathione and soluble protein concentrations generally displayed exponential, quadratic or other non-linear increases in response to increasing soil La concentrations. Maximum La accumulations in shoots of Gn9 and Eg5 in the pot experiment were 0.46 and 0.57% of soil La, respectively, with corresponding accumulations in the plot experiment of 87 and 80 mg plant^{-1}. Most bioconcentration and translocation factors in shoots were less than the critical value (1.0). These findings indicate that the two species may be potentially useful for the remediation of La-polluted soil.

1. Introduction

Because of their special physical and chemical properties, rare earth elements (REEs) are used in the industrial production of technological devices and in agricultural fertilizers (Kobayashi et al., 2007). While some researchers have reported that low concentrations of REEs are beneficial for plant growth (d'Aquino et al., 2009), and excessive amounts may have significant negative effects on plant growth and human health (Kobayashi et al., 2007). In recent years, high global demand for REEs has resulted in the exploitation and abuse of REE sources (Zeng et al., 2006). As a result, the accumulation of REEs in the environment has become an international concern (d'Aquino et al., 2009).

In China, which supplies most of the world's REEs, excavation of REEs since the 1970s has generated large amounts of waste rocks and mine tailings with high lanthanum (La) concentrations and increased the extent of La-polluted land. Furthermore, rare earth fertilizers with high La content have been extensively applied to soils in the past 30 years (Zeng et al., 2006). The advancement of technologies for the remediation of La-contaminated soils is therefore important.

Compared with conventional remediation methods, phytoremediation is an environmentally sustainable remediation strategy because of its operational simplicity and large-scale applicability (Kumar et al., 2013; Babu et al., 2014). Some researchers have suggested that this method may be useful for the remediation of La-polluted soil (Wei et al., 2005), followed by recycling of La after incineration of plants. Because only a few REE accumulator or hyperaccumulator species, such as *Carya cathayensis* (Robinson, 1943) and *Dicranopteris dichotoma* (Wei et al., 2006), have been identified, additional species suitable for phytoremediation are needed.

Eucalyptus, which is indigenous to Australia, is widely cultivated worldwide because of its rapid growth, broad adaptability, and high productivity. Some studies have revealed that *Eucalyptus* can accumulate heavy metals (King et al., 2008), but no reports have appeared on the use of *Eucalyptus* for the restoration of La-polluted soil.

In this study, we investigated two *Eucalyptus* species that we hypothesized might have strong La tolerance and accumulation capabilities. Our objectives were to assess the La tolerance of the

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two species in La-polluted soil and to evaluate their capabilities for La uptake and accumulation using pot and plot experiments.

2. Materials and methods

The plant materials used for experiments were tube seedlings of Guanglin 9 (*Eucalyptus grandis* × *Eucalyptus urophylla*) (Gn9) and *E. grandis* 5 (Eg5), both obtained from Guangzhou Fangji Flower Limited Liability Company (Guangzhou, China).

The pot experiment was conducted in Yaan, Sichuan, China (102°59′ E and 29°59′ N). This area is in the subtropical zone and experiences a moist climate (Zhang et al., 2013). The experimental soil was yellow soil, with clay, silt, sand, organic matter, and total N contents of 475, 339, 186, 29.54, and 1.06 g kg^{-1}, respectively. Concentrations of available N, P, and K and total La were 79.4, 16.6, 141.8, and 13 mg kg^{-1}, respectively, and the pH was 5.17. Exchangeable, reducible, oxidizable, and residual La fractions in the soil were 2, 2, 4, and 5 mg kg^{-1}, respectively. Each plastic pot (20 cm diameter and 35 cm height) was filled with 8.0 kg of ground soil that had been filtered through a 4-mm mesh, mixed with a La solution (prepared by dissolving analytical grade $LaCl_3 \cdot 6H_2O$) to give a supplemental concentration of 0 (control), 10, 30, 100, 200, 350, or 500 mg La kg^{-1} soil, and incubated for 4 weeks. Two uniform tube seedlings (10–12 cm height) of Gn9 or Eg5 with measured La concentrations of 0.26 and 0.29 mg kg^{-1}, respectively, were transplanted into each pot. Four replicates were run for each treatment. The seedlings were harvested 180 days following transplantation.

The plot experiment was conducted in cement pools comprising three plots, each with an area of 2.0 m^2 (2 m × 1 m). All plots were filled with La-contaminated mine soil to a depth of 60 cm. Eight 10–12-cm high tube tree seedlings of Gn9 or Eg5, which contained La of 0.26 and 0.29 mg kg^{-1}, respectively, were planted in each plot. Clay, silt, sand, organic matter, and total N contents of the mixed mine soil were 185, 393, 422, 24.75, and 1.12 g kg^{-1}, respectively; pH and available N, P, and K were 5.33 and 120.5, 16.7, and 138.4 mg kg^{-1}, respectively. The following REE and heavy metal concentrations (mg kg^{-1}) were also recorded: 241 (La), 116 (Ce), 2.13 (Cd), and 47 (Pb). Soil fractions of La, Ce, Cd, and Pb were determined using the BCR sequential extraction procedure. Exchangeable, reducible, oxidizable, and residual fractions of these elements in soil were respectively 27, 31, 96, and 87 mg kg^{-1} for La, 20, 15, 40, and 41 mg kg^{-1} for Ce, 0.32, 0.31, 0.74, and 0.76 mg kg^{-1} for Cd, and 7, 8, 16, and 16 mg kg^{-1} for Pb. Four plants in each plot were randomly sampled for investigation 365 days after seedling transplantation.

The seedlings that were sampled in the pot and plot experiments were thoroughly washed with running tap water followed by distilled water. Washed seedlings were divided into roots, stems, and leaves, and their fresh weights were immediately determined. Some of the fresh samples in the pot experiments were used for the determination of physiological characteristics, with the remaining samples dried at 75 °C for 72 h and then finely ground and sieved through a 1-mm nylon sieve. Plant samples (0.3 g) were digested with a mixture of HNO_3 (5 ml) and $HClO_4$ (1 ml). In both pot and plot experiments, soil samples were randomly taken from the rhizosphere, air-dried at room temperature for 15 days, and ground to pass through a 2-mm nylon sieve. The soil samples (1.0 g) were digested with a mixture of HNO_3 (5 ml), HF (1 ml), and $HClO_4$ (1 ml) (Zhou et al., 2011). La concentrations in plant and soil were determined by inductively coupled plasma–atomic emission spectroscopy (IRIS Intrepid II; Thermo Electron Corporation, Milford, MA, USA). $LaNO_3$ from Beijing Gaoke Limited Liability Company (Beijing, China) was used as a standard material for quality assurance. Soil basic physicochemical properties were measured using the methods described by Bao (2008).

Table 1
Plant growth and photosynthesis parameters of the two *Eucalyptus* species (Gn9 and Eg5) in the pot experiment. P_n, net photosynthetic rate; Cond, stomatal conductance; C_i, intercellular CO_2 concentration; T_r, transpiration rate. Data are means ± S.D. (n = 4). Means with different letters are significantly different from each other (P < 0.05).

Treatment (mg kg^{-1})	Plant height (cm)	Root length (cm)	Root biomass (g DW)	Stem biomass (g DW)	Leaf biomass (g DW)	Shoot biomass (g DW)	P_n (μmol m^{-2} s^{-1})	Cond (mol m^{-2} s^{-1})	C_i (μmol mol^{-1})	T_r (mmol m^{-2} s^{-1})
Gn9										
0	135.50 ± 9.15b	13.00 ± 0.82b	14.41 ± 3.11	44.42 ± 3.89c	21.60 ± 1.14b	66.01 ± 4.75bc	13.23 ± 1.21	0.076 ± 0.013	76.15 ± 19.52	4.692 ± 0.467
10	155.50 ± 12.50c	13.25 ± 2.22b	15.46 ± 2.29	49.65 ± 3.63 cd	24.96 ± 2.32b	74.61 ± 4.63 cd	13.40 ± 0.72	0.082 ± 0.006	74.16 ± 3.05	4.693 ± 0.229
30	162.00 ± 5.94c	13.25 ± 0.96b	15.99 ± 1.85	47.79 ± 6.18c	23.11 ± 7.16b	70.90 ± 2.48 cd	13.60 ± 0.91	0.085 ± 0.012	72.53 ± 2.98	4.714 ± 0.432
100	153.50 ± 7.59c	14.25 ± 1.71b	16.03 ± 1.84	53.83 ± 1.94 d	25.93 ± 4.08b	79.83 ± 5.38 d	13.72 ± 0.81	0.084 ± 0.004	71.82 ± 16.17	4.711 ± 0.290
200	164.67 ± 7.02c	12.17 ± 2.36ab	16.13 ± 2.43	45.45 ± 1.17c	25.66 ± 2.79b	61.12 ± 2.16b	13.28 ± 1.40	0.077 ± 0.007	70.69 ± 13.17	4.527 ± 0.495
350	145.00 ± 12.76bc	12.00 ± 1.00ab	14.52 ± 0.83	37.44 ± 4.67b	22.61 ± 4.10b	60.06 ± 3.22b	13.39 ± 1.12	0.073 ± 0.007	71.79 ± 4.60	4.324 ± 0.393
500	122.75 ± 9.03a	9.35 ± 2.63a	13.52 ± 0.69	13.60 ± 1.46a	15.44 ± 1.53a	29.04 ± 1.50a	12.99 ± 1.52	0.074 ± 0.004	73.04 ± 0.71	4.283 ± 0.448
	F = 9.804	F = 3.111	F = 0.790	F = 53.200	F = 3.443	F = 31.365	F = 0.182	F = 1.190	F = 0.081	F = 0.833
	P = 0.000	P = 0.027	P = 0.590	P = 0.000	P = 0.018	P = 0.000	P = 0.979	P = 0.363	P = 0.997	P = 0.558
Eg5										
0	130.0 ± 12.8ab	19.9 ± 1.1c	17.21 ± 1.86b	53.72 ± 3.18bc	24.99 ± 2.25bc	78.71 ± 4.32b	11.02 ± 0.78bc	0.0814 ± 0.005	138.57 ± 11.85ab	4.188 ± 0.196
10	140.0 ± 8.7b	16.0 ± 1.4b	17.36 ± 2.56b	49.79 ± 1.73b	25.38 ± 0.87bc	75.17 ± 2.50b	11.63 ± 0.72c	0.0819 ± 0.004	124.01 ± 6.92a	4.198 ± 0.218
30	140.3 ± 10.2b	14.0 ± 2.9ab	18.28 ± 1.90b	49.62 ± 3.78b	26.11 ± 1.03c	75.73 ± 3.27b	12.11 ± 1.13c	0.0821 ± 0.014	122.96 ± 10.69a	4.229 ± 0.611
100	145.0 ± 7.5b	13.9 ± 0.1ab	20.63 ± 1.00b	55.41 ± 4.12c	26.31 ± 2.13c	81.72 ± 5.21b	11.20 ± 0.75bc	0.0816 ± 0.006	156.43 ± 12.35c	4.221 ± 0.131
200	128.8 ± 9.5ab	14.0 ± 0.8ab	20.30 ± 3.32b	53.64 ± 2.52bc	26.34 ± 4.25c	79.98 ± 3.95b	9.66 ± 1.32ab	0.0817 ± 0.018	193.27 ± 10.01 d	4.205 ± 0.763
350	138.3 ± 13.2b	12.3 ± 2.2a	17.46 ± 2.73b	49.95 ± 1.20b	22.34 ± 1.95b	72.30 ± 1.92b	9.22 ± 1.30a	0.0810 ± 0.004	152.18 ± 13.09bc	4.186 ± 0.340
500	117.7 ± 4.7a	12.0 ± 0.8a	13.87 ± 0.96a	28.76 ± 0.81a	17.94 ± 1.12a	46.70 ± 1.25a	9.03 ± 1.18a	0.0808 ± 0.010	156.90 ± 9.37c	4.152 ± 0.390
	F = 2.953	F = 11.186	F = 4.190	F = 41.737	F = 7.748	F = 48.098	F = 5.459	F = 0.016	F = 20.047	F = 0.120
	P = 0.031	P = 0.000	P = 0.006	P = 0.000	P = 0.000	P = 0.000	P = 0.002	P = 0.832	P = 0.000	P = 0.851