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Lead, zinc and iron (Fe^{2+}) tolerances in wetland plants and relation to root anatomy and spatial pattern of ROL

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ABSTRACT

Metal (Pb, Zn and Fe^{2+}) tolerances, root anatomy and profile of radial oxygen loss (ROL) along the root (i.e., spatial pattern of ROL) were studied in 10 emergent wetland plants. The species studied could be classified into three groups. Group I included *Alternanthera philoxeroides*, *Beckmannia syzigachne*, *Oenanthe javanica* and *Polypogon fugax*, with high ROL along the whole length of root ('partial barrier' to ROL). Group II included *Cyperus flabelliformis*, *Cyperus malaccensis*, *Juncus effusus*, *Leersia hexandra* and *Panicum paludosum*, ROL of which was remarkably high just behind the root apex, but decreased significantly at relatively basal regions ('tight barrier' to ROL). Group III consisted of only *Neyraudia reynaudiana*, with extremely low ROL along the length of root. The results indicated that metal tolerance in wetland plants was related to root anatomy and spatial pattern of ROL. Co-evolution of metal (Fe and Zn) tolerance and flood tolerance possibly developed in wetland plants since species showing a 'tight barrier' to ROL (a common trait of flood-tolerant species) in basal root zones had higher Fe and Zn tolerances than those showing a 'partial barrier'. Root anatomy such as lignin and suberin deposition contributed to a 'tight barrier' in root and conferred to exclusion ability in tolerant species.

1. Introduction

Wetland plants have the ability to accumulate heavy metals from polluted water and substrates (Zhu et al., 1999; Qian et al., 1999; Deng et al., 2004), which makes them more attractive and interesting in phytoremediation of metal mine drainage and tailings (McCabe and Otte, 2000; Ye et al., 2004). Understanding metal tolerances and mechanism in wetland plants will provide important hints for species selection in wetland remediation system construction. Metal tolerance is generally evolved in populations living in soils enriched with metals and populations from heavy-metal-polluted soils are usually more resistant to the relevant metals than populations of the same species from normal soils (Baker, 1987). The evolution of metal tolerance has been well documented in terrestrial plants, such as in species of *Festuca rubra* (Davies et al., 1991), *Silene vulgaris* (Bringezu et al., 1999), *Arabidopsis halleri* and *Thlaspi caerulescens*, hyperaccumulators of Zn and Cd (Küpper et al., 2000; Whiting et al., 2000). However, different from terrestrial plants, wetland plants are generally characterized by constitutive tolerance in metals, i.e., contaminated populations have similar metal tolerance with control populations from areas free of contamination (McNaughton et al., 1974; Taylor and Crowder, 1984; Ye et al., 1997a,b, 2003; McCabe and Otte, 2000; Matthews et al., 2004, 2005; Deng et al., 2006). Meanwhile, their metal tolerances (e.g. Fe and Zn) do differ greatly with species (Snowden and Wheeler, 1993; Matthews et al., 2005). Therefore, the question has been raised as to what is crucial for metal tolerance in wetland plants. Since their tolerance is not strongly related to metal contamination in their habitat as is found in terrestrial plants, other features unique to wetland plants must be underlying their metal tolerances.

To grow in a waterlogged environment, a striking strategy exhibited by wetland plants is to form extensive aerenchyma, which provides a low-resistance-pathway for root oxygen transportation (Jackson and Armstrong, 1999). Oxygen may diffuse from roots (termed radial oxygen loss, ROL) to create an oxidized rhizosphere in the otherwise anoxic substrate (Armstrong et al., 1992; Mainiero and Kazda, 2004) and protect wetland plants from reduced soil toxins (Armstrong and Armstrong, 2005; Soukup et al., 2007; Van der Welle et al., 2007). Many flood-tolerant species prevent excessive oxygen loss from the basal root zones by forming roots with a strong 'barrier' to ROL in their epidermis, exodermis or subepider-

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mal layers (Armstrong et al., 2000; Visser et al., 2000; McDonald et al., 2001; Colmer, 2003a) to inhabit in reduced environments. It has been suggested that the existence of a physical barrier against ROL may meanwhile impair nutrient absorption and uptake by roots (Hose et al., 2001; Insalud et al., 2006; Soukup et al., 2007). Snowden and Wheeler (1993) have indicated that Fe^{2+} tolerance in wetland plants is significantly related to root porosity, root oxidizing ability and flood tolerance. Coincidently, flood resistant species such as *Eriophorum angustifolium* and *J. effusus* being very tolerant to Fe (Snowden and Wheeler, 1993) are also proved to be highly tolerant to Zn (Matthews et al., 2005). Will the tolerance of other metals (such as Pb and Zn) share the same mechanism with that of Fe^{2+} in wetland plants, such as root anatomy, oxidizing ability and flood tolerance? We hypothesize that flooding would impose natural selection pressures on wetland plants to develop adaptations. For instance, changes in root anatomy might be one of the adaptations for efficient oxygen transport to apex, and also for improving tolerances of wetland plants to metals. In the present study, 10 wetland species were compared with regard to their metal (Pb, Zn and Fe^{2+}) tolerances, root anatomy and ROL. Most of the species selected have been found to grow well in sediments containing extremely elevated Pb, Zn and Fe (Deng et al., 2004). Field observations also indicate that these species inhabit submerged sediment with variable water levels and redox potential, exhibiting different abilities in waterlogging tolerance. Additionally, Fe plaque is formed in varying degrees and is deposited unevenly on root surfaces. Here we have specifically performed the following investigations:

(1) Evaluating Pb, Zn and Fe^{2+} tolerance and uptake among different species.
(2) Examining profiles of ROL and root anatomy of wetland plants with different metal tolerance.
(3) Studying the relationship between metal tolerance and root anatomy and ROL.

The results will have significance in understanding the mechanisms of metal tolerance in wetland plants, which can in turn provide direction for species selection in wetland remediation construction.

2. Materials and methods

2.1. Plant material and preparation

Table 1 lists the names and site information of the 10 wetland species studied. Tillers/seedlings of populations living in non-contaminated soil were used except for *J. effusus* and *C. malaccensis* for which seeds from clean sites were not available when the experiment was carried out. Tillers of *A. philoxeroides*, *L. hexandra*, *O. javanica* and *P. paludosum* were grown from vegetative propagation while seedlings of the other six species were grown from seeds. Cuttings of the four vegetative species were rooted in sand and watered with 20% Rorison solution (Hewitt, 1966). As for the others, seeds were soaked in deionized water for 30 min and germinated at 25 °C in sand. Germination occurred after 7–14 days and seedlings were then watered with 20% Rorison solution. Considering the different growth rates of the species studied, seedlings/tillers with similar size (about 15–20 cm in shoot height) were subjected to the following experiments, i.e., 4-week-old for the four vegetative species, 6-week-old for *B. syzigachne*, *P. fugax* and *C. flabelliformis* and 8-week-old for *N. reynaudiana*, *J. effusus* and *C. malaccensis*.

Table 1
Species information for Pb, Zn, Fe tolerance and root aeration experiments.

Species	Site description of the population collected and general distribution
Alternanthera philoxeroides (Mart.) Griseb	Clean site in Shaoguan, Guangdong Province. Distributed in submerged soil with E_h from −50 to −100 mV.
Beckmannia syzigachne (Steud.) Fernald	Clean site in Sanmen County, Zhejiang Province. Distributed in saturated soil with E_h 0–50 mV.
Cyperus flabelliformis Rottb.	Clean site from Hong Kong Mai Po Nature Reserve. Waterlogged salt mash.
Cyperus malaccensis Lam.	Constructed wetland receiving effluents from Pb/Zn mine in Fankou Pb/Zn mine, Guangdong Province, with high Pb and Zn in sediment. Waterlogged sediment with E_h ranging from −150 to −50 mV.
Juncus effusus L.	Semi-waterlogged wetland receiving wastewater from the multi-metal mine in Jin Chuantang, Hunan Province. High Pb and Zn in sediment. Distributed in sediment with E_h range from 100 to −150 mV.
Leersia hexandra Swartz.	Clean site in Fankou, Guangdong Province. Submerged paddy soil. Grown in wetlands with sediment E_h range from −150 to −50 mV.
Neyraudia reynaudiana (Kunth) Keng	Clean site in Laidong village, Hong Kong. Drained farmland.
Oenanthe javanica (Bl.) DC	Clean site in Sanmen County, Zhejiang Province. Distributed in saturated soil or running shallow water with E_h around 0–50 mV in substrate.
Panicum paludosum Roxb.	Clean site in Shaoguan, Guangdong Province. Submerged paddy field with E_h from −50 to −100 mV in substrate.
Polypogon fugax Steud.	Clean site in a residential area of Qiaokou County, Hunan Province. Distributed in semi-waterlogged soil.

2.2. Hydroponic experiment for Pb, Zn and Fe^{2+} tolerance

Lead, Zn and Fe^{2+} tolerance was inferred by measuring the elongation of the longest root, which is indicated to be the most sensitive to toxins (Wilkins, 1978) upon metal exposure in hydroponics. Uniform seedlings/tillers mentioned above were selected and transplanted to 8 L (35 cm × 20 cm × 13 cm; length × width × height) lightproof plastic containers. Seedlings/tillers were mounted on floating polyfoam board so that their roots could suspend in the solution. Before metal exposure, roots were cut to 1 cm in length and left in full strength Rorison solution (pH 5.5) for 1 week until a new root system was developed. After that, seedlings/tillers were exposed to 20 mg l^{-1} Pb (supplied as $Pb(NO_3)_2$), 4.0 mg l^{-1} Zn (supplied as $ZnSO_4$) and 25 mg l^{-1} Fe (supplied as $FeSO_4$) in 20% Rorison solutions (pH 5.5) in addition to control treatment for 21 days. The concentrations for metal exposure were based on a preliminary test (Deng et al., 2006). All species were cultured in the same container for the same treatment, with 6 seedlings/tillers per species and 60 in total in each container. Although it is pseudoreplication to some extent, the experiment was set up in this way to control the growing conditions, such as metals, nutrients and dissolved oxygen, for different species, to look at their metal tolerance and radial oxygen loss. There were three replicates for each metal treatment. As for the controls, four replicates were conducted because one of