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Distribution correlations of cadmium to calcium, phosphorus, sodium and chloridion in mangrove *Aegiceras corniculatum* root tissues

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ABSTRACT

Nutriment distributions might influence Cd distribution and Cd tolerance in mangrove plant roots. To demonstrate this, *Aegiceras corniculatum* was stressed by Cd, and the distributions of Cd, Ca, P, Na and Cl in plant roots were detected with the aid of SEM-EDX. It was found that endodermis, pith and xylem were the predominant tissues for retardation and regional enrichment of Cd. Na and Cl distributions suggest a critical role of salt resistance tissues on Cd tolerance in roots. P participated in Cd retardation and regional enrichment of endodermis and xylem. P, Na, Cl and Ca distribution had a high correlation to that of Cd in roots. The synergetic accumulation between Ca and Cd could be a crucial mechanism for Cd tolerance in *A. corniculatum* roots. In conclusion, the research of Cd and nutriment distributions in *A. corniculatum* roots deepens the understanding on Cd tolerance in mangrove plants.

1. Introduction

As critical ecosystems located in coastal wetland of tropics and subtropics, mangroves play significant roles in maintaining coastal ecological balance. Mangroves can function as filtration and precipitation to the sewage discharged into sea due to their specific traits and locations of junction between sea and land. However, in the wake of development of modern industry and agriculture, increasing contaminants are poured into soil and water bodies causing heavy metals pollution aggravation. The filtration and precipitation to the sewage make mangroves the “sink” of heavy metals, and excess accumulation of contaminants seriously threaten the health of mangrove ecosystems (J. Li et al., 2016). As a pioneer mangrove species in the southeast of China, *A. corniculatum* with great tolerance to heavy metal pollutants distributes widely along coastal and estuarine areas in China (Jiang et al., 2017). Knowledge on mangrove protection and restoration would get enhancement through research on the distribution correlations between heavy metals and nutrient elements in the anatomical structure of *A. corniculatum* roots.

Mangroves are distributed in estuarine and coastal wetlands with waterlogged saline sediments. Halophytes are resistant to salt and heavy metals and this resistance partly relies on common physiochemical mechanisms (Sruthi et al., 2017). In the recretohalophyte *Avicennia marina*, one species of mangrove plants, salt filtration through roots is the most important salt-rejecting mechanism. The salts are carried towards root surface by transpiration stream, among which 80% are hindered into the stem. Then the remaining salts enter the root xylem and reach the leaves (Waisel et al., 2010). Salts, particularly NaCl, enhance Cd accumulation and affect several physiological and biochemical processes, such as plasma membrane permeability and transpiration rate in plants (Mei et al., 2014). This effect is closely related to the uptake and translocation and tolerance of Cd. Such as in *A. marina*, Cd and Pb are excreted through salt glands as a possible metal detoxification mechanism (Sruthi et al., 2017).

Phosphorus (P) is an essential macroelement for plant growth. It could mitigate the toxicity from heavy metals both in soil environments and the plants (Madhavi et al., 2006; Barka et al., 2012). P as the essential element not only dilutes heavy metals content in plants by promoting biomass increase, but also decreases the toxicity by disturbing the absorption and bioavailability of heavy metals (Karblane, 1994). Furthermore, P could develop into insoluble phosphate with heavy metals via chelation or complexation in plants (Dai et al., 2017), and this reduces the damage of heavy metals to cell functions, such as signal transduction, transmembrane transport and energy metabolism. For instance, in the cell walls of mangrove plant *A. marina*, heavy metal Zinc (Zn) precipitates with phosphate by chelation and complexation,

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and this process reduces the concentration of free Zn^{2+} in protoplast and alleviates Zn toxicity to plant metabolism (Macfarlane and Burchett, 1999).

Calcium (Ca) acts a crucial role in maintaining the physiological and biochemical process of cells. Under the stress condition, Ca ion concentration increases in the cells, and activates relevant ion channels, thus the selectivity to ion absorption gets improved in protoplast (Mendoza et al., 1994). In addition, Ca^{2+} channel is the main pathway for Cd into plants (Li et al., 2015). Ca absorption facilitates the competition and antagonistic effects between Ca and heavy metals and promotes antioxidase activity and plant tolerance to heavy metals (Li et al., 2006). Moreover, crystals containing calciate or oxalate form in the cell walls when plants are exposed to heavy metals; this inactivates heavy metals and enhances the tolerance of plants (Rauser and Ackerley, 1987; Pr et al., 2001).

Compared with Pb, Cr, etc. heavy metals, Cd possesses the highest biotoxicity (Basic et al., 2006), and even low concentration of Cd can lead to intense ecological effect (Schützendübel and Polle, 2002). In systems of soil and plant, Cd owns high mobility and concentrates in plant tissues greatly. Absorbed and accumulated in plants, Cd could restrain plant growth, cell division and metabolism, thus affects genetic expression (Kovalchuk et al., 2005), inhibits DNA replication (Banerjee and Floresrozas, 2005), reduces photosynthesis and decreases the uptake of water and nutrients in plant directly (Kahle, 1993). Furthermore, Cd can disturb enzyme activity due to its high affinity with the sulfydryl in protein and other organic compounds (Braude et al., 2010). In ecosystems, Cd gets enriched from primary producers to consumers and the organisms maintain high Cd vestigial in vivo through food chain or food web. As a result, Cd not merely threatens plant development but also becomes a human health hazard (Veltman et al., 2008; Li et al., 2017). Taken together, Cd pollution has become an urgent problem and aroused wide concern among research workers.

In the current research, the hypothesis was presented that nutriment distributions might influence Cd distribution in the roots of mangrove plant *A. corniculatum* and influence this species tolerance to Cd. To demonstrate this, distribution traits of Ca, P, Na, Cl were analyzed in *A. corniculatum* roots under the stressed condition of Cd to 1) reveal the distribution correlation between Ca, P, Na, Cl and Cd in the tissues of this species root, and 2) explore the potential alleviation mechanism of these elements to Cd pollution.

2. Materials and methods

2.1. Samples culture

A. corniculatum propagules were collected from Jiulong River Mangrove Natural Reserve (24°24′ N, 117°55′ E), Zhangzhou City, Fujian Province, China. Complete undamaged propagules of high vitality were chosen for pre-cultivation in sea sand containing Hoagland nutrient solution (Li et al., 2015). Sea sand was pre-washed with concentrated HCl and rinsed thoroughly with tap water (Liu et al., 2009). The Hoagland nutrient solution contained 15‰ NaCl (adjusted pH to 7.0 ± 0.1). Pre-cultivation duration was one year, and nutrient solution with 15‰ NaCl was renewed every one week. After pre-cultivation, comparable size seedlings were chosen for the following treatment. One *A. corniculatum* seedling was inserted into a 2 l polyethylene seedling pot filled with Hoagland nutrient solution with 4 g/l Cd (used $CdCl_2$) and 15‰ NaCl (adjusted pH to 7.0 ± 0.1). The seedlings were stressed in the polyethylene seedling pots for 4 days with five replicates.

2.2. Determination method and instrument

The Scanning Electron Microscopy (SM-6390LV, Japan) and Energy Dispersive X-ray Detector (JED-2300EDS, Japan) were used in this research (SEM-EDX). Root tissue element levels were detected with the method of MacFarlane and Burchett (2000) with slight modifications. In order to investigate more details of the Cd distribution within root tissues, Cd concentration was increased further to 4 g/l to ensure tissue metal levels would exceed the detection limit for SEM X-ray microanalysis. After 4-d Cd treatment the *A. corniculatum* roots were gathered. Next the root samples were fixed in the 2.5% glutaraldehyde phosphate buffer precooled in 4 °C and then kept in darkness for 24 h. After fixation, the root samples were washed out for 2–3 times with phosphate buffer precooled in 4 °C. Then the dehydration processes were undertaken by 30%, 50%, 70%, 90%, 95% and 100% ethanol respectively. After that, ethanol was replaced by tertiary butanol and the samples were dried in a vacuum freeze drier (JFD-310). Then conducting layer was formed by metal spraying on the dried root samples. Running conditions for the instrument were as follows: accelerating voltage of 30 kV, working distance of 20 mm, beam diameter of 10 mm, X-ray radiological diameter of 1 μm and depth of 1 μm. A high accelerating voltage was chosen to minimize sample charging and to obtain a sufficient signal to noise ratio for X-ray line profiles (MacFarlane and Burchett, 2000).

2.3. Statistical analysis

All statistical analyses were performed by the aid of SPSS statistical software version 19.0 (Armonk, NY: IBM Corp.). Differences of element intensities among tissues were determined using a one-way ANOVA with Tukey's honestly significant difference test among treatment means (MacFarlane and Burchett, 2000). Two-sided test of Pearson correlation were applied in the correlation analysis of the element distributions in plant roots.

3. Results and discussion

3.1. The distribution traits of Cd in A. corniculatum root tissues

Through the analysis of SEM-EDX, Cd was detected from the epidermis and exodermis, the cortex, the endodermis of the xylem, the phloem and the pith, and this might be a result of radial transport across these tissues (Fig. 1). It was found that Cd was mainly accumulated in the endodermis, pith and xylem tissues. Besides, the cortex and phloem contained the least Cd. While the greatest Cd content was detected in the endodermis and was significantly higher than other tissues ($p < 0.05$). Following endodermis, the root pith contained higher Cd than the epidermis and exodermis, the cortex and the phloem ($p < 0.05$), but it showed no significant difference with the xylem

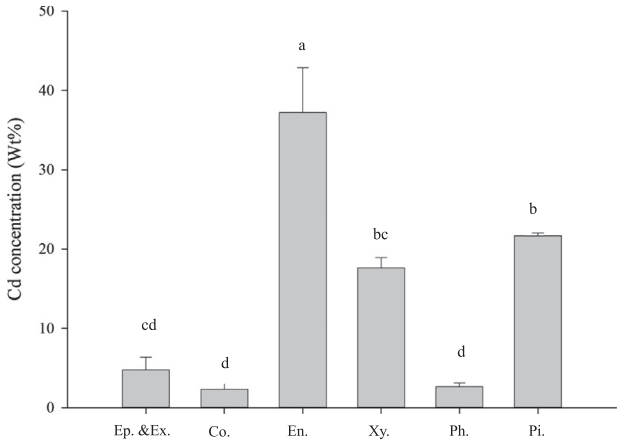

Fig. 1. Distribution of Cd in the root tissues of *A. corniculatum* under Cd stress. Values with the same letters are equivalent and different letters denote significant differences ($p < 0.05$) based on one-way ANOVAs. Note: Ep.: epidermis; Ex: exodermis; Co.: cortex; En.: endodermis; Xy.: xylem; Ph.: phloem; Pi.: pith.