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Roles of root cell wall components and root plaques in regulating elemental uptake in rice subjected to selenite and different speciation of antimony

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ABSTRACT

Root plaques and cell wall components play important roles in regulating the uptake of heavy metals in rice plants. Proper doses of selenium (Se) can reduce heavy metal uptake in plants. Whether Se can influence both the formation of root plaques and the contents of root cell wall components to affect heavy metal uptake in plants is unknown. A hydroponic culture system was set up using rice plants (Yangdao No.6) to mainly investigate the roles of root plaques and cell wall components in regulating the uptake of antimony (Sb) and Se in rice plants subjected to Se and different speciation of antimony [antimonite (Sb(III)) and antimonate (Sb(V))]. Se alone or plus different speciation of Sb showed a negative effect on plant growth. Addition of Se did not counteract the toxic effect of inhibiting rice plant growth exerted by Sb, despite the addition of Se to the solution containing 20 $mg\,L^{-1}$ Sb(III) or Sb(V) significantly reduced Sb concentration in the roots. Addition of Se stimulated the formation of Mn plaque and addition of 5 $mg\,L^{-1}$ Sb(III) or Sb(V) increased the formation of Fe plaque. When compared to the 20 $mg\,L^{-1}$ Sb(III) treatment, the addition of 0.8 $mg\,L^{-1}$ Se significantly increased the Fe plaque, but did not result in more accumulation of Sb on the root plaques. Other levels of Se did not show positive effects on the formation of Fe plaque on the roots of this rice plant when exposed to Sb(III) or Sb(V). In many cases, the single addition of Se, Sb(III) or Sb(V), especially for Se, can significantly increase the production of pectin, hemicelluloses and lignin. Additional Se helped rice plants exposed to Sb(III) or Sb(V) generate more pectin, hemicelluloses and lignin, especially when exposed to Sb(V). In summary, we speculate that Se inhibits, in most cases, the uptake of Sb in rice plants by affecting the root cell components rather than the formation of root plaques.

1. Introduction

Selenium (Se) is an essential nutrient element for humans (Shenkin, 2009), and an appropriate amount of Se is also beneficial for plant growth (Natasha Shahid et al., 2018). Research has shown that a low dose of Se ($< 0.8\,mg\,L^{-1}$) could lower the uptake and toxicity of many heavy metals (metalloids) in plants including arsenic, cadmium, lead, chromium, copper, mercury and antimony (Sb) (Belokobylsky et al., 2004; Fargasová et al., 2006; Yathavakilla and Caruso, 2007; Lin et al., 2012; Malik et al., 2012; Wu et al., 2017). Current researchers have paid their concerns mainly on: 1) responses of antioxidant systems (triggering the activities of some antioxidative enzymes and reducing the oxidative damages resulting from heavy metal or metalloid exposure) (Malika et al., 2012); 2) repairing the damaged cell membrane (Filek et al., 2009); 3) improving the photosynthesis system (Filek et al., 2009; Malik et al., 2012); 4) and the rebalance of essential element uptake in stressed plants (Lin et al., 2012). However, the mechanisms on why and how the addition of Se can reduce the uptake of so many heavy metals (metalloids) in different plants are unclear.

It is well documented that plants can lower the uptake of heavy metals (metalloids) through a series of stress responses (Huang et al., 2018), such as, increasing their root diameter and decrease the number and proportion of fine roots, thereby reducing antimonite [Sb(III)] uptake (Wu et al., 2017). In addition, under submerged growth conditions, plants can form iron plaques on the root surface to bind high amounts of heavy metals (metalloids) including Sb (Batty et al., 2000;

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Chen et al., 2005; Huang et al., 2012; Ren et al., 2014; Tripathi et al., 2014). Furthermore, plants can immobilize heavy metal cations in the cell wall via positively affecting the concentrations of cell wall components, such as cellulose, hemicellulose, pectin, and structural proteins which containing numerous negatively charged groups (e.g., hydroxyl, carboxyl, aldehyde, amino, and phosphate) (Leita et al., 1996; Haynes, 1980). The binding of heavy metals to cell wall will reduce the entrance of heavy metals into the protoplast through transmembrane transport and attenuate the interference of normal physiological activities in plants under metal stress (Memon and SchrÖder, 2009).

China possesses the major mineral resource and industrial production of Sb in the world, especially in the southern provinces of China such as Hunan, Guizhou, Guangxi, and Yunnan (He et al., 2012). Due to mining and other human activities, antimony pollution has become a serious concern (Flakova et al., 2012; Fu et al., 2010, 2011). The background Sb content in terrestrial vascular plants generally ranges from 0.2 to 50 μg kg^{-1} (Murciego et al., 2007). Many studies have showed that Sb(V) can also exist in flooded paddy soil (Xiao et al., 2017) and it is relatively stable even under reducing conditions (Okkenhaug et al., 2012). In soils, Sb(III) is much more available for plant uptake than Sb(V), such as in *Lolium perenne, Trifolium repens, Plantago lanceolata* and *Rumex obtusifolius* (Hockmann et al., 2018). Excessive Sb can affect the normal growth of crops and influence human health through the food chain (Cai et al., 2016; Huang et al., 2012).

Our previous studies have shown that the addition of Se can change the root morphology of rice roots to control the uptake of Sb and cadmium (Cd) (Ding et al., 2014; Wu et al., 2017). Reports have shown Fe and Mn affected the chemical behavior and bioavailability of nutrients and heavy metals in soil through adsorption and co-precipitation, thereby influencing plant uptake of various elements (Dong et al., 2000; Tripathi et al., 2014). The cell wall components, such as pectin and hemicellulose, play crucial roles in binding heavy metals (Hossain et al., 2006; Polec-Powlak et al., 2007). In addition, a more recent study by Cui et al. (2018) pointed out that Se could activate the related gene expression involved lignin synthesis (*OsPAL*, *OsCoMT* and *Os4CL3*) in rice suspension cells (*Oryza sativa* L. *japonica*) subjected to Cd exposure. Therefore, in this study, the results of our previous studies and the studies by other researchers let us hypothesize that Se might reduce the uptake of heavy metals (metalloids) in plants via exerting influences on the formation of root plaques or on the concentrations of some cell wall components in rice plants. To indentify the above hypothesis, we performed this hydroponic experiment using the rice variety Yangdao 6 subjected to selenite and different speciation of Sb(III) and Sb(V).

2. Materials and methods

2.1. Plant cultivation and management procedure

Plump seeds (Yangdao No.6) of even size were selected for this experiment. The seeds were disinfected with a 2% (v/v) NaClO solution for 20 min, and were then thoroughly rinsed with tap water followed by de-ionized water. Thereafter, the seeds were sprouted in a moist mixture of perlite and vermiculite (1:1, v/v). A 50% Hoagland-Arnon nutrient solution (HN) was used drop-wise to supply the seedlings with nutrients for natural growth. The de-ionized water was also timely applied to maintain the moisture content of the culture medium. The seeds were cultured in a glass greenhouse with natural light.

The seedlings with two true leaves and a uniform size were selected for the hydroponic experiment, and their roots were carefully washed with de-ionized water. After that, the seedlings were adapted for two weeks in a 50% HN nutrient solution, which comprised of 2.5 mM KNO_3, 0.5 mM NH_4NO_3, 0.5 mM $NH_4H_2PO_4$, 2 mM $Ca(NO_3)_2{\cdot}4H_2O$, 1 mM $MgSO_4{\cdot}7\ H_2O$, 4.5 μM $MnCl_2{\cdot}4H_2O$, 23 μM H_3BO_3, 0.4 μM $ZnSO_4{\cdot}7H_2O$, 0.15 μM $CuSO_4{\cdot}5H_2O$, 0.05 μM H_2MoO_4, and 4.5 μM EDTA-Fe. After two weeks, selenite (analytically pure) and different speciation of antimony (analytically pure) were added into the solution. Se, Sb(III) and Sb(V) were added in the form of sodium selenite (Na_2SeO_3), antimony potassium tartrate ($KSbC_4H_4O_7$), and potassium pyroantimonate ($K_2H_2Sb_2O_7{\cdot}4H_2O$), respectively.

There were a total of fifteen treatments in this study, including the control treatment (CK, without Se and different speciation of antimony), 0.2 mg L^{-1} Se (Se0.2), 0.8 mg L^{-1} Se (Se0.8), 5 mg L^{-1} Sb(III) [Sb(III)5], 20 mg L^{-1} Sb(III) [Sb(III)20], 5 mg L^{-1} Sb(V) [Sb(V)5], 20 mg L^{-1} Sb(V) [Sb(V)20], 0.2 mg L^{-1} Se plus 5 mg L^{-1} Sb(III) [Se0.2 + Sb(III)5], 0.2 mg L^{-1} Se plus 20 mg L^{-1} Sb(III) [Se0.2 + Sb(III) 20], 0.8 mg L^{-1} Se plus 5 mg L^{-1} Sb(III) [Se0.8 + Sb(III)5], 0.8 mg L^{-1} Se plus 20 mg L^{-1} Sb(III) [Se0.8 + Sb(III)20], 0.2 mg L^{-1} Se plus 5 mg L^{-1} Sb(V) [Se0.2 + Sb(V)5], 0.2 mg L^{-1} Se plus 20 mg L^{-1} Sb(V) [Se0.2 + Sb(V)20], 0.8 mg L^{-1} Se plus 5 mg L^{-1} Sb(V) [Se0.8 + Sb(V)5], 0.8 mg L^{-1} Se plus 20 mg L^{-1} Sb(V) [Se0.8 + Sb(V)20]. Each treatment was replicated thrice, and the nutrient solution volume was 1 L and replaced every three days in each pot in which two seedlings were cultivated. The pH value of the nutrient solution was adjusted at 5.5 by 0.1 mol L^{-1} NaOH and HNO_3. The growth conditions of the seedlings were as follows: a light/dark cycle of 16/8 h, a 100 μmol m^{-2} s^{-1} light intensity, a 20–25 °C temperature range and a 60–70% relative humidity. Two weeks later, the plant samples were collected.

The seedlings were first washed with tap water and then with de-ionized water. After that, the filter papers were used to blot the water adhering to the surface of the seedlings, and then the plants were separated into the shoots and roots. The fresh weights of the shoots and roots were recorded at the same time. One part of fresh root samples were gathered for the immediate extraction of root plaques, and the remaining part of fresh root samples were gathered, quickly frozen in liquid nitrogen and then stored at −80 °C for the determination of cell wall components. The shoot and root samples after root plaque extraction were oven-dried at 70 °C to a constant weight for 48 h in order to determine the elemental concentrations.

2.2. Extraction of root plaques

Fresh root samples were immersed in de-ionized water for 24 h. Thereafter, the roots were removed from the water and rinsed with de-ionized water twice. The water attached to the root systems was removed using filter papers. A portion of fresh root samples was placed in 50 mL of DCB solution [containing 0.03 mol L^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), 0.125 g sodium bicarbonate ($NaHCO_3$), and 0.5 g sodium dithionite ($Na_2S_2O_4$)] and extracted at room temperature for 1 h (Liu et al., 2011). The extract was filtered through a 0.45 μM filter and stored in a 50 mL volumetric flask for determining the concentrations of Fe, Mn and other elements.

2.3. Extraction and determination of lignin in the root cell wall

Approximately 0.15 g of fresh rice roots were weighed and ground with 5 mL of 95% ethanol in a mortar (Bhaskara Reddy et al., 1999). The homogenate slurry was transferred into a 10 mL centrifuge tube and centrifuged at 10,000 rpm for 5 min and then the supernatant was discarded. The precipitate was washed twice with 95% ethanol (the supernatant was discarded), followed by two washes with a mixture of ethanol and hexane (1:2, v/v). The precipitate was allowed to air dry, and the dry precipitate was rinsed with 1 mL of 25% (v/v) acetyl bromide (dissolved in acetic acid), incubated in a 70 °C water bath for 30 min, and allowed to cool to room temperature. Subsequently, 0.9 mL of 2 mol L^{-1} NaOH and 0.1 mL of 7.5 mol L^{-1} hydroxylamine-HCl were added to the cooled centrifuge tube and diluted to a volume of 10 mL with acetic acid. The mixture was centrifuged at 10,000 rpm for 5 min. The precipitate was discarded, and the absorbance of the supernatant was measured at 280 nm.

2.4. Extraction and determination of pectin and hemicellulose (I and II) in the root cell wall

Approximately 0.15 g of rice roots were weighed, washed with 0.5 mmol L^{-1} $CaCl_2$ solution and rinsed twice with de-ionized water. Subsequently, 0.5 mL of pre-cooled 75% ethanol (4 °C) was added to the roots for grinding (Zhong and Lauchli, 1993). The homogenate slurry was transferred into a 10 mL centrifuge tube and centrifuged at 10,000 rpm for l0 min. The supernatant was discarded, and the precipitate was washed twice with l mL of 75% ethanol, then was washed with 1 mL mixture of methanol: chloroform (1:1, v/v) for one time, and finally was washed with 1 mL of acetone for one time. The precipitate was freeze-dried and regarded as the root cell wall. Then, 2 mL of 0.5% ammonium oxalate buffer solution was added to the extracted cell wall, and the mixture was incubated in a boiling water bath for 1 h, followed by centrifugation at 10,000 rpm for 10 min. The above procedure was repeated twice, and the supernatant was combined as pectin. Subsequently, 2 mL of 4% KOH was added to the precipitate for 12 h of extraction. The extract was centrifuged at 10,000 rpm for 10 min, and the supernatant was collected. The above procedure was repeated twice, and the supernatant was combined as hemicellulose I. Finally, 2 mL of 24% KOH was added into the precipitate for 12 h of extraction. The extract was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. The above procedure was repeated twice, and the supernatant was combined as hemicellulose II. The absorbance of the supernatant was also measured at 280 nm.

2.5. Sample analysis

The shoot and root samples were digested using an ED54 DigiBlock digestion system (Lab Tech, Inc., Hopkinton, MA, USA) according to the method described in Liao et al. (2016). The Fe and Mn root plaques were measured using an atomic absorption spectrometer (ZEEnit 700 P, Analytikjena, Germany). The concentrations of other elements were detected via inductively coupled plasma mass spectrometry (iCAP Qc ICP-MS, Thermo Fisher, USA). Standard reference materials (bush leaves, GBW07603, GSV-2) were obtained from the Center for Standard Reference of China and used to ensure the accuracy of the elemental analysis.

2.6. Data processing and analysis

In order to compare the significant differences between different treatments ($p \leq 0.05$), univariate variance analysis combining multiple comparisons (Tukey's test) were used. Multivariate variance analysis was performed to assess the interaction between Se and Sb. All results were the means of three replications (n = 3). SPSS18.0 statistical software was used to analyze the data, and Sigmaplot software was employed to draw the charts.

3. Results and discussion

In this study, we attempted to investigate the roles of root plaques and cell wall components in regulating element uptake in rice plants. The results showed that there were, in general, significant interactive effects of Se/Sb(III) and Se/Sb(V) on the shoot and root biomass, the concentrations of Se and Sb in the shoots, roots and root plaques, and the contents of pectin, hemicelluloses I and II and lignin (Table 1). The interaction of Se and Sb(V) showed a limited effect on the concentrations of root Se, shoot Sb, root Mn and root Cu; in addition, the interaction of Se and Sb(III) did not significantly affect the root Mn content (Table 1).

3.1. Responses of plant growth

In this study, the single addition of Se or Sb(III) significantly lowered the shoot and root biomass, whereas addition of Sb(V) only significantly lowered the shoot biomass (Fig. 1). The supplementation of Se to 5 or 20 mg L^{-1} Sb(III) [Sb(V)] treatment did not show a significantly beneficial effect on the growth of rice plants despite that 0.8 mg L^{-1} Se mitigated a treatment of 20 mg L^{-1} Sb(III) and increased the shoot and root biomass up to 37.3% and 89.2%, respectively (data not shown) when compared to the 20 mg L^{-1} Sb(III) treatment alone (Fig. 1). The above results were not completely consistent with the results of our previous study where 0.2 mg L^{-1} Se alone and the addition of low doses of Se to Sb(III) treated plants could stimulate the growth of rice (Ding et al., 2014). The above inconsistencies may be attributable to different rice varieties and the rice plant used in this study might be sensitive to Se. Similarly, He and Yang (1999) reported that Sb(III) and Sb(V) could both significantly inhibit the growth of the shoots and roots of rice plants. However, the shoot and root biomass of rice subjected to the 20 mg L^{-1} Sb(V) treatment was 3.8- and 2.8-fold (data not shown) higher than that in the 20 mg L^{-1} Sb(III) treatment, respectively. This indicated that Sb(V) exhibited a relatively low toxicity to rice relative to Sb(III).

3.2. Concentrations of Se and Sb in shoots and roots of rice plants

Within a certain concentration range, Se was shown to reduce plant uptake of many heavy metals (metalloids) (Zhang et al., 2012). In the present study, the addition of Se also antagonized uptake of Sb when plants were exposed to 20 mg L^{-1} of Sb(III) and Sb(V) (only in the roots) (Tables 2 and 3). However, when compared to the 5 mgL^{-1} Sb(V) treatment, the addition of Se to the 5 mgL^{-1} Sb(V) treatment significantly increased Sb uptake in the rice roots (Table 3). The above results indicated that the interaction between Se and Sb(III), as well as the interaction between Se and Sb(V), had different effects on Sb uptake. Our previous results also showed that when rice plants were exposed to Sb(V), supplementation of Se promoted Sb uptake in the shoots and roots of rice plants (Wu et al., 2017). Different speciation of Sb has various uptake pathways in plants (Tschan et al., 2009), which might be the reason for the different effects of Se on the uptake of different speciation of Sb in this study and our previous study (Wu et al., 2017).

3.3. Formation of root plaques and their roles in regulating elemental uptake in rice plants

Reports have shown that rice plants grown under submerged conditions for a long period of time will form reddish-brown iron oxide plaques on their root surface (Liu et al., 2011; Ren et al., 2014). These plaques were shown to adsorb nutrient elements (e.g., P and Zn), beneficial elements (e.g., Se), and contaminating elements (e.g., Cd and As), increasing their accumulation on the root surface (Batty et al., 2000; Greipsson, 1995; Tripathi et al., 2014; Wang and Peverly, 1999). Ren et al. (2014) revealed that substantial amount of Sb was accumulated on Fe plaque, especially under Sb(III) stress. In addition, Huang et al. (2012) revealed that the high DCB-extractable Sb was about 70–90% of the total Sb in rice. Similar results were also observed in this study when the Sb(III) or Sb(V) was added alone (Table 4). In this study, root plaques (DCB-extracted) were also formed on the surface of rice roots when selenite and different forms of antimony were added into the solution (Fig. 2a, b). However, when compared to the control, 5 mg L^{-1} Sb(III) or Sb(V) alone significantly enhanced the Fe plaque, and 0.2 mg L^{-1} Se alone significantly reduced the Fe plaque (Fig. 2a, b). The formation of iron plaque needs an oxidation driver, and a decrease of radial oxygen loss (ROL) of rice roots was reported to result in iron plaque less formation (Fleck et al., 2011). Reports also showed that antimony could generate oxidative stress in plants (Feng et al., 2009). Therefore, the increase in Fe plaque by addition of 5 mg L^{-1} Sb(III) or Sb(V) might be due to the resulting oxidative stress from these compounds themselves, driving the formation of Fe plaque. In Sb(III)-treated maize, peroxidase (POD) activity was enhanced at low Sb levels

Table 1
The two-way ANOVA for the interactive effects between Se and different speciation of Sb on the biomasses of shoots and roots, the absorbance of pectin, hemicellulose I, hemicellulose II and lignin, and the concentrations of Se, Sb and essential elements in the iron plaque, shoots and roots of rice plants.

Source of variation	Biomass		Se		Sb		Pectin	HLI	HL II	Lignin				
	Shoots	Roots	Shoots	Roots	Shoots	Roots								
Se*Sb(III)	34.81**[1]	8.51**	349.1**	4.24*	3.59*	2.94*	12.04**	23.87**	44.59**	23.67**				
Se*Sb(V)	10.42**	6.15**	7.07**	**0.86ns**	**0.93ns**	85.89**	4.03*	24.35**	20.65**	20.81**				
Source of variation		Mg	K	Ca	Mn	Fe	Cu	Zn	Se	Sb				
Se*Sb(III)	Within	130.4**	107.1**	336.9**	43.26**	13.74**	83.95**	123.8**	165.9**	6.20**				
Se*Sb(V)	iron plaque	8.25**	137.3**	276.1**	39.26**	7.83**	124.5**	80.28**	109.6**	36.54**				
Source of variation	Mg		K		Ca		Mn		Fe		Cu		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Se*Sb(III)	137.5**	104.4**	20.85**	241.9**	27.21**	757.8**	426.1**	**1.48ns**	7.15**	24.94**	27.38**	11.29**	12.64**	4.39*
Se*Sb(V)	50.58**	94.21**	3.08*	22.64**	101.2**	45.07**	78.09**	**1.77ns**	9.93**	340.0**	14.95**	**0.64ns**	64.77**	18.29**

[1] F-values for the Se* Sb(III) interaction, Se*Sb(V) interaction, Se treatment, Sb(III) treatment, Sb(V) treatment. ns: not significant F ratio ($p \leq 0.05$); * and ** indicate significant at $p \leq 0.05$ and 0.01, respectively.

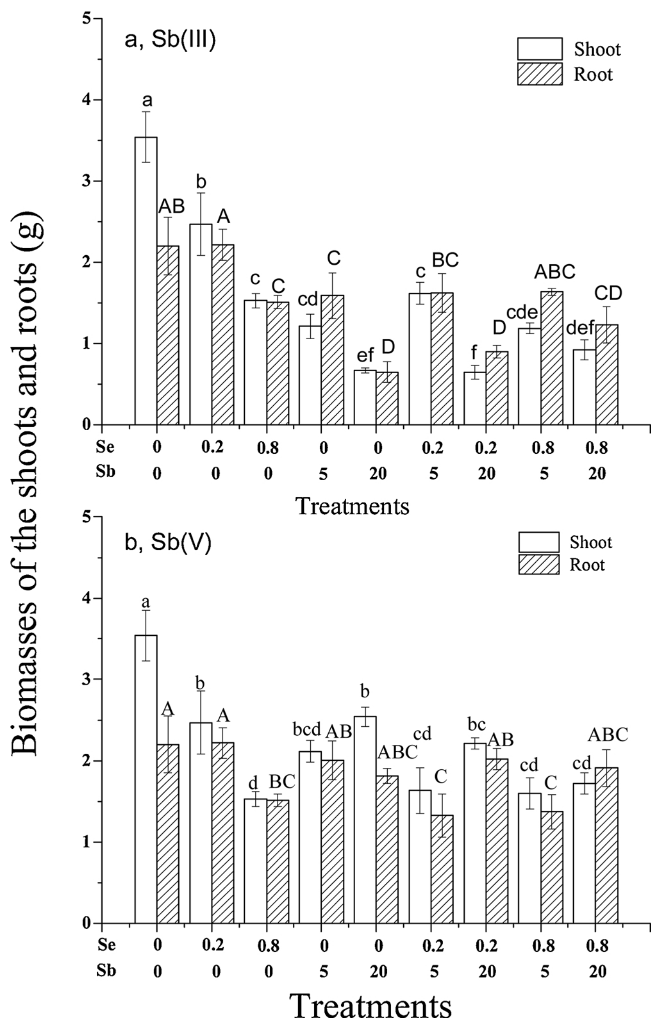

Fig. 1. Effects of Se and different forms of Sb on the fresh shoot and root biomasses of paddy-rice. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$. Lowercase letters denote significance within the treatments for shoots. Capital letters denote significance within the treatments for roots. There were fifteen treatments in this study and Sb was added in the forms of antimony potassium tartrate [Sb(III)] and potassium pyroantimonate [Sb(V)] at 0, 5 and 20 mg L^{-1}. Se was added in the forms of sodium selenite at 0, 0.2 and 0.8 mg L^{-1}. The same below for the treatment levels of Se, Sb(III) and Sb(V) in the following figures.

but inhibited at high Sb levels; simultaneously, the CAT activity increased with increasing amounts of Sb in soils (Pan et al., 2011). Reports have shown that catalase and peroxidase can oxidize Fe^{2+} to Fe^{3+} and thereafter facilitate the formation of Fe plaque on the root surface of rice plants (Ando and Nishiyama, 1983). In this study, more subsequent addition of Sb(III) or Sb(V) alone did not further enhance the Fe plaque when compared to the control. In addition, the singular addition of Sb(V) significantly enhanced, but Sb(III) significantly reduced, the Mn plaque (Fig. 2a, b). Low dosages of selenium are considered as an antioxidant (Xue et al., 2001; Seppänen et al., 2003). So, the decreased Fe plaque in this study could be attributed to the antioxidative function of low doses of selenium. However, the antioxidative role of Se cannot be used to explain the significantly enhanced Mn plaque when Se was added alone into the solution. Here, we do not know the exact reasons of how the unchanged Fe plaque at high levels of Sb(III) or Sb(V) and the different variations for Mn plaque could be affected by Sb(V) and Sb(III). These unresolved issues merit more investigations.

In the combined treatments of this study, the addition of Se to the solution containing different speciation of Sb was shown to unlikely reduce the uptake of Sb by binding Sb on root plaques. This is because: 1) the DCB-extracted root plaques were stimulated when 0.2 mg L^{-1} Se was added to the solution containing 5 (Mn plaque) or 20 mg L^{-1} Sb(V) (Mn plaque); or Se was added to the solution containing 20 mg L^{-1} Sb (III) (Fe plaque) (Fig. 2); 2) At a Sb(III) concentration of 20 mg L^{-1}, additional Se significantly lowered the root Sb concentration (Table 3), but did not affect the Sb concentration in the root plaques [except for the 0.2 mg L^{-1} Se plus 20 mg L^{-1} Sb(III) treatment, which significantly increased the Sb concentration on the root plaques compared to 20 mg L^{-1} Sb(III)] (Table 4); 3) Furthermore, under the Sb(V) treatment, supplementation of Se lowered, rather than increased, the Sb concentration in the root plaques (Table 4); meanwhile, supplementation of Se increased the root Sb concentration subjected to the Sb(V)5 treatment (Table 3). The reasons for why selenite did not affect the Sb uptake by binding of Sb in the root plaques might be partially explained by the predominant species of selenite, antimonite and antimonate in the solution, which are all anions (Huang et al., 2012; He et al., 2018). The increasing selenite concentrations could compete with different species of Sb for adsoption sites of root plaques.

The addition of Se showed different effects on the concentrations of Sb and Fe in the different tissues of plants and root plaques, which might be dose- and speciation-dependent. For example, 1) when plants were exposed to Sb(V) or 5 mg L^{-1} Sb(III), the addition of Se did not significantly affect the concentration of Sb in the shoots, but under a 20 mg L^{-1} Sb(III) treatment, the addition of Se significantly reduced the Sb concentration in the shoots (Table 2). 2) In the roots, the addition of Se significantly reduced the Sb concentration in the shoots only upon the 20 mg L^{-1} Sb(III) or Sb(V) treatment (Table 3). However, when plants were subjected to 5 mg L^{-1} Sb(V), the addition of Se significantly enhanced the concentration of Se in the roots (Table 3). 3) Under a

Table 2
The concentrations of some elements in the shoots of rice plants subjected to different levels of Se, Sb(III) and Sb(V).

Treatments	Mg ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Se ($mg\ kg^{-1}$)	Sb ($mg\ kg^{-1}$)
Control	5689 ± 156.3a[1]	30180 ± 775.9a	54432 ± 1220de	561.1 ± 0.03a	247.2 ± 6.55a	29.36 ± 0.04a	52.84 ± 0.54ab	0.29 ± 0.16e	2.85 ± 0.36d
Se0.2	3208 ± 132.9e	29670 ± 1200a	54557 ± 1169de	155.5 ± 20.55 cd	247.4 ± 26.50a	15.52 ± 1.48bcd	44.26 ± 1.60bc	54.64 ± 3.25c	2.48 ± 1.76d
Se0.8	3418 ± 70.03de	28375 ± 1289a	63948 ± 2473bc	228.9 ± 5.70b	231.7 ± 27.85ab	17.41 ± 0.68bc	40.12 ± 5.22 cd	144.5 ± 5.03a	4.20 ± 0.98d
Sb(III)5	3980 ± 49.60b	12954 ± 827.7c	48891 ± 466.5e	160.5 ± 4.90 cd	174.9 ± 1.83 cd	13.95 ± 0.63 cd	54.79 ± 7.12a	2.45 ± 0.48e	67.48 ± 19.25c
Sb(III)20	3491 ± 108.3cde	9930 ± 29.44d	65519 ± 765.1bc	151.0 ± 1.03 cd	152.7 ± 3.73d	18.72 ± 1.25b	30.88 ± 0.60d	2.68 ± 0.11e	131.9 ± 19.25a
Se0.2 + Sb(III)5	3523 ± 123.9cde	16903 ± 544.2b	59370 ± 115.8 cd	139.8 ± 4.89de	227.1 ± 0.40ab	13.57 ± 1.25d	43.91 ± 1.54bc	15.74 ± 1.32d	59.97 ± 1.44c
Se0.2 + Sb(III)20	3604 ± 57.09 cd	9474 ± 356.5d	83358 ± 4872a	115.8 ± 5.13e	157.4 ± 11.07 cd	13.88 ± 0.24 cd	37.59 ± 2.24 cd	14.78 ± 0.62d	96.59 ± 1.91b
Se0.8 + Sb(III)5	3842 ± 56.74bc	18385 ± 503.1b	62175 ± 2057c	169.7 ± 14.82c	235.7 ± 20.31ab	15.89 ± 1.13bcd	35.55 ± 2.21 cd	71.10 ± 2.45b	64.59 ± 5.36c
Se0.8 + Sb(III)20	4195 ± 236.7b	8737 ± 536.5d	68490 ± 2424b	164.5 ± 13.18 cd	201.3 ± 8.17bc	15.05 ± 2.88bcd	37.88 ± 3.50 cd	58.11 ± 0.56c	99.02 ± 10.54b
Control	5689 ± 156.3A	30180 ± 775.9A	54432 ± 1220C	561.1 ± 0.03B	247.2 ± 6.55BC	29.36 ± 0.04A	52.84 ± 0.54BC	0.29 ± 0.16D	2.85 ± 0.36B
Se0.2	3208 ± 132.9E	29670 ± 1200AB	54557 ± 1169C	155.5 ± 20.55G	247.4 ± 26.50BC	15.52 ± 1.48E	44.26 ± 1.60CD	54.64 ± 3.25C	2.48 ± 1.76B
Se0.8	3418 ± 70.03E	28375 ± 1289ABC	63948 ± 2473A	228.9 ± 5.70F	231.7 ± 27.85BC	17.41 ± 0.68CDE	40.12 ± 5.22DE	144.5 ± 5.03A	4.20 ± 0.98B
Sb(V)5	5791 ± 184.5A	29720 ± 826.3AB	67654 ± 3066A	754.9 ± 25.08A	277.2 ± 24.95AB	28.31 ± 4.15A	80.78 ± 6.50A	1.15 ± 0.02D	29.60 ± 8.26A
Sb(V)20	5204 ± 195.6B	26951 ± 186.1C	36843 ± 519.5E	467.09 ± 37.57C	247.8 ± 5.29BC	21.71 ± 0.48BCD	40.35 ± 3.15DE	1.41 ± 0.35D	30.80 ± 2.33A
Se0.2 + Sb(V)5	3862 ± 38.08D	28820 ± 1259ABC	48276 ± 1053D	473.9 ± 4.46C	324.2 ± 3.07A	25.18 ± 1.68AB	54.37 ± 0.99B	55.75 ± 3.25C	26.50 ± 5.40A
Se0.2 + Sb(V)20	4000 ± 84.77D	28272 ± 506.9ABC	49522 ± 562.9D	332.5 ± 31.40D	268.6 ± 21.26BC	22.05 ± 3.59BC	33.03 ± 0.77E	48.67 ± 5.70C	30.98 ± 5.00A
Se0.8 + Sb(V)5	3403 ± 48.48E	26543 ± 321.0C	58901 ± 815.4B	270.9 ± 1.75EF	221.8 ± 1.47C	15.85 ± 0.25DE	35.56 ± 0.62DE	131.8 ± 4.90B	37.25 ± 6.89A
Se0.8 + Sb(V)20	4497 ± 77.21C	27338 ± 1058BC	56021 ± 103.6BC	299.4 ± 10.83DE	279.2 ± 7.19AB	20.43 ± 1.57BCDE	50.69 ± 1.06BC	124.6 ± 4.55B	37.11 ± 3.29A

[1] Values are means ± SD (n = 3). The lowercases letters and capital letters in the same column indicate significant differences among different treatments, respectively ($p \leq 0.05$).

Table 3
The concentrations of some elements in the roots of rice plants subjected to different levels of Se, Sb(III) and Sb(V).

Treatments	Mg ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Se ($mg\ kg^{-1}$)	Sb ($mg\ kg^{-1}$)
Control	1515 ± 71.32a[1]	16417 ± 23.66a	185316 ± 2552e	32.28 ± 4.13ab	1513 ± 182.8b	56.90 ± 3.71a	63.23 ± 1.59 cd	7.16 ± 1.07d	9.58 ± 0.14c
Se0.2	781.5 ± 35.82 cd	14237 ± 270.01b	216159 ± 48.77 cd	27.24 ± 2.60abc	1203 ± 117.0c	45.50 ± 6.13bc	47.75 ± 1.73d	126.8 ± 13.39b	18.11 ± 1.72c
Se0.8	924.4 ± 62.42c	9994 ± 325.0c	198370 ± 1863de	34.61 ± 4.98a	766.8 ± 170.3d	37.84 ± 3.55 cd	57.97 ± 2.94 cd	240.2 ± 3.25a	13.46 ± 2.44c
Sb(III)5	1331 ± 43.75b	3611 ± 147.6f	187568 ± 711.1de	26.21 ± 3.14abc	1239 ± 10.24bc	32.90 ± 1.55d	66.05 ± 9.94bcd	4.92 ± 2.28d	776.2 ± 31.53ab
Sb(III)20	680 ± 63.99d	4332 ± 21.29e	611893 ± 25702a	22.62 ± 4.89abc	2445 ± 4.46a	38.53 ± 2.74 cd	106.8 ± 19.15a	5.34 ± 1.04d	931.2 ± 273.9a
Se0.2 + Sb(III)5	776.3 ± 3.43 cd	5197 ± 362.5d	237548 ± 1803c	19.41 ± 1.09c	569.8 ± 39.49de	37.62 ± 3.66 cd	78.37 ± 16.28abc	96.09 ± 7.82bc	521.9 ± 93.11b
Se0.2 + Sb(III)20	1275 ± 127.5b	4001 ± 1.44ef	373655 ± 7061b	24.29 ± 5.90abc	1312 ± 34.49bc	51.16 ± 3.47ab	73.20 ± 9.83bcd	83.68 ± 3.76c	668.8 ± 69.99ab
Se0.8 + Sb(III)5	849.2 ± 10.61 cd	4995 ± 224.7d	191437 ± 12225de	21.89 ± 1.04bc	298.8 ± 94.84e	35.14 ± 0.35 cd	61.44 ± 8.68 cd	260.7 ± 21.86a	518.1 ± 4.64b
Se0.8 + Sb(III)20	828.9 ± 32.94 cd	2630 ± 225.4 g	63326 ± 7036f	21.85 ± 6.95bc	672.4 ± 18.89d	42.93 ± 5.60bcd	93.04 ± 5.78ab	245.9 ± 27.40a	586.6 ± 11.04b
Control	1515 ± 71.32A	16417 ± 23.66A	185316 ± 2552C	32.28 ± 4.13A	1513 ± 182.8C	56.90 ± 3.71AB	63.23 ± 1.59BC	7.16 ± 1.07C	9.58 ± 0.14C
Se0.2	781.5 ± 35.82BC	14237 ± 270.01B	216159 ± 48.77A	27.24 ± 2.60A	1203 ± 117.0C	45.50 ± 6.13BCD	47.75 ± 1.73C	126.8 ± 13.39B	18.11 ± 1.72C
Se0.8	924.4 ± 62.42B	9994 ± 325.0C	198370 ± 1863B	34.61 ± 4.98A	766.8 ± 170.3D	37.84 ± 3.55CDE	57.97 ± 2.94BC	240.2 ± 3.25A	13.46 ± 2.44C
Sb(V)5	689.8 ± 58.90CD	10882 ± 1055C	81088 ± 1332F	30.31 ± 2.85A	329.5 ± 7.63E	58.98 ± 7.44A	77.82 ± 4.70B	1.33 ± 0C	138.4 ± 18.84B
Sb(V)20	880.6 ± 50.88B	13444 ± 206.5B	55654 ± 3714G	39.84 ± 14.25A	744.4 ± 105.4D	50.78 ± 1.16ABC	79.19 ± 4.28B	2.44 ± 0.07C	373.0 ± 53.80A
Se0.2 + Sb(V)5	914.2 ± 16.85B	9694 ± 1149C	134957 ± 6899D	39.22 ± 8.27A	1520 ± 250.6C	41.92 ± 3.69CD	131.4 ± 23.24A	147.0 ± 42.63B	354.6 ± 42.38A
Se0.2 + Sb(V)20	523.3 ± 17.85D	13712 ± 120.6B	82154 ± 421.0F	47.87 ± 4.35A	2784 ± 10.49B	36.47 ± 1.70DE	75.08 ± 3.87B	127.2 ± 5.01B	131.3 ± 24.67B
Se0.8 + Sb(V)5	1454 ± 148.2A	7629 ± 214.9D	105033 ± 3999E	48.94 ± 3.31A	3843 ± 34.20A	40.56 ± 7.51CDE	75.60 ± 4.66B	275.7 ± 28.54A	406.6 ± 12.59A
Se0.8 + Sb(V)20	548.5 ± 20.29D	6154 ± 70.26D	112243 ± 9169E	40.79 ± 15.99A	408.1 ± 119.4DE	28.24 ± 0.99E	79.51 ± 6.00B	234.3 ± 42.95A	160.7 ± 3.07B

[1] Values are means ± SD (n = 3). The lowercases letters and capital letters in the same column indicate significant differences among different treatments, respectively ($p \leq 0.05$).

Table 4
The concentrations of some essential elements, Se and Sb in the iron/manganese plaques of roots subjected to different levels of Se, Sb(III) and Sb(V).

Treatments	Mg (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Se (mg kg^{-1})	Sb (mg kg^{-1})
Control	424.8 ± 19.77b[1]	9861 ± 4.93b	5410 ± 397.5e	2.35 ± 0.17bc	8.60 ± 0.18f	0.87 ± 0.06e	4.69 ± 0.23d
Se0.2	373.4 ± 9.08bc	10621 ± 237.9a	8911 ± 257.7c	1.60 ± 0.29c	12.67 ± 0.71e	524.3 ± 18.57b	10.67 ± 0..46d
Se0.8	286.1 ± 4.65d	8923 ± 283.6c	9215 ± 176.7bc	6.73 ± 0.26a	9.86 ± 0.09f	751.1 ± 17.84a	3.65 ± 0.37d
Sb(III)5	420.5 ± 25.73b	7379 ± 6.74d	4569 ± 337.8f	6.77 ± 0.77a	32.88 ± 1.14a	6.07 ± 0.78e	3794 ± 73.45c
Sb(III)20	313.9 ± 5.21d	3694 ± 113.2 h	9950 ± 322.8b	4.01 ± 1.08b	25.79 ± 2.47b	8.99 ± 0.73e	8154 ± 819.3b
Se0.2 + Sb(III)5	312.0 ± 4.88d	6405 ± 294.2e	4548 ± 331.7f	1.50 ± 0.13c	18.30 ± 0.11d	258.3 ± 8.14d	4545 ± 59.42c
Se0.2 + Sb(III)20	615.4 ± 34.60a	4479 ± 140.2 g	14290 ± 45.80a	8.47 ± 1.60a	32.91 ± 0.12a	287.3 ± 8.67d	9573 ± 317.9a
Se0.8 + Sb(III)5	275.9 ± 24.45d	6180 ± 87.09ef	8128 ± 193.5d	1.72 ± 0.01c	21.35 ± 0.07c	470.3 ± 18.19c	3881 ± 6.69c
Se0.8 + Sb(III)20	325.6 ± 11.26 cd	5754 ± 97.64f	7429 ± 14.78d	1.52 ± 0.03c	21.77 ± 0.62c	537.5 ± 9.52b	8059 ± 48.04b
Control	424.8 ± 19.77A	9861 ± 4.93B	5410 ± 397.5B	2.35 ± 0.17CD	8.60 ± 0.18D	0.87 ± 0.06G	4.69 ± 0.23E
Se0.2	373.4 ± 9.08B	10621 ± 237.9A	8911 ± 257.7A	1.60 ± 0.29D	12.67 ± 0.71BC	524.3 ± 18.57D	10.67 ± 0..46E
Se0.8	286.1 ± 4.65D	8923 ± 283.6C	9215 ± 176.7A	6.73 ± 0.26B	9.86 ± 0.09CD	751.1 ± 17.84B	3.65 ± 0.37E
Sb(V)5	329.7 ± 24.21C	10215 ± 251.7AB	3963 ± 122.4C	3.50 ± 0.48C	23.58 ± 1.47A	5.04 ± 0.42G	2673 ± 174.1B
Sb(V)20	293.6 ± 14.15CD	8969 ± 161.2C	5078 ± 80.08B	2.40 ± 0.40CD	12.88 ± 0.47B	2.64 ± 0.22G	4197 ± 108.4A
Se0.2 + Sb(V)5	263.6 ± 11.47DE	8403 ± 133.7D	3828 ± 278.1C	10.95 ± 1.02A	14.38 ± 1.25B	635.0 ± 24.32C	1219 ± 74.58D
Se0.2 + Sb(V)20	239.2 ± 11.45EF	7721 ± 10.26E	2063 ± 11.48E	5.91 ± 0.06B	22.13 ± 1.93A	228.5 ± 5.36F	2134 ± 441.2C
Se0.8 + Sb(V)5	163.8 ± 19.21G	4944 ± 118.1F	3035 ± 269.9D	3.50 ± 0.06C	11.86 ± 0.18BC	919.0 ± 49.15A	1213 ± 199.3D
Se0.8 + Sb(V)20	213.0 ± 1.99F	4642 ± 0.71F	736.3 ± 174.3F	2.54 ± 0.83CD	12.38 ± 0.77BC	419.9 ± 28.67E	2053 ± 70.26C

[1] Values are means ± SD (n = 3). The lowercases letters and capital letters in the same column indicate significant differences among different treatments, respectively ($p \leq 0.05$).

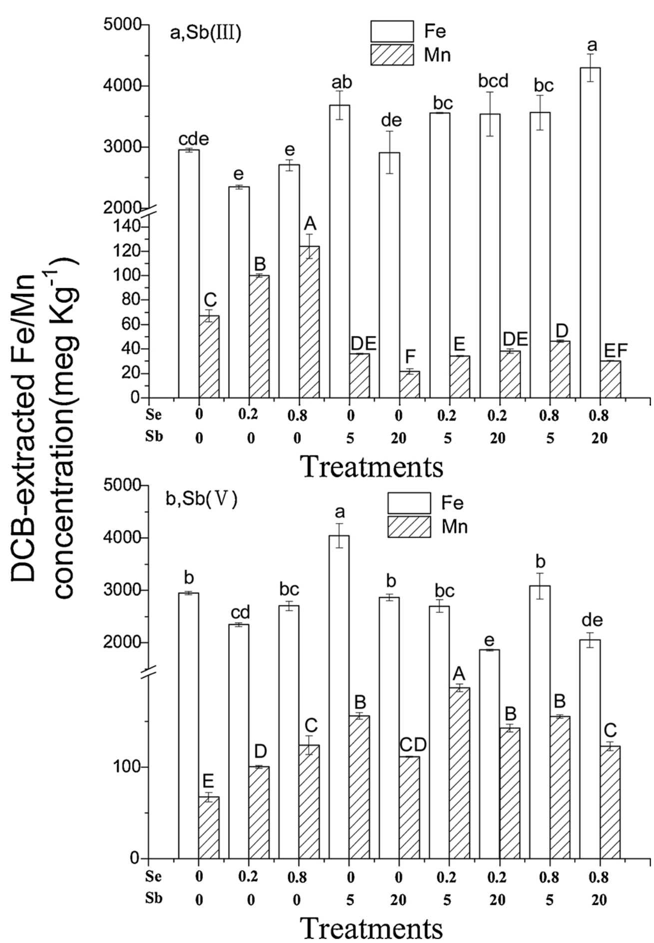

Fig. 2. Effects of Se and different forms of Sb on the DCB-extracted iron/manganese concentrations in the root iron/manganese plaques of paddy-rice. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$. Lowercase letters denote significance within the treatments for Fe concentration. Capital letters denote significance within the treatments for Mn concentration.

20 mg L^{-1} Sb(III) treatment, 0.2 mg L^{-1} significantly enhanced the Sb concentration in root plaques, but under Sb(V) exposure, the addition of Se significantly reduced the Sb concentration in root plaque (Table 4). The reason for the enhanced Sb concentration in roots treated with 5 mg L^{-1} Sb(V) after the addition of Se is unknown.

In the present study, Se alone inhibited the uptake of Mg (shoots and roots), K (roots), Mn (shoots), Fe (roots), Cu (shoots and roots) and Zn (shoots) in rice (Tables 2 and 3). Meanwhile, Se alone significantly increased the Ca, Mn, and Cu concentrations in the root plaques (Table 4). The above results might mean that the decrease in the Mn and Cu concentrations in the rice roots under Se treatment was possibly due to more Mn and Cu being restrained on the root plaques. There was no obvious causal relationship between the root concentrations of other nutrients and their corresponding concentrations on the root plaques (Tables 3 and 4). This suggested that Se was unlikely to affect the root uptake of most essential nutrient elements by influencing the formation of root plaques in rice exposed to different speciation of Sb. In the present study, some levels of Se added to the 5 mg L^{-1} Sb(III) treatment increased the K, Ca and shoot Fe concentrations (Tables 2 and 3), while they lowered the root Mg, root Fe, and shoot Zn concentrations.

3.4. Roles of root cell wall components and their roles in regulating elemental uptake in this rice plant

As a key protective barrier, the plant root cell wall plays an important role in blocking the uptake of heavy metals (Macfie and Welbourn, 2000). The cell wall can be in its turn affected by heavy metals via altering the biosynthesis and composition of cell wall (Krzesłowska, 2011). The results of the present study showed that 20 mg L^{-1} Sb(III) alone significantly increased the absorbance of hemicellulose I and II (indicating elevated concentration, the same below) (Figs. 4, 5). In contrast to Sb(III), the single addition of 20 mg L^{-1} Sb(V) only significantly increased the hemicellulose II concentration, whereas Se alone generally increased the pectin (Fig. 3), hemicelluloses I (Fig. 4), hemicellulose II (Fig. 5) and lignin (Fig. 6, at 0.2 mg L^{-1} Se) concentrations. The above results indicated that 1) pectin, hemicellulose I, and hemicellulose II play different roles in regulating the uptake of Se and Sb when plants were exposed to the single addition of Se, Sb(III) and Sb(V); 2) Hemicellulose II plays a more important role than pectin and hemicelluloses I. Cell wall components such as pectin and hemicellulose also play a key role in regulating the uptake of other heavy metals, including Al (Schmohl and Horst, 2000; Yang et al., 2011), Cd, Cr and Cu (Konno et al., 2005; Zeng et al., 2011). Although Sb exists in the environment mainly as oxoanions, but researches have shown that the cell wall in plants could sequestrate a large amount of Sb (Ding et al., 2015). Also, *Hydrilla verticillata* (L.f.)

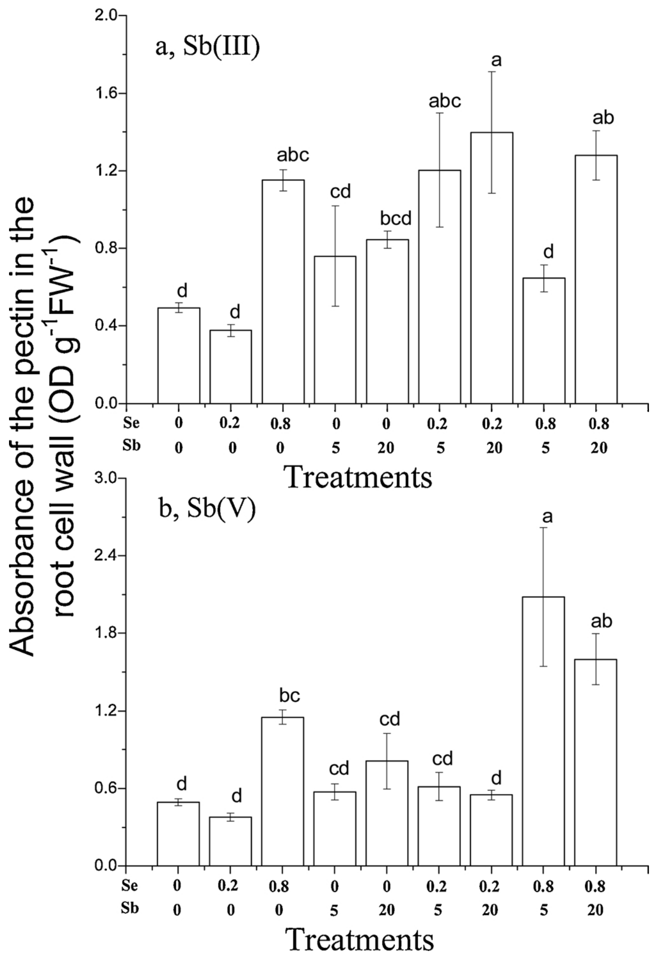

Fig. 3. Effects of Se and different forms of Sb on the pectin absorbance of the root cell wall. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$.

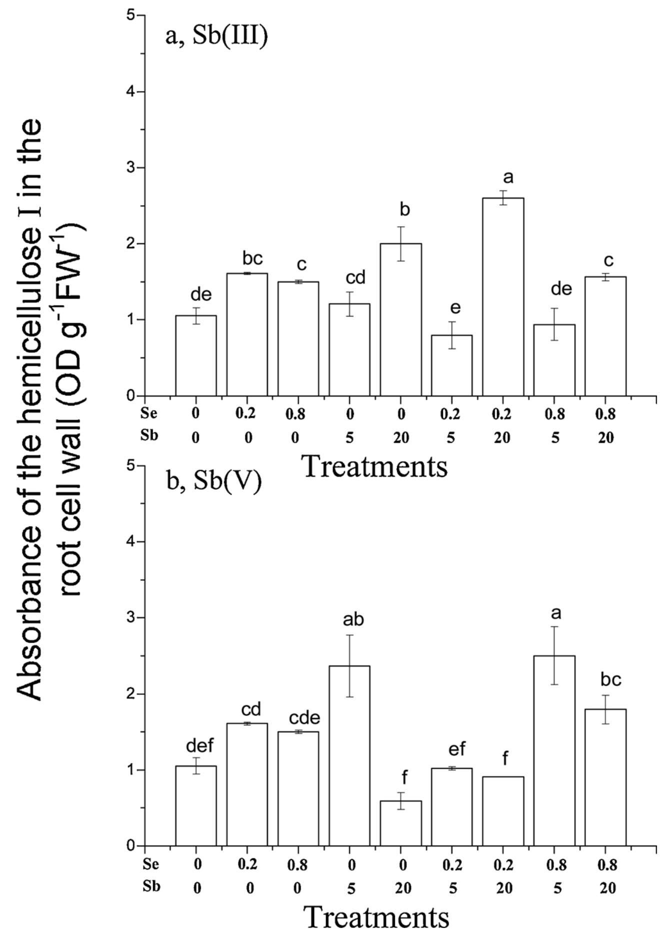

Fig. 4. Effects of Se and different forms of Sb on the absorbance hemicelluloses I in the root cell wall. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$.

Royle was reported to accumulate a major of arsenic (also mainly exists in the environment as oxoanions) in its root cell wall (Xue and Yan, 2011). Tian et al. (2012) suggested that As(V) mainly interacted with functional groups such as polysaccharides, flavin derivates, C-N, COO- and CH_2 on cell walls of arsenic resistant bacteria via surface-enhanced Raman scattering analysis. However, in their study, the direct proof on the molecular structure of As(V) with the groups in the cell wall was scarce. In this study, we also did not know which parts or functional groups of cell wall components would exert their roles to bind with Sb. This merits to be further studied.

In the present study, when the plants were exposed to 20 mg L^{-1} Sb (V), the addition of Se significantly enhanced the pectin (Fig. 3), hemicellulose (I and II) (Figs. 4, 5) and lignin contents (Fig. 6); and under 20 mg L^{-1} Sb(III) exposure, additional Se significantly increased the pectin, hemicellulose (I and II) and lignin contents (all at 0.2 mg L^{-1} Se) (Figs. 3,4,5,6). At the same time, when the plants were subjected to 20 mg L^{-1} Sb(III) or 20 mg L^{-1} Sb(V), additional Se significantly reduced the uptake of Sb in the roots (Table 3). The above results might suggest that additional Se induced more production of pectin, hemicellulose (I and II) and lignin, and promoted the aging of plant root cells thereby reducing Sb uptake. The enhanced lignin content in the rice roots might be associated with the enhanced expression of genes responsible for lignin synthesis (such as *OsPAL*, *OsCoMT* and *Os4CL3*) (Cui et al., 2018). In addition, under Sb(III) stress, a low dose of Se (0.2 mg L^{-1}) was shown to be superior to a high dose of Se (0.8 mg L^{-1}) to induce increased synthesis of pectin, hemicellulose (I and II) and lignin in the roots of rice plants. However, under Sb(V) stress, the conditions reversed, especially regarding the synthesis of pectin and hemicellulose (I and II). Reports also showed that Sb(III) was more toxic compared to Sb(V) in plants (He and Yang, 1999) and high levels of Se would deteriorate the growth of rice plant subjected to high levels of Cd exposure (Ding et al.,2014). Above results let us to speculate that Se could regulate the formations of pectin, hemicellulose (I and II) and lignin in the roots of rice plants, however, this may depend on the undergoing stress levels of plants and/or Se dosages.

In addition, enhanced lignification in rice roots was believed to result in a decrease of ROL of rice roots, therefore reduce iron plaque formation (Fleck et al., 2011). However, just as mentioned in the study of Fleck et al. (2011), other researchers observed an increased deposition of Fe and Mn oxides on the root surface treated with silicic acid (Okuda and Takahashi, 1964), which was not inconsistent with the decreased ROL found in the study of Fleck et al. (2011). Similarly, in this study enhanced lignin content and increased Fe plaque or Mn plaque were simultaneously observed. For example, the single addition of 0.2 mg L^{-1} Se significantly enhanced the Mn plaque and lignin content (Figs. 2a, b and 6 a, b) when compared to the control; Under 5 mg L^{-1} Sb(V) exposure, additional 0.2 mg L^{-1} Se significantly increased the lignin content (Fig. 6b) and Mn plaque (Fig. 2b) when compared to the 5 mg L^{-1} Sb(V) treatment. Other unknown oxidation drivers might exist and be responsible for the formation of root plaques.

4. Conclusions

The single addition of Se, Sb (III), and Sb(V) had adverse effects on the growth of rice plants, and Sb(V) showed less toxicity than Sb(III) to

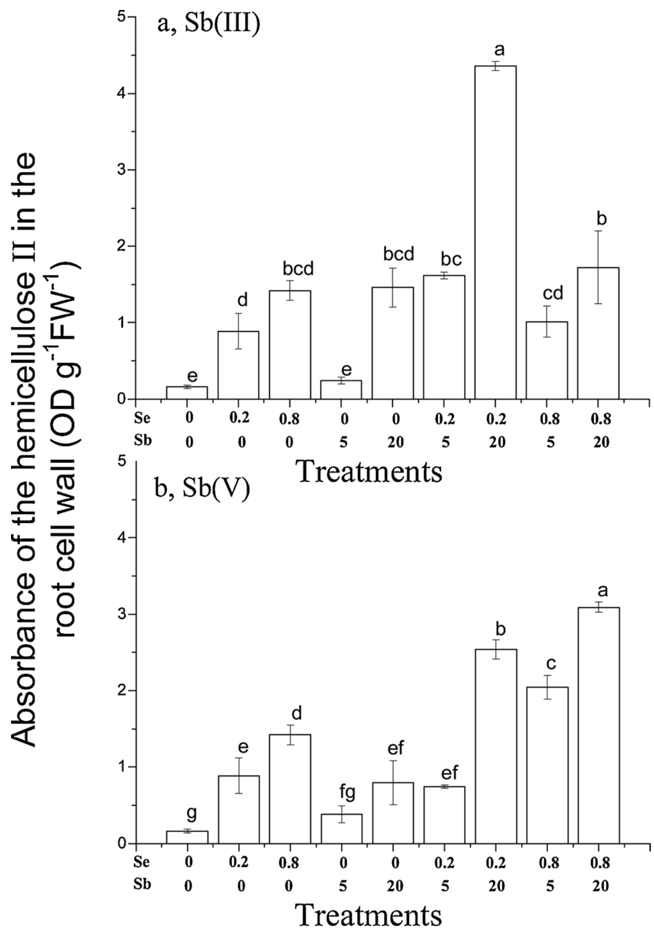

Fig. 5. Effects of Se and different forms of Sb on the absorbance hemicelluloses II in the root cell wall. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$.

rice. The addition of Se and different speciation of Sb could to some extent stimulate the formation of root plaques. However, the Sb retained on the plaques was not enhanced correspondingly when Se was added to the treatments containing different speciation of Sb. When rice plants were subjected to different speciations of Sb, Se could not regulate the uptake of Sb and most essential elements via affecting the formation of root plaques to bind them. In this study, Se could control the uptake of Sb in rice plants exposed to different speciation of Sb by affecting the aging process of rice plant roots, in particularly for Sb(V) exposure.

Author statement

All authors are in agreement with the content of the manuscript. We confirm that none of the material in this manuscript has not been published or is under consideration for publication elsewhere. No other author has reported a potential conflict of interest relevant to this article

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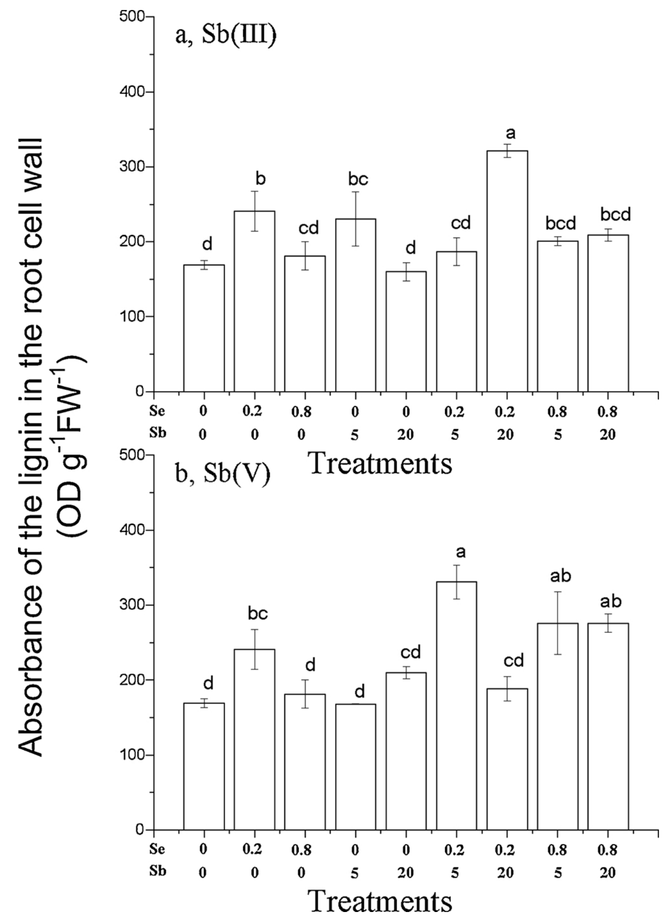

Fig. 6. Effects of Se and different forms of Sb on the absorbance lignin in the root cell wall. Bars are means and standard errors for three replications. Different letters above bars indicate significant difference at $p \leq 0.05$.

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