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Sequestration of As by iron plaque on the roots of three rice (*Oryza sativa* L.) cultivars in a low-P soil with or without P fertilizer

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Abstract

A pot experiment was carried out in a greenhouse to investigate the sequestration of As in iron plaques on root surface of three rice (*Oryza sativa* L.) cultivars. Phosphate (P) fertilization increased both plant biomass and tissue P concentrations significantly, indicating that the soils used in this study was highly P-deficient. Results from this study confirmed that low P supply improved the formation of iron plaque on rice roots. As a consequence, arsenic (As) concentrations in DCB-extracts with no P addition were significantly higher than those with P fertilization. Arsenic was highly sequestered in iron plaque; arsenic concentration in iron was up to nearly 120 mg kg⁻¹, while arsenic concentrations in roots were just several mg kg⁻¹. Both arsenic and phosphate concentrations in iron plaque were highly positively correlated with the amounts of iron plaque (DCB-extractable Fe). Contrary to normal understanding that increasing P supply could reduced As accumulation in plants, results from the present study showed that P fertilization did not inhibit the As uptake by plants (As accumulation in aboveground), which was probably due to the fact that iron plaque formation was improved under low P conditions, thus leading to more As sequestration in the iron plaque. Thus results obtained in this study indicated that the iron plaque may inhibit the transfer of As from roots to shoots, and thus alter the P–As interaction in plant As uptake processes.

Introduction

Arsenic (As) is a highly toxic and carcinogenic ubiquitous metalloid and is widely distributed in the environment through both natural and anthropogenic pathways. Natural background levels of As are 5–6 mg kg⁻¹ dry soil on average (Yan-Chu 1994). However, anthropogenic activities, such as pesticide use (Maclean & Langille 1981), mining (Galbraith *et al.* 1995), or irrigation with contaminated groundwater (Abedin *et al.* 2002) have significantly enhanced As levels in the environment in some locations. Arsenic contamination of groundwater has been reported from many countries, with the most severe problems

occurring in Bangladesh (Dhar *et al.* 1997; Biswas *et al.* 1998; Nickson *et al.* 1998; Chowdhury *et al.* 1999), West Bengal and India (Mandal *et al.* 1996, 1997), and China (Huang *et al.* 1992; Smith *et al.* 1992; Liangfang & Jianghong 1994; Chen *et al.* 1995). The people of these regions not only drink contaminated groundwater but also use it for crop irrigation. In Bangladesh, irrigation is mostly dependent on groundwater and 75% of the total cropped area and 83% of the total irrigated area are irrigated using groundwater for rice cultivation (Dey *et al.* 1996). It has been reported that As levels in soils may reach over 80 mg kg⁻¹ after irrigation with contaminated water (Ullah 1998) and may lead to a ten-fold elevation in grain As

level (Meharg 2004). The local population may therefore be exposed to As contamination through two principal pathways, namely groundwater-soil-plant-human and groundwater-human (Juhaz *et al.* 2003).

Rice (*Oryza sativa*) is the staple food in many parts of the world, particularly in Southeast Asia. Rice grown on As-contaminated soils in China had high As levels in the grains (Xie & Huang 1998). It was reported that in the vicinity of an As mine in Hunan, China, up to 35% of the local population had severe arsenism and the proportion increased with age (Wang *et al.* 1999). Epidemiological studies further demonstrated that there were significant correlations between As concentrations in human hair and those in local rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and soils (Lin *et al.* 2001). For populations living on subsistence rice diets the As contamination of rice grains contributes greatly to dietary As exposure. When drinking water levels of As are at the World Health Organization (WHO) limit of $10 \mu\text{g L}^{-1}$, 0.05 mg kg^{-1} arsenic in rice contributes $\sim 60\%$ of dietary As exposure. Rice with an As level of 1.8 mg kg^{-1} has been recorded in the As-affected tubewell areas of Bangladesh (Meharg 2004). Even at the very dangerous level of As in drinking water of 1 mg L^{-1} , a rice arsenic level of 1.8 mg kg^{-1} contributes around 30% of dietary As intake (tips). Thus, As uptake by rice plants plays an important role in food chain transfer of this toxic element and poses health risks to human beings.

Iron plaque is commonly formed on the roots of aquatic plants such as *Oryza sativa*, *Typha latifolia* and *Phragmites communis*. The iron plaque may be amorphous or crystalline (Bacha & Hossner 1977; Chen *et al.* 1980). It is composed mainly of ferrihydrite (63%) with lesser amounts of goethite (32%) and minor levels of siderite (5%) (Hansel *et al.*, 2001). It has been shown that iron plaque may be a barrier to the uptake of heavy metals such as Cu, Ni, Mn and Cd (Taylor & Crowder 1983a; Otte *et al.* 1989; Greipsson 1994; Ye *et al.* 1998; Wang & Peverly 1999). However, the overall effect of iron plaque on plant uptake of nutrients and/or contaminants may depend on the amounts of iron plaque formed on the roots (Otte 1987, 1989; Zhang *et al.* 1998). The functional groups of iron hydroxides may sequester some cations and anions (Kuo 1986; Otte *et al.* 1989).

Iron hydroxides in soil or solution had very strong binding affinity for As (V) (Meng 2002). Otte *et al.* (1991) showed that iron plaque played an important role in the uptake of As by the salt marsh plant *Aster tripolium*. Although the role of iron plaque on the roots has been investigated in recent years, the relationship between iron plaque and plant uptake of As is still under investigation.

In rice plants, iron plaque can be formed in both natural and laboratory conditions (Chen *et al.* 1980; Greipsson & Crowder 1992; Greipsson 1994, 1995). The role of iron plaque on root surfaces of rice plants may be important for the development of practical approaches to reduce As uptake. Our previous experiments showed that iron plaque had a very strong affinity to As and enhanced uptake, but reduced the translocation of As from roots to shoots (Liu *et al.* 2004a, b). It has also been demonstrated that under solution culture conditions, low phosphate (P) supply may improve the formation of iron plaque leading to the sequestration of As on the root surface (Liu *et al.* 2004a). The aim of the present experiment was to investigate the effects of P fertilization on the amounts of iron plaque on the roots of three rice cultivars, on As sequestration in iron plaque and on As accumulation in the aerial plant parts.

Materials and methods

Soil preparation

A pot experiment was conducted using a highly weathered red soil taken from Huangshi, Hubei province, central China. After transport to the laboratory, the soil was air-dried and sieved ($< 2 \text{ mm}$, stainless steel mesh) prior to analysis of chemical and physical properties. Selected soil properties analyzed according to the standard methods recommended by the Soil Science Society of China (Lu 1999) are listed in Table 1. The soil was uniformly supplied with both N and K fertilizers at amounts equivalent to 200 mg N kg^{-1} soil (as urea) and $133 \text{ mg K}_2\text{O kg}^{-1}$ (as K_2SO_4). Two P treatments were included in the experiment, namely no P addition ($-P$) and P addition at 100 mg kg^{-1} as CaHPO_4 ($+P$), and the P fertilizer was thoroughly mixed in powder form with the

Table 1. Physicochemical properties of the soil.

Soil parameters		
pH	5.21	
Soil organic matter (g kg ⁻¹)	13.36	
Olsen P (mg kg ⁻¹)	1.10	
Cation exchange capacity (cmol (+) kg ⁻¹)	19.66	
Free iron oxide (g kg ⁻¹)	3.88 g kg ⁻¹	
Soil texture	<0.002 mm	46.6%
	0.02–0.002 mm	24.9%
	2–0.2 mm	28.5%

soil. Soil was then placed in plastic pots (600 g capacity) lined with plastic bags.

Plant culture and analysis

Seeds of three rice (*Oryza sativa* L.) cultivars, 94D22, KY1360 and YY1 were obtained from Professor Damo Li, Institute of Subtropical Regional Agriculture, Chinese Academy of Sciences. Seeds were disinfected in 30% (v/v) H₂O₂ solution for 15 min, followed by thorough washing with de-ionized water. The seeds were germinated in moist Perlite. After 3 weeks, uniform seedlings were selected and two seedlings transplanted into each pot of soil. The pots were randomly arranged in the greenhouse and rearranged several times during the growth period. Each pot was watered with de-ionized water to saturation after transplantation. After initial watering, each pot received 30 ml As-contaminated water (1 mg kg⁻¹) every other day until harvest. The mean growth temperature was 25 °C. The plants were harvested 4 weeks after transplanting.

At harvest, iron plaque on fresh root surfaces was extracted using dithionite-citrate-bicarbonate (DCB) by the method of Taylor and Crowder (1983b) and Otte *et al.* (1991). The whole root system of each seedling was incubated for 60 min at room temperature (20–25 °C) in 40 ml of a solution containing 0.03 M sodium citrate (Na₃C₆H₅O₇ · 2H₂O) and 0.125 M sodium bicarbonate (NaHCO₃), with the addition of 0.6 g sodium dithionite (Na₂S₂O₄). Roots were rinsed three times with deionized water that was added to the DCB-extracts. The resulting solution was made up to 100 ml with deionized water. After DCB extraction, roots and shoots were oven dried at 70 °C for 3 days and weighed.

Plant analysis

Dried plant material was ground and about 0.25 g weighed accurately into clean, dry digestion tubes (100 ml) (FOSS digestion tube). Concentrated HNO₃ (5 ml) was added and allowed to stand overnight. On the following day, the tubes were placed on a heating block (Foss Tecator 2006 Digestion System) and the temperature was raised to 80 °C for 1 h and then to 120–130 °C for 20 h. A reagent blank and standard reference plant material (GBW07605 from the Chinese National Center for Standard Materials) were included to verify the accuracy and precision of the digestion procedure and subsequent analysis. After digestion the solutions were cooled, diluted to 50 ml with ultra-pure water (Easy-pure, Dubuque, Iowa, USA) and filtered into acid-washed plastic bottles. The concentrations of P and Fe in the root DCB-extracts and the plant acid digests were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 2000 DV and As by atomic fluorescence spectrometry using a Beijing Ruili Analytical Instrument Co. AF-610A, respectively. An internal standard was included and negligible matrix effects were observed for both ICP-OES and atomic fluorescence spectrometry.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the GENSTAT package (Version 6.0, NAG Ltd, England).

Results

Plant growth

There were significant differences in both root and shoot biomass among the cultivars and P treatments (Table 2). Addition of P fertilizer resulted in a 3–4-fold increase in plant biomass in all three cultivars. Cultivar 94D-22 had the lowest biomass irrespective of P fertilization.

Plant P concentrations

The application of P increased tissue P concentrations in all cultivars ($P < 0.001$, Table 3), with

Table 2. Shoot and root biomass (g pot⁻¹) of three rice cultivars grown in a low-P soil with or without P fertilizer.

Cultivar	Shoot		Root	
	-P	+P	-P	+P
94D-22	0.49 ± 0.05	1.64 ± 0.18	273 ± 28.5	517 ± 18.4
KY1360	0.62 ± 0.02	2.43 ± 0.06	0.18 ± 0.02	0.75 ± 0.05
YY1	0.61 ± 0.02	2.09 ± 0.10	0.24 ± 0.02	0.63 ± 0.05

Analysis of variance

P treatment (P)	$p < 0.001$	$p < 0.001$
Cultivar (C)	$p < 0.01$	$p < 0.001$
P × C	$p < 0.05$	$p < 0.01$

Mean ± 1 SD.

Table 3. P concentrations in shoot and root (mg kg⁻¹) of three rice cultivars grown on a low-P soil with or without P fertilizer.

Cultivar	Shoot		Root	
	-P	+P	-P	+P
94D-22	495 ± 11.6	865 ± 13.8	273 ± 28.5	517 ± 18.4
KY1360	448 ± 10.1	784 ± 26.2	312 ± 28.2	438 ± 10.8
YY1	489 ± 11.3	968 ± 63.9	284 ± 16.8	493 ± 26.6

Analysis of variance

P treatment (P)	$p < 0.001$	$p < 0.001$
Cultivar (C)	$p < 0.05$	NS
P × C	NS	$p < 0.05$

Mean ± 1 SD.

shoot P concentrations roughly doubling with P application compared with the control treatment, and root P concentrations increasing to a lesser extent with P application. There were significant differences in shoot P concentration between cultivars but differences in root P concentration were marginal, possibly due to the large variation between replicates.

Iron plaque formation and P and As concentrations in iron plaque

Roots of all plants appeared reddish at harvest, indicating the formation of iron plaque on the root surface. Addition of P fertilizer decreased the amounts of iron plaque (DCB-extractable Fe, DCB-Fe hereafter) on the root surface ($P < 0.001$, Figure 1), and with P fertilization the amounts of iron plaque were less than half of those without P fertilization. There were significant dif-

ferences between cultivars in the amounts of iron plaque, with cultivar 94D-22 having the most and KY136 having the smallest amount of iron plaque irrespective of P fertilization, although the differ-

Fig. 1. DCB-extractable Fe concentration on the roots of three rice cultivars grown on a low-P soil with or without P fertilizer. Bars: ± 1 SD.

ence in the zero-P controls was rather small (albeit statistically significant).

Concentrations of P associated with iron plaque (DCB-extractable P, DCB-P hereafter) were much lower with P fertilization than without P ($P < 0.001$, Figure 2). There were significant differences among cultivars in DCB-P. Concentrations of As associated with iron plaque (DCB-extractable As, DCB-As hereafter) were significantly lower with P fertilization than without P (Figure 3). Cultivar 94D-22 had the highest amounts of DCB-As irrespective of P fertilization.

Tissue as concentrations

P fertilization did not have any significant effect on shoot As concentrations (Table 4) but there were significant differences among cultivars, with

Fig. 2. DCB-extractable P concentration on the roots of three rice cultivars grown on a low-P soil with or without P fertilizer. Bars: ± 1 SD.

Fig. 3. DCB-extractable As concentration on the roots of three rice cultivars grown on a low-P soil with or without P fertilizer. Bars: ± 1 SD.

cultivar KY1360 always having the lowest shoot As concentration irrespective of P fertilization. Concentrations of As in roots of cultivars 94D-22 and YY1 were significantly lower with P fertilization than without P fertilization, but P fertilization had no effect on root As concentrations in cultivar KY1360. No significant differences in As concentrations of roots were observed between the three cultivars.

Discussion

The soil used in the present study was very low in P (Table 2) and without P fertilization plant growth was severely restricted, with tissue P concentrations being well below optimum levels (Marschner 1995). Even with the P fertilization,

Table 4. As concentrations in shoot and root (mg kg^{-1}) of three rice cultivars grown on a low-P soil with or without P fertilizer.

Cultivar	Shoot		Root	
	-P	+P	-P	+P
94D-22	1.16 $\pm$ 0.10	1.23 $\pm$ 0.32	7.52 $\pm$ 1.89	2.74 $\pm$ 0.36
KY1360	0.85 $\pm$ 0.12	0.68 $\pm$ 0.11	4.81 $\pm$ 0.66	4.34 $\pm$ 1.18
YY1	1.04 $\pm$ 0.18	1.01 $\pm$ 0.11	7.55 $\pm$ 1.27	2.31 $\pm$ 0.24

Analysis of variance

P treatment (P)	$p = 0.05$	$p < 0.01$
Cultivar (C)	NS	NS
P $\times$ C	NS	NS

Mean ± 1 SD.

tissue P concentrations were at the lower end of normal range for plant tissues. In accordance with our previous results (Liu *et al.* 2004a), low P conditions enhanced the formation of iron plaque as there was an almost two-fold increase in the amounts of iron plaque (DCB-Fe) under low P level compared to high P level in the soil (Figure 1). Despite the low P level in the non-fertilized soil, concentrations of DCB-P were much higher than in the P-amended soil, and the concentrations of DCB-P were highly correlated with the amounts of iron plaque (Figure 4). This strongly indicates that the iron plaque was a major factor controlling P deposition on the root surface. It is well known that phosphate has a high affinity for iron oxide surfaces, thus iron plaque on the root surface may sequester phosphate, and act as a 'buffer' for P in the soil-plant system.

In this study the soil was irrigated with As (V)-contaminated water, and a substantial proportion of As would have remained as As (V) despite the flooded conditions during the experimental period due to the oxidizing status of the rhizosphere (Belzile & Tessier 1990; Abedin *et al.* 2002). Both As (V) and As (III) may be adsorbed (sequestered) by the iron plaque on the root surface. A linear relationship between DCB-Fe and DCB-As was also observed (Figure 5). Although it has been shown that As in the form of arsenate has a higher affinity for iron oxide than phosphate (Meng *et al.* 2002), P concentrations in the soil solution were presumably much higher than those of As, therefore As concentration in the iron plaque was much lower than those of P. Nevertheless, despite the high P concentration in the soil with P fertilization, there was only a marginal difference in the

Fig. 4. Relationship between concentrations of DCB-extractable P and DCB-extractable Fe in the roots of three rice cultivars grown on a low-P soil with or without P fertilizer.

Fig. 5. Relationship between concentrations of DCB-extractable As and DCB-extractable Fe in the roots of three rice cultivars grown on a low-P soil with or without P fertilizer.

ratio of DCB-Fe to DCB-As, indicating a strong association between As and iron plaque (data not shown). Due to the strong As sequestration in iron plaque, DCB-As was 10 to 100 times higher than As concentration in the plant tissues (Table 4 and Figure 3).

It has been shown that As (V) uptake by various plants is through high-affinity phosphate transporters because arsenate and phosphate are analogous (Meharg & Macnair 1990) and As (V) uptake by rice was shown by Abedin *et al.* (2002) to be inhibited by P. In this study it seems to show that P fertilization significantly reduced As concentrations in the roots, and the higher As concentrations in the roots in the zero-P treatment may be partly due to residual As on the root surface following extraction with DCB. Despite the significantly higher concentrations of As in roots and in DCB-extracts in the zero-P treatment, difference in shoot As concentrations was not observed. These results indicate that iron plaque may restrict the translocation of As from roots to shoots, and confirm our previous finding that the formation of iron plaque may alter the interactions between P and As in soil-rice plant systems (Liu *et al.* 2004a). However, the quantitative relationships between P nutrition, and As accumulation in iron plaque on rice roots remain unclear, and further investigation is warranted.

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