Environmental Pollution 162 (2012) 369–373

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

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

Effects of silicon on the distribution of cadmium compartmentation in root tips of *Kandelia obovata* (S., L.) Yong

Juan Ye, Chongling Yan*, Jingchun Liu, Haoliang Lu, Tao Liu, Zengfeng Song

Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, and School of Life Sciences, Xiamen University, 422# South Siming Road, Xiamen, 361005 Fujian Province, China

ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form
16 October 2011
Accepted 1 December 2011

Keywords:
Silicon
Kandelia obovata (S., L.) Yong
Cadmium
Cell wall
Compartmentation

ABSTRACT

The Effects of silicon (Si) on the distribution of cadmium (Cd) compartmentation in root tips of *Kandelia obovata* (S., L.) Yong were investigated by pot experiments. Cd concentrations in the apoplastic saps and symplastic fractions of the root tips of *K. obovata* seedlings were decreased at both Si-supplied treatments. Si addition reduced the concentrations of $BaCl_2$-extractable cell-wall-Cd in root tips, but increased the concentrations of Na_3citrate-extractable cell-wall-Cd and HCl-extractable cell-wall-Cd in root tips. The total root-tip contents of Cd were mainly distributed in the apoplast and most of the Cd in the apoplast was bound to the cell wall. Our experiment found that Si increased the ratio of apoplast Cd (>87.08%) and reduced the ratio of Cd in the symplast (<12.92%). This suggested that Si enhanced binding of Cd to the cell walls and restricted the apoplastic transport of Cd.

1. Introduction

Mangrove forests are diverse communities distributed in the inter-tidal zones of tropical to subtropical coastal rivers, estuaries and bays. The sediments in such areas have a large capacity to retain heavy metals from tidal waters, fresh water rivers and storm water runoff, and they often act as sinks for heavy metals (Tam and Wong, 2000; Zhou et al., 2010). Despite their potential exposure to metal contaminated sediments, mangroves seem to possess a great tolerance to high levels of heavy metal pollution (Joshi et al., 2007; MacFarlane et al., 2007). The mangrove plants have developed various mechanisms such as exclusion, chelation, compartmentalization and sequestration for heavy-metal tolerance. These mechanisms decrease heavy metal uptake and accumulation in mangroves from the environment, restrict translocation of heavy metals from roots to shoots, and thus improve the survival rate of mangroves in the polluted habitats. The tolerance of mangroves to high concentrations of heavy metals may be due to metal combination with sulfides in the roots or on their surfaces (Walsh et al., 1979; Lacerda et al., 1993) or formation of Fe-plaques and Mn-plaques on root surfaces (Che, 1999; Machado et al., 2005). One of the detoxic mechanisms for *Avicennia marina* (Forsk.) Vierh preventing normal metabolism of the plants from interference of metal ions is through metals bound to polygalacturonic acids and carbohydrates of the cell wall decreasing metal ion concentrations in the cytoplasm (MacFarlane and Burchett, 2000). Mangrove tolerance to toxicity of heavy metals appears to be in conjunction with salt-tolerant mechanisms, heavy metals being excreted through salt glands on the adaxial surface of leaf tissue (MacFarlane and Burchett, 1999). However, the mechanisms involved and environmental impact factors are still only partially understood.

Si is the second most abundant element in both the surface of the Earth's crust and in the soils. Although Si has not been considered as an essential element for higher plants, it has been proved to be beneficial for the healthy growth and development of many plant species (Ma et al., 2001; Ma and Yamaji, 2008). One of these benefits is to enhance tolerance of some plant species to toxic metals, including manganese (Mn) (Rogalla and Romheld, 2002; Shi et al., 2005a), aluminum (Al) (Wang et al., 2004; Prabagar et al., 2011) and zinc (Zn) (Neumann and zur Nieden, 2001). Similarly, Si was described as an effective substance for alleviation of Cd toxicity in some plants (Shi et al., 2005b; Nwugo and Huerta, 2008; Zhang et al., 2008).

The relationship between Si and tolerance to heavy metals in plants has been widely studied. Previous studies have suggested that there are many strategies by which Si affects heavy metal tolerance in plants. Kidd et al. (2001) suggested that an enhanced exudation of phenolic compounds leading to complexation and

* Corresponding author.
E-mail addresses: yejuan168121@163.com (J. Ye), ycl@xmu.edu.cn (C. Yan), liujingchun@xmu.edu.cn (J. Liu), luhl@xmu.edu.cn (H. Lu), liutao19860907@163.com (T. Liu), songzfeng1987@163.com (Z. Song).

0269-7491/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2011.12.002

thus detoxification of Al is responsible for the Si-mediated enhanced Al tolerance in maize. The coprecipitation of Si and metals in the cell wall of plants was regarded as a tolerance mechanism to cope with metal toxicity (Neumann and zur Nieden, 2001; Prabagar et al., 2011). The role of Si in tolerance of plants to metal stress has been attributed particularly to modification of cell wall properties. Shi et al. (2005b) and da Cunha and do Nascimento (2009) showed that Si might reduce the cell wall porosity of the endodermis and restrict the apoplastic transport of Cd. Horst et al. (1999) and Iwasaki et al. (2002a,b) showed that Si-enhanced tolerance of Mn in cowpea (*Vigna unguiculata*) is a consequence of the enhanced adsorption of Mn on the cell and a reduction of Mn concentrations in the apoplastic washing fluids.

Before reaching the coastal sea, riverine Si passes through estuaries which act as filters for land derived material (Humborg et al., 2003; Roubeix et al., 2008). The liberation experiments of superficial sediments in the Pearl River Estuary show that there is a logarithmic relationship between dissolved Si concentration and agitation time and there is a decreasing trend of silicon release with increasing pH (Qin and Weng, 2006). Mangrove wetlands are important types of ecosystems distributed in the tropical and subtropical coastal estuaries. Mangrove sediments are composed of fine particles with a high organic matter content and low pH (sulphide oxidation), and they are periodically agitated by tide (Peters et al., 1997; Marchand et al., 2004). So there may be a relatively high concentration of available Si both in mangrove forest sediments and mangrove tissues.

It has been well documented that Si can enhance tolerance of some plant species to heavy metals. However, the role of Si in cadmium (Cd) tolerance of mangrove plants and their involved mechanisms has not yet been reported. The tolerance mechanisms of mangroves to heavy metals and impact factors of detoxic process are still not comprehensively revealed. It is assumed that mangrove tolerance to heavy metals stress may be in relation to relatively high concentrations of Si both in mangrove forest sediments and mangrove tissues. The objective of this study was: to gain further information on the influence of Si-induced modification of the Cd binding properties on the cell walls; on the Cd concentration in the apoplast and the symplast; and on the distribution of Cd compartmentation. It was hoped that the study would provide the theoretical basis for an understanding of the tolerance mechanisms of mangroves to heavy metals and key factors of detoxic processes.

2. Materials and methods

2.1. Plant culture and experimental treatments

Mature *Kandelia obovata* propagules were collected from from the Jiulong River Estuary (24°29′N, 117°55′E), Fujian China, in April 2009. Only complete, undamaged propagules with testa intact and no emergent hypocotyl or radicles were selected for planting. The propagules selected were planted in vermiculite in the greenhouse. After 10 months, the seedlings were removed from the vermiculite and washed carefully under tap water to remove any adhering particles. Seedlings of uniform size were selected and transplanted into 2-L plastic pots (3 seedlings per pot) filled with Hoagland nutrient solution that was prepared using distilled water. Two weeks later, a $CdCl_2 \cdot 2.5H_2O$ solution was added to the growth medium to final concentrations of 0, 0.5 and 5.0 mg L^{-1}Cd and a $Na_2SiO_3 \cdot 9H_2O$ solution was added to final concentrations of 0, 70, and 100 mg L^{-1}Si. The nutrient solution was renewed once each week, and the pH was adjusted to 6.5 using 0.1 M NaOH or l M HCl. The treatments were arranged in a fully randomized design with three replicate pots (3 seedlings per pot) of each treatment. The seedlings were harvested to determine Cd concentrations in different fractions (apoplastic sap, symplastic sap and cell wall) of root tips when the old leaves of 0 mg L^{-1}Si treated plants became slightly yellow (20 days).

2.2. Fraction of Cd in root tips

2.2.1. Filtration of centrifugal forces

Malate dehydrogenase (MDH) is a cellular enzyme and at relatively low apoplastic concentrations, suitable as a cytoplasmic contamination marker (Yu et al., 1999; Lohaus et al., 2001). Cellular contamination of the apoplastic sap was quantified by comparing the activity of cytoplasmic marker enzymes (MDH) in the apoplastic sap with root tip extracts (Terry and Bonner, 1980; Yu et al., 1999). When using eight successive steps of centrifugation, the activities of MDH was determined (Fig. 1). The increase in MDH activity is steady from 500 to 4000 g and then sharply increases with increasing centrifugal force. This indicates that MDH is coming mostly from the cell wall with only a small amount of cytoplasmic contamination at low centrifugal forces. Above 4000 g, the plasmalemma no longer serves as a barrier to the contents of the cell. As the specific activity of MDH remains fairly constant from 500 to 4000 g and then increases, below 4000 g, much of the total protein released comes from the cell wall space. Above 4000 g, more protein, a greater portion of which is MDH, is released due to increased cell permeability and to the breakage of cells causing the specific activity to increase. The activity of MDH in apoplastic saps extracted from root segments of *K. obovata* seedlings at 4000 g was 2.1% of the total activity in root segment homogenates (Table 1), indicating a low degree of cytosolic contamination.

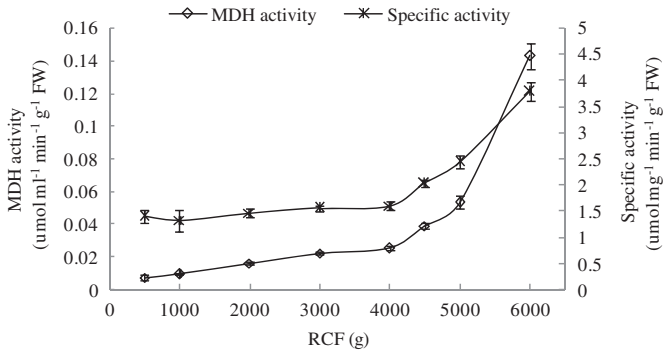

Fig. 1. Malate dehydrogenase (MDH) activity and specific activity detected in apoplastic sap extracted from root tips of *K. obovata* centrifuged at increasing relative centrifugal forces (RCF). Error bars indicate standard deviations ($n = 4$).

2.2.2. Extraction of apoplastic and symplastic sap

The apoplastic and symplastic saps of the root segments were collected by infiltration and centrifugation, according to the method described by Yu et al. (1999) with some modifications (Iwasaki et al., 2002a,b). Briefly, freshly excised 1 g, 2 cm root segments from root tips were arranged in 10-ml needle tubes with the cut ends facing down. The root segments were infiltrated twice with 50 mM MES-Tris buffer solution (pH 6.5) in a suction flask reducing the pressure to −3.5 kPa, followed by slow relaxation to atmospheric pressure over a 120 s period. Most of the surface water was drawn off by momentary suction applied to the tip of each needle tube. Then needle tubes were placed in 85 ml plastic centrifuge tubes (Nalgene, American) and centrifuged at 4000 g at 4 °C for 15 min. After collecting the apoplastic saps, the root segments were frozen at −20 °C. The symplastic1 fraction was recovered from the frozen-thawed samples by centrifugation at 4000 g at 4 °C for 15 min. The samples were homogenized in 1 mL of ethanol for 3 min. After centrifugation, the supernatant and pellet were separated, and washed again with ethanol. The combined two supernatants represents the symplastic2 fraction. The pellet consisted of the cell wall material (CW).

2.3. Extracting Cd from cell wall and residue

Cd was extracted from the isolated cell walls by a sequential procedure using solutions of 50 mM $BaCl_2$, pH 4.3, 33 mM Na_3citrate, pH 5.8, and HCl (0.5 mM) (Rogalla and Romheld, 2002; Wang et al., 2004). Every washing step was repeated once, each time for 15 min. Finally, the remaining residue was dry-ashed and dissolved in 6 M HCl with 15 g L^{-1}hydroxylammoniumchloride (Iwasaki et al., 2002a).

Table 1
The ratio of malate dehydrogenase activity in apoplastic sap extracted from tips of *K. obovata* centrifuged at increasing relative centrifugal forces (RCF).

RCF (g)	The ratio of MDH activity in apoplastic sap (%)
500	0.61
1000	0.80
2000	1.32
3000	1.84
4000	2.10
4500	3.21
5000	4.41
6000	11.79