



## Oxic and anoxic conditions affect arsenic (As) accumulation and arsenite transporter expression in rice



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### HIGHLIGHTS

- Rice root and shoot total As were lower in oxic treatments than anoxic treatments.
- Rice arsenite concentrations in oxic treatments were lower than anoxic treatments.
- Lsi1 and Lsi2 expressions were down-regulated in oxic than anoxic treatments.
- Lsi1 and Lsi2 expressions were not affected by As addition and different genotypes.

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### ABSTRACT

Arsenic (As) exposure from rice consumption has now become a global health issue. This study aimed to investigate the effects of rice rhizosphere oxic conditions on silicate transporter (responsible for arsenite transportation) expressions, and on As accumulation and speciation in four rice genotypes, including two hybrid genotypes (Xiangfengyou9, Shenyou9586) and two indica subspecies (Xiangwanxian17, Xiangwanxian12). Oxic and anoxic treatments have different effects on root length ( $p < 0.001$ ) and weight ( $p < 0.05$ ). Total As concentrations in roots were dramatically lower in oxic treatments (88.8–218 mg/kg), compared to anoxic treatments (147–243 mg/kg) ( $p < 0.001$ ). Moreover, root and shoot arsenite concentrations in oxic treatments were lower than that in anoxic treatments in arsenite treatments. The relative abundance of silicate transporter expressions displayed a trend of down-regulation in oxic treatments compared to anoxic treatments, especially significantly different for Xiangwanxian17, Xiangwanxian12 in Lsi1 expressions ( $p < 0.05$ ), Xiangfengyou9, Shenyou9586, Xiangwanxian17 in Lsi2 expressions ( $p < 0.05$ ). However, there were no significant differences of transporter expressions in different As treatments and genotypes. It may be a possible reason for low As accumulation in rice growing aerobically compared to flooded condition and a potential route to reduce the health risk of As in rice.

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### 1. Introduction

Arsenic (As) is ubiquitous in the environment and classed as a chronic carcinogen to humans (Dong et al., 2014; Shi et al., 2014; Li et al., 2015). Arsenic exposure from water and diet has now become

a global health issue (Berg et al., 2001; Liao et al., 2005; Seyfferth et al., 2014). Over the past decades, due to anthropogenic activities such as mining, smelting and irrigation of As-contaminated groundwater, large areas of paddy soils have become contaminated with As (Wu et al., 2015; Zhu et al., 2016; Xue et al., 2016). Furthermore, among cereal crops, rice is much more efficient at accumulating As due to its waterlogged growth conditions which enhance As mobility (Su et al., 2009; Somenahally et al., 2011; Jia et al., 2014). Rice is a staple food for half the world's population

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(Stone, 2008; Pan et al., 2014; Seyfferth et al., 2014), with its consumption being a primary source of dietary As (Meharg et al., 2009; Batista et al., 2011; Halder et al., 2014), especially in some countries of southeast Asia where rice contains a higher percentage of inorganic arsenic (iAs) (Williams et al., 2005; Pan et al., 2014; Rahman et al., 2014).

The As species present in soils and rice plants are mainly inorganic As [arsenite (AsIII) and arsenate (AsV)] and organic As [monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)], with inorganic As being the predominant species (Zhao et al., 2010; Zheng et al., 2013; Wu et al., 2015). Studies have demonstrated that oxic conditions in soils play an important role in As uptake and speciation in rice plants (Xu et al., 2008; Li et al., 2009a; Somenahally et al., 2011). In aerobic conditions, arsenate [As(V)] predominates, with low bioavailability, due to it being strongly adsorbed by Fe and Al (hydr)oxides (Xu et al., 2008; Zhao et al., 2010; Yamaguchi et al., 2014). When a soil is flooded, large concentrations of As, mainly as arsenite [As(III)], are released into the soil solution due to reductive dissolution of Fe (hydr)oxides and the reduction of As(V) to weakly adsorbed As(III) (Takahashi et al., 2004; Xu et al., 2008; Li et al., 2009a; Zhao et al., 2010), thereby making As(III) the predominant As species in flooded paddy soils. Xu et al. (2008) and Li et al. (2009a) observed that growing rice aerobically significantly reduced total As concentrations in the soil solution, whilst also markedly reducing total As and iAs concentrations in rice grain compared to those grown in flooded conditions. A field-scale experiment by Somenahally et al. (2011) revealed that intermittent flooding alleviated total As and iAs accumulation in rice grains compared to continuous flooding.

Rice, similar to most wetland plants, have developed extensive aerenchyma in their roots to adapt to flooded soil conditions (Colmer, 2003a, b). The aerenchyma supply O<sub>2</sub> which is required for root respiration, maintenance and nutrient uptake; however approximately 30–40% O<sub>2</sub> is lost to the surrounding soil (Colmer, 2003a, b), in a process called radial oxygen loss (ROL) (Colmer et al., 2006; Wu et al., 2012). The rate of ROL varies greatly amongst genotypes and exerts a significant effect on rhizospheric chemical processes such as iron plaque formation on root surfaces (Wu et al., 2012, 2016a; Mei et al., 2012; Jia et al., 2014). Genotypes with greater rates of ROL lead to increased concentrations of iron plaque formation on root surface of rice and as a result sequester As (Mei et al., 2012; Wu et al., 2012). The rate of ROL significantly influences As bioavailability and As uptake by rice roots (Mei et al., 2012; Hartley et al., 2010; Wu et al., 2015, 2016b), with some studies indicating that total As and iAs concentrations in rice grains were negatively correlated with the rate of ROL (Wu et al., 2012, 2016a).

Arsenic transfer into rice differs as a result of its speciation (Zheng et al., 2013; Zhao et al., 2010, 2013). In flooded paddy soils, AsIII predominates as uncharged H<sub>3</sub>AsO<sub>3</sub> at pH < 8 (Zhao et al., 2010; Seyfferth and Fendorf, 2012). As a silicic acid analogue, AsIII is transported into rice roots through the nodulin26-like intrinsic proteins (NIPs) responsible for the uptake of a range of small neutral molecules including Si (Ma et al., 2008; Li et al., 2009b; Wang et al., 2015). Lsi1 (OsNIP2; 1) is highly expressed in rice roots and a major route to facilitate AsIII and silicic acid transport bidirectionally (Ma et al., 2006, 2008). Subsequently, the efflux of AsIII from root cells to the stele is regulated by Lsi2 and this process is considered to be crucial for AsIII translocation into rice shoots (Zhao et al., 2010). In aerobic environments AsV is the predominant As species, but studies have indicated that AsIII is the predominant As species in plant tissues although AsV is initially supplied to the plant (Zhao et al., 2010). Methylated As species present in rice are mainly MMA and DMA (Williams et al., 2005; Zavala and Duxbury, 2008; Pan et al., 2014), and studies have shown that rice plants lack

the ability to methylate As; methylated As species in rice originate from microbial methylation in soils (Jia et al., 2012; Lomax et al., 2012; Zhao et al., 2013). Studies have indicated that MMA and DMA can be taken up by rice roots via aquaporin channel Lsi1 (OsNIP2; 1) (Li et al., 2009b; Zheng et al., 2013).

It is reported that growing rice aerobically could significantly reduce As accumulation in rice (Xu et al., 2008; Li et al., 2009a). And the root oxidation ability of rice such as ROL rates could affect As accumulation as well (Wu et al., 2012, 2016a). However, the effects of rice rhizosphere aeration conditions on As accumulation and speciation in different rice genotypes were uncertain, still uncertain about the related mechanisms such as the relationship with arsenite transporter expressions. This study aimed to investigate the effect of root rhizosphere oxygenation conditions on arsenite transporter expressions and to establish the effect rhizosphere oxygenation conditions has on As uptake, translocation and speciation in rice plants.

## 2. Materials and methods

### 2.1. Materials

Four rice genotypes were selected as follows: two hybrid subspecies Xiangfengyou9 ('XFY-9'), Shenyong9586 ('SY-9586') and two indica subspecies Xiangwanxian17 ('XWX-17'), Xiangwanxian12 ('XWX-12'). Radial oxygen loss of XFY-9, SY-9586, XWX-17 and XWX-12 were 9.55, 10.83, 19.7 and 27.0 μmol O<sub>2</sub> g<sup>-1</sup> root dry weight h<sup>-1</sup> respectively (Wu et al., 2015). Seeds were all obtained from Hunan Agricultural University. Seeds were surface sterilized with a 30% H<sub>2</sub>O<sub>2</sub> solution for 15 min, and then thoroughly washed with deionized water three times. The seeds were germinated in culture dishes on moist filter paper and germinated rice seedlings were then cultured in Kimura B nutrient solution for 2 weeks (Ma et al., 2001).

### 2.2. Anoxic treatment

After 2 weeks in the nutrient solution, uniform seedlings were selected and transplanted to 10-L plastic vessels (six vessels, twelve plants per vessel) with Kimura B nutrient solution. The plants were then subjected to stagnant conditions (a deoxygenated nutrient solution containing 0.1%, w/v, agar, which more closely resembles the flooded soil than either semi-stagnant or N<sub>2</sub>-flushed solutions, because dilute agar prevents convective movement in the solution) (Wu et al., 2012). Furthermore the stagnant solutions were bubbled with N<sub>2</sub> gas for 24 h to ensure deoxygenation before experimental use. Nutrient solution pH was maintained at approximately 5.8, with KOH or HCl, and renewed once every 5 d. Vessels were placed randomly in a greenhouse (maintained at 25 °C during the day and 20 °C at night, with 70% relative humidity) and natural light was supplemented with sodium light (1200 Lux), providing a photoperiod of 12 h light/12 h dark. Plants were cultured for 60 days.

### 2.3. Arsenic treatment

Following the stagnant treatment (60 d), plants were transplanted to 2-L plastic vessels (four plants per vessel) with Kimura B nutrient solution and contained either no arsenic or 4 μM arsenite (NaAsO<sub>2</sub>). The plants added to solutions containing no arsenic served as controls. Half the plants were aerated using an air pump for the entire growth period (oxic treatments), while the other half were treated as stagnant as previously described (anoxic treatments). Treatments were designated as Anoxic-As (stagnant with no As addition), Oxic-As (aerated with no As addition), Anoxic + AsIII (stagnant with arsenite addition) and Oxic + AsIII

(aerated with arsenite addition). There were three replicates for each treatment, with four plants for each replicate (vessels). The nutrient solution was renewed everyday due to the arsenite remained stable within the solution in one day (Ma et al., 2008). Vessels were randomly arranged in the aforementioned greenhouse and plants were grown for 10 days. Though aeration treatments were air pumped for the whole treatment time, solution arsenite in the arsenite treatments only slightly changed to arsenate, with average 96% arsenite in the nutrient solutions in the 24 h time.

#### 2.4. Plant analysis for total As

Plants were harvested after 10 days and washed carefully using deionized water, then divided into roots and shoots. Root and shoot length were measured, fresh root weight determined and 0.5 g fresh root was collected for RNA extraction. The remaining root and shoot samples were divided equally and either oven-dried at 70 °C to a constant weight, or freeze-dried and stored at –20 °C prior to As species determination.

For determination of total As concentrations, 0.5 g sample was weighed into a conical flask (100 ml), and 5 ml concentrated nitric acid added. The samples were left to digest overnight at room temperature (25 °C), then placed on an electric hot plate (120 °C) until the solution became clear. After digestion, samples were filtered and diluted to 20 ml with deionized water into colorimetric tubes (Wu et al., 2015, 2016a, 2016b). The total As concentration (root and shoot) was determined using HG-AFS (AFS-8230, Beijing Jitian Instruments Co., China) (Wu et al., 2015). For quality control, a certified reference material (bush branches and leaves, GBW07603) was used and As recovery ranged from 85.5% to 93.5%.

#### 2.5. Plant analysis for As speciation

For determination of As species, samples were ground under liquid N<sub>2</sub> to ensure stabilization (Shi et al., 2013; Wu et al., 2015). Milled rice plants (1.0 g) was added to centrifuge tubes (50 ml), then 20 ml nitric acid (1%) was added and subsequently heated to 95 °C for 1.5 h. After the samples had cooled to room temperature (25 °C), the extracting solution was centrifuged at 5000r/min for 10 min and the supernatant filtered (0.22 μm). Arsenic speciation was determined using HPLC-HG-AFS (HPLC, Shimadzu LC-15C Suzhou Instruments Co., China; HG-AFS, AFS-8230, Beijing Jitian Instruments Co., China) (Shi et al., 2013; Wu et al., 2016b). NIST CRM 1568a rice flour was used to validate the method (Wu et al., 2015). The mean total recovery [(sum of species detected in the method/total As from acid digestion) × 100%] ranged from 81% to 109%.

#### 2.6. RNA isolation and RT-PCR

The total RNA was extracted from roots using an RNA extraction kit (RNeasy Plant Mini Kit, Qiagen, Germany). Total RNA (500 ng) was used for first-strand cDNA synthesis using SuperScript III Reverse Transcriptase (Invitrogen, USA). One-tenth of the reaction volume was used as the template for silicate transporters (Lsi1 and Lsi2) (Ma et al., 2008) and actin (internal control) amplification using PowerUp SYBR Green Master Mix (Life Technologies, USA) for real-time polymerase chain reaction.

The primer sequences of the different genes are as follows:

Lsi1, 5'-ATCTACTTCTGGGCCAGT-3' (forward) and 5'-AGGAGAGCTTCTGGGAGGAG-3' (reverse); Lsi2, 5'-ATCACCTTCCC-CAAGTCC-3' (forward) and 5'-CAGCTCCCTCCAGTACATGC-3' (reverse); Actin, 5'-GACTCTGGTGATGGTGTGTCAGC-3' (forward) and 5'-GGCTGGAAGAGGACCTCAGG-3' (reverse).

qRT-PCR was carried out in a StepOnePlus instrument (Applied Biosystems, USA) and relative expression normalized against Actin using the comparative CT method recommended by the instrument manufacturer. Experiments were repeated at least three times for statistical analysis of each individual experimental set. All values in the experiments were expressed as mean ± SD.

#### 2.7. Data analysis

All data was analyzed in EXCEL 2007 and SPSS 19.0. Figures were created in Origin 8.0.

### 3. Results

#### 3.1. Plant growth in different treatments

Root length, root weight and shoot length were measured as plant growth parameters (Table 1). There were significant genotypic effects on root length ( $p < 0.001$ ), root weight ( $p < 0.001$ ) and shoot length ( $p < 0.001$ ) of rice plants, with the greatest value of root length, 29.14 cm, from hybrid genotype XFY-9 in Stagnant + AsIII treatment and lowest value, 19.3 cm, from hybrid genotype SY-9586 with Stagnant + AsIII treatments. With the exception of indica genotype XWX-12, shoot length was not significantly affected by different oxalic treatments ( $p > 0.05$ ). In addition, oxalic treatments had significant effects on root length ( $p < 0.001$ ) and root weight ( $p < 0.05$ ), being greatest for hybrid genotype XFY-9 in Stagnant + AsIII treatment and indica genotype XWX-17 in Stagnant + AsIII treatment respectively (Table 1). Additionally, shoot length were relatively smaller following arsenite treatments than with no As treatments (Table 1).

#### 3.2. Arsenic accumulation of rice in different treatments

Arsenic was undetectable in plants grown in As-free treatments (Table 2). Analysis of variance revealed significant genotypic effects on total As concentrations of rice roots ( $p < 0.001$ ) and shoots ( $p < 0.001$ ) in the same treatments. The hybrid genotypes (SY-9586

**Table 1**

Root, shoot length (cm) and fresh root weight (g/plant) in four rice genotypes grown in different treatments (mean ± SD, n = 3).

Genotype	Treatment	As addition	Root length	Shoot length	Root weight
XFY-9	Stagnant	–As	27.98 ± 2.73	61.30 ± 3.18	3.96 ± 0.35
		As(III)	29.14 ± 2.27	58.74 ± 3.08	3.49 ± 1.04
	Aeration	–As	27.80 ± 3.58	62.60 ± 4.92	3.46 ± 0.66
		As(III)	28.87 ± 2.42	59.0 ± 4.20	3.92 ± 0.76
SY-9586	Stagnant	–As	21.78 ± 2.30	45.90 ± 2.62	2.95 ± 0.38
		As(III)	19.30 ± 2.37	45.40 ± 3.38	3.16 ± 0.89
	Aeration	–As	25.40 ± 3.36	53.20 ± 3.70	3.18 ± 0.56
		As(III)	20.93 ± 1.61	47.50 ± 4.16	2.86 ± 0.64
XWX-17	Stagnant	–As	22.10 ± 2.49	55.90 ± 2.61	3.62 ± 1.21
		As(III)	19.76 ± 3.37	53.59 ± 2.56	4.08 ± 0.73
	Aeration	–As	24.70 ± 1.82	56.04 ± 2.99	3.85 ± 0.76
		As(III)	22.30 ± 1.43	56.37 ± 3.36	3.15 ± 0.48
XWX-12	Stagnant	–As	20.50 ± 2.59	38.83 ± 1.33	2.31 ± 0.20
		As(III)	21.77 ± 1.37	37.87 ± 2.38	2.20 ± 0.40
	Aeration	–As	22.94 ± 1.97	35.94 ± 0.81	2.01 ± 0.15
		As(III)	22.53 ± 1.86	36.47 ± 1.46	2.27 ± 0.58
Analysis of variance					
Genotype (G)			P < 0.001	P < 0.001	P < 0.001
Treatment (T)			P < 0.001	NS	P < 0.05
Arsenic (A)			NS	P < 0.05	NS
G*T			P < 0.05	P < 0.001	NS
G*A			P < 0.05	NS	NS
T*A			NS	NS	NS
G*T*A			NS	NS	NS

**Table 2**

Arsenic concentrations in rice roots and shoots grown in different aeration treatments when treated with arsenite (mg/kg, mean  $\pm$  SD).

Genotype	Aeration treatment	Total As (mg/kg)	
		Root	Shoot
XFY-9	Stagnant	228 $\pm$ 30.9	57.2 $\pm$ 11
	Aeration	218 $\pm$ 61.6	36.3 $\pm$ 7.6
SY-9586	Stagnant	147 $\pm$ 24.0	40.3 $\pm$ 0.30
	Aeration	143 $\pm$ 8.70	34.1 $\pm$ 11
XWX-17	Stagnant	212 $\pm$ 4.80	76.9 $\pm$ 8.4
	Aeration	210 $\pm$ 2.0	50.1 $\pm$ 10
XWX-12	Stagnant	243 $\pm$ 23.8	27.3 $\pm$ 4.8
	Aeration	88.8 $\pm$ 7.50	23.4 $\pm$ 5.7
Analysis of variance			
Genotype (G)		P < 0.001	P < 0.001
Treatment (T)		P < 0.001	NS
G*T		P < 0.05	NS

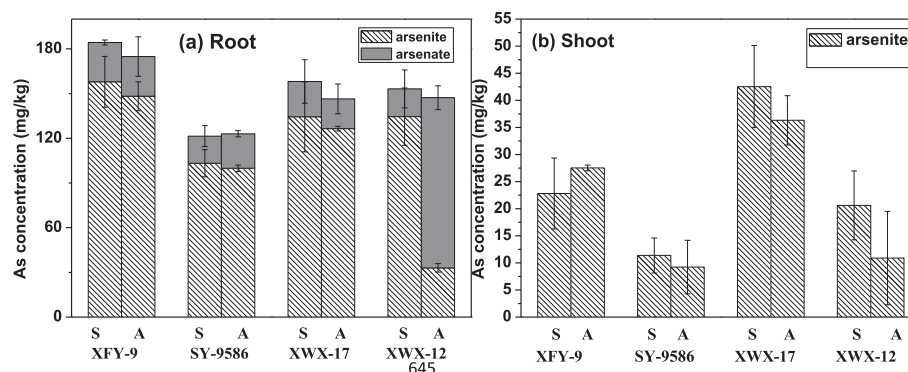
and XFY-9), with lower ROL, accumulated slightly greater As concentrations than indica genotypes (XWX-12 and XWX-17). Total As concentrations in rice roots were dramatically reduced following oxic treatments, (88.8–218 mg/kg) compared to anoxic treatments (147–243 mg/kg) ( $p < 0.001$ ). In addition, total As concentrations in rice shoots were not significantly different between oxic and anoxic treatments, but oxic treatments slightly reduced total As concentrations in shoots compared to anoxic treatments.

### 3.3. Arsenic species of rice in different treatments

Methylated As species (MMA and DMA) were not detected in rice roots or shoots in any treatment (Fig. 1). Arsenite was the predominant As species in roots, except for genotype XWX-12 grown with aeration in arsenite treatments. Arsenate concentrations were undetectable in shoots, with only arsenite detected. Results indicated that there were genotypic differences in arsenite accumulation in roots and shoots. Compared to anoxic treatments, oxic treatments slightly increased arsenate concentrations, but reduced arsenite concentrations in roots. Moreover, root and shoot arsenite concentrations in oxic treatments were lower than that in anoxic treatments in arsenite treatments (Fig. 1). Arsenite concentrations ranged from 11.4 to 42.5 mg/kg in shoots, with lowest value found in genotype SY-9586 (Aeration + AsIII treatment) and greatest concentrations in genotype XWX-17 (Stagnant + AsIII treatment).

### 3.4. Arsenic transporter expression of rice in different treatments

Arsenite transporter (Lsi1 and Lsi2) expressions are presented in



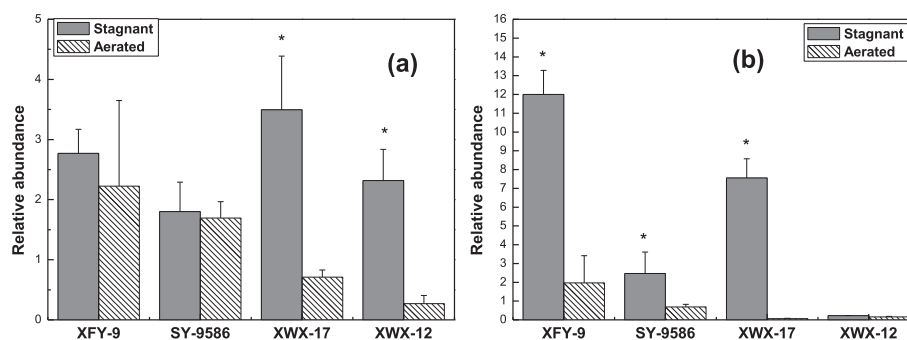
**Fig. 1.** Arsenic speciation in roots (a) and shoots (b) of different rice genotypes in the arsenite treatment, S replicates anoxic (stagnant) treatment, and A replicates oxic (aeration) treatment. Data are mean  $\pm$  S.D. (n = 3).

Fig. 2. The relative abundance of Lsi1 and Lsi2 expressions presented a trend of down-regulation in oxic treatments compared to anoxic treatments, significantly different for XWX-17, XWX-12 in Lsi1 expressions, and XFY-9, SY-9586, XWX-17 in Lsi2 expressions ( $p < 0.05$ ). Different As treatments did not exert significant differences in Lsi1 and Lsi2 expressions (Table 3). Furthermore, there were no significant genotypic differences on Lsi1 and Lsi2 expressions regardless of hybrid or conventional indica genotypes.

## 4. Discussion

Root and shoot length and fresh root weight were significantly different between the four genotypes, which is in agreement with previous studies (Wu et al., 2012, 2015). However, variation between As treatments did not exert any significant difference in either root length or weigh. Only in shoot length; to some extent arsenic treatments displayed decreasing effects in shoot length, which was different to other studies (Marin et al., 1993; Abedin et al., 2002). However, addition of arsenate has not revealed any significant reductions in previous studies (Marin et al., 1993; Carbonell et al., 1998; Wu et al., 2015), possibly due to different growing conditions and genotypes. In addition, there were significant differences in fresh root length and weight between aerated and stagnant treatments; studies have revealed that improved aeration may enhance root growth (Comis, 1996; Wu et al., 2012).

It was also revealed that genotypic effects significantly affected As concentrations in rice roots and shoots, which is in agreement with previous studies (Wu et al., 2012; Zhao et al., 2013). Oxic treatments showed significantly lower As accumulations in roots compared to stagnant treatments (Table 2). Norton et al. (2013) showed that flooding conditions substantially increased grain As content. Hu et al. (2015) demonstrated in a pot experiment, that constant and intermittent flooding treatments gave 3–16 times greater As concentrations in the soil solution, than in aerobic conditions. The effect of water management in rice paddies was investigated to determine As concentrations in Japanese rice grains (Arao et al., 2009), revealing that flooding increased As in rice straw and grains. In a greenhouse experiment, Xu et al. (2008) investigated the dynamics of As speciation in the soil solution under both flooded and aerobic conditions, comparing As accumulation in rice shoots and grain; they revealed that As concentrations in the soil solution were 4–16 times greater under flooded conditions, whilst straw, husk and grain As concentrations were elevated, grain As being 10–15 times greater than the aerobically grown rice. Flooding may reduce soil redox potential, causing As desorption from soil particles, which greatly increases As bioavailability in both greenhouse (Xu et al., 2008; Guo et al., 2009; Hartley et al., 2010) and



**Fig. 2.** Relative expressions of arsenic transporters under different treatments, relative expressions of Lsi1 in different aeration conditions in the arsenite treatment of different genotypes (a), relative expressions of Lsi2 in different aeration conditions in the arsenite treatment of different genotypes (b). \*\*\* indicate significant difference ( $p < 0.05$ ). Data are mean  $\pm$  S.D. ( $n = 3$ ).

**Table 3**

Analysis of variance of different transporter expression abundance in different treatments.

Analysis of variance	Lsi1	Lsi2
Genotypes (G)	NS	NS
Arsenic treatments (A)	NS	NS
Aeration treatments (T)	$P < 0.05$	$P < 0.05$

\*NS, not significant at  $P < 0.05$  level.

field studies (Takahashi et al., 2004). In addition, compared to permanently flooded soil, intermittent flooding reduced As in porewater by 86%, root plaque by 55% and grain by 41% (Somenahally et al., 2011). In the present study, unlike previous investigations (Wu et al., 2015, 2016a), hybrid genotypes with lower ROL did not reveal significantly increased As accumulation compared with indica genotypes with higher ROL (Table 2). The difference may due to that in the pot studies, the increased iron plaque formation which sequestered more As in iron plaque, reduced As transportation to rice roots, or oxidized rhizosphere arsenite species, and caused more As sorption in rhizosphere soils (Wu et al., 2012; Hartley et al., 2010).

It has been shown that AsV, AsIII, and DMA were the predominant As species detected in rhizosphere-soils and root-plaque, porewater and grain samples, respectively (Somenahally et al., 2011). Flooded conditions increased concentrations of dimethylarsinic acid (DMA) in grains compared to aerobic conditions (Arao et al., 2009). Likewise, the concentration of inorganic As was 2.6–2.9 times greater in grain from flooded treatments than in those from aerobic treatments (Arao et al., 2009). The present study also demonstrated that arsenite and arsenate accumulation were both lower in rice roots and shoots in oxic conditions than in anoxic conditions (Fig. 1). Organic As species were undetectable in both roots and shoots, which is in agreement with other studies (Chen et al., 2012).

Ma et al. (2008) showed that OsNIP2; 1 (Lsi1, a silicon influx transporter) is involved in arsenite uptake to root cells, while Lsi2 is involved in arsenite transport out of root cells towards the stele. However, the expression of Lsi2 in wild-type roots was abundant and not affected by arsenite exposure (Ma et al., 2008), which is in agreement with the present study (Table 3). Expressions of Lsi1 and Lsi2 were also not affected by different As treatments (Table 3). Meanwhile, mRNA expressions of Lsi1 and Lsi2 in the present study resulted in a significant down-regulation in oxic treatments compared to anoxic treatments (Table 3), which led to the reduction of total As and inorganic As in rice roots. Chen et al. (2012) also found that mycorrhizal inoculation reduced Lsi1 and Lsi2 expression in rice roots. It was also demonstrated that high sulfur (S)

concentrations resulted in reduced transcript levels of Lsi2, and it is probable that low shoot As accumulation resulted from S application (Dixit et al., 2015).

The differences in Lsi1 and Lsi2 expressions (Fig. 2) may be also due to the differences in anoxia stress on the signaling pathways, especially on the transcriptional factors, which require further research. Plant cells following abiotic stress signals can transduce them through various signaling pathways, and regulate the gene expression by different transcription factors, which results in the expression of stress-responsive genes for the tolerance to the environmental stress (You and Cha, 2015; Muhammad et al., 2016). Dolferus et al. (1997) have identified two sets of alcoholic fermentation pathway genes in *Arabidopsis thaliana* (L.), each of which might play a different role in the adaptive response of the plant to anoxia. One set is strongly induced by low oxygen stress mainly in the roots, while the other set is expressed constitutively in both roots and leaves. In addition, expression of the maize alcohol dehydrogenase 1 (Adh1) gene is transcriptionally regulated under conditions of anaerobic stress (Walker et al., 1987). Moreover, the natural variations in expression of regulatory and detoxification related genes under limiting phosphate and arsenate stress in *Arabidopsis thaliana* were investigated by Shukla et al. (2015); it was found that differential regulation by transcription factors may be responsible for the natural variation in *Arabidopsis* in response to nutrient deficiency and As(V) stress. A possible reason for differences in transporter expressions (Fig. 2) may be due to differences in oxygen on the As accumulation related genes and its related signaling pathways, especially on the transcriptional factors, which require further research.

## 5. Conclusion

The effects of rice rhizosphere oxic conditions on As accumulation and speciation in different rice genotypes (including conventional indica and hybrid genotypes) were investigated, and the related mechanisms such as the relationship with arsenite transporter expressions were also discussed in the present study. Results showed that: aeration treatments have significant effects on root length ( $p < 0.001$ ) and root weight ( $p < 0.05$ ). In the same As treatment, the total As concentrations in rice roots were dramatically lower in oxic treatment, with values being 88.8–218 mg/kg compared to 147–243 mg/kg in anoxic treatments ( $p < 0.001$ ). Moreover, root and shoot arsenite concentrations in oxic treatments were lower than that in anoxic treatments. The relative abundance of Lsi1 and Lsi2 expressions presented a trend of down-regulation in oxic treatments compared to anoxic treatment, especially significantly different for XWX-17, XWX-12 in Lsi1 expressions, and XFY-9, SY-9586, XWX-17 in Lsi2 expressions

( $p < 0.05$ ). Moreover, there were no significant As treatment or genotypic differences on Lsi1 and Lsi2 transporter expressions regardless of hybrid or conventional indica genotypes. It may be a possible reason for low As accumulation in rice growing aerobically compared to flooded condition, and be potential for finally decreasing the health risks posed by As contamination in rice.

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