Effects of silicon on growth, root anatomy, radial oxygen loss (ROL) and Fe/Mn plaque of Aegiceras corniculatum (L.) Blanco seedlings exposed to cadmium

Qiong Zhang¹,², Jingchun Liu¹, Haoliang Lu¹, Suzheng Zhao¹, Wenyun Wang¹, Jingna Du¹, Chongling Yan¹,²

¹ Key Laboratory of Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiang'an South Road, Xiangan District, Xiamen, 361102 Fujian Province, PR China
² School of Biological Science and Biotechnology, Minnan Normal University, Zhangzhou, 363000 Fujian Province, PR China

ABSTRACT

Biomass, root anatomy, the location of Cd, radial oxygen loss (ROL) and Fe/Mn plaque in Aegiceras corniculatum (L.) blanco were investigated under Si and Cd treatments. The results revealed that Si alleviated the inhibition of growth due to Cd stress. Furthermore, Si prompted the development of apoplastic barriers in roots under Cd stress. Promotion of the apoplastic barrier caused the reduction of ROL. The effect of Si on the formation of Fe/Mn plaque was contrary, but Si reduced the content of Fe plaque and increased the content of Mn plaque. However, the content of Cd on Fe/Mn plaque was significantly increased by Si, which could possibly block the absorption of Cd in the root from the growth media. The present study proposed new evidence of adaptive strategy on metal tolerance and the effect of Si on metal tolerance by A. corniculatum seedlings.

© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Mangrove ecosystems are distributed in tropical and subtropical coastal regions, where they play a key role in the ecological balance of estuaries and seashores. However, it has been reported that more metallic contaminants have been input into mangrove forests with the development of economies and populations. Research into response and tolerance mechanisms with respect to heavy metal in mangroves has been a major topic in the past three decades. Some research studies have suggested that the rhizosphere processes of mangrove plants can affect the bioavailability and mobility of heavy metals while limiting heavy-metal uptake (Lu et al., 2007; Zhou et al., 2011; Xie et al., 2012; Cheng et al., 2012a,b). For example, the radial oxygen loss (ROL) of the root induces Fe/Mn plaque formation on the root surface of mangrove (Pi et al., 2010). The Fe/Mn plaque can alter the forms of heavy metals in the rhizosphere through a series of physical and chemical process, which affect heavy-metal transfer. The root of mangrove can excrete low molecular weight organic acid, which plays a key role in reducing the input of heavy metals in mangrove plants (Xie et al., 2012).

Silicon is not listed among the higher plant essential elements, but the direct and indirect beneficial effects of Si on plant growth and development are well known. Many studies have reported that Si may be involved in metabolic or physiological and/or structural activity in higher plants that are exposed to abiotic and biotic stresses (Liang et al., 2003; Shen et al., 2010). It has been reported that Si increases some plant species’ tolerance to toxic metals such as manganese (Mn), aluminum (Al), cadmium (Cd), zinc (Zn) and arsenic (As) (Liang et al., 2007). The strategies of silicon-mediated alleviation to heavy-metal stress vary with plant species. Some studies have suggested that Si decreases heavy-metal intake through an external or internal mechanism and alleviates the toxicity (Kidd et al., 2001; Shi et al., 2010; Zhang et al., 2013). In some plants, Si treatment can alter the sub-cellular distribution of heavy metals and increase the binding of heavy metals to the cell walls, thereby decreasing the heavy metals’ toxicity to the cell (Ye et al., 2012; Zhang et al., 2014; Shi et al., 2010). Some studies have found that Si stimulated antioxidant systems and reduced membrane lipid peroxidation under heavy-metal stress (Shi et al., 2005, 2010).

http://dx.doi.org/10.1016/j.enmm.2015.04.001
2215-1532/© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
It is thought that there are high concentrations of available Si in mangrove wetlands due to sediments, which are composed of fine particles with high organic matter content but low pH and are periodically agitated by tides (Peters et al., 1997; Marchand et al., 2004; Qin and Weng, 2006; Ye et al., 2012). The effect of Si on mangrove has been studied recently. Ye et al. (2012) and Zhang et al. (2014) investigated the amelioration by Si of Cd stress of Kandelia obovata (S., L.) Yong and A. marian, respectively. They suggested Si enhanced the binding of Cd to the cell walls in the root tips and restricted its apoplastic transport in K. obovata (S., L.) Yong and A. marian, thus playing an important role in the amelioration of Cd toxicity. Zhang et al. (2013) found that the amelioration by Si of Cd toxicity was related to the alteration of the anatomy of the roots and the increased ROL of A. marian seedlings. Aegiceras corniculatum is also an important mangrove plant species in southeastern China. The effect of Si on A. corniculatum has not yet been reported. The aim here was to investigate whether Si ameliorates the toxic effects of Cd and whether the effect of Si is correlated with the alteration of root-anatomy structure and ROL.

2. Materials and methods

2.1. Plant culture and experimental treatments

Healthy propagules of A. corniculatum were planted in vermiculite in the greenhouse, with average day/night temperature of 25 °C/20 °C and average relative humidity of 75%. After three months of growing, the uniform seedlings were transplanted to plastic buckets containing 1.5L Hoagland’s solution, which was prepared using distilled water. After two weeks of hydroponics, two levels of Cd (control and 2 mg L⁻¹) and two levels of Si (control and 100 mg L⁻¹) were developed, being CdCl₂ and Na₂SiO₃, respectively. A completely randomized design was used. Each treatment had three replicates, and the solution was changed every three days. The initial pH of nutrient solutions was adjusted to 6.5 by adding 0.1 M HCl or 0.1 M NaOH. Seedlings were harvested after eight weeks of growth.

2.2. Measurements of plant biomass

The seedlings were rinsed thoroughly with distilled water and divided into the roots and shoots. The plant materials were oven-dried for 15 min at 105 °C, then at 70 °C until constant weight. Data was recorded in regard to the dry weights of the shoots and roots.

2.3. Observation of root anatomy

The anatomical structures of the healthy roots of different treatments were studied using fresh sections of different distances behind the root tip (0.8–1.0, 3.8–4.0 cm from the tip). Fresh root sections were collected and immediately fixed in FAA (formalin–acetic acid–alcohol) at 4 °C for 48 h. Samples were dehydrated in a graduated solution series of TBA (tert-butyl alcohol) and were then embedded in paraffin. Root sections with a thickness of 10 μm were obtained using the RM2125RT rotary microtome. Berberine-aniline blue staining was used to observe the root anatomy and detect Casparian bands using the method described by Brundrett et al. (1988). Specimens were analyzed using a fluorescence microscope (Olympus IX81, Japan) and light micrographs were acquired by a digital camera (Olympus DP-50, Japan). The images were analyzed for the thickness of the epidermis, exodermis, endodermis and Casparian band, as well as the cross-sectional area of the xylem and the central cylinder using the Image-Pro Plus 6.0 analysis software.

2.4. The localization of Cd

Dithizone was used to locate Cd, based on the ability of dithizone in producing a reddish color compound after reacting with cadmium (da Cunha and do Nascimento, 2009; Seregin and Ivanov, 1997). The location of Cd in the sections of the root tips was observed, and photos were taken immediately after they were dyed in a solution of dithizone (30 mg diphenylcarbazone dissolved in 60 mL of acetone and 20 mL of distilled water) for about 2 h.

2.5. Measurement of radial oxygen loss (ROL) from entire roots

ROL from entire roots was analyzed with titanium(III) citrate buffer calorimetrically. Each of the entire roots was inserted into a beaker with the nutrient solution purged with N₂ gas for 1200 s. The layer of paraffin oil about 20 mm thick covered the solution.
Fig. 3. Cross-sections of root tip (1 cm from the root tip) of *A. corniculatum* (cross sections with thickness of 10 μm were made and photographed).

Fig. 4. The ratio of the cross-sectional width of epidermis, exodermis, central cylinder and endodermis to the root diameter and the Casparian band to the endodermis in root 2 cm section along lateral roots of *A. corniculatum* (%). *Note*: Exodermis – the ratio of the cross-sectional width of the exodermis to the root diameter; epidermis – the ratio of the cross-sectional width of the epidermis to the root diameter; central cylinder – the ratio of the cross-sectional width of the central cylinder to the root diameter; endodermis – the ratio of the cross-sectional width of the endodermis to the root diameter; Casparian band – the ratio of the cross-sectional width of the Casparian band to the endodermis. Values are means (X) ± SD (n = 3). Different letters mean significant differences between the treatments at 0.05 level.

To inhibit contamination by O₂, Titanium(III) citrate buffer (30 mL) was then injected into each of the beakers with a syringe. Control treatments without plants were done simultaneously. The absorption of solution was measured at 527 nm after incubation 6 h. ROL was calculated with the following formula (Klundze et al., 1994):

$$\text{ROL} = \frac{c(y - z)}{4}$$

- \(c\) = initial volume of Ti³⁺-citrate (L);
- \(y\) = concentration of Ti³⁺ of blank (without plants) (μmol L⁻¹);
- \(z\) = concentration of Ti³⁺ after incubation for 6 h (μmol L⁻¹).

2.6. Determination of Fe/Mn plaque formation

Roots were extracted in cold DCB (dithionite–citrate–bicarbonate) containing 40 mL 0.3 M Na₂G₆H₆O₇·2H₂O, 5 mL 1.0 M NaHCO₃ and 3 g Na₂S₂O₄ about 3 h for the measurement of Fe/Mn plaque (Taylor and Crowder, 1983; Deng et al., 2009; Pi et al., 2010). The concentrations of Fe⁺⁺, Mn and Cd in the DCB extract were measured using ICP–AES (inductively coupled plasma–atomic emission spectrometry). The concentrations of Fe/Mn plaque and Cd on the root surfaces were calculated (Taylor and Crowder, 1983):

The concentration of Fe plaque

$$= \frac{0.1591 \times [\text{Fe}^{II+}]}{\text{Root dry weight (mg/g root d. wt)}}$$

Fig. 5. Cross-sections of root tip (2 cm from the root tip) of *A. corniculatum* (cross – sections with thickness of 10 μm were made and photographed).
The concentration of Mn plaque
\[
\frac{\text{[Mn]}}{\text{Root dry weight (mg/g root d. wt)}}
\]

The concentration of Cd on Fe/Mn plaque
\[
\frac{\text{[Cd]}}{\text{Root dry weight (mg/g root d. wt)}}
\]

3. Result

3.1. Effects of silicon on the growth of A. corniculatum

Fig. 1 shows the biomass of A. corniculatum treated with Si (Si 0 mg L\(^{-1}\), Si 100 mg L\(^{-1}\)) and Cd (Cd 0 mg L\(^{-1}\), Cd 2 mg L\(^{-1}\)). Addition of Si did not significantly affect the total biomass, shoot biomass and root biomass of A. corniculatum seedlings under Cd 0 treatment. However, the total biomass, shoot biomass and root biomass were significantly increased by the addition of Si under the toxicity of 2 mg L\(^{-1}\) Cd. This suggested that the inhibition effect of Cd on the growth was alleviated by Si.

3.2. Effect of Si on root anatomy

The effect of Si on root anatomy varied with the Cd treatment used (Figs. 2 and 3). Under Cd 0 treatment, addition of Si did not significantly affect two root sections anatomy. Under Cd 2 treatment, the ratio of the cross-sectional width of the epidermis to the root diameter in the 1 cm root section was not affected by Si, but the ratio of the cross-sectional width of the exodermis to the root diameter in the 1 cm root section was significantly increased by 35.7% in Si treatment. This suggested that the addition of Si significantly induced the development of the exodermis. Under Cd 2 treatment, the ratio of the cross-sectional width of the central cylinder to the root diameter in 1 cm root section was significantly reduced by the addition of Si. In the 2 cm root section, addition of Si significantly increased the ratio of the cross-sectional width of the exodermis and endodermis to the root diameter under Cd 2 treatment (Figs. 4 and 5). Moreover, the ratios increased by 40.5% and 16.8%, respectively.

3.3. Total ROL from entire roots

Fig. 6 shows the total amount of ROL from entire roots of A. corniculatum seedlings in four treatments (treated with Si or without Si under the different Cd treatments). The addition of Si reduced ROL by 23.4% and 28.3% under the Cd 0 and Cd 2 treatments, respectively (P < 0.05).

3.4. Effect of Si on Fe/Mn plaque on the roots surface

Fig. 7 shows that Si significantly reduced the concentration of Fe plaque on the roots surface in both of the two Cd treatments. On the contrary, Si significantly increased the concentration of Mn plaque and the Cd content on Fe/Mn plaque in both of the two Cd treatments. Si reduced the Fe plaque by 31.6% and 62.7%, increased the Mn plaque by 108.4% and 34.0%, and increased the Cd content on Fe/Mn plaque by 20.7% and 85.7% under Cd 0 treatment and Cd 2 treatment, respectively.

3.5. Localization of Cd in roots

Fig. 8 shows that the Cd accumulated in the roots of A. corniculatum seedlings was generally located in the endodermis cell wall. There was not obvious difference between Si 0 treatment and Si 100 treatment.

4. Discussion

Some research studies have reported that Si alleviated the heavy-metal phytotoxicity of plants. For example, Zhang et al. (2013, 2014) reported Si alleviated the reduction of growth of Avicennia marina and Cd stress, which was consistent with the results from this study (Fig. 1). The mechanism of Si alleviation varied with plant species, heavy metals and cultivation method. Some studies have suggested that the effect of Si on organ anatomical structure was related to Si alleviation. In their study, da Cunha and do Nascimento (2009) observed significant structural alterations on xylem diameter, mesophyll and epidermis thickness, and the transversal area occupied by collenchyma and midvein treated with Si, which played an important role on the maize tolerance to Cd and Zn stress. Gong et al. (2005) suggested that thicker leaves in wheat plants treated with Si resulted from Si precipitation on the surface of the epidermis cell wall. Vaculik et al. (2009) reported that Si increased the cell-wall extensibility and delayed the endodermal
development of maize root in Cd treatments. Zhang et al. (2013) reported that Si increased the ratio of the cross-sectional width of the exodermis to the root diameter and the ratio of the epidermis to the root diameter in the root tips of A. marina seedlings in Cd stress. They inferred that promotion of the development of the apoplastic barriers in roots may be an important mechanism in the Si-induced reduction of Cd uptake. Similarly, this study showed that Si significantly increased the ratio of the cross-sectional width of the exodermis to the root diameter in the root tips of A. corniculatum seedlings under Cd 2 treatment in the current experiment. However, Si did not affect the root sections anatomy structure under Cd 0 treatment, which suggested that the effect of Si on root-anatomy structure varied growth environment. Moreover, the effect of Si on the development of apoplastic barrier was different in the roots of maize under different Cd treatments.

The location of Cd in the root varied with plant species. The Cd that accumulated in the roots of maize was mostly located in the endodermis cell wall but less in the exodermis. For the common reed, Cd deposits were detected in the exodermis (Ederli et al., 2004). The Casparian strips in the endodermis and exodermis could block apoplastic transportation and reduced the absorption of toxic elements. In this study, Cd accumulated in the 1 cm root section of A. corniculatum seedlings was generally located in the exodermis, which could hinder the translation of Cd form roots to shoots. It was thought that the transition of heavy metals from the roots to the shoots in mangroves may be related to the thicker exodermis and endodermis in the root of mangroves.

Some studies have reported that the effect of Si on the location of heavy metal was significant, and that heavy metals and Si have precipitated the same tissues and form Si-metal deposits, which seemed to contribute to the cell detoxification (da Vieira and do Nascimento, 2009; Iwasaki et al., 2002). However, our study found no difference of the location of Cd in the 1 cm root section between non-Si treated and Si treated A. corniculatum seedlings. It is, therefore, necessary to further study whether there is a difference in the location of Cd in other root sections. Moreover, the location of Si in the root was not part of the study. Further study must be given to the question of whether the co-precipitation of Si–Cd complex exists in the root of A. corniculatum seedlings as one of mechanisms of the cell detoxification.

The apoplastic barrier could not only reduce the absorption of Cd but may also affect the ROL of roots. ROL could oxidize the reductive substance and alter both microbial and chemical processes in the rhizosphere, which is of adaptive significance for wetland plants. The amount of ROL was related to many factors, in which the thickness and shape of the exodermis of roots as a barrier to ROL has great influence on the ROL. Cheng et al. (2010, 2012a) reported that heavy metals thickened the exodermis of root of mangrove so that the content of ROL as well as the intake of heavy metals by roots was decreased. In this study, Si significantly increased the ratio of the exodermis which prevent excessive heavy metals into the root and also decreased the ROL in the Cd 2 treatment. Nevertheless, Si did not affect the ratio of the exodermis in the Cd 0 treatment but reduced the amount of ROL.

The oxygen-penetration capabilities of wetland plants can make the rhizospheric micro-environment under oxidation conditions, resulting in the formation of Fe/Mn plaques on root surface. The plaques can alter the forms of heavy metals and nutrients in the rhizospheric micro-environment through a series of physical and chemical processes. In our study, Si significantly affected the Fe/Mn plaque while Si decreased the amounts of Fe plaque but increased Mn plaque. Similarly, Zhang et al. (2013) reported that the amount of Fe plaque on the surface of roots of A. marina seedlings was reduced but that Mn plaque was increased by Si. The formation of Fe/Mn plaque mainly depends on the rhizospheric micro-environment oxidized state and the concentration of Fe/Mn in the media. The ROL and the root exudate oxides determine the rhizospheric oxidation condition. It was thought that the more ROL would induce more Fe/Mn plaque formation on the root surfaces of wetland plants (Otte et al., 1991; Pi et al., 2010). In turn, the Fe/Mn plaque as the barrier may prevent the release of ROL (Møller and Sand-Jensen, 2008). Moreover, the factors affecting the formation of Fe/Mn plaque had a time effect (Pi et al., 2010). The effect of Si on the formation of Fe/Mn plaque was contrary in this study. The mechanism of effect of Si on the Fe and Mn plaque may be different and should be studied in the future.

Some studies have reported that the content of Fe plaque on the surface of the root was far higher than Mn plaque (Crowder and Coltman, 1993; Ye et al., 2001; Bacha and Hossner, 1977). This is consistent with our study. Mn plaque, with its greater surface activity and catalytic ability, could absorb and accumulate more heavy metal than Fe plaque (Ye et al., 2001; St-Cyr and Crowder, 1990). Thus, the content of Cd on Fe/Mn plaque was significantly increased by Si, which could possibly block the absorption of Cd in the root from the growth media.

The present result found that the total biomass, shoot biomass and root biomass were significantly increased by the addition of Si under Cd 2 treatment. The addition of Si significantly induced the development of the exodermis and endodermis under Cd 2 treatment. The low permeability, thus, induced may be related to the reduction of the amount of ROL and probably could block the absorption of Cd. Si significantly increased the Cd content on Fe/Mn plaque in both of the two Cd treatments, which led to lower Cd entrance to the their tissues. It was concluded that Si alleviated the
toxicity of Cd on A. corniculatum and that this alleviation was related to the lower permeability of roots, the reduction of the amount of ROL, the higher concentration of Mn plaque and the higher Cd content on Fe/Mn plaque. These results were important as the means to more fully understand the mechanisms of the tolerance of heavy metal in mangrove.

Conflict of interest

None.

Acknowledgements

This work was jointly supported by National Important Scientific Research Program of China (2013CB956504) and National Natural Science Foundation of China (31370516, 31170471).

References


