Ecotoxicology and Environmental Safety 139 (2017) 272–279

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Phosphorus mediation of cadmium stress in two mangrove seedlings *Avicennia marina* and *Kandelia obovata* differing in cadmium accumulation

Minyue Dai[a], Haoliang Lu[a], Wenwen Liu[a], Hui Jia[a], Hualong Hong[a], Jingchun Liu[a], Chongling Yan[a,b,*]

[a] Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361102, China
[b] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361102, China

ARTICLE INFO

Keywords:
Phosphorus
Cadmium
Avicennia marina
Kandelia obovata
Detoxification mechanism
Adaptive strategies

ABSTRACT

Mangrove ecosystems are vulnerable to environmental threats. In order to elucidate the effect of phosphorus (P) on cadmium (Cd) tolerance and physiological responses in mangroves under Cd stress, a mangrove specie with salt exclusion *Kandelia obovata* and a specie with salt secretion *Avicennia marina* were compared in a hydroponic experiment. The results showed that most Cd was accumulated in mangrove roots and that P addition induced Cd immobilisation in them. Cd stress significantly increased malonaldehyde content, whereas P significantly decreased malonaldehyde in mangroves. Phosphorus positively regulated the photosynthetic pigment, proline content and synthesis of non-protein thiols, glutathione and phytochelatins in the leaves under Cd stress conditions. The results suggest different adaptive strategies adopted by two mangroves in a complex environment and *A. marina* showed a stronger Cd tolerance than *K. obovata*. The study provides a theoretical basis for P mediated detoxification of Cd in mangrove plants.

1. Introduction

Mangrove forests are transitional coastal ecosystems distributed at subtropical and tropical coastal regions (Woodroffe et al., 2016). They have a variety of ecological worth with nearly 70 species of mangroves, in 27 genera and 20 families around the world (Lin, 1999). However in recent years, the world's mangrove forests have become a threatened ecosystem, which have degenerated and disappeared between 0.16% and 0.39% per year since 2000 (Hamilton and Casey, 2016). The relevant studies have shown that heavy metal pollution is an important cause of mangrove ecosystem degradation (Zhang et al., 2014), which has received extensive attention and become a pressing research topic nowadays (Lewis et al., 2011; Cabral et al., 2016).

Cadmium (Cd) frequently appears in the environment and is considered as a toxic element to plants, which causes stunted growth (Gogorcena et al., 2011), impairs photosynthesis (Gill et al., 2012), and leads to plant nutrient absorption imbalances (Gallego et al., 2012). Cd can also alter water status (Zouari et al., 2016), damage membrane structure and induce oxidative damage (Anjum et al., 2015). At the same time, plants can use different strategies to alleviate the harm by Cd. For example, proline (Pro) can improve the antioxidant effect and counteract Cd inhibitory effects (Zouari et al., 2016). Under Cd stress, glutathione (GSH) in plants can remove ROS and be the precursor of phytochelatins synthesis (Jozefczak et al., 2015). In addition, phytochelatins are the most suitable heavy metal chelating agent, especially in the presence of Cd (Greger et al., 2016).

Phosphorus (P) is one of the indispensable elements to biological growth which is taken up by plants in the form of $H_2PO_4^-$ or HPO_4^{2-} (Shen et al., 2011). In addition, P is the component of many important compounds (e.g. nucleic acid, nucleoprotein, phospholipids and some enzymes) in plants, which participate in carbohydrate metabolism, lipometabolism and nitrogen metabolism (Blank, 2012; Gupta et al., 2014). Large quantities of P could improve plant resistance and the ability to adapt to external environmental conditions, promote the formation, development and growth of plant tissues (Wang et al., 2010). In addition, P can also affect the accumulation of heavy metals (Shi et al., 2015).

However, in plants the co-existence of Cd and P is quite complicated and shows synergistic or antagonistic effects. Yu and Zhou (2009) found sufficient amounts of P can improve plant growth and decrease Cd accumulation. Qiu et al. (2011) reported that P supplements could fix more Cd to the cell wall, thus inhibiting the absorption of Cd. Yin et al. (2016) found P may change the chemical forms of Cd, thus affecting the bioavailability of spinach (*Spinacia oleracea* L.) cultivars. In contrast, some studies showed that the increase of P promoted the absorption of Cd in mangrove plants (Du et al., 2014). However, the exact physio-

* Corresponding author at: Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361102, China.
E-mail address: ycl@xmu.edu.cn (C. Yan).

http://dx.doi.org/10.1016/j.ecoenv.2017.01.017
Received 24 August 2016; Received in revised form 5 January 2017; Accepted 6 January 2017
Available online 07 February 2017

logical mechanism underlying Cd stress tolerance in response to P supply remains elusive.

Mangroves have higher productivity at the land-sea interface and play an important role in biogeochemical cycles (Ward et al., 2016). Different mangroves have different salt tolerance mechanism which associate with heavy metal regulation (MacFarlane et al., 2007). *Avicennia marina* (Forsk.) Vierh and *Kandelia obovata* (S., L.) Yong are common mangroves with differences in salt balance strategy. *K. obovate* is one of a species of salt exclusion mangroves, while *A. marina* is a salt secretion species. In order to elucidate how P mediates Cd tolerance and the involved physiological mechanism, we hypothesize that P is considered a crucial factor that influences the Cd tolerance mechanism within the plant tissues and, therefore, the extent of Cd accumulation in the salt balance strategy of mangroves.

2. Materials and methods

2.1. Plant materials and culture conditions

Mature *A. marina* and *K. obovata* propagules used in the experiment were obtained from a Natural Mangrove Reserve (23°53′45″~23°56′00″N, 117°24′07″~117°30′00″E) in the Zhangjiang estuary, Fujian, China. Propagules were disinfected with 10‰ $KMnO_4$, followed by washing with distilled water. Complete undamaged and high vitality propagules were chosen for planting. These propagules were randomly placed in an experimental green house and irrigated with Hoagland nutrient solution with 10‰ NaCl every 3 days. After the second leaf had been fully developed, seedlings were removed from the sand and washed carefully to remove any residual sand. Seedlings of consistent size and growth were selected and transplanted into 2.0 L black plastic pots, then cultured in Hoagland nutrient solution with 10‰ NaCl.

2.2. P and Cd treatments of seedlings in hydroponic culture

After adapting to the nutrient solution for two weeks, a hydroponic experiment was conducted to explore the interaction between P and Cd on the two mangrove seedlings. Nine different treatments were performed for each plant species. Three levels of Cd (as $CdCl_2{\cdot}2.5H_2O$, guaranteed reagent, 99.9% purity) (0, 0.5, 5 mg kg^{-1} Cd) was added to the nutrient solution with 10‰ NaCl combined with three levels of P (as KH_2PO_4, guaranteed reagent, 99.9% purity) (0, 30, 90 mg kg^{-1} P) indicated by Cd0, Cd0.5, Cd5 and P0, P30, P90, respectively. Solution pH was adjusted to 6.5 with 1 M HCl or 1 M NaOH. There were three plants in each pot and three replicates were made of each treatment. The pots were completely randomly arranged in the experimental green house (60–80% relative humidity, 25 ± 5 °C temperature and 12 h light/dark at 800–1400 μmol photons m^{-2} s^{-1}) (Li et al., 2015). The hydroponic medium was renewed every 3 days. After 30 days of treatments, plant samples were harvested for further analyses.

2.3. Determination of Cd and P concentration

After harvest, the plants were thoroughly washed in distilled water and separated into roots, stems and leaves, then dried for 20 min at 105 °C and then at 70 °C in an oven to constant weights. For analysis of total Cd concentration, dried plant material was powdered and digested in HNO_3 and H_2O_2 then diluted to 30 mL with ultrapure water and filtered on a 0.45 μm nylon membrane. Cd concentration was determined by inductively coupled plasma mass spectrometry (Agilent 7500cx). Certified reference material (CRM) of plant GBW-07603 (provided by National Research Centre for Standards, China) was used for quality assurance and quality control (QA/QC) in the experimental process. The recovery rate was 92–101%. Translocation factor (TF) of root systems and aerial parts (stems + leaves) was calculated as: TF = cadmium concentration in the aerial parts (mg kg^{-1})/cadmium concentration in the roots (mg kg^{-1}) (Zacchini et al., 2009). Total P in plants tissues was determined after digestion with H_2SO_4 and H_2O_2 using the method of the molybdenum blue (Lu, 1999). The increase in plant height was recorded during the experiment.

2.4. Assays of lipid peroxide, proline and photosynthetic pigments

The level of lipid peroxidation in leaf samples was expressed as malondialdehyde (MDA) content according to the method of Heath and Packer (1968) with some modifications. Briefly, fresh leaves were homogenized and extracted in 10% (w/v) trichloroacetic acid (TCA). After centrifuging, 2 mL 0.5% thiobarbituric acid (TBA) was added into 2 mL supernatant. This was boiled for 20 min followed by quick-cooling. The tubes were centrifuged and the absorbance of the resulting supernatant was measured at 532, 600 and 450 nm wavelengths.

Proline content was determined by the method described by Bates et al. (1973) with some modifications. Fresh leaves were extracted with 3% (w/v) sulphosalicylic acid and boiled for 10 min. After the samples were cooled and filtered, the reaction mixture contained supernatant, glacial acetic acid and ninhydrin reagent (2 mL each respectively). This was then boiled for 30 min. Toluene was added into the mixture, and the organic phase was quantified by using an ultraviolet spectrophotometer at 520 nm.

Chlorophyll and carotenoids were calculated using the formula of Lichtenthaler and Wellburn (1983). Fresh leaves were cut into pieces with small amounts of quartz and calcium carbonate added, homogenized in 95% ethanol until the tissue became white. The homogenate was filtered and diluted to 25 mL in a volumetric flask with deionized water. The absorbance of the mixture was measured spectrophotometrically at 470, 649 and 665 nm.

2.5. Determination of glutathione (GSH), non-protein thiols (NPT) and phytochelatins (PCs) contents

Glutathione content was measured following the procedures by Guri (1983) with some modifications. In brief, fresh plant leaf was ground in EDTA-TCA, then diluted to 25 mL in a volumetric flask with EDTA-TCA. 1 M NaOH was added to 2 mL filtrate and pH was adjusted to 6.5–7.0, then 0.1 mL potassium phosphate buffer and 0.1 mL DTNB were added. The absorbance was measured at 412 nm.

Non-protein thiols content was measured using the methods described by Del Longo et al. (1993). Fresh leaf was ground in 5% (w/v) sulfosalicylic acid and stored in a cryogenic tank for 30 min. The homogenate was centrifuged at 8000*g* for 15 min. The reaction mixture now containing 200 μL supernatant, 2 mL 0.2 M Tris-HCl pH 8.2 and 0.15 mL 10 mM 5, 5′-dithiobis (DTNB) was incubated for 30 min. After incubation, the absorbance was measured at 412 nm.

The content of PCs was assayed by subtracting the amount of total GSH from the amount of total non-protein in the SH compounds (Bhargava et al., 2005).

2.6. Statistical analysis

All statistical analyses between the different treatments were evaluated at 0.05 probability level using two-way analysis of variance (ANOVA) followed by a Tukey HSD with JMP 10.0 statistical software (SAS Institute 2012). The relationships between variables were determined via correlation analysis. All graphs were plotted using SigmaPlot (version 10.0). A log transformation was used in order to visualize data of Cd concentration (Fig. 2).

3. Results

3.1. Plant growth and element uptake by different mangrove seedlings

The plant height increment in the test mangroves treated with P or without P under the Cd stress are shown in Fig. 1. Cd treatments