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The role of Ca pathway in Cd uptake and translocation by the hyperaccumulator *Sedum alfredii*

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ABSTRACT

Effect of Ca on plant growth, Cd uptake and translocation in the hyperaccumulator *Sedum alfredii* was investigated, as to reveal the possible pathway of Cd entry into the plants system. High Ca increased plant growth under Cd stress after 7 d, and significantly affected the total Cd influx and translocation rate. Short-term kinetics of ^{109}Cd influx performed using radiotracers confirmed a significant inhibition of ^{109}Cd influx into the roots induced by high Ca. Under exposure of 5.0 mM Ca, K_m of ^{109}Cd influx into roots was 2-fold higher in the hyperaccumulator, although the V_{max} value remained at similar level, when compared with the treatments of 0.5 mM Ca. Calcium concentrations in xylem sap of the hyperaccumulator decreased with the increasing Cd levels and significant negative correlationship between the two elements was observed. However, increased xylem loading of Cd was observed in the hyperaccumulator in response to the increasing exogenous Ca level from 0.5 to 4.0 mM, but reverse effect was observed when higher Ca levels (8–32 mM) were presented in the solutions. These results suggest that Cd uptake and translocation in the hyperaccumulator *S. alfredii* plants is positively associated with Ca pathway.

1. Introduction

The hyperaccumulators, defined as being capable of accumulating minimum 100-fold metals in shoots [1], are valuable for understanding fundamental aspects of metal-ion homeostasis and accumulation in plant cells [2–4], and the potential use in phytoextraction of metal-contaminated soils [5–9]. Cadmium (Cd) hyperaccumulator, however, is very rare due to the high toxicity of the metal. To understand the physiology mechanisms of Cd accumulation in the hyperaccumulators, it is helpful to trace the pathway of metal movement from soil to the aboveground parts of the plants.

As a non-essential element for plants [10], cadmium has been assumed to be taken up by transporters for essential elements as a consequence of a lack of specificity of the transporters [11]. Although a specific Cd transporter has been proposed to be located in the root plasma membrane of the hyperaccumulator *Thlaspi caerulescens* (Granges) [12], interactions of Cd and other elements, such as zinc (Zn) and iron (Fe), have been frequently reported in plants. Competitions between Cd and Zn during the uptake processes have been reported in both regular plants [13], and hyperaccumulators [14–16]. Most Cd hyperaccumulators identified so far, including *T. caerulescens*, *Arabidopsis halleri*, and *Sedum alfredii*, are also able to hyperaccumulate Zn [12,16–19], implying a possibility of the similar pathway of Zn and Cd accumulation in plants. In the Cd hyperaccumulator *A. halleri*, several reports suggest that the uptake and translocation of Zn and Cd in the plants may share the same pathway to certain extent [14–16]. And a metal transporter in the ZIP family, ZNT1, from the Zn hyperaccumulator *T. caerulescens* Prayon population, was able to mediate high-affinity uptake of Zn as well as low-affinity uptake of Cd [20]. Similarly, interactions of Fe and Cd were also reported in the Cd hyperaccumulators. In both *T. caerulescens* (Ganges) [21] and *A. halleri* [16], root uptake of Cd was up-regulated by low iron status, which may be related to an up-regulation in the expression of genes encoding for Fe^{2+} uptake. It was also found that iron deficiency dramatically increased Cd concentration in the xylem sap of *A. halleri* [15]. However, to date, there is no report on interaction of Cd and Ca in the Cd hyperaccumulators, although competitive uptake of the two elements was reported in some regular plants [22,23].

S. alfredii is a newly discovered Cd/Zn co-hyperaccumulator native to China [18,19], with the mechanisms involved in its accumulation rarely known. Our previous studies showed that addition of high Ca or ion channel inhibitor (La) significantly suppressed the Cd influx into its roots [24], implying Cd uptake by the plant species is probably regulated by Ca transporters or channels in root cell

Abbreviations: HE, hyperaccumulating ecotype; NHE, non-hyperaccumulating ecotype.

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plasma membranes. This indicates that *S. alfredii*, the only discovered Cd hyperaccumulator belonging to Crassulaceae family, does not use the same mechanisms as the other Brassica Cd hyperaccumulators like *A. halleri* and *T. caerulescens*. A better understanding of the physiological mechanisms by which the hyperaccumulator *S. alfredii* uptake and translocate Cd, may facilitate both the basic understanding of Cd hyperaccumulation in plants and the development of the plant species for phytoremediation purposes, especially in China. The objectives of the present study were to investigate the possible role of Ca pathway in Cd uptake and translocation in the hyperaccumulating ecotype (HE) *S. alfredii*, by comparison with its relative nonhyperaccumulating ecotype (NHE).

2. Materials and methods

2.1. Plant materials and growth conditions

Seedlings of the hyperaccumulator *S. alfredii* Hance were cultivated hydroponically according to Lu et al. [24]. Plants were originally obtained from an old Pb/Zn mine area in Zhejiang Province, China, and chosen to grow in non-contaminated soil for several generations to minimize the internal metal contents, then uniform and healthy shoots were selected and cultivated in the basal nutrient solution containing 2 mM Ca^{2+}, 4 mM NO_3^-, 1.6 mM K^+, 0.1 mM $H_2PO_4^-$, 0.5 mM Mg^{2+}, 1.2 mM SO_4^{2-}, 0.1 mM Cl^-, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6$ Mo_7O_{24}, 100 μM Fe-EDTA. Nutrient solution pH was adjusted daily to 5.5–5.8 with 0.1 M NaOH or HCl. Plants were grown under glasshouse conditions under photo flux density of 400 μmol m^{-2} s^{-1}, light/dark period of 16/8 h, day/night temperature of 26/20 °C and day/night humidity of 70/85%. The nutrient solution was continuously aerated and renewed every 3 d.

2.2. Effects of exogenous Ca on Cd uptake, translocation and plant growth

Intact 4-week-old seedlings of HE and NHE *S. alfredii* were treated with 100 and 10 μM $CdCl_2$, respectively, with different concentrations of Ca^{2+} (0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 mM) presented in nutrient solution. Ca was added as a form of $CaCl_2$. In a preliminary experiment, the possible confounding effects of Cl on Cd uptake and plant growth were investigated by addition of equal molar NaCl (1.0–64 mM, data not shown). The results showed that no significant effect of Cl^- on plant growth and Cd accumulation were observed, although slight reduced Cd accumulation were noted at a very high Cl^- level (64 mM). This suggested that the effects of elevated $CaCl_2$ levels in the uptake solutions would be mainly a consequence of the Ca ions. $NaNO_3$ was added to keep NO_3^- concentration at 4.0 mM level in the solution. Each treatment was replicated 3 times. Nutrient solution pH was adjusted daily to 5.5–5.8 with 0.1 M NaOH. The nutrient solution with different treatments were continuously aerated and renewed every 3 d. Plants were harvested after 7 d exposure, and rinsed, separated into roots and shoots, oven-dried and weighed. Cadmium in plant tissues were analyzed by ICP-MS (Agilent 7500a, USA) after digestion with HNO_3-$HClO_4$.

2.3. Effect of high Ca on ^{109}Cd uptake kinetics

The kinetics of ^{109}Cd uptake was determined using intact seedlings of HE and NHE *S. alfredii* according to the method described by Lu et al. [24] and Zhao et al. [12]. Intact 4-week-old seedlings were treated with a pretreatment solution containing 2 mM MES-Tris (pH 5.8) and 0.5 mM $CaCl_2$ [21,24]. After 12-h pretreatment, the seedlings were exposed to eight concentrations of $CdCl_2$ (0.5–80 μM) labelled with ^{109}Cd (2.0 μCi L^{-1}), with additional of 0.5 mM or 5.0 mM $CaCl_2$, each treatment was replicated four times. After 60-min uptake, the seedlings were quickly rinsed, and then transferred to identical vessels containing ice-cold desorption solutions (2 mM MES-Tris, pH 5.8, 5 mM $CaCl_2$, and 100 μM $CdCl_2$). After 15 min, the seedlings were separated into roots and shoots, blotted dry and weighed. Roots and shoots were transferred into radioactivity counting vials, ^{109}Cd was assayed by gamma spectroscopy (Canberra Packard Auto Gamma 5780).

2.4. Xylem sap collection and analysis

Plants of HE and NHE *S. alfredii* grown hydroponically for 8 weeks were used for xylem sap collection. Plants were decapitated 3–4 cm above the roots after treatment with different Cd (0, 10, 100 μM) for 4 h in the nutrient solution. Treatments were replicated nine times, and six plants in the same pot were treated as one replicate. Xylem sap was collected according to Lu et al. [24]. Briefly, 12 plants from each treatment were de-topped using sharp blades at about 3.0 cm above the junction point of root and shoot. Immediately after de-topping, each stem was rinsed with deionized water and blotted with absorbent paper to remove contaminants from cut cells. After discarding approximately 0.3 mL of sap, each cut surface was blotted again and silicon tubing was fitted over the stem. Sap flowing from the tubing was collected in sterile vials for 1 h. At the end of the collection period, xylem sap samples collected from four plants in each culture vessel were pooled and immediately frozen at −20 °C. A subsample of 0.5 mL xylem sap was mixed with 2.5 mL of 2% (w/v) nitric acid. The concentrations of Ca and Cd in the samples were determined by ICP-MS (Agilent 7500a, USA).

In a separate experiment, intact 8-week-old seedlings of HE *S. alfredii* were treated with 100 μM $CdCl_2$, with addition of different treatments of Ca (0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 mM) in nutrient solution. Xylem sap from the plants was collected and analyzed as described above.

2.5. Statistical analysis of data

All data were statistically analyzed using the SPSS package (Version #11.0), analysis of variance (ANOVA) was performed on the data sets, with the mean and S.E. of each treatment calculated.

3. Results

3.1. Cd uptake, translocation and growth in response to Ca status

The growth of both ecotypes of *S. alfredii* was increased significantly ($P < 0.05$) by high Ca conditions (Fig. 1). Plants grown in solution containing less than 1.0 mM Ca showed severe wilting and chlorosis, whereas higher Ca treatments resulted in healthier growth of both ecotypes (Fig. 1(a) and (b)). Compared with the 0.5 mM Ca treatment, the root and shoot biomass of HE *S. alfredii* increased by 2.1- and 1.5-fold, respectively, when Ca was present in solution at a concentration of 8.0 mM (Fig. 1(c)). Similarly, elevated Ca levels in solution also resulted in the increased plant growth of NHE *S. alfredii*, although the effect was less pronounced (Fig. 1(d)). However, elevated Ca in solution did not always increase the growth of the plants. Both root and shoot biomass of the HE plants decreased significantly when Ca concentration in solution increased from 8.0 to 32 mM ($P < 0.05$) (Fig. 1(c)), and no further increase of biomass in the NHE was observed at Ca concentrations above 8.0 mM (Fig. 1(d)). Despite of the 10 times higher treatment of Cd (100 μM), the biomass of HE plants was significantly higher than NHE, showing the greater tolerance of Cd toxicity of the former.

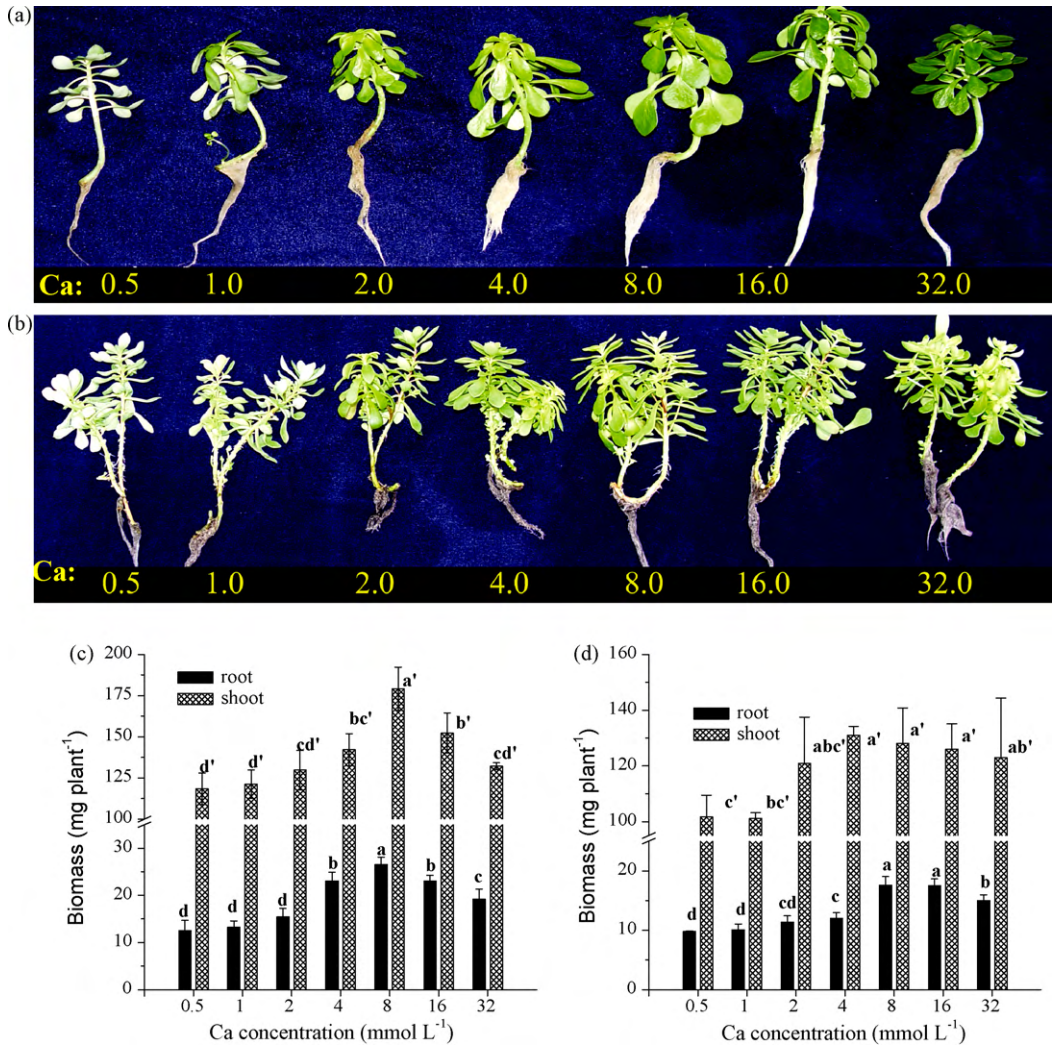

Fig. 1. Growth responses and biomass of HE (a and c) and NHE (b and d) *Sedum alfredii* at different Ca treatments under Cd stress. Plants were treated with Cd (HE, 100 μM; NHE, 10 μM) for 7 d in hydroponics with a range of Ca concentrations. Data points and error bars represent mean and S.E. of three replicates. Different letters indicate significant difference between different treatments at $P < 0.05$.

Inhibition of Cd influx by elevated Ca in the solution was observed in both ecotypes (Fig. 2(a)). The uptake of Cd by HE decreased significantly when Ca in the solution increased from 2.0 to 32 mM ($P < 0.05$). For example, the influx of Cd was 35,547 μg g root DW^{-1} at 2.0 mM Ca, but decreased to only 9951 μg g root DW^{-1} at 32.0 mM Ca. However, the total Cd uptake in HE increased by more than 2-fold when Ca in solution increased from 0.5 to 2.0 mM. This was probably because of the extreme nutrient-deficient conditions in this treatment that may have impaired the uptake processes. Total Cd influx in the NHE plants decreased consistently with the increasing concentrations of Ca in the solution ($P < 0.05$). When Ca in the solution increased from 0.5 to 32 mM, Cd uptake by the NHE plants decreased by 72%. The two ecotypes showed a significant difference in terms of the root-to-shoot translocation rate (Fig. 2(b)). The translocation rate of Cd was 1.1–1.9-fold higher in the HE than that of the NHE at a range of Ca treatments from 0.5 to 32 mM ($P < 0.05$, Fig. 2(b)). Significant increase of Cd translocation rate was induced by elevated Ca treatments from 0.5 to 2.0 mM ($P < 0.05$), and slightly more Cd was translocated to shoots when Ca in the solution continuously increased to 32 mM, although not reached at significant level. However, the variation of Cd translocation rate in the NHE in response to Ca treatments followed an opposite pattern in comparison with that of the HE (Fig. 2(b)). It was observed that significant decrease of root-to-shoot Cd translocation in the NHE plants in response to the increasing Ca concentration until 4.0 mM (Fig. 2(b)). When plants were treated with Ca levels of above 4.0 mM, less than 60% Cd was translocated in to the shoots of NHE, whereas up to 97% Cd absorbed by HE was transport to the shoots.

3.2. Effect of high Ca on concentration-dependent uptake kinetics of ^{109}Cd

The kinetics of ^{109}Cd influx into the roots of both *S. alfredii* ecotypes showed curvilinear patterns, but the addition of high Ca resulted in a marked decrease of ^{109}Cd uptake ($P < 0.05$) (Fig. 3(a) and (b)). The uptake curves could be mathematically resolved into a Michaelis–Menten saturable component and a linear component (Fig. 3(c) and (d)) ($R^2 = 0.99$–1.00, see Table 1), according to [25]. The linear component was believed to represent the apoplastically bound fraction that was not removed by the desorption procedure, and the hyperbolic parts was the real symplastic uptake by the plants [24,25].

Analysis of the kinetic constants for the saturable components showed the different response of the two ecotypes to the high Ca treatments (Table 1). In the NHE plants, the V_{max} value decreased

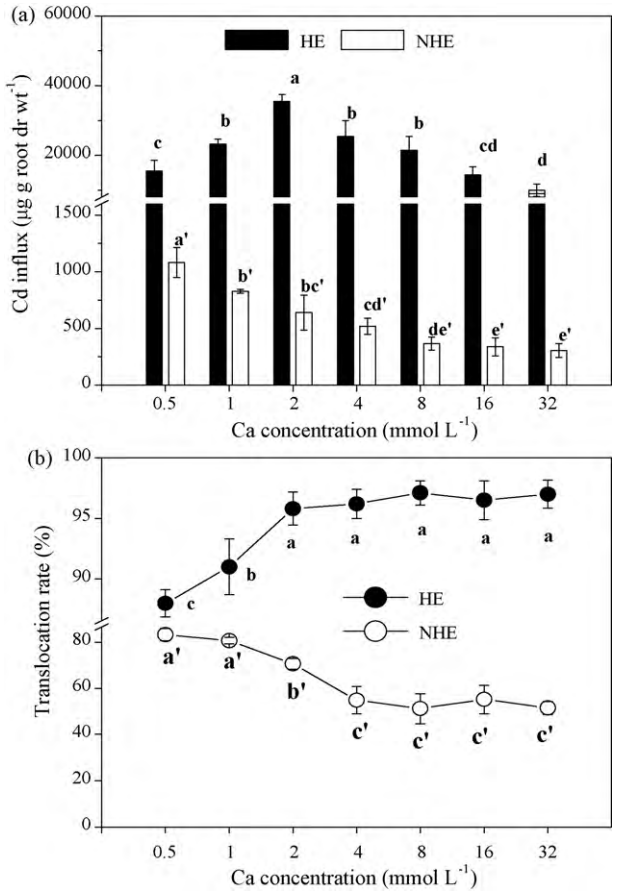

Fig. 2. Total Cd influx (a) and root-to-shoot translocation (b) of HE (black symbols) or NHE (white symbols) *S. alfredii* under Cd stress with different Ca treatments. Plants were treated with Cd (HE, 100 μM; NHE, 10 μM) for 7 d in hydroponics with a range of Ca concentrations. Data points and error bars represent mean and S.E. of three replicates. Different letters indicate significant difference between different treatments at $P < 0.05$.

from 1.06 to 0.27 μmol g DW^{-1} h^{-1} when high Ca (5.0 mM) was present in the uptake solution. However, in case of HE, the decrease of V_{max} value in response to high Ca treatment was not observed. The maximal Cd influx (V_{max}) in HE was higher either low or high Ca condition. For instance, the V_{max} for Cd was about 1.9 times higher ($P < 0.01$) in the HE than that of the NHE when the plants were grown in normal Ca condition (0.5 mM), and this difference increased to over 7 times under exposure of high Ca ($P < 0.01$). Under low Ca condition, the saturable component of the Cd influx was characterized by similar K_m values, estimated to be 4.51 and 5.35 μM for HE and NHE, respectively, with no significant differences between the ecotypes. However, very different responses of K_m to high Ca exposure were noted in the two ecotypes. When plants were exposed to high Ca level, a 2-fold increase of K_m was observed in the HE ($P < 0.05$) as compared with the control, whereas the increase of K_m was much less pronounced in the NHE plants and statistically insignificant.

3.3. Correlation between Cd and Ca in xylem sap under Cd stress

Cadmium concentration in xylem sap of HE and NHE *S. alfredii* increased significantly with increasing Cd levels in the external solution (Fig. 4(a)). Under Cd exposure of 10 or 100 μM, the concentrations of Cd in the xylem sap of HE were approximately 3-fold higher than those of NHE, whereas the content of Ca was significantly lower in the former ($P < 0.05$ and $P < 0.01$, respectively), regardless of Cd treatments (Fig. 4(a)). Cadmium exposure significantly decreased concentrations of Ca in xylem sap of HE ($P < 0.05$) (Fig. 4(b)), whereas the effect was not pronounced in the NHE plants (Fig. 4(b)). Correlations between Cd and Ca in the xylem sap were statistically analyzed. Significantly negative correlation between Cd and Ca concentration were observed in the xylem sap of HE ($r^2 = 0.15$; $P < 0.05$) (Fig. 5), whereas no such relationship were noted in that of the NHE (data not shown).

3.4. Effects of exogenous Ca on Cd in xylem sap

Furthermore, the effect of exogenous Ca on Cd content in xylem sap of HE were investigated (Fig. 6). Interestingly, the presence of Ca in the external solution did not significantly inhibit the Cd transport in the xylem sap of HE (Fig. 6). Significant increase of Cd concentration in xylem sap of HE was induced by elevated Ca treatments from 0.5 to 4.0 mM ($P < 0.05$). More than 3-fold higher Cd concentration in the xylem sap of the plants exposed to 4.0 mM Ca was noted, when compared with the level of 0.5 mM (Fig. 6). However, when Ca concentration in the external solution increased from 4.0 to 32 mM, dose-dependent decrease of Cd concentrations in the xylem sap was observed (Fig. 6). The Cd level in the xylem sap at Ca treatment of 32.0 mM was almost as same as low Ca exposure level (1.0 mM).

4. Discussion

Calcium shares many physical similarities with Cd, notably charge and ionic radius (Ca-99 pm; Cd-97 pm). It has been reported that Cd competed with Ca for uptake through ion channels in bacteria [26] and in animal cells [27] and to replace Ca in bone structure [28,29]. Clemens et al. [30] showed that a Ca transport pathway could be involved in the uptake of Cd, albeit with a low affinity. Cadmium may also permeate through calcium channels from guard cells [23] and root cells [22]. In previous paper, we investigated the effects of divalent ions on Cd influxes into roots of HE and NHE *S. alfredii*, and the results revealed a competitive interaction between Cd and Ca, confirming that the two elements may share a common transport system at the root cell plasma membrane in both ecotypes [24]. To get more insights into the role of Ca pathway in Cd uptake and translocation in the hyperaccumulator *S. alfredii*, the present study further investigates the competitive interactions of Cd and Ca in both ecotypes.

Table 1
Parameters of the linear component and Michaelis–Menten model as shown in Fig. 2. V_{max} and K_m values of saturable components were calculated by fitting a hyperbolic curve function to the saturable points.

Ecotype	Treatments	V_{max} (μmol g DW^{-1} h^{-1})	K_m (μmol)	a (μmol g DW^{-1} h^{-1} μM^{-1})	R^2
HE	Control	1.99 ± 0.12a**	4.51 ± 0.64b	0.0517 ± 0.002a	0.99
	High Ca	1.95 ± 0.22a**	8.89 ± 1.77a*	0.0240 ± 0.002b	0.99
NHE	Control	1.06 ± 0.07a	5.35 ± 0.74a	0.0575 ± 0.001a	1.00
	High Ca	0.27 ± 0.03b	5.80 ± 1.22a	0.0192 ± 0.000b	1.00

Means marked with one or two asterisks indicate significant difference between HE and NHE at $P < 0.05$ or $P < 0.01$, respectively. Different letters indicate significant between different treatments at $P<0.05$.

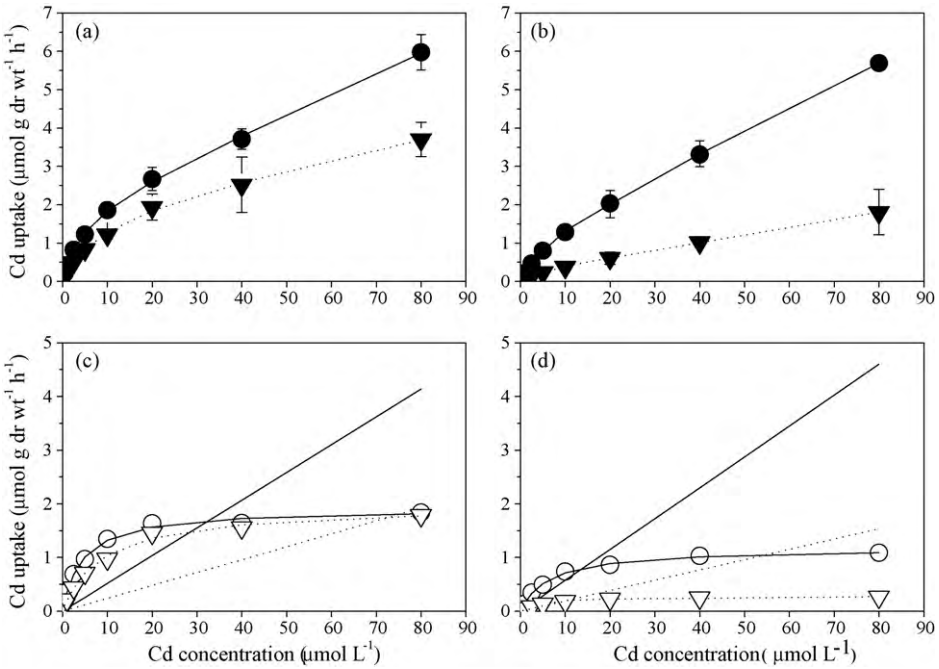

Fig. 3. Concentration-depended kinetics of Cd uptake in roots of HE (a) and NHE (b) *S. alfredii* in radiolabelled Cd uptake solution (solid lines) or with addition of high Ca (dotted line). The lines in (a) and (b) represent the best fit of the data using a Michaelis–Menten plus linear model. (c) and (d) show the dissected Michaelis–Menten and linear components separately. Data points and error bars represent mean and S.E. of four replicates. Means marked with one asterisks indicate significant difference between treatments and control at $P < 0.05$.

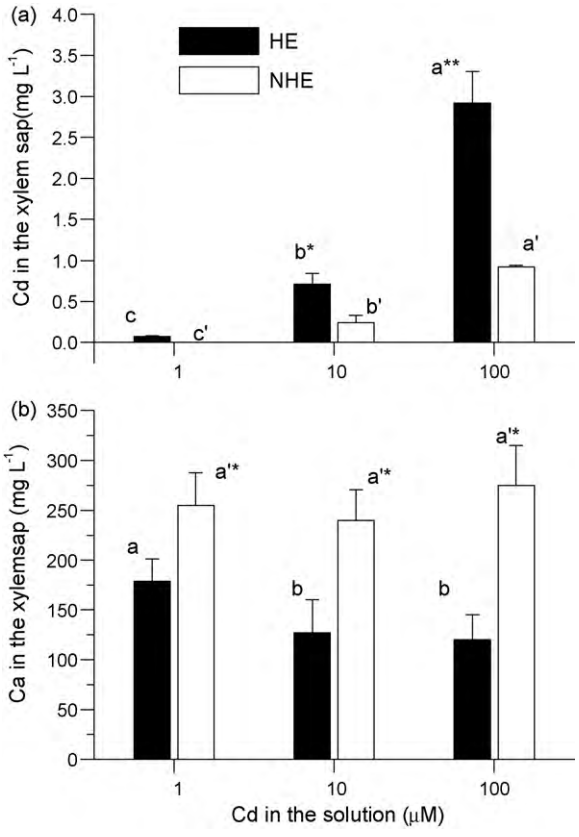

Fig. 4. Cadmium (a) and Ca (b) concentrations in the xylem sap of HE (black column) and NHE (white column) *S. alfredii* under different Cd supplies. Data points and error bars represent mean and S.E. of nine replicates. Means marked with different letters mean significant different between different treatments at $P < 0.05$, one or two asterisks indicate significant difference between two ecotypes at $P < 0.05$ or $P < 0.01$, respectively.

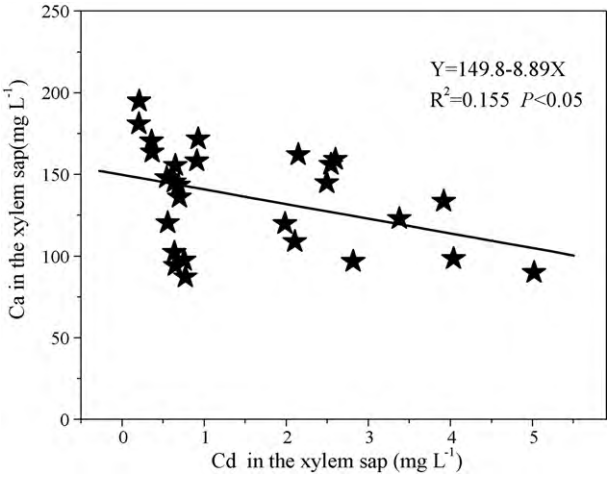

Fig. 5. The correlationship of Ca with Cd concentration in the xylem sap of HE *S. alfredii* after different Cd exposure (1, 10 and 100 μM).

The results of the long-term accumulation experiment clearly showed the alleviation of Cd toxicity in both HE and NHE by increasing Ca in the solution (Fig. 1). But Cd accumulation patterns were different between the two ecotypes (Fig. 2). It is clear that Cd influx and translocation in the NHE were consistently inhibited by the increasing Ca levels in the external solution, which is in agreement with our previous results [24]. Similarly, in the HE plants, higher Ca levels (>2.0 mM) significantly decreased its influx into the HE plants (Fig. 2(a)), although increasing Ca from 0.5 to 2.0 mM resulted in elevation of Cd accumulation, which is probably due to the healthier status of the plants under higher Ca conditions. Increasing $CaCl_2$ concentration in the uptake solution increased the concentration of Cl^-. This would decrease the activity of free Cd^{2+} as a result of complexation of Cd^{2+} with Cl^-. However, our previous results indicated that increasing Cl^- (as NaCl) from 1.0 to 10.0 mM had no significant effect on Cd influx by the plants [24], indicating that the

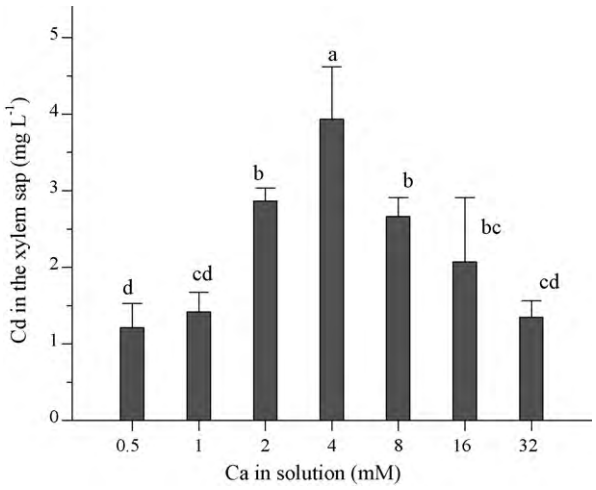

Fig. 6. The impacts of exogenous Ca on Cd concentration in the xylem sap of HE *S. alfredii*. Data points and error bars represent mean and S.E. of nine replicates. Different letters among treatments indicate significant differences at $P < 0.05$.

effect of elevated $CaCl_2$ levels in the uptake solutions was mainly a consequence of the Ca ions, which was also suggested by Zhao et al. [12].

Inhibition of Cd uptake by Ca was confirmed by the results from short-term uptake studies of the kinetics of Cd influx performed using radiotracers (Fig. 3(a) and (b)). As suggested by Lasat et al. [25] and Cohen et al. [31], the saturable components of the uptake curves generally represent the true transport mediated by transporters in the plasma membrane. The Cd^{2+} transport system in roots of NHE under both low and high Ca conditions exhibited similar K_m values, for saturable Cd influx, whereas the V_{max} value for Cd uptake was approximately 4 times higher in the plants grown in normal Ca condition, as compared with those in high Ca condition (Table 1). This suggested that Cd influx into the roots of NHE was probably through Ca channels or transporters. In HE plants, when elevated Ca was presented in the external solution, the Cd influx into the roots was inhibited. Although the V_{max} value for Cd influx did not change, its K_m value increased 2-fold for the plants grown under high Ca condition, when compared with those at 0.5 mM Ca level. This suggests that Cd may use the same transport systems with Ca, however, with different affinities.

By xylem sap analysis, we found that the exogenous supply of Cd in the nutrient solution reduced the contents of Ca in xylem sap of HE (Fig. 4), and significantly negative correlationship of Cd with Ca were noted in the xylem sap of HE (Fig. 5). To confirm the above results of xylem sap analysis, we investigate the effect of exogenous supply of Ca on Cd concentration in xylem sap of HE *S. alfredii*, but unexpected results were observed. Increasing levels of Ca in the medium did not simply resulted in decreasing Cd contents in the xylem sap. Modest supply of Ca significantly increased the Cd concentration in the xylem sap (Fig. 6). In our previous studies, we demonstrated that Cd uptake, and xylem loading in the HE *S. alfredii* are active processes [32], thus Cd content in the xylem of the plants largely depends on three processes including root symplastic uptake, vacuolar compartmentation, and xylem loading. The capacity of the HE roots to release metal from vacuolar compartmentation, is essential for its subsequent loading into the xylem and translocation to the shoot [33]. It has been suggested that Cd competes with Ca not only for the same uptake systems but also for intracellular Ca-binding sites [34]. In the present study, Ca is also likely to compete with Cd for the intracellular transporters and binding sites, thus to increase the dissociated Cd ions in the root symplasm, subsequently enhance its loading into the xylem (Fig. 6) and translocation in to the shoots (Fig. 2(b)). However, when too high Ca were presented in the medium, the competition for the uptake systems plays the dominant role in their interactions with Cd, resulting in decreased root influx (Fig. 2(a)) and loading into the xylem of HE (Fig. 6).

In conclusion, the present study suggests that Cd uptake and translocation in the hyperaccumulator *S. alfredii* plants is positively associated with Ca pathway, although the processes are fairly complicated. Further studies focused on these aspects would shed more light on the possible mechanisms of Cd uptake and translocation in the hyperaccumulator *S. alfredii*, thus to promote its potential use in phytoremediation of contaminated sites.

Acknowledgements

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