

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Effects of selenium and silicon on enhancing antioxidative capacity in ramie (*Boehmeria nivea* (L.) Gaud.) under cadmium stress

Hui Tang · Yunguo Liu · Xiaomin Gong ·
 Guangming Zeng · Bohong Zheng · Dafei Wang ·
 Zhichao Sun · Lu Zhou · Xiaoxia Zeng

Received: 18 October 2014 / Accepted: 29 January 2015 / Published online: 11 February 2015
 © Springer-Verlag Berlin Heidelberg 2015

Abstract Hydroponic experiments were performed to investigate the ameliorating effects and mitigation mechanisms of selenium and silicon on Cd toxicity in *Boehmeria nivea* (L.) Gaud. Metal accumulation, chlorophyll content, activities of antioxidant enzymes, and antioxidant contents in ramie were evaluated. The results revealed that cadmium was mainly accumulated in the roots of plants rather than in the aerial parts. Additionally, under 5 mg L⁻¹ Cd stress, both Se (1 μmol L⁻¹) and Si (1 mmol L⁻¹) treatments decreased the Cd concentrations in plants. Besides, the treatments also inhibited the translocation ability of Cd from roots to the aboveground parts, which might be related to the decline of generation of reactive oxygen species (ROS). The application of Se and/or Si ameliorated Cd toxicity via stimulating the activities of antioxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD), and ascorbate peroxidase (APX), which resulted in the significant decrease of the contents of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) in ramie leaves. In addition, the content of nonenzymatic antioxidant such as glutathione (GSH) was increased significant-

ly through the addition of selenite and silicate. Also, ascorbate (AsA) and vitamin E played a crucial role in scavenging excess ROS within plants. On the whole, appropriate doses of Se and Si were found to benefit plant growth and enhance the ability of ramie to alleviate Cd-induced stress. Moreover, the effects of combination of Se and Si appeared to be more superior compared to addition separately in response to Cd stress.

Keywords Ramie · Selenium · Silicon · Antioxidative system · GSH · Vitamin E

Introduction

Cadmium (Cd) is one of the most toxic and harmful heavy metals with high mobility in the current environment due to its toxicity to plants, animals, and humans. Although not essential to plant growth, cadmium ions are readily taken up by roots and transported into the aerial organs in many plant species (He et al. 2011; Shi et al. 2010; Zacchini et al. 2009). In a very general way, cadmium exposure could result in the reduction of chlorophyll concentrations, decreases of photosynthesis in plants, depressed water and nutrient uptake (He et al. 2011; Zacchini et al. 2009). Cadmium can modify cell wall structure and degenerate cellular organelles such as chloroplasts and mitochondria (He et al. 2013a). Additionally, previous reports suggested that Cd induced oxidative damage, which was characterized by the accumulation of lipid peroxides, and oxidized proteins as a result of the inhibition of the antioxidant system in plants (Ekmekçi et al. 2008).

Cd stress has been illustrated to disturb the cellular metabolic process by producing excessive reactive oxygen species (ROS) (He et al. 2011). In this case, plants have developed

Responsible editor: Philippe Garrigues

H. Tang · Y. Liu · X. Gong · G. Zeng · D. Wang · Z. Sun · L. Zhou ·
 X. Zeng

College of Environmental Science and Engineering, Hunan
 University, Changsha 410082, People's Republic of China

H. Tang · Y. Liu (✉) · X. Gong · G. Zeng · D. Wang · Z. Sun ·
 L. Zhou · X. Zeng

Key Laboratory of Environmental Biology and Pollution Control
 (Hunan University), Ministry of Education, Changsha 410082,
 People's Republic of China
 e-mail: yunguo_liu_hnu@163.com

B. Zheng
 School of Architecture and Art, Central South University,
 Changsha 410083, People's Republic of China

defensive and detoxifying mechanism to reduce Cd activity and alleviate its toxicity. First, the apoplast acts as a barrier for cadmium entry because Cd^{2+} binds to polyuronic acids and pectin in plant cell walls and stimulates increased lignification (He et al. 2013a). Second, secretions in plants chelate with cadmium. Some researchers believed that the Cd-chelated complexes with root secretion like organic acids could decrease the amount of Cd uptake in soils (Dong et al. 2007). Others demonstrated that the production of phytochelatin (PCs), which is a family of metal-induced peptides, was the main detoxifying strategy of plants contaminated by heavy metals (Dai et al. 2014). Moreover, some reports suggested that two types of antioxidants were triggered in plants to balance the ROS levels. One type is the antioxidant enzymes, such as, superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GSH-Px), and glutathione reductase (GR). The other is the low molecular weight substances, such as glutathione (GSH), ascorbate (AsA), and tocopherols (vitamin E) (Aravind and Prasad 2005; Gajewska and Skłodowska 2007). All these antioxidants can react with ROS directly or indirectly via enzyme catalysis; thus, they could relieve the metal toxicity (Feng et al. 2013).

The ascorbate–glutathione cycle contributes to the plant stress tolerance (Aravind and Prasad 2005). As a chelating metabolite and a substrate for PC biosynthesis, GSH is essential in metal homeostasis. It is also important to the detoxification of metal-induced oxidative stress as an antioxidant (He et al. 2015; Seth et al. 2012). Additionally, GSH functions as a stress indicator of oxidative stress, which can react directly with ROS, promote the regeneration of AsA, and participate in signal regulation. It plays a crucial role not only in antioxidative defending and redox signaling but also in plant growth and development (Munné-Bosch 2005; Seth et al. 2012). Also, AsA plays a crucial role in protecting cells against oxidative stress in plants (Aravind and Prasad 2005). Furthermore, it appears that vitamin E, AsA, and GSH are interdependent in the control of ROS levels in chloroplasts, and vitamin E could be reduced by AsA and GSH (Munné-Bosch 2005; Packer 1991). Vitamin E, also known as tocopherols, is supposed to function nonenzymatically in cooperation with other antioxidants. α -Tocopherol, the major vitamin E compound found in leaf chloroplasts, plays a great part in reducing ROS levels in photosynthetic membranes and limiting the extent of lipid peroxidation by reducing the lipid peroxy radicals to the corresponding hydroperoxides (Munné-Bosch 2005). Previous study supports the hypothesis that intracellular GSH levels are related to vitamin E protection against oxidation-induced cell damage, and vitamin E is crucial to maintain protein thiols and cell viability (Rana and Verma 1996). However, little is known about the correlation between vitamin E and the ascorbate–glutathione cycle, and their combination function in response to heavy metal-evoked stress in plants.

During the past decade, many experiments have been conducted to alleviate heavy metal toxicity in plants by applying exogenous materials such as nitric oxide and spermine (Groppa et al. 2007; Wang et al. 2014). Recent studies indicated that exogenous selenium and silicon enhanced the tolerance of rice and maize against Cd stress and also promoted the plant growth (Lin et al. 2012; Malčovská et al. 2014). However, the effect of Se under oxidative stress is not unanimous, owing to the differences in Se concentrations. Although the role of Se as an essential nutrient to higher plants remains controversial, it has been widely proved that at low concentrations, Se acts as an antioxidant which protects plants against oxidative stress and delays senescence (Cartes et al. 2005; Djanaguiraman et al. 2005), while at high concentrations, it can exert toxic effects such as suppression of growth and the appearance of chlorosis on leaves (Krystofova et al. 2010). However, the mechanism of Se alleviation on heavy metal-induced toxicity is still unclear and equivocal. Some researchers believed that the alleviation effect of Se on Cd toxicity was related to reduced Cd uptake and ROS accumulation, balanced nutrients, and increased H^+ - and Ca^{2+} -ATPase activities (Lin et al. 2012). Some researchers thought that this alleviation might be due to the functions of decreasing the translocation of Cd toward the shoots (Pedrero et al. 2007), while some believed that it might be owing to forming nontoxic Se-metal complexes (Vorobets and Mykiyevich 2000). Others regarded that Se had protected garlic against Cd toxicity mainly through (i) removing Cd from metabolically active cellular sites, (ii) inducing Se to harm the Cd-induced free oxygen radical, and (iii) regulating Se to phytochelatin activity (Sun et al. 2010).

Silicon, the second most abundant element both on the surface of the Earth's crust and in the soils, has been proved beneficial to the healthy growth and development of many plant species such as barley, cotton, and maize (Ali et al. 2013; Farooq et al. 2013; Malčovská et al. 2014). To date, it has been proved that silicon increased the resistance to biotic as well as abiotic stresses including salinity, heat, drought, freezing, UV radiation, and metal toxicity (Guntzer et al. 2012; Ma 2004). Si application mitigated the adverse effects of Cd stress by reducing electrolytic leakage (EL), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2) contents and improving activities of antioxidant enzymes (Farooq et al. 2013). The amelioration of Si on metal-induced stress might be correlated with a reduction in Cd uptake and alteration of Cd subcellular distribution (Farooq et al. 2013; Zhang et al. 2014). Besides, it has been reported that Si supplement stimulated antioxidant systems in plants and promoted the complexation or coprecipitation of metals with Si (Neumann and Zur Nieden 2001), which may be partially responsible for the alleviating effect of Si on heavy metal stress.

Some kinds of species of vegetables and crops have been studied for phytoremediation. Although these species may act well in scavenging free Cd ion in soil, it is hard to popularize

using vegetables applying for phytoremediation because of the high cost and little economic benefits. However, ramie is considered to be a potential plant for restoring Cd-contaminated soils. As a nonfood and economic crop, ramie is widely grown in south China, which is famous for its large biomass and fast growth rate. Above all, ramie not only can survive under Cd stress but also can create economic values without any damages to human bodies. Studies on ramie growth and resistance against Cd toxicity have been reported recently (Liu et al. 2007; Sun et al. 2014; Wang et al. 2014). However, it has been also revealed that ramie growth was markedly inhibited under high concentration of Cd treatment, which restrained the application of ramie in phytoremediation. Therefore, it is necessary to explore how to elevate the tolerance of ramie under Cd stress. Few studies focused on a combined effect between selenium and silicon on cadmium stress. It is noteworthy to find whether external Se and Si could function efficiently on enhancing antioxidant ability in response to oxidative stress induced by Cd. Therefore, the main objective of the present study was (i) to assess the effects of Se and Si on metal uptake and translocation under Cd stress and (ii) to evaluate the roles of Se and Si in alleviating Cd-induced oxidative stress by regulating the antioxidant defense system. The present work puts an emphasis on the antioxidant systems, including the enzymatic and nonenzymatic reaction, in ramie under Cd stress. The uptake and translocation of cadmium in plants were also studied.

Materials and methods

Plant materials and treatment

One-month-old ramie seedlings were provided by the Institute of Bast Fiber Crops, Chinese Academy of Agricultural

Sciences. In the first stage, in order to adapt to the hydroponic environment, plants were grown in 2-L plastic pots (two plants per pot) with 1/8 density Hoagland nutrient solution with aeration for 2 weeks. During the training, all nutrient solutions were renewed every 2 days and the pH was maintained in the range of 6.0 ± 0.5 . In the second stage, these plants were divided into five different groups. There were three replicates in each treatment. The treatments are presented in Table 1. Except for the controls, the others were exposed to 5 mg L^{-1} CdCl₂ during the whole experiments. The optimum concentrations of Se and Si (from solutions of Na₂SeO₃ and Na₂SiO₃, respectively) were $1 \text{ } \mu\text{mol L}^{-1}$ and 1 mmol L^{-1} . These concentrations were based on the pre-experiments (data not shown). All nutrient solutions were renewed every 2 days. Plants were grown under a 16-h-day (25 °C)/8-h-night (20 °C) condition. Leaves, stems, and roots were collected after 7 days. All plant organs were washed with deionized water and stored in liquid nitrogen.

Cadmium uptake and TF

First, plant roots were soaked in 20 mM EDTA-2Na for 15 min to displace extra metals on the surface. After rinsed with deionized water, tissues were dried at 70 °C for 48 h. Then, dried tissues were ground and digested in concentrated HNO₃/HClO₄ (3:1, v/v). Cadmium contents were determined by atomic absorption spectroscopy (AAAnalyst 300, PerkinElmer, Germany).

Translocation factor (TF) defines shoot/root ratio and represents the ability of plants to transfer the metal from roots to shoots. It is measured in the ratio of Cd concentration in the aerial parts (mg kg^{-1} DW) to Cd concentration in the roots (mg kg^{-1} DW) (He et al. 2013b). In this research, TF was expressed as follows:

$$\text{TF} = \frac{\text{Stem Cd concentration} \times \text{biomass of stems} + \text{leaf Cd concentration} \times \text{biomass of leaves}}{(\text{biomass of stems} + \text{biomass of leaves}) \times \text{root Cd concentration}}$$

Determination of lipid peroxidation and chlorophyll content

The malondialdehyde (MDA) content represents the level of lipid peroxidation in plants. It was detected using the thiobarbituric acid method (Chaoui et al. 1997). Concentration of chlorophyll in the leaves reflected photosynthesis intuitively in plant. Leaves were extracted in 80 % chilled acetone in dark and estimated by the method of He et al. (2011). Fresh leaf materials (0.2 g) were used in the detection of different index.

For determination of H₂O₂ concentration, fresh leaf tissues (0.2 g) were extracted with 3 mL 0.1 % (w/v) trichloroacetic acid (TCA) in an ice bath and centrifuged at 12,000 rpm for

15 min (Velikova et al. 2000). Supernatant (0.5 mL) was mixed with 0.5 mL of phosphate buffer (pH 7.0) and 1 mL

Table 1 Experiment design

NO.	Treatment
CK	No added CdCl ₂ and Na ₂ SeO ₃ and Na ₂ SiO ₃
Cd	5 mg L^{-1} CdCl ₂
Cd+Se	5 mg L^{-1} CdCl ₂ + $1 \text{ } \mu\text{M}$ Na ₂ SeO ₃
Cd+Si	5 mg L^{-1} CdCl ₂ + 1 mM Na ₂ SiO ₃
Cd+Se+Si	5 mg L^{-1} CdCl ₂ + $1 \text{ } \mu\text{M}$ Na ₂ SeO ₃ + 1 mM Na ₂ SiO ₃

of 1 mol L⁻¹ KI. The absorbance was read at 390 nm ($E=0.28 \mu\text{M}^{-1} \text{cm}^{-1}$). The amount was expressed as micromoles per gram (μmolg^{-1}) FW.

Analysis of antioxidant enzyme activities

Fresh leaves (0.2 g) were homogenized in 4-mL ice-cold 50 mM phosphate buffer (PH 7.0, 4 % PVP). After the centrifugation at 14,000 rpm at 4 °C for 15 min, the supernatant was taken for detection. Antioxidative capacity in plants plays an important role against the abiotic stress. Activities of antioxidant enzymes including SOD, POD, APX, and GR were determined.

The activities of antioxidant enzymes (SOD, POD) were determined with an assay kit purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

APX (EC1.11.1.11) could catalyze the reaction of reduced ascorbic acid and hydrogen peroxide. Its activity was detected by following the method of Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer, 0.1 mM H₂O₂, 0.5 mM sodium ascorbate, and the supernatant. Decrease in absorbance at 290 nm was measured at 25 °C for 3 min ($E=2.8 \text{mM}^{-1} \text{cm}^{-1}$).

For measurement of GR (EC1.6.4.2) activity, the method of Anderson (1984) was used. The reaction mixture consisted of 0.5 mM oxidized glutathione (GSSG) and 0.15 mM NADPH in 50 mM potassium phosphate buffer (pH 7.8). The GR activity was monitored by the decrease in absorbance at 340 nm ($E=6.2 \text{mM}^{-1} \text{cm}^{-1}$) over 2.5 min.

Detection of antioxidative compound concentrations

GSH and AsA are the most important indexes in the ascorbate–glutathione cycle. The method of Anderson (1984) was used to determine GSH content. The assay was based on the sequential oxidation of GSH by DTNB to produce TNB and reduction of GSSG by NADPH in the presence of GR. The content of GSH was assayed in a reaction mixture contained 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), K-phosphate buffer (pH 7.6), EDTA, NADPH, and BSA, and the absorbance was read at 412 nm. The AsA concentration was measured according to the method of Wang et al. (1991). Fresh leaves (0.2 g) were homogenized in 1-mL ice-cold 5 % (1:5, w/v) TCA and then centrifuged at 20,000 rpm at 4 °C for 10 min. The supernatant was mixed with solution containing 1 mL 5 % TCA, 1 mL alcohol, 0.5 mL 0.4 % (w/v) H₃PO₄-alcohol, 1 mL 0.5 % (w/v) BP-alcohol, and 0.5 mL 0.03 % (w/v) FeCl₃-alcohol. After the mixture reacted for 90 min at 30 °C, the absorbance was measured at 534 nm. Vitamin E content was determined with an assay kit purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China. The mechanism is based on the reduction of Fe³⁺ to Fe²⁺ in the presence of tocopherols and production of colored complex with

bathophenanthroline. The content of vitamin E could be calculated via colorimetric analysis.

Statistical analysis

Results are presented as mean values±SE of three replications. Statistical analyses were performed by one-way analysis of variance (ANOVA). To determine the significant difference between treatments, least significant difference (LSD) was estimated, taking $P<0.05$ as the significant level.

Results

Plant growth and concentration of cadmium

Uptake and distribution of Cd in ramie tissues and dry biomasses are given in Table 2. Cd inhibited plant growth and caused 50.5 % loss of root dry weights and 44.8 % loss of shoot dry weights compared to control. However, both Se and Si prompted the growth of seedlings and reduced the Cd concentration in roots, stems, and leaves of *Boehmeria nivea* (L.) Gaud. In comparison to Cd treatment, Cd+Se treatment increased the dry biomasses of roots and shoots (stems and leaves) by approximately 24.6 and 16.9 %, respectively; Cd+Si treatment improved the dry biomasses of roots and shoots by 14.2 and 28 %, respectively; and Cd+Se+Si treatment elevated the dry biomasses of roots and shoots by 32.8 and 40.2 %, respectively. Besides, the results also indicated that cadmium was mainly accumulated in roots rather than in the aerial parts. Compared to Cd-treated group, concentrations of Cd in different organs (including roots, stems, and leaves) were decreased more or less in all the other groups (Cd+Se, Cd+Si, Cd+Se+Si). In comparison to Cd treatment, additional Se, Si, and Se+Si treatment decreased Cd concentrations by approximately 19, 15, and 28 % in roots, 28, 54, and 38 % in stems, and 55, 7, and 70 % in leaves, respectively. Besides, either Se or Si treatment inhibited the TF. TF of Cd+Se, Cd+Si, and Cd+Se+Si group was respectively 15.5, 38.3, and 20.4 % smaller than that of the Cd group.

Lipid peroxidation and plant antioxidant responses

Cd uptake by plants has been reported to damage membrane integrity, therefore induced lipid peroxidation (Dong et al. 2007). It is well established that MDA is an important production of lipid peroxidation. Cd-induced oxidative damage was measured as MDA content. In comparison with control, the content of MDA in leaves of ramie was increased by about 66.5 % when exposed to Cd alone (Fig. 1a), while visible reductions of MDA content appeared in Cd+Se (20.9 %), Cd+Si (14.3 %), and Cd+Se+Si (25.5 %) treatments compared to Cd group. The results indicated that Cd+Se+Si

Table 2 Uptake and distribution of Cd and dry biomasses in ramie tissues exposed to Cd stress

Treatment	Cd content (mg kg ⁻¹ DW)			TF	Dry biomass (g)	
	Roots	Stems	Leaves		Roots	Shoots
CK	–	–	–	–	2.43±0.27 a	3.82±0.21 a
Cd	341.43±25.29 a	211.75±19.05 a	125.08±12.36 a	0.567	1.34±0.45 d	1.76±0.12 d
Cd+Se	276.55±21.21 ac	151.73±9.21 b	56.36±10.79 b	0.479	1.67±0.13 c	2.21±0.23 c
Cd+Si	291.39±12.73 b	97.78±12.71 c	115.98±13.08 a	0.350	1.53±0.21 c	2.42±0.36 c
Cd+Se+Si	246.08±17.59 d	130.52±11.06 b	37.5±4.04 c	0.451	1.78±0.33 b	2.65±0.21 b

Different letters mean significance of difference between the treatments ($p < 0.05$)

treatment provided a more efficient ability for remitting lipid peroxidation.

Moreover, accumulation of ROS is regarded as an important approach of Cd to induce cellular oxidative stress (Collén et al. 2003). Moreover, increase in H₂O₂ content is supposed to be probably responsible for Cd-induced lipid peroxidation (Lin et al. 2012). In this study, Cd caused a drastic increase in H₂O₂ content in leaves (60 % higher compared to control), while both Se and Si decreased H₂O₂ content by 31.5 and 40 %, respectively, when compared to Cd treatment alone (Fig. 1b). Additionally, Se+Si provided a more powerful reduction (54.8 %) with respect to Se and Si separately under Cd stress. Results above showed that either Se or Si could

alleviate the oxidative stress induced by Cd. Furthermore, the effect was magnified by the coaction between Se and Si.

The decrease in chlorophyll content is one of the most commonly observed consequences of Cd toxicity (Ekmekçi et al. 2008). On the whole, exposure to Cd stress diminished the chlorophyll content by about 27.5 % compared to control (Fig. 2). However, Cd+Se treatment markedly increased the chlorophyll content by 48.7 % compared to Cd treatment, while Cd+Si and Cd+Se+Si treatment increased it by 10.9 and 21 %, respectively.

The effects of Se, Si, and Cd supplementation on antioxidative enzymes in ramie leaves are presented in Table 3. Compared to control, the presence of Cd in the nutrient

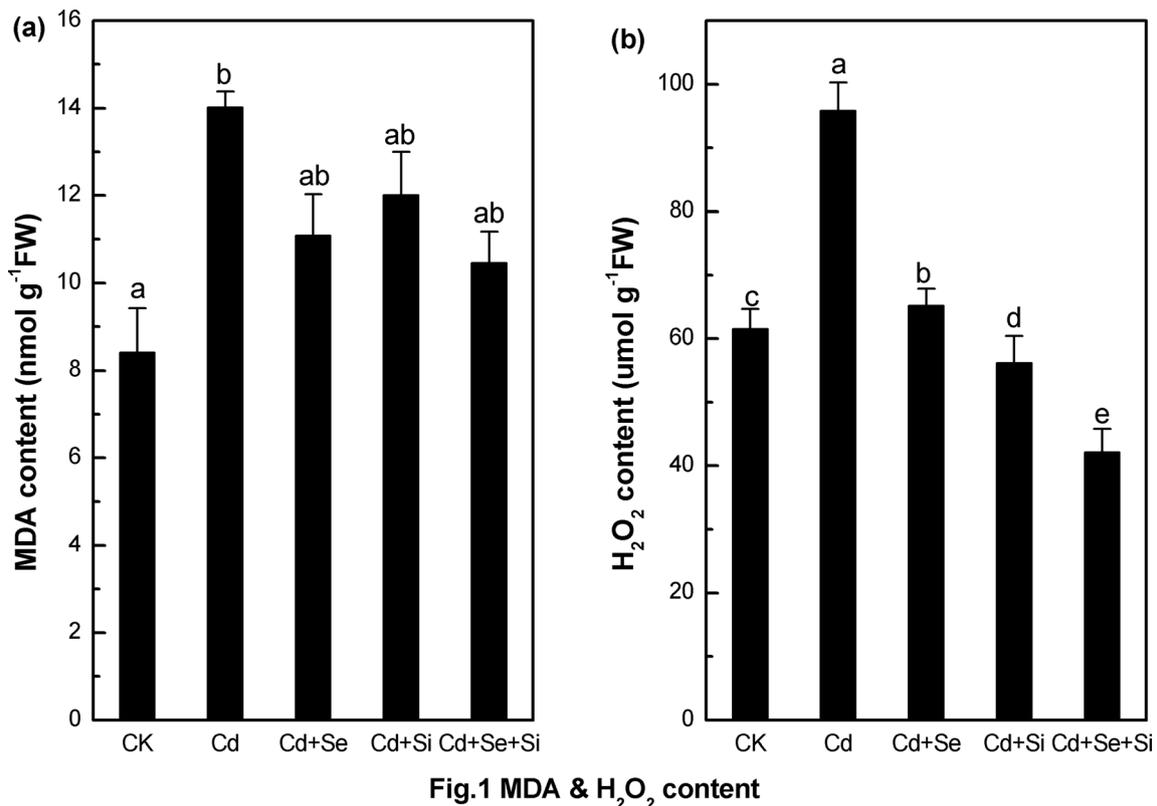


Fig. 1 Effects of different treatments on the **a** MDA and **b** H₂O₂ content in ramie leaves subjected to Cd stress. Bars with different letters mean significance of difference between the treatments ($p < 0.05$). Error bars (⊎) show SE

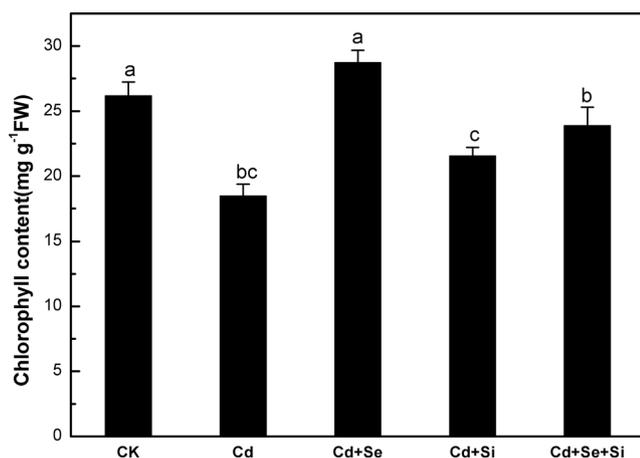


Fig. 2 Effects of different treatments on chlorophyll content in ramie leaves subjected to Cd stress. Bars with different letters mean significance of difference between the treatments ($p < 0.05$). Error bars (\top) show SE

solution ($5 \text{ mg L}^{-1} \text{ Cd}$) led to significant decrease in the activities of the antioxidative enzymes, by about 34.1 % for SOD, 19.1 % for POD, and 61.7 % for APX, respectively. However, this decrease was significantly reversed by Se and/or Si effects. Compared to Cd treatment, Cd+Se, Cd+Si, and Cd+Se+Si treatment significantly increased SOD activity by approximately 32.3, 42.4, and 83 %, respectively. Moreover, almost all the studied antioxidative enzymes were expressed to enhance activities in the plants exposed to Cd together with Se and/or Si treatments. Besides, the activities of all the antioxidative enzymes were increased by a large margin in Cd+Se+Si treatment ($p < 0.01$) in contrast with Cd treatment. The activity of SOD and POD was 83 and 56 % higher, respectively, and the increase of APX activity and GR activity even reached to 93 and 113 % in Cd+Se+Si group. Besides, APX was detected almost twofold in control group more than that in Cd treatment, while GR was 42 % less. Compared to Cd treatment, addition of Se or Si elevated APX (34 and 7 %, respectively) and GR activity (20 and 46 %, respectively). Moreover, when Se and Si appeared simultaneously, the improvement of APX and GR activity was more marked.

It is well known that the ascorbate–glutathione cycle plays a vital role in the detoxification of ROS, and vitamin E can protect cell membranes from peroxidative damage (Aravind

and Prasad 2005). As shown in Fig. 3, compared to control, Cd addition alone significantly increased the GSH and AsA contents by 164 and 69 %, respectively. After all, GSH content was significantly increased in Cd+Si, Cd+Se, and Cd+Se+Si groups by 113, 27.7, and 39.2 %, respectively, compared to Cd treatment. Besides, AsA contents in Cd+Se, Cd+Si, and Cd+Se+Si group were, respectively, 35.6, 25.7, and 15.6 % smaller than that in Cd group.

Besides, exposure of the ramie seedlings to Cd stress led to a 36.6 % increase in vitamin E content in the leaves compared to control (Fig. 3c), which contributed to protection by reducing ROS levels and inhibiting lipid peroxidation. This enhancement was found reversed in the presence of Se or Si. The vitamin E content was reduced by 50.9 % in Cd+Se group, by 36.3 % in Cd+Si group and by 30 % in Cd+Se+Si group compared to Cd group.

Discussion

Plant growth and Cd uptake

The data showed that Cd alone inhibited the plant growth (Table 2). Nevertheless, both Se and Si prompted the growth of seedlings. Meanwhile, they reduced the Cd concentration in organs of *Boehmeria nivea* (L.) Gaud. That Se affected plant growth promotion might be the result of increased starch accumulation in chloroplasts (Pennanen et al. 2002). As shown in Table 2, Cd was more likely to accumulate in the roots than in the aerial parts, which might be a natural protective response of plants to Cd toxicity (Liu et al. 2007; Munné-Bosch 2005). Ramie roots might act as a barrier against heavy metal translocation, while the beneficial effect of Se or Si was greatly related to the reduction in Cd uptake as well as its translocation toward the shoot in plants (Pedrero et al. 2007; Zhang et al. 2014). Under Cd exposure, additional Se and/or Si declined the content of Cd as well as the TF value in ramie plants (Table 2). In fact, previous research studies have illustrated the functions of Se or Si on restraining uptake of heavy metals in different plants. Saidi et al. (2014) found that the addition of selenate to sunflower (*Helianthus annuus*) plants under Cd stress markedly decreased Cd contents in roots and

Table 3 Effects of Se or Si treatments on SOD, POD, APX, and GR activity in ramie leaves subjected to Cd stress

Treatment	SOD ($\text{U g}^{-1} \text{ prot}$)	POD ($\text{U g}^{-1} \text{ prot}$)	APX ($\text{U g}^{-1} \text{ prot}$)	GR ($\text{U g}^{-1} \text{ prot}$)
CK	11,330.72±241.11 a	8.17±0.34 c	1.15±0.17 a	0.22±0.02 e
Cd	7466.36±141.11 c	6.61±0.13 d	0.44±0.1 d	0.39±0.01 d
Cd+Se	9878.76±233.59 b	9.81±0.04 b	0.59±0.05 c	0.47±0.1 c
Cd+Si	10,633.95±73.98 b	11.66±0.25 a	0.47±0.05 d	0.58±0.01 b
Cd+Se+Si	13661.78±341.2 a	10.3±0.13 b	0.85±0.04 b	0.83±0.01 a

Different letters mean significance of difference between the treatments ($p < 0.05$)

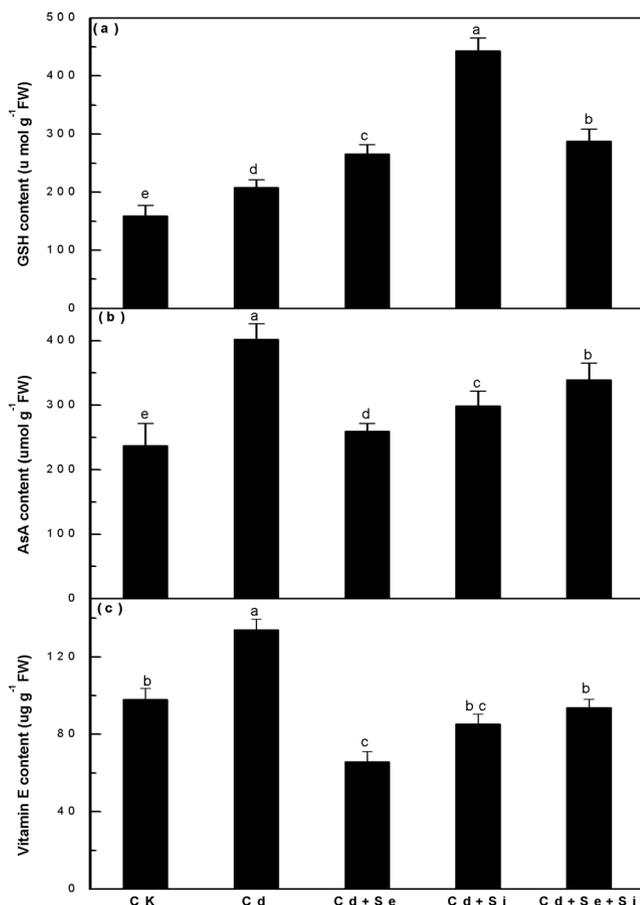


Fig. 3 Effects of different treatments on the **a** GSH, **b** AsA, and **c** vitamin E content in ramie leaves subjected to Cd stress. Bars with different letters mean significance of difference between the treatments ($p < 0.05$). Error bars ($\bar{\tau}$) show SE

leaves. In the research on durum wheat (Rizwan et al. 2012), the application of Si increased plant biomass and plant Si concentrations, which might reduce the available Cd in the soil and the Cd translocation to shoots. The results of this study revealed that Se or Si supplement not only decreased Cd uptake and TF but also increased plant biomass, despite its small difference.

Oxidative stress

In the wide condition, the generation and elimination of ROS are kept in balance within plants. When plants are exposed to external environmental stress, ROS production may be greatly triggered, which can induce oxidative damage to plants. Under Cd stress, inhibition occurs in most of the physiological and biochemical processes in plants, such as chlorophyll synthesis, photosynthesis, and nutrient uptake, thus resulting in retardation of growth and low yield (Ali et al. 2013). Besides, as a consequence of the generation of ROS, increased levels of H_2O_2 and MDA are considered to be correlated with the growth inhibition (Collén et al. 2003). More seriously, a rapid

increase in H_2O_2 , in turn, may result in the increase of mechanical strength and decrease of the extensibility of plant cell walls, which rapidly terminate growth (Schützendübel et al. 2002).

The significant increase in MDA and H_2O_2 contents in Cd treatment alone might result from the oxidative stress. However, in Cd-stressed plants treated with Se and/or Si, the H_2O_2 and MDA contents were much lower than those in plants treated with Cd only (Table 2). It was observed that Cd failed to raise MDA content in leaves in the presence of Se or Si. Se supplement was supposed to protect ramie by lowering the levels of H_2O_2 and MDA, because Se stimulated the spontaneous dismutation of $O_2 \cdot^-$ into H_2O_2 and regulated the production and quenching of ROS. The finding was confirmed by Cartes et al. (2010), who investigated the response of ryegrass to Al stress. In ramie plants, Si alleviation on Cd toxicity is mainly attributed to the decrease in Cd concentration of shoots and the stimulation to antioxidant systems. Nevertheless, Si application decreases the uptake of heavy metal in the culture and integrates into nontoxic Si-metal complexes in roots (Ma 2004). It can partially account for the Si-induced improvement in defensive function in Cd-stressed plants.

Photosynthesis, which is an important process for plant growth and biomass production, has been shown to be very sensitive to Cd in higher plants (Ekmekçi et al. 2008). Results revealed that chlorophyll content was decreased as a consequence of Cd exposure (Fig. 2), which may well explain part of the decrease of photosynthetic rate of plants (Xue et al. 2014). Furthermore, decrease in chlorophyll content and reduction in mesophyll CO_2 assimilation ability induced by Cd, further decreased the consumption of ATP and NADPH, leading to accumulation of NADPH on the acceptor sides of the PSI. Then, the feedback inhibited electron transport in chloroplasts (Xue et al. 2014).

Antioxidant defense

To scavenge excess ROS in cells, plants have developed a complex enzymatic and nonenzymatic antioxidant system, protecting the cells from oxidative stress (Kumar et al. 2010; Lin et al. 2012). In ramie leaves, Cd treatment decreased activities of SOD, POD, and APX (Table 2). The decrease of enzyme activities was due to the fact that heavy metal disturbed plant metabolism such as the synthesis of enzyme protein (Vögeli-Lange and Wagner 1990). Ascent in H_2O_2 and MDA contents along with suppression in enzyme activities in Cd treatment indicated that plants suffered from Cd toxicity. By contrast, Se and/or Si addition remitted Cd-induced changes in the activities of enzymes in different degrees. Se was proved to increase the glutathione peroxidase (GSH-Px) activity, and a positive relation between the increase and H_2O_2 scavenging efficiency was observed in soybean and sorghum

(Djanaguiraman et al. 2005, 2010). In ramie leaves, Cd-induced increase in H_2O_2 content affected antioxidant enzymes activity. The reason could be attributed to that a high H_2O_2 level might inactivate enzymes by oxidizing their thiol groups (Saidi et al. 2014). The enhanced antioxidative enzyme activities accelerated the elimination to H_2O_2 and ROS provoked by Cd stress in the presence of Se or Si. It is supposed that exogenous Se or Si improved the antioxidant capacity of plants. Furthermore, it is noteworthy that the enhancement in antioxidant system seemed to be greater in plants treated with Se and Si concurrently than separately. It is possible that Se and Si exhibited a cooperate effect, thus helping plants to adjust the adverse environment preferably.

The research of Liu et al. (2007) indicated that the tolerance of ramie to Cd stress attributed to the efficient operation of the ascorbate–glutathione cycle. GSH and AsA are important antioxidants involved in this cycle, and APX and GR are enzymes involved in H_2O_2 detoxification. Data obtained from the research also suggested that ramie plants strengthened antioxidant abilities to offsetting the Cd-caused stress, as the GSH and AsA levels increased sharply in Cd treatment (Fig. 3). It can be inferred that a visible increase in GSH and AsA contents under Cd stress was helpful to the mitigation on oxidative damage in plants. Besides, GSH synthesis was driven by increasing demand for GSH in response to oxidative stress.

Due to the high affinity of APX toward H_2O_2 , the increase in APX activity substantially contributed to scavenging intracellular H_2O_2 level (Caretto et al. 2002). Cd-stressed plants treated with Se improved APX activity, which suggested that Se might benefit the integrity of the structure and the function of the chloroplast (Saidi et al. 2014). On the other hand, the role of GR is crucial for maintaining of the optimal GSH level which is not only required for the synthesis of PCs and functioning of ascorbate–glutathione cycle but also needed as a reductant in many biochemical reactions (Pawlik-Skowrońska et al. 2007). The high activities of APX and GR indicated the high efficiency of the ascorbate–glutathione cycle, ensuring that the ROS scavenging and production were kept in balance.

Cd toxicity causes damage to plasmid membrane and induced ROS accumulation, thus leading to enhancement of tocopherol (vitamin E) content to prevent plants from injury of cell membranes (Munné-Bosch 2005). Interestingly, there was a strong relationship between chlorophyll and tocopherol concentrations. In the research of Gajewska and Skłodowska (2007) on Ni-treated wheat, a loss of chlorophyll content was found in parallel with an increase in tocopherol concentration. The results in this study also demonstrated this relation. Both tocopherols and chlorophyll contain a phytol moiety as part of their molecules and chlorophyll-derived phytol has been suggested to be utilized for the biosynthesis of tocopherols (Rise et al. 1989). The enhanced level of vitamin E was resulted

from chlorophyll degradation. Furthermore, when photosynthetic activity is high, α -tocopherol may be turning over rapidly (Rise et al. 1989), which could partly account for the less in vitamin E content than that in Cd treatment.

It is noteworthy that GSH, AsA, and vitamin E cooperate in scavenging oxygen radicals and protecting cell metabolism (Caretto et al. 2002). In cell metabolism, tocopherols are the main lipid-soluble antioxidants, whereas AsA and GSH are hydro-soluble antioxidants (Caretto et al. 2002). While GSH was increased to scavenge Cd-induced ROS, vitamin E content was increased correspondingly in Cd treatment (Fig. 3a–c). As AsA and GSH are required for the regeneration of tocopherol from its α -cromanoxy radical, they improve tocopherol efficiency in limiting lipid peroxidation. Moreover, GSH is the electron donor for the reduction of dehydroascorbate (DHA), the fully oxidized form of AsA (Caretto et al. 2002). In addition, some previous studies support the hypothesis that intracellular GSH level is associated with vitamin E protection against oxidation-induced cell damage (Rana and Verma 1996). The formation of GSH reduces the generation of free radicals, and vitamin E helps maintaining the GSH content. When exposed to Cd stress, GSH and AsA were triggered to eliminate redundant ROS. In this case, effects of vitamin E as an antioxidant were suppressed. Anyway, after Se or Si treatment, GSH content of Cd-stressed plants was increased while vitamin E level was reduced. The antioxidative effects of vitamin E may function via regulating the GSH level. High levels of tocopherols, AsA, and GSH have been reported to be correlated with a high tolerance to oxidative stress caused by adverse environmental conditions (Caretto et al. 2002).

Combination effects of Se and Si

Both Se and Si are thought to be nutrient elements to plants and beneficial to plant growth and development. There is a wealth of evidence that both low concentrations of Se and Si enhance the growth of plants and both are implicated in the activation of plant defense systems. Based on the results, both Se and Si treatment promoted the plant biomasses and decreased the uptake of Cd in plants. Moreover, the combination of Se and Si exhibited a pronounced effectiveness. It might be due to the fact that exogenous Se and Si triggered a higher induction of lipid peroxidation and the antioxidant system. Exactly a certification was the enhancement of chlorophyll and GSH levels. Under Cd stress, silicates are able to convert the soluble and exchangeable fractions of metals in soil into stable chemical forms, decreasing the metal bioavailability (Patrícia Vieira da Cunha et al. 2008). Such a reduction in metal bioavailability could alleviate the metal deleterious effects on plants (Neumann and Zur Nieden 2001). During heavy metal stress, Se and Si might prevent its toxic effect in plants. On the one hand, plant defenses are stimulated

through the production of phenolic compounds, lignin, and phytoalexins in the presence of Si (Ma and Yamaji 2006). On the other hand, Se acts as an antioxidant and inhibits lipid peroxidation via increased levels of thiols and GSH (Srivastava et al. 2009). Besides, Se ions are co-transported with Cd ions by the same protein carriers. Both Se and Cd ions are bound to thiol groups of cysteine and the competition for the specific binding sites in proteins partly explains the protective effect of Se against Cd toxicity (Filek et al. 2008). Additionally, the alleviation of Se or Si on metal toxicity may be based on the formation of nontoxic metal chelation (Neumann and Zur Nieden 2001; Vorobets and Mykiyevich 2000). Nevertheless, the detailed mechanisms of contribution of Se and Si to the antioxidative defenses against Cd toxicity need to be addressed by further studies.

In conclusion, the results showed that both low dosages of Se and Si supplementation effectively mitigated the adverse effects of Cd on ramie plants, and enhanced the biomasses and chlorophyll content while decreased the contents of MDA and H₂O₂. Overall, it was proposed that selenium and silicon relieved Cd toxicity via decreasing the uptake of cadmium and improving the ability of the antioxidant system in plants. Under Cd pollution, Se and Si decreased the Cd concentration in plants and the translocation ability from roots to the above-ground parts, which might be related to the decline of generation of ROS. The promotion of antioxidant enzymes (SOD, POD, APX, and GR) activities contributed much to defending the Cd-caused damages in ramie. Additionally, Se or Si counteracted Cd-induced alterations of antioxidant enzymes. Furthermore, the evaluated vitality of the ascorbate–glutathione cycle played an essential role in scavenging excess ROS and decreasing Cd uptake and transport in plant organs. Besides, both Se and Si assisted the plants in the adaption via regulating the levels of GSH and vitamin E. Moreover, the effects of combination of Se and Si appeared to be more superior compared to addition separately in response to Cd stress. Consequently, the combination effects of Se and Si provided a broader approach to ramie tolerance under Cd stress. It is important to pay attention to the role of Se or Si in alleviating Cd toxicity in ramie plants.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant Nos. 41271332 and 51478470) and the Natural Science Foundation of Hunan province (Grant No. 11JJ2031).

References

- Ali S et al (2013) The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. *Ecotoxicol Environ Saf* 89:66–72
- Anderson ME (1984) Determination of glutathione and glutathione disulfide in biological samples. *Method Enzymol* 113:548–555
- Aravind P, Prasad MNV (2005) Modulation of cadmium-induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate–glutathione cycle and glutathione metabolism. *Plant Physiol Biochem* 43:107–116
- Caretto S, Paradiso A, D'Amico L, De Gara L (2002) Ascorbate and glutathione metabolism in two sunflower cell lines of differing α -tocopherol biosynthetic capability. *Plant Physiol Biochem* 40:509–513
- Cartes P, Gianfreda L, Mora M (2005) Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. *Plant Soil* 276:359–367
- Cartes P, Jara A, Pinilla L, Rosas A, Mora M (2010) Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. *Ann Appl Biol* 156:297–307
- Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Sci* 127:139–147
- Collén J, Pinto E, Pedersén M, Colepicolo P (2003) Induction of oxidative stress in the red macroalga *Gracilaria tenuistipitata* by pollutant metals. *Arch Environ Contam Toxicol* 45:337–342
- Dai H-P, Shan C, Wei Y, Liang J-G, Yang T-X, Sa W-Q, Wei A-Z (2014) Subcellular localization of cadmium in hyperaccumulator *Populus × canescens*. *Afr J Biotechnol* 11:3779–3787
- Djanaguiraman M, Devi DD, Shanker AK, Sheeba JA, Bangarusamy U (2005) Selenium—an antioxidative protectant in soybean during senescence. *Plant Soil* 272:77–86
- Djanaguiraman M, Prasad P, Seppanen M (2010) Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. *Plant Physiol Biochem* 48:999–1007
- Dong J, Mao W, Zhang G, Wu F, Cai Y (2007) Root excretion and plant tolerance to cadmium toxicity—a review. *Plant Soil Environ* 53:193
- Ekmekçi Y, Tanyolac D, Ayhan B (2008) Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. *J Plant Physiol* 165:600–611
- Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z (2013) Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicol Environ Saf* 96:242–249
- Feng R, Wei C, Tu S (2013) The roles of selenium in protecting plants against abiotic stresses. *Environ Exp Bot* 87:58–68
- Filek M, Keskinen R, Hartikainen H, Szarejko I, Janiak A, Miszalski Z, Golda A (2008) The protective role of selenium in rape seedlings subjected to cadmium stress. *J Plant Physiol* 165:833–844
- Gajewska E, Skłodowska M (2007) Relations between tocopherol, chlorophyll and lipid peroxides contents in shoots of Ni-treated wheat. *J Plant Physiol* 164:364–366
- Groppa MD, Tomaro ML, Benavides MP (2007) Polyamines and heavy metal stress: the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves. *Bio Metals* 20:185–195
- Guntzer F, Keller C, Meunier J-D (2012) Benefits of plant silicon for crops: a review. *Agron Sustain Dev* 32:201–213
- He J et al (2011) Net cadmium flux and accumulation reveal tissue-specific oxidative stress and detoxification in *Populus × canescens*. *Physiol Plant* 143:50–63
- He J et al (2013a) A transcriptomic network underlies microstructural and physiological responses to cadmium in *Populus × canescens*. *Plant Physiol* 162:424–439
- He J et al. (2013b) Cadmium tolerance in six poplar species. *Environ Sci Pollut Res* 20:163–174
- He J et al (2015) Overexpression of bacterial γ -glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. *New Phytol* 205:240–254
- Krystofova O, Adam V, Babula P, Zehnalek J, Beklova M, Havel L, Kizek R (2010) Effects of various doses of selenite on stinging nettle (*Urtica dioica* L.). *Inter JEnv Res Pub Heal* 7:3804–3815

- Kumar M, Kumari P, Gupta V, Anisha P, Reddy C, Jha B (2010) Differential responses to cadmium induced oxidative stress in marine macroalga *Ulva lactuca* (Ulvales, Chlorophyta). *Bio Metals* 23:315–325
- Lin L, Zhou W, Dai H, Cao F, Zhang G, Wu F (2012) Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. *J Hazard Mater* 235:343–351
- Liu Y, Wang X, Zeng G, Qu D, Gu J, Zhou M, Chai L (2007) Cadmium-induced oxidative stress and response of the ascorbate–glutathione cycle in *Beckmeria nivea* (L.) Gaud. *Chemosphere* 69:99–107
- Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci Plant Nutr* 50:11–18
- Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. *Trends Plant Sci* 11:392–397
- Malčová SM, Dučaiová Z, Maslaňáková I, Bačkor M (2014) Effect of silicon on growth, photosynthesis, oxidative status and phenolic compounds of maize (*Zea mays* L.) grown in cadmium excess. *Water Air Soil Pollut* 225:1–11
- Munné-Bosch S (2005) The role of α -tocopherol in plant stress tolerance. *J Plant Physiol* 162:743–748
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- Neumann D, Zur Nieden U (2001) Silicon and heavy metal tolerance of higher plants. *Phytochemistry* 56:685–692
- Packer L (1991) Protective role of vitamin E in biological systems. *Am J Clin Nutr* 53:1050S–1055S
- Patrícia Vieira da Cunha K, Williams Araújo do Nascimento C, José da Silva A (2008) Silicon alleviates the toxicity of cadmium and zinc for maize (*Zea mays* L.) grown on a contaminated soil. *J Plant Nutr Soil Sci* 171:849–853
- Pawlik-Skowrońska B, Pirszel J, Brown MT (2007) Concentrations of phytochelators and glutathione found in natural assemblages of seaweeds depend on species and metal concentrations of the habitat. *Aquat Toxicol* 83:190–199
- Pedrero Z, Madrid Y, Hartikainen H, Cámara C (2007) Protective effect of selenium in broccoli (*Brassica oleracea*) plants subjected to cadmium exposure. *J Agr Food Chem* 56:266–271
- Pennanen A, Xue T, Hartikainen H (2002) Protective role of selenium in plant subjected to severe UV irradiation stress. *J Appl Bot* 76:66–76
- Rana S, Verma S (1996) Protective effects of GSH, vitamin E, and selenium on lipid peroxidation in cadmium-fed rats. *Biol Trace Elem Res* 51:161–168
- Rise M, Cojocar M, Gottlieb HE, Goldschmidt EE (1989) Accumulation of α -tocopherol in senescing organs as related to chlorophyll degradation. *Plant Physiol* 89:1028–1030
- Rizwan M, Meunier J-D, Miche H, Keller C (2012) Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination. *J Hazard Mater* 209:326–334
- Saidi I, Chtourou Y, Djebali W (2014) Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings. *J Plant Physiol* 171:85–91
- Schützendübel A, Nikolova P, Rudolf C, Polle A (2002) Cadmium and H₂O₂-induced oxidative stress in *Populus×canescens* roots. *Plant Physiol Biochem* 40:577–584
- Seth C et al (2012) Phytoextraction of toxic metals: a central role for glutathione. *Plant Cell Environ* 35:334–346
- Shi G, Liu C, Cai Q, Liu Q, Hou C (2010) Cadmium accumulation and tolerance of two safflower cultivars in relation to photosynthesis and antioxidative enzymes. *Bull Environ Contam Toxicol* 85:256–263
- Srivastava M, Ma LQ, Rathinasabapathi B, Srivastava P (2009) Effects of selenium on arsenic uptake in arsenic hyperaccumulator *Pteris vittata* L. *Bioresour Technol* 100:1115–1121
- Sun H-w, Ha J, Liang S-X, Kang W-J (2010) Protective role of selenium on garlic growth under cadmium stress. *Commun Soil Sci Plan* 41:1195–1204
- Sun Z, Liu Y, Huang Y, Zeng G, Wang Y, Hu X, Zhou L (2014) Effects of indole-3-acetic, kinetin and spermidine assisted with EDDS on metal accumulation and tolerance mechanisms in ramie (*Boehmeria nivea* (L.) Gaud.). *Ecol Eng* 71:108–112
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci* 151:59–66
- Vögeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves implication of a transport function for cadmium-binding peptides. *Plant Physiol* 92:1086–1093
- Vorobets N, Mykiyevich I (2000) Single and combined effects of lead and selenium on sunflower seedlings. *Sodininkystė ir Daržininkystė* 19:390–395
- Wang SY, Jiao HJ, Faust M (1991) Changes in ascorbate, glutathione, and related enzyme activities during thidiazuron-induced bud break of apple. *Physiol Plant* 82:231–236
- Wang D et al. (2014) Effect of exogenous nitric oxide on antioxidative system and S-nitrosylation in leaves of *Boehmeria nivea* (L.) Gaud under cadmium stress. *Environ Sci Pollut Res*:1–9
- Xue Z, Gao H, Zhao S (2014) Effects of cadmium on the photosynthetic activity in mature and young leaves of soybean plants. *Environ Sci Pollut Res* 21:4656–4664
- Zacchini M, Pietrini F, Mugnozza GS, Iori V, Pietrosanti L, Massacci A (2009) Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. *Water Air Soil Pollut* 197:23–34
- Zhang Q, Yan C, Liu J, Lu H, Duan H, Du J, Wang W (2014) Silicon alleviation of cadmium toxicity in mangrove (*Avicennia marina*) in relation to cadmium compartmentation. *J Plant Growth Regul* 33:233–242