

Relationships Between Root Oxidation Characteristics and Plant Pb Uptake in Different Rice Cultivars

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Abstract

In order to understand some mechanisms on the variations among rice cultivars in plant Pb accumulations, the relationships between root oxidation characteristics and Pb uptake and translocation were studied with six rice cultivars at different soil Pb levels. The results showed that the variation rates among the rice cultivars were 27.2%–49.6% in root Pb concentrations, 69.7%–122.1% in shoot Pb concentrations, 14.3%–41.0% in root radial oxygen loss (ROL) per root weight, 172.4%–240.6% in total ROL per pot, and 35.9%–47.2% in soil redox potentials (Eh). The magnitudes of the variations for the parameters followed a same trend: 1000 mg kg⁻¹ soil Pb treatment > 500 mg kg⁻¹ soil Pb treatment > the control. Root ROL (per root weight and per pot) correlated positively and significantly ($P < 0.05$ or 0.01) with root Pb concentrations, but insignificantly ($P > 0.05$) with shoot Pb concentrations. Soil Eh correlated positively and highly significantly ($P < 0.01$) with Pb concentrations of roots and shoots. Soil Eh correlated positively and partially significantly ($P < 0.05$ or 0.01) with root ROL. It can be concluded that root ROL influences Pb uptake by rice root highly, but Pb translocation from root to shoot is little related to root ROL. Pb uptake and translocation to aerial parts are highly and directly affected by soil redox status. By affecting soil Eh, root ROL may indirectly influence Pb uptake and translocation in rice plants.

Keywords

Lead (Pb), Rice (*Oryza sativa* L.), Cultivar, Root Oxidation, Soil Redox Potential

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1. Introduction

Heavy metal pollution in field soil is serious in China. The sources of heavy metal include mining, municipal and industrial wastes, agricultural activities (chemical and fertilizer applications, wastewater irrigation), etc. [1, 2]. It was reported that in the sediments from an acid leaching site of e-waste in China, average concentrations of Cu, Zn, Cd, Sn, Sb and Pb reached as high as 4820, 1260, 10.7, 2660, 5690 and 2570 mg/kg, respectively [3]. Widespread and considerable levels of metal pollution in arable lands have raised great concerns on food safety, especially of rice [4, 5].

Lead (Pb) is one of the most important heavy metal pollutants for its widespread and highly toxic to organism, and it has been attracting great public concerns [6]. Toxic effects of Pb on plant growth include interfering with nutrient absorption, respiration, protein metabolism, photosynthesis and the activities of antioxidant enzymes [7-9]. Excessive exposure to Pb will pose severe damages on circulatory, skeletal, nervous, immune, endocrine, and enzymatic systems in human body [10]. The toxic effects are persistent and irreversible. Therefore, Pb has been listed as a pollutant for priority control by many countries and organizations, and Pb pollution has attracted much concern around the world [11].

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Former researches indicated that great genotypic variations in metal absorption and translocation have been found between and within plant species [12, 13]. Rice is the first staple food for Asian, and also one of the most important crops all over the world. Significant variations among rice cultivars and genotypes in Pb uptake, translocations and accumulations were found in our previous studies [14]. The achievements can provide a possible way to select or breed the rice cultivars and genotypes with low levels of Pb in the grains, while grown in Pb-contaminated soils. However, the mechanisms on the differences among rice cultivars and genotypes in Pb uptake and translocation are poorly understood.

The uptake and transport of trace elements by plants are influenced by their chemical forms in the rhizospheres [15]. Redox potential in the rhizospheric soils is assumed as one of the main factors controlling metal bioavailability in the soils. Furthermore, Root activities can change redox conditions of rhizospheric soils, and hence influence the chemical form, mobility and availability of metals, specifically for wetland plants. Paddy rice, as a kind of wetland plants, can transfer oxygen from the aerial parts to the roots and release oxygen and oxidants to the rhizosphere by adapting to waterlogged conditions. Literature reports indicated that the differences among rice cultivars and genotypes in Cd and As uptake and accumulation were related to the variations in rhizosphere oxidation conditions [16, 17]. However, the relationships between root oxidation abilities and plant Pb uptake and accumulations for different rice cultivars and genotypes are not understood sufficiently.

2. Materials and Methods

2.1. Soil Preparation

In this study, the soil was obtained from an unpolluted field. The soil was air-dried and sieved through 2 mm screen. The following properties were determined: soil texture, pH, cation exchange capacity (CEC), organic matter (OM) content and Pb concentration. It is a sandy loam (sand 58.2%, silt 21.7%, clay 20.1%), with pH 6.7, CEC 12.6 cmol kg⁻¹, OM 27.3 g kg⁻¹, and Pb content 37.5 mg kg⁻¹.

Pot experiments were applied in this research. Four kilogram of the prepared soil was put into each pot (diameter 18 cm, height 20 cm) for rice cultivation. Two levels of soil Pb treatments, e.g. 500 mg Pb kg⁻¹ soil (moderate pollution) and 1000 mg kg⁻¹ (heavy pollution), were designed with lead acetate solution. The soil without adding Pb served as control. Before rice cultivation, all the pot soils were submerged under water (water deep 2–3 cm) for more than a month.

2.2. Rice Plant Materials and Experimental Design

Six rice cultivars differing in Pb uptake and accumulation were chosen for this research. There were two high Pb accumulators Liangyoupeiiju (abbr. C01) and Shanyou 63 (C02), two moderate Pb accumulators CV6 (C03) and Yangdao 6 (C04), and two low Pb accumulators Wuyunjing 7 (C05) and Yu 44 (C06). Rice seeds were submerged in water for 48 h at room temperature (23–26°C), germinated at 32°C for 30 h. The sprouted seeds were grown in unpolluted soil for 30 days. Then, similar seedlings were fetched and transplanted into the prepared pots (three seedlings for each pot). During rice growth, the pot soils were submerged under water (water deep 2–3 cm). The pots of four replicates were placed in open air with a randomized arrangement.

2.3. Sample Analyses

Determination of Pb Concentrations in Rice Plants

Rice plants were sampled at the 40th day after seedling transplant (tillering stage) to test Pb concentrations in the roots and shoots. The whole rice plants were washed with tap water and deionized water, and separated as root and shoot samples. The samples were dried to constant weights at 70°C in an oven. The dried samples were ground with a grinder, and sieved with a 100-mesh screen. Pb concentrations of the samples were tested with AAS after digesting with HNO₃-HClO₄.

Determination of Root Radial Oxygen Loss (ROL)

The rates of root radial oxygen loss (ROL) of the rice roots were estimated using the Ti³⁺-citrate buffer method [18]. Intact rice plants were sampled at the 40th day after seedling transplant, and washed with tap water and deionized water. The intact root was submerged in 50 mL of 20% strength Kimura B solution containing Ti³⁺-citrate. The solution was covered with 2 cm of paraffin oil to prevent atmospheric O₂ penetration. All the preparations were carried out under N₂ gas. The control treatment of no plant was done synchronously. Six hours later, the absorbance of the partly oxidized solution was read with a UV/Vis spectrophotometer (Perkin-Elmer 3, Germany) at 527 nm.

Determination of Soil Redox Potential

From the 40th day after seedling transplant, redox potentials (Eh) of the pot soils were measured at 10 cm under soil surface with Pt electrodes, once daily for five consecutive days.

3. Results

3.1. Variations Among Rice Cultivars in Plant Pb Accumulation

There were significant ($P < 0.05$) variations among the rice

cultivars in root and shoot Pb concentrations, and the magnitudes of variation differed with parts of plants and soil Pb levels (Table 1). The variation rates in root Pb concentrations were 27.2%, 38.8% and 49.6% [(the value of the highest cultivar – the value of the lowest cultivar)/the value of the lowest cultivar × 100%] for the control, 500 and 1000 mg kg⁻¹ soil Pb treatment, respectively. The variation rates in shoot Pb concentrations were 69.7%, 98.6% and 122.1% respectively. Apparently, the magnitudes of variation were larger in the shoots than in the roots, and also increased with the rise of soil Pb levels.

3.2. Variations Among Rice Cultivars in Root Oxidation Abilities

The variations among the rice cultivars in root radial oxygen loss (ROL) per root weight and in total ROL per pot were also significant ($P < 0.05$) (Table 2). The variation rates in ROL per root weight ranged from 14.3% to 41.0%, and increased with soil Pb levels. The variation rates in total ROL per pot were largely higher than the variation rates in ROL per root weight, and they were 172.4%, 224.3% and 240.6% for the control, 500 and 1000 mg kg⁻¹ soil Pb treatment, respectively.

Table 1. Pb Concentrations in the Roots and Shoots of Six Rice Cultivars (mg kg⁻¹).

Rice Cultivars	Control		Pb500 ^a		Pb1000 ^b	
	Root	Shoot	Root	Shoot	Root	Shoot
C01	23.31	6.18	1824.42	44.76	3566.32	76.95
C02	27.42	6.50	2035.55	63.18	4055.46	97.78
C03	23.63	5.26	1755.31	46.39	3350.76	62.53
C04	22.05	4.75	1747.63	46.99	3191.79	70.98
C05	23.09	3.90	1466.11	31.81	2930.75	48.57
C06	21.55	3.83	1531.24	33.69	2711.56	44.03
Average	23.51	5.07	1726.71	44.47	3301.11	66.81
LSD _{0.05}	2.15	0.61	189.33	3.39	340.60	5.14

^a Soil Pb treatment of 500 mg kg⁻¹.

^b Soil Pb treatment of 1000 mg kg⁻¹.

Table 2. Variations Among Rice Cultivars in Root Radial Oxygen Loss (ROL) at Different Soil Pb Levels.

Rice Cultivars	Control		Pb500		Pb1000	
	Root ROL (μmol g ⁻¹ h ⁻¹)	Total Root ROL (μmol pot ⁻¹ h ⁻¹)	Root ROL (μmol g ⁻¹ h ⁻¹)	Total Root ROL (μmol pot ⁻¹ h ⁻¹)	Root ROL (μmol g ⁻¹ h ⁻¹)	Total Root ROL (μmol pot ⁻¹ h ⁻¹)
C01	14.28	76.08	15.51	84.35	14.23	82.83
C02	15.39	82.03	17.17	80.88	16.36	79.13
C03	13.47	74.10	13.43	59.36	12.42	61.34
C04	15.14	79.98	13.72	73.17	11.60	51.73
C05	14.07	30.11	13.69	26.01	12.88	29.34
C06	13.52	46.77	12.94	42.21	12.34	24.32
Average	14.31	64.89	14.41	61.00	13.31	54.78
LSD _{0.05}	0.86	6.21	1.03	7.28	1.23	6.19

The variations among the rice cultivars in soil Eh were large and significant ($P < 0.05$) (Table 3). The largest differences (between the largest cultivar and the lowest cultivar) were 29.21 mV (variation rate 35.9%), 35.76 mV (44.1%) and

42.16 mV (47.2%) for the control, 500 and 1000 mg kg⁻¹ soil Pb treatment, respectively. So the variation rates also increased with soil Pb levels.

Table 3. Variations Among Rice Cultivars in Soil Redox Potentials (mV) at Different Soil Pb Levels.

Rice Cultivars	Control	Pb500	Pb1000
C01	-98.57	-94.35	-98.53
C02	-81.39	-81.03	-89.10
C03	-94.24	-102.70	-108.33
C04	-107.76	-107.16	-117.62
C05	-101.91	-116.79	-125.82
C06	-110.60	-114.39	-131.26
Average	-99.08	-102.74	-111.78
LSD _{0.05}	12.47	6.83	8.06

Table 4. Correlation Coefficient Between Root Radial Oxygen Loss (ROL) and Plant Pb Concentrations.

		Root ROL			Total Root ROL		
		Control	Pb500	Pb1000	Control	Pb500	Pb1000
Root Pb Concentration	Control	0.5468			0.4255		
	Pb500		0.8400*			0.8999*	
	Pb1000			0.8275*			0.9203**

		Root ROL			Total Root ROL		
		Control	Pb500	Pb1000	Control	Pb500	Pb1000
Shoot Pb Concentration	Control	0.5181			0.8099		
	Pb500		0.8075			0.8069	
	Pb1000			0.7629			0.8904*

*, ** Significant at $P < 0.05$, 0.01 , respectively.

3.3. Relationships Between Root Oxidation Abilities and Plant Pb Concentrations

Correlation analyses showed that the relationships between root ROL and plant Pb concentrations differed with soil Pb levels and plant parts (Table 4). For the control, the correlations between root ROL and plant Pb concentrations (irrespective in roots and shoots) were all insignificant ($P >$

0.05). Under soil Pb treatments (500 and 1000 mg kg⁻¹), root ROL (irrespective ROL per root weight or total ROL per pot) correlated positively and significantly ($P < 0.05$ or 0.01) with root Pb concentrations, but insignificantly ($P > 0.05$) with shoot Pb concentrations generally, except for the correlation between total ROL per pot and shoot Pb concentration under 1000 mg kg⁻¹ soil Pb treatment.

Table 5. Correlation Coefficient Between Soil Redox Potentials and Plant Pb Concentrations.

		Root Pb Concentration			Shoot Pb Concentration		
		Control	Pb500	Pb1000	Control	Pb500	Pb1000
Soil Redox Potentials	Control	0.9734**			0.7978		
	Pb500		0.9704**			0.9290**	
	Pb1000			0.9860**			0.9362**

The soil Eh correlated positively and highly significantly ($P < 0.01$) with root Pb concentrations irrespective of soil Pb levels (Table 5), and positively and highly significantly ($P < 0.01$) with shoot Pb concentrations under soil Pb treatments (500 and 1000 mg kg⁻¹), but insignificantly ($P > 0.05$) with shoot Pb

concentrations of the control.

The correlations between root ROL and soil Eh were all positive, but only partially significant ($P < 0.05$ or 0.01) (Table 6).

Table 6. Correlation Coefficient Between Soil Redox Potential and Root Radial Oxygen Loss (ROL).

		Root ROL			Total Root ROL		
		Control	Pb500	Pb1000	Control	Pb500	Pb1000
Soil Redox Potential	Control	0.4157			0.4729		
	Pb500		0.9239**			0.8312*	
	Pb1000			0.8071			0.9639**

4. Discussion

As a kind of wetland plants, rice plant has developed massive aerenchyma in the roots for adapting to waterlogged environment. About 30–40% of the O₂ supplied via the root aerenchyma will be lost to the soil, and the process is called radial oxygen loss (ROL) [18, 19]. Former studies showed that ROL decreased metal mobility by causing oxidation and precipitation of heavy metals in the rhizosphere soils and on the surface of plant roots [20]. By limiting As mobility in the rhizosphere and the translocation to aerial parts, the rice cultivars with higher ROL accumulated less As in the shoots than the cultivars with lower ROL [21].

The results of our present studies showed that the rice cultivars varying largely in Pb uptake and accumulation differed greatly in root ROL per root weight, total root ROL per pot, and in soil Eh. Under different soil Pb levels, the variation rates of the parameters followed a similar trend, e.g. 1000 mg kg⁻¹ soil Pb treatment $>$ 500 mg kg⁻¹ soil Pb treatment $>$ the control.

Therefore, the diversities among the rice cultivars in Pb accumulation and root oxidation abilities were promoted by soil Pb stress.

The present research also indicated that the root ROL correlated positively and significantly ($P < 0.05$ or 0.01) with root Pb concentrations under soil Pb treatments (500 and 1000 mg kg⁻¹), but insignificantly ($P > 0.05$) with shoot Pb concentrations generally under all the soil Pb levels. The results indicate that root ROL of rice plant influences Pb uptake and accumulation in the roots highly, but the translocation of Pb from the roots to the shoots is little related to root ROL. Our research also presented that Pb concentrations of rice plants (roots and shoots) correlated positively and highly significantly ($P < 0.01$) with the soil Eh under all the soil Pb levels generally. The results suggest that there were direct and close relationships between soil redox status and plant Pb uptake, and the translocation to aerial parts. Root ROL may indirectly influence plant Pb uptake and translocation by affecting soil Eh. The results of this research also proved that the soil Eh correlated positively and partially

significantly ($P < 0.05$ or 0.01) with root ROL.

It was reported that root-induced effects of plant Cd uptake in different rice cultivars were very important, for it influenced chemical speciation of Cd in the soils. But the detail processes involved remain uncertain [22]. Therefore, the relationships between root oxidation characteristics and metal uptake and translocation in rice plants may be metal-dependent, and still need much more investigations.

5. Conclusions

The rice cultivars differing significantly in Pb uptake and accumulation varied largely in root ROL and soil Eh. The magnitudes of variation increased with the rise of soil Pb levels. So the diversities among rice cultivars in Pb accumulations and root oxidation abilities were elevated by soil Pb stress. The root ROL correlated positively and significantly ($P < 0.05$ or 0.01) with root Pb concentrations, but insignificantly ($P > 0.05$) with shoot Pb concentrations. The soil Eh correlated positively and highly significantly ($P < 0.01$) with Pb concentrations of rice roots and shoots. The soil Eh correlated positively and partially significantly ($P < 0.05$ or 0.01) with root ROL. Therefore, it can be concluded that root ROL of rice plant influences Pb uptake and accumulation of the root highly, but Pb translocation from root to shoot is little related to root ROL. Pb uptake and translocation to aerial parts in rice plant are highly and directly affected by soil redox status. Root ROL may indirectly influence Pb uptake and translocation by affecting soil Eh.

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