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Root Morphology and Zn^{2+} Uptake Kinetics of the Zn Hyperaccumulator of *Sedum alfredii* Hance

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Abstract: Root morphology and Zn^{2+} uptake kinetics of the hyperaccumulating ecotype (HE) and non-hyperaccumulating ecotype (NHE) of *Sedum alfredii* Hance were investigated using hydroponic methods and the radiotracer flux technique. The results indicate that root length, root surface area, and root volume of NHE decreased significantly with increasing Zn^{2+} concentration in growth media, whereas the root growth of HE was not adversely affected, and was even promoted, by 500 μmol/L Zn^{2+}. The concentrations of Zn^{2+} in both ecotypes of *S. alfredii* were positively correlated with root length, root surface area and root volumes, but no such correlation was found for root diameter. The uptake kinetics for $^{65}Zn^{2+}$ in roots of both ecotypes of *S. alfredii* were characterized by a rapid linear phase during the first 6 h and a slower linear phase during the subsequent period of investigation. The concentration-dependent uptake kinetics of the two ecotypes of *S. alfredii* could be characterized by the Michaelis-Menten equation, with the V_{max} for $^{65}Zn^{2+}$ influx being threefold greater in HE compared with NHE, indicating that enhanced absorption into the root was one of the mechanisms involved in Zn hyperaccumulation. A significantly larger V_{max} value suggested that there was a higher density of Zn transporters per unit membrane area in HE roots.

Key words: phytoremediation; *Sedum alfredii*; translocation; uptake kinetics; zinc (Zn).

Heavy metal contamination is a major environmental problem facing the modern world (Nriagu and Pacyan 1988; Zhang and Ke 2004). Phytoremediation has emerged as an alternative technique for removing toxic metals from the soil and offers the benefits of being performed *in situ*, cost-effective, and environmentally sustainable (Salt *et al*. 1995; Raskin *et al*. 1997; McGrath and Zhao 2003). Unfortunately, most of the hyperaccumulator species have a small biomass and slow growth rate, resulting in limited usefulness of the technique for the large-scale decontamination of polluted soils (Ebbs and Kochian 1997). An understanding of the basic biochemical, physiological, and molecular mechanisms involved in heavy metal accumulation in plant species is very important for exploring new metal hyperaccumulating plant species that are tolerant to high levels of metals or multiple metals, have a large biomass, extensive adaptation, and are easy to propagate (Salt *et al*. 1998). Some progress has been made towards an understanding of plant internal processes associated with metal hyperaccumulation, but little is known about the role of rhizosphere processes. There have been many studies on root morphology, but most have focused on the relationship between root morphology and yield. Little information is available regarding the relationship between root morphology and hyperaccumulation (Schwarz 2003).

Uptake kinetics and translocation characteristics of

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zinc (Zn) in the hyperaccumulating plant species have not been studied extensively (Kochian 1991, 1993). Studies on *Thlaspi caerulescens* showed that the time-dependent kinetics for $^{65}Zn^{2+}$ uptake could be resolved into two components: the concentration-dependent kinetics of root $^{65}Zn^{2+}$ absorption over a 0–100 μmol/L concentration range showed that Zn uptake followed Michaelis-Menten kinetics (Lasat *et al*. 1996). *Sedum alfredii* Hance has been identified as a new Zn-hyperaccumulator plant native to China (Yang *et al*. 2002). Not only has it shown exceptional abilities to tolerate and accumulate high concentrations of Zn, but it also has the characteristics of a large biomass, fast growth, asexual propagation, and it is perennial. So, *S. alfredii* is an ideal plant for investigating the mechanism responsible for hyperaccumulation and the practice of phytoremediation (Yang *et al*. 2002; Ye *et al*. 2003). Both a tolerance to high levels of Zn in nutrient solution and high Zn accumulation have been reported previously for *S. alfredii* (Yang *et al*. 2001, 2002, 2004; He *et al*. 2002; Long *et al*. 2002). However, these studies provided little information regarding the mechanisms of Zn uptake and translocation that results in Zn hyperaccumulation in *S. alfredii* and on the relationship between Zn uptake and root morphology. Thus, the aims of the present study were to: (i) compare root morphology differences between two ecotypes of *S. alfredii*; and (ii) characterize the root $^{65}Zn^{2+}$ uptake kinetics and its translocation to the shoot in the hyperaccumulating ecotype (HE), as well as in a related species, the non-hyperaccumulating ecotype (NHE), of *S. alfredii*.

1 Materials and Methods

1.1 Plant materials

The HE of *Sedum alfredii* Hance was obtained from an old Pb/Zn mined area in the Zhejiang Province of China, whereas the NHE of *S. alfredii* was obtained from a tea garden in Hangzhou, Zhejiang Province, China.

Healthy and equal-sized shoots of the two ecotypes of *S. alfredii* were chosen and grown for 2 weeks in basic nutrient solution (composition (in μmol/L): Ca $(NO_3)_2 \cdot 4H_2O$ 2000; KH_2PO_4 100; $MgSO_4 \cdot 7H_2O$ 500; KCl 100; K_2SO_4 700; H_3BO_3 10; $MnSO_4 \cdot H_2O$ 0.5; $ZnSO_4 \cdot 7H_2O$ 1; $CuSO_4 \cdot 5H_2O$ 0.2; $(NH_4)_6$ Mo_7O_{24} 0.01; Fe-EDTA 100). The pH of the nutrient solution was adjusted daily to 5.5 with 0.1 mol/L NaOH or 0.1 mol/L HCl. The nutrient solution was aerated continuously and renewed every 4 d.

1.2 Zn accumulation in roots and shoots of *S. alfredii*

The seedlings of *S. alfredii* were precultured for 14 d for the initiation of new roots before plants were exposed to treatments with different concentrations of Zn. The treatments were composed of control (1 μmol/L Zn) and 10, 50, 250, and 500 μmol/L Zn, which was applied as sulfate. Each treatment was replicated three times. Each 3-L container had 18 plants. The nutrient solution was aerated continuously and renewed every 4 d. The pH of the nutrient solution was maintained at 5.5. Plants were harvested after exposure to metal treatment for 20 d and root morphology parameters and root activity were determined. At harvest, plant roots were soaked in 20 mmol/L Na_2-EDTA for 15 min to remove metal ions adhering to the root surface (Yang *et al*. 1996). Harvested plants were separated into leaves, stems, and roots and oven dried at 65 °C. Fresh and dry weights were recorded.

1.3 Radiotracer (^{65}Zn) uptake experiments

Seedlings (20-day-old) were used in all radiotracer studies. One day prior to the uptake experiment, seedling roots were immersed in aerated pretreatment solution consisting of 2 mmol/L Mes-Tris buffer (pH 6.0) and 0.5 mmol/L $CaCl_2$.

1.3.1 Time-course of $^{65}Zn^{2+}$ accumulation in *S. alfredii* Roots of the HE and NHE of *S. alfredii* were immersed in 1 L aerated uptake solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and 10 μmol/L $^{65}Zn^{2+}$ (44.4 kBq/L). At different times (0, 10, 20, 30, 60, 120, and 240 min and 6, 12, 24, 48, and 72 h), one plant from each species was harvested and the roots were desorbed for 20 min in ice-cold solution containing 5 mmol/L Mes-Tris (pH 6.0), 5 mmol/L

$CaCl_2$, and 100 μmol/L $ZnCl_2$ and then separated from the shoots. Excised roots were blotted, roots and shoots were oven-dried at 65 °C and weighed, and root ^{65}Zn radioactivity was measured using a gamma detector (γ Spectrum Instruments model GR2519; CANBERRA; 1115.39 keV)

1.3.2 Concentration-dependent kinetics of $^{65}Zn^{2+}$ influx Pretreatment solution (500 mL; 2 mmol/L Mes-Tris, pH 6.0, and 0.5 mmol/L $CaCl_2$) was added to individual Plexiglas uptake apparatus. Subsequently, Zn was added as $ZnCl_2$ to each uptake well to yield a final Zn concentration of 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 μmol/L, and then 44.4 kBq/L $^{65}ZnCl_2$ was added to each well. After 5 min equilibration, roots of HE and NHE were immersed in the 500 mL pretreatment solution. After a 30-min uptake period, uptake wells were refilled with ice-cold desorption solution. Following a 20-min desorption period, seedlings were harvested and their roots excised, blotted, oven-dried at 65 °C and weighed, and ^{65}Zn was quantified via gamma detection.

1.4 Plant analysis

Root length, root surface area, root diameter, and root volume were determined with a root automatic scan apparatus (MIN MAC, STD1600$^+$), equipped with WinRHIZO software (Regent Instruments). Root activity was determined using the TTC method and root activity was expressed as the amount of triphenyl formazan (TPF) deoxidized by TTC. Dried plant materials were ground with a stainless-steel mill and passed through a 0.25-mm sieve for element analysis. A 0.1 g dry sample for each treatment group was washed in a muffle furnace at 550 °C for 5 h. The ash was dissolved in 5 mL of 1 ∶ 1 HCl. The solution was transferred to a 50-mL volumetric flask, made up to volume and filtered through Whatman No. 40 filter paper. The concentration of Zn in the filtrates was determined using a flame atomic absorption spectrophotometer (AA 6800; Shimadzu, Kyoto, Japan). Statistical analysis of the data was performed by using the SPSS v. 10.0 statistical package (SPSS, Chicago, IL, USA).

2 Results

2.1 Growth response of two ecotypes of *S. alfredii* to Zn

Within the concentration range of 1–500 μmol/L Zn^{2+}, the HE of *S. alfredii* grew normally and there were no toxic symptoms observed. However, growth of the NHE of *S. alfredii* was inhibited significantly when the Zn level was > 50 μmol/L (Tables 1, 2). After 20 d growing in solutions containing different concentrations of Zn^{2+} (1–500 μmol/L), no differences were seen in the leaf and stem weights of HE with increasing Zn^{2+} concentrations, whereas in NHE leaf and stem weights decreased (Table 1). In the solution containing 500 μmol/L Zn^{2+}, the dry weight of the leaves and stems of NHE decreased by 42.8% and 34.5%, respectively. The root growth of NHE was significantly inhibited when the concentration of Zn^{2+} was $\geqslant$50 μmol/L ($P < 0.05$); in contrast, a significant increased in the dry weight of HE roots was found when in the presence of 500 μmol/L Zn^{2+}.

2.2 Differences in root morphology between the two ecotypes of *S. alfredii*

In order to compare root morphology characteristics of the two ecotypes following exposure to different concentrations of Zn, root length, root surface area, root diameter, and root volume were measured. The effects of Zn on the root morphology characteristics of *S. alfredii* varied between the two ecotypes; root length, root surface area, and root volume were significantly affected by the presence of Zn, whereas there was no effect on root diameter (Table 2). Root length, root surface area, and root volume of the NHE decreased significantly with increasing concentrations of Zn^{2+} and the difference was significant, compared with control, when the concentration of Zn^{2+} was $\geqslant$50 μmol/L ($P < 0.05$). After 20 d growth in solution containing 500 μmol/L Zn^{2+}, root length, root surface area, and root volume of the NHE were decreased by 66.2%, 67.9%, and 78.3%, respectively, whereas the root surface area and root volume of the HE increased by 14.4% and 15.8%, respectively, compared with

Table 1 Effects of Zn treatment on the yields (dry weight) of two ecotypes of *Sedum alfredii*

Zn treatment (μmol/L)	Leaf weight (g/plant dry weight)		Stem weight (g/plant dry weight)		Root weight (g/plant dry weight)	
	NHE	HE	NHE	HE	NHE	HE
1	0.56a	0.59a	0.29ab	0.33a	0.109a	0.112b
10	0.54a	0.61a	0.31a	0.31a	0.102a	0.111b
50	0.50ab	0.62a	0.27ab	0.29a	0.091b	0.112b
250	0.44b	0.58a	0.25b	0.30a	0.075c	0.123ab
500	0.32c	0.66a	0.19c	0.32a	0.057d	0.132a

All data are the means of three replicates. In a column, values followed by different letters are significantly different at $P < 0.05$. HE, hyperaccumulating ecotype of *S. alfredii*; NHE, non-hyperaccumulating ecotype of *S. alfredii*.

Table 2 Effects of Zn treatment on the root morphology of the two ecotypes of *Sedum alfredii*

Zn treatment (μmol/L)	Root length (cm/plant)		Root surface area (cm^2/plant)		Root diameter (mm)		Root volume (cm^3/plant)	
	NHE	HE	NHE	HE	NHE	HE	NHE	HE
ck	558.6a	568.8a	151.8a	149.3b	0.81a	0.89a	3.28a	3.15b
10	527.6a	558.1a	129.9a	159.7ab	0.74a	0.82a	2.97a	3.21b
50	403.2b	575.8a	88.8b	150.1b	0.72a	0.78a	1.55b	3.49ab
250	237.6c	561.2a	56.7c	164.3ab	0.76a	0.80a	1.08c	3.67a
500	189.9d	581.6a	48.7c	170.8a	0.73a	0.81a	0.71d	3.65a

All data are the means of three replicates. In a column, values followed by different letters are significantly different at $P < 0.05$. HE, hyperaccumulating ecotype of *S. alfredii*; NHE, non-hyperaccumulating ecotype of *S. alfredii*.

control. As for root diameter, no differences between the two ecotypes were observed for any of the treatments.

2.3 Effect of Zn on root activity of the two ecotypes of *S. alfredii*

The root is the primary organ plants using to absorb nutrients and water and, therefore, the toxic symptoms of heavy metals would be reflected in root activity first. For the HE, root activity was not influenced with increasing concentrations of Zn^{2+}; however, for the NHE, root activity decreased significantly when Zn concentrations were >10 μmol/L. For example, at 500 μmol/L, Zn decreased root activity by 52% in the NHE compared with control, and the root activity of the HE was 2.3-fold higher than that of the NHE (Fig. 1).

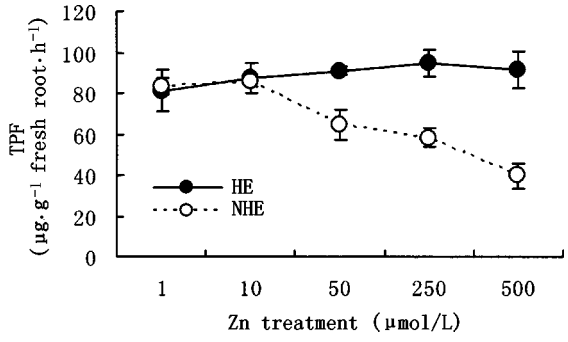

Fig. 1. Root activity difference of two ecotypes *Sedum alfiedii* exposed to different levels of Zn in nutrient solution. Data points and error bars represent means ± *SE* of three replicates. HE and NHE in figures are the same as in Table 1.

2.4 Zn accumulation in *S. alfredii*

The Zn concentration in shoots of the HE and the roots of both ecotypes of *S. alfredii* increased with an increase in the amount of Zn^{2+} supplied. After 20 d growth in solutions containing different Zn^{2+} concentrations (1–500 μmol/L), the NHE accumulated more Zn^{2+} in the roots, whereas most of the Zn was translocated to the shoots in the HE. When the growth solution contained 500 μmol/L Zn^{2+}, the shoots of HE accumulated 17.3-fold more Zn compared with the NHE, whereas 1.8-fold more Zn was accumulated in the roots of NHE than of HE (Fig. 2).

2.5 Time-course of $^{65}Zn^{2+}$ accumulation in *S. alfiedii*

Accumulation of $^{65}Zn^{2+}$ was significantly different in desorbed intact roots of the two ecotypes of *S. alfredii*. For the roots of both ecotypes, $^{65}Zn^{2+}$ accumulation during the first 6 h was characterized by a rapid linear phase, whereas during the subsequent period uptake was represented by a slower linear phase (Fig. 3a'). During the first 4 h, $^{65}Zn^{2+}$ uptake kinetics in roots could also be resolved into two components, with an initial rapid linear stage during the first 20 min followed by a second, slower linear stage over the subsequent 220 min (Fig. 3a'). After a 4 h absorption period, 1.3-fold more $^{65}Zn^{2+}$ was accumulated in the roots and 2.1-fold more $^{65}Zn^{2+}$ was translocated in the shoots of the HE compared with the NHE (Fig. 3a', b'). During the first 4 h, little $^{65}Zn^{2+}$ was translocated from roots to shoots in both ecotypes, the $^{65}Zn^{2+}$ concentration ratio for roots/shoots was 37 and 61 for the HE and the NHE, respectively. Accumulation of $^{65}Zn^{2+}$ in the shoots of the HE increased quickly whereas, that in the NHE increased slightly with increasing treatment time. At the end of a 72 h uptake period, 26% more radioactive $^{65}Zn^{2+}$ had accumulated in the roots of the NHE compared with the HE, whereas, Zn translocation to the shoot was approximately 7.4-fold greater in the HE compared with the NHE (Fig. 3b').

It is interesting to note that, in the first 30 min, the $^{65}Zn^{2+}$ concentration in the roots of the NHE was greater than that in the roots of the HE (Fig. 3a'). Presumably, after 20 min desorption in a solution containing a high level of non-labeled Zn^{2+} (100 μmol/L) and Ca^{2+} (5 mmol/L), 80% of the $^{65}Zn^{2+}$ accumulated during the uptake period was desorbed into the external solution. However, at the end of desorption, more $^{65}Zn^{2+}$ remained in the roots of the NHE than the HE (data not shown).

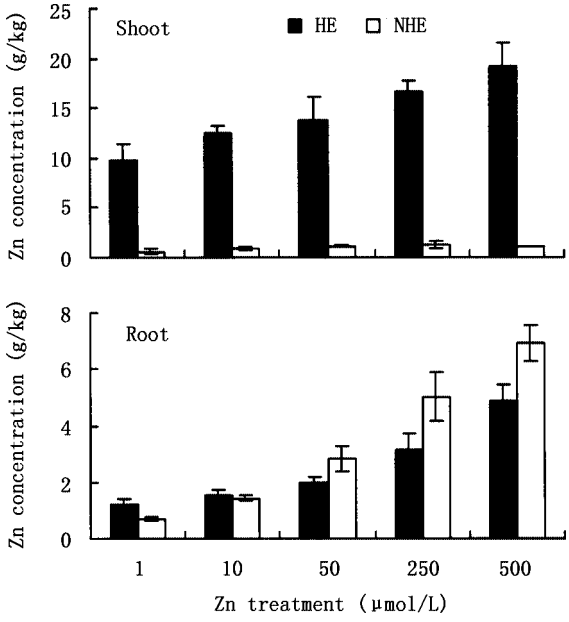

Fig. 2. Zn accumulation in two ecotypes *Sedum alfiedii* exposed to different level of Zn^{2+} in nutrient solution. Data points and error bars represent means ± *SE* of three replicates.

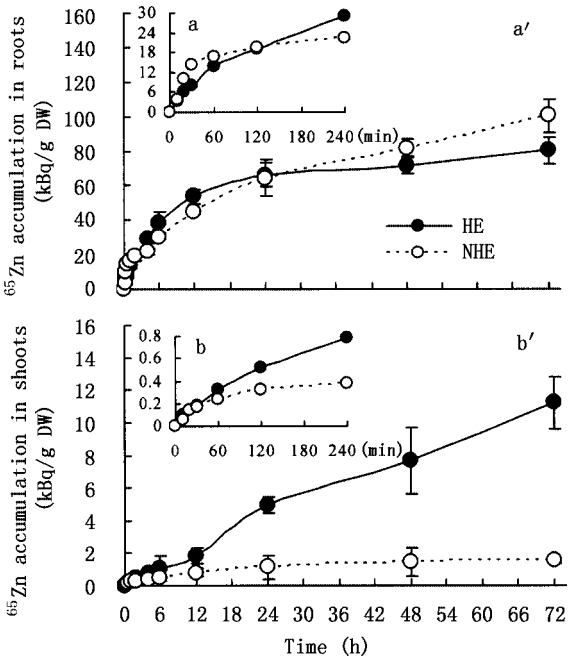

Fig. 3. Time course of Zn^{2+} accumulation in roots (**a'**) and shoots (**b'**) of two ecotypes of *Sedum alfiedii*. Seedlings were immersed with roots in uptake solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and 10 μmol/L $^{65}Zn^{2+}$ (1.2 mCi/L). The curve shown in **a** was derived from the data points in **a'**. The curve in **b** was similarly derived from the curve in **b'**. Data points in **a'** represent means ± *SE* of three replicates.

2.6 Concentration-dependent kinetics of Zn^{2+} uptake into the roots of the two ecotypes of *S. alfredii*

The concentration-dependent uptake kinetics for $^{65}Zn^{2+}$ influx in the HE were characterized by a non-saturable curve that approached linearity at externa1 concentrations above 25 μmol/L; however, for NHE, uptake kinetics were biphasic, with an initial rapid component followed by a slower, saturable phase of uptake at external concentrations above 50 μmol/L (Fig. 4). The uptake kinetics of the two ecotypes could be characterized by the Michaelis-Menten equation, with apparent K_m values of 34.83 and 20.43 μmol/L for the HE and NHE, respectively. The V_{max} values were 27.77 and 9.21 μmol/L Zn^{2+}·g^{-1} FW·h^{-1} for the HE and NHE, respectively, indicating that Zn^{2+} influx in the HE roots was more than threefold higher compared with influx into the NHE roots.

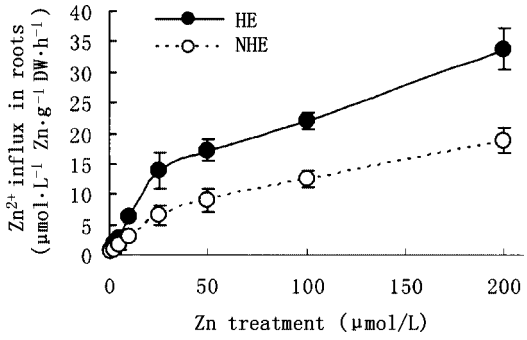

Fig. 4. Concentration-dependent kinetics of Zn^{2+} uptake into roots of two ecotypes *Sedum alfredii*. Roots were immersed in a solution containing 2 mmol/L Mes-Tris (pH 6.0), 0.5 mmol/L $CaCl_2$, and $^{65}Zn^{2+}$ (1.2 mCi/L) at the concentrations of 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 100.0, 200.0 μmol/L. Data points and error bars represent means ± *SE* of three replicates.

3 Discussion

The uptake of water, minerals, and heavy metals is directly affected by root morphological characteristics (Marschner 1995). Results from the present study showed that roots of the HE of *S. alfredii* had a greater ability to tolerate and absorb Zn^{2+}. Root length, root surface area, and root volumes of the NHE decreased significantly, whereas those of the HE were not affected by increasing concentrations of Zn^{2+} in the growth medium. Furthermore, 500 μmol/L Zn^{2+} significantly increased root surface area and root volumes of the HE. All these contrasting root morphological responses of the two ecotypes of *S. alfredii* to Zn treatment may be responsible, in part, for the different abilities of the two ecotypes to accumulate Zn. Correlation analysis proved that root length, root surface area, and root volumes had a significant positive correlation with Zn concentration in the shoot ($P < 0.05$), root activity ($P < 0.01$), and the plant biomass of *S. alfredii* ($P < 0.01$), but no such correlation was found for root diameter. This indicates that root length, root surface area, and root volumes contribute more to Zn accumulation in *S. alfredii* than root diameter does. That is, the greater the root length, root surface area, and root volume of an ecotype, the higher the concentrations of Zn accumulated in the tissue. This may explain, in part, why the HE of *S. alfredii* could hyperaccumulate Zn.

Studies of the accumulation of several different cations in roots have demonstrated that the time-dependent cation uptake kinetics are biphasic, with an initial rapid component followed by a slower, linear phase of uptake (Veltrup 1978; Hart *et al*. 1992). The rapid

Table 3 Correlation coefficients between root morphology parameters and root activity, plant biomass, and Zn concentrations in different tissues

Root parameters	Zn concentration		Root activity	Biomass		
	Shoot	Root		Leaf	Stem	Root
Length	0.651 5*	–0.775 9**	0.957 4**	0.925 9**	0.902 4**	0.933 1**
Surface area	0.713 2*	–0.653 2*	0.964 8**	0.948 5**	0.891 9**	0.971 7**
Diameter	0.353 4	0.150 3	0.553 3	0.602 7	0.617 6	0.623 9
Volume	0.695 6*	–0.653 1*	0.973 1**	0.921 6**	0.863 0**	0.950 3**

*, $P < 0.05$; **, $P < 0.01$; $r_{0.05}(8)=0.632$, $r_{0.01}(8)=0.765$.

component of cation uptake has generally been interpreted to represent accumulation in the apoplasm, whereas the slower, linear phase is thought to represent transport across the plasma membrane. In the present study, the time-dependent kinetics for $^{65}Zn^{2+}$ uptake in *S. alfredii* roots of both ecotypes was characterized by a rapid, linear phase during the first 6 h, followed by a slower, linear phase during the subsequent experimental period owing to Zn translocation to the shoots. The uptake of $^{65}Zn^{2+}$ during the first 4 h could also be resolved into two components, with an initial rapid, linear stage during the first 20 min, followed over the subsequent 220 min with a second slower, linear stage (Fig. 3a'). We interpret the initial rapid component to represent cell wall-associated $^{65}Zn^{2+}$ not removed by the desorption treatment. The results indicate that the slower, linear phase of accumulation that dominates the uptake curve at exposures longer than 20 min is due primarily to $^{65}Zn^{2+}$ transport into the symplasm, with a minor component due to undesorbed cell wall $^{65}Zn^{2+}$. These results are consistent with those of studies performed in *T. caerulescens*, one of the best-known Zn hyperaccumulators (Lasat *et al.* 1996).

Investigations into *T. caerulescens* have found that Zn transport at the root cell plasma membrane is mediated by protein (Lasat *et al*. 1996, 2000). In the present study, the apparent K_m values were 34.83 and 20.43 μmol/L for the HE and NHE, respectively, and the V_{max} for Zn^{2+} influx were threefold greater in the HE compared with the NHE (Fig. 4). These results suggested that, in *S. alfredii*, transport across the plasma membrane was mediated by proteins with different affinities, in which the protein affinities of the HE of *S. alfredii* were lower than those of the NHE of *S. alfredii*, but the capacity for influx was much greater in the roots of the HE. A significantly larger V_{max} value suggested that there was a higher density of Zn transporters per unit membrane area in roots of the HE roots.

During the first 4 h of the time-course uptake experiments, there was a greater influx of Zn into the HE root symplasm compared with influx observed in the NHE (Fig. 3a). The maximum initial velocity for $^{65}Zn^{2+}$ influx was threefold greater in the HE compared with the NHE. These results indicate that Zn influx was enhanced into the root symplasm in the HE. However, during the course of this series of experiment, little Zn was translocated from roots to shoots. At the end of a 72-h uptake period, the NHE retained more Zn in the roots than the HE, whereas the HE translocated considerably more Zn to the shoots (Fig. 3b'). Similarly, long-term exposure to high concentrations of Zn^{2+} resulted in greater metal accumulation in the shoots of the HE compared with the NHE (Fig. 2). These results indicate that Zn transport sites other than influx into the root are also altered in the HE and contribute to the marked increase in translocation of Zn to the shoot.

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