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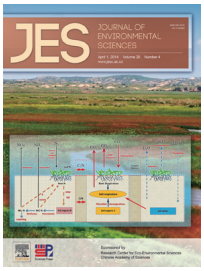

Arsenic dynamics in the rhizosphere and its sequestration on rice roots as affected by root oxidation

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ABSTRACT

A pot experiment was conducted to investigate the effects of root oxidation on arsenic (As) dynamics in the rhizosphere and As sequestration on rice roots. There were significant differences ($P < 0.05$) in pH values between rhizosphere and non-rhizosphere soils, with pH 5.68–6.16 in the rhizosphere and 6.30–6.37 in non-rhizosphere soils as well as differences in redox potentials ($P < 0.05$). Percentage arsenite was lower (4%–16%) in rhizosphere soil solutions from rice genotypes with higher radial oxygen loss (ROL) compared with genotypes with lower ROL ($P < 0.05$). Arsenic concentrations in iron plaque and rice straw were significantly negatively correlated ($R = -0.60$, $P < 0.05$). Genotypes with higher ROL (TD71 and Yinjingruanzhan) had significantly ($P < 0.001$) lower total As in rice grains (1.35 and 0.96 mg/kg, respectively) compared with genotypes with lower ROL (IAPAR9, 1.68 mg/kg; Nanyangzhan 2.24 mg/kg) in the As treatment, as well as lower inorganic As ($P < 0.05$). The present study showed that genotypes with higher ROL could oxidize more arsenite in rhizosphere soils, and induce more Fe plaque formation, which subsequently sequestered more As. This reduced As uptake in aboveground plant tissues and also reduced inorganic As accumulation in rice grains. The study has contributed to further understanding the mechanisms whereby ROL influences As uptake and accumulation in rice.

Introduction

Contamination of groundwater by arsenic has been frequently reported in the scientific literature (Stone, 2008; Zhu et al., 2008a, 2008b). In the arsenic-affected areas of Bangladesh, groundwater contains up to 2 mg As/L compared to the WHO recommended provisional limit of 0.01 mg As/L. The irrigated soils of Bangladesh generally contain 4–8 mg/kg As, while in areas where arsenic-contaminated water is used for irrigation, the soil As concentration can be as high as 83 mg/kg (Abedin et al., 2002). Rice grains collected in districts of As-contaminated soils in Bangladesh had concentrations that were 10-fold higher than the average concentration of 0.1 mg As/g (Meharg and Rahman, 2003). Rice is the staple diet of 3 billion people in the world, predominantly those in Asia. However, there is extensive As contamination of paddy soils around the world, as a result of irrigation using As-contaminated groundwater (Meharg, 2004; Liao et al., 2005) or mining activities around rice cultivation areas (Zhu et al., 2008a; Williams et al., 2009). The rice collected from mine-impacted regions in China were found to be highly enriched with As, reaching concentrations of up to 624 ng/g (Zhu et al., 2008b). For populations living on subsistence rice diets, As contamination in rice grain contributes greatly to their dietary As exposure. It has been reported that when drinking water levels of As

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are at the WHO's 10 mg/L limit, 0.05 mg/kg As in rice contributes to 60% of the dietary As exposure (Meharg et al., 2009; Williams et al., 2009). Inorganic arsenic species are of particular concern, as they are associated with various internal cancers and other health problems (IARC, 2004). Therefore, the food that sustains half of the world's population also increases a health risk (Stone, 2008). It is crucial that the physiology and genetics of rice uptake of As is better understood to counteract this widespread contamination of the food chain (Meharg, 2004).

Arsenic chemistry in the rhizosphere is complex and is controlled by several factors (Fitz and Wenzel, 2002). Under paddy field conditions, inorganic As is inter-converted between the reduced inorganic species arsenite (As(III)) and the oxidized species arsenate (As(V)) (Marin, 1993). Soil microbes can also methylate inorganic As to produce monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMA) (Turpeinen et al., 1999). In roots, oxygen transported within the root aerenchyma is consumed by adjacent tissue cells, or diffused towards the root apex or the rhizosphere; the transfer of oxygen from aerenchyma to the rhizosphere is termed radial oxygen loss (ROL) (Colmer, 2003a, 2003b). ROL can oxidize rhizosphere soil elements (e.g. Fe^{2+} to Fe^{3+}) and cause precipitation of toxic metals in rhizosphere soil and on root surfaces (Otte et al., 1989; Smolders and Roelofs, 1996), subsequently altering rhizosphere metal mobility. Caetano and Vale (2002) reported that iron-rich concretions are frequently found around plant roots in the Tagus estuary where radial delivery of O_2 takes place. Furthermore, the oxidizing ability of the plant roots is considered the most important biotic factor controlling iron plaque formation (Mendellsohn et al., 1995). Rice plants develop aerenchyma to transfer O_2 from the aerial parts of the plant to the roots, resulting in the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), and the precipitation of Fe oxides or hydroxides (Fe-plaque) on the root surfaces (Chen et al., 1980). Iron plaque can sequester metals on wetland plant roots (Hansel et al., 2001; Blute et al., 2004) and prevent translocation of As from roots to shoots (Liu et al., 2004a, 2004b). Therefore rhizosphere interactions play a key role in controlling As bioavailability to crop plants (Hinsinger, 2001; Fitz and Wenzel, 2002).

A previous study noted that there was a significant correlation between root aeration (ROL) and As tolerance and accumulation in rice (Mei et al., 2009; Wu et al., 2011). However, the mechanism involved in terms of effects of ROL on As tolerance and accumulation is unclear, and may due to its effects on the rhizosphere. This study focuses on root oxidation and the effect it has on As dynamics in the rhizosphere and subsequent As sequestration on rice roots. In this investigation, we studied (1) the effects of root oxidation on As dynamics in soil solutions; and (2) the effects of root oxidation on As sequestration by rice roots, and uptake and translocation in rice plants.

1 Materials and methods

1.1 Hydroponic investigation

Genotypes IAPAR, Nanyangzhan, TD71 and Yinjingruanzhan were chosen for this study with porosities of 19%, 12.4%, 31.1% and 26.3% and ROL's of 14.7, 5.3, 27.1 and 22.1 μmol O_2/(g dry weight · day) respectively (Wu et al., 2011). All seeds were sterilized in 30% H_2O_2 for 15 min, then washed with deionized water. The seeds were germinated in petri dishes each containing a moist filter paper in a controlled chamber (28°C and relative humidity 70%). After 1 week, uniform seedlings were transplanted to 2-L plastic vessels (four plants per vessel) with deoxygenated nutrient solution containing 0.1% (*W*/*V*) agar ('stagnant' treatment/solution, which more closely resembled the waterlogged soil than either semi-stagnant or N_2-flushed solution, because dilute agar prevents convective movements in solution) (Wiengweera et al., 1997). The nutrient solution contained 40 mg N/L as NH_4NO_3, 10 mg P/L as $NaH_2PO_4{\cdot}H_2O$, 40 mg K/L as K_2SO_4, 40 mg Ca/L as $CaCl_2{\cdot}2H_2O$, 40 mg Mg/L as $MgSO_4{\cdot}7H_2O$, and traces of Mn, B, Zn, Cu, and Fe (Yoshida et al., 1976). Solution pH was maintained at 5.8 with KOH. The nutrient solutions were renewed every five days.

All the vessels were arranged randomly in a greenhouse (25°C during the day and 20°C during the night, relative humidity 70%. Natural light was supplemented with sodium light (1200 Lux), providing a photoperiod of 12 hr light/12 hr dark per day. Measurement of ROL was determined at stem elongation and flowering stages using the titanium(III) citrate buffer method (Kludze et al., 1994). The method was described in more detail in our previous study (Wu et al., 2011).

1.2 Pot investigation

1.2.1 Plant culture

The same four genotypes used in the hydroponic experiment were used in the pot investigation. Rice seeds of the genotypes were germinated as previously mentioned and grown in Yoshida Nutrient solution for two weeks. Soils were collected in a paddy field on the campus of South China Agricultural University (sandy clay, pH 6.43, low As concentration 8.6 mg/kg, particle size distribution: clay 33%, silt 36% and sand 31%). The soils were air dried and sieved through a 2 mm sieve. A rhizobag system with two compartments was established using 3.5 L plastic pots (150 mm diameter × 200 mm height). These consisted of a central compartment in which the plants roots were grown as rhizosphere conditions and an outside compartment that served as the bulk soil. The compartments were separated using 30 μm nylon mesh cloths mounted on cylindrical plastic frames (60 mm diameter × 200 mm height). There were 500 g soil in the central compartment and 2500 g in

the outside compartment, giving a total of 3000 g soil per pot.

Arsenic was added to the soils as arsenate ($Na_2HASO_4{\cdot}7H_2O$) with concentrations of 0 and 50 mg As/kg dry weight. Arsenate was added as a solution and mixed thoroughly with soils by hand. Soils without arsenate amendment were supplied with the same volume of distilled water. All the soils were equilibrated for two weeks in plastic trays. The As concentration in the contaminated soil increased slightly above the maximum As concentration limit of 30 mg/kg for soils set by the Ministry of Environmental Protection (MEP, 1995); nevertheless, this was within the contaminated paddy soil level in China (Liao et al., 2005). The soil was kept submerged in water for 2 weeks before planting the rice. After 2 weeks, two 2-week-old rice seedlings were planted in the central compartment of each pot. There were two treatments for each genotype: (1) control and (2) 50 mg As/kg treatments, with 6 replicates in each treatment and each genotype.

Plants were allowed to grow under flooded conditions until maturity in the same aforementioned greenhouse.

1.2.2 Soil analysis

Once filled with soil, deionized water was added to maintain the level in the pots, which was kept constant at 2 cm above the soil surface. Soil pH and redox potential were determined at 60 days (stem elongation stage), and 90 days (flowering stage) after transplanting. pH and redox potential were measured using a portable pH/ISE/mV meter (Model 290A, Orion Research, Boston, USA). Soil solution samples were collected using 'rhizon' soil pore water samplers inserted at a depth of 10 cm in the central compartment. The samples were collected at 20, 30, 40, 50, 60, 70, 80, 90, 100 and 110 days after transplanting and the contents analyzed for total As and As speciation. Samples were stored at 4°C in a refrigerator and analyzed within 24 hr of collection. Total As concentrations in solutions were determined by ICP-MS (PerkinElmer, Elan 9000), and As speciation was determined by HPLC-ICP-MS (HPLC, Agilent Technologies 1100 series) (Wu et al., 2011).

1.2.3 DCB-extraction of iron plaque

At harvest (after a growth period of 120 days), after washing with deionized water, 0.5 g root tissue was incubated for 60 min at room temperature (20–25°C) in 40 mL of 0.03 mol/L sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), 0.125 mol/L sodium bicarbonate ($NaHCO_3$) and 0.6 g sodium dithionite ($Na_2S_2O_4$). After incubation, the roots were rinsed three times with deionized water and added to the DCB-extract to make up the resulting solution to 100 mL for analysis (Liu et al., 2006).

1.2.4 Plant analysis for total As

Sampling procedures followed those described by Abedin et al. (2002). At maturity (after a growth period of 120 days), after measuring plant height, half of the plants were harvested, carefully washed with deionized water, and separated into grains, straws, and roots. The plant tissues were then oven-dried at 50°C. The remaining plants were freeze-dried and stored at −20°C for As speciation analysis. After recording the dry weight, all plant samples were ground to a fine powder using a stainless steel mill, and digested in 5 mL concentrated HNO_3 on a hot plate (120°C) until the digestion solution became clear. The certified reference material ((CRM) 1568a rice flour from the National Institute of Standards and Technology, USA (NIST)) was used to verify the accuracy of metal(loid) determinations. The acid digests of plant materials (grains, straws and roots) and DCB-extracts were analyzed for total As and Fe (Inductively Coupled Plasma Spectrometer (ICP), PerkinElmer, Elan 9000) (Allen, 1989). The recoveries of As in 1568a ranged from 105.1% to 107.3%.

1.2.5 Plant analysis for As speciation

Soil solutions and grain from the four genotypes were used in this investigation. To speciate As in rice, a TFA (trifluoroacetic acid) extraction method was used (Heitkemper et al., 2001; Williams et al., 2005). Arsenic speciation was determined by HPLC-ICP-MS (HPLC, Agilent Technologies 1100 series, USA); detailed information relating to the procedures is described in Wu et al. (2011).

All analyses were performed within 24 hr of sample extraction to minimize any changes in speciation that may occur during prolonged storage. NIST CRM 1568a rice flour was used to validate the method, and was also used to characterize speciation (Wu et al., 2011). The mean total recovery ((sum of species recovered from the TFA extraction/total As from acid digestion) × 100%) ranged from 83% to 111%, which was consistent with other studies (Heitkemper et al., 2001; Williams et al., 2005).

1.3 Statistical analyses

Analysis of variance (ANOVA) was performed using the statistical package SPSS 13.0 for Windows (SPSS Inc., USA).

2 Results

2.1 Radial oxygen loss, pH and redox potentials of soils

Rice plants showed relatively higher rates of ROL at day 30 compared with day 90, showing the decrease of ROL at the mature stage in comparison to the stem elongation stage. There were significant differences ($P < 0.05$) in total ROL in all genotypes at these two stages. TD71 showed the greatest ROL, 27.1 and 16.7 μmol O_2/(g dry weight·day) at the two stages respectively, while Nanyangzhan had the lowest ROL, 9.5 and 5.3 μmol O_2/(g dry weight·day) respectively (**Table 1**).

Table 1 Values of pH and Eh in different soil zones and ratio of radial oxygen loss (ROL) for four genotypes at day 30 and 90 after transplanting

Genotype	Day 30	Day 90	
	Rhizosphere	Rhizosphere	Non-rhizosphere
ROL (μmol O_2/(g dry weight·day))			
IAPAR9	14.7±0.6	4.7±0.7	
Nanyangzhan	9.5±1.0	5.3±0.84	
TD71	27.1±1.3	16.7±2.2	
Yinjingruanzhan	22.1±0.2	13.0±3.0	
pH			
IAPAR9	6.60±0.06	6.12±0.24	6.36±0.09
Nanyangzhan	6.34±0.03	6.05±0.12	6.37±0.09
TD71	5.89±0.14	5.68±0.65	6.30±0.07
Yinjingruanzhan	6.10±0.25	5.86±0.09	6.34±0.03
Eh (mV)			
IAPAR9	7.0±9.5	−129±3.6	−175±25.3
Nanyangzhan	16±5.5	−148±12.1	−160±17.8
TD71	55±8.5	−105±16.7	−119±12.1
Yinjingruanzhan	28±10.2	−112±9.4	−193±2.5

Soil redox potentials and pH at day 30 and 90 for rhizosphere and non-rhizosphere soils are presented in **Table 1** for all genotypes. At day 90, there were significant differences in pH between rhizoshpere and non-rhizosphere soils ($P < 0.05$); rhizosphere soils had lower pH than non-rhizosphere soils. Genotype TD71 demonstrated the greatest degree of decline in pH (0.62) between rhizosphere and non-rhizosphere soils. Furthermore, there were significant differences ($P < 0.05$) in pH values of rhizosphere soils between different genotypes at the two stages. Genotypes with higher ROL (TD71 and Yinjingruanzhan) had lower pH in rhizosphere soils than genotypes with lower ROL (IAPAR9 and Nanyangzhan) at the two stages (**Table 1**). However, there were no significant differences ($P > 0.05$) between different genotypes in pH for the non-rhizosphere soils. For rhizosphere soils, there were significant differences ($P < 0.05$) in Eh between the two stages for the genotypes, with the highest in TD71 (55 and −105 mV respectively) and lowest in IAPAR9 (7.0 and −129 mV respectively). For all genotypes, Eh in rhizosphere soils was higher than in non-rhizosphere soils ($P < 0.05$) on day 90.

2.2 Effect of root oxidation on As dynamics in soil solutions and As sequestration

Arsenic concentrations in rhizosphere soil solution under flooded conditions in the control and As treatments during the growing period of 120 days are presented in **Fig. 1a, b**. Flooding resulted in a large mobilization of As into the soil solution, increasing with the growth period ($P < 0.001$). In the control and As treatment, As concentrations in rhizosphere soil solutions of genotypes with higher ROL (TD71 and Yinjingruanzhan) were relatively higher than for genotypes with lower ROL (IAPAR9 and Nanyangzhan) ($P < 0.01$).

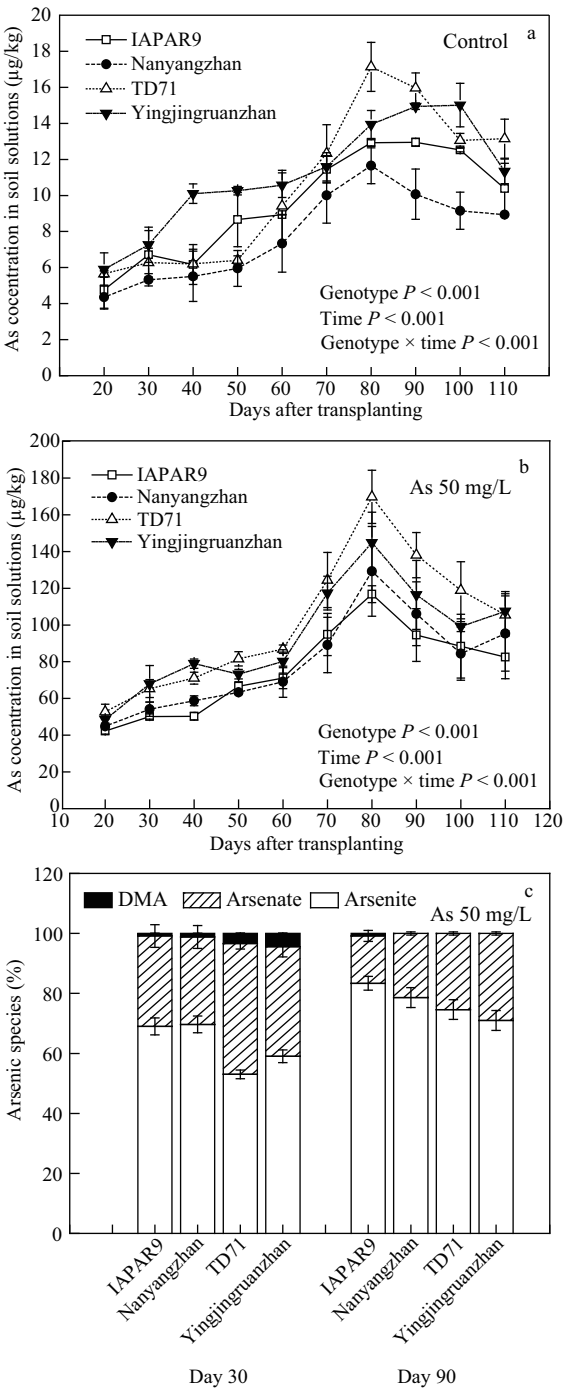

Fig. 1 Arsenic concentrations (a, b) and As species percentages (c) in soil solution during the growth period (120 days) of rice plants grown in soils amended with different concentrations of arsenic (a: control; b, c: 50 mg/kg). All data are shown as means ± standard deviation.

Figure 1c shows As speciation results from soil solutions of the four genotypes on day 30 and 90. Arsenite accounted for 53%–83% of the total As in the soil solutions (**Fig. 1c**) while arsenate accounted for 15%–43% of the total As. There were significant genotypic differences in

percentages of arsenite and arsenate ($P < 0.05$) at the two stages. Rhizosphere soil solutions from genotype TD71 showed the lowest percentage of arsenite (53.0%), while genotype Nanyangzhan showed the highest percentage of arsenite (69.6%) on day 30. Furthermore, on day 90, rhizosphere soil solutions of genotype Yinjingruanzhan showed the lowest percentage of arsenite (70.9%), while genotype IAPAR9 showed the highest percentage (83.3%). Flooding increased the percentage of arsenite and decreased the percentages of arsenate and organic As (**Fig. 1c**). Rhizosphere soil solutions of genotypes TD71 and Yinjingruanzhan with a relatively higher degree of ROL showed a higher percentage of arsenate and lower percentage of arsenite than genotypes IAPAR9 and Nanyangzhan (**Fig. 1c**).

2.3 Plant growth and As accumulation and speciation in rice plants

Root biomass varied from 1.9 to 4.6 g/pot, with the highest recorded in the control treatment of Nanyangzhan and the lowest in the As treatment of TD71. There were significant genotypic effects on root biomass ($P < 0.001$), but no significant treatment effects on root biomass ($P > 0.05$, **Table 2**). Application of As significantly ($P < 0.01$) changed straw yield. Straw biomass varied from 3.3 to 9.5 g/pot, with the lowest observed for the As treatment of IAPAR9 and the highest in the control treatment of TD71. There were significant genotypic effects on grain yield ($P < 0.001$), and significant As treatment effects on grain yield ($P < 0.01$, **Table 2**).

Application of As exerted significant effects on root and straw As concentrations ($P < 0.001$). There were significant genotypic variations of As concentrations in iron plaque, roots and straws of rice ($P < 0.01$). Genotype TD71 (1.35 mg/kg) and Yinjingruanzhan (0.96 mg/kg) had a significantly ($P < 0.001$) lower As accumulation in rice grains, compared with genotype IAPAR9 (1.68 mg/kg) and Nanyangzhan (2.24 mg/kg) in the As treatment (**Table 3**).

Arsenic species As(III), As(V), DMA and MMA were analyzed in rice grains for all genotypes. Due to the fact that TFA might reduce arsenate to arsenite, the concentrations of total inorganic As were adopted instead of the concentrations of As(III) and As(V) (Liu et al., 2006). The majority of As species in grains was present as the inorganic form for genotypes IAPAR9, TD71 and Yinjingruanzhan and accounted for 41%–55% of total As. However, in genotype Nanyangzhan, the organic As species dimethyl As was detected, accounting for 53% of total As. There were also significantly ($P < 0.05$) lower concentrations of inorganic As in TD71 and Yinjingruanzhan, compared

Table 2 Four different genotypes of rice grown of root, straw, and grain biomass in soils amended with different concentrations of arsenic

Genotype		Root dry mass (g/pot)	Straw dry mass (g/pot)	Grain yield (g/pot)
Nanyangzhan	Control	4.6±0.5	6.8±1.2	1.8±0.68
	As (50 mg/kg)	4.2±1.3	6.1±1.1	1.6±0.50
IAPAR9	Control	2.1±0.3	5.4±1.3	4.1±0.78
	As (50 mg/kg)	2.0±0.2	3.3±0.9	1.9±0.31
TD71	Control	2.3±0.06	9.5±0.4	5.4±2.3
	As (50 mg/kg)	1.9±0.5	7.3±0.8	3.6±1.3
Yinjingruanzhan	Control	2.5±0.4	4.4±0.6	5.3±0.29
	As (50 mg/kg)	2.4±0.2	3.7±0.5	4.7±1.0
Analysis of variance				
Genotypes (G)		$P < 0.001$	$P < 0.001$	$P < 0.001$
Arsenic treatment (A)		NS	$P < 0.01$	$P < 0.01$
G×A		NS	NS	NS

NS indicates no significance at $P < 0.05$.

Table 3 Proportions of arsenic species in grains for four genotypes grown in soils amended with 50 mg As/kg using trifluoroacetic acid (TFA) extraction and HPLC-ICP-MS determination

Genotype	Total As (mg/kg)	Inorganic As (mg/kg)	Dimethyl As (mg/kg)	Monomethyl As (mg/kg)	Inorganic As[a] (%)	Dimethyl As[b] (%)	Recovery[c] (%)
IAPAR9	1.68	0.97±0.05	0.77±0.10	nd	55	44	105
Nanyangzhan	2.24	0.93±0.33	1.21±0.46	0.12±0.01	41	53	92
TD71	1.35	0.74±0.09	0.73±0.44	0.03±0.003	50	49	102
Yinjingruanzhan	0.96	0.35±0.21	0.33±0.35	nd	51	48	69
NIST CRM 1568a	0.29	0.11±0.005	0.16±0.02	nd	40	59	97

[a]Inorganic arsenic = (conc. of inorganic As/conc. of species sum) ×100%, [b]DMA = (conc. of DMA/conc. of species sum) ×100%, [c] recovery = (conc. of species sum/conc. of total As) × 100%; nd means not detected.

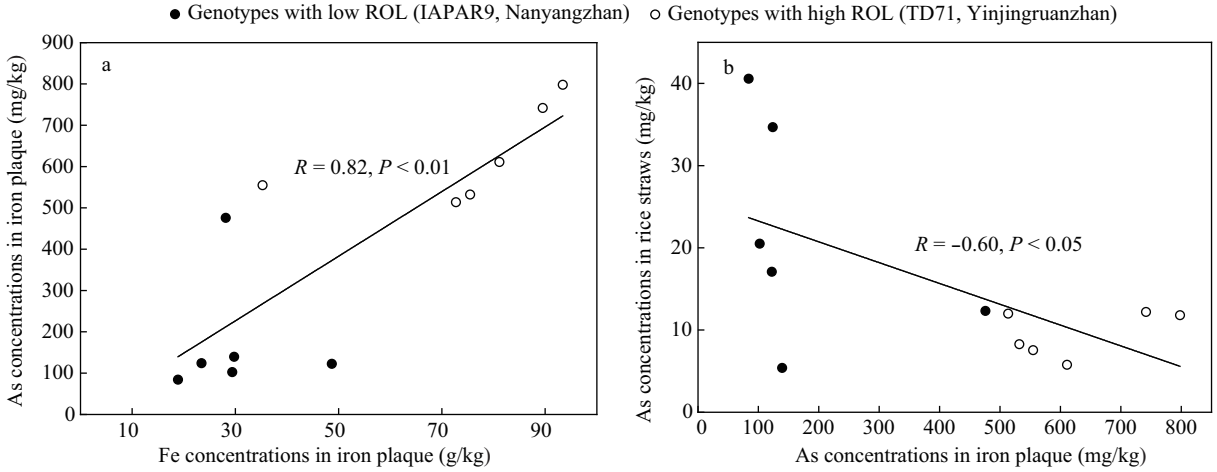

Fig. 2 Correlations of Fe and As concentrations in iron plaque (a), As concentrations in iron plaque and in rice straws (b) of the four genotypes amended with 50 mg As/kg.

with IAPAR9 and Nanyangzhan (**Table 3**).

2.4 Effect of root oxidation on As sequestration in iron plaque and As accumulation in rice plants

Iron plaque was clearly visible as reddish coatings on root surfaces when harvested. There were significant genotypic effects on As and Fe concentrations in iron plaque ($P < 0.01$). Significant correlations also existed between Fe and As concentrations in iron plaque of the four genotypes ($R = 0.82$, $P < 0.01$). Moreover, genotypes with higher ROL had greater Fe and As sequestration in iron plaque (**Fig. 2**). In addition, As concentrations in iron plaque were negatively correlated with As concentrations in rice straws ($R = -0.60$, $P < 0.05$).

3 Discussion

The process of root oxidation may release ions and reduce soil pH (Wright and Otte, 1999). It is known that wetland plants can alter the redox conditions, pH and organic matter content of sediments, and therefore affect the chemical speciation and mobility of trace elements (Jacob and Otte, 2003). Moreover, Kludze et al. (1994) found that the decrease of Eh could enhance aerenchyma formation and ROL. In the present study, pH values of the rhizosphere were lower than that of the non-rhizosphere in the four genotypes, which demonstrated that root oxidation may affect pH. Moreover, genotype TD71 and Yinjingruanzhan produced a more radical decrease in pH between rhizosphere and non-rhizosphere compared with the other two genotypes. Yinjingruanzhan and TD71 also had a more dramatic effect on redox conditions, increasing Eh potentials between the rhizosphere and non-rhizosphere; this may be associated with their higher capacity for root oxidation. It has been demonstrated that metal speciation in soils varies with soil Eh and pH change (Weis and Weis, 2004). It has also been shown that flooding reduced soil redox potentials, and increased As mobility and solubility during continuously flooded rice growth studies (Li et al., 2009; Xu et al., 2008) and also in the field (Takahashi et al., 2004). In this study, flooding resulted in a large mobilization of As in the soil solution, increasing with growth period (**Fig. 1**). There were significant genotypic differences in arsenite and arsenate ($P < 0.05$) in soil solutions between the two stages, with genotypes of higher ROL having a lower percentage of arsenite but higher percentage of arsenate. This has demonstrated that root oxidation may affect arsenic speciation in the rice rhizosphere.

It has been established that ROL and root oxidation of wetland plants may affect metal mobility in soils, by increasing or decreasing metal mobility under different circumstances. Recently, there have been a number of reports regarding the effects of ROL on heavy metal mobility. Vigneault et al. (2001) revealed that ROL of wetland plants may induce oxidation of metal sulfides, thus increasing metal mobility in rhizosphere soils. Roden et al. (1996) revealed that ROL of wetland plants may well oxidize rhizosphere Fe and cause co-precipitation with heavy metals to reduce metal mobility in the rhizosphere. The present study has shown that there was a slightly higher As concentration in rhizosphere soils than in non-rhizosphere soils. Moreover, genotype TD71 and Yinjingruanzhan, with higher ROL, also showed greater As concentrations in rhizosphere soil solutions than IAPAR9 and Nanyangzhan; this further demonstrates that root oxidization has a marked influence on As mobility in soils.

Furthermore, As sequestration in iron plaque was greater in genotype TD71 and Yinjingruanzhan compared with genotype IAPAR9 and Nanyangzhan; also As concentrations in the roots and shoots of genotype TD71 and Yinjingruanzhan were lower than in IAPAR9 and

Nanyangzhan. This demonstrates that rice genotypes with a higher ROL may sequester more As in the rhizosphere and iron plaque, and help to reduce As accumulation in the above-ground parts of rice. However, the effects of ROL on rhizosphere soils may vary under different growth stages and at different times of the same stage, which therefore requires further investigation.

It was observed that rates of ROL were negatively correlated with As concentrations in straws (Mei et al., 2009) and roots of rice (Wu et al., 2011) between different genotypes. ROL of different genotypes was also significantly correlated with inorganic As in rice grains, as well as total grain As (Wu et al., 2011). In the present study, As concentrations in iron plaque were significantly negatively correlated with As concentrations in rice straws. This demonstrated that rice genotypes with higher ROL may sequester more As on iron plaque, reduce its transfer into rice roots, and subsequently transportation to aboveground parts of rice and accumulation in grains.

Meharg (2004) demonstrated that the dynamics of As in the rhizosphere were controlled by regulation of Fe plaque formation and also feedback regulation of As(III) uptake; this was regulated by oxidation or reduction and methylation of As, producing MMA and DMA, which are poorly transported across the plasma-membrane of root epidermal cells. Inorganic As is considered more toxic and poses greater risk to human health than organic forms of As (Abedin et al., 2002; Norton et al., 2009a). TD71 and Yinjingruanzhan contained less inorganic As, as well as total As, in their grains than IAPAR9 and Nanyangzhan. Both environment and genotype differences may affect As uptake and speciation in rice (Norton et al., 2009a, 2009b). Our previous study revealed that genetic variation of rice genotypes has a pronounced influence on As accumulation and speciation in rice (Wu et al., 2011). In the present study, the genotypes revealed different percentages of As species within their tissues, with inorganic As being the principal species. The exception to this was genotype Nanyangzhan, having mainly DMA present in its tissues. Inorganic As concentrations were significantly different between different genotypes ($P < 0.05$), while the difference between organic As concentrations was not significant ($P > 0.05$). Zhao et al. (2013) suggested that while rice plants lack the ability to methylate As, grain As speciation was primarily attributed to environmental factors and methylated As species in rice were derived from the soil. The genotypic variation of As speciation in this study may be due to variation in root uptake or internal translocation efficiency of different As species by the different genotypes (Zhao et al., 2013). Many archaea, bacteria, fungi, and eukaryotic algae are able to methylate As; Lomax et al. (2012) discovered several sulfate-reducing bacteria and methanogens that are likely to be particularly active in terms of As methylation under anaerobic conditions of flooded paddy soils. It is possible that different rice genotypes may modify the rhizosphere in their own way, thus affecting As methylation microbes in the rhizosphere and indirectly influencing the accumulation of DMA by rice (Zhao et al., 2013). The percentage of As species varied between this and the previous study (Wu et al., 2011) and may be due to the different growing conditions encountered between the two.

4 Conclusions

The present study has demonstrated that genotypes with higher ROL may induce more Fe plaque formation and sequester more As in iron plaque and rhizosphere soils. This may lead to the reduction of As uptake in aboveground parts of rice and less inorganic As accumulation in rice grains. This study has contributed to further understanding the mechanisms whereby ROL influences As uptake and accumulation in rice.

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