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The role of radial oxygen loss and root anatomy on zinc uptake and tolerance in mangrove seedlings

H. Cheng [a], Y. Liu [b], N.F.Y. Tam [c], X. Wang [a], S.Y. Li [d], G.Z. Chen [d], Z.H. Ye [a,*]

[a] State Key Laboratory for Bio-control, and School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, PR China
[b] Ministry of Agriculture Key Laboratory of Mariculture Ecology and Products Quality and Safety, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, PR China
[c] Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, PR China
[d] School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, PR China

Mangrove seedlings with a 'tighter barrier' in ROL spatial pattern exhibit higher Zn tolerance.

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ABSTRACT

Root anatomy, radial oxygen loss (ROL) and zinc (Zn) uptake and tolerance in mangrove plants were investigated using seedlings of *Aegiceras corniculatum*, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*. The results revealed that *B. gymnorrhiza*, which possessed the 'tightest barrier' in ROL spatial patterns among the three species studied, took up the least Zn and showed the highest Zn tolerance. Furthermore, zinc significantly decreased the ROL of all three plants by inhibition of root permeability, which included an obvious thickening of outer cortex and significant increases of lignification in cell walls. The results of SEM X-ray microanalysis further confirmed that such an inducible, low permeability of roots was likely an adaptive strategy to metal stress by direct prevention of excessive Zn entering into the root. The present study proposes new evidence of structural adaptive strategy on metal tolerance by mangrove seedlings.

1. Introduction

Mangrove ecosystems possess important ecological and economical value and are one of the major types of natural wetlands in tropical and subtropical coastal regions (Peters et al., 1997; Tam and Wong, 1997). Despite their importance, mangrove wetlands have experienced significant contaminant input, aggravated by rapid urban development (MacFarlane et al., 2007). Among these anthropogenic pollutants, heavy metals (e.g., Zn) have received increasing attention in recent years and are often found in high concentrations in polluted estuarine zones, including mangrove wetlands (Irvine and Birch, 1998). Most mangrove plants, however, have adapted in numerous ways to overcome this threat and can survive in relatively high metal conditions (MacFarlane and Burchett, 1999; Zhang et al., 2007; Caregnato et al., 2008). Unfortunately, the mechanisms involved in metal tolerance by mangrove plants are still poorly understood (MacFarlane et al., 2007); therefore, it is crucial to understand the internal or external factors of mangrove plants that may play important roles in metal uptake and tolerance.

Success of mangrove plants growing in intertidal zones, where sediments are often characterised by a shortage of oxygen, accumulation of soluble phytotoxins (e.g., Fe^{2+}, Mn^{2+}, H_2S and CH_4) and deficiency of essential nutrients (Ponnamperuma, 1984), is generally ascribed to anatomical adaptations that allow sufficient oxygen (O_2) can be transported to below-ground roots (Koncalová, 1990; Kludze et al., 1993; Youssef and Saenger, 1996). A part of this O_2 is used for aerobic metabolism in roots, while excessive O_2 may diffuse into rhizosphere, a process defined as radial oxygen loss (ROL) (Armstrong et al., 1992). ROL from roots is important for aerobic microbial activity and can cause the oxidation and/or immobilization of potential phytotoxins in rhizosphere to avoid the toxicity to roots (Armstrong et al., 1988; Taggart et al., 2009).

In order to better tolerate anaerobic condition, mangrove plants often develop a barrier to ROL in subapical root zones (Pi et al., 2009), which typically show significantly lower ROL rates. Reduced O_2 leakage from subapical root zones enhances longitudinal O_2 diffusion towards root tips (Visser et al., 2000; Vasellati et al., 2001; Colmer et al., 2006). It is also generally accepted that the performance of this barrier is mainly related to hypodermal structure, as well as quantitative variations in suberin composition

* Corresponding author. Tel.: +86 20 39332858.
E-mail address: lssyzhh@mail.sysu.edu.cn (Z.H. Ye).

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and distribution within exodermal cell walls (Soukup et al., 2007). Previous studies illustrate that the root anatomical features, especially exodermal and hypodermal structure, are various among different mangrove plants (Youssef and Saenger, 1996; Liu, 2009; Pi et al., 2009), which indicates that the performances of this barrier in mangrove plants are species specific (Soukup et al., 2007).

However, this barrier will also bring some negative issues to plants (e.g., reduce the capacity for nutrient uptake) (Armstrong and Armstrong, 2001, 2005). Additionally, it has been reported that exterior environmental factors (e.g., some phytotoxins like oil, organic acid and sulfide) can regulate ROL from roots by changing their root anatomical structure (Armstrong and Armstrong, 2001, 2005; Armstrong et al., 2009) or morphology (Taggart et al., 2009). However, few researchers have reported the effects of heavy metals (e.g., Zn) on the permeability and ROL of roots and/or the correlations between permeability and ROL of roots and metal uptake and tolerance by plants.

Furthermore, ROL would alert mobility and bioavailability of heavy metals, both on root surface and in rhizosphere, by process of oxidation (Youssef and Chino, 1989; Jacob and Otte, 2003) and by altering pH, redox potential, microbial populations and by formation of iron-plaque, which could eventually affect metal uptake and tolerance by plants (Ye et al., 1997; Tao et al., 2003).

Until now, few articles have reported on the important mechanisms involved in metal uptake and tolerance in mangrove plants as related to root anatomy and ROL. The aims of this current study are, therefore, to investigate the relationships among ROL, root anatomy and Zn uptake and tolerance in three mangrove seedlings, in order to provide new evidence regarding metal tolerant mechanisms in mangrove plants. The present study not only provides a better understanding of the mechanisms involved in metal tolerance, but it also highlights information which is also very significant in mangrove management, as well as the selection of mangrove plant species with high metal tolerance for the bioremediation of metal-contaminated waters or soils.

2. Materials and methods

2.1. Plant materials and preparation

Mature and healthy propagules or seeds of six species of mangrove plants, including *Aegiceras corniculatum* (L.) Blanco, *Avicennia marina* (Forsk.) Vierh, *Bruguiera gymnorrhiza* (L.) Poir, *Kandelia candel* (Linn.) Druce, *Rhizophora stylosa* Griff and *Sonneratia apetala* Buch.-Ham, were collected from a national nature reserve in Shenzhen, Guangdong Province, P.R. China. The propagules or seeds were then cultivated in clean river sand irrigated by 0.2 strength Hoagland nutrient solution (containing 10‰ NaCl) under glasshouse conditions. The seedlings were kept in a glasshouse with a temperature of 25°C ± 5 °C, on a 16/8 h day/night cycle, with a light intensity of 480 μmol m^{-2} s^{-1} throughout the reported experiments.

2.2. Selection of mangrove seedlings with different ROL spatial pattern

Uniform, one-year-old seedlings of the above six mangrove species were selected for the measurement of ROL using root-sleeving O_2 electrodes as described by Armstrong and Wright (1975). Each mangrove seedling was placed with its roots in a cylindrical perspex vessel (12 cm diameter and 15 cm high) with a cover. The vessel was fitted with a rubber bung to erect the plant and minimize re-oxygenation of the medium by the atmosphere. Before ROL measurement, 1.25 L freshly deoxygenated 0.05% (W/V) aqueous agar containing 7 mM NaCl was prepared and added into the vessel. ROL rates along lateral roots (with similar diameter in basal root region, 0.8–0.9 mm) were measured at the points 1, 3, 5, 7 cm from root tips. Four seedlings were used per treatment and two lateral roots were examined per plant. Using our ROL dates, three mangrove species with significantly different net reductions in ROL from root tip to basal zone were selected in current study, e.g., low (*A. corniculatum*; with 42% reduction in ROL in root base when compared to that in root tip), intermediate (*R. stylosa*; 62%) and high (*B. gymnorrhiza*; with the largest reduction, 75%) (Table 1).

2.3. Pot trial with addition of Zn

Uniform, one-year-old seedlings of the three selected mangrove species were transplanted into plastic pots (20 cm diameter and 25 cm high, two seedlings of same species per pot) with 4 kg prepared soil (50% silty clay loam, 40% clean river sand, 10% organic peat moss) per pot. This mixed soil was determined to have sufficient nutrients for the mangrove seedlings to grow and no additional fertilizers were added during the experiment. Zinc was prepared by the addition of 0 (control, CK), 200, 400, 600, 800 mg as $ZnCl_2$ per kg dry soil (Burchett et al., 1984). Eight replicates were used per treatment.

Table 1
ROL at different positions (apical root regions, 1 cm from root tip; basal root regions, 7 cm from root tip) along lateral roots in six mangrove plants (mean ± SE, $n = 4$, different letters in the same column indicate significant differences at $P < 0.05$ as determined by LSD test).

Species	ROL rate in apical root regions (ng O_2 cm^{-2} min^{-1})	ROL rate in basal root regions (ng O_2 cm^{-2} min^{-1})	Ratio of reduced ROL in root base (%)
A. corniculatum	22.64 ± 1.31c	13.09 ± 0.64bc	42c
A. marina	32.95 ± 0.81b	14.40 ± 0.31b	56b
B. gymnorrhiza	49.26 ± 1.10a	12.21 ± 0.25bc	75a
K. candel	29.23 ± 1.79b	13.20 ± 1.00bc	55b
R. stylosa	30.27 ± 0.84b	11.65 ± 0.31c	62b
S. apetala	30.07 ± 1.70b	16.95 ± 0.97a	43c

After 120 days exposure to Zn, the seedlings were harvested and washed by deionized water. Half of them were used for growth measurements and metal analysis. Growth parameters included seedling height, diameter of the stem base and the number of fully-expanded leaves per plant. These measurements were taken immediately after harvest ($n = 4$). The seedlings were then dried for one week at 60 °C, to a constant weight, for the calculation of the total biomass (which is a summation of root, stem and leaf, but without propagules). The oven-dried samples (root and leaf, 0.2–0.3 g) were digested by a mixture of concentrated nitric acid and hydrogen peroxide (MacFarlane and Burchett, 2002). The concentrations of Zn in the digests were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) ($n = 4$). Blanks and standard plant materials (GBW-07063, Gsv-2, China Standard Materials Research Center, Beijing) were tested for quality assurance, and the average recovery rate of Zn was 91%.

The remaining seedlings were used for the measurement of ROL (using the same method described above) and root anatomy. For root anatomy, transverse sections in apical root regions and basal root regions, 1 cm and 7 cm from root tips, respectively, were made from the same lateral roots as ROL measurements. Fresh cross-sections were cut by hand with a sharp razor. All sections were stained with phloroglucinol and concentrated hydrochloric acid to detect lignification (Armstrong and Armstrong, 2001, 2005). Finally, specimens were examined and photographed using an Olympus BX40 photomicroscope.

2.4. Scanning electron microscopy with X-ray microanalysis (SEM X-ray microanalysis)

In order to investigate in more detail the internal distribution of Zn within root tissues, it was necessary to further increase tissue metal level to 5%, to reach the detection limits for SEM X-ray microanalysis. Thus, a four-day uptake experiment was undertaken following MacFarlane and Burchett (2000). Whole plant roots were placed in separate 500 ml beakers containing 4 g Zn L^{-1} in synthetic seawater (10‰ NaCl); synthetic seawater, alone, was used for controls ($n = 4$). All profiles were obtained using an accelerating voltage of 20 kV with a probe current ranging from 0.5 to 1.0 nA. A high accelerating voltage was chosen to minimize hydrated sample charging and to obtain a sufficient signal to noise ratio for X-ray line profiles.

2.5. Statistical analysis

Data on plant performances were tested for their normality and variance prior to one-way analysis of variance (ANOVA), and no transformation was needed. If the difference among plant species for each Zn treatment, or among different Zn treatments for each plant species, was significant at the 5% probability level, the least significant difference (LSD) was calculated as the post-hoc test to determine where the difference lay. The statistical analyses were performed using the SPSS 13.0 statistical packages. All figures were created using the PC-based Origin 6.1 program.

3. Results

3.1. ROL and root anatomy in three selected mangrove plants

ROL rates at root tips among all three seedlings were significantly higher than those in basal regions ($P < 0.05$), which indicated a barrier to ROL in root base. However, net reductions in ROL