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Influence of Iron Fertilization on Cadmium Uptake by Rice Seedlings Irrigated with Cadmium Solution

H. J. LIU,[1,2] J. L. ZHANG,[1] P. CHRISTIE,[1,3] AND
F. S. ZHANG[1]

[1]Key Laboratory of Plant Nutrition, Ministry of Agriculture; Key Laboratory of Plant–Soil Interactions, Ministry of Education; and College of Resources and Environmental Sciences, China Agricultural University, Beijing, China
[2]College of Soil and Environmental Sciences, Shenyang Agricultural University, Shenyang, China
[3]Agricultural and Environmental Science Department, Queen's University Belfast, Belfast, United Kingdom

A pot experiment investigated the effects of iron (Fe) fertilization on cadmium (Cd) uptake by rice seedlings irrigated with Cd solution. Shoot dry weight was significantly affected by Fe addition, and root dry weight was affected by Cd addition. Iron supply was the dominant factor affecting the length of the longest leaf and the soil and plant analyzer development (SPAD) value. Cadmium concentrations were much greater in roots than in dithionite–citrate–bicarbonate (DCB) extracts or shoots, and a significant correlation was found between shoot Fe and Cd concentrations. Enhanced Cd uptake observed at high Fe supply implies that enhanced Fe nutrition may counteract the adverse effects of Cd on plants.

Keywords Cadmium, iron fertilization, iron plaque, *Oryza sativa*

Introduction

Rice is the second staple cereal after wheat and is therefore an important food source for the world population. It is widely cultivated in many Asian countries. However, rice is also considered to be the leading dietary source of cadmium (Cd) intake among Asian populations, particularly in Japan (Shimbo et al. 2001), China (Zhang et al. 1998), and Korea (Moon et al. 1995). Cadmium is readily taken up by rice plants and accumulates in rice grains. Consequently, Cd accumulation in the human body over long time periods can lead to a risk of chronic Cd toxicity, which is detrimental to human health. It is therefore desirable to limit Cd concentrations in rice and other crops to reduce potential risks of Cd to human health (Grant et al. 2008).

Cadmium-related health risks associated with the long-term consumption of Cd-contaminated rice grain are exacerbated by the fact that rice-grain iron (Fe), zinc (Zn), and calcium (Ca) contents are insufficient for human health needs (Pedersen and

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Address correspondence to J. L. Zhang, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China. E-mail: junlingz@cau.edu.cn

Eggum 1983; Simmons et al. 2003). Increases in the concentrations of essential trace elements such as Fe and Zn in the grain can therefore reduce the bioavailability of Cd (Grant et al. 2008). Iron is an important microelement that is very relevant to plant nutrition and human health. Interactions between Cd and Fe have attracted much attention, and antagonistic effects between the two elements have often been reported (Cohen et al. 1998; Vert, Briat, and Curie 2003; Yoshihara et al. 2006; Shao et al. 2008). Cadmium has been shown to compete efficiently with Fe and other elements (e.g., manganese [Mn] and Zn) for transporters (such as OsIRT1 in rice) to move across plant membranes (Hall and Willams 2003). The presence of Cd was shown to reduce Fe uptake and cause Fe deficiency in tobacco plants (Yoshihara et al. 2006). However, enhanced Fe nutrition can decrease Cd uptake and relieve Cd toxicity to plants (Liu et al. 2008), and hence Fe fertilization has been suggested as one promising technology to control Cd accumulation in rice when grown in Cd-polluted soil (Shao et al. 2008). Recently, it was found that phytosiderophores released by maize plants enhanced plant Fe uptake in the presence of Cd and thereby contributed to Cd tolerance of plants (Meda et al. 2007). In addition, the formation of ferromanganese oxide (Fe plaque) on the root surfaces of rice as a result of oxidization of Fe^{2+} to Fe^{3+} is a common feature of rice plants, and Fe plaque has been shown to affect uptake and accumulation of heavy metal(loid)s in plants (Greipsson and Crowder 1992; Greipsson 1995; Liu, Zhu, and Smith 2005; Bravin et al. 2008), although the role of Fe plaque in Cd uptake by rice is in doubt (Liu et al. 2008).

The present experiment was designed to investigate the effects of Fe fertilization and the formation of Fe plaque on Cd uptake by rice seedlings. Plants were first grown in soil amended with Fe at different supply levels and were then irrigated with Cd solution to simulate Cd pollution via sewage irrigation because irrigation with sewage and other industrial effluents has been one source of Cd pollution in some Chinese paddy soils. For example, in the Zhangshi sewage-irrigated region in Shenyang, Liaoning Province, soils and sediments were polluted with Cd, and Cd concentrations in sediments of irrigation channels exceeded 16 $mg\,kg^{-1}$ (Junta et al. 1998). Our hypothesis was that Fe fertilization and the resulting formation of Fe plaque on root surfaces may lower Cd uptake and accumulation by rice seedlings, and plant Fe nutrition may therefore mediate Cd phytotoxicity.

Materials and Methods

Precultivation of Rice Seedlings

Seeds of rice (*Oryza sativa* L. cv. 'II You 718') were surface-sterilized in 30% v/v hydrogen peroxide (H_2O_2) for 30 min, washed thoroughly with deionized water, immersed in saturated calcium sulfate ($CaSO_4$) solution for 6 h, and then germinated in acid-washed quartz sand for 10 days. Uniform seedlings were selected, transferred to a 1.2-L ceramic container, and grown in a half-strength nutrient solution for 3 days and thereafter in a full-strength solution for 16 days. The solution was composed of ($\mu mol\,L^{-1}$) ammonium nitrate (NH_4NO_3) 500, sodium phosphate (NaH_2PO_4-$2H_2O$) 60, potassium sulfate (K_2SO_4) 230, calcium chloride ($CaCl_2$) 210, magnesium sulfate ($MgSO_4$-$7H_2O$) 160, Fe–ethylenediaminetetraacetic acid (EDTA) 10, zinc sulfate ($ZnSO_4$-$7H_2O$) 0.5, manganese chloride ($MnCl_2$-$4H_2O$) 0.5, ammonium molybdate [$(NH_4)_6Mo_7O_{24}$-$4H_2O$] 0.05, boric acid (H_3BO_3) 0.2, and copper sulfate ($CuSO_4$-$5H_2O$) 0.01. The nutrient solution was adjusted to pH 5.0 with sodium hydroxide (NaOH) and hydrochloric acid (HCl) and was changed every 3 days. The seedlings were then transplanted into soil with the designated treatments.

Soil Culture and Experiment Setup

Soil was collected from a rice field in Sichuan Province, southwest China. The soil is classified as an aquorizem and was developed from an alluvial deposition of the Minjiang River. After removing root debris and stones, the soil was air-dried and sieved (<3 mm). The soil had the following properties: pH (10:1 distilled water–soil) 6.2, organic matter [potassium dichromate ($K_2Cr_2O_7$)-H_2SO_4] 19.6 g kg^{-1}, total N (semiquantitative titration) 1.84 g kg^{-1}, Olsen P [0.5 M sodium bicarbonate ($NaHCO_3$)] 17.9 mg kg^{-1}, available K [1.0 M ammonium acetate (NH_4OAc)] 34 mg kg^{-1}, diethylenetriaminepentaacetic acid (DTPA)–Fe (2:1 extract solution–soil) 74.9 mg kg^{-1}, and DTPA-Cd 0.227 mg kg^{-1}. The soil was supplied with 0.2 g N kg^{-1} soil as ammonium sulfate [$(NH_4)_2SO_4$], 0.15 g P_2O_5 kg^{-1} soil as potassium phosphate (KH_2PO_4), and 0.20 g K_2O kg^{-1} soil as potassium sulfate (K_2SO_4) as basal fertilizers. Afterward, the soil was amended with Fe at rates of 0, 1, and 2 g Fe kg^{-1} soil (Fe sulfate; $FeSO_4$-$7H_2O$). All reagents were dissolved in solution and then mixed thoroughly with the soil. After growing for 30 days, rice seedlings were irrigated daily with 150 mL Cd solution at the concentrations of 0, 1.0, or 5.0 mg L^{-1} Cd (as cadmium sulfate; $CdSO_4$-$8H_2O$) for 8 days. The total rates of Cd applied to the soil were 0, 2.0, and 10 mg Cd kg^{-1}. The plants were grown for a further 6 days and then harvested. There were nine treatments in total: Fe0Cd0, Fe1Cd0, Fe2Cd0, Fe0Cd2, Fe1Cd2, Fe2Cd2, Fe0Cd10, Fe1Cd10, and Fe2Cd10, and there were five replicates of each treatment.

Plant Growth

The plastic plant pots used were round and tapered with a mean diameter of 7.5 cm and a height of 15 cm. A total of 600 g of soil was placed in each pot by placing a small amount (about 200 g) in the pot, and then a bag made of nylon net (8 cm wide × 15 cm long) of 30-µm pore size was placed upright in the center of the pot. The nylon net was used to restrict root growth within the bag so that the roots could be easily separated from the soil at harvest. Finally, the remaining soil (about 400 g) was divided into two equal parts, which were placed on either side of the bag. Seedlings were grown in mesh bags containing no substrate but surrounded by soil to avoid the difficulty of separating roots from soil and to minimize losses of root material at harvest. The soil in each pot was then moistened with deionized water and allowed to equilibrate for 2 weeks. Two rice seedlings were placed in each nylon net, and the pots were then flooded with deionized water. The water level was maintained at about 1–2 cm above the soil surface during whole growth period of the plants.

The seedlings were grown in a greenhouse at the College of Resources and Environmental Sciences, China Agricultural University, Beijing. The average day/night regime was 15/9 h with a temperature regime of 35/15 °C. During plant growth, the length of the longest leaf of each seedling was measured by ruler on 5, 12, 19, and 26 July and 3 August. The SPAD (soil and plant analyzer development) values of the newly mature leaves were also determined on 22, 25, 28, and 31 July and 3 August.

Harvest and Chemical Analysis

At harvest, the nylon nets were removed from the pots. Seedlings were separated into shoots and roots. Each complete root sample was first used for DCB extraction, and afterward the roots and shoots were oven dried at 70 °C for 3 days. Oven-dried root and shoot samples were ground, and subsamples (0.25 g) were weighed into digestion tubes and moistened overnight with a mixed acid solution composed of 5 mL concentrated HNO_3 and 1 mL mixed concentrated acid [HNO_3/perchloric acid ($HClO_4$) = 1:1]. On the following day, the samples were heated on a digestion block at 90 °C for 3 h, then at 140 °C for 5 h, and 180 °C for a further 2 h until a little solution remained in the tubes. After cooling, the digests were transferred to 25-mL flasks with deionized water and filtered into plastic bottles. A reagent blank and a standard reference material (tomato, GSB 07-1264-2000, Chinese National Certified Reference Material) were included to verify the accuracy and precision of the digestion procedure and subsequent analysis. Iron plaque on the surface of the fresh roots was extracted using a modified DCB method (Otte et al. 1989). The concentrations of Fe and Cd in the DCB extracts and in the acid digests were measured by inductively coupled plasma–optical emission spectrometry (ICP-OES, Optima 3300 DV, Perkin-Elmer, Waltham, Mass., USA). The proportions of Cd in DCB extracts, roots, and shoots were calculated as described by Liu et al. (2008).

Statistical Analysis

Data were analyzed by two- or three-way (for the length of longest leaf and SPAD values of leaves) analysis of variance (ANOVA) using SAS for Windows (version 8.2, SAS Institute Inc., Cary, N.C., USA). Data presented are means ± SD (n = 5), and the means were compared using least significant difference (LSD) at the 5% level.

Results and Discussion

Plant Dry Weights, Length of the Longest Leaf, and Leaf SPAD Values

Shoot dry weights were significantly affected by Fe addition but not by Cd addition, whereas root dry weights showed the opposite pattern of response (Table 1). Root dry weights at Cd2 and Cd10 were heavier than at Cd0. Few effects of Cd pollution on rice growth and yields were also reported from other studies, suggesting that evaluation of Cd toxicity based on growth and yield alone should be avoided (Yu et al. 2006). The stimulatory effect of Cd on root growth agrees with some previous studies (Meda et al. 2007) but disagrees with others. For example, the dry weight of roots of 12-day-old rice seedlings decreased at 0.5, 1, and 1.5 mmol L^{-1} $CdCl_2$ (Hsu and Kao 2005). The contrasting results may be due to various factors such as plant species and cultivar, Cd supply level, and other differences in the experimental conditions.

Although no visual symptoms of Fe toxicity were observed in the Fe2 treatment, shoot dry weights under Fe2 were significantly less than with Fe0 or Fe1 (except treatment Fe2Cd10), indicating that the rice seedlings may have experienced chronic Fe toxicity, as also shown by the reduced leaf length at Fe2 (Figure 1) and high shoot Fe concentration at Fe2. Prior to irrigation with Cd solution, the leaf lengths decreased significantly with increasing Fe supply ($P < 0.001$; Table 2, Figure 1), but the differences among Fe treatments became smaller after addition of Cd. No

Table 1. Dry weight of shoots and roots of rice seedlings

Cd supplied (mg/kg)	Fe supplied (g/kg)	Shoot dry weight (g)	Root dry weight (g)
0	0	4.27 ± 0.41 ab	1.60 ± 0.16 a
	1	4.74 ± 0.42 a	1.47 ± 0.13 a
	2	4.00 ± 0.32 b	1.41 ± 0.16 a
2	0	4.60 ± 0.35 a	1.90 ± 0.10 a
	1	5.12 ± 0.39 a	1.62 ± 0.15 ab
	2	3.95 ± 0.49 b	1.52 ± 0.32 b
10	0	4.18 ± 0.48 a	1.74 ± 0.14 a
	1	4.74 ± 0.62 a	1.72 ± 0.38 a
	2	4.52 ± 0.44 a	1.86 ± 0.27 a
Significance[a]			
Cd		NS	**
Fe		***	NS
Cd × Fe		NS	NS

[a] By analysis of variance.

Notes. ***$P < 0.001$; **$P < 0.01$; NS, not significant. Plants were grown in soil amended with different levels of Fe for 30 days, irrigated with Cd solution for 8 days, and irrigated thereafter with water for another 6 days. Data are means ± SD (n = 5). Within each group of three values, any two means sharing a lowercase letter are not significantly different by least significant difference at the 5% level.

significant effect of Cd or Fe additions was observed on the leaf lengths or dry weights of shoots or roots. The leaf lengths increased over time and were significantly affected by plant growth period, Fe and Cd additions, and their two-way interactions.

The SPAD values of leaves were significantly affected by plant growth period, Fe and Cd additions, and time × Fe interaction ($P < 0.05$, Table 2). The SPAD values of leaves at Fe1 and Fe2 were significantly greater than that at Fe0 regardless of Cd supply level (Figure 2). At Fe0 and Fe1, the SPAD values of leaves decreased gradually with the duration of the plant growth period, whereas at Fe2 the value no longer decreased from 28 July onward. Addition of 10 mg kg^{-1} Cd had no clear effect on the SPAD values of the leaves. External Cd supply often led to leaf chlorosis. Concentrations of chlorophyll pigments in the fourth leaf of maize plants decreased when Cd was supplied at concentrations ranging from 1.7 to 25 μmol L^{-1} (Lagriffoul et al. 1998). Our results are somewhat different than those of previous studies but again suggest that parameters other than growth index [as shown by Lagriffoul et al. (1998) in maize plants], for example, measurement of enzyme activities, might be useful as early biomarkers in a plant bioassay to assess the phytotoxicity of Cd contamination to rice plants.

Iron and Cd Concentrations in DCB Extracts and in Roots and Shoots

The concentrations of Fe in the DCB extracts and in the roots and shoots of rice were significantly affected by Fe supply level but not by Cd supply level in the irrigation solution and Fe × Cd interaction (Table 3). It is likely that the duration of Cd irrigation was too short to affect plant Fe uptake. Iron concentrations in DCB

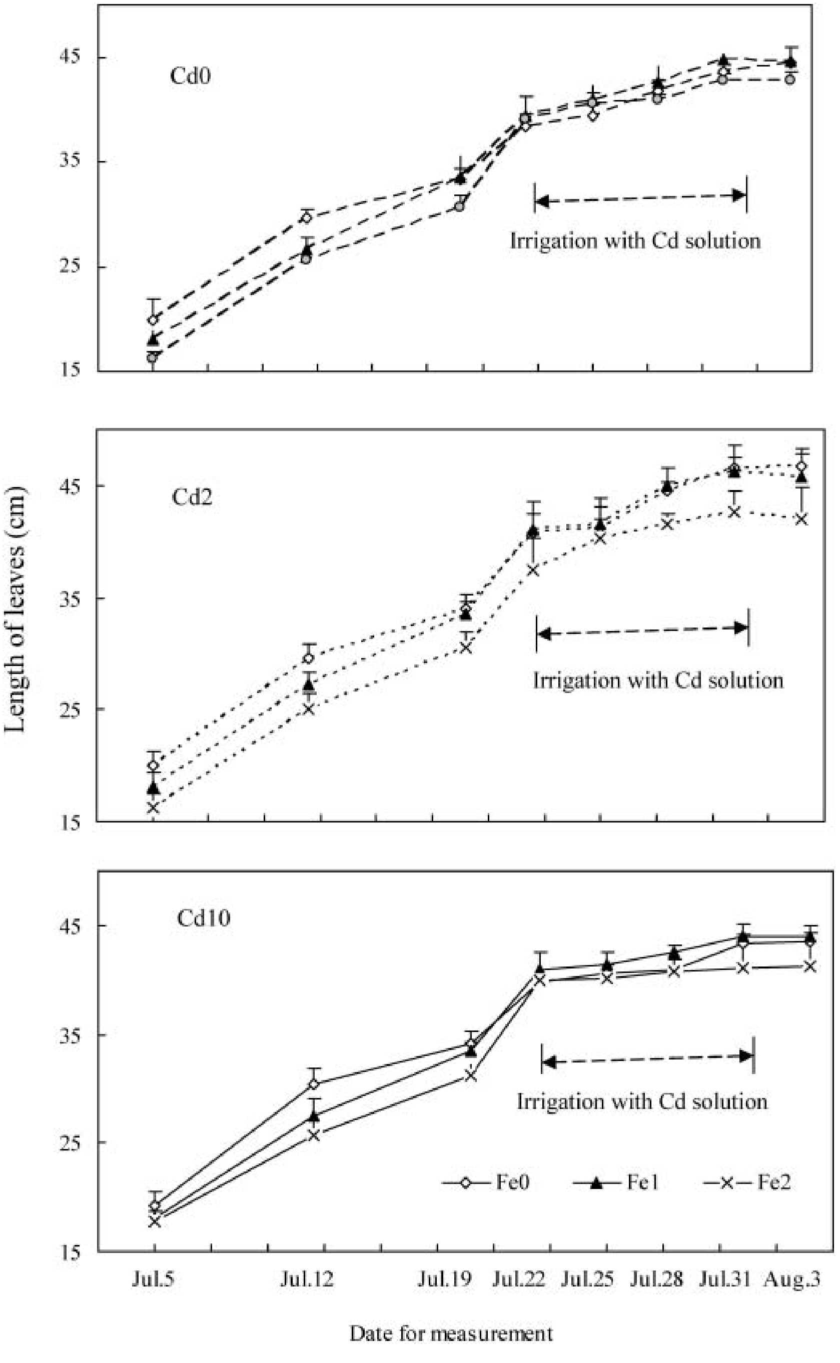

Figure 1. Length (cm) of the longest leaf of rice seedlings. Plants were grown in soil amended with different levels of Fe for 30 days and then irrigated with Cd solution for 8 days and thereafter with water for another 6 days.

Table 2. Analysis of variance for Figures 1 and 2

Significance[a]	Length of leaves	SPAD value
Time	***	***
Cd	***	**
Fe	***	***
Time × Cd	***	NS
Time × Fe	***	***
Cd × Fe	***	NS
Time × Cd × Fe	NS	NS

[a] By analysis of variance.

Notes. ***P < 0.001; **P < 0.01; NS, not significant.

extracts and roots were significantly greater when Fe was supplied than when it was not, and there was no significant different between Fe concentrations in DCB extracts and roots at Fe1 and Fe2. Shoot Fe concentrations at Fe2 were much greater than that at Fe1 or Fe0, but there was no significant difference between Fe concentrations at Fe1 and Fe0 (Table 3). Formation of Fe plaque on root surfaces of rice and other aquatic plants is considered to be one of the mechanisms by which the plants acclimate to anaerobic conditions, particularly to strong concentrations of elements such as Fe^{2+} and Mn^{2+} in the soil. Our results show that the amounts of Fe in Fe plaque (Fe concentrations in DCB extracts) were much higher than in roots and shoots, indicating that Fe plaque is a barrier to excessive Fe uptake by rice plants (Greipsson and Crowder 1992). Similarly, Fe plaque can sequester metal(loid)s such as arsenic (As) and aluminum (Al) on root surfaces of rice and thus depress their translocation to the shoots (Chen et al. 2006; Bravin et al. 2008). In comparison to greater Fe concentrations in Fe plaque, Cd concentrations in rice

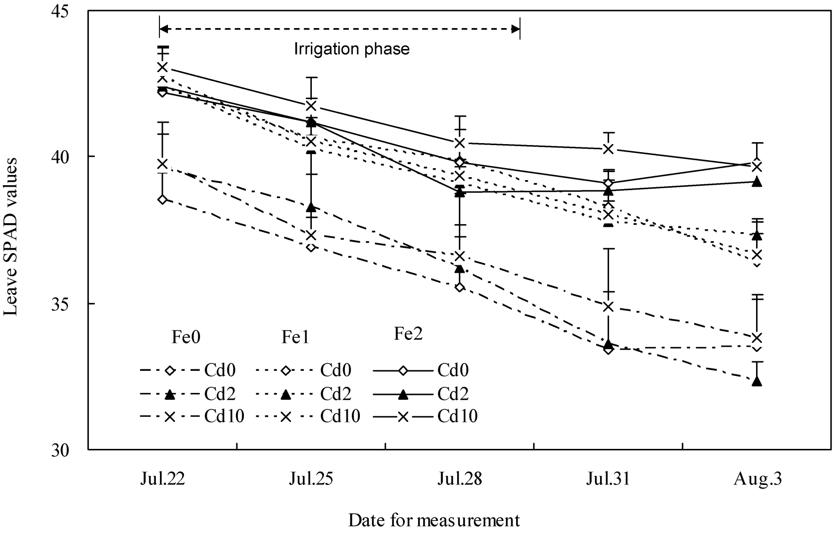

Figure 2. SPAD values of leaves of rice seedlings. Plants were grown in soil amended with different levels of Fe for 30 days and then irrigated with Cd solution for 8 days and thereafter with water for another 6 days.

Table 3. Mean Fe and Cd concentrations in DCB extracts and in roots and shoots of rice seedlings

Cd supplied $(\mathrm{mg\,kg^{-1}})$	Fe supplied $(\mathrm{g\,kg^{-1}})$	Fe in DCB $(\mathrm{mg\,kg^{-1}})$	Fe in roots $(\mathrm{mg\,kg^{-1}})$	Fe in shoots $(\mathrm{mg\,kg^{-1}})$	Cd in DCB $(\mathrm{mg\,kg^{-1}})$	Cd in roots $(\mathrm{mg\,kg^{-1}})$	Cd in shoots $(\mathrm{mg\,kg^{-1}})$
0	0	4435 ± 1077 b	2109 ± 1044 b	225.4 ± 9.4 b	0.55 ± 0.08b	2.45 ±0.49a	0.46 ± 0.09a
	1	37769 ± 6303 a	9346 ± 2104a	302.6 ± 26.4 b	1.13 ± 0.40 a	0.95 ±0.12 b	0.21 ± 0.10 b
	2	35359 ± 5732 a	10583 ± 624 a	646.9 ± 89.8 a	1.15 ± 0.21 a	1.27 ±0.56 b	0.16 ± 0.06 b
2	0	4054 ± 1135 b	2037 ± 1027 ab	224.1 ± 17.2 b	3.98 ± 0.83 a	79.9 ±10.2 b	7.77 ± 0.83 b
	1	41960 ± 8194 a	9180 ± 2270 a	306.6± 34.6 b	3.79 ± 0.55 a	81.0 ±5.28 b	8.45 ± 0.59 b
	2	32356 ± 9122 a	9483 ± 1625 a	669.3 ± 135.2 a	3.56 ± 0.40 a	92.4 ±2.22 a	12.2 ± 1.27 a
10	0	6189 ± 1987 b	1819 ± 833 b	223.4 ± 15.6 b	27.4 ± 2.89 a	256 ±16.2b	25.0 ± 1.67 b
	1	32455 ± 6253 a	10963 ± 2137 a	322.9 ± 15.7 b	16.9 ± 3.41 b	348 ±53.7 b	26.7 ± 1.18 b
	2	27310 ± 4777 a	11758 ± 3904 a	653.2 ± 137.2 a	10.2 ± 1.99 c	541 ±138 a	34.4 ± 1.94 a
Significance[a]							
Cd		NS	NS	NS	***	***	***
Fe		***	***	***	***	***	***
Cd × Fe		NS	NS	NS	***	***	***

[a] By analysis of variance.

Notes. ***$P < 0.001$; NS, not significant. Plants were grown in soil amended with different levels of Fe for 30 days and then irrigated with Cd solution for 8 days and thereafter with water for another 6 days. Data are means ± SD ($n = 5$). Within each group of three values, any two means sharing a lowercase letter are not significantly different by least significant difference at the 5% level.

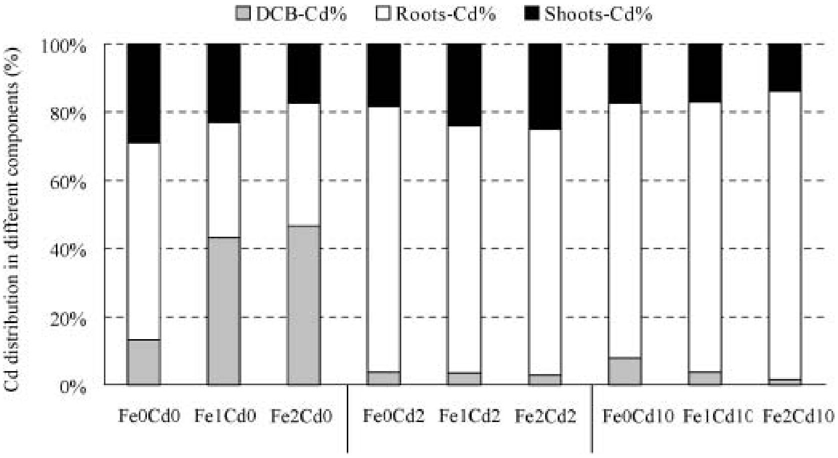

Figure 3. Cadmium percentages in DCB extracts and in roots and shoots of rice seedlings. Plants were grown in soil amended with different levels of Fe for 30 days and then irrigated with Cd solution for 8 days and thereafter with water for another 6 days.

roots were much more than in DCB extracts and shoots (Table 3). The proportions of Cd in DCB extracts and in roots and shoots were approximately 2–4, 72–84, and 14–25%, respectively (Figure 3). Our results are consistent with our previous conclusion that Fe plaque may be of minor importance for uptake and accumulation of Cd in rice plants, and roots may be the main tissue retaining Cd in rice (Liu et al. 2008).

The Cd concentrations in DCB extracts and in roots and shoots were significantly affected by Cd and Fe supply levels and their interactions (Table 3). Cadmium concentrations were very low when no Cd was added and increased markedly with increasing Cd addition. The concentrations of Cd in DCB extracts at Cd2 were not significantly different among different Fe supply levels, and at Cd10 the Cd concentrations in DCB extracts decreased with increasing Fe supply level. The concentrations of Cd in shoots and roots were significantly greater at Fe2 than that at Fe1 or Fe0, but no significant difference was observed between Fe1 and Fe0 (Table 3). High Fe concentrations in plants can confer tolerance to excessive heavy metals (Chien et al. 2001) and have been shown to be beneficial to plants grown in excessive Cu and/or Ni (Greipsson 1995) because Fe competes with the heavy metals for sensitive metabolic sites within leaves (Taylor and Crowder 1983). A recent study showed that under Fe-deficiency conditions the release of phytosiderophores by roots of maize stimulated Fe uptake and that the high Fe uptake may confer plant tolerance to Cd (Meda et al. 2007). Cadmium toxicity in rice leaves caused by the supply of $CdCl_2$ may require the participation of Fe (Chien et al. 2001). Recently, Shao et al. (2008) reported that the concentrations of Cd in roots, shoots, and grain of rice all increased significantly when the soil was amended with $FeSO_4$ or when foliar $FeSO_4$ was applied, but Cd concentrations decreased when the soil was amended with EDTA Na_2Fe. In the present experiment, Cd concentrations in shoots were positively related to Fe concentrations at both Cd2 and Cd10 (Figure 4), implying that Fe nutrition might be important in mediating Cd toxicity to the plants. Adequate Fe nutrition in shoots might inhibit the expression of high-affinity Fe transporters (Vert, Briat, and Curie 2003) and in the long term may attenuate Cd

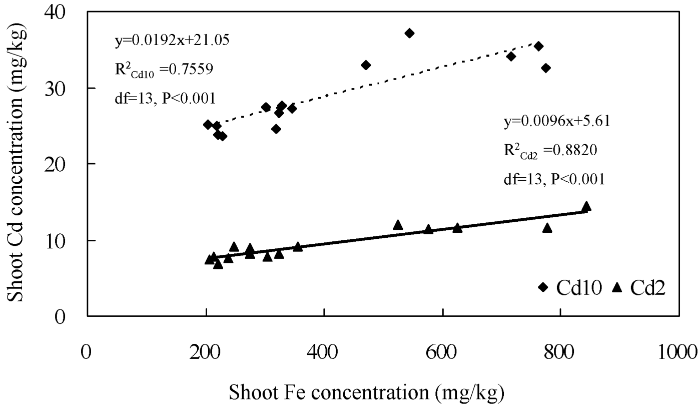

Figure 4. Correlation between concentrations of Fe and Cd in shoots of rice seedlings.

uptake into plant roots. In addition, the stimulatory effect of Fe nutrition on Cd uptake in the present experiment may also be partly attributed to the enhanced Cd uptake by the larger and newly grown roots at Fe2 (Table 1).

In conclusion, our results indicate that growth parameters may not be a valid index to evaluate Cd toxicity in rice plants. Iron plaque has been shown to mediate Fe uptake in rice plants but may be of little relevance to Cd uptake by rice seedlings. Soil Fe supply enhances plant Cd uptake, but the underlying mechanism of the interaction between Cd and Fe in the plants warrants further investigation.

Acknowledgments

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