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RESEARCH ARTICLE

Underlying mechanisms and effects of hydrated lime and selenium application on cadmium uptake by rice (*Oryza sativa* L.) seedlings

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Abstract A pot experiment was conducted to investigate the effects of selenium (Se) and hydrated lime (Lime), applied alone or simultaneously (Se+Lime), on growth and cadmium (Cd) uptake and translocation in rice seedlings grown in an acid soil with three levels of Cd (slight, mild, and moderate contamination). In the soil with 0.41 mg kg^{-1} Cd (slight Cd contamination), Se addition alone significantly decreased Cd accumulation in the root and shoot by 35.3 and 40.1%, respectively, but this tendency weakened when Cd level in the soil increased. However, Se+Lime treatment effectively reduced Cd accumulation in rice seedlings in the soil with higher Cd levels. The results also showed that Se application alone strongly increased Cd concentration in the iron plaque under slight Cd contamination, which was suggested as the main reason underlying the inhibition of Cd accumulation in rice seedlings. Se+Lime treatment also increased the ability of the iron plaques to restrict Cd uptake by rice seedlings across all Cd levels and dramatically decreased the available Cd concentration in the soil. These results suggest that Se application alone would be useful in the soil with low levels of Cd, and the effect would be enhanced when Se application is combined with hydrated lime at higher Cd levels.

Keywords Cadmium · Soil · Iron plaque · Lime · Selenium · Rice seedling

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Introduction

Currently, soil contamination by heavy metals and metalloids represents a serious problem in China. According to the national soil pollution survey report of China released in 2014, cadmium (Cd) ranks first in the percentage of sampling sites (7%) that exceeded the soil quality standards, among which 93% of sites were classified as slightly to moderately contaminated (0.3–1.5 mg kg^{-1}) (MEP and MLR 2014). Rice (*Oryza sativa* L.) is a major staple crop that is widely cultivated in China, and its average consumption rate is 219 g/capita/day which is almost 50% higher than the global average (148 g/capita/day) (Hu et al. 2016). Compared with other cereals, rice tends to accumulate more Cd and is the principal source of dietary Cd intake in the rice-eating population (Arao et al. 2009; Meharg et al. 2013). Excess Cd accumulation in the human body can induce many incurable diseases: such as itai-itai disease, cancer, and kidney disease among others (Baba et al. 2013; Skroder et al. 2015; Valko et al. 2006). Therefore, feasible measures are urgently needed to reduce Cd uptake and improve rice growth in Cd-contaminated soils.

To ensure sufficient food production, many slightly to moderately Cd-contaminated paddy soils in China are cultivated with appropriate agronomic management practices. Many alkaline materials and mineral fertilizers are used to inhibit Cd uptake by plants in acid soils (Kabir et al. 2016; Tang et al. 2015; Wang et al. 2014b), among which the application of selenium (Se) and hydrated lime has been proven to be an effective agronomic management practice in previous studies (Bolan et al. 2003; Hu et al. 2014). Se is an essential element for animals including humans; it can promote selenoprotein synthesis and release peroxide pressure in the cell (El-Demerdash and Nasr 2014), counterbalance Cd-induced oxidative stress (Lin et al. 2012), and inhibit the toxicity and accumulation of Cd in plants (Haghighi and da Silva 2016; Hu et al. 2014; Khan et al. 2015; Liu et al. 2015). In addition, Se added to soil can decrease Cd uptake by removing this element from metabolically active cellular sites in rape seedlings and by inhibiting upward translocation in plants (Ding et al. 2014; Filek et al. 2008; Hu et al. 2014). Hu et al. (2014) reported that Se applied to soil reduced Cd accumulation in brown rice by decreasing Cd bioavailability in soil. However, the mechanism through which Se decreases Cd bioavailability in soil remains unknown. Iron plaque on the rice root surface is considered as an efficient way by which Cd uptake is inhibited. Chang et al. (2013) found that Se reduced Cd accumulation in rice by increasing Cd adsorption by iron plaques, but Hu et al. (2014) reported that Se had no impact on the ability of iron plaques to restrict Cd uptake by rice. These studies focused primarily on the mature stage of rice, while iron plaques mainly formed during the early growth stage and decreased at later growth stage (Wang et al. 2013); consequently, the effect of Se on Cd adsorption by iron plaques was uncertain at mature stage. Therefore, the impacts of Se on Cd adsorption by iron plaques during early rice growing periods should be examined.

In previous studies, hydroponic experiments were generally conducted to determine the role of Se in alleviating Cd toxicity (Ding et al. 2014; Lin et al. 2012; Wang et al. 2014a), while its effect in actual Cd-contaminated soil was rarely studied. In addition, the efficiency of Se in reducing Cd uptake by rice may be affected by soil pH, because soil pH exerts significant effects on Se availability (Eich-Greatorex et al. 2007). As a traditional agricultural practice, hydrated lime application offers the most cost-effective and widely used means of immobilizing heavy metals in soils by increasing the pH value, and its efficiency is positively correlated with its dose (Bolan et al. 2003; Wang et al. 2015). However, excessive hydrated lime can damage soil, e.g., through soil hardening or excessive pH values, which can reduce the uptake of certain nutrient elements and therefore inhibit plant growth (Tyler and Olsson 2001). Thus, hydrated lime is usually applied to Cd-contaminated soil as a low dose and mixed with other materials. Little knowledge of the effects of Se and low doses of hydrated lime, added to soil simultaneously, with respect to reducing Cd uptake by rice could be found in previous studies. Accordingly, it was hypothesized that Se and a low dose of hydrated lime, applied simultaneously to an acid soil, could increase Se availability and promote its effect with respect to decreasing Cd bioavailability.

In this work, we aimed to study the effects of Se and hydrated lime, applied alone and simultaneously, on growth and Cd uptake by rice seedlings and to investigate changes in the available Cd concentrations in the soil, Cd adsorption by iron plaques, and Cd translocation in rice seedlings to clarify the underlying mechanisms associated with the effect of Se and lime application in the soil on Cd uptake by rice.

Materials and methods

Soil preparation and pot trial

Slightly Cd-contaminated paddy soil (0–20 cm) was collected in Xiangyin, Hunan province, China. The principal soil properties were as follows: pH (H_2O), 5.02; organic matter (OM), 29.63 g kg^{-1}; cation exchange capacity (CEC), 10.55 cmol kg^{-1}; total Cd, 0.41 mg kg^{-1}; total Se, 0.37 mg kg^{-1}; and soil texture: sand (38.4%), silt (41.0%), and clay (20.6%). After air-drying and passing through a 2-mm sieve, the soil was mixed with 0, 0.3, or 0.9 mg kg^{-1} Cd ($3CdSO_4 \cdot 8H_2O$) to simulate slightly (0.3–0.6 mg kg^{-1}), mildly (0.6–0.9 mg kg^{-1}) and moderately (0.9–1.5 mg kg^{-1}) Cd-contaminated soils, respectively, which were aged for 3 months. The soil humidity was maintained at 80% of the field water-holding capacity during this period. After additional air-drying and passing through another 2-mm sieve, Cd-contaminated soil was used in a pot trial. Cd-contaminated soil containing 0.68 and 1.28 mg kg^{-1} was obtained after the addition of 0.3 and 0.9 mg kg^{-1} Cd, respectively.

The pot trial was carried out in the greenhouse of the Institute of Soil Science Chinese Academy of Sciences, Nanjing, Jiangsu province, China. Each pot (30 cm diameter and 29 cm high) was filled with 8 kg soil. The soil was mixed with a base fertilizer: 0.2 g N kg^{-1} ($CO(NH_2)_2$), 0.15 g P_2O_5 kg^{-1} (CaH_2PO_4 H_2O), and 0.2 g K_2O kg^{-1} (KCl). The following treatments were applied to each Cd-contaminated soil ($Cd_{0.41}$ 0.41 mg kg^{-1}, $Cd_{0.68}$ 0.68 mg kg^{-1}, $Cd_{1.28}$ 1.28 mg kg^{-1}): control treatment (CK), 0.5 mg kg^{-1} Se (Na_2SeO_3) (Hu et al. 2014), referred to as Se, 1 g kg^{-1} hydrated lime ($Ca(OH)_2$ in powder form, conventional dosage used for Cd-contaminated farmland, referred to as Lime, and 0.5 mg kg^{-1} Se and 1 g kg^{-1} hydrated lime applied simultaneously (Se+Lime). Each treatment had three replicates. The soils were kept moist for 7 days before the rice was planted.

Plant culture and collection

The japonica rice cultivar Suxiangjing 1 (obtained from the Suzhou Academy of Agricultural Sciences, China) was selected for this study. After surface sterilization in a 15% H_2O_2 solution for 15 min followed by rinsing with deionized water, seeds were soaked in deionized water in the dark for 24 h at 25 °C. Then, the seeds were germinated on moist gauze for

24 h. Uniform seeds were sown in the pots directly on May 20, 2015. Three holes were formed in each pot, and five seeds were placed in each hole. After emerging, the seedlings were thinned to nine per pot, and the soils were continuously submerged in water (2–3 cm deep). The pots were arranged randomly.

When the rice seedlings grew to the three-leaf stage (approximately 30 days), the plants and soil in all pots were collected. The seedlings were rinsed three times with tap water followed by deionized water. The height of the plants and the length of the roots were measured using a ruler. Then, the plants were separated into roots and shoots, and the shoots were oven-dried at 75 °C for 3 days. The roots were used to extract iron plaques; then, they were oven-dried at 75 °C for 3 days, and the shoots and roots were weighed after oven-drying. After air-drying and passing through a 2-mm sieve, the soil was used to extract available Cd.

Chemical analysis

Iron plaques on the root surface of the rice were extracted using the dithionite–citrate–bicarbonate (DCB) method (Otte et al. 1989; Taylor and Crowder 1983): the entire root systems from each pot was placed in a flask with 40 mL 0.03 mol L^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) and 0.125 mol L^{-1} sodium bicarbonate ($NaHCO_3$) solution, to which 1.0 g sodium dithionite ($Na_2S_2O_4$) was added, followed by incubation for 70 min after slight shaking. Then, the roots were rinsed with deionized water, and the water was collected and added to the extracting solution. After filtration, the extracting solution was made up to a final volume of 100 mL finally, and two drops of concentrated nitric acid were added for conservation and measurement of Cd.

Oven-dried shoots and roots (without iron plaques) were milled; approximately 0.2 g samples were weighed into polytetrafluoroethylene tubes, to which 4 mL high purity HNO_3 was added for cold digestion overnight. Then, 2 mL high purity H_2O_2 was added to the tubes for digesting using a high-pressure digestion system at 140 °C for 4 h. After the system cooled, the tubes were removed and 2 mL HCl (1:1) was added. Finally, the solution was transferred to centrifuge tubes after acid evaporation for the measurement of Cd and Se.

Available Cd in the soil was extracted using a 0.01 M $CaCl_2$ solution (Houba et al. 2000). Soil pH was measured at a soil/water ratio of 1:2.5 using a pH meter (Orion Star A211, Thermo, USA).

Cd was measured using atomic absorption spectroscopy (SpectrAA 220Z, Varian, USA). Se was measured by atom fluorescence spectroscopy (AFS-930, JiTian Instruments, Beijing).

Data analysis

Cd concentration in the iron plaque ($Cd_{\text{iron plaque}}$), translocation factors (TFs) of Cd from the roots to shoots, and the distribution rates in different parts of the rice seedling were calculated by equation as follows:

$$\mathrm{Cd}_{\textit{iron plaque}} = \left[\mathrm{T}_{\textit{iron plaque}-\mathrm{Cd}}\right]/[\mathrm{DW}_{\textit{root}}]$$

$$\mathrm{TF} = [\mathrm{Cd}_{\textit{shoot}}]/\left[\mathrm{Cd}_{\textit{root}} + \mathrm{Cd}_{\textit{iron plaque}}\right]$$

$$\mathrm{Cd}\ \textit{distribution rate in iron plaque}\ (\%) = \left[\mathrm{Cd}_{\textit{iron plaque}} \times \mathrm{DW}_{\textit{root}}\right] / \left[\mathrm{Cd}_{\textit{iron plaque}} \times \mathrm{DW}_{\textit{root}} + \mathrm{Cd}_{\textit{root}} \times \mathrm{DW}_{\textit{root}} + \mathrm{Cd}_{\textit{shoot}} \times \mathrm{DW}_{\textit{shoot}}\right]$$

$$\mathrm{Cd}\ \textit{distribution rate in root}\ (\%) = [\mathrm{Cd}_{\textit{root}} \times \mathrm{DW}_{\textit{root}}] / \left[\mathrm{Cd}_{\textit{iron plaque}} \times \mathrm{DW}_{\textit{root}} + \mathrm{Cd}_{\textit{root}} \times \mathit{DW}_{\textit{root}} + \mathit{Cd}_{\textit{shoot}} \times \mathrm{DW}_{\textit{shoot}}\right]$$

$$\mathrm{Cd}\ \textit{distribution rate in shoot}\ (\%) = [\mathrm{Cd}_{\textit{shoot}} \times \mathrm{DW}_{\textit{shoot}}] / \left[\mathrm{Cd}_{\textit{iron plaque}} \times \mathrm{DW}_{\textit{root}} + \mathrm{Cd}_{\textit{root}} \times \mathrm{DW}_{\textit{root}} + \mathrm{Cd}_{\textit{shoot}} \times \mathrm{DW}_{\textit{shoot}}\right]$$

where $T_{\text{iron plaque-Cd}}$ is the total content of Cd in the iron plaque. $Cd_{\text{iron plaque}}$, Cd_{shoot}, and Cd_{root} are the concentrations of Cd in the iron plaque, shoots, and roots (without iron plaque), respectively. DW_{root} and DW_{shoot} are the dry weight of root and shoot.

Statistical analysis

The statistical differences among treatments in the same Cd level were determined by one-way analysis of variance (ANOVA); the effects of treatments, Cd levels in soil, and their interactions on Cd and Se concentrations in rice were determined by two-way ANOVA using the least significant difference (LSD) ($P < 0.05$). The data were expressed as means ± SD ($n = 3$). The statistical analyses were performed using SPSS 19.0, and all figures were generated using SigmaPlot 10.0 software.

Results

Effects of exogenous Se and hydrated lime on the growth of the rice seedlings

The growth indices of rice seedlings are presented in Fig. 1. In the soil with 1.28 mg kg^{-1} Cd, compared with CK, Se, and Se+Lime treatments significantly increased the weight of seedling roots (26.6 and 37.2%) and shoots (22.5 and 24.3%) (Fig. 1a, b). However, no significant differences were found for the dry weights of the roots and shoots, the root length, and shoot height among treatments when rice

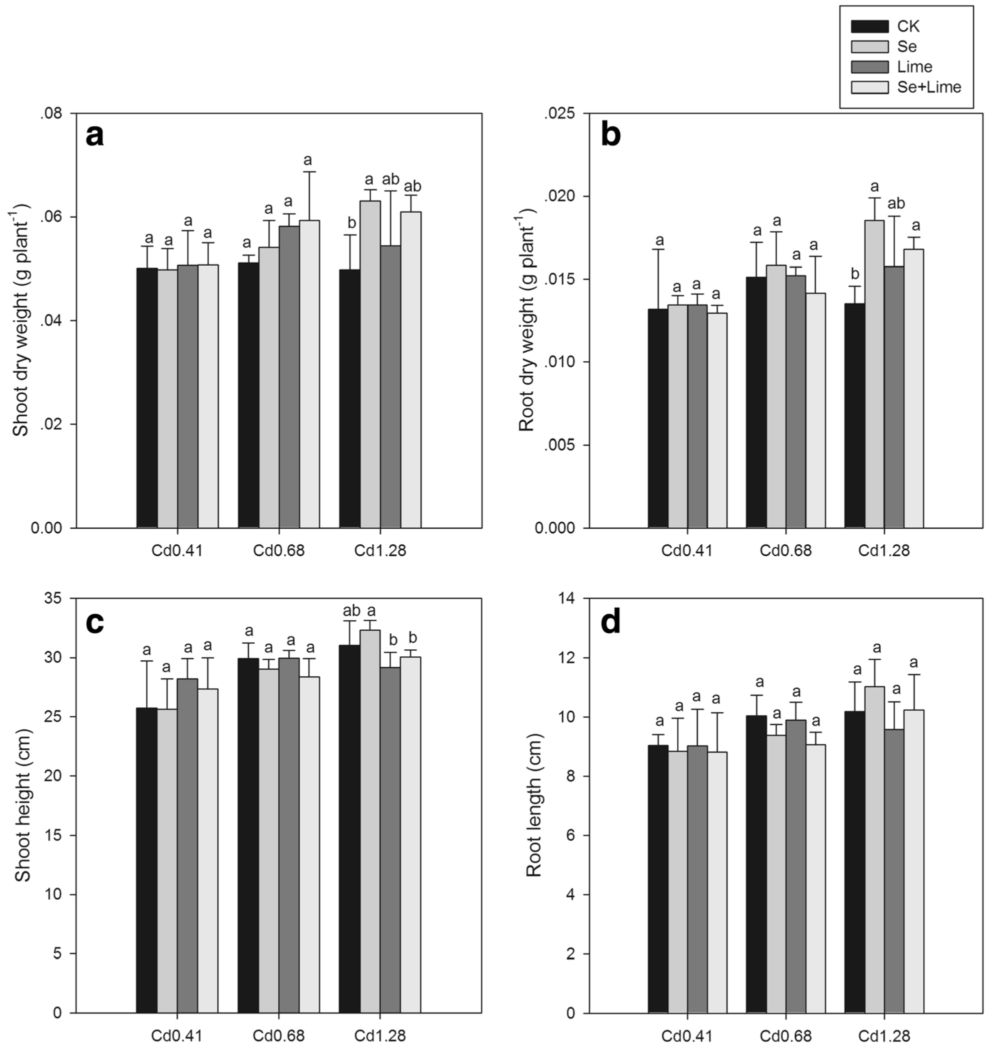

Fig. 1 Changes of the shoots dry weight (DW) (**a**) and height (**c**), roots dry weight (**b**) and length (**d**) of rice seedlings exposed to three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) when selenium (Se) and hydrated lime (Lime) were applied alone or simultaneously (Se+Lime). Values are means with standard deviations shown by the *vertical bars* ($n = 3$). *Bars with the same letter(s)* indicate no significant differences between different treatments at $P < 0.05$

seedlings were planted in the soils with 0.41 and 0.68 mg kg^{-1} Cd (Fig. 1).

Effects of exogenous Se and hydrated lime on Cd and Se uptake by rice seedlings

Concentrations of Se and Cd in rice seedling roots and shoots are shown in Fig. 2. One-way ANOVA showed that the concentration of Cd in rice seedling was significantly affected by Se and hydrated lime application (Fig. 2a, b). In the soil with 0.41 mg kg^{-1} Cd, Se application alone significantly decreased Cd concentration in the roots by 35.3% with respect to the control (CK) (Fig. 2a), which was the highest reduction rate. The Lime and Se+Lime treatments decreased Cd concentrations in seedling roots by 12.8 and 22.6%, respectively, at this level of Cd contamination (Fig. 2a). In the soils with 0.68 and 1.28 mg kg^{-1} Cd, the reduction rates of Cd concentration in the roots were the highest when Se and lime were applied together (Fig. 2a). When Se was applied alone, the reduction rates were only 16.8 and 12.4% in the soils with 0.68 and 1.28 mg kg^{-1} Cd, respectively (Fig. 2a), while the Lime treatment decreased Cd concentrations in the root by 10.9 and 22.3% in the soils with 0.68 and 1.28 mg kg^{-1} Cd (Fig. 2a), respectively (Fig. 2a). Similar results were observed for the shoots of rice seedlings. Compared with CK, all treatments significantly decreased Cd concentrations in the shoots at all Cd levels (Fig. 2b). When compared from the lowest to the highest Cd concentrations assayed, the respective reduction rates were 40.1, 25.4, and 20.6% for Se treatment; 11.2, 14.7, and 25.7% for the Lime treatment; and 27.5, 29.8, and 44.3% for Se+Lime treatment.

Exogenous Se strongly increased Se concentrations of rice seedlings (Fig. 2c, d). With Se application alone, Se accumulation in the roots and shoots reached 1.74–2.82 and 0.26–0.31 mg kg^{-1} (Fig. 2c, d), respectively, nearly 5–10 times that of the control. However, the simultaneous application of hydrated lime and Se markedly decreased Se uptake (Fig. 2c, d). In addition, Se accumulation in rice seedlings was strongly affected by Cd level in the soil, as Cd level in the soil increased, Se concentrations in both rice roots and shoots decreased in all treatments.

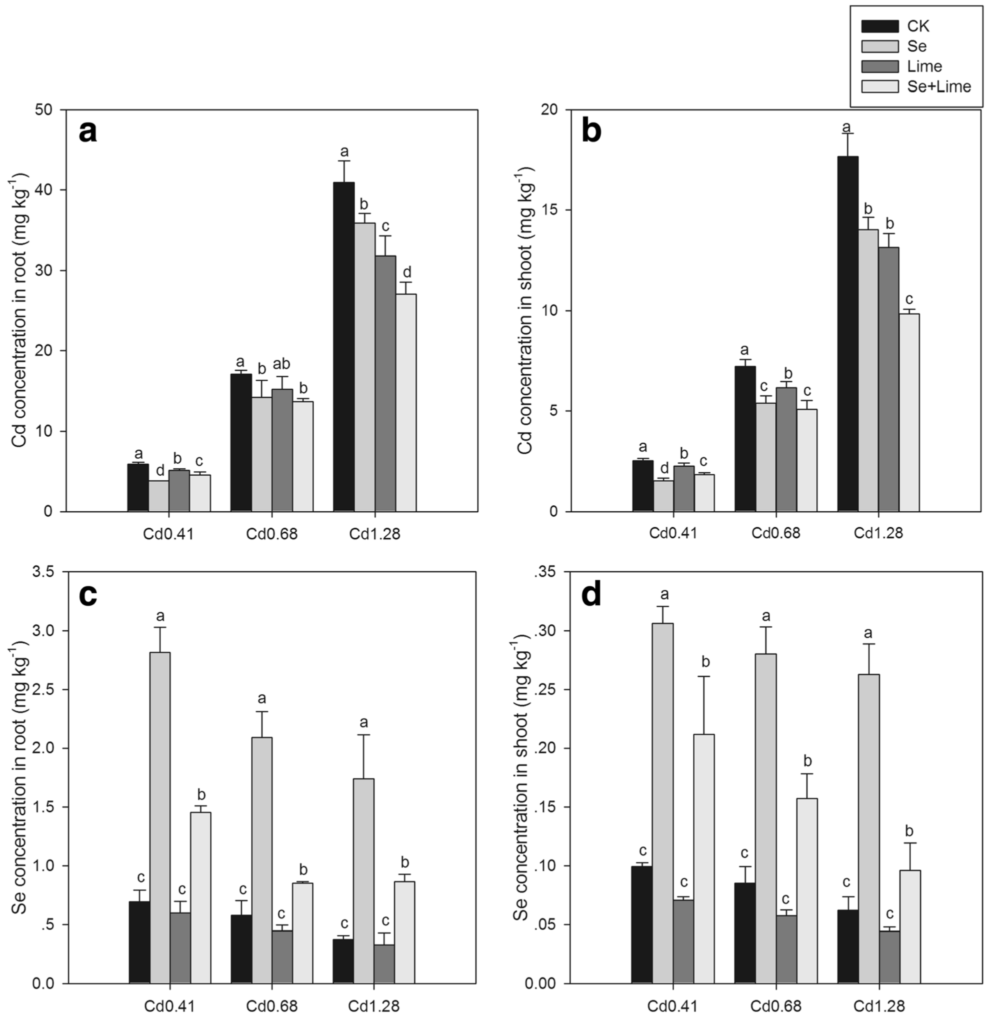

Fig. 2 Changes of Cd accumulation in the roots (without iron plaque) (**a**) and shoots (**b**), Se accumulation in the roots (without iron plaque) (**c**) and shoots (**d**) of rice seedlings exposed to three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) when selenium (Se) and hydrated lime (Lime) were applied alone or simultaneously (Se+Lime). Values are means with standard deviations shown by the *vertical bars* ($n = 3$). *Bars with the same letter(s)* indicate no significant differences between different treatments at $P < 0.05$

Two-way ANOVA showed that the concentrations of Cd and Se in seedling roots and shoots were not only significantly ($P < 0.01$) affected by soil Cd level and treatment, but also significantly ($P < 0.01$) affected by their interaction (Table 1). Although the contribution of individual factor or their interaction to the total variation (sum of squares, SS) varied between roots and shoots, Cd levels in soil consistently exerted the greatest effect on Cd concentrations in rice seedlings; while Se application exerted the greatest effect on Se concentrations in rice seedlings.

Effects of exogenous Se and hydrated lime on Cd accumulation in root iron plaque

Table 1 revealed that Cd concentration in the iron plaques depended both on the treatment considered and Cd level in the soil, as indicated by the significant interaction term. In the soil with 0.41 mg kg^{-1} Cd, Se application alone significantly increased Cd concentration in the iron plaque (Fig. 3), which reached to 52.65 mg kg^{-1} and was twice as high as that measured in the control, while this effect was weakened as Cd level in the soil increased. In the soils with 0.68 and 1.28 mg kg^{-1} Cd, when Se was applied alone, Cd concentrations in the iron plaque were increased by only 39.7 and 14.4%, respectively, but this effect was potentiated when lime was applied together with Se (i.e., Cd concentrations in the iron plaque were increased by 100.8 and 57.5% with respect to the control, respectively). Lime addition alone had no significant effect on Cd concentration in the iron plaque (Fig. 3).

Effects of exogenous Se and hydrated lime on Cd distribution and translocation factors in rice seedling tissues

The allocation of Cd in rice seedlings exposed to the different treatments is shown in Fig. 4. In the soil with 0.41 mg kg^{-1} Cd, Cd occurred mainly in the iron plaques, accounting for 57.5–84.8%, whereas only 9.1–26.5% of Cd was distributed to the shoots. However, the proportion of Cd in the iron plaque decreased with increasing Cd in the soils. In terms of the treatments, Se application alone dramatically increased Cd levels in the iron plaques and decreased Cd allocation to the

Table 1 Two-way ANOVA results showing the main effects of treatment (i.e., Se and/or hydrated lime addition), Cd level in soil and their interaction on the concentration of Cd and Se in rice seedlings)

		SS	MS	F
Cd concentration in iron plaque	Treatment	1301	325.3	27.93***
	Cd level in soil	782.6	391.3	33.60***
	Treatment × Cd level in soil	1888	236.1	20.27***
	Error	349.4	11.65	
Cd concentration in root	Treatment	225.9	56.49	14.53***
	Cd level in soil	5874	2937	755.3***
	Treatment × Cd level in soil	231.2	28.90	7.432***
	Error	116.7	3.889	
Cd concentration in shoot	Treatment	67.21	16.80	52.52***
	Cd level in soil	886.4	443.2	1385***
	Treatment × Cd level in soil	72.91	9.114	28.49***
	Error	9.597	.320	
Se concentration in root	Treatment	20.48	5.121	258.8***
	Cd level in soil	1.882	.9410	47.56***
	Treatment × Cd level in soil	.9440	.1180	5.967***
	Error	.5940	.0200	
Se concentration in shoot	Treatment	.3600	.0900	247.5***
	Cd level in soil	.0140	.0070	19.78***
	Treatment × Cd level in soil	.0120	.0010	4.074**
	Error	.0110	.0000	

SS sum of squares, *MS* mean square; **, ***: significant at the level $P < 0.01$, $P < 0.001$, respectively

roots and shoots with respect to the control in the soil with 0.41 mg kg^{-1} Cd. In the soils with 0.68 and 1.28 mg kg^{-1} Cd, the proportion of Cd allocated to the iron plaques in the Se+Lime treatment was 44.7 and 34.5%, respectively, which were much higher than that of the other treatments (Fig. 4).

The translocation factors (TFs) of Cd from roots (including iron plaques) to shoots were calculated (Table 2), and different impacts on the TFs were found among treatments. In the soil with 0.41 mg kg^{-1} Cd, the addition of Se alone was more effective in reducing the TF than the remaining treatments. In the soils with 0.68 and 1.28 mg kg^{-1} Cd, Se+Lime treatment had the lowest TFs among all treatments and the reduction rates were 47.8 and 44.8%, respectively. The Lime treatment had no effect on the TFs in these two Cd-contaminated soils (Table 2).

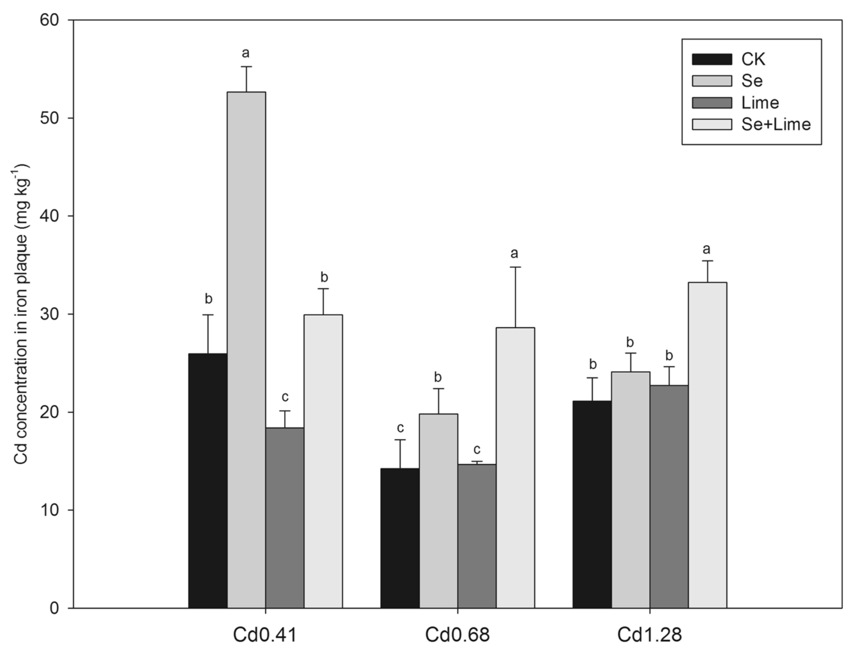

Fig. 3 Changes in Cd concentration in the iron plaque after extraction from the root surface of rice seedlings exposed to three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) when selenium (Se) and hydrated lime (Lime) were applied alone or simultaneously (Se+Lime). Values are means with standard deviations shown by the *vertical bars* ($n = 3$). *Bars with the same letter(s)* indicate no significant differences between different treatments at $P < 0.05$

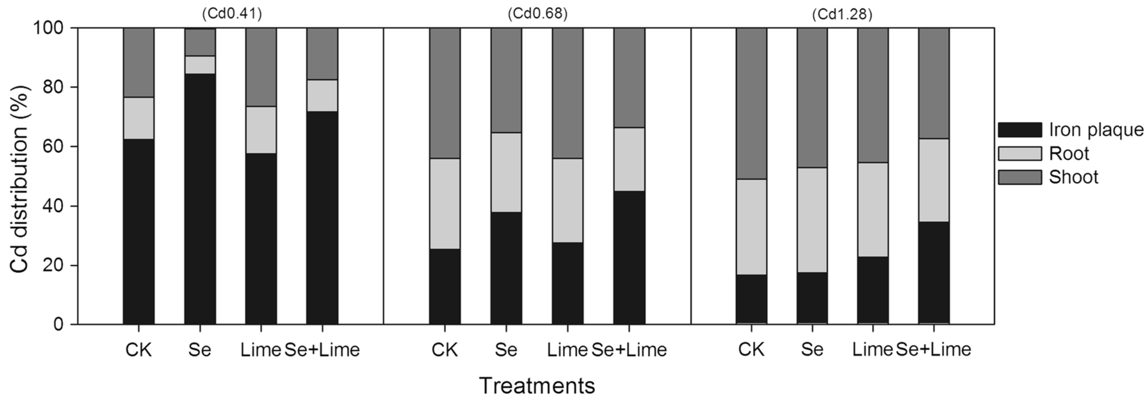

Fig. 4 Allocation of Cd in rice seedlings (iron plaque, root, and shoot) exposed to three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) when selenium (Se) and hydrated lime (Lime) were applied alone or simultaneously (Se+Lime). Values are means of three replicates

Effects of exogenous Se and hydrated lime on $CaCl_2$-extracted available Cd in the soil

As shown in Fig. 5, the concentration of available Cd in the soil increased with exogenous Cd addition. The concentrations of available Cd were significantly decreased by 11.4 to 33.5% when Se was applied alone, with no significant change in soil pH value. Lime treatment significantly increased pH values of soils and reduced the concentrations of available Cd by 41.9 to 65.9% with respect to the control. This effect was potentiated when Se and lime were applied together, with a decrement of 61.9 to 78.4% of available Cd (Fig. 5).

Discussion

Physiological toxicity and inhibition of plant growth generally occur in the heavy Cd-polluted fields. It has been reported that rice growth was significantly inhibited in the soil with 100 mg kg^{-1} Cd (Jalloh et al. 2009), and in aqueous solution containing 50 μM Cd (Lin et al. 2012). In slightly to moderately Cd-contaminated soil, for example, below 2 mg kg^{-1}, no impacts occurred on rice growth (Aziz et al. 2015), which was consistent with the results of the present work. In China, Cd contamination in the farmland mostly is slight to moderate (0.3–1.5 mg kg^{-1}) (MEP and MLR, 2014), so the present work mainly focused on Cd uptake by rice in this Cd contamination level.

Se and hydrated lime are two common passivators that are used to reduce Cd accumulation in many plants (Bolan et al. 2003; Feng et al. 2013; Haghighi and da Silva 2016; Lim et al. 2013; Liu et al. 2015). Previous studies revealed the suppressive effect of Se on Cd uptake and accumulation in rice (Ding et al. 2014; Feng et al. 2013; Hu et al. 2014), and the effect might depend on Cd level in the soil. Feng et al. (2013) reported that the effect of Se on reducing Cd uptake by rice was more efficient at low rather than high Cd levels and this has also been demonstrated by the present results (Fig. 2a). Furthermore, we found that Se application alone reduced Cd accumulation in the root mainly by increasing Cd restriction ability of the iron plaques (Fig. 3). Iron plaques on the root surfaces are generally regarded as heavy metal barriers (Dong et al. 2016; Hossain et al. 2009; Li et al. 2016; Zhou et al. 2015). Ding et al. (2014) found that moderate Se application to soil contaminated with low levels of Cd could decrease the proportion of thick roots; consequently, the root surface area increased, which resulted in more Cd adhering to iron plaques on the root surface. However, the proportion of medium-sized roots would increase with an increase in Cd concentration of the soil; consequently, the effect of Se treatment on Cd concentration of the root surface would gradually decrease under high levels of Cd (Fig. 3). The bioavailability of Cd is strongly affected by soil pH (Garau et al. 2007; Wharfe 2004). Previous studies showed that hydrated lime application increased the soil pH value and decreased the available Cd concentration in the soil (Li et al. 2015; Wang et al. 2015), which is consistent with the results of the present study (Fig. 5). The reduction rate of available Cd in the soil with 1.28 mg kg^{-1} Cd was higher than that measured under lower Cd levels (Fig. 5); thus, Lime treatment seems to have a beneficial effect particularly at moderate to high exogenous Cd levels. It is known that Se can also affect the mobility of heavy metals in soil. Previous studies have reported that Se applied to mercury

Table 2 Translocation factors (TFs) of Cd in rice seedlings grown in soils with three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) after exposure to the different Se and hydrated lime treatments)

Treatments	$Cd_{0.41}$	$Cd_{0.68}$	$Cd_{1.28}$
CK	0.08 ± 0.01a	0.23 ± 0.02a	0.29 ± 0.02a
Se	0.03 ± 0.01c	0.16 ± 0.02b	0.23 ± 0.01b
Lime	0.09 ± 0.01a	0.20 ± 0.01a	0.27 ± 0.02a
Se+Lime	0.05 ± 0.01b	0.12 ± 0.02c	0.16 ± 0.01c

Data are presented as means ± standard errors (n = 3). Means with the same letter within each line are not significantly different between different treatments at $P < 0.05$

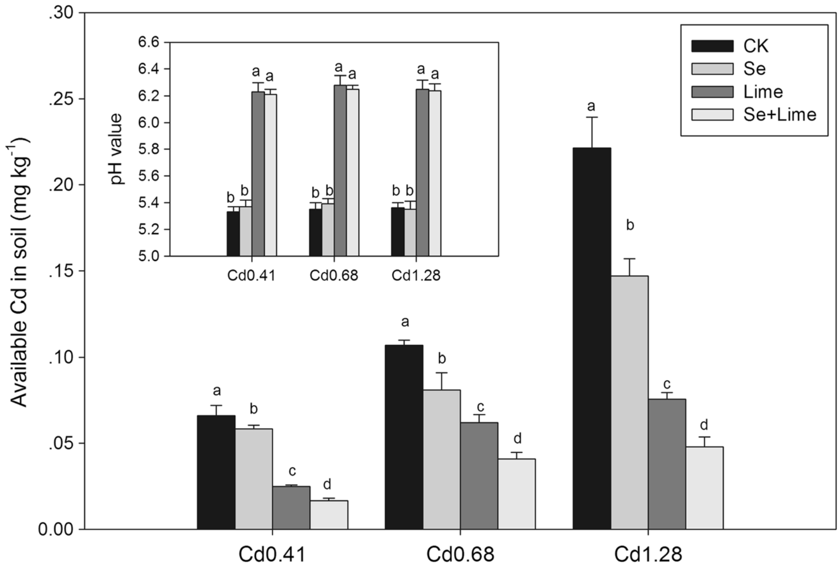

Fig. 5 Changes of $CaCl_2$-extracted available Cd and pH value in the soil with three Cd levels (0.41, 0.68, and 1.28 mg kg^{-1}) when selenium (Se) and hydrated lime (Lime) were applied alone or simultaneously (Se+Lime). Values are means with standard deviations shown by the *vertical bars* ($n = 3$). *Bars with the same letter(s)* indicate no significant differences between different treatments at $P < 0.05$

(Hg)-contaminated soil decreased Hg availability by inducing Hg-Se complexes (Wang et al. 2016). In this work, Se application slightly decreased the available Cd concentration in the soil, which indicated that Se can also inhibit Cd mobility in soil. A previous study showed that an insoluble compound (CdSe) could be formed by sodium selenite and cadmium chloride, and the synthesis was enhanced by increasing Cd concentration when the ratio of sodium selenite and cadmium chloride was lower than 1:3 (Brooks and Lefebvre 2016); this might explain why in the present work the reduction of Cd availability was enhanced as Cd level in the soil increased, when 0.5 mg kg^{-1} Se was applied alone (Fig. 5). Furthermore, the mobility of Se depends on soil properties, which include pH value, redox potential (Eh), organic matter, and carbonate contents (Eich-Greatorex et al. 2007; Stroud et al. 2010; Zhao et al. 2005); thus, the mobility would change when Se is applied to soil in combination with hydrated lime. Eich-Greatorex et al. (2007) found that Se availability in a loam soil increased with increasing pH value within a range of 5–7. The experimental soil used in the current study was loam soil, and the pH value was 5.33. The soil pH reached 6.21 in Se+Lime treatment (Table 2). The main Se species in neutral to alkaline soil is Se^{6+}, which is more mobile in soil than Se^{4+} (Barrow and Whelan 1989); thus, Se availability in Se+Lime treatment could be higher than in Se treatment, and greater contact may be made with Cd. Therefore, the reduction in the available Cd concentrations in the soil was enhanced when Se was applied simultaneously with hydrated lime. However, Huang et al. (2015) found that iron plaques on the root surface decreased Se^{6+} uptake by rice seedlings but enhanced Se^{4+} uptake, with the result that Se concentrations in the rice seedling tissues in Se+Lime treatment were lower than those measured when Se was applied alone (see also Fig. 2c, d). In addition, available Cd concentrations were dramatically reduced by Se+Lime treatment (Fig. 5), which increased Cd restriction ability of the iron plaques by changing the rice root morphology in the soils with higher Cd levels.

Cd concentration in the roots, shoots, and iron plaques was significantly affected by the different treatments and Cd level in the soil ($P < 0.01$) (Table 1). Se and Se+Lime treatments inhibited Cd accumulation in the rice tissues by increasing Cd concentration in the iron plaques on the root surface; then, the TFs of Cd from the root to shoot decreased (Table 2). Many studies indicated that Se can inhibit upward translocation of Cd in many ways (Abd Allah et al. 2016; Pang et al. 2014). Pang et al. (2014) found that more Cd was sequestered in rice root cell walls so that Cd upward translocation was inhibited. Se can also increase the synthesis of glutathione (GSH) in plant tissues (Abd Allah et al. 2016); the mobility of Cd might be decreased due to GSH-chelated Cd in plant tissues (Fan et al. 2010), with the result that more Cd is distributed to the root. As shown in Fig. 3, Cd concentration in the iron plaque did not increase with the increase of soil Cd level, but the proportion of Cd in the iron plaque decreased with the increase of soil Cd level (Fig. 4). Accordingly, it was speculated that Cd adsorption by the iron plaques had a threshold (approximately 53 mg kg^{-1}) at the rice seedling stage (Fig. 3). When Cd concentration in the iron plaques reaches the limit, they might not prevent upward Cd migration anymore; a further investigation needs to be conducted to verify this speculation. With respect to the effect of Se on the ability of the iron plaques to restrict Cd uptake by rice at the mature stage, varying results were found in previous studies (Chang et al. 2013; Hu et al. 2014). The differences were mainly attributed to the fact that a considerable part of the iron plaques is lost from the root surface at the mature stage due to a decrease in radial oxygen loss (ROL) (Wang et al. 2013). Consequently, the results from the mature stage would differ from those obtained at the seedling stage.

Conclusion

Se, Lime, and Se+Lime treatments reduced Cd uptake by the rice seedlings. Se treatment represented the highest reduction rate in the soil with a slightly contaminated Cd level, mainly by increasing the ability of the iron plaque to restrict Cd uptake by rice seedlings. In the soil with higher Cd levels, Se application alone slightly inhibited Cd accumulation in rice seedlings mainly by decreasing the available Cd concentration in the soil. However, Se+Lime treatment strongly inhibited the availability of Cd in the soil and increase the ability of the iron plaques to restrict Cd uptake. The present results suggest that Se could be applied alone to a soil with a low level of Cd contamination; however, in soil with a relatively high level of Cd contamination, Se should be applied in combination with hydrated lime.

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