Environ Sci Pollut Res (2013) 20:8334–8341
DOI 10.1007/s11356-013-2083-1

Effects of As levels on radial oxygen loss and As speciation in rice

Chuan Wu · Hui Li · Zhihong Ye · Fuyong Wu · Ming Hung Wong

Received: 12 March 2013 / Accepted: 21 August 2013 / Published online: 5 September 2013

Abstract Greenhouse experiment was conducted to examine effects of arsenic (As) on iron plaque formation, radial oxygen loss, As accumulation, and speciation in rice. Three genotypes were grown in soil with three different concentrations of As. The stress of As caused a slight increase of iron plaque formation ($P>0.05$) and a decrease in the rates of radial oxygen loss (ROL; $P<0.01$). The results of As speciation showed that the percentages of DMA increased from 19–28 % to 53–58 %, while the percentages of inorganic As decreased from 53–58 % to 36–42 % with the increasing soil As concentrations, indicating a strong environmental influence on As species in rice grain. The present study showed that elevated soil As may induce As toxicity towards rice plants, leading to the decrease of ROL; environmental factors could influence As methylation or As species transportation. Our study provided useful information on As tolerance and accumulation in rice which may contribute to reducing the health risk posed by As contamination in rice.

Keywords Arsenic · Radial oxygen loss · Rice · Speciation

Responsible editor: Vera Slaveykova

C. Wu · H. Li · F. Wu (✉) · M. H. Wong (✉)
Croucher Institute for Environmental Sciences, and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, People's Republic of China
e-mail: wfy09@163.com
e-mail: mhwong@hkbu.edu.hk

C. Wu
Institute of Environmental Engineering, School of Metallurgical Science and Engineering, Central South University, Changsha 410083, People's Republic of China

Z. Ye
State Key Laboratory for Bio-control, and School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, People's Republic of China

Introduction

Arsenic (As) contamination of groundwater has been frequently reported (e.g., Stone 2008; Zhu et al. 2008a, b; Meharg and McGrath 2009). In the As-affected areas of Bangladesh, groundwater contains up to 2 mg As L^{-1} as compared to the WHO recommended provisional limit of 0.01 mg As L^{-1}. In areas where the arsenic-contaminated water is used for irrigation, soil As concentration can be up to 83 mg kg^{-1} (Abedin et al. 2002a, b). Three billion people, predominantly in Asia, eat rice as a staple. However, rice grown on As-contaminated soils usually contain high As levels in shoots (including grains) (Meharg 2004). Rice grains with As levels of 1.8 mg kg^{-1} have been recorded in the arsenic-affected tube well areas of Bangladesh (Zhu et al. 2008b; Williams et al. 2009). Therefore, the food that sustains half of the world's population also increases the risk of cancer (Stone 2008). It is crucial that the physiology and genetics of rice uptake of As is understood to counteract this widespread contamination of the food chain (Meharg 2004).

Waterlogging is a typical characteristic growth condition for wetland plants including paddy rice, resulting in deficiency of oxygen and essential nutrients, low redox potential, and accumulation of Fe^{2+}, Mn^{2+}, H_2S, S^{2-}, HS^-, and organic acids (McDonald et al. 2001). On the other hand, wetland plants have developed some special features, such as root anatomy (i.e., aerenchyma), radial oxygen loss (ROL), and the formation of iron (Fe) plaque on root surface, to cope with the adverse environmental conditions (Armstrong 1979; Colmer 2003a, b). The formation of Fe plaque could be an adaptation to stressed environments, as Fe hydroxides forming on roots of wetland plants are capable of reacting with metals and may therefore immobilize phytotoxic metals (Kuo 1986; Deng et al. 2010; Pi et al. 2010). Transport of oxygen (O_2) from shoot to root via aerenchyma can occur in wetland plants and O_2 can diffuse from root to rhizosphere, which is termed as

radial oxygen loss (Armstrong and Armstrong 1994; Comer et al. 2006). ROL from root to the rhizosphere is essential for the detoxification of phytotoxins by direct oxidation or indirectly by oxidizing aerobic microorganisms maintained in the rhizosphere regions (Revsbech et al. 1999), which presumably contribute to the waterlogging tolerance of wetland plants (Colmer 2003a, b).

It has been found that Fe plaque and ROL are related with As tolerance and uptake in rice (Liu et al. 2004a, b; Chen et al. 2005; Mei et al. 2009). However, most of the previous studies have focused on As uptake, root anatomy, and Fe plaque conducted in solution culture (Liu et al. 2004a, b; Deng et al. 2010), which is substantially different from the real rhizosphere condition in the environment (Fitz and Wenzel 2002; Liu et al. 2006). Moreover, As toxicity depends not only on its total contents, but also on its chemical speciation, with inorganic As considered much more toxic than organic forms (Meharg et al. 2008; Norton et al. 2009a, b). It is therefore crucial to investigate As speciation in rice plants and grains to assess the associated human health risks.

The objectives of the present study were (1) to investigate the effects of As levels (low, medium, and high) on growth, Fe plaque formation, and the amount of ROL in rice; (2) to determine As accumulation, distribution, and speciation in rice plants grown in soils contaminated with low, medium, and high levels of As.

Materials and methods

Plant culture

Rice seeds of three genotypes (Nanyangzhan, Yuxiang youzhan, CNT 87059-3) were germinated on moist filter papers and grown in Yoshida Nutrient solution (Yoshida et al. 1976) for 2 weeks. The genotypes were chosen based on their differences on radial oxygen loss and As accumulation levels according to the previous study (Wu et al. 2011). The plants were then transplanted into bags (30 μm nylon mesh, 6 cm diameter, 9 cm height, with one plant per bag) filled with acid-washed quartz sand. The sand–soil culture system was employed to mitigate the damage to rice roots for determination of ROL and Fe plaque, and to simulate the soil condition (Chen et al. 2005). The nylon bags were then transferred to a PVC pot (10 cm diameter, 14 cm height, with one plant per pot) and the gap between nylon bag and PVC pot was filled with 1.5 kg soil, previously air dried and sieved through a 2-mm sieve. Figure 1 shows the rhizobag design of the experiment. Soils were collected from a paddy field in the campus of South China Agricultural University (sandy clay with pH of 6.43 and average As concentration of 8.6 mg kg^{-1}). Arsenic was added as arsenate ($Na_2HAsO_4 \cdot 7H_2O$) with concentrations of 0, 50, and 100 mg As kg^{-1} dry weight (treatments designated as control, As 50, and As 100). Arsenate was added as a solution and mixed thoroughly with soils. Soils without arsenate amendment received the same volume of distilled water. All the soils were equilibrated for 2 weeks. There were six replicates in each treatment and each genotype.

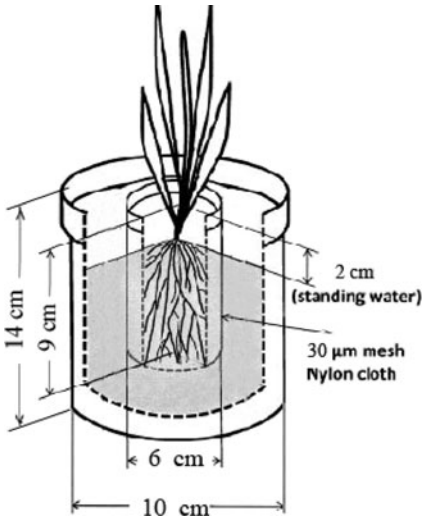

Fig. 1 Design of the rhizobag system

Plants were allowed to grow under submerged conditions until maturity in a greenhouse (with a temperature of 25 °C during the day and 20 °C during the night, relative humidity of 70 %, and the natural light supplemented with sodium light [1,200 lx], with a photoperiod of 12:12 h [day/light]).

Iron plaque extraction

Upon harvest and before drying, half of root samples were extracted using dithionite–citrate–bicarbonate (DCB) (Otte et al. 1989) to measure As and Fe on the root surface, while the other half were used for determination of ROL. After washing in deionized water, 0.5 g root tissue was incubated for 60 min at room temperature (20–25 °C) in 40 ml solution consisting of 0.03 M sodium citrate and 0.125 M sodium bicarbonate, with addition of 0.6 g sodium dithionite. After incubation, the roots were rinsed three times with deionized water which was added to the DCB extract. The resulting solution was made up to 100 ml with deionized water for analysis.

Measurement of ROL

ROL measurements were determined 60 days (stem elongation stage) and 90 days (grain filling stage) after transplanting. Each nylon bag was removed carefully from the PVC pot, and roots were washed under tap water to remove any quartz particles adhering to the root surface. Care was taken during this process to ensure the roots were intact and exposed to open air for a minimal period for the determination of ROL.

The ROL rates of the entire root system of rice plants were measured using the titanium (III) (Ti^{3+}) citrate buffer method

(Kludze et al. 1994). The method was described in more detail in previous studies (Mei et al. 2009; Wu et al. 2011). The released O_2 was calculated using the following formula (Kludze et al. 1994):

$$\mathrm{ROL} = c(y-z)$$

where ROL = radial oxygen loss, μmol O_2 plant^{-1} day^{-1}, c = initial volume of Ti^{3+} added to each test tube, L; y = concentration of Ti^{3+} in solution of control (without plant), μmol Ti^{3+} L^{-1}; and z = concentration of Ti^{3+} in solution after 6 h treatment with plants, μmol Ti^{3+} in solution plant^{-1} L^{-1}.

$$\text{Rate of ROL} = c(y-z)/G$$

where rate of ROL = rate of radial oxygen loss, μmol O_2 g^{-1} dry weight day^{-1}; G = root dry weight, g.

Plant analysis

Plant analysis for total As

Sampling procedures followed those described by Abedin et al. (2002a) and Liu et al. (2006). At maturity, after measuring plant height, half of the plants were harvested, carefully washed, and separated into grains, straws, and roots, then oven-dried at 50 °C. Another half of plants were freeze-dried, and stored at −20 °C for the analysis of As speciation. After recording the dry weight, all plant samples were ground to fine powder, and digested with 5 ml HNO_3 until the digestion solution became clear. The certified reference material [(CRM) 1568a rice flour from National Institute of Standards and Technology, USA (NIST)] was used to verify the accuracy of metal determination. The acid digests of plant material (grains, straws, and roots) and DCB extracts were analyzed for total As and Fe [determined by inductively coupled plasma spectrometer (ICP), PerkinElmer, Elan 9000] (Allen 1989). The recoveries of As in 1568a ranged from 105.1 to 107.3 %.

Plant analysis for As speciation

Two genotypes (Nanyangzhan and Yuxiangyouzhan) were used in this investigation. The speciation method is described in more detail in Wu et al. (2011). Trifluoroacetic acid (TFA) extraction method was used (Heitkemper et al. 2001; Williams et al. 2005). Milled subsamples (0.2 g) were weighed into quartz digestion tubes and 2 mL of 2 M TFA was added. The mixture was allowed to stand overnight. The tubes were then placed on a heating block at 100 °C for 6 h. The digest was evaporated to dryness at 120 °C. The residues were resuspended in distilled water and filtered through a 0.45 μm filter (cellulose nitrate, Micro Filtration Systems, California, USA), then made up to 10 mL with ultrapure (>18 MΩ) deionized water before analysis.

A Hamilton PRP-X100 10-μm anion-exchange column (4.1×150 mm) with an appropriate precolumn (containing the same material) and an Agilent 1100 series HPLC system (Agilent Technologies) were used for all analyses. The mobile phase employed for anion-exchange chromatography, consisted of ultrapure (>18 MΩ) deionized water and 50 mM ammonium bicarbonate (from Aldrich Chemical Co.).

Each analysis was performed within 24 h of sample extraction to minimize any changes in speciation during prolonged storage. Post-column element-specific detection of arsenic was achieved using an ICP-mass spectrometer (PerkinElmer, Elan 9000). NIST CRM 1568a rice flour was used to validate the method, which was also used to characterize its speciation (Williams et al. 2005; Liu et al. 2006). The mean total recovery [(sum of species recovered from the TFA extraction/total As from acid digestion)×100 %] ranged from 83–111 %, which was consistent with other studies (Heitkemper et al. 2001; Williams et al. 2005).

Statistical analyses

Analysis of variance (ANOVA) on plant biomass, concentrations of As and Fe, and rates of ROL was performed using the statistical package SPSS 13.0 for Windows (SPSS Inc., USA).

Results

Effects of As on growth, iron plaque formation, and ROL of entire roots in three genotypes of rice

Plant height varied from 84 to 98 cm among the three genotypes. The As treatments did not exert a significant effect on the height of rice plants ($P>0.05$); and there was no significant genotypic effect on the height of rice plants ($P>0.05$). Root biomass varied from 2.8 to 5.9 g pot^{-1}, with the highest root biomass in As 100 treatment of genotype Nanyangzhan (Table 1). Straw biomass varied from 6.1 to 8.2 g pot^{-1} (Table 1). Application of As did not significantly ($P>0.05$) change the biomass of root and straw, while there was a significant genotypic effects ($P<0.05$) on the biomass of root and straw. There were significant genotypic effects on grain yield ($P<0.01$), but there were no significant As treatment effects on grain yield ($P>0.05$, Table 1).

Iron plaque was clearly visible as reddish coatings on the root surface when harvested. There were no significant differences ($P>0.05$) in the amounts of Fe plaque formed for different genotypes and for different treatments (Table 2). However, the increase of As led to a slight increase ($P>0.05$) of Fe plaque formed on root surface. Arsenic concentration in Fe plaque ranged between 29 mg kg^{-1} in control

Table 1 Biomass (g pot^{-1}, DW) and As concentrations (mg kg^{-1}) in roots, straws, and grains of three genotypes of rice grown in soils amended with different concentrations of arsenic (0, 50, and 100 mg kg^{-1})

Genotypes		Root dry mass (g)	Straw dry mass (g)	Grain yield (g)
Nanyangzhan	Control	4.3±1.3	6.1±1.1	1.6±0.50
	As 50	4.6±0.53	6.9±1.3	1.8±0.68
	As 100	5.9±1.9	7.0±0.63	1.9±0.31
CNT 87059-3	Control	2.9±0.39	7.8±0.02	4.1±0.78
	As 50	2.8±0.43	7.4±0.61	5.4±2.3
	As 100	4.0±0.62	8.0±1.2	3.6±1.3
Yuxiangyouzhan	Control	3.3±0.53	7.4±1.3	5.3±0.29
	As 50	4.9±1.2	7.5±0.69	4.7±1.0
	As 100	3.9±1.1	8.2±0.76	5.2±0.96
Analysis of variance				
Genotypes (G)		$P<0.01$	$P<0.05$	$P<0.01$
Arsenic treatment (A)		NS	NS	NS
G×A		NS	NS	NS
Genotypes		As in roots	As in straws	As in grains
Nanyangzhan	Control	10±2.4	7.8±1.2	0.15±0.02
	As 50	30±9.7	13.4±4.2	1.8±0.46
	As 100	46±13	29.5±9.6	1.7±0.09
CNT 87059-3	Control	16±0.59	10±2.6	0.25±0.08
	As 50	35±11	20±1.5	1.4±0.78
	As 100	358±152	17±4.5	1.1±0.47
Yuxiangyouzhan	Control	28±5.2	15±0.50	0.09±0.01
	As 50	104±18	35±11	1.5±0.45
	As 100	169±8.2	39±16	1.6±0.30
Analysis of variance				
Genotypes (G)		NS	$P<0.01$	$P<0.05$
Arsenic treatment (A)		$P<0.001$	$P<0.01$	$P<0.01$
G×A		$P<0.05$	NS	NS

treatment and 665 mg kg^{-1} in As 100 treatment (Table 2). There were significant differences in As concentration in Fe plaque between As treatments ($P<0.01$), while no significant difference between genotypes ($P>0.05$).

Rice plants showed relatively higher rate of ROL on day 60 compared with day 90, showing the decrease of ROL in mature stage of rice compared with stem elongation stage. Yuxiangyouzhan showed the highest total amount of ROL in all treatment groups, while Nanyangzhan and CNT 87059-3 had relatively lower amounts in all treatments. On days 60 and 90, increasing As concentration caused a decrease in total ROL rates ($P<0.01$; Fig. 2). This suggested that total ROL was inhibited by arsenic stress. Total ROL on entire roots varied significantly referred to genotypes × treatments combination ($P<0.05$) according to the two-way ANOVA (Fig. 2). There were significant differences between genotypes in ROL rates only in As 100 treatment ($P<0.05$).

Arsenic uptake and speciation in rice plants

Arsenic concentration in root increased significantly ($P<0.001$) with the increase of soil As concentration, and As concentrations in rice straws or grains showed significant

Table 2 Concentrations of Fe (g kg^{-1}) and As (mg kg^{-1}) in DCB extracts of three genotypes of rice grown in soils amended with different concentrations of arsenic (0, 50, and 100 mg kg^{-1})

Genotypes		Fe (g kg^{-1})	As (mg kg^{-1})
Nanyangzhan	Control	41±5.6	44±12
	As 50	35±9.3	77±8.3
	As 100	46±16	143±15
CNT 87059-3	Control	32±2.0	33±8.2
	As 50	42±6.3	335±3.1
	As 100	41±21	400±304
Yuxiangyouzhan	Control	19±7.6	29±5.4
	As 50	39±5.8	177±14
	As 100	33±11	665±391
Analysis of variance			
Genotypes (G)		NS	NS
Arsenic treatment (A)		NS	$P<0.01$
G×A		NS	NS

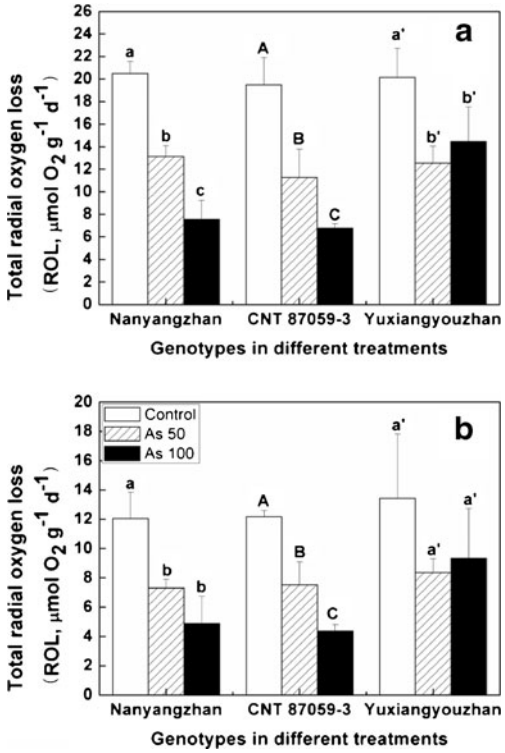

Fig. 2 Total amounts of radial oxygen loss (*ROL*) from entire root systems of three genotypes of rice growing in treatments amended with different concentrations of arsenic (0, 50, and 100 mg kg^{-1}) for 60 days (**a**) and 90 days (**b**). All data are shown as means ± standard deviation; *different letters* in each genotype indicated that they were significantly different in three treatments at $P<0.05$ determined by Tukey's HSD test

($P<0.01$) differences between As treatments. Arsenic concentration in grains remained statistically similar in As 50 and As 100 treatments, which implicated a lower translocation from straws to grains for rice as a protection process. There were significant genotypic effects on As concentrations in the straw and grain but not root ($P<0.05$; Table 1).

According to the World Health Organization's 10 μg l^{-1} limit for As in drinking water, 0.05 mg kg^{-1} As in rice contributes about 60 % of dietary As exposure (WHO 2001). The maximum contaminant levels for inorganic As in rice grains was set at 0.2 mg kg^{-1} in China (Chinese Food Standards Agency 2012). In this investigation, As concentrations in grains of rice grown in soils spiked with different As levels exceeded 0.2 mg kg^{-1}. It reflects a great risk when growing rice with As-contaminated soils in the field. However, the situation in the field may be different, due to the combination of different environmental factors, soil As speciation and behavior, water management, cultural practices, and genetic differences.

Arsenic species, As(III), As(V), DMA, and MMA were analyzed in different parts of rice for two genotypes (Yuxiangyouzhan and Nanyangzhan). There were genotypic differences ($P<0.05$) in levels of DMA and inorganic As (As_i) in the grain, with Yuxiangyouzhan having higher inorganic As in grains than Nanyangzhan. The percentages of inorganic As decreased with increasing As concentrations, while percentages of DMA increased (Fig. 3). Inorganic As was the predominant As species in the root and straw, accounting for 94–99 % of the total As, whereas grains contained substantially higher DMA, accounting for 53–70 % of the total As in As 100 treatment (Fig. 3).

Discussion

Lower shoot biomass of rice subjected to high arsenate treatment has been observed by Marin et al. (1993) and Abedin et al. (2002a). However, stimulation of growth by arsenate addition has also been reported for rice (Marin et al. 1992; Carbonell et al. 1998). The present study showed no significant reduction in plant biomass when subjected to different As levels, possibly because of the different growth conditions. Arsenate addition may displace phosphate from the soil in certain situations, increasing plant P availability (Jacobs et al. 1970).

The waterlogged anoxic conditions of wetland plants would cause the accumulation of potentially toxic, reduced solutes, such as Fe^{2+}, Mn^{2+}, and Pb^{2+} in the pore water. On the

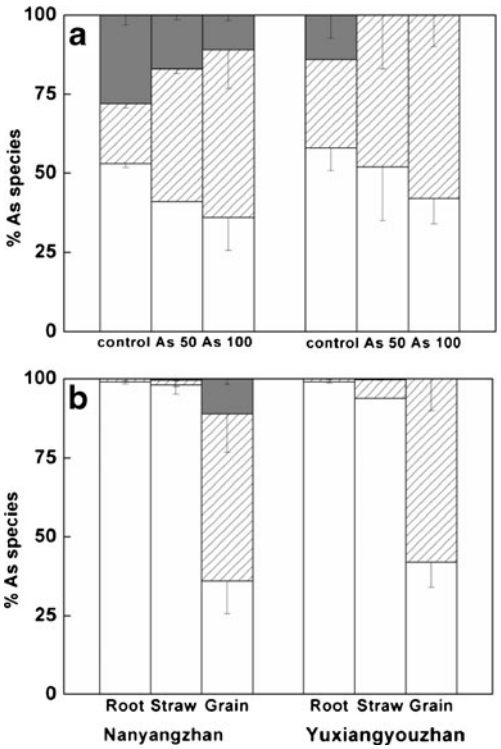

Fig. 3 As speciation **a** in grains of rice plants grown in soils amended with different concentrations of arsenic (0, 50, and 100 mg kg^{-1}); **b** in different parts of rice plants grown in soils with 100 mg As kg^{-1}. *White bar* inorganic arsenic, *hatched bar* DMA, *gray bar* MMA. All data are shown as means − standard deviation

other hand, wetland plants can develop some special features, such as radial oxygen loss, root anatomy, and the formation of Fe plaque on root surface, to cope with the adverse environments (Armstrong 1979; Visser et al. 2000). Iron plaque could serve as a barrier to prevent excess pollutants entering roots of wetland plants (Pi et al. 2010; Liu et al. 2004a, b). A slight increase of Fe plaque formed in As added treatments was observed in the present study. This might be due to the tolerance strategy demonstrated by wetland plants and rice to cope with pollutants (Pi et al. 2010). Pi et al. (2009) observed significantly higher amounts of Fe plaque formed on the root surface in two mangrove plants treated with wastewater discharge. This was due to aerobic degradation of nutrients and organic matter by microorganisms, leading to a more anaerobic soil environment and induced more ROL around the rhizosphere to oxidize ferrous ions, thus formed more Fe plaque. Hu et al. (2007) reported the sulfur-induced enhancement of plaque formation, probably due to an increase in concentrations of Fe^{2+} and Mn^{2+} resulting from S transformation in soil. In the present study, the stress of As caused a slight increase of iron plaque formation, but there was no significant difference between As treatments and control. It may be due to the fact that As has influenced the rhizosphere microbial activities. However, more investigations are needed to further our understanding of the mechanism. Garnier et al. (2010) have shown that the As contents of roots and Fe plaque raise to 1,000–1,500 mg kg^{-1} towards the middle of the growth season, then decline to ~300 mg kg^{-1} in the field. The As contents in roots and Fe plaque in the present study were within this range in the field (Table 2).

ROL could be affected by environmental factors, such as the oxygen content, light condition, redox potential, and microbial oxygen demand (Laskov et al. 2006). Rahman et al. (2007) revealed that the content of photosynthetic pigments were reduced, and subsequently affect photosynthesis, under As treatments. Furthermore, Connell et al. (1999) indicated that photosynthesis can affect ROL of *Halophila ovalis* roots. The decrease of ROL might be due to the decrease of the photosynthetic pigments and photosynthesis, which further affected rates of ROL in rice. However, this should be clarified further in future studies. Cheng et al. (2010) suggest that ROL from root tip is a potential biomarker of environmental pollution (such as heavy metals). The decreases of ROL observed in rice are mainly related to the alteration of root anatomical structure and decreased root porosity induced by heavy metals (Liu et al. 2009; Mei et al. 2009; Cheng et al. 2010). The reduced permeability of the roots which are induced by the pollutants seems to be a defense response to prevent excessive toxins entering the root and causing possible fungal infection (Hose et al. 2001; Armstrong and Armstrong 2005).

The concentration of Fe plaque is profoundly influenced by the amount of the ROL of plant roots, the more ROL present around the rhizosphere would induce more Fe plaque formation on the root surface of wetland plants (Armstrong 1979; Otte et al. 1991). However, Møller and Sand-Jensen (2008) found that if excessive Fe plaque formed on the root surface of *Lobelia dortmanna*, it may act as a 'barrier' and prevent oxygen from being released from the root, leading to lower ROL around rhizosphere. Another study also showed that the concentration of Fe plaque formed in two mangrove plants was negatively correlated with the rate of ROL along the lateral root when treated with wastewater (Pi et al. 2010). The present results of the significantly decreased ROL and slightly increased Fe plaque formation in As treatments indicated that the increased Fe plaque might inhibit oxygen release from the roots.

Zavala and Duxbury (2008) speculated that As speciation in rice grain is under genetic control. However, it has been demonstrated that As speciation in rice grain can be strongly influenced by the environmental conditions such as watering regime and As bioavailability in soils (Xu et al. 2008; Arao et al. 2009; Li et al. 2009). Zhao et al. (2013) reviewed past literature and indicated that grain As speciation are primarily attributed to environmental factors, and methylated As species in rice are derived from the soil, while rice plants lack the As methylation ability. The present study demonstrated that As speciation varied between different genotypes and As treatments. The genotypic variation of As speciation may be due to the variation in the root uptake or the internal translocation efficiency of methylated As of different genotypes (Zhao et al. 2013).

Studies showed that the majority of As present in rice grain were DMA or inorganic As, while in roots and straws the majority of As were inorganic As (Smith et al. 2008; Zavala et al. 2008; Zheng et al. 2011). Zheng et al. (2011) reported that the unloading of inorganic As and DMA into rice grain are different, with the latter accumulating mainly in the caryosis before flowering and inorganic As mainly transported into the caryopsis during grain filling. Moreover, inorganic As is considered more toxic than methylated As (Abedin et al. 2002a, b; Zhu et al. 2008a, b; Zhao et al. 2009), indicating As speciation exerts important implications for human health.

Conclusions

The stress of As caused a slight increase of iron plaque formation ($P > 0.05$), a decrease in the rates of ROL ($P < 0.01$). The results of As speciation showed that the percentages of DMA increased from 19–28 to 53–58 %, while the percentages of inorganic As decreased from 53–58 % to 36–42 % with the increasing soil As concentrations, indicating a strong environmental influence on As species in rice grain. The present study provided useful information on As tolerance and accumulation in rice to reduce the health risk posed by As contamination in rice.

Acknowledgments We sincerely thank Dr. Haitao Zhu (South China Agriculture University) and Guangdong Rice Research Institute, Guangzhou, Peoples Republic of China, for providing the rice seeds. Financial support from Research Grants Council of Hong Kong [HKBU261407 and HKBU262009] and National Natural Science Foundation of China (no. 30770417) is gratefully acknowledged.

References

Abedin MJ, Cresser MS, Meharg AA, Feldmann J, Cotter-Howells J (2002a) Arsenic accumulation and metabolism in rice (*Oryza sativa* L.). Environ Sci Technol 36:962–968

Abedin MJ, Feldmann J, Meharg AA (2002b) Uptake kinetics of arsenic species in rice plants. Plant Physiol 128:1120–1128

Allen SE (1989) Chemical analysis of ecological materials, 2nd edn. Blackwell Science, Oxford

Arao T, Kawasaki A, Baba K, Mori S, Matsumoto S (2009) Effects of water management on cadmium and arsenic accumulation and dimethylarsenic acid concentrations in Japanese rice. Environ Sci Technol 43:9361–9367

Armstrong W (1979) Aeration in higher plants. In: Woolhouse HW (ed) Advances in botanical research. Academic Press, London, pp 225–332

Armstrong W (1994) Polarographic oxygen electrodes and their use in plant aeration studies. Proc Royal Soc Edinburgh 102B:511–527

Armstrong J, Armstrong W (2005) Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. Ann Bot 96:625–638

Carbonell AA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick WHJ (1998) Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition. Sci Total Environ 217:189–199

Chen Z, Zhu YG, Liu WJ, Meharg AA (2005) Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. New Phytol 165:91–97

Cheng H, Liu Y, Tam NFY, Wang X, Li SY, Chen GZ, Ye ZH (2010) The role of radial oxygen loss and root anatomy on zinc uptake and tolerance in mangrove seedlings. Environ Pollut 158:1189–1196

Chinese Food Standards Agency. (2012) Maximum levels of contaminants in food. GB 2762–2012.

Colmer TD (2003a) Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). Ann Bot 91:301–309

Colmer TD (2003b) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell Environ 26:17–36

Comer TD, Cox MCH, Voesenek LACJ (2006) Root aeration in rice (*Oryza sativa*): evaluation of oxygen, carbon dioxide, and ethylene as possible regulators of root acclimatizations. New Phytol 170:767–777

Connell EL, Colmer TD, Walker D (1999) Radial oxygen loss from intact roots of *Halophila ovalis* as a function of distance behind the root tip and shoot illumination. Aquat Bot 63:219–228

Deng D, Wu SC, Wu FY, Deng H, Wong MH (2010) Effects of root anatomy and Fe plaque on arsenic uptake by rice seedlings grown in solution culture. Environ Pollut 158:2589–2595

Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. J Biotechnol 99:259–278

Garnier JM, Travassac F, Lenoble V, Rose J, Zheng Y, Hossain MS, Chowdhury SH, Biswas AK, Ahmed KM, Cheng Z, Geen AV (2010) Temporal variations in arsenic uptake by rice plants in Bangladesh: the role of iron plaque in paddy fields irrigated with groundwater. Sci Total Environ 408:4185–4193

Heitkemper DT, Vela NP, Stewart KR, Westphal CS (2001) Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. J Anal At Spectrom 16:299–306

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W (2001) The exodermis: a variable apoplastic barrier. J Exp Bot 52:2245–2264

Hu ZY, Zhu YG, Li M, Zhang LG, Cao ZH, Smith FA (2007) Sulfur (S)-induced enhancement of iron plaque formation in the rhizosphere reduce arsenic accumulation in rice (*Oryza sativa* L.) seedlings. Environ Pollut 147:387–393

Jacobs LW, Keeney DR, Walsh LM (1970) Arsenic residue toxicity to vegetable crops grown on plainfield sand. Agron J 62:588–591

Kludze HK, DeLaune RD, Patrick WH (1994) A colorimetric method for assaying dissolved oxygen loss from container-grown rice roots. Agron J 60:616–621

Kuo S (1986) Concurrent sorption of phosphate zinc, cadmium, or calcium by a hydrous ferric oxide. Soil Sci Soc Am J 50:1412–1419

Laskov C, Horn O, Hupfer M (2006) Environmental factors regulating the radial oxygen loss from roots of *Myriophyllum spicatum* and *Potamogeton crispus*. Aquat Bot 84:333–340

Li RY, Stroud JL, Ma JF, McGrath SP, Zhao FJ (2009) Mitigation of arsenic accumulation in rice with water management and silicon fertilization. Environ Sci Technol 43:3778–3783

Liu WJ, Zhu YG, Smith FA, Smith SE (2004a) Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? J Exp Bot 55:1707–1713

Liu WJ, Zhu YG, Smith FA, Smith SE (2004b) Do phosphorus nutrition and iron plaque alter arsenate (As) uptake by rice seedlings in hydroponic culture? New Phytol 162:481–488

Liu WJ, Zhu YG, Hu Y, Williams PH, Gault AG, Meharg AA, Charnock JM, Smith FA (2006) Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (*Oryza sativa* L.). Environ Sci Technol 40:5730–5736

Liu Y, Tam NFY, Yang JX, Pi N, Wong WH, Ye ZH (2009) Mixed heavy metals tolerance and radial oxygen loss in mangrove seedlings. Mar Pollut Bull 58:1843–1849

Marin AR, Masscheleyn PH, Patrick WHJ (1992) The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant Soil 139:175–183

Marin AR, Masscheleyn PH, Patrick WH (1993) Soil redox–pH stability of arsenic species and its influence on arsenic uptake by rice. Plant Soil 152:245–253

McDonald MP, Galwey NW, Colmer TD (2001) Waterlogging tolerance in the tribe Triticeae: the adventitious roots of *Cristesion marinum* have a relatively high porosity and a barrier to radial oxygen loss. Plant Cell Environ 24:585–596

Meharg AA (2004) Arsenic in rice—understanding a new disaster for South-East Asia. Trends Plant Sci 9:415–417

Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants. New Phytol 181:777–794

Meharg AA, Lombi E, Williams PN, Scheckel KG, Feldmann J, Raab A, Zhu Y, Islam R (2008) Speciation and localization of arsenic in white and brown rice grains. Environ Sci Technol 42:1051–1057

Mei XQ, Ye ZH, Wong WH (2009) The relationship of root porosity and radial oxygen loss on arsenic tolerance and uptake in rice grains and straw. Environ Pollut 157:2550–2557

Møller CL, Sand-Jensen K (2008) Iron plaques improve the oxygen supply to root meristems of the freshwater plants, *Lobelia dortmanna*. New Phytol 179:848–856

Norton GJ, Islam MR, Deacon CM, Zhao FJ, Stroud JL, McGrath SP, Islam S, Jahiruddin M, Feldmann J, Price AH, Meharg AA (2009a) Identification of low inorganic and total grain arsenic rice cultivars from Bangladesh. Environ Sci Technol 43:6070–6075

Norton GJ, Duan G, Dasgupta T, Islam MR, Lei M, Zhu YG, Deacon CM, Moran AC, Islam S, Zhao FJ, Stroud JL, McGrath SP,

Feldmann J, Price AH, Meharg AA (2009b) Environmental and genetic control of arsenic accumulation and speciation in rice grain: comparing a range of common cultivars grown in contaminated sites across Bangladesh, China, and India. Environ Sci Technol 43:8381–8386

Otte ML, Rozema J, Koster L, Haarsma MS, Broekman RA (1989) Iron plaque on root of *Aster tripolium* L.: interaction with zinc uptake. New Phytol 111:309–317

Otte ML, Dekkers MJ, Rozema J, Broekman RA (1991) Uptake of arsenic by Aster tripolium in relation to rhizosphere oxidation. Can J Bot 69:2670–2677

Pi N, Tam NFY, Wu Y, Wong MH (2009) Root anatomy and spatial pattern of radial oxygen loss of eight true mangrove species. Aquat Bot 90:222–230

Pi N, Tam NFY, Wong WH (2010) Effects of wastewater discharge on formation of Fe plaque on root surface and radial oxygen loss of mangrove roots. Environ Pollut 158:381–387

Rahman MA, Hasegawa H, Rahman MM, Islam MN, Miah MAM, Tasmen A (2007) Effect of arsenic on photosynthesis, growth and yield of rive widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere 67:1072–1079

Revsbech NP, Pedersen O, Reichardt W, Briones A (1999) Microsensor analysis of oxygen and pH in the rice rhizosphere under field and laboratory conditions. Biol Fertil Soils 29:379–385

Smith E, Juhasz AL, Weber J, Naidu R (2008) Arsenic uptake and speciation in rice plants grown under greenhouse conditions with arsenic contaminated irrigation water. Sci Total Environ 392:277–283

Stone R (2008) Arsenic and paddy rice: a neglected cancer risk. Nature 321:184–185

Visser EJW, Colmer TD, Blom CWPM, Voesenek LACJ (2000) Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. Plant Cell Environ 23:1237–1245

WHO (2001) World Health Organization: arsenic fact sheet http://www.who.int/mediacentre/factsheets/fs372/en/index.html

Williams PN, Price AH, Raab A, Hossain SA, Feldmann J, Meharg AA (2005) Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ Sci Technol 39:5531–5540

Williams PN, Islam S, Islam R, Jahiruddin M, Adomako E, Soliaman ARM, Rahman GKMM, Lu Y, Deacon C, Zhu YG, Meharg AA (2009) Arsenic limits trace mineral nutrition (selenium, zinc, and nickel) in Bangladesh rice grain. Environ Sci Technol 43:8430–8436

Wu C, Ye ZH, Shu WS, Zhu YG, Wong WH (2011) Arsenic accumulation and speciation in rice are affected by root aeration and variation of genotypes. J Exp Bot 62:2889–2898

Xu XY, McGrath SP, Meharg AA, Zhao FJ (2008) Growing rice aerobically markedly decreases arsenic accumulation. Environ Sci Technol 42:5574–5579

Yoshida S, Forno DA, Cock J, Gomez KA (1976) Laboratory manual for physiological studies of rice, 3rd edn. IRRI, Los Banos

Zavala YJ, Duxbury JM (2008) Arsenic in rice: I. Estimating normal levels of total arsenic in rice grain. Environ Sci Technol 42:3856–3860

Zhao FJ, Ma JF, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants. New Phytol 181:777–794

Zhao FJ, Zhu YG, Meharg AA (2013) Methylated arsenic species in rice: geographical variation, origin, and uptake mechanisms. Environ Sci Technol 47:3957–3966

Zheng MZ, Cai C, Hu Y, Sun GX, Williams PN, Cui HJ, Li G, Zhao FJ, Zhu YG (2011) Spatial distribution of arsenic and temporal variation of its concentration in rice. New Phytol 189:200–209

Zhu YG, Sun GX, Lei M, Teng M, Liu YX, Chen NC, Wang LH, Carey AM, Deacon C, Raab A, Meharg AA, Williams PN (2008a) High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. Environ Sci Technol 42:5008–5013

Zhu YG, Williams PN, Meharg AA (2008b) Exposure to inorganic arsenic from rice: a global health issue? Environ Pollut 154:169–171