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RESEARCH PAPER

Arsenic accumulation and speciation in rice are affected by root aeration and variation of genotypes

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Abstract

Root aeration, arsenic (As) accumulation, and speciation in rice of 20 different genotypes with regular irrigation of water containing 0.4 mg As l^{-1} were investigated. Different genotypes had different root anatomy demonstrated by entire root porosity (ranging from 12.43% to 33.21%), which was significantly correlated with radial oxygen loss (ROL) (R=0.64, P<0.01). Arsenic accumulation differed between genotypes, but there were no significant differences between Indica and Japonica subspecies, as well as paddy and upland rice. Total ROL from entire roots was correlated with metal tolerance (expressed as percentage mean of control straw biomass, R=0.69, P<0.01) among the 20 genotypes; total As concentration (R=–0.67, P<0.01) and inorganic As concentration (R=–0.47, P<0.05) in rice grains of different genotypes were negatively correlated with ROL. There were also significant genotype effects in percentage inorganic As (F=15.8, P<0.001) and percentage cacodylic acid (F=22.1, P<0.001), respectively. Root aeration of different genotypes and variation of genotypes on As accumulation and speciation would be useful for selecting genotypes to grow in areas contaminated by As.

Key words: Aerenchyma, arsenic, inorganic As, porosity, radial oxygen loss, rice.

Introduction

Arsenic (As) contamination in the environment has aroused considerable recent attention (Stone, 2008; Zhu *et al.*, 2008*a*). Inorganic species are of particular concern, as they are associated with various internal cancers and other health problems (IARC, 2004). There is extensive As contamination of groundwater around the world, especially in Asia (Nickson *et al.*, 1998; Chowdhury *et al.*, 2000; Smedley and Kinniburgh, 2002; Williams *et al.*, 2006). Irrigation using As-contaminated groundwater has led to elevated As in paddy soils (Roychowdhury *et al.*, 2003; Meharg, 2004; Liao *et al.*, 2005). Mining activities around rice cultivation areas has aggravated the contamination of paddy soils (Zhu *et al.*, 2008*a*; Williams *et al.*, 2009). Rice is the staple food for 3 billion people (Stone, 2008); however, rice grains grown in regions with As contamination of paddy soils has high As concentrations (Meharg, 2004; Stone, 2008; Zhu *et al.*, 2008*b*). Unfortunately, flooding of rice paddies greatly elevates As concentrations in rice (Xu *et al.*, 2008), and enhances As shoot assimilation compared with other crops, such as wheat and barley (Williams *et al.*, 2007). There is an urgent need to understand the mechanism of As tolerance and uptake by rice, in order to finally reduce As accumulation in rice grains.

It has been demonstrated that with uniform soil As concentration, there is a large variation in total As in grains of different genotypes (Liu *et al.*, 2006). Field and market surveys showed that As accumulation and speciation varies in different grain types with origins in Bangladesh, and elsewhere (Williams *et al.*, 2005, 2006; Hossain and Yanai, 2009). Both environment and genotype difference affect As

Abbreviations: As, arsenic; DMA, cacodylic acid; MMA, methylarsonic acid; ROL, radial oxygen loss; TFA, trifluoroacetic acid.

uptake and speciation in rice (Norton *et al.*, 2009*b*), and there appears to be considerable variation in rice grown in different countries, in different genetic variation, and probably in different seasons. Variation in grain As level and As speciation could be accounted for by soil redox, pH, phosphate concentration, rhizosphere iron plaque formation, microbial activity, and rice variety (Marin *et al.*, 1993; Chen *et al.*, 2005; Williams *et al.*, 2006). The oxidative rhizosphere in paddy soil alters As speciation associated with rice root surface (Liu *et al.*, 2006), but the behaviour of As in the rhizosphere is still unclear.

Wetland plants have adapted to waterlogged conditions by developing extensive aerenchyma in the roots (Justin and Armstrong, 1987; Deng *et al.*, 2009; Pi *et al.*, 2009; Deng *et al.*, 2010). Rice roots constitutively form aerenchyma, and the amount is enhanced by waterlogging (Evan, 2003) or O_2-deficient conditions (Colmer, 2003*a*,*b*; Colmer *et al.*, 2006); porosity (gas volume/tissue volume) in plant tissues results from the intercellular gas-filled space formed as a constitutive part of development, which is enhanced by the formation of aerenchyma (Drew *et al.*, 2000) . In roots, O_2 is obviously required for respiration to provide sufficient energy for growth, maintenance, and nutrient uptake processes, and up to 30–40% of the O_2 supplied via the root aerenchyma is being lost to the soil (Colmer, 2003*a*,*b*), a process called radial oxygen loss (ROL). Formation of aerenchyma and ROL presumably contribute to the waterlogging tolerance of wetland species, including rice (Colmer, 2003*a*,*b*).

A previous study noted that there was a significant correlation between ROL and As tolerance and accumulation in rice (Mei *et al.*, 2009). However, the differences in As accumulation and tolerance between Indica and Japonica subspecies, and between upland and paddy rice are not clear. Moreover, it will be interesting to investigate root uptake and translocation of As from straws to grains, as Mei *et al.* (2009) only focused on the As accumulation in the above-ground parts of rice (grains and straws). In addition, as inorganic As is considered more toxic and may pose greater risks for human health than organic As (Abedin *et al.*, 2002*a*,*b*; Meharg *et al.*, 2008; Norton *et al.*, 2009*a*,*b*), the study of As speciation of rice grains is crucial and needs to be investigated further.

Clearly, it is therefore important to focus on rice breeding programmes that result in reduction in grain total As and inorganic As, while still retaining a high yield. The aim of the present investigation is to investigate: (i) the quantitative relationships between porosity of roots, the rate of ROL, and levels of As tolerance and accumulation (grain, husk, straw, and root); (ii) As translocation in rice plants; and (iii) As accumulation and speciation related to genotype variation.

Materials and methods

Hydroponic experiment

Plant culture. Seeds of 20 rice genotypes (CNT87059-3, Dongnong413, Erjiufeng, Guinongzhan, Handao, Handao1, Handao3, Hejiang16, IAPAR9, IR72, Kinmaze, Nanyangzhan, PSB RC70, TD71, TNAU98, TORO2, Xijing7, Xiushui11, Yingjingruanzhan, Yuxiangyouzhan) were obtained from the National Rice Research Institute, Hangzhou and Guangdong Rice Research Institute, Guangzhou, PR China. There were half Indica and half Japonica subspecies, including five upland rice species.

All seeds were sterilized in 30% H_2O_2 solution 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 (with a temperature of 28 °C and relative humidity of 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 waterlogged soil than either semi-stagnant or N_2-flushed solution because dilute agar prevents convective movements in the solution) (Wiengweera *et al.*, 1997). The nutrient solution contained 40 mg l^{-1} N as NH_4NO_3, 10 mg l^{-1} P as $NaH_2PO_4H_2O$, 40 mg l^{-1} K as K_2SO_4, 40 mg l^{-1} Ca as $CaCl_22H_2O$, 40 mg l^{-1} Mg as $MgSO_47H_2O$, 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 once every 5 d.

All the vessels were arranged randomly in a greenhouse [at a temperature of 25 °C during the day and 20 °C during the night, with natural light supplemented with sodium light (3000 lux), with a photoperiod of 12:12 h (day:night), and relative humidity of 70%] for a growth period of 30 d.

Observation of root aerenchyma

Seeds of Yingjingruanzhan were used in this investigation. Seedlings were prepared by growing in aerated solution or stagnant solution for 30 d. The nutrient solution was prepared as previously mentioned. Fresh cross-sections of roots were cut by hand with a sharp razor at 20 mm behind the root tip after vacuum infiltrating the roots with water. Sections were examined using scanning electron microscopy (SEM, Philips XL30_ESEM_FEG).

Measurement of porosity

Porosity (percentage gas space per unit tissue volume) was measured on samples of adventitious roots from each genotype by the pycnometer method described by Visser and Bogemann (2003). From two 35-d-old plants taken from each pot containing deoxygenated nutrient solution, the adventitious roots were excised and those 100–150 mm in length were selected, cut into 50-mm segments for the following measurement (Colmer, 2003*a*), with four replicates for each genotype and each treatment.

A 25-ml pycnometer (1861#, Gay-Lussac, Guangzhou, China) was filled with water and weighed. The samples were then gently blotted dry on a paper towel and introduced into the water-filled pycnometer and reweighed. The roots were then vacuumed for 10 min three times under water and returned quantitatively to the pycnometer for reweighing. The porosity of roots was determined using the formula:

$$POR = \frac{[(p\&vr)-(p\&r)]}{[(p+r)-(p\&r)]} \times 100$$

Where POR=root porosity (air space), in %:
r=mass of roots, in g;
p=mass of water-filled pycnometer, in g;
p & r=mass of pycnometer with roots and water, in g;
p & vr=mass of pycnometer with vacuumed roots and water, in g.

Measurement of ROL

The ROL rates of the entire root of seedlings were measured using the titanium(III) citrate buffer method (Kludze *et al.*, 1994). There were four replicates for each genotype. Several methods have been

used to evaluate ROL from roots. The titanium(III) citrate method, making use of reducing agents (Ti^{3+}-citrate) added to solutions bathing intact root systems, can measure the entire root system and mimic the negative redox potential of flooded soils (Kludze *et al.*, 1994; Sorrell and Armstrong, 1994). Compared with the O_2-depleted method that was commonly used as quantification of ROL, it could prevent O_2 re-adsorption by roots (Sorrell and Armstrong, 1994; Laskov *et al.*, 2006).

Stock solutions of the reduced titanium(III) citrate buffer were prepared using commercially available titanium(III) chloride in HCl solution (Aldrich Chemical Co., Taufkirchen, Germany). Ti^{3+}-citrate was prepared under an N_2 atmosphere according to Zehnder and Wuhrmann (1976). The plants were extracted carefully from the vessels, and their roots were washed in tap water to remove all agar before rinsing in deionized water. Afterwards, the intact roots were gently blotted on tissue paper to dry, with the stem base of each plant coated with parafilm, and finally immersed (one plant per tube) in 50 ml of Yoshida nutrient solution (Yoshida *et al.*, 1976). The solution had previously been bubbled with N_2 gas for 2 h in order to ensure an oxygen-free condition. A 5-ml aliquot of Ti^{3+}-citrate was injected into each tube with a plastic syringe. The surface of the solution was covered with a layer of paraffin oil to prevent re-aeration from the atmosphere. Tubes with plants were then put into a growth chamber with a temperature of 28 °C, five sodium lights (3000 lux), and relative humidity of 70%.

After 6 h, small samples of the solution were then withdrawn through a plastic syringe and the absorbance of the partially oxidized Ti^{3+}-citrate solution was read at 527 nm on a Shimadzu UV–visible spectrophotometer. Released O_2 from the whole-plant root system was determined by extrapolation of the measured absorbance to a standard curve from a dilution series of the Ti^{3+}-citrate solution being used. The released oxygen was calculated using the following formula (Kludze *et al.*, 1994):

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

where ROL=radial oxygen loss, μmol O_2 $plant^{-1}$ d^{-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 l^{-1} Ti^{3+}; and z=concentration of Ti^{3+} in solution after 6 h of treatment with plants, μmol Ti^{3+} in solution $plant^{-1}$ l^{-1}.

Pot experiment

Plant culture. The same 20 rice genotypes were used in this pot trial. Rice seeds were germinated (as described above) and grown in Yoshida nutrient solution for 30 d. Soils were collected in a paddy field at South China Agricultural University (sandy clay with a pH of 6.59 and average As concentration of 8.6 mg kg^{-1}), then air dried and sieved through a 2-mm sieve. Phosphorus as $CaH_2PO_4H_2O$ at 0.15 g kg^{-1} P_2O_5, K as KCl at 0.2 g kg^{-1} K_2O, and N as CO $(NH_2)_2$ at 0.2 g kg^{-1} N were thoroughly mixed as solutions with soil at the start of the experiment to ensure adequate mineral nutrition for the growth of rice seedlings. The soils were equilibrated for 2 weeks.

Selected uniform seedlings were transplanted to 1-l plastic pots (without drainage holes), uniformly packed with 1.1 kg of dry soil (Abedin *et al.*, 2002*a*). All the pots were randomly placed in the same greenhouse. After transplantation, the seedlings were grown under flooded conditions with a layer of water ~2–3 cm above the soil surface. Arsenate was supplied as a solution of $Na_2HA_SO_47H_2O$ in distilled water, at a concentration of 0 (control treatment) or 0.4 mg l^{-1} until the rice grain was ripe (Abedin *et al.*, 2002*a*). Plants were irrigated with 500 ml of 0.4 mg l^{-1} As solution every week for 14 weeks. The concentration of As (0.4 mg l^{-1}) chosen was based on As levels in wastewater or groundwater in As-contaminated areas commonly observed in China (Liao *et al.*, 2005; Liu *et al.*, 2005, 2006).

There were five replicates in each treatment. All the pots were arranged randomly in the same greenhouse, and rearranged every week. Rice plants were grown in flooded conditions until maturity (105–150 d), which varied between genotypes.

Plant analysis for total As

Sampling procedures followed those described by Abedin *et al.* (2002*a*) and Liu *et al.* (2006). At maturity, after measuring plant height, plants were harvested, carefully washed, and separated into grain, husk, straw, and root. For straws, the base parts that were immersed in the contaminated irrigation water were discarded to avoid contamination from irrigation water. Straws and roots were oven dried at 70 °C. Spikelets were freeze-dried, then divided into grains and husks using a pestle and mortar, and stored at –20 °C for analysis.

After recording the dry weight, all plant samples were ground to fine powder for analysis. For concentrations of total As, milled subsamples (0.5 g) were weighed into quartz glass digestion tubes, steeped in 6 ml of nitric acid, and allowed to predigest/stand overnight at room temperature (25 °C). The samples were digested at 120 °C, until the solution became clear. The resulting solution was made up to 25 ml with deionized water for analysis. The certified reference material (CRM) 1568a rice flour from NIST (National Institute of Standards and Technology, USA) and blanks were included for quality assurance.

The recovery of As in 1568a ranged from 105.1% to 107.3%. An inductively coupled plasma mass spectrometry (ICP-MS, Elan 9000; PerkinElmer, Fair Oaks, CA, USA) was used to determine total As concentration of acid digests of the plant material (grains, husks, straws, and roots) (Allen, 1989). As sensitivity was monitored daily and optimized when required. Concentration was determined using a five-point calibration of 0, 10, 25, 50, and 100 μg l^{-1} As. Subsamples were randomized prior to analysis.

Plant analysis for As speciation

Trifluoroacetic acid (TFA) (98%) and methylarsonic acid (MMA) were purchased from Wako Pure Chemical Industries, Ltd. Arsenic(V) oxide, arsenic(III) oxide, and cacodylic acid (DMA) were purchased from Aldrich Chemical Co.

To speciate As in rice, the 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, Dublin, CA, 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, Santa Clara, CA, USA) were used for all analyses. The chromatographic columns were connected to a four-way valve (10-μl sample loop), and an HPLC pump. The auto-sampler was set to 4 °C. Injection volume was set at 100 μl of sample and the HPLC mobile phase flow rate was maintained at 1.5 ml min^{-1}. The mobile phase employed for anion-exchange chromatography, consisted of ultrapure (>18 MΩ) deionized water and 50 mM ammonium bicarbonate (pH 7.5, chemicals from Aldrich Chemical Co.).

Samples were injected directly onto the HPLC column. Each analysis was performed within 24 h of sample extraction to minimize any changes in speciation during prolonged storage, and the extraction solution was stored at –20 °C after the extraction to minimize the transformation of species. Post-column, element-specific detection of As was achieved using an ICP-mass spectrometer (Elan 9000). The outlet of the HPLC column was connected directly to a concentric nebulizer of ICP-MS, allowing continuous

transportation of the sample to the argon plasma of ICP-MS. Standard plasma conditions were used.

Retention time for the As species was determined using a species mix comprising standards of 50 μg l^{-1} As(III), As(V), DMA, and MMA. Peaks of different As species were identified by comparison with the retention times of individual standard compounds of As(III), As(V), DMA, and MMA. The concentrations were determined using a five-point calibration of As(III), As(V), DMA, and MMA (0, 2, 5, 10, and 25 μg l^{-1} As) with peak areas.

The detection limit for As species was 1 μg l^{-1} As standard solution for the present analysis. 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% to 111%, which was consistent with other studies (Heitkemper *et al.*, 2001; Williams *et al.*, 2005).

Data analyses

All statistical analyses were performed using the statistical package SPSS 13.0 for Windows (SPSS Inc., College Station, TX, USA). The level of significance of various genotypes on porosity, ROL, As concentrations, and speciation in rice was set at $p<0.05$, using one-way analysis of variance (ANOVA). Correlation coefficient analyses were conducted using SigmaPlot 10.0. Peak analysis in As speciation analysis was performed by Origin 7.5.

Results and discussion

Porosity and ROL of different genotypes

Compared with transverse sections under aerated treatment (Fig. 1a), transverse sections of rice under stagnant treatment (Fig. 1b) showed enlarged aerenchyma. In the stagnant treatment, large lacunae that extended radially from the endodermis to the outer cortex were separated by narrow sections of parenchyma cells, while roots grown under aerated conditions exhibited broad parenchymatous cortex with smaller airspaces (Fig. 1). This is in accordance with other studies that showed that porosity and ROL of rice roots could be enhanced by hypoxia (Kludze *et al.*, 1994; Colmer *et al.*, 1998). Enhanced root porosity of rice roots in stagnant conditions clearly has adaptive value in anaerobic environments.

The porosity of different genotypes was significantly different ($p<0.01$), ranging from 12.4% to 33.2% (Table 1). There were neither significant differences between Indica and Japonica subspecies ($p>0.05$), nor between upland and paddy rice ($p>0.05$). ROLs of different genotypes were between 5.30 and 27.8 μmol O_2 g^{-1} DW h^{-1} (Table 1). There were significant differences ($p<0.01$) in terms of ROL and porosity between different genotypes, but no significant differences between Indica and Japonica subspecies, or between upland and paddy rice (both $p>0.05$). There was, however, a positive correlation ($R=0.64$, $p<0.01$) between ROL and entire root porosity (Supplementary Fig. S1, available at *JXB* online).

ROL to the soil and consumption in the rhizosphere by microorganisms or by the oxidation of reduced chemicals can be a potentially greater sink for oxygen. Moreover, it is considered that ROL from root to the rhizosphere is essential for the detoxification of phytotoxins such as Fe^{2+}, Mn^{2+}, H_2S, S^{2-}, HS^-, and organic acids by direct oxidation or by the agency of oxidizing aerobic microorganisms maintained in the rhizosphere regions (Begg *et al.*, 1994; Revsbech *et al.*, 1999). The rice genotypes studied in our experiments responded to oxygen-deficient conditions by increasing adventitious root porosity. This acclimation increased the longitudinal diffusion of oxygen in the roots, which may increase the resistance of rice plants to anoxic conditions in anaerobic soils (Jackson and Colmer, 2005).

Plant growth during the pot experiment

There were no significant ($P>0.05$) differences in straw biomass between rice irrigated with 0 and those with 0.4 mg l^{-1} As in most genotypes, possibly due to the relatively low As concentration used. Only five genotypes, Handao1, PSB RC 70, TORO2, Xijing7, and Xiushui11 showed significant differences in straw biomass between control and As treatments (Table 2). Lower shoot biomass and reduced plant height of rice subjected to higher arsenate treatment were observed by Marin *et al.* (1993) (0.8 mg l^{-1} arsenite and MMAA) and Abedin *et al.* (2002*a*) (1–8 mg l^{-1} As). However, when rice was subjected to a lower concentration (0.2–0.8 mg l^{-1}) of arsenate, there was no significant reduction in plant height or straw biomass (Marin *et al.*, 1993; Abedin *et al.*, 2002*a*).

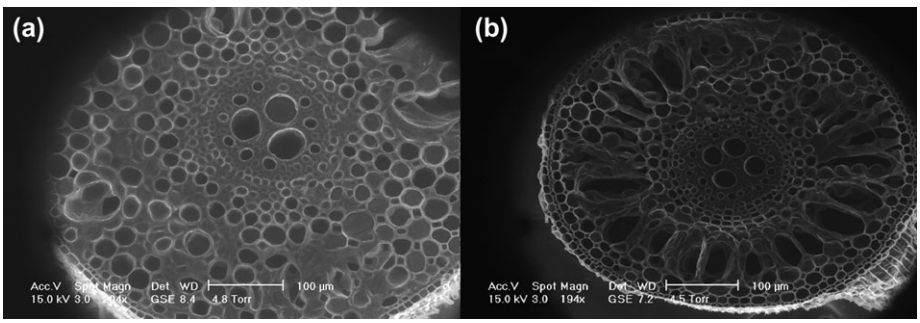

Fig. 1. Fresh cross-section at 2 cm behind the apex of adventitious roots of rice (genotype Yingjingruanzhan). (a) Aerated solution, (b) stagnant solution. Plants were grown in aerated or stagnant solution [deoxygenated nutrient solution containing 0.1% (w/v) agar] for 30 d before the sections were taken.

Table 1. Characteristics of genotypes used in this investigation with porosity (%) and ROL (μmol O_2 g^{-1} DW h^{-1}) (values are means±SD) of 20 different genotypes of rice subjected to stagnant solutions for 30 d

Genotype	Subspecies	Origin	Type	Porosity	ROL
CNT87059-3	Indica	Thailand	Paddy	18.8±1.5	7.0±0.55
TD71	Indica	Thailand	Paddy	31.1±0.1	27.1±1.3
IR72	Indica	Philippines	Paddy	20.4±2.3	20.3±2.9
PSB RC 70	Indica	Philippines	Paddy	23.5±1.8	5.8±0.49
TNAU 98	Indica	India	Paddy	21.9±1.7	15.5±1.1
Erjiufeng	Indica	Zhejiang, China	Paddy	26.9±7.5	23.0±1.2
Xiushui11	Indica	Zhejiang, China	Paddy	20.7±4.8	11.8±0.6
Guinongzhan	Indica	Guangdong, China	Paddy	19.3±0.9	24.5±0.2
Yingjingruanzhan	Indica	Guangdong, China	Paddy	26.3±5.6	22.1±0.2
Yuxiangyouzhan	Indica	Guangdong, China	Paddy	28.2±3.8	17.5±3.1
Kinmaze	Japonica	Japan	Paddy	26.3±1.8	15.4±1.7
TORO 2	Japonica	USA	Paddy	18.8±1.7	22.8±2.8
Dongnong413	Japonica	Heilongjiang, China	Paddy	24.1±8.1	18.0±3.1
Hejiang16	Japonica	Heilongjiang, China	Paddy	30.7±11.8	23.4±1.4
Nanyangzhan	Japonica	Guangxi, China	Paddy	12.4±4.3	5.3±0.84
IAPAR9	Japonica	Brazil	Upland	19.0±5.0	14.7±0.6
Handao	Japonica	Shanxi, China	Upland	33.2±2.8	18.0±1.3
Handao3	Japonica	Shanxi, China	Upland	29.0±4.9	25.8±1.5
Handao1	Japonica	Shaanxi, China	Upland	32.6±1.2	27.8±0.4
Xijing7	Japonica	Shaanxi, China	Upland	15.3±1.2	8.3±0.23
Analysis of variance					
Genotypes				$P<0.01$	$P<0.01$
Subspecies				NS	NS
Types				NS	NS

Table 2. Straw biomass (g pot^{-1}, DW) and arsenic concentrations (mg kg^{-1}, DW) in roots, straws, husks, and grains of 20 different genotypes of rice grown in soils regularly irrigated with 0.4 mg l^{-1} As-contaminated water

Genotype	Straw biomass	As in roots	As in straws	As in husks	As in grains
CNT87059-3	11.8±0.67	478±30	7.29±1.6	1.83±0.12	0.70±0.26
TD71	7.20±2.2	180±48	1.40±0.36	1.05±0.18	0.46±0.11
IR72	12.5±3.5	175±29	2.36±0.57	0.89±0.21	0.61±0.33
PSB RC 70	9.70±2.2*	234±53	6.05±0.51	3.42±0.02	1.17±0.25
TNAU 98	8.01±1.6	18.0±9.2	2.93±0.28	0.45±0.13	0.19±0.05
Erjiufeng	1.35±0.77	18.6±1.8	1.67±0.07	0.55±0.12	0.22±0.05
Xiushui11	10.6±2.5*	164±44	4.55±0.58	1.61±0.19	0.78±0.04
Guinongzhan	9.5±1.2	51.0±4.9	0.64±0.41	0.31±0.02	0.22±0.05
Yingjingruanzhan	8.43±0.17	66.4±8.8	2.13±0.81	1.37±0.16	0.48±0.08
Yuxiangyouzhan	11.6±4.2	60.6±4.0	2.29±0.17	0.55±0.10	0.27±0.06
Kinmaze	11.7±2.7	376±48	6.90±0.80	1.49±0.40	0.40±0.04
TORO 2	16.4±5.1*	109±17	1.91±0.45	0.80±0.06	0.30±0.07
Dongnong413	4.14±0.15	80.5±10	0.82±0.03	0.55±0.15	0.22±0.06
Hejiang16	3.66±0.21	55.2±13	1.68±0.73	0.57±0.11	0.22±0.08
Nanyangzhan	11.9±0.9	179±75	8.22±2.4	1.67±0.66	0.70±0.05
IAPAR9	8.10±2.2	134±33	6.85±0.76	0.48±0.14	0.28±0.07
Handao	8.41±2.9	92.8±23	1.53±0.18	0.87±0.08	0.34±0.03
Handao3	4.93±0.68	41.0±3.5	2.50±0.64	0.93±0.21	0.22±0.01
Handao1	10.4±1.7*	91.4±36	1.59±0.45	0.62±0.02	0.47±0.09
Xijing7	13.1±3.9*	266±37	16.3±0.99	2.71±0.70	0.79±0.08
Analysis of variance					
Subspecies		NS	NS	NS	NS
Types		NS	NS	NS	NS

*Indicates that this species shows significant difference in biomass between control and As treatment.

In the present study, there was a significant positive correlation between the total ROL from entire roots and As tolerance (expressed as percentage mean of control straw biomass, $R=0.69$, $P<0.01$) among the 20 different genotypes (Fig. S2, at *JXB* online). Mei *et al.* (2009) similarly reported that rice with higher ROL resulted in higher As tolerance. Waterlogged conditions can cause reduction of toxic metals and damage plant roots (McDonald *et al.*, 2001, 2002), but wetland plants can evolve some special features, such as enlarged root porosity and ROL, to facilitate their survival in the anoxic environment (Liu *et al.*, 2009; Pi *et al.*, 2009). Tolerance of wetland plants to waterlogged conditions is associated with aeration ability of roots (McDonald *et al.*, 2001, 2002). Liu *et al.* (2009) found that there was significant positive correlation between the amount of ROL from the roots of seedlings and metal tolerance in three species of mangrove. Deng *et al.* (2009) also found that ROL and co-evolution of Fe/Zn tolerance was significantly correlated in 10 species of wetland plant.

Effects of ROL on As accumulation in different genotypes of rice

At maturity, As concentration in different parts of rice among all genotypes followed the trend: root>straw>husk>grain (Table 2). There were significant differences ($P<0.05$) in As concentrations in rice roots, straws, husks, and grains for all genotypes, while there were no significant differences between Indica and Japonica subspecies, and paddy and upland rice (both $P>0.05$). The highest As of 1.17 mg kg^{-1} in rice grains was recorded in PSB RC 70, whereas the lowest, of 0.19 mg kg^{-1}, was in TNAU 98.

There were significant correlations between rice grains and rice straws ($R=0.57$, $P<0.01$), in terms of As concentration. Other studies (Abedin *et al.*, 2002*a*) also showed that roots contained the highest As. Storing relatively larger amounts of As in their roots, and to a certain extent, straws seemed to serve as a protection mechanism for lowering As concentration in rice grains. Figure 2 shows that Japonica subspecies had lower assimilation and transportation of As from straws to grains than Indica subspecies. This was also the case for upland rice when compared with paddy rice. These differences between specific rice varieties would be of interest to rice breeders.

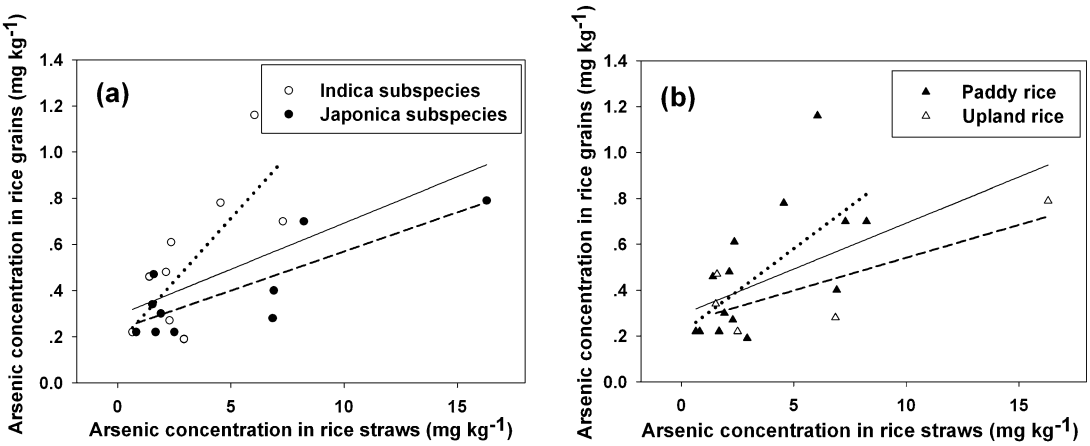

Fig. 2. Correlations of As concentrations between straws and grains (y=0.0401x+0.0292, R=0.57, P<0.01). (a) Linear correlation in Indica and Japonica subspecies (dotted line represents Indica subspecies; short dashed line represents Japonica subspecies; solid line represents all genotypes). (b) Linear correlation in paddy and upland rice (dotted line represents paddy rice; short dashed line represents upland rice; solid line represents all genotypes). White circles represent Indica subspecies, y=0.1089×+0.1679, R=0.75; black circles represent Japonica subspecies, y=0.0339×+0.2301, R=0.81; black triangles represent paddy rice, y=0.0738×+0.2119, R=0.67; white triangles represent upland rice, y=0.0286×+0.2553, R=0.79.

Among all the genotypes, the rate of ROL was negatively correlated with As concentration in rice roots (R=–0.60, P<0.01), straws (R=–0.74, P<0.001), husks (R=–0.71, P<0.001), and grains (R=–0.67, P<0.01). It has been found that rates of ROL are negatively correlated with As concentrations in above-ground parts of rice (Mei *et al.*, 2009). In this study, a negative correlation was also found in underground parts of rice (R=–0.60, P<0.01), which further demonstrated that ROL could decrease As uptake in rice plants.

The fact that genotypes with higher ROL accumulated less As in grains indicates the potential of breeding rice genotypes that will accumulate less As even if irrigated with As-contaminated water. Mei *et al.* (2009) observed that three Japonica cultivars out of the 25 selected cultivars possessed higher porosity and rates of ROL, higher grain and straw biomass (percentage of control), but lower As concentration in grains and straws than Indica cultivars. However, there were no significant differences in ROL or As accumulation between Indica and Japonica subspecies in the present study.

ROL can oxidize rhizosphere soil substances and cause precipitation of toxic metals in rhizosphere soil and root surface, demonstrating the ability of plant roots to release oxygen from the aerenchyma to the rhizosphere (Otte *et al.*, 1989; Smolders and Roelofs, 1996), which reduces metal bioavailability in soil. The different genotypes of rice have different porosity and ROL, leading to different capacities to release oxygen to the soil. Rice plants with higher ROL can release more oxygen to the soil, leading to a higher degree of oxidization in the rhizosphere soil, and further lower bioavailability of As.

In addition, a number of wetland plants, including rice, are known to form iron plaque on their roots by oxidizing Fe^{2+} to Fe^{3+}, resulting from the oxidizing activity of the plant roots and associated microorganisms (Crowder and St-Cyr, 1991). Both abiotic and biotic factors control the presence and degree of iron plaque formation. Oxidizing capacity of the plant root is considered the most important biotic factor controlling plaque formation (Mendelssohn *et al.*, 1995). It has been reported that iron plaque can affect As accumulation in rice (Liu *et al.*, 2004, 2006; Chen *et al.*, 2005). In the present study, ROL was correlated with As accumulation, which may be because ROL affects iron plaque formation. Further research is needed to clarify the relationship between ROL and iron plaque formation.

As speciation in different genotypes

The recovery of As speciation for different genotypes ranged from 64% to 111%. The relatively large difference in recovery may be due to the different types and qualities of grains. As(III), As(V), DMA, and MMA were detected in rice grains for all genotypes. Due to the fact that TFA might reduce arsenate to arsenite, the levels of total inorganic As were adopted instead of the concentrations of As(III) and As(V) (Liu *et al.*, 2006). The predominant species detected were inorganic As and DMA (Table 3). MMA was detected only in certain genotypes with trace amounts. For genotypes CNT87059-3, Dongnong413, Erjiufeng, Guinongzhan, Handao1, Handao3, Hejiang16, IAPAR9, IR72, Kinmaze, Nanyangzhan, TD71, TNAU98, TORO2, Xijing7, Xiushui11, Yingjingruanzhan, and Yuxiangyouzhan, inorganic As was the predominant species, and the proportions of inorganic As and DMA were 57–96% and 2–42%, respectively. However, for genotypes Handao and PSB RC70, DMA was the main species with proportions of 81% and 60%, respectively, in accordance with the NIST CRM 1568a rice flour, which contained a 59% proportion of DMA (Table 3). Smith *et al.* (2008) found that DMA comprised the major As species in rice

Table 3. Proportions of arsenic species in grains for 20 genotypes using TFA extraction and HPLC-ICP-MS measurement.

Genotype	Total As (mg kg^{-1})	Inorganic As (mg kg^{-1})	DMA (mg kg^{-1})	MMA (mg kg^{-1})	Inorganic As[a] (%)	DMA[b] (%)	Recovery[c] (%)
CNT87059-3	0.70±0.26	0.57±0.06	0.01±0.001	0.02±0.004	95	2	87
TD71	0.46±0.11	0.35±0.03	0.15±0.02	nd	69	30	109
IR72	0.61±0.33	0.53±0.1	0.02±0.003	nd	96	3	91
PSB RC 70	1.16±0.25	0.45±0.05	0.71±0.04	0.02±0.003	38	60	102
TNAU 98	0.19±0.05	0.11±0.02	0.02±0.007	0.005±0.000	82	15	72
Erjiufeng	0.22±0.05	0.08±0.002	0.06±0.001	nd	57	42	64
Xiushui11	0.78±0.04	0.47±0.002	0.14±0.02	nd	77	22	78
Guinongzhan	0.22±0.05	0.19±0.01	0.02±0.001	nd	90	9	101
Yingjingruanzhan	0.48±0.08	0.22±0.03	0.13±0.01	nd	63	37	71
Yuxiangyouzhan	0.27±0.06	0.14±0.02	0.01±0.001	0.02±0.001	82	6	66
Kinmaze	0.40±0.04	0.3±0.03	0.14±0.02	nd	68	32	111
TORO2	0.30±0.07	0.22±0.007	0.08±0.005	nd	73	26	76
Dongnong413	0.22±0.06	0.11±0.01	0.04±0.005	0.01±0.004	69	25	75
Hejiang16	0.22±0.08	0.16±0.01	0.04±0.003	nd	80	20	99
Nanyangzhan	0.70±0.05	0.41±0.009	0.23±0.03	0.01±0.004	63	35	92
IAPAR9	0.28±0.07	0.19±0.02	0.1±0.03	nd	65	34	101
Handao	0.34±0.03	0.07±0.004	0.29±0.05	nd	19	81	105
Handao3	0.22±0.01	0.15±0.01	0.03±0.005	nd	83	17	81
Handao1	0.47±0.09	0.35±0.02	0.02±0.001	nd	95	5	69
Xijing7	0.79±0.08	0.34±0.001	0.19±0.01	nd	64	35	67
NIST CRM 1568a	0.29	0.11±0.005	0.16±0.02	nd	40	59	97

[a] Inorganic arsenic (%)=[(inorganic As]/(species sum)]×100.
[b] DMA (%)= [(DMA)/(species sum)] ×100.
[c] Recovery (%)= [(species sum]/(total As)] ×100.

grain in a greenhouse experiment. The difference with this study may be due to the differences in rice genotype and growth conditions. In addition, CNT87059-3, IR72, and Handao1 had very low DMA content even though the total grain levels were elevated. The relatively large variation in grain speciation of different genotypes of rice is in agreement with other findings (Williams *et al.*, 2005; Liu *et al.*, 2006). Zavala *et al.* (2008) speculated that As speciation in rice grain is under genetic control, and classified rice grain into inorganic or DMA type. However, Xu *et al.* (2008) demonstrated that As speciation in rice grain can be strongly influenced by environmental conditions such as the watering regime and As bioavailability in soil. The pathway, enzymology, and genetic basis for As methylation in rice, however, were not well elucidated (Zhao *et al.*, 2009). Enhanced uptake and accumulation of As but a small proportion of methylation in genotype CNT87059-3, IR72, and Handao1 may be due to the difference in As metabolism between genotypes. The mechanism for As methylation in different genotypes needs further investigation.

There were significant genotype effects in percentage inorganic arsenic (F=15.8, P<0.001) and percentage DMA (F=22.1, P<0.001), but there was no significant difference (P>0.05) between Indica and Japonica subspecies, and between paddy and upland rice (P>0.05) in terms of percentage of As species. There was a significant positive correlation between total grain As and inorganic As (R=0.81, P<0.0001), and DMA (R=0.70, P<0.001). ROL of different genotypes was also significantly correlated with inorganic As in rice grains (R=–0.47, P<0.05), as well as total grain As (R=–0.67, P<0.01) (Fig. 3). The percentage of inorganic As decreased as total grain As increased (Fig. 4), along with increasing percentage of DMA, which is in line with other studies (Meharg *et al.*, 2008; Xu *et al.*, 2008). However, at the lower total As concentration range (0–0.8 mg kg^{-1}), the percentage of inorganic As or DMA remained relatively constant with increasing total As concentration in rice grains, demonstrated in Fig. 4. It was found that arsenate was the predominant As species in rice straws (Abedin *et al.*, 2002*a*). Presumably the major As species transported from straws to grains was inorganic As, hence higher As accumulation in rice grains may induce more methylation of inorganic As for detoxificaiton of plant cells, because inorganic As is more toxic than the organic species (Meharg and Hartley-Whitaker, 2002). Another reason is due to genetic differences between genotypes with regard to their capacity for methylation of inorganic As. The mechanism needs further investigation.

The Maximum Contaminant Levels (MCLs) for inorganic As in rice grains was set at 0.15 mg kg^{-1} in China (Chinese Food Standards Agency, 2005). The standard is comparable to the drinking water MCLs of 50 μg l^{-1} adopted by many countries, and the daily consumption of 400 g d^{-1} weight of rice with a concentration of 150 mg kg^{-1} inorganic As could result in six times the exposure for US citizens from drinking water at their MCL (Zhu *et al.*, 2008*a*). Inorganic As concentration of most genotypes exceeded this standard, with a mean concentration of 0.27 mg kg^{-1} in the range 0.07–0.57 mg kg^{-1}. This demonstrates a great risk when growing rice with As-contaminated water in the field.

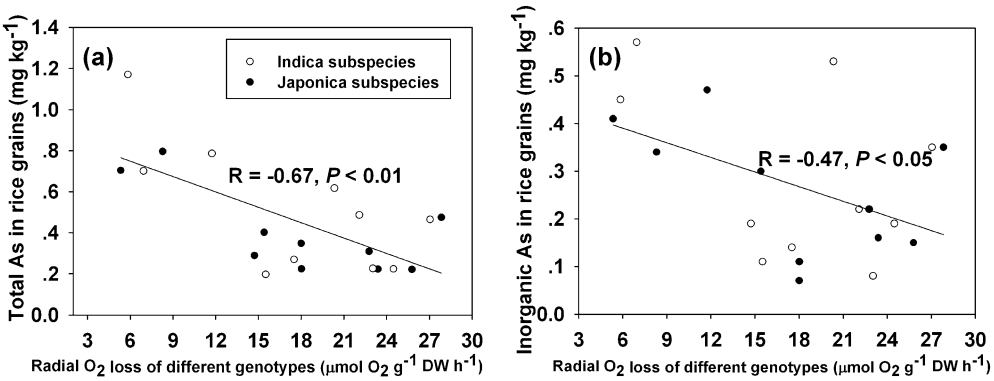

Fig. 3. Correlations of ROL and total As (a), inorganic As (b) in grains of 20 different genotypes of rice grown in soils regularly irrigated with 0.4 mg l^{-1} As-contaminated water. White circles represent Indica subspecies, and black circles represent Japonica subspecies.

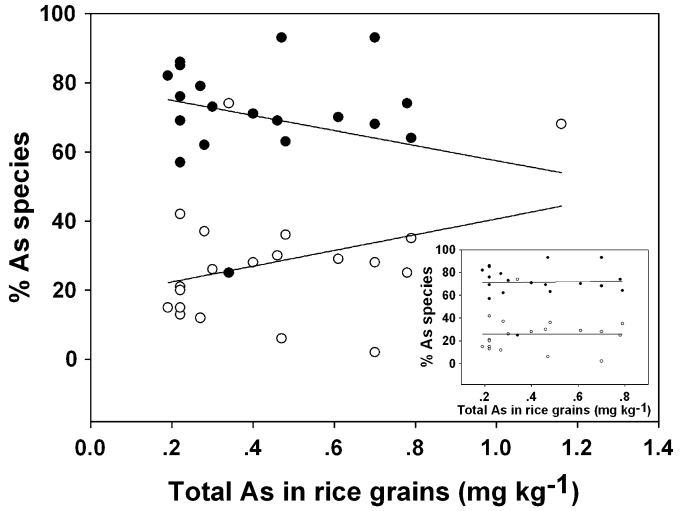

Fig. 4. Correlation of total As concentrations with percentage inorganic As (black circles) and DMA (white circles) concentrations.

In soils, arsenate can be transformed to arsenite due to redox conditions in flooded soils and methylation through microbial activity (Abedin *et al.*, 2002*a*,*b*). Arsenate can also be readily converted to arsenite, and further metabolized to methylated species in plants (Meharg and Hartley-Whitaker, 2002). Therefore, the arsenite and methylated As in rice grains may be transported from soil solution or through metabolism in rice plants. The genotype differences in As accumulation and speciation observed in the present study may be due to differences in genotypes on alteration of soil rhizosphere conditions or metabolism of As in rice plants.

Genotypes with higher ROL resulted in lower grain total As and inorganic As. There was a significant correlation between total As and inorganic As (R=0.81, P<0.0001); rice grains with higher total As tended to contain more inorganic As. Rice genotypes with higher ROL accumulated less total As, which in turn reduced inorganic As accumulation in grains. Potential health risk for As in rice grains depends mainly on inorganic As, because inorganic As is considered more toxic than methylated As (Byrd *et al.*, 1996; Abedin *et al.*, 2002*a*,*b*; Zhu *et al.*, 2008*a*,*b*). Breeding of rice genotypes seems complicated and should take into account both total and inorganic As concentrations. Some genotypes with low grain As and inorganic As could be used in breeding programmes and genetic studies aiming to identify genes that could decrease grain total As and inorganic As. Mechanisms of ROL to mitigate As accumulation in rice and its application for breeding rice genotypes accumulating less inorganic As in grains need further investigation.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Fig. S1 shows correlations of ROL and porosity of roots in 20 different genotypes subjected to stagnant solution for 30 d.

Supplementary Figure S2 shows correlations of ROL in 20 different genotypes subjected to stagnant solution for 30 d and As tolerance.

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