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Variations among rice cultivars on root oxidation and Cd uptake

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Abstract: In order to understand the mechanisms on the variation between rice cultivars in Cd uptake and accumulation, two pot soil experiments were conducted with typical rice cultivars that varied greatly in soil Cd uptake. The experiments with six rice cultivars showed that the root oxidation abilities of rice differed with rice cultivars and also with types of the cultivars, the cultivars with indica consanguinity were significantly higher than the cultivars with japonica consanguinity. Root oxidation abilities of the rice cultivars correlated positively and significantly (P<0.01) with their Cd concentrations and Cd quantity accumulations in rice plants. The experiments with two rice cultivars showed that significant differences also existed between the two cultivars in pot soil redox potentials, which of Shan you 63 (higher soil Cd accumulator) were significantly higher than that of Wu yun jing 7 (lower soil Cd accumulator) under different soil Cd levels, but the degrees of the differences varied with soil Cd levels. The differences were larger under soil Cd treatments than the control. The results indicate that root oxidation ability, especially in Cd contaminated soil, is one of the main mechanisms which dominate Cd uptake and accumulation by rice plant.

Keywords: cadmium(Cd); uptake and accumulation; root oxidation; soil redox potential

Introduction

Of all the metals in the environment, Cd is one of the most important to be considered in terms of food-chain contamination, because it is readily taken up by plant and translocated to different parts of plant (Li *et al.*, 1995). It is also absorbed by rice plant strongly and transported to grain considerably (Wu *et al.*, 1999). Researches indicated that the uptake of Cd can vary greatly among plant species and also among cultivars within a species (Penner *et al.*, 1995), so the strategy of selecting crop species and varieties which are tolerant to Cd, and absorb or translocate less Cd to edible parts from Cd contaminated soil has been proposed for crops and applied successfully to sunflower and durum wheat(Penner *et al.*, 1995; Li *et al.*, 1995). Our previous studies (Liu *et al.*, 2003; Liu *et al.*, 2005) and other studies (Wu *et al.*, 1999) showed that rice cultivars varied significantly in Cd uptake and accumulation. However, limited information is available about the mechanisms of this variation between rice cultivars.

The ability of roots to absorb Cd may depend on both the activity of roots and the interaction between roots and its located soil environments. The proportions of soluble or bio-available Cd and minerals vary considerably with the redox potential in paddy soil, corresponding to the reduction of sulfate to sulfides, bound to organism, binding with iron and manganese oxides, and absorbed by soil granule (Kashem and Singh, 2001). Rice is a wetland plant, and developed the special ability to translocate oxygen from the aerial parts to roots under anaerobic conditions. Different rice cultivars possess different oxidation abilities in root, thus may creating different rhizospheric environments and accessing different amount of plant-available Cd and mineral nutrients, especially in submerged paddy soil (Liu *et al.*, 2000). But the relations between root oxidation characteristics and Cd uptake in different rice cultivars remain unclear.

Based on our previous studies (Liu *et al.*, 2003; Liu *et al.*, 2005), typical rice cultivars with high or low Cd accumulation abilities were used in the present experiments. The aim was to investigate the relations between Cd uptake abilities and root oxidation characteristics, and rhizosphere redox potentials (Eh) in different rice cultivars, so as to understand some mechanisms of the variations between rice cultivars in Cd uptake and accumulation.

1 Materials and methods

There were two experiments, the one on root oxidation ability was conducted in Yangzhou, Jiangsu Province (experiment 1) while the other one on soil redox potential was conducted in Hong Kong (experiment 2).

1.1 Soil preparation

For the experiment of root oxidation ability in Yangzhou, 10 kg of soil was placed in each pot(25 cm

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in diameter and 30 cm in height). Cadmium in the form of $CdCl_2$ was added into the soil to obtain a Cd level of 100 mgCd/kg soil (dw). This created a highly Cd-contaminated soil without severe inhibition to rice growth (Liu *et al.*, 2003). For the experiment of soil redox potential in Hong Kong, 4 kg of soil was placed in each pot (18 cm in diameter and 20 cm in height). Cadmium in the form of $CdCl_2$ was added to the soil to obtain Cd levels of 10 and 50 mgCd/kg soil(dw). The soils without adding Cd were served as controls.

1.2 Rice plant materials and experimental design

Based on our previous studies (Liu *et al.*, 2003, 2005), six typical rice cultivars of different types with significant variable Cd accumulation abilities were used in root oxidation ability experiment. They were Liang you pei jiu(hybrid indica; V01), CV6(New Plant Type; V02), Shan you 63 (hybrid indica; V03), Yang dao 6(indica; V04), Wu yun jing 7(japonica; V05) and Yu 44(japonica; V06). Two typical cultivars with high (Shan you 63) and low (Wu yun jing 7) Cd accumulation abilities were used in soil redox potential experiment. Rice seeds were submerged in a water bath for about 48 h at room temperature(20—25℃) and germinated under moist condition (seeds were covered with two layers of moist gauze cloth) at 32℃ for another 30 h and the germinated seeds were grown in uncontaminated soils. After 30 d, the seedlings with 2—3 tillers were transplanted into the pots (3 plants per pot). The pot soil was maintained under flooded conditions (with 2—3 cm of water above soil surface) during the whole growth period of 110—115 d(differed with cultivars).

The experiments were carried out in greenhouses, with a 16 h light period and a day/night temperature of 32—35℃/23—25℃. The pots were arranged in a randomized complete block design with five replicates.

1.3 Sample preparation and analytical methods

1.3.1 Cd uptake and accumulation in rice plants

The whole rice plants were harvested at tillering stage(the day 40 after seedling transplant) and panicle heading stage (the day 70 after seedling transplant). The plant samples were washed thoroughly with tap water and deionized water, and oven-dried at 70℃ to a constant weight. The oven-dried samples were ground with a stainless steel grinder (FW-100, China) to pass through a 100-mesh sieve. The Cd concentrations of the samples were determined with an atomic absorption spectrophotometer (Solaar S4 + Graphite Furnace System 97, Thermo Elemental, USA) following HNO_3-$HClO_4$ (4 : 1) digestion procedures (Allen, 1989).

1.3.2 Determination of root oxidation ability

At tillering stage (the day 40 after seedling transplant) and panicle heading stage (the day 70 after seedling transplant), whole rice plant samples were dug up from the pots and washed thoroughly with tap water and deionized water. Root oxidation abilities were tested with a method of α-NA oxidation described by Zhang (1992). About 1 g of fresh roots (whole roots) was cut from the rice plant, submerged in 25 ml α-NA solution of 40 mg/L, shaked for 3 h in 25℃, and then the amount of oxidated α-NA was tested. After the test, the roots were washed and oven-dried at 70℃ to constant weight for dry weights.

1.3.3 Determination of pot soil redox potential

From the day 40 (at the end of tillering stage) after seedling transplant, the pot soil redox potential at 10 cm under soil surface was measured with Pt electrodes, once daily for five consecutive days.

Data were analyzed with the statistical package SPSS10.0 and EXCEL'2000 for Win. The significant levels 0.05 and 0.01 were used in presenting the results.

2 Results

2.1 Variations among rice cultivars in root oxidation abilities and Cd uptake

The properties of the soil used in this experiment are shown in Table 1. It was a sandy loam with a high portion of sand and a neutral pH. It also contained moderate level of organic matter, CEC and nitrogen content, and a low level of Cd.

Figs.1 and 2 show that the differences among the six rice cultivars were significant($P<0.05$) in both root oxidation abilities per unit of dry weight and total root oxidation abilities per pot at both tillering and panicle heading stages. Generally, the root oxidation abilities of Shan you 63 (hybrid indica, V03) were the highest, and the two japonica cultivars (Wu yun jing 7, V05 and Yu 44, V06) were the lowest. The root oxidation abilities of the cultivars with indica consanguinity (V01—V04) were significantly higher than the cultivars with japonica consanguinity (V05 and V06). The ranges of the differences among the cultivars were larger in total root oxidation abilities per pot than in root oxidation abilities per unit of dry weight, and also larger at tillering stage than at panicle heading stage. The differences among the cultivars of indica consanguinity varied with the two root oxidation indicators and with rice growth stages, and the differences between the two cultivars of japonica consanguinity were generally not significant.

The differences among the six rice cultivars were

Table 1 Selected properties of the soil used in this experiment

Soil type	Soil texture	Particle size, %			PH	OM[a], g/kg	CEC[b], cmol/kg	Total N, g/kg	Total Cd, mg/kg
		Sand	Silt	Clay					
Experiment of root oxidation ability in Yangzhou									
Paddy soil	Sandy loam	62.7	18.5	18.8	7.87	28.5	11.6	1.20	0.16
Experiment of soil redox potential in Hong Kong									
Paddy soil	Silty loam	18.4	42.7	38.9	4.71	26.2	15.9	1.25	ND[c]

Notes: a. Organic matter; b. cation exchange capacity; c. not detected

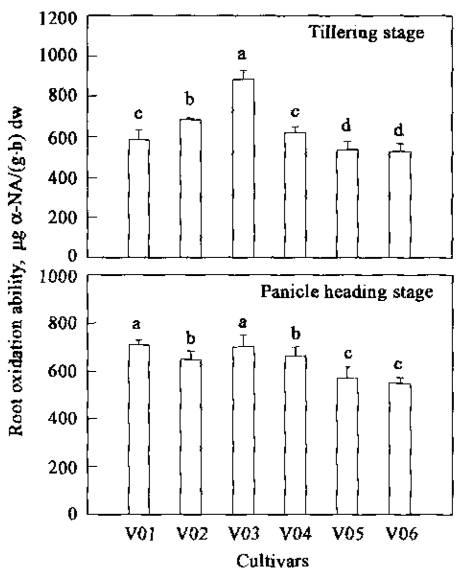

Fig.1 Differences among rice cultivars in root oxidation abilities per unit of root dry weight(experiment 1)

Different letters indicate significant differences at the 0.05 level based on *LSD* test

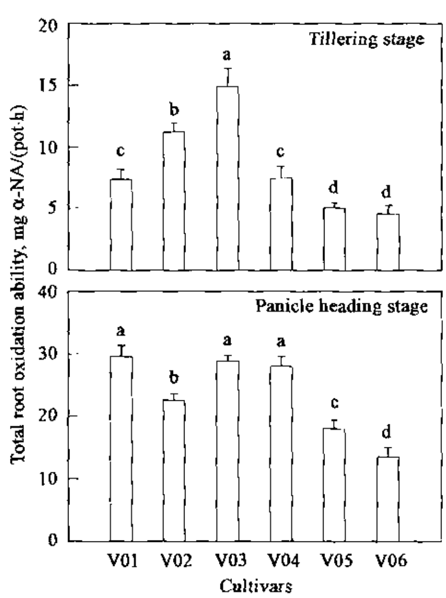

Fig.2 Differences among rice cultivars in total root oxidation abilities per pot(experiment 1)

also significant in both Cd concentrations (Fig.3) and Cd quantity accumulations(Fig.4) in rice plants at both tillering and panicle heading stages. The orders of the differences follow similar trends of the differences in root oxidation abilities.

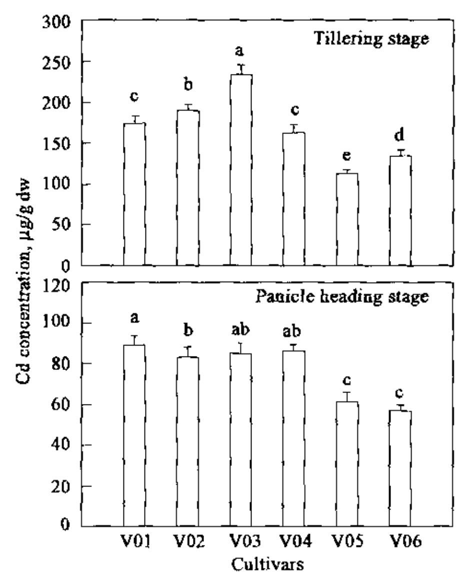

Fig.3 Differences among rice cultivars in plant Cd concentrations (experiment 1)

2.2 Relations between root oxidation abilities and Cd uptake in rice plants

Regress analysis showed that there were positive and significant correlations between root oxidation abilities per unit of root weight and Cd concentrations of rice plants (Fig.5), and also between total root oxidation abilities per pot and plant Cd quantity accumulations per pot (Fig.6) at both tillering and panicle heading stage. The correlations between root oxidation abilities per unit of root weight and Cd concentrations were linear at the two stages. The correlations between total root oxidation abilities per pot and Cd quantity accumulations per pot was linear at tillering stage, but exponential at panicle heading stage in which Cd accumulation increased slowly with total root oxidation abilities per pot under 25, but fastly with it above 25.

2.3 Variations between rice cultivars in soil redox potential and the relation with Cd uptake

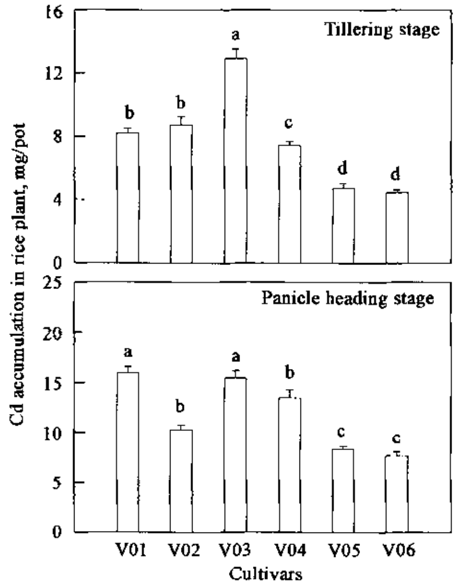

Fig.4 Differences among rice cultivars in plant Cd quantity accumulations(experiment 1).

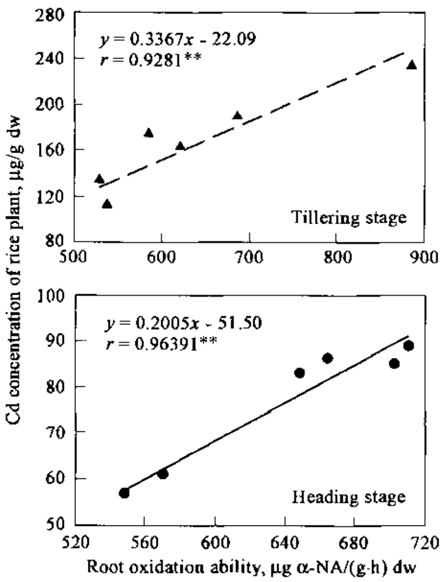

Fig.5 Correlations between root oxidation abilities per unit of root weight and Cd concentrations(experiment 1)

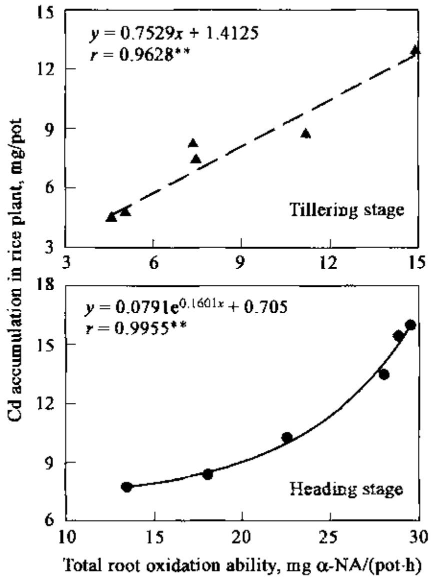

Fig.6 Correlations between total root oxidation abilities and plant Cd quantity accumulations per pot(experiment 1)

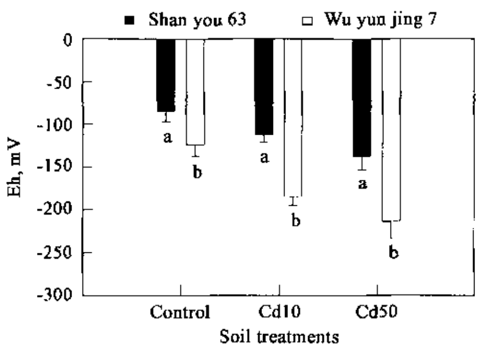

Fig.7 Differences between rice cultivars in soil redox potential (Eh) (experiment 2)

The properties of the soil used in this experiment are showed in Table 1. It was a silty loam with a lower portion of sand and an acidic pH. It contained moderate level of organic matter, CEC and nitrogen content, and a very low level of Cd.

Fig.7 shows the redox potentials (Eh) of the pot soils in which two rice cultivars, Shan you 63 (V03) and Wu yun jing 7(V05), were planted under different soil Cd levels. Shan you 63 is a typical rice cultivar with high Cd uptake, but Wu yun jing 7 a typical rice cultivar with low Cd uptake. The Eh of Wu yun jing 7 were significantly lower than that of Shan you 63 under all the soil Cd levels. But the ranges of the differences between the two cultivars varied with soil Cd levels, and were in the order of: 10 mg/kg soil Cd treatment (V05 is 64.2% lower than V03)>50 mg/kg soil Cd treatment (V05 is 55.0% lower than V03) >control (V05 is 46.6% lower than V03). The Eh of control were higher than that of soil Cd treatments for the two cultivars.

The differences between the two cultivars in plant Cd concentrations and Cd quantity accumulations were also obvious, with Shan you 63 significantly higher than Wu yun jing 7 under all soil Cd levels except for the difference of plant Cd concentrations of control (Fig.8). The ranges of the differences in plant Cd concentrations and Cd quantity accumulations were in the order of: 10 mg/kg soil Cd treatment (V03 is 50.2%, 111.0% higher than V05 in Cd concentration, Cd quantity accumulation respectively)>50 mg/kg soil Cd treatment (V03 is 32.1%, 74.7% higher than V05 respectively)>control (V03 is

16.2%, 62.8% higher than V05 respectively). It was consistent with the order of the differences between the two cultivars in pot soil redox potentials.

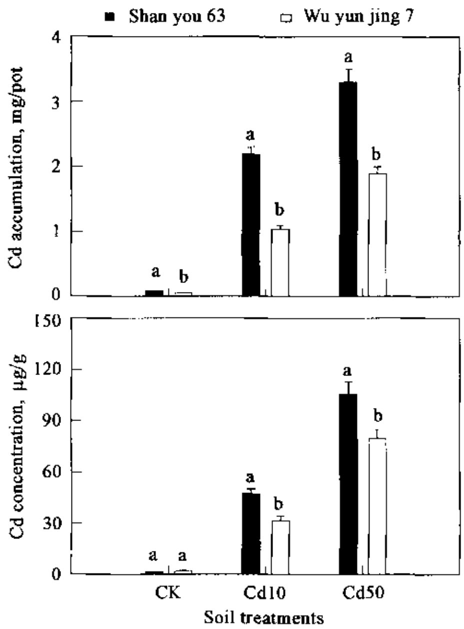

Fig.8 Differences between rice cultivars in plant Cd concentrations and Cd quantity accumulations (experiment 2).

3 Discussion and conclusions

The uptake and accumulation of trace elements can be enhanced through the changes in metal availability in the rhizosphere, and redox potential is considered as one of the key factors controlling metal mobility in the rhizosphere (Lombi *et al.*, 2001). Root-induced changes of redox conditions can influence the speciation, and hence the bioavailability of trace metals to plant (Hinsinger, 2001). But the differences between rice cultivars in root oxidation characteristics, rhizosphere oxidation-reduction conditions, and the relations with soil Cd uptake by rice plant were rarely studied.

In this study there existed significant differences in root oxidation abilities and rhizosphere redox potentials between the rice cultivars which varied greatly in Cd uptake and accumulation. Root oxidation abilities varied with cultivars and types(the cultivars with indica consanguinity were significantly higher than the cultivars with japonica consanguinity). The relations were positive and signicant between root oxidation abilities and Cd accumulations, and between pot soil redox potentials and Cd accumulations. The cultivars with stronger root oxidation abilitiy and higher pot soil Eh showed higher Cd concentrations and more Cd quantity accumulation in plant, such as Shan you 63. On the contrary, the cultivars with weaker root oxidation abilitiy and lower pot soil Eh accumulated less Cd in plant, such as Wu yun jing 7.

It was presented by former researches that when the redox potential of paddy soil decreased to about -140 mV, the proportion of soluble Cd was reduced, corresponding to the reduction of sulfate to sulfides. As a result, much lower Cd was absorbed by rice on submerged soil than on the soil drained after the tillering stage (Kabata-Pendias and Pendias, 2001). The results were consistent with the former reports and could be explained by the description.

From our experiments, we can presume a mechanism on the differences between rice cultivars in Cd uptake and accumulation. It is that the cultivar with stronger root oxidation ability; higher redox potential in rhizosphere; more soluble Cd in rhizosphere soil; more Cd uptake by rice plant.

The relations between root oxidation characteristics of rice cultivars and speciation and phytoavailability of Cd in rhizosphere soil need further research.

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