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Full Length Article

Effect of Iron Plaque and Selenium on Cadmium Uptake and Translocation in Rice Seedlings (*Oryza sativa*) Grown in Solution Culture

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Abstract

This paper investigated the effects of iron (Fe) plaque of rice root and selenium (Se(VI)) on cadmium (Cd) uptake and translocation within rice (*Oryza sativa* L.) seedlings grown in nutrient solution. Rice was grown in solution culture with or without Se(VI) (0.5 mg/L) for 3 weeks. To produce different amounts of Fe plaque, ferrous Fe at the levels of 0, 20, 50, 100 mg/L Fe^{2+} were added to the nutrient solution for 24 h. Nutrient solution with 1.0 mg/L Cd was then supplied for rice seedlings for 3 days. The results showed that Se(VI) stimulated rice growth and increased the dry weight of roots and shoots. The iron concentrations in shoots, roots and root surfaces increased gradually in parallel with the increasing amounts of iron plaque, both with and without Se(VI). The presence of iron plaque caused no significant changes of the Cd concentrations in the roots of rice without Se(VI). However, in the shoots and the DCB extracts, the Cd concentrations reflected decreasing and increasing trends respectively. Se(VI) did not affect the Cd concentrations in root surfaces and roots, but reduced shoots Cd concentrations compared to those without Se(VI). These results indicate that iron plaque and Se(VI) block Cd translocation and alleviate Cd toxicity to rice seedlings.

Keywords: Cadmium; Rice; Selenium; Iron plaque; Translocation

Introduction

Cadmium (Cd) is toxic heavy metal entering the environment from anthropogenic activities such as mining, smelting, electroplating etc., and is also commonly used as a component of pigments (Buxbaum and Pfaff, 2005; Scoullos *et al*., 2001; Dahmani-Muller *et al*., 2000). The presences of Cd in the environment can adversely affect plant growth and human health (Hussain *et al*., 2010, 2012). It was found that growth of rice seedling crown roots, which are essential for maintaining their normal growth and development, decreased under Cd stress (Xiong *et al*., 2009; Ahmad *et al.,* 2013). Cd has also been reported to inhibit germination, root and coleoptile growth of barley, and reduced grain yield of rice (Munzuroglu and Zengin, 2006; Liu *et al*., 2007). In contaminated regions of Japan, the chronic exposure of humans to Cd resulted in the occurrence of an epidemic of osteomalacia (Itai-Itai disease), and triggered the dysfunction of their renal tubules when concentration of Cd reached or exceeded 200 μg/g (Mao *et al*., 2011). China produced almost one-fifth of the world Cd and is the top producer of Cd (Brown *et al*., 2010). This immensely increased the chance of plants exposure to Cd and posed serious challenges to human health. For example, Zhang *et al*. (2014) found that Cd levels in Dayu county which was a Cd-contaminated area in Jiangxi Province of China, were higher than those in 1987, and renal dysfunction of residents became worse mainly due to rice consumption and smoking (local cultivated tobacco leaves); the average Cd concentration in rice from polluted areas was increased from 0.23 mg/kg in 1987 to 0.59 mg/kg in 2006.

Rice (*Oryza sativa* L.) is the world's dominant staple food, and represents 30% of the world cereal production and will be supplied for 4.6 billion people for their daily nourishment in 2025 (Gnanamanickam, 2009). Several studies had reported that rice was directly or indirectly subjected to Cd pollution in the environment (Watanabe *et al*., 2000; 2004). In addition, rice is a wetland crop plant that is often precipitated by iron (Fe) oxides on root surfaces called "iron plaque" arising from oxygen loss from roots under anaerobic conditions. Studies on the effects of Fe plaque have been reported on adsorbing arsenic (As), nickel (Ni), zinc (Zn) and copper (Cu), and alleviating their toxicities to plants (Liu *et al*., 2004a; Du *et al*., 2009).

Selenium (Se) is one of the essential micronutrient elements for human health. Suboptimal dietary Se intakes have been shown to increase the risk of developing cancer and heart disease, and to cause hypothyroidism and immune system dysfunctions (Combs, 2000). In regions of the world

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wherein the concentrations of Se in the soil are very low, especially in some parts of China (Ellis and Salt, 2003; Fang *et al*., 2003), Se is added to fertilizers to avoid problems caused by Se deficiency in agriculture production (Grant *et al*., 2007). Several studies have been carried out regarding Se detoxification of heavy metals in humans or animals (Drasch *et al*., 1996; Novoselov *et al*., 2005; Cabanero *et al*., 2006; Schrauzer, 2009). There are also several documents mentioned about the interaction between Se and heavy metals in plants (Zembala *et al*., 2010; Feng *et al*., 2011). However, there are no studies that investigate the response of rice with selenium (Se(VI)) in the presence of Fe plaque exposed to Cd. Therefore the aim of the present study was to investigate the effects of Fe plaque of rice root with or without Se(VI) on Cd uptake and translocation in rice seedlings grown in nutrient culture.

Materials and Methods

Pre-culture of Plants

The seeds of rice (TY180, local varieties of Fujian Province, People's Republic of China) were sterilized in 30% v/v H_2O_2 for 10 min and washed five times using deionized water. The seeds were germinated for 10 days in quartz sand which was soaked in 5% v/v hydrochloric acid overnight and then washed thoroughly using deionized water until washing was neutral. 24 seedlings growing uniformly were selected and divided into two groups which were respectively grown in 1/3 full strength Hoagland solution with or without Se(VI) (0.5 mg/L) for 3 weeks. The nutrient composition of full strength solution was: 1181 mg/L $Ca(NO_3)_2{\cdot}4H_2O$, 510 mg/L KNO_3, 490 mg/L $MgSO_4{\cdot}7H_2O$, 136.09 mg/L KH_2PO_4, 5.56 mg/L Fe(Ⅱ)-ethylenediaminetetraacetic acid (EDTA), 2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2{\cdot}4H_2O$, 0.08 mg/L $CuSO_4{\cdot}5H_2O$, 0.22 mg/L $ZnSO_4{\cdot}7H_2O$, 0.09 mg/L $H_2MoO_4{\cdot}4H_2O$. The pH of nutrient solution was adjusted to 5.5 with 0.1 M sodium hydroxide (NaOH) or hydrochloric acid (HCl) and renewed every 3 days. The experiment was carried out in a greenhouse with 70% humidity, temperature varying between 25-35ºC and light exposure varying between 12-14 h/day during the experiment period.

Experimental Treatments

After 3 weeks duration, rice seedlings were grown in deionized water for 1 day to eliminate element interferences with iron followed by exposure to 0, 20, 50 and 100 mg/L Fe^{2+} for 24 h (Fe^{2+} as $FeSO_4{\cdot}7H_2O$). Rice seedlings were transferred to 1/3 full strength Hoagland solution for another 2 days adaptive growth and then subjected to 1.0 mg/L Cd^{2+} (Cd^{2+} as $CdSO_4{\cdot}4H_2O$) for 3 days.

Chemical Analysis of Plant Samples

After 3 days, plants were harvested and separated into root and shoot material and thoroughly washed using deionized water. Dithionite–citrate–bicarbonate method (DCB) was used for extracting Cd and Fe in iron plaque of rice root according to Otte *et al*. (1989). Briefly, fresh root material of rice plants was incubated in 30 mL 0.125 M sodium bicarbonate ($NaHCO_3$), 0.03 M sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 0.8 g sodium dithionite ($Na_2S_2O_4$) for 1h at room temperature (25ºC). Roots were washed three times using deionized water and the residual liquid was mixed with the 30 mL DCB extracts. The mixed liquid was transferred to 100 mL volumetric glass for analysis. Shoots and roots were transferred to drying oven at 70^{o}C to achieve constant weight. Dried shoot and root material were ground for digestion, weighed and placed into digestion tubes with5 mL nitric acid (HNO_3) into tubes overnight. To each tube, 5 mL HNO_3 and 2 mL H_2O_2 were added and heated on a digestion block at 90^{o}C for 3 h, 120^{o}C for 3 h, and 160^{o}C for 2 h until about 1 mL solution in tubes was remained. The digests were transferred to 25 mL volumetric glass after cooling. A reagent blank and a standard reference material (bush twigs and leaves, GBW07603, Chinese National Certified Reference Material) were used in this process.

Statistical Analysis

Total uptake and distributions of the elements in rice seedlings were calculated according to Liu *et al*. (2004a). Analysis of variance (ANOVA) was used to test for statistically significant changes in dry weights and metal concentrations using SPSS 13 system for Windows. Means and standard deviation were used for data which were analyzed using least significant difference at the 5% level.

Results

Formation of Iron Plaque and Plant Growth

Rice roots became reddish from white after 24 h pre-treatment of ferrous Fe, indicating that Fe plaque was precipitated on rice root surface (data not shown). Iron plaque showed no significant effect on the biomass of rice shoots and roots after 3 weeks of cultivation in nutrient solution with or without Se and 24 h of treatment of iron plaque, however, Se(VI) significantly stimulated rice growth and increased the dry weight of shoots and roots (Table 1).

Fe Uptake and Translocation

The Fe concentrations in shoots, roots and DCB extracts of rice seedlings significantly increased in parallel with increasing Fe^{2+} supply, both with and without Se(VI) (Table 2). Se(VI) supply significantly reduced Fe concentrations in shoots and DCB extracts, but significantly increased Fe concentrations in roots (Table 2). Rice root surfaces accumulated significantly higher Fe concentration compared with shoots and roots (Table 2). The highest proportion of

Table 1: Mean dry weight (g/plant) of roots and shoots of rice seedlings grown in nutrient solution supplied with various levels of Se(VI) and Fe

Se supplied (mg/L)	Fe supplied (mg/L)	Shoot Dry Weight (g/plant)	Root Dry Weight (g/plant)
0	0	0.331 ±0.022	0.077 ±0.003
	20	0.297 ±0.062	0.066±0.009
	50	0.324 ±0.045	0.074 ±0.014
	100	0.323 ±0.074	0.068 ±0.018
0.5	0	0.453±0.009	0.124 ±0.001
	20	0.466 ±0.031	0.127 ±0.007
	50	0.452±0.004	0.130 ±0.009
	100	0.458±0.067	0.137±0.021
Analysis of variance			
Se		$p<0.001$	$p<0.001$
Fe		*ns*	*ns*
Se×Fe		*ns*	*ns*

Data are means ± SD ($n = 3$). The "*ns*" means non-significant

Table 2: Concentration of Fe (mg/kg) in dithionite-citrate-bicarbonate (DCB) extracts on the root surface, roots and shoots of rice seedlings grown in nutrient solution with various levels of Se(VI) and Fe

Se supplied (mg/L)	Fe supplied (mg/L)	Shoot-Fe (mg/kg)	Root-Fe (mg/kg)	DCB-Fe (mg/kg)
0	0	230.15 ±24.99	192.75±10.38	7034.98±625.35
	20	340.63 ±16.58	489.67±32.02	55264.17±6359.62
	50	442.36±82.97	720.20 ±61.74	65995.03±4286.65
	100	673.03±169.94	1096.48±161.17	80523.94±6277.81
0.5	0	133.55±28.43	140.12 ±38.45	5933.86±150.79
	20	276.01 ±66.58	624.34 ±98.62	49332.12±857.29
	50	355.00 ±58.78	892.32 ±139.29	61007.46±7883.86
	100	567.93±27.38	1377.46 ±89.99	62625.08±559.00
Analysis of variance				
Se		$p = 0.012$	$p = 0.003$	$p<0.001$
Fe		$p<0.001$	$p<0.001$	$p<0.001$
Se×Fe		*ns*	$p = 0.046$	$p = 0.029$

Data are means ± SD ($n = 3$). The "*ns*" means non-significant

Table 3: Percentages of Fe (%) in different parts of rice seedlings grown in nutrient solution supplied with various levels of Se(VI) and Fe

Se supplied (mg/L)	Fe supplied (mg/L)	Shoot-Fe (%)	Root-Fe (%)	DCB-Fe (%)
0	0	12.10 ±1.61	2.35±0.20	85.55±1.62
	20	2.65±0.24	0.87 ±0.16	96.48±0.40
	50	2.87±0.73	1.05±0.09	96.08±0.79
	100	3.79±0.66	1.29 ±0.09	94.92±0.64
0.5	0	7.41±1.29	2.12±0.49	90.47 ±1.69
	20	1.97 ±0.35	1.23 ±0.19	96.81 ±0.30
	50	1.97 ±0.18	1.41±0.13	96.62±0.23
	100	2.88±0.13	2.09±0.15	95.03 ±0.01
Analysis of variance				
Se		$p<0.001$	$p = 0.00255$	$p = 0.001$
Fe		$p<0.001$	$p<0.001$	$p<0.001$
Se×Fe		$p = 0.002$	$p = 0.009$	$p<0.001$

Data are means ± SD ($n = 3$). The "*ns*" means non-significant.

Fe was concentrated in DCB extracts (85.6-96.8%) in comparison with that in shoots (0.9-2.4%) and roots (2.0-12.1%; Table 3). Fe supply significantly decreased the percentages of Fe in shoots and roots. Similarly, Se(VI) supply significantly decreased the percentages of Fe in shoots (Table 3).

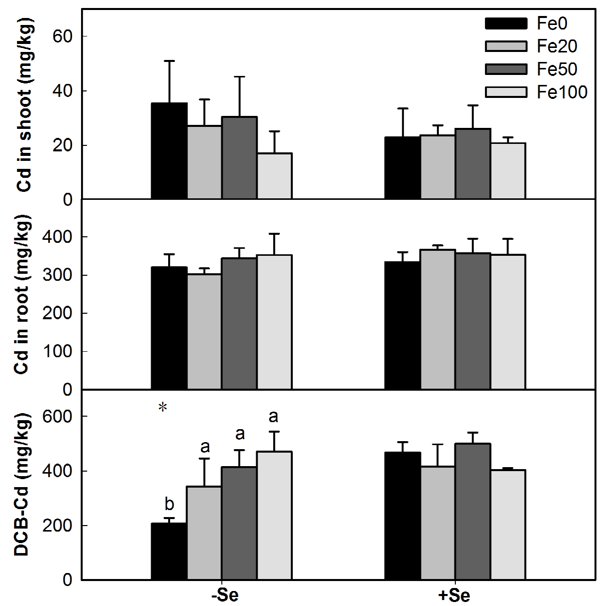

Fig. 1: Concentration of Cadmium (Cd) concentrations (mg/kg) in dithionite-citrate-bicarbonate (DCB) extracts, roots and shoots of rice seedlings grown in nutrient solution with various levels of selenium (Se(VI) and iron (Fe). Data are means ± SD ($n = 3$). Different letters above bars indicate significant differences among Fe treated levels at $p<0.05$ in different plant part. The "*" labeled bars mean significant differences between –Se(VI) and +Se(VI)induction at $p< 0.05$ at certain Fe level

Cd Uptake and Translocation

The concentration of Cd in DCB extract with Se(VI) significantly increased in Fe0 compared to that without Se (VI) in Fe0 (Fig. 1). Se(VI) supply significantly increased Cd concentrations in DCB extracts, but caused no significant changes of Cd concentrations in rice roots. Although no significant differences were observed, Cd concentrations in shoots were reduced with Se(VI) in comparison with those without Se(VI). The percentages of Cd in shoots (8.4-10%)with Se(VI) also showed a decreasing trend compared to shoot without Se(VI) (8.7-22.4%), however, more Cd was mainly concentrated in roots (37.5-42.7%) and Fe plaque (47.8%-52.9%) with Se(VI) or roots (38.7-47.1%) and Fe plaque (30.5-52.6%) without Se(VI) (Fig. 2). In addition, Fe plaque reduced Cd concentrations in shoots without Se(VI) although there was no statistical difference and significantly increased Cd concentrations on root surfaces (Fig. 1). Cd concentrations in DCB extracts gradually increased in parallel with the increasing amounts of iron plaque with a good linear relationship between DCB-Fe and DCB-Cd without Se (VI), but not with Se(VI) (Fig. 3).

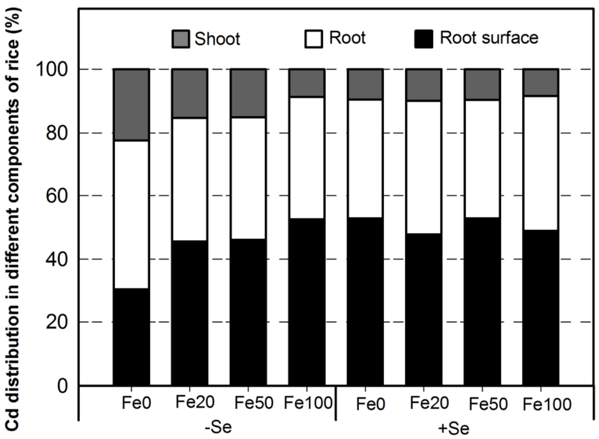

Fig. 2: Percentages of Cd(%)in different components of rice seedlings grown in nutrient solution with various levels of Se(VI) and Fe

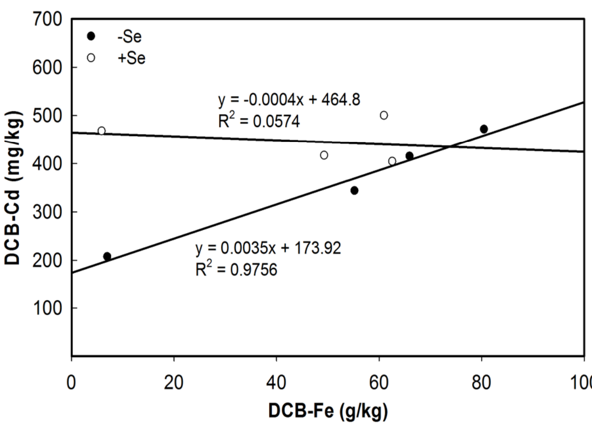

Fig. 3: Relationship between Cd concentration (mg/kg) and Fe concentration (mg/kg)in dithionite-citrate-bicarbonate (DCB) extracts on the root surface of rice seedlings grown in nutrient solution with various levels of Se(VI) and Fe

Discussion

The results in this study revealed that rice growth was not significantly affected by iron plaque. Similar findings were reported by Liu *et al.* (2004b) and Xu and Yu (2013) in hydroponic studies, however, Fe plaque was found to reduce rice growth in soil culture (Liu *et al.*, 2008). Different concentrations of Fe addition in culture matrix and exposure period between these studies may have resulted in the observed differences. Our results showed Se(VI) supply could enhance the growth of rice seedlings. These findings are similar to those reported by Dhillon and Dhillon (2009) who found 1.25 mg/kg Se(VI) could play a positive role on biomass of *Chenopodium album* L., however, elevated Se (VI) supply (2.5 mg/kg) decreased its biomass. Studies by Yao *et al.* (2009) and Ramos *et al.* (2011) showed that Se supply could increase the dry weight of wheat and lettuce. The biomass of wheat seedlings were promoted by 1 and 2 mg/L Se(VI), and 1, 2 and 3 mg/L Se(VI) could significantly increase root activities (Yao *et al.*, 2009). 15 µM Se(VI) treatment stimulated lettuce (Line: Shining Star, Ruby, Prize head, Pavane and Eruption) growth and increased shoot biomass by 13% (Ramos *et al.*, 2011). Se as an essential element nutrient for organisms is beneficial to plants growth at low dose, however, can inhibit growth at high dose.

We report that Fe plaque can accumulate more Fe on rice root surface compared with control. Ye *et al.* (1997) and Liu *et al.* (2008) reported root surfaces of *Typha latifolia* and rice accumulated the highest concentrations and proportion of Fe in the presence of iron plaque. *Typha latifolia* in a hydroponic system accumulated 20959 mg/kg Fe on root suface under 15 mg/L Fe^{2+} for 1 week in comparison with 113 mg/kg of control (Ye *et al.*, 1997). More than 30000 mg/kg Fe precipitated on root sufaces of rice cultivated in soilunder 1 and 2 g/kg Fe^{2+} treatment for 42 days, and only less than 8000 mg/kg Fe was concentrated on root suface of control (Liu *et al.*, 2008). Our results suggest that the regular pattern of Fe concentrations in DCB extracts was interrupted by Se (VI) supply which also decreased Fe concentrations and proportion in shoots. These results suggested that Se(VI) could play essential roles in alleviating the adverse effect of excess Fe on rice growth. Feng *et al.* (2012) indicated that Se could regulate Fe level in *Pteris vittata* L. to defend to environmental stress. They found the consistent changes in the Fe concentration, MDA content and SOD activity; they decreased at low dose Se (2 mg/L) but increased at high dose Se (20 mg/L).

The Cd concentrations and percentages in rice shoots showed decreasing trends with Se(VI) supply, indicating that Se(VI) could alleviate Cd toxic effect on rice plant to some extent. There have been many reports on alleviating toxicity of heavy metals to plants by Se. For example, Feng *et al.* (2011) showed that Se supply could reduce the concentrations of Se in rice and Cr in spinach. Zembala *et al.* (2010) also found that Se played protective roles in decreasing Cd toxicities to rape and wheat. Nevertheless, the role of Se in alleviating toxicity of heavy metals and increasing biomass of plants depended on ionic species, Se dose, Se valence and plant species. For example, Se enhanced Cd and Cu uptake and toxicity in different plants (Landberg and Greger, 1994; Liang *et al.,* 2012). A study by Muñoz *et al.* (2007) showed that Se(IV) or Se(VI) did not play beneficial effect on detoxifying Ag in mycelia of *Pleurotusos treatus*, and Se(IV) revealed strong beneficial effect in mycelia exposed to Cd in comparison to Se(VI).

Our results demonstrate that Fe plaque decreased rice shoot Cd concentrations and proportion by sequestering Cd on root surface, suggesting that Fe plaque could play an essential role in alleviating Cd toxicity to rice plants. Jiang *et al.* (2009) and Liu *et al.* (2004b) also found the concentrations of Ca, Cu, Zn, P and As in the Fe plaques were significantly correlated with the amounts of iron plaque. It was also found that Fe plaque of rice root could reduce Cd concentrations in shoots (Liu *et al*., 2008). However, our results contrast with those of Liu *et al*. (2007) showing that root surfaces of rice seedlings with Fe plaque accumulated less Cd under 1.0 mg/L Cd stress in comparison with that without Fe plaque. This could be due to different genotypes of the rice studied. Liu *et al*. (2004b) found arsenic (As) concentrations in shoots differed significantly between three genotypes of rice. Similarly, Geng *et al*. (2005) showed different accumulative abilities of root surfaces of rice seedlings to adsorb Fe, As and P between genotype 94D-54 and 94D-64.

Furthermore, Se(VI) supply decreased the Cd concentrations in rice shoots with or without Fe plaque compared to those without Se(VI) supply except for the Fe100 treatment. In addition, Fe concentrations in shoots and DCB extracts with Se(VI) supply were less than those without Se (VI) supply. These results indicate that Se(VI) could block Cd and Fe translocation to rice shoots. An earlier study has demonstrated that Se(VI) affected the leaf tissue concentrations of P, K, Ca, Mg, S, B, Cu, Mn and Mo in kale (Lefsrud and Kopsell, 2006). Similarly, Kopsell *et al*. (2000) also found Se(VI) supply reduced the content of B, Fe and P and increased the content of S and K in leaf tissue of *Brassuca oleracea*. These earlier findings confirmed our results, indicating Se(VI) effect involved in detoxifying Cd in rice plants. We suggest that Se(VI) may compete with Fe for some ligands or sites binding to some Fe related proteins, resulting in decreased Fe concentrations in shoots and root surfaces observed. Further studies are required to confirm this.

In conclusion, Fe plaque precipitated on root surfaces of rice seedlings grown in nutrient solution can effectively accumulate Cd. Iron plaque blocks Cd uptake and translocation to above-ground parts and alleviates Cd toxicity to rice plants without Se(VI). Se(VI) supply decreases the Cd concentrations and percentages in shoots, indicating that Se(VI) can play protective roles in the detoxification of Cd to rice plants. Further research is required to determine the roles of Fe plaque and Se(VI) on Cd translocation to rice grain.

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