

## Effects of Se on the uptake of essential elements in *Pteris vittata* L.

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**Abstract** Selenium has been proven to be an antioxidant in plants at low dosages. To understand better the mechanisms of Se toxicity and benefit to plants, more investigations about effects of Se on the uptake of essential elements in plants would be desirable. In this study, hydroponic (nutrient solution culture) and pot (soil culture) experiments were simultaneously conducted to investigate the effects of Se on the uptake and distribution of essential elements in *Pteris vittata* L. (Chinese brake fern), an arsenic (As)-hyperaccumulator and a selenium (Se)-accumulator. Chinese brake fern took up much more Se in nutrient solution culture than in soil culture, with the highest Se content recorded as 1,573 mg kg<sup>-1</sup> in the roots, demonstrating remarkable tolerance to Se. In soil culture, Chinese brake fern also accumulated high content of Se, with the highest

content measured as 81 mg kg<sup>-1</sup> and 233 mg kg<sup>-1</sup>, in the fronds and roots, respectively. In soil culture, the addition of Se suppressed the uptake of most measured elements, including magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), copper (Cu) and zinc (Zn). In nutrient solution culture, when the Se content in the tissues of Chinese brake fern was relatively low, the supplementation of Se suppressed the uptake of most essential elements; however, with the increase of Se content, stimulation effects of Se on the uptake of Ca, Mg, K were observed. An initial decrease followed by a rapid increase of Fe content in the fronds of Chinese brake fern was found with Se addition and tissue Se content increasing in nutrient solution culture, suggesting antagonistic and synergic roles of Se on these elements under low and high Se exposure, respectively. We suggest that Ca, Mg, K may be involved in the tolerance mechanism of Se, and that the regulation of Fe accumulation by Se in the fronds might be partially due to the dual effects of Se on Chinese brake fern.

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### Introduction

Selenium is a non-essential element for plants. It has dual effects, i.e., high dosage of Se may be toxic whereas low dosage of Se is a natural antioxidant in plants (Hartikainen et al. 2000). After being absorbed

by plants,  $\text{SeO}_3$  is rapidly transformed into organic Se species and stored mainly in the roots (Zayed et al. 1998). The accumulation of organic Se species in plants may enhance ethylene production, increase membrane permeability and influence the uptake of essential elements, such as K (Xue et al. 2001).

Except for the enhancement of membrane permeability derived from the accumulation of organic Se species, the interactions of Se and other elements may also affect the uptake of essential and non-essential elements. To date, the interactions between Se and other elements are mainly documented in humans and animals (Su et al. 2008), these interactions are either antagonistic or synergistic; however, in some cases the reversed interaction is seen, i.e., antagonism to synergism (Hamilton 2004). To date, little attention is paid on such interactions in plants. Low dosages of Se can protect plants through reducing the uptake of some deleterious elements, such as arsenic (As) (Khattak et al. 1991), mercury (Hg) (Yathavakilla and Caruso 2007), lead (Pb) (He et al. 2004) and cadmium (Cd) (Ebbs and Leonard 2001; He et al. 2004). High dosages of Se may be toxic to plants and compete with some essential elements, such as phosphorus (P) (Broyer et al. 1972; Hopper and Parker 1999; Khattak et al. 1991) and sulphur (S) (Hopper and Parker 1999; White et al. 2004), resulting in the deficiency of these two elements in plants. On the contrary, supplementation of Se did not significantly affect the uptake of iodine (I) (Zhu et al. 2004) and molybdenum (Mo) (Khattak et al. 1991).

Information about effects of Se on the uptake of other essential elements in plants is insufficient. According to current reports, the antagonistic and synergistic operations of Se to some essential elements both simultaneously operate even for the same element. It is generally considered that absorption of elements such as Mn, Zn, Cu and Fe is inhibited by increasing Se levels (Kabata-Pendias and Pendias 2001; Fargašová et al. 2006), however, in some cases, the stimulating effects of Se on Cu and Zn uptake in plants were also observed (Arvy et al. 1995).

When plants are exposed to Se, either stimulation or suppression of Fe uptake is also reported. For instance, in Chinese cabbage (*Brassica rapa*) and lettuce (*Lactuca sativa* L.) grown in pots, He et al. (2004) found that the addition of only  $1 \text{ mg kg}^{-1}$  Se significantly inhibited the uptake of Fe and Cu in both vegetables, however in a field plot test treated with  $75 \text{ mg m}^{-2}$  Se, the uptake of Fe by lettuce was found

to be enhanced. Wu and Huang (1992) reported that Fe content increased with the increasing tissue Se content in white clover (*Trifolium repens* L.), even under severe growing inhibition conditions, and application of Se could enhance the accumulation of Cu, Mn and Zn in this plant. It has been established that catalytic Fe and Cu may produce high reactive hydroxyl radical through Fenton reaction to damage the lipid membrane. Furthermore, some reports have also shown that low dosages of Se may protect but high dosages of Se may damage the lipid membrane (Cartes et al. 2005; Hartikainen et al. 2000; Mora et al. 2008). According to the above reports, there may be some relationships among Se, iron and lipid peroxidation in plants, and we suppose that the dual effects of Se on plants may occur with the down-regulation of Fe by low doses of Se and up-regulation of Fe by high doses of Se in plants.

*Pteris vittata* L. (Chinese brake fern) has been identified as an As-hyperaccumulator (Ma et al. 2001; Chen et al. 2002) and a Se-accumulator (Srivastava et al. 2005). However, the uptake regulation manners of essential elements in Chinese brake fern to resist Se toxicity have not been elucidated yet. In addition, growth medium may possibly affect the uptake of essential elements by Chinese brake fern. Consequently, the objective of this study was to explore the effects of Se on the uptake of essential elements in Chinese brake fern, under both hydroponic (nutrient solution culture) and pot experimental (soil culture) conditions. The results were expected to give insight on the mechanisms for both beneficial and toxic effects of Se to plants.

## Materials and methods

Chinese brake ferns were cultivated at temperatures ranged from  $25^\circ\text{C}$  to  $28^\circ\text{C}$ , with a relative humidity of ca. 75%. A fourteen-hours photoperiod with an average photon flux density of  $820 \mu\text{mol m}^{-2} \text{s}^{-1}$  was supplied with cool-white fluorescent lamps. Healthy and uniform ferns with 8–12 fronds were selected for both two experiments. Details for fern germination and cultivation were described in our previous published paper (Feng et al. 2009).

### Nutrient solution culture (experiment I)

After being rinsed with tap water and de-ionized water, ferns were transplanted to 20% strength Hoagland-

Arnon nutrient solution to acclimate for 2 weeks (Hoagland and Arnon 1938). The 20% strength Hoagland-Arnon nutrient solution comprised of 1 mM KNO<sub>3</sub>, 0.2 mM NH<sub>4</sub>NO<sub>3</sub>, 0.2 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 0.8 mM Ca(NO<sub>3</sub>)<sub>2</sub>•4H<sub>2</sub>O, 0.4 mM MgSO<sub>4</sub>•7 H<sub>2</sub>O, 1.8 μM MnCl<sub>2</sub>•4H<sub>2</sub>O, 9.2 μM H<sub>3</sub>BO<sub>3</sub>, 0.16 μM ZnSO<sub>4</sub>•7H<sub>2</sub>O, 0.06 μM CuSO<sub>4</sub>•5H<sub>2</sub>O, 0.02 μM H<sub>2</sub>MoO<sub>4</sub>, and 1.8 μM EDTA-Fe. The nutrient solution was replaced once a week with vigorous aeration. Two weeks later, the fern plants were transplanted to an opaque plastic pot containing one liter of treatment solution. Se was added in the form of Na<sub>2</sub>SeO<sub>3</sub> at the rates of 0, 1, 2, 5, 10, 20 mg L<sup>-1</sup>. One plant was placed in a hole on a styro-foam sheet as the cover of each pot, each treatment was replicated three times. The pH of the solution was adjusted to 6.5 with diluted HCl or NaOH.

### Soil culture (experiment II)

A silt loam soil collected from field without amending with Se fertilizers in Tongzhou district, Beijing, China, was used for soil culture experiment. Selected physical and chemical properties of the soil are shown in Table 1. Soils were air-dried, sieved (< 0.9 mm) and homogenized. A single factorial design with three replications for each treatment was employed. For each replication, one plant was transplanted to the pot containing with one kilogram of soils. Selenium was added in the form of Na<sub>2</sub>SeO<sub>3</sub> at rates of 0 (control),

10, 20, 50, 100 mg kg<sup>-1</sup>. Base fertilizers of N, P, K for each replication were comprised of 0.57 mg kg<sup>-1</sup> NH<sub>3</sub>NO<sub>3</sub>, 0.17 mg kg<sup>-1</sup> CaHPO<sub>4</sub>•2H<sub>2</sub>O and 0.33 mg kg<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub>, no other fertilizers with micro-nutrients were added. All chemicals were of analytical grads. After being mixed with the base fertilizers and the designated amounts of Se salts, the soils were placed in a 1-L plastic pot, fully watered with de-ionized water and then incubated for 1 week. One fern plant was transplanted into each pot to let it grow for 30 d under Se exposure, plants were watered when necessary with de-ionized water throughout.

### Determination of elements

After 14 d growth in nutrient solution and 30 d in soils containing various concentrations of spiked Se, plants were harvested. They were then separated into above-ground (fronds) and below-ground (roots plus rhizomes) portions, thoroughly rinsed with tap water and de-ionized water, dried and pulverized.

The pulverized plant tissues were digested using concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (Wei et al. 2006). Se concentrations were determined using a hydride generation atomic fluorescence spectrometer (AFS820, Beijing Titan Instruments Co., China). Concentrations of other elements (Ca, Mg, K, P, Fe, Mn, Cu, Zn) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Optima-2000 DV, PerkinElmer, Co, USA). Accuracy of element analysis was checked by standard reference material from the Center for Standard Reference of China.

**Table 1** Selected physical and chemical properties of the soil

Properties	Values
pH	7.59
Organic matter content (g kg <sup>-1</sup> )	13.10
Total N (mg kg <sup>-1</sup> )	980.0
Total P (mg kg <sup>-1</sup> )	666.30
Available P (mg kg <sup>-1</sup> )	10.10
K <sub>2</sub> O (%)	0.623
Available K(mg kg <sup>-1</sup> )	95.80
CaO (%)	2.767
MgO (%)	1.831
Fe <sub>2</sub> O <sub>3</sub> (%)	2.865
Mn (mg kg <sup>-1</sup> )	391.90
Cu (mg kg <sup>-1</sup> )	12.78
Zn (mg kg <sup>-1</sup> )	57.20

### Data analysis

One-way ANOVA with multi-comparisons by Tukey's test was employed to compare the means among different treatments at  $P \leq 0.05$  in the SPSS statistical package. All results were expressed as means with corresponding standard errors.

## Results

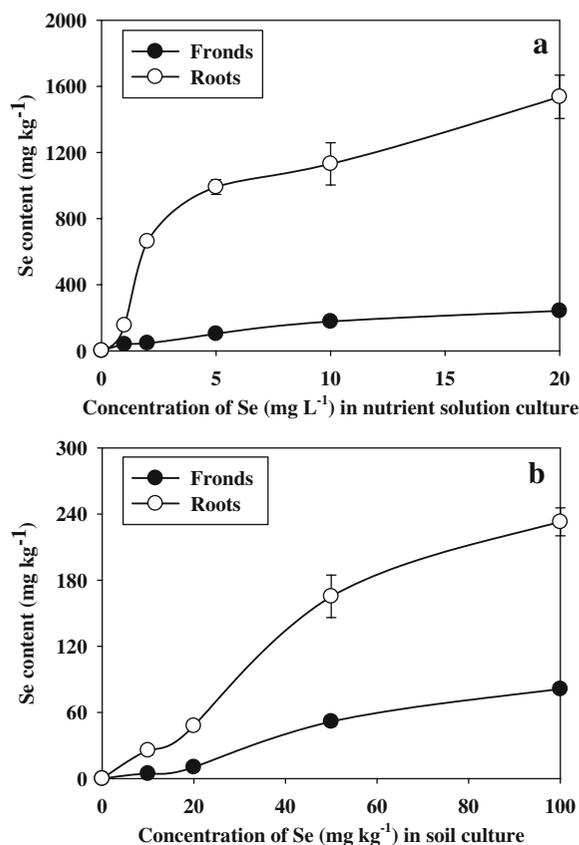
### Se uptake in Chinese brake fern

After 14 d Se exposure in nutrient solution and 30 d exposure in soils, no visible toxic symptoms were observed in Chinese brake fern, suggesting this fern

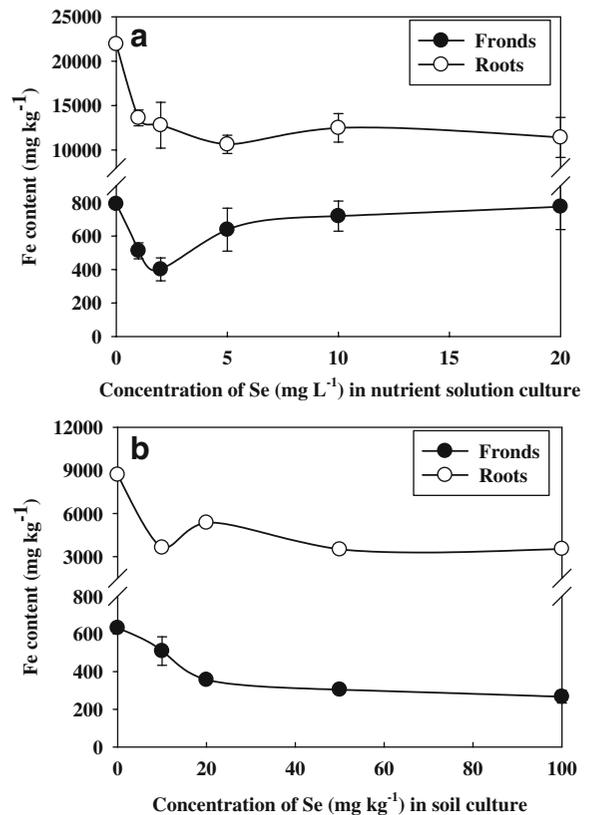
possessed an uncommon tolerance to Se. The uptake of Se by Chinese brake fern increased significantly with the addition of Se, with more Se being taken up by roots than fronds (Fig. 1a, b). Ferns in nutrient solution took up remarkably higher content of Se than those in soils, with the highest Se content measured as 1,573 mg kg<sup>-1</sup> and 263 mg kg<sup>-1</sup> in nutrient solution culture, as compared to those of 233 mg kg<sup>-1</sup> and 81 mg kg<sup>-1</sup> in soil culture, in the roots and fronds, respectively (Fig. 1a, b). In both the fronds and roots of Chinese brake fern, the highest Se content in soil culture was even lower than the Se content at the 5 mg L<sup>-1</sup> Se exposure in solution culture.

#### Effects of Se on the uptake of Fe

In nutrient solution culture, the content of Fe in the fronds encountered significant decreases at the Se



**Fig. 1** Selenium content in the fronds and roots of Chinese brake fern grown in nutrient solution and soils spiked with different doses of selenite. Bars on the curves are standard errors for the mean of three replications



**Fig. 2** Fe content in the fronds and roots of Chinese brake fern grown in nutrient solution and soils spiked with different doses of selenite. Bars on the curves are standard errors for the mean of three replications

exposure levels of 1–2 mg L<sup>-1</sup>, however, with the exposure levels exceeding 2 mg Se L<sup>-1</sup>, the Fe content in the fronds became to gradually increase, and returned to the initial level as the control (Fig. 2a). On the contrary, the frond Fe content in soil culture was gradually suppressed by the addition of Se (Fig. 2b). This was also the case for the root Fe content in both nutrient solution and soil cultures, with only significantly decreases in their contents being observed (Fig. 2a, b).

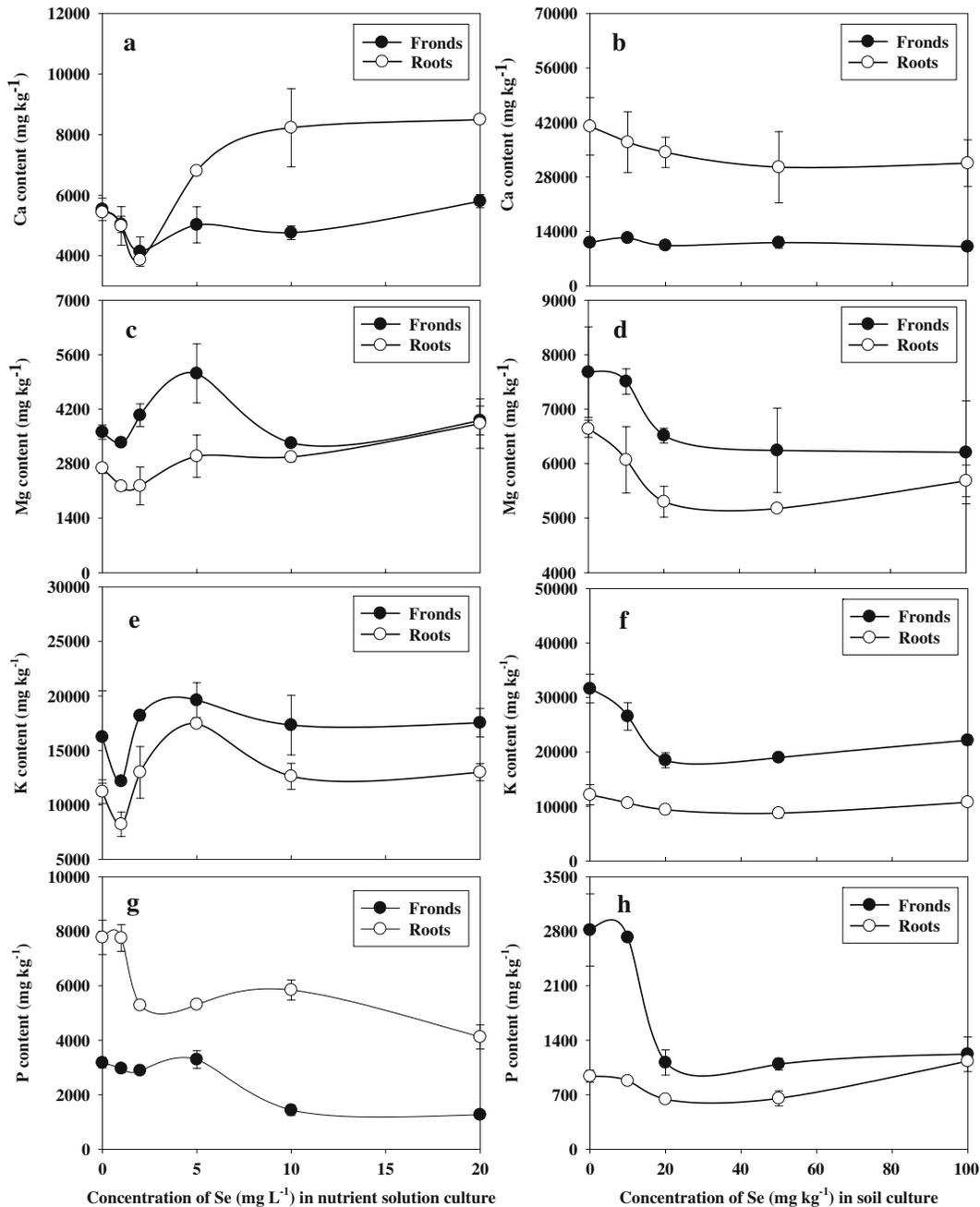
#### Effects of Se on the uptake of Ca

In nutrient solution culture, the Ca content in the fronds significantly decreased with Se addition lower than 2 mg Se L<sup>-1</sup>, and then it became to increase up to the initial content as the control. This is consistent with the variation pattern of Fe. Different with root Fe content in nutrient solution culture, rapid increase for

root Ca content in nutrient solution culture was observed when Se exposure level was greater than  $2 \text{ mg L}^{-1}$ . The root Ca content at 5 and  $10 \text{ mg Se L}^{-1}$  exposure was 25% and 52% greater than that of the control, respectively (Fig. 3a). In soil culture, only

slight decrease was observed for Ca content with Se addition, both in the fronds and roots (Fig. 3b).

Interestingly, in nutrient solution culture, the initial content of Ca in the fronds of Chinese brake fern was almost equal to that in the roots, however, with the



**Fig. 3** Ca, Mg, K and P contents in the fronds and roots of Chinese brake fern grown in nutrient solution and soils spiked with different doses of selenite. Bars on the curves are standard errors for the mean of three replications

treated levels of Se increasing, the Ca content of roots became higher than that of fronds (Fig. 3a). Whereas under soil culture, the Ca content of roots was always higher than that of fronds (Fig. 3b).

#### Effects of Se on the uptake of Mg

The frond Mg content firstly slightly decreased with Se addition up to  $1 \text{ mg L}^{-1}$ , and then became to increase significantly, with peak values appeared at the treatment of  $5 \text{ mg Se L}^{-1}$ , and finally they gradually decreased to the initial content as the control (Fig. 3c). In soil culture, the addition of Se up to  $20 \text{ mg kg}^{-1}$  gradually inhibited the uptake of frond Mg, whereas with Se addition greater than  $20 \text{ mg kg}^{-1}$ , no further such inhibition was observed (Fig. 3d).

In nutrient solution culture, the root Mg content firstly gently decreased with Se addition up to  $2 \text{ mg Se L}^{-1}$  and then became to gradually increase, especially at the treatment concentration of  $20 \text{ mg Se L}^{-1}$ , where a significant increase in root Mg content was observed (Fig. 3c). In soil culture, only significant decreases were observed with Se addition (Fig. 3d).

#### Effects of Se on the uptake of K

The variation patterns of K in the fronds and roots with Se addition were, to some extent, similar to those of Mg, both in nutrient solution and soil cultures (Fig. 3e, f). However, in nutrient solution culture, the increasing extents of K content in both the fronds and roots with Se addition greater than  $1 \text{ mg L}^{-1}$  were less than those of Mg content, and no significant peak values like those of K were observed (Fig. 3e).

#### Effects of Se on the uptake of P

In nutrient solution culture, the addition of Se significantly inhibited phosphorus (P) uptake in the roots of Chinese brake fern when Se addition was lower than  $2 \text{ mg L}^{-1}$ . This was consistent with those of Fe, Ca, Mg and K uptakes. When Se levels were lower than  $5 \text{ mg L}^{-1}$ , the frond P content was not evidently affected by the addition of Se; however, with Se levels exceeding  $5 \text{ mg L}^{-1}$ , significant inhibition of P uptake was observed (Fig. 3g). In soil culture, the variation pattern of P in the fronds of

Chinese brake fern was similar to those of Mg and K. The only difference lied in that, with Se addition up to  $20 \text{ mg kg}^{-1}$ , a much deeper suppression of P than those of Mg and K was observed (Fig. 3h). Simultaneously, it was worthy to note that in nutrient solution culture, the content of P in the roots was much higher than that in the fronds; whereas the reverse was seen in soil culture (Fig. 3g, h).

#### Effects of Se on the uptake of Mn, Cu, Zn

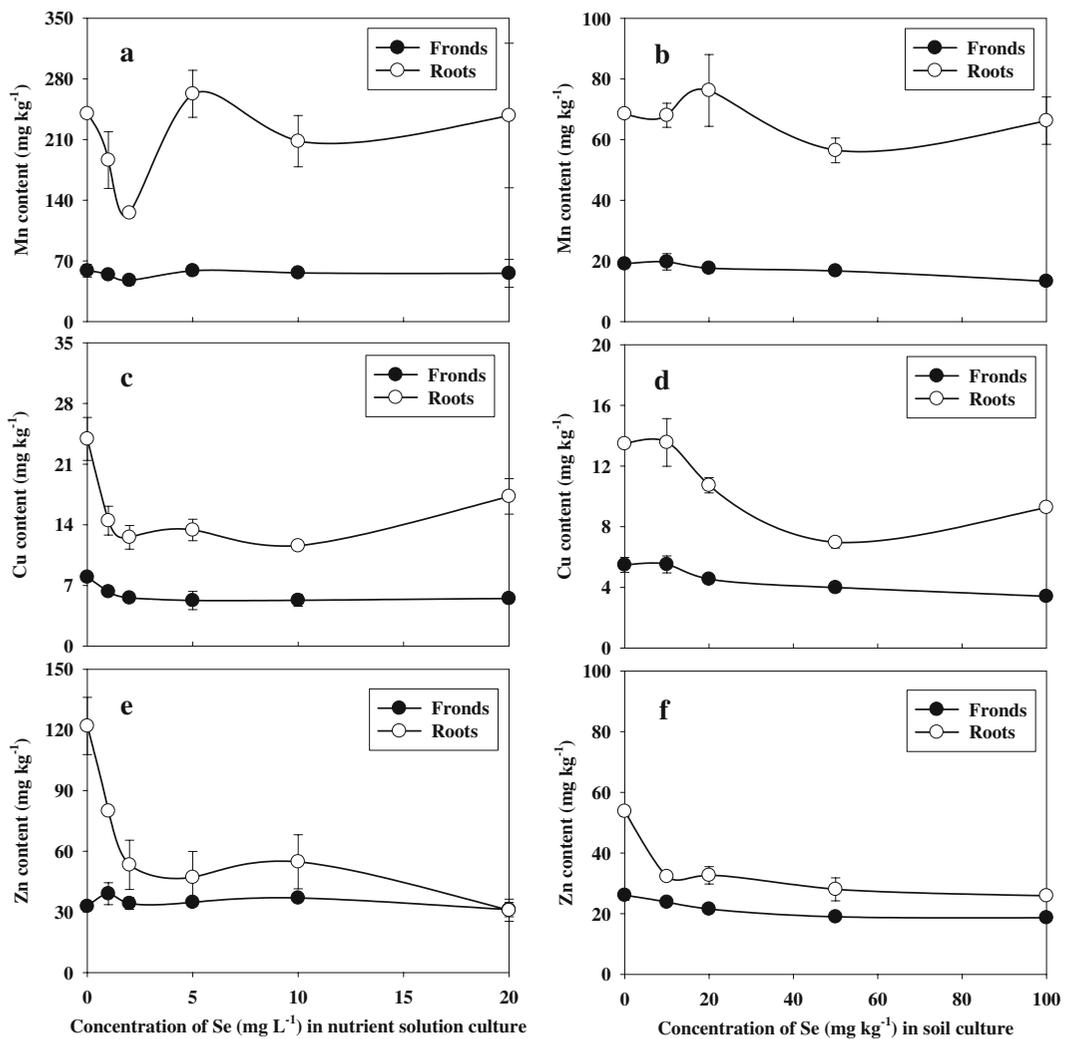
In both nutrient solution and soil culture, no significant changes was observed for the frond Mn content in Chinese brake fern with Se addition (Fig. 4a, b). In nutrient solution culture, Mn uptake by the roots of Chinese brake fern was only suppressed with Se addition up to  $5 \text{ mg L}^{-1}$  (Fig. 4a), whereas in soil culture, only very slight changes in Mn content in the roots with Se addition were observed (Fig. 4b).

The variation patterns in the content of Cu and Zn in the fronds of Chinese brake fern were generally similar, no significant changes was observed for plant uptakes of these two elements with Se addition. When Se addition was low, the root Cu uptake was significantly inhibited; however, with Se addition increasing to be higher, slight increases in Cu uptake of roots were observed, both in nutrient solution and soil culture (Fig. 3d). Zinc content in the roots of Chinese brake fern was also greatly suppressed with lower levels of Se addition, in both nutrient solution and soil culture (Fig. 3e, f). In addition, more Cu and Zn were accumulated in the roots than that in the fronds of Chinese brake fern (Fig. 4c-f).

## Discussion

#### Chinese brake fern as a Se-accumulator

In nutrient solution culture, the greater Se accumulation in roots than that in fronds, plus high content of Se in both parts of Chinese brake fern, suggest a remarkable tolerance to Se for this fern. This fern can be categorized as a Se-accumulator, according to the standards suggested by Baker (1981). Simultaneously, the higher Se accumulation for Chinese brake fern in nutrient solution culture than that in soil culture indicate that selenite might probably be adsorbed in soils (Rajan and Watkinson 1976). The adsorption of



**Fig. 4** Mn, Cu and Zn contents in the fronds and roots of Chinese brake fern grown in nutrient solution and soils spiked with different doses of selenite. Bars on the curves are standard errors for the mean of three replications

Se in soils may significantly retard the uptake of Se by Chinese brake fern.

#### Uptake of essential elements under Se exposure in Chinese brake fern

The present study examined the effects of Se on the uptake of essential elements in Chinese brake fern. Generally, two opposite traits of inhibition and stimulation were found, which might depend on the tissue Se content. When Se content was lower than 104 mg kg<sup>-1</sup> in fronds and 991 mg kg<sup>-1</sup> in roots, slight to remarkable suppressions of most tested elements by the addition of Se were observed, suggesting the antagonistic effects of Se on these

elements. However, elevated exposure to Se could stimulate the uptakes of Fe, Ca, Mg, K and Mn to various extents, especially in the roots.

As expected, the addition of Se lower than  $\leq 2$  mg L<sup>-1</sup> in the nutrient solution significantly inhibited the uptake of Fe in the fronds of Chinese brake fern, whereas the addition of higher amounts did not. However, the Se effect of regulation on the Fe uptake was not observed in the soil culture (Fig. 2a, b). This might be because an active adsorption reaction between selenite and Fe<sub>2</sub>O<sub>3</sub> existed in the soils (Dhillon and Dhillon 1999), or due to the very low content of Se in the fronds and roots of Chinese brake fern in soil culture as compared to those in nutrient solution culture.

Consequently, it is speculated that low Se content might decrease, whereas high Se content might enhance, the content of Fe (possible catalytic iron) in the fronds of Chinese brake fern. Such changes in Fe content then could result in the variation of lipid peroxidation from alleviation to enhancement, exhibiting dual effects of Se on plants. Previous reports have found the relationship between Fe content in plant tissues and oxidative stress. For instance, Iturbe-Ormaetxe et al. (1995) found that in pea plant (*Pisum sativum* L. cv. Frilene), the deficiency of Fe did not cause the accumulation of oxidatively damaged lipids in the leaves. In the chlorotic leaves of Fe-starved mulberry (*Morus alba* L. cv. Kanva-2), maize (*Zea mays* L. cv. GSF-2), and cauliflower (*Brassica oleracea* var. *botrytis* L. cv. Snowball-16), the MDA content was significantly lower than that in green leaves or non-chlorotic leaves (Tewari et al. 2005). Under drought stress, the concentrations of catalytic iron in pea plants increased by 1.5 fold with simultaneous oxidized lipids and proteins accumulation in leaves (Moran et al. 1994).

Similar to Fe, the changes from inhibition to stimulation effect of Se on the uptakes of Ca, Mg and K were also observed (Fig. 3a-f). The uptake stimulations of Ca, Mg and K at high concentration of Se exposure might be associated with the Se tolerance in Chinese brake fern. The increase of Ca content in plant tissues was also observed by He et al. (2004) and by Wu and Huang (1992) under selenate stress. Similarly, under arsenic stress, increasing Ca content was found in *Spartina alterniflora* (Carbonell et al. 1998) and winter wheat (*Triticum aestivum* L.) (Liu et al. 2008). Increases in Mg content in this study was in line with the results of He et al. (2004) in the field experiment. The alleviating roles of Mg to aluminium (Al) (Keltjens and Tan 1993) and Cd (Kashem and Kawai 2007) toxicities were also documented. Under As exposure, the enhancement of K content in Chinese brake fern was found by Tu and Ma (2005).

The increases in Ca, Mg and K content with Se addition in Chinese brake fern might suggest an element regulation mechanism existence for Se tolerance. This is because that these three elements can play important physiological roles in plants. For instances, Ca can maintain the membrane integrity (Kirkby and Pilbeam 1984); Mg appears to provide a protective function in maintaining plant tissue integrity (Wilkinson et al. 1990); K can provide a correct

ionic environment in cytoplasm for metabolic processes, thereafter maintaining the normal osmotic pressure in cells and inhibiting the production of reactive oxygen species (ROS) (Leigh and Wyn Jones 1984; Ismail 2005). Since high level exposure of Se can cause the enhancement of membrane permeability, damage the membrane integrity and result in high oxidative stress in plants (Hartikainen et al. 2000; Xue et al. 2001), the enhanced uptake of Ca, Mg and K by Chinese brake fern might indicate the involvements of these three elements in the tolerance mechanism of Se, particularly in its roots where Se mainly stored (Fig. 1a, b).

In terms of P, addition of Se invariably suppressed its uptake in both parts of Chinese brake fern in the nutrient solution and soil cultures (Fig. 3g, h). This might be derived from the competition effect between  $\text{SeO}_3^{3-}$  and  $\text{PO}_4^{3-}$ . Evidence from the report of Belokobylsky et al. (2004) indicates that when anion of  $\text{SeO}_3^{3-}$  penetrates inside the cell by specific channels of anion transport, the competition between  $\text{SeO}_3^{3-}$  and  $\text{PO}_4^{3-}$  presents. In this study, most P was stored in the roots of Chinese brake fern in nutrient solution culture, contrary to the general principle that P is stored mainly in the shoots of plants, therefore, more obvious inhibition on the uptake of P by Se in the roots might be expected (Fig. 3g). The inhibition of Se to P found in Chinese brake fern is in agreement with that of Hopper and Parker (1999), who found the uptakes of P were inhibited by Se in perennial ryegrass (*Lolium perenne* L. cv. Evening Shade) and strawberry clover (*Trifolium fragiferrum* L. cv. O'Conner).

Mg, Cu and Zn, although essential, may also produce toxicity to plants at high content (Kabata-Pendias and Pendias 2001). These elements were mostly accumulated in the roots of Chinese brake fern, both in nutrient solution and soil cultures (Fig. 4a-f). Consequently, Se produced evident inhibition to Mg, Cu and Zn in the roots, whereas in the fronds, no such effects were observed, suggesting great antagonistic role of Se to these three elements.

The results of the present study suggest that the uptake regulations of some essential elements by Se may indicate the involvement of Ca, Mg and K in the tolerance mechanism of Se. Fe might possibly act as an important factor to influence the extent of lipid peroxidation in Chinese brake fern exposed to Se. However, further investigations are needed to substantiate this hypothesis. The finding of this study that

Se may regulate the uptake of some major nutrient elements may shed light on the tolerance mechanisms of Se in Chinese brake fern.

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