RESEARCH ARTICLE

Alleviation of cadmium toxicity in cucumber (*Cucumis sativus*) seedlings by the application of selenium

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Abstract

In the present study, the role of selenium in cadmium toxicity was investigated in cucumber seedlings by hydroponic experiments. The application of Se for cucumber exposed to Cd significantly reduced Cd accumulation in all tissues, elevated Cd-depressed chlorophyll content, and improved photosynthetic performance. External Se significantly reduced $\cdot$OH, H_2O_2 and malondialdehyde content. Exogenous Se balanced Cd-depressed elements (*e.g.*, Se enhanced Cd-induced decreases in root Zn, leaf/stem/root Mn concentrations) and carbohydrate contents. External Se also significantly decreased the Cd-induced increases in Na^+K^+-, $Ca^{2+}Mg^{2+}$- and total ATPase activities, which recovered almost to control level. Results indicate that application of Se can alleviate Cd toxicity in cucumber seedlings by reducing Cd uptake and reactive oxygen species (ROS) accumulation, moreover protecting photosynthetic machinery from damaging, balancing elements and carbohydrate contents, and improving ATPase activities in cucumber.

Introduction

Cadmium (Cd) is an unessential element for plants. When occurs in excess in the growth medium it becomes toxic to plants, which can naturally occur either in soil or by the anthropogenic activities, such as industrial mining, phosphate fertilizers application etc. (Loganathan & Hedley, 1997). Gallego *et al.* (2012) indicated that out of an estimated 30,000 tonnes of annual Cd addition to the environment, 4,000-13,000 tonnes are contributed by human activities. Moreover, plant roots can easily absorb Cd and transfer it to their edible parts; hence, Cd is also a toxic trace pollutant for humans,

animals and plants via food chain (Bur *et al.*, 2010). Once in plants, Cd can bring a host of biochemical and physiological consequences. Cd-accrued inhibited photosynthesis and impaired growth have been reported in several plants including barley, cucumber (Gallego *et al.*, 2012; Janicka-Russak *et al.*, 2012; Cao *et al.*, 2014a; Asgher *et al.*, 2015). Cd is a non-redox active metal, it can induce generation of reactive oxygen species (ROS), lead to the oxidative burst. Thus, it's imperative to find effective and low-cost strategies to bring down Cd toxicity for the sake of humans health.

Selenium (Se) has also been found to be beneficial to plants, and it is an essential nutrient for humans

(Thomson, 2004; Liu *et al.*, 2015). Vegetables grown in Se-enriched media could improve their photooxidative stress tolerance (Breznik *et al.*, 2005; Pedrero *et al.*, 2008). Such as, Se supplementation could significantly decrease lettuce Cd and Pb accumulation, and Se is also capable of abating rice Cd accumulation (He *et al.*, 2004; Lin *et al.*, 2012). Meanwhile, Liu *et al.* (2015) reported that Cd suppressed plant growth, chlorophyll contents and photosynthetic performance, as well as nitrogen contents of tobacco plants were increased when grown in the media containing Se. Our previous study (Sun *et al.*, 2013) also indicated that Se could alleviate Cd-inhibited maize plant growth and lower its Cd concentration. Moreover, some researches on vegetal species demonstrated that Se has the ability of decreasing toxic metals accumulation and could prevent their oxidative stress (Thangavel *et al.*, 1999; Muñoz *et al.*, 2007), but the literature is limited, and further researches are required.

In China, Se deficient is detected in approximately two-thirds of cultivated soils, so the people living at these regions often leads to Se lack (Zhang *et al.*, 2014). In addition, cucumber is one of the vital vegetables at present; however, a large area of cucumber fields is contaminated by Cd (Feng *et al.*, 2010). Therefore, the question is whether the addition of Se in Chinese cultivated cucumber genotypes could alleviate Cd toxicity and depress Cd accumulation, and to clarify the possible mechanisms. The present research used a pot experiment to make clear the possible function of exogenous Se in alleviating Cd-induced plant growth inhibition, Cd and other microelements uptake and distribution, carbohydrate contents, oxidative stress and ATPase activities in cucumber seedlings. We aimed to find an acceptable and cost-effective approach to reduce cucumber Cd toxicity and to investigate its underlying physiological and biochemical mechanisms for its further commercial application.

Material and methods

Plant material and experimental designs

A pot experiment was undertaken in growth season in the greenhouse of Taiyuan University of Science and Technology, Taiyuan, China, using the common cultivated cucumber ˙Jinyan 4˙. Soil was collected from the experimental farm (depth 0–15 cm) in the campus. Air-dried soil was sieved (the mesh diameter is 1 cm) and 4.5 kg of the soil was loaded in a plastic pot (5 L, 20 cm height). The soil in each pot was fertilized with 1 L of basal nutrient solution (BNS). The composition of the BNS was described in Sun *et al.* (2013). Cd (as

CdCl$_2$) and Se (as Na$_2$SeO$_3$) were added to the corresponding pots to form four treatments: (1) control, BNS; (2) Se, BNS+6 μM Se; (3) Cd, BNS+100 μM Cd; and (4) Cd+Se, BNS+100 μM Cd+6 μM Se. There were 3 replicates for each treatment. Healthy cucumber seeds were sown in the above mentioned pots 20 days after application of BNS, Cd and Se. One week after emergence, each pot was thinned to four seedlings. After one month of treatment, plants from both control and treatments were sampled for physiological parameters measurements, and biochemical analysis.

Chlorophyll contents, photosynthesis and chlorophyll fluorescence parameters analysis

The topmost fully expanded leaves from three random plants of each treatment were sampled for measurement of chlorophyll content. Chl was extracted with 80% acetone, analyzed on a spectrophotometer (UV-1750, Shimadzu, Japan) according to the method of Lichtenthale & Wellburn (1983). The net photosynthetic rate (*Pn*), stomatal conductance (*Gs*), transpiration rate (*Tr*) and intercellular CO$_2$ concentration (*Ci*) were measured using a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). The chlorophyll fluorescence parameters were measured with an IMAGINGPAM (chlorophyll fluorometer) system (Zhang *et al.*, 2015).

Plant growth, Cd and other microelement determination

After one month of treatment, plants were uprooted and separated into roots, stems and leaves, and plant height of each plant was measured. Roots were soaked in 20 mM Na$_2$EDTA for 20 min to eliminate adsorbed ions and possible chemical contamination on surfaces and then rinsed in deionized water; then all samples were dried at 70°C until constant weight, and weighed, respectively. Dried samples were powdered and digested in acid mixture (HNO$_3$:HClO$_4$, 5:1, v/v). Cd, Zn, Mn and Cu concentrations were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPS 1200 VR, Seiko Co., Ltd., Japan).

Carbohydrate contents measurements

Carbohydrates were extracted from 0.2 g of freeze-dried samples with 50 mL of 80% ethanol (v/v); the supernatant was used for analysis of hexose, sucrose,

and total soluble sugars using a modified phenolsulfuric acid method (Buysse & Merckx, 1993). The residue was boiled for 3 hours in 10 mL of 2% HCl (v/v) to hydrolyze starch, and the supernatant was analyzed for soluble sugars released from starch by acid hydrolysis (Yu *et al.*, 2004).

·OH, H$_2$O$_2$ and lipid peroxidation determination

The hydroxyl free radical (·OH) and H$_2$O$_2$ contents were determined using a ·OH detection kit and a hydrogen peroxide assay kit, respectively (Liu *et al.*, 2015). The level of lipid peroxidation was quantitated by the amount of malondialdehyde (MDA) which was determined by thiobarbituric acid reaction in all treatments (Wu *et al.*, 2003).

Determination of ATPase activities

The frozen cucumber tissues (0.4 g) were homogenized in 8 mL of 50 mM phosphate buffer saline (pH 7.8) using a pre-chilled mortar and pestle; the homogenate was centrifuged for 15 min at $10000 \times g$ at 4 °C, then the supernatant was used to assays of activities of enzymes. ATPase activities were determined by measuring the release of inorganic phosphate (Pi) using the activity assay kit (Jiancheng Bioengineering Institutes, China).

Table 1. Effect of external Se addition on microelement concentration in leaves, stems and roots of cucumber seedlings exposed to Cd for one month.

Treatment	Element concentration (mg/kg DW)			
	Cd	**Zn**	**Mn**	**Cu**
Leaf				
Control	0.04[c]	125.31[b]	321.14[a]	8.53[b]
Se	0.04[c]	120.16[b]	326.86[a]	8.31[b]
Cd	11.96[a]	149.84[a]	111.23[c]	11.64[a]
Cd+Se	6.84[b]	151.25[a]	299.80[a]	7.23[b]
Stem				
Control	0.06[c]	267.29[c]	434.33[a]	34.79[b]
Se	0.07[c]	242.17[c]	416.03[a]	36.63[b]
Cd	45.61[a]	635.54[a]	273.69[c]	54.18[a]
Cd+Se	33.88[b]	478.61[b]	362.65[b]	39.89[b]
Root				
Control	0.09[c]	683.86[a]	587.68[a]	186.20[b]
Se	0.10[c]	629.53[ab]	563.08[a]	175.72[b]
Cd	345.55[a]	553.97[b]	226.59[c]	218.09[a]
Cd+Se	264.33[b]	662.92[a]	360.58[b]	188.46[b]

Different uppercase letters indicate significant differences ($p \le 0.05$) among the four treatments. DW represents dry weight.

Statistical analysis

Each experiment was repeated three times. All statistical analyses were carried out with Data Processing System (DPS) statistical software package using ANOVA, followed by Duncan's Multiple Range Test (SSR) with statistical significance of $p \le 0.05$.

Results

Cd accumulation in cucumber seedlings under Cd and Se application

Cadmium concentrations of all tissues are shown in Table 1. In control condition, Cd concentration was lower than 0.1 mg/kg DW, and it was within the safe range. Cd treatment positively affected the cucumber seedlings Cd concentration in all tissues, especially in the roots (Table 1). Contrary to Cd-alone treatment, application of Se negatively affected the Cd concentration, and significantly decreased by 42.8%, 25.7% and 23.5% in leaves, stems and roots, respectively.

Alleviatory effect of exogenous Se on plant growth, chlorophyll content, photosynthetic and chlorophyll fluorescence parameters under Cd stress

Under non-stress conditions, Se addition had no significant effect on the growth of cucumber plants (Fig. 1). Cd treatment significantly decreased plant height, root, stem and leaf DW in cucumber seedlings. Moreover, under Cd treatment, Se addition (Cd+Se) significantly increased these parameters by 20.3%, 61.3%, 34.7% and 26.2%, respectively, and improved plant growth. In addition, compared with control, Cd treatment significantly decreased both chlorophyll a and b content, also chlorophyll a+b and a/b ratio. Significantly, plants for Cd+Se treatment showed higher chlorophyll a and b content than those for Cd treatment alone. Also, the addition of Se (Cd+Se) greatly alleviated Cd-induced reductions in chlorophyll a+b, which were 25.4% higher than those for Cd alone-treated plants (Fig. 2).

Exposure to Cd stress significantly depressed the values of *Pn*, *Tr* and *Gs* by 44.9%, 39.1% and 27.6% respectively, compared to the control (Fig. 3). The addition of Se (Cd+Se) greatly alleviated the inhibitory effect of Cd toxicity in photosynthetic parameters, such as *Pn* increased by 39.8% compared with Cd stress merely (Fig. 3a). Furthermore, the *Ci* increased
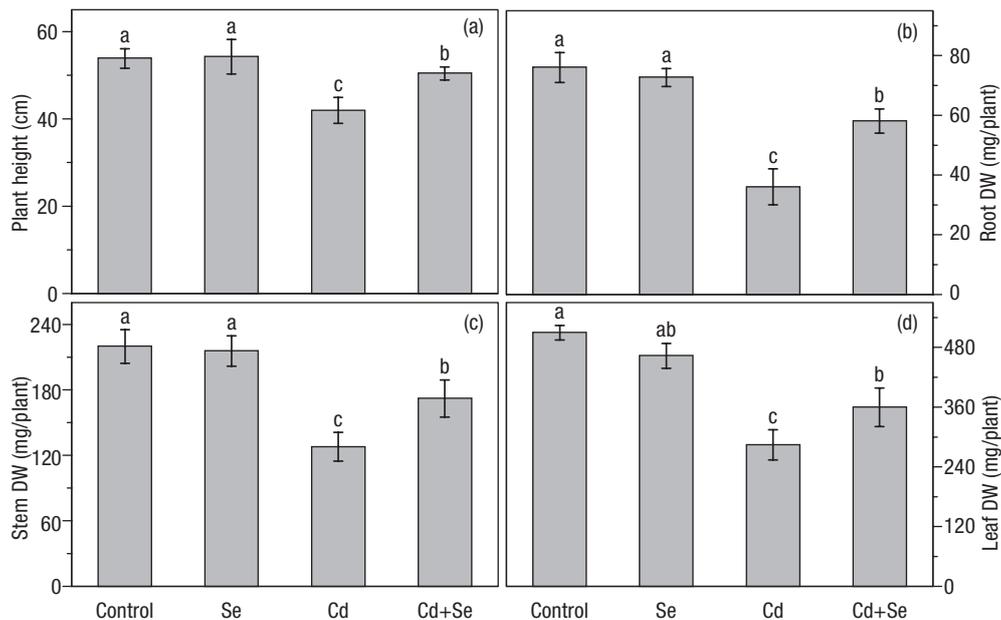
Figure 1. Effect of external selenium addition on plant height and biomass of cucumber seedlings exposed to cadmium for one month. DW, dry weight; pl, plant. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.
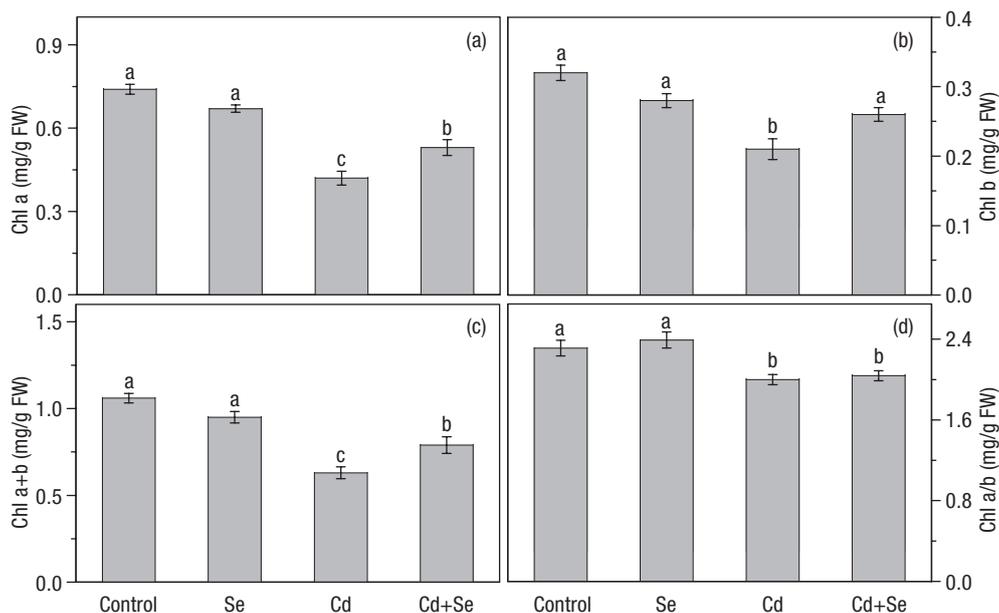

Figure 2. Effect of external selenium addition on chlorophyll content in leaves of cucumber seedlings exposed to cadmium for one month. FW, fresh weight. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.

significantly after Cd treatment, while decreased after addition of Se (Fig. 3d). And there were no significant differences between plants treated with Se alone and the control plants (Fig. 3).

The maximum efficiency of photosystem II photochemistry (Fv/Fm) decreased under Cd stress; however, initial fluorescence (F_0) increased significantly.

Addition of Se improved Fv/Fm, and distinctly suppressed Cd-induced increase in the F_0 by 22.5% (Fig. 4a,b). There were no significant changes between Se alone and the control plants. With respect to quantum yield of non-regulated energy dissipation $Y(NO)$, there was no significant difference among the four treatments (Fig. 4c).
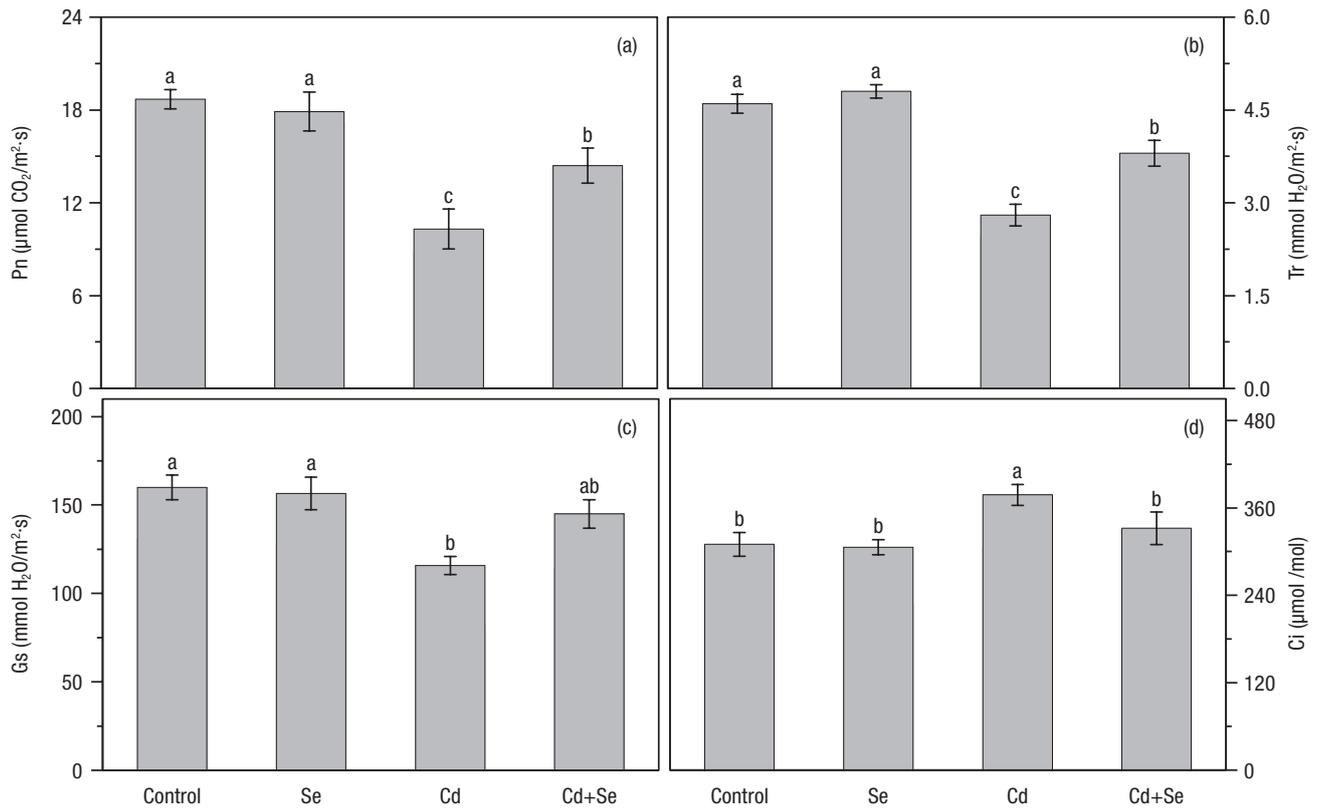
Figure 3. Effect of external selenium addition on photosynthesis parameters of cucumber seedlings exposed to cadmium for one month. *Pn,* net photosynthetic rate; *Tr,* transpiration rate; *Gs,* stomatal conductance; *Ci,* intercellular CO_2 concentration. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.
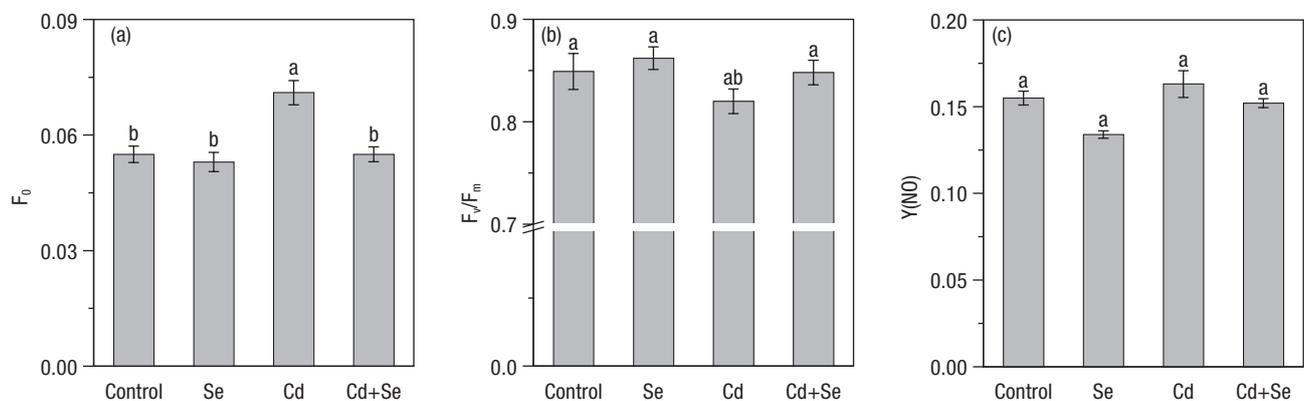

Figure 4. Effect of external selenium addition on chlorophyll fluorescence parameters (*Fo, Fv/Fm, Y(NO)*) of cucumber seedlings exposed to cadmium for one month. *Fv/Fm* and *Y(NO)* measurements were taken after leaves had 20 min of dark adaption. F_0, initial fluorescence; *Fv/Fm*, maximum efficiency of photosystem II photochemistry; *Y(NO)*, quantum yield of nonregulated energy dissipation. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.

Effect of Se and Cd on micro-elements

Compared with control, Cd treatment significantly decreased Zn concentration in root, and Mn concentration in leaf, stem and root. Moreover, these reductions in mineral concentrations were lower in plants in the Cd+Se treatment than Cd-alone treatment, and exogenous Se distinctly brought root Zn and leaf Mn concentration back to the control level. Such as, leaf Mn and root Zn concentration under Cd+Se was 169.5% and 19.7 higher than those under Cd alone treatment. With respect to the stems and roots Mn, the concentrations were intermediate between those of control and Cd treated plants (Table 1).

On the other side, plants under Cd treatment showed higher Zn concentration in leaf and stem, and Cu concentration in all tissues (leaf, stem and root) than those under control. However, exogenous Se (Cd+Se) significantly suppressed Cd-induced increase in stem Zn, leaf, stem and root Cu concentration, e.g. Cu concentrations in Cd+Se being 37.9%, 26.4% and 13.6% in leaves, stems and roots, respectively, lower than the Cd alone treatment (Table 1).

Effect of Se and Cd on carbohydrate contents

Cadmium treatment significantly increased total soluble sugar, sucrose and hexose contents. The change of sucrose was the most significant; the sucrose content for Cd treated plants were 49.1%, 57.9% and 84.8% higher than those for control plants in leaves, stems and roots, respectively (Table 2). Furthermore, the combination of Se and Cd completely inhibited the induction of these three parameters by Cd alone, and brought them back to the control level except for the root hexose which was higher than the control. In terms of starch contents, root starch under Cd treatment was 39.6% lower than control, while exogenous Se markedly increased the content by 23.8% compared with Cd-alone treatment. However, no significant difference was detected in leaf and stem starch among the four treatments (Table 2).

Effect of Se and Cd on ·OH, H_2O_2 and lipid peroxidation contents

A distinct increase in the accumulation of ·OH was observed in plants under Cd exposure (Fig. 5), Se addition in 100 μM Cd markedly reduced Cd-induced ·OH accumulation, c.f. Se + Cd treatment being 38.4%, 20.4% and 16.2% in leaves, stems and roots, respectively, lower than Cd stress (Fig. 5a-c). The response of leaves and roots H_2O_2 to Cd and Se resembled to that of ·OH (Fig. 5d-f). Cd stress also increased MDA accumulation, Se addition effectively inhibited the Cd-induced MDA ac-

cumulation in shoots (c.f. 54.5 % and 29.9 lower in leaves and stems than the Cd-alone treatment, respectively), while there was no significant effect on root MDA content. In addition, no significant change in MDA content was found in seedlings treated with Se (without Cd) in comparison with control (Fig. 5g-i).

Responses of ATPase activities to Cd and Se application

Activities of ATPase under each treatment are shown in Fig. 6. There was a significant difference among different cucumber tissues, and the highest value was observed in the roots, followed by stems and leaves, simultaneously, a significant difference was also found between Cd and Se-mediated effects on ATPase activity. Cucumber seedlings treated with Cd showed significant increase in Na^+K^+-ATPase, being 52.1%, 30.1% and 11.1% higher than those in control. Se addition (Cd+Se) counteracted Cd-mediated increase of Na^+K^+-ATPase activity, giving values 23.1% in leaves, 12.0% in stems and 21.7 % in roots lower than those in Cd alone (Fig. 5a-c). Similar tendencies were shown in $Ca^{2+}Mg^{2+}$-ATPase and stem and root total-ATPase activities. However, Cd+Se and Cd alone treatment induced no significant effects on leaf total-ATPase activity in comparison with control (Fig. 6).

Discussion

The global problem of soil Cd contamination is serious and Cd causes abiotic stress to plants (Uraguchi & Fujiwara, 2012), strategies for reducing contamination are urgently desired. For animals and humans Se is an indispensable element that is also beneficial to plants under low concentration, however Se deficiency is general in some countries, such as China, India and Egypt where it is a common problem (Bañuelos et al., 2011; Feng et al., 2013). To counteract this problem, Se content in the edible crops organs has been increased through base Se addition or foliar sprays of Se compounds (e.g. selenate

Table 2. Effect of external Se addition on carbohydrate contents (mg/g dry weight) in leaves, stems and roots of cucumber seedlings exposed to Cd for one month.

Treatment	Total soluble sugar			Sucrose			Hexose			Starch		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Control	33.2[b]	13.9[b]	26.9[c]	16.9[b]	9.5[b]	16.4[c]	19.7[b]	10.4[b]	15.9[c]	0.44[a]	0.23[a]	1.39[a]
Se	31.7[b]	13.4[b]	25.3[c]	18.6[b]	8.9[b]	16.2[c]	20.1[b]	9.9[b]	14.6[c]	0.46[a]	0.25[a]	1.44[a]
Cd	36.9[a]	18.4[a]	43.8[a]	25.2[a]	15.0[a]	30.3[a]	24.9[a]	14.6[a]	27.7[a]	0.35[ab]	0.18[ab]	0.84[b]
Cd+Se	30.1[b]	14.1[b]	31.6[b]	17.8[b]	8.2[b]	23.4[b]	20.1[b]	11.4[b]	20.3[b]	0.40[a]	0.21[a]	1.04[a]

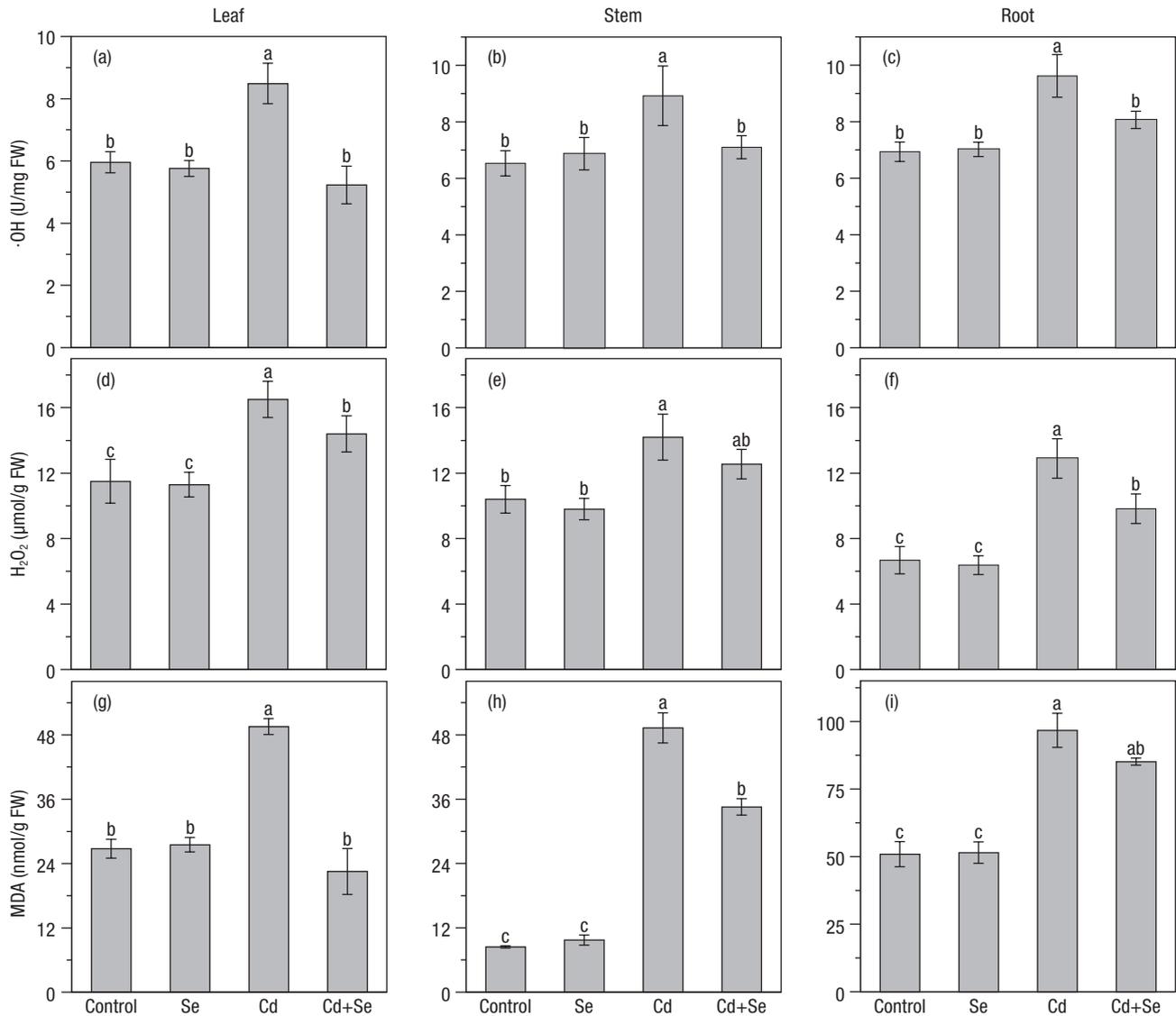Different uppercase letters indicate significant differences ($p \leq 0.05$) among the four treatments.

Figure 5. Effect of external selenium addition on ·OH, H_2O_2 and MDA contents in leaves (*left*), stems (*middle*) and roots (*right*) of cucumber seedlings exposed to cadmium for one month. FW, fresh weight. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.

and selenite) (Hartikainen, 2005). In addition, Feng *et al.* (2013) and Saidi *et al.* (2014) found that Se could counteract various abiotic stresses in plants; however, the associated mechanisms are still to be little elucidated. In this paper, we have concentrated on identifing the possible mechanisms and the influence of Se in conferring tolerance of cucumber seedlings against Cd toxicity.

Exogenous Se decreases Cd accumulation

Increasing evidence has indicated that heavy metals such as Cd, As, Hg and Pb toxic symptoms to plants were alleviated by Se via decreasing their uptake (Ebbs & Leonard, 2001; He *et al.*, 2004; Muñoz *et al.*, 2007; Yathavakilla & Caruso, 2007; Lin *et al.*, 2012; Sun

et al., 2013; Saidi *et al.*, 2014; Zhang *et al.*, 2014; Liu *et al.*, 2015). And several reports have also demonstrated that Se is poorly translocated to shoots in selenite-treated plants (Hopper & Parker, 1999); similarly, the present experiment demonstrates that most of Cd taken up by plants is accumulated in roots (Table 1). This response implies a low translocation of Cd within the cucumber plant under the Cd+Se treatment, which may be a mechanism of cucumber to defend itself toward Cd toxicity. Meanwhile, cucumber plants grown on Cd+Se medium showed the lower Cd concentration in all tissues (Table 1) in comparison with Cd treatment alone. The reduced Cd uptake might be attributable to the formation of a complex between Se and Cd which may hinder Cd uptake by root cells. Hawrylak-Nowaka *et al.* (2014) investigated complex
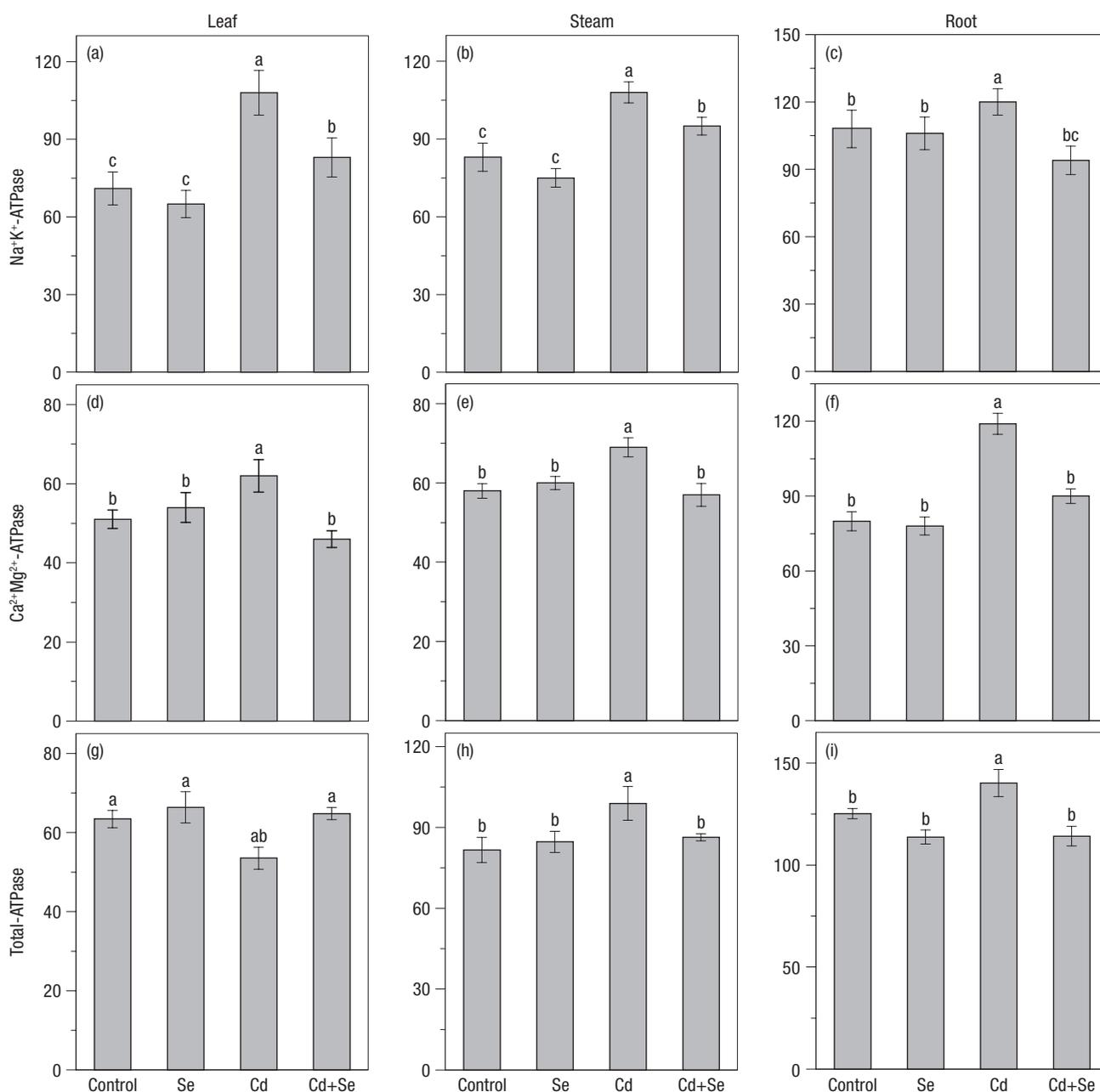
Figure 6. Effect of external selenium addition on ATPase activity (μmol Pi/g·h fresh weight) in leaves (*left*), stems (*middle*) and roots (*right*) of cucumber seedlings exposed to cadmium for one month. Error bars refer to the SD (n = 3). Different letters indicate significant differences ($p \leq 0.05$) among the four treatments.

formation between Cd and Se in aqueous solutions and found reduced glutathione (GSH) and phytochelatins (PCs) were formed in cucumber plants.

Se alleviates Cd-induced suppression in plant growth, chlorophyll content, photosynthetic and chlorophyll fluorescence parameters

In the present pot experiment, 6 μM Se supply to 100 μM Cd elevated Cd-induced reduction in plant growth

as indicated by plant height and biomass (Fig. 1). The study by Terry *et al*. (2000) has demonstrated that assimilation of Se, which requires reducing power from NADPH and GSH, occurs in the chloroplast and can alter the redox state of chloroplasts, therefore affecting biomass production. Hawrylak-Nowak *et al*. (2014) reported that the effect of Se on the Cd-stressed plants greatly depended on the proportion of these two elements in the nutrient solution. The current results provide strong evidence that Se can effectively alleviate Cd-induced cucumber seedlings plant growth inhibition,

suggesting that low micromoles of Se can exert beneficial effects on plants, although it is toxic at high concentrations (Terry *et al.*, 2000; Lin *et al.*, 2012; Haghighi & da Silva, 2016). On the other hand, it is known that one of the most universal symptoms of Cd stress in plants is leaf chlorosis; furthermore, the reduced pigment could result in photosynthetic capacities decrease. In the present study, chlorophyll a and b content, chlorophyll a+b and chlorophyll a/b ratio significantly decreased under Cd stress comparing with control (Fig. 2). On the other side, cucumber seedlings treated with Cd and Se together showed similar chlorophyll b content with control, the Cd-induced growth inhibition was greatly alleviated compared with those under Cd alone treatment. Meanwhile, addition of Se (Cd+Se) increased chlorophyll a, chlorophyll a+b content. Moreover, significant decrease in *Pn* was simultaneously accompanied by marked reduction of *Tr* and *Gs* (Fig. 3), nevertheless, the *Ci* level was significant higher under Cd stress compared with control. Addition of Se (Cd+Se) recovered cucumber photosynthetic ability, which might likely link to protective mechanisms maintain the integrity of the photosynthetic machinery. Similar results were reported by Haghighi & da Silva (2016), treatments Se1 (2 mg/L) and Se2 (4 mg/L) improved photosynthesis in Cd1 (5 μM) in cucumber (*Cucumis sativus* cv. 4200) at an early growth stage in a hydroponic system.

Recently, chlorophyll a fluorescence measurements have been used to rapidly estimate the operating quantum efficiency of electron transport through PSII in leaf plants (Feng *et al.*, 2010), and it can be as a probe of heavy metal stress in plants, as it can also reflect photosynthetic activities in a complex manner (Mateos-Naranjo *et al.*, 2008). In the present experiment, considerable increased *Fo* and reduced *Fv/Fm* were observed in cucumber under Cd stress. Similar results were observed in rice, soybean, tobacco and barley (Kao *et al.*, 2003; Cai *et al.*, 2011; Wang *et al.*, 2011; Liu *et al.*, 2015), however, there was no significant different in *Y(NO)* under Cd stress in comparison with control (Fig. 4). Generally, F_0 is also fluorescent when the reaction center of PSII is open, and the increase in F_0 under Cd stress indicates the injury of PSII (Kitajima & Butler, 1975). The decrease in *Fv/Fm* is a sign of photoinhibition; on the other side, the decrease of *Fv/Fm* under Cd stress also indicates that the photochemistry of PSII and its ability to reduce the primary acceptor Q_A are also affected by Cd; this reduction under Cd exposure may be attributed to the inhibition of chlorophyll content biosynthesis or due to an altered stoichiometry between PSI and PSII (Babani & Lichtenthaler, 1996; Ding *et al.*, 2008). A non-considerable change of *Y(NO)* under Cd stress indicates that there is no severe irreversible photodamage to PSII (Kramer *et al.*, 2004). On the contrary, addition of Se (Cd+Se) significantly enhanced Fv/Fm, but decreased Fo under Cd stress (Fig. 4a,b), indicating that cucumber plants under Cd stress could keep higher activity of reaction center by the addition of Se, the effect of Se has also been observed in Cd-treated tobacco (Liu *et al.*, 2015). Taken together, these results suggested that Se mediated photosynthesis improvement partly because of the protection of PS II reaction centers and the increase of chlorophyll synthesis, which are important for improving Cd tolerance.

Exogenous Se counteracts Cd-induced changes in micro-elements

Absorption and distribution of essential microelements, and the interaction with Cd has been reported, although there are some contradicting results (Zhang *et al.*, 2002; Wu *et al.*, 2005). In present research, Cd treatment elevated leaf/stem Zn and leaf/stem/root Cu, however decreased root Zn, leaf/stem/root Mn concentration. Se addition markedly down regulated Cd induced increase in stem Zn and Cu concentration in all tissues (Table 1), which leads to mineral deficiencies and imbalance in cucumber. Se application on Cd-treated plants reversed the tendency and counterbalanced Cd induced decrease in root Zn and leaf/stem/root Mn concentration, indicating Se could mitigate Cd toxicity through balance elements metabolism in plants. The possible reason for the conflicting results could be the differences in the methods of growth, plant species, and interactions between cultivars and metals (Lin *et al.*, 2012).

Se balances Cd-induced changes in carbohydrate contents

Photosynthesis is also limited by inorganic phosphate supply to chloroplasts, which is accompanied by triosephosphate transport and also sucrose metabolism. Therefore, we determined the effect of Se and Cd on carbohydrate metabolism. In this study, cucumber seedlings under Cd stress had higher total soluble sugar, sucrose, hexose, especially in roots, accompanied by lower root starch as compared with those under control condition (Table 2). These results suggested that Cd might regulate carbohydrate metabolism at transcriptional levels. Application of Se resulted in the decrease of above carbohydrate contents while increase root starch. These results indicated that there is an equilibrium shift between photosynthesis and respiration as previously observed under Cd excess condition (Kováčik *et al.*, 2011), and

demonstrated that Se could increase starch accumulation, thus promotes plant growth.

Se counteracts Cd-induced changes in ·OH, H_2O_2 and MDA contents

The Cd-induced accumulation of leaf ·OH, H_2O_2 and MDA in all tissues was markedly reduced by Se addition, especially leaf $O_2^{·-}$ and ·OH (Fig. 4), Se-subdued ·OH, H_2O_2 and MDA formation were supported by several recent reports (Chen *et al.*, 2010; Lin *et al.*, 2012; Sun *et al.*, 2013; Liu *et al.*, 2015), which have illustrated Se could regulate ROS production and quenching, this control in plants may be a key mechanism for counteracting environmental stress.

Se alleviates Cd-reduced ATPase activities in cucumber

ATPase could facilitate ions, organic and inorganic solutes transport in and out of the plant cells. Theoretically, preventing Cd ions from entering the cytosol through the plasma membrane is one of the best defense mechanisms (Lin *et al.*, 2012). Duby & Boutry (2009) reported Ca^{2+}-ATPase was involved in the homeostasis of Ca^{2+} signal in plant cells and was very sensitive to many abiotic stresses. In current study, Cd treatment significantly increased leaf/stem/root Na^+K^+-, $Ca^{2+}Mg^{2+}$- and stem/root total ATPase activities (Fig. 5). Similar results were found in Cd stressed (Cao *et al.*, 2014b) and salt/drought-stressed barley (Ahmed *et al.*, 2013), Cd stressed maize (Li *et al.*, 2016). Exogenous Se mitigated these Cd-induced increased in ATPase activities, which indicated that Se ameliorated Cd toxicity by modulating ATPase activity. Nevertheless, further studies should be carried out to understand the mechanism by which Se alleviates Cd toxicity by regulating ATPase activity.

In summary, our results illustrated that exogenous Se application may protect cucumber against Cd toxicity and partially alleviate its destructive physiological effects on plants. The mechanism involved in the prevention of Cd toxicity is mainly linked to the decreased Cd concentration in leaves, stems and roots and dramatically depressed ·OH, H_2O_2 and MDA accumulation in cucumber seedlings in comparison with Cd treatment alone. In addition, the results also suggested that Se could activate protective mechanisms which can ameliorate Cd-induced reduction in chlorophyll content, Fv/Fm ratio, and repair photosynthetic machinery. Furthermore, the alleviating effect of Se was associated with improved ATPase activity, and balanced nutrients and carbohydrate contents in cucumber plants. However, detailed studies on molecular mechanism of Se-mediated tolerence to Cd stress, such as identify Cd tolerant related proteins and genes, and their post translational modification may facilitate a better understanding of the mechanisms involved in Cd-tolerence of cucumber.

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