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Tolerance mechanism of *Triarrhena sacchariflora* (Maxim.) Nakai. seedlings to lead and cadmium: Translocation, subcellular distribution, chemical forms and variations in leaf ultrastructure

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ABSTRACT

Hydroponic experiments were conducted to assess the accumulation, translocation, and chemical forms of lead (Pb) and cadmium (Cd) in the roots, stems, and leaves of *Triarrhena sacchariflora* seedlings and the associated variation in leaf ultrastructure. The leaves and leaf ultrastructure showed no significant symptoms of toxicity with 0.05 mM Pb or 0.01 mM Cd exposure for 10d. Chlorosis and wilting were observed in leaves when the Pb and Cd concentration was higher than 0.1 and 0.05 mM in the medium, respectively, as demonstrated by severe ultrastructural modifications at higher concentration in the leaves, such as plasmolysis, cell wall detachment, chloroplast swelling, nuclear condensation, and even nuclear fragmentation. The Pb and Cd concentrations in the roots was significantly higher than those in the stems and leaves. This indicated low Pb and Cd translocation from the roots to the aboveground parts. Subcellular distribution analysis showed that the majority of Pb and Cd was bound to the cell wall, especially in the roots, indicating that the cell wall likely constitutes a crucial storage site for Pb and Cd. This mechanism decreases the translocation of Pb and Cd across membranes and is more effective than vacuolar compartmentation. The majority of Pb and Cd exited in form of insoluble Pb/Cd-pectate or -oxalate complexes in the plant. In conclusion, higher concentrations of Pb or Cd induced premature senescence. High Pb and Cd enrichment was observed in the roots, which decreased the translocation of Pb and Cd from the roots to the aboveground tissues. The immobilization of Pb or Cd by the cell wall is important for plant detoxification and can protect protoplasts from Pb or Cd toxicity. Pb and Cd mainly existed in insoluble Pb/Cd-phosphate or -oxalate complexes, exhibiting low activity and thereby limiting symplastic transport and suppressing toxicity.

1. Introduction

Lead (Pb) and cadmium (Cd) are widely spread and common non-nutritive heavy metal (HM) pollutants in aquatic ecosystems. Aquatic plants inescapably suffer from HM toxicity due to its strong biotoxicity, resistance to degradation, and chemical activity. Previous studies have demonstrated that Pb and/or Cd can disturb chlorophyll biosynthesis, damage chloroplast structures, and also decrease critical enzyme activities in the Calvin Cycle, such as RuBPcase and 3-PGA kinase, thereby interfering with photosynthesis and consequently resulting in plant growth inhibition or even death (Seregin et al., 1997; Han et al., 2006; Singh and Prasad, 2014; Deng et al., 2014; Dezhban et al., 2015). Meanwhile, Pb and/or Cd at higher concentrations will induce the generation of reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals (·OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), which otherwise should have been controlled at normal levels (Farooq et al., 2016; Li et al., 2017; Deng et al., 2017). Pb and/or Cd can cause excessive ROS occurrence, leading to oxidative stress, including membrane lipid peroxidation (Biteur et al., 2011; Singh and Prasad, 2014), enzyme activity inhibition (Singh et al., 2008), and DNA damage (Siddiqui, 2015), thereby disturbing the physiological and biochemical processes in plant cells. Additionally, Pb and Cd can lead to an imbalance in water and ionic metabolization in the guard cells, resulting in plants becoming dehydrated and wilted, and can also inhibit root growth by decreasing the absorption of mineral nutrients (Muradoglu et al., 2015; Alemayehu et al., 2015). Most importantly, Pb and/or Cd constitute a danger to the health of humans and other organisms through their accumulation in the food chain and biological amplification (Sugiyama, 1994; Grant et al., 2008; Lim et al., 2016).

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The absorption and accumulation of HMs at higher concentrations in plants will result in ultrastructural alterations, including plasmolysis, chromatin condensation, plasmodesmata fragmentation (Xu et al., 2013; Li et al., 2016), chloroplast swelling, decreased cristae number, intercellular space constriction (Farooq et al., 2016; Baruah et al., 2017), or even cell death (Souza Vânia et al., 2011; Cadelas et al., 2012). Several defense mechanisms, such as HM exclusion, cell wall binding, organic molecular chelation, and vacuolar compartmentalization, are involved in HM tolerance and detoxification in plants. The binding of HM to the cell wall and compartmentalization in vacuoles could be identified as a morphophysiological mechanism for detoxification (Seregin and Ivanov, 1997; Sharma et al., 2016), which also plays a significant role in HM accumulation. Cd is mainly sequestrated in the root cell wall in *Nasturtium officinale* (Wang et al., 2015a, 2015b, 2015c) and *Ipomoea aquatica* (Xin et al., 2013, 2014), where Cd can bind to pectin, polysaccharides, and proteins (Wojcik et al., 2005). The vacuoles contain the highest amount of Cd in the leaves of *Thlaspi caerulescens* (Ma et al., 2005), *Sedum plumbizincicola* (Cao et al., 2014), and *Arenaria orbiculata* (Zu et al., 2015), where the Cd binds to proteins, organic acids, and alkalis (Krämer, 2010), thereby protecting metabolically active tissues from toxicity (Zhang et al., 2014). Meanwhile, the different chemical forms of HMs are also key factors for tolerance in plants, and influence HM migration and accumulation across the entire plant. Different chemical forms of Pb and Cd can be gradually extracted by ethanol, distilled water, sodium chloride (NaCl), acetic acid (HAc), and hydrochloric acid (HCl), and exhibit different toxicities and migratory patterns in plants. The chemical forms of Cd in the roots of *Athyrium wardii* (Zhang et al., 2014) and soybean (Wang et al., 2015a, 2015b, 2015c) were principally HAc- and NaCl-extractable forms, while HAc- and HCl-extractable Pb were the dominant chemical forms in *Arenaria orbiculata* (Zu et al., 2015).

To date, Pb and/or Cd contamination in some large-scale rivers and major lakes have exceeded the V class (0.24×10^{-3} mM ≤ Pb ≤ 0.48×10^{-3} mM, 5×10^{-3} mM ≤ Cd ≤ 10×10^{-3} mM) of the Environmental Quality Standard for Surface Water (Ministry of Environmental Protection of the People's Republic of China and General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2002). The Pb and Cd concentrations were reported to range from 18.3 to 44.1 mg kg^{-1} and 0.12–0.75 mg kg^{-1} in the surface sediments of the Yangtze River intertidal zone (Zhang et al., 2009); from 42.8 to 143 mg kg^{-1} and 0.28–1.23 mg kg^{-1} (Qu et al., 2001) in Taihu Lake; and from 1.25 to 29.69 mg $kg^{-1}$and 0.05–0.54 mg kg^{-1} (Luo et al., 2008) in the branch sediments of Poyang Lake, respectively. And, more remarkably, soil Pb and/or Cd contamination in some mines is even worse, and has exceeded the II class (25 mg L^{-1} ≤ Pb ≤ 300 mg kg^{-1}, 0.2 mg kg^{-1} ≤ Cd ≤ 0.3 mg kg^{-1}) of the Environmental Quality Standard for Soils (Ministry of Environmental Protection of the People's Republic of China, General Administration of Quality Supervision and Inspection and Quarantine of the People's Republic of China, 1995). Similar environmental contamination has also been found in some areas in other countries, such as Korea (Myung, 2001; Li et al., 2016), Japan (Arao et al., 2010; Yuri et al., 2015), and Russia (Mandzhieva et al., 2016; Galitskaya et al., 2017). HM contamination not only causes significant financial losses, but is also seriously toxic to animals, plants, and humans (Park et al., 2014; Sato et al., 2016). Unsurprisingly, increasing attention has been paid to the remediation of HM contamination. Furthermore, various techniques, mainly physical, chemical, and biological methods, have been employed to remediate soil and/or water contaminated by HMs. As an *in situ* remediation strategy, phytoremediation, which has the advantages of being low in cost, environmentally friendly, and with easily controllable secondary pollution, constitutes one type of bioremediation that is popular in HM contamination processes.

China is the world distribution center of plants in the genus *Triarrhena*, which originated in northeast, north, and northwest, as well as eastern China, and is also distributed in Korea, Japan, and Russia. *Triarrhena saccharifloral* (Maxim.) Nakai, an emergent aquatic herb in the Poaceae family and a C4 plant with high photosynthetic efficiency, is usually associated with *Phragmites communis* growing on grassy hillsides, plains, riparian wetlands, rivers, and lakes, and is regarded as an environmentally friendly plant with ornamental and economic value ascribed to its fast growth, large biomass, strong propagation, beautiful inflorescence, and extensive adaptability as well as developed root system. These traits make it useful in landscape construction, environmental protection, water conservation, soil amelioration, and bioenergy, as well as a substitution for wood, plastic products, and textiles. We previously reported that *T. saccharifloral* exhibited strong HM accumulation and constitutes an HM-tolerant plant (Tian et al., 2013). However, the tolerance mechanisms of this plant to HMs were not fully assessed. The main objectives of the present study were thus to (1) identify the translocation characteristics of Pb and Cd from the roots to the aboveground parts of *T. saccharifloral*, (2) evaluate the subcellular distribution of Pb and Cd, (3) investigate the chemical forms of Pb and Cd in the plant, and (4) determine the ultrastructural changes induced by Pb and Cd using histological sectioning and transmission electron microscopy (TEM) in order to understand its tolerance mechanisms to Pb and Cd, and to also provide a theoretical basis for the restoration of Pb- and/or Cd-contaminated environments.

2. Materials and methods

2.1. Plant material

Seeds of *T. saccharifloral* were collected from a tree farm in Dafeng, Yancheng, Jiangsu (N33°03', E120°44'). Healthy, uniform seeds were surface sterilized in 1% sodium hypochlorite for 10 min after soaking in deionized water for 48 h, and then rinsed repeatedly with tap water and the surface dried using filter paper. The seeds were evenly grown in plastic crates (50 × 33 × 26 cm) filled with sterilized moor soil, and then cultivated in the greenhouse at the Nanjing Forestry University, Nanjing, Jiangsu (32°04′ N,118°78′ E). When the seedlings had grown 4–5 leaves, uniform plants were selected and transported to flasks containing 500 mL $^1/_2$ Hoagland's nutrient solution for cultivation for 20 d.

The seedlings were rinsed with tap water and then transported to flasks containing the nutrient solution and Pb^{2+} (0, 0.05, 0.1, 0.25, 0.5 and 1 mM) supplied in the form of Pb $(NO_3)_2$, and Cd^{2+} (0, 0.01, 0.05, 0.1, 0.2 and 0.5 mM) supplied in the form of $CdCl_2{\cdot}2.5H_2O$. Each flask was filled with 500 mL Pb^{2+} or Cd^{2+} solution before uniform seedlings were placed into the flasks. The Pb^{2+} concentrations in the solution were 100, 200, 500, and 1000 times the V class of the Environmental Quality Standard for Surface Water (GB3838-2002), and the Cd^{2+} concentrations were 100, 500, 1000, 2000, and 5000 times the II class of the Environmental Quality Standard for Soils (GB15618-1995). Each treatment contained 15 seedlings and was carried out in triplicate. The nutrient solution was renewed every 5 d, and the water was replenished in the solution and the pH adjusted to 6.0–7.0 every other day. The plants were harvested after being exposed to Pb and Cd for 10 d.

2.2. Ultrastructural observations

Leaf samples from the top of the plants treated with Pb and Cd were initially removed the main veins and than fixed in 3% glutaraldehyde, and then post-fixed in osmic acid. Fixed samples were dehydrated in an ethanol series of increasing concentration until 100%, and ultimately embedded in Epon812 epoxy resin. Ultrathin sections were sliced using an ultratome (LKB, France) and were then observed using TEM (Hitachi7650, Japan) after being stained in uranyl acetate and lead citrate.

2.3. Separation of subcellular fractions

The subcellular fractions in the fresh samples of the roots, stems, and leaves were separated according to the method of Weigel and Jager (1980) and Gabbrielli et al. (1990), with minor modifications. Each sample was homogenized in precooled extraction buffer (250 mM sucrose, 1 mM Dithioerythritol, and 50 mM Tris-HCl, pH 7.5) with chilled mortars and pestles. The homogenate was centrifuged (Sigma 4K15, Germany) at 300