Arsenic uptake is suppressed in a rice mutant defective in silicon uptake

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Abstract

Possible mechanisms of the effects of silicon (Si) on arsenic (As) uptake were explored using a wild-type rice and its low-Si mutant (*lsi1*). Hydroponic experiments were carried out to investigate the effects of internal and external Si on the As accumulation and uptake by rice in excised roots (28 d-old seedlings) and xylem sap (61 d-old plants). The presence of Si significantly decreased the As concentrations in both shoots and roots of the wild type but not in the mutant with 13.3 μM –arsenite or 10/20 μM –arsenate treatments. The Si-defective mutant rice (*lsi1*) also showed a significant reduction in arsenite or arsenate uptake. Moreover, As concentrations in xylem sap of the wild type were reduced by 51% with 1 mM Si– and 15 μM –arsenate treatments, while Si had no effect on As concentrations in the xylem sap of the mutant. Arsenic-species analysis further indicated that the addition of 1 mM Si significantly decreased As(III) concentrations but had little effect on As(V) concentrations in the xylem sap of the wild type with 15 μM –arsenate treatments. These results indicated that external Si-mediated reduction in arsenite uptake by rice is due to the direct competition between Si and arsenite during uptake. This is because both share the same influx transporter Lsi1. In addition, internal Si-mediated reduction in arsenite uptake by rice is due to competition of the Si/arsenite efflux transporter Lsi2 during the As(III)-transportation process. Silicon also inhibited arsenate uptake by rice. It is proposed that this could actually be due not to the inhibition of arsenate uptake *per se* but rather the inhibition of arsenite transformed from arsenate, either in the external solution or in rice roots.

Key words: arsenic species / *Oryza sativa* / silicon transporter / xylem sap

Accepted August 13, 2009

1 Introduction

Arsenic (As) is a widespread environmental contaminant posing serious health risks to humans (Mandal and Suzuki, 2002). Arsenic may accumulate in soil and water from natural and anthropogenic sources. Recent research has found that paddy rice is particularly efficient in As accumulation compared with other cereal crops (Williams et al., 2007) and a large proportion can be the toxic inorganic form of As (Williams et al., 2005; Zhu et al., 2008). For example, in Chenzhou, Hunan province of China, As in rice was found to range from 0.5 to 7.5 $\mu\text{g g}^{-1}$ with a mean of 1.7 $\mu\text{g g}^{-1}$ (Liao et al., 2005). The maximal permissible value of inorganic As in rice is 0.15 $\mu\text{g g}^{-1}$ in China (CHM, 2005). Rice is the staple food for more than half the world's population, and the As contained in rice can eventually pose long-term health risks to people consuming large amounts of rice in their diet (Zhu et al., 2008). Thus, knowledge of As accumulation in rice may be helpful in improving the quality of rice grown in As-contaminated soils and in minimizing the health impact of As contamination of soil and water.

Silicon (Si) is the second-most prevalent element in soils. Although Si is not considered to be an essential element for higher plants (Epstein, 1999), it has been demonstrated to be beneficial for the growth of plants, especially in the family Gramineae (Matoh et al., 1991). Furthermore, the benefits of Si are especially obvious in plants subject to both biotic and abiotic

stresses (Ma, 2004). In recent years, the ability of Si to ameliorate metal toxicities has received increasing attention. It has been shown that Si can enhance the resistance to Al (Liang et al., 2001), Mn (Iwasaki et al., 2002), Cd (Liang et al., 2005), and Zn toxicity in plants (Neumann and Niesen, 2001).

Rice (*Oryza sativa*) is a typical Si-accumulating plant species, and it requires high Si concentrations (up to 10% of shoot dry weight) to maintain healthy growth and high productivity (Liang et al., 1994). It has been shown previously that applying Si to arsenate pretreatment solution or an uptake solution can significantly decrease shoot and root As concentrations in rice seedlings (Guo et al., 2005, 2007). These results suggest that both internal and external Si concentrations can inhibit As uptake by rice.

A low-Si rice mutant (*lsi1*) defective in active Si uptake was isolated from M_2 seeds mutagenized with sodium azide and was designated as low-Si rice mutant (*lsi1*) (Ma et al., 2004). Ma et al. (2006) described the low-silicon rice1 (*Lsi1*) gene, which controls silicon accumulation in rice, due to its primary function as a silicon transporter. Recently, Ma et al. (2008) have further identified *Lsi1* as a major pathway for the entry of arsenite into rice roots, indicating that arsenite shares the Si transport pathway for entry into rice-root cells. Bogdan and Schenk (2008) observed that the silicic acid concentration in

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soil solution and the As content in straw and grain was strongly negatively correlated, which indicated that endogenous silicic acid in the soil solution had a clear inhibitory effect on As uptake. Therefore, soils with high plant-available Si contents resulted in low plant As contents, and Si application to soils may decrease the As content of rice. To reduce As transfer into rice, it seems essential to understand the mechanisms of Si effects on As uptake by rice. While previous studies have shown that Si can decrease arsenate uptake by rice (Guo et al., 2005, 2007), the mechanisms by which Si reduces the arsenate uptake are still unknown. The availability of a rice mutant defective in Si uptake provides the opportunity to investigate Si-mediated reduction in As uptake by rice. The aims of the present study were therefore to investigate: (1) whether internal Si affects arsenite uptake, (2) whether *Isi1* accumulates less arsenate, and (3) to study possible mechanisms of internal and external Si effects on As uptake by rice.

2 Materials and methods

2.1 Growing conditions

All experiments were carried out in a growth chamber with 14 h/10 h light/dark cycle. Light intensity was about $280 \mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature was kept at 25°C during the day and 20°C during the night. The relative humidity was 70%.

2.2 Plant material

Seeds of wild-type rice (*Oryza sativa* L. cv Oochikara) and the low-silicon rice mutant (referred to as *Isi1*) were kindly provided by Prof. JianFeng Ma (Research Institute for Biore-sources, Okayama University, Japan). Seeds of both wild-type and mutant rice were sterilized in 2.94 M H_2O_2 solution for 10 min, followed by thorough-washing in deionized water, and then germinated on moist perlite. The 2 week-old seedlings were removed from the perlite and washed carefully with tap water to remove any adhering particles. Seedlings of uniform size were selected and transplanted in PVC pots (one seedling per pot) containing 500 mL nutrient solution with the components as follows: 1.68 mM NH_4NO_3 , 0.67 mM K_2SO_4 , 0.50 mM MgSO_4 , 1.33 mM CaCl_2 , 0.44 mM KH_2PO_4 , 25 μM Fe (II)-EDTA, 0.5 μM CuSO_4 , 0.5 μM ZnSO_4 , 2.5 μM MnSO_4 , 5 μM H_3BO_3 , 0.25 μM Na_2MoO_4 , 0.1 μM CoSO_4 , and 50 μM NaCl (pH was adjusted to 5.5 using 0.1 M KOH or HCl). The nutrient solutions were renewed every 3 d. There were four replicates in a fully randomized design, and the pots were rerandomized daily during the growth period.

2.3 Treatment application

2.3.1 Experiment 1: Arsenite uptake with different Si treatments

After 2 weeks, half of the seedlings (28 d old) were transferred to nutrient solution containing 1 mM Si as $\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$ (+Si pretreatment) and the other half continued to grow in Si-free nutrient solution (–Si). After 9 d, the seedlings of –Si and +Si treatments were both divided into two groups and

transferred to 2-morpholinoethanesulfonic acid monohydrate (MES, 5.0 mM) buffered nutrient solution containing 13.3 μM As (NaAsO_2) with or without 1 mM Si ($\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$, eight treatments). MES was added to maintain the nutrient solution at a pH of 5.5. In the absence of rice seedlings, 10%–20% of the arsenite was oxidized to arsenate in the nutrient solution within 24 h. The nutrient solutions were renewed every 24 h after arsenite addition and the 40 d-old rice plants were harvested after 3 d of As treatment.

2.3.2 Experiment 2: Arsenic uptake by excised roots with different Si treatments

Two week-old seedlings of the wild and mutant rice prepared as described above were transferred to PVC pots (one seedling per pot) containing 500 mL nutrient solution (pH 5.5). After 2 weeks, roots were excised at the basal node and used to measure As uptake with different Si concentrations. Care was taken to ensure the roots were exposed to air for a minimal period.

Excised roots of the 28 d-old seedlings were incubated in deionized water for 20 min at room temperature. They were then transferred to conical flasks containing 1000 mL test solution and incubated for further 30 min according to Meharg and Jardine (2003) and Chen et al. (2005). The test solution contained 50 μM arsenite or arsenate with different Si concentrations (0, 0.4, 0.8, 1.2, 1.6 mM, $\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Potassium (as 1.6 M KCl solution) was added to give all test solutions the same K concentration. A stock solution of As was prepared from sodium arsenite (NaAsO_2) or sodium arsenate ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$). All test solutions contained 5.0 mM MES and 0.5 mM $\text{Ca}(\text{NO}_3)_2$ (pH 5.5). Finally, the roots were thoroughly rinsed with an ice-cold solution containing 1 mM K_2HPO_4 , 5 mM MES, and 0.5 mM $\text{Ca}(\text{NO}_3)_2$ for 20 min to remove the As residue in the free space of the roots. The roots were oven-dried at 70°C for 72 h; subsamples were digested, and As concentrations were determined.

2.3.3 Experiment 3: Arsenate uptake with different Si treatments

Three week-old seedlings (2 weeks in perlite and 1 week in nutrient solution) of the wild and mutant rice were transferred to a PVC pot (one seedling per pot) containing 500 mL nutrient solution (pH 5.5). After 18 d, the 40 d-old rice plants were transferred to the nutrient solution containing different concentrations of arsenate (0, 10, and 20 μM , $\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) and Si (0 and 1 mM, $\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Potassium (as 0.4 M KCl solution) was supplemented to maintain the same K concentration in all the treatments. The nutrient solutions were renewed every 3 d (pH 5.5). The 62 d-old rice plants were harvested after 23 d As and Si treatment applications.

2.3.4 Experiment 4: Effect of Si on As concentrations and As species in xylem sap

Three week-old seedlings (2 weeks in perlite and 1 week in nutrient solution) of the wild and mutant rice were transferred to PVC pots (six seedlings per pot) containing 1.2 L nutrient

solution (pH 5.5). After 40 d, the 61 d-old rice plants were transferred to the nutrient solution containing 15 μM arsenate ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) with or without 1 mM Si ($\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). In addition, the phosphate concentration was lowered to 50 μM , and potassium (as 2 M KCl solution) was supplemented to maintain the same K concentration in the growth medium. The nutrient solutions were renewed every 24 h after arsenate treatments. After 5 d As and Si treatments, the stem of the 66 d-old rice plants was severed about 2 cm above the root base, and the xylem sap was collected every 30 min with micropipettes for 4 h. The xylem sap was immediately diluted to 4 mL with distilled water and stored at 4°C in a refrigerator. The whole process of collection was carried out in the dark. The Si and As concentrations and As species in xylem sap were determined immediately after collection.

2.4 Chemical analyses

2.4.1 Analysis of Si and As concentrations of plant tissues

Dried plant material was finely ground to powder. Subsamples were placed in 100 mL Teflon tubes and digested in 5 mL of high-purity nitric acid using the CEM Microwave Sample Preparation System (Matthews, NC, USA). The temperature was raised to 180°C within 15 min and maintained for 15 min. After digestion, the solutions were cooled and diluted to 25 mL in plastic volumetric flasks with ultrapure water (Easypure). One milliliter of 22.5 M HF solution was added to the flasks. The solution was shaken overnight and made up to 50 mL with distilled water. A reagent blank and standard reference material (tea leaves obtained from the National Research Center for standards in China) were included to verify the accuracy and precision of digestion and subsequent analysis. Concentrations of Si and As in the digestion solution and xylem sap were analyzed using the colorimetric molybdenum blue method described by *van der Vorm* (1987) and atomic-fluorescence spectrometry (A2022; Beijing Haiguang Analytical Instrument Co., Beijing, China), respectively.

2.4.2 Analysis of arsenic species in xylem sap

Arsenic species were analyzed using ionic chromatography coupled with hydride generation atomic-fluorescence spectrometry (IC-HGAFS). The IC system consisted of a GS50 gradient pump and an LC30 oven (30°C) with a 250 μL injection loop (Dionex, USA). The column used for separation was an AS16 strong anion-exchange column (250 mm $\times$ 2 mm) equipped with an AG16 guard column (50 mm $\times$ 2 mm, Dionex, USA), and the mobile phase used was gradient sodium hydroxide at a flow rate of 0.25 mL min⁻¹.

The AFS (atomic-fluorescence spectrometry) used was an AFS-610D system (Rayleigh, China) developed for hyphenation. The operating conditions were as follows: the voltage was set to 320 V; the lamp current was set to 80 mA; the flow rates of carrier and auxiliary argon gas were 500 and 800 mL min⁻¹. The reductant was a solution of 0.53 M sodium borohydride at a flow rate of 4 mL min⁻¹. The acid used was 3 M hydrochloric acid at a flow rate of 3 mL min⁻¹. The detection limits of IC-HGAFS for dimethyl arsenic acid (DMA), As(III),

monomethyl arsenic acid (MMA), and As(V) were 1.1, 1.2, 0.8, and 1.1 $\mu\text{g L}^{-1}$, respectively. Standard solution containing the four As species was used to control the analysis quality.

2.5 Statistical analysis

Analysis of variance (ANOVA) on plant biomass and concentrations of As and Si were performed using Windows-based Genstat (6th edition, VSN Ltd, England).

3 Results

3.1 Arsenite uptake with different Si treatments

The As accumulation by the mutant was significantly lower than that by the wild-type rice with 13.3 μM arsenite and in the absence of Si (Fig. 1, $p < 0.001$). For the wild type, the addition of 1 mM Si to the pretreatment solution or 13.3 μM –arsenite uptake solution significantly decreased shoot and root As concentrations compared with the no Si treatments (Fig. 1, $p < 0.001$). Shoot and root As concentrations of the wild-type rice were reduced by 49.6%, 45%, 26.7%, and 9.1% with internal and external Si treatments, respectively. However, the addition of Si to the pretreatment solution and arsenite uptake solution had no effect on shoot and root As concentrations of the mutant rice (Fig. 1).

Figure 1: Shoot and root As concentrations of the wild and mutant rice treated with 13.3 μM As (NaAsO_2), with or without 1 mM Si ($\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Different letters indicate significant differences by LSD test (0.05). Bars present standard errors of the mean ($n = 4$).

3.2 As uptake by excised roots with different Si treatments

Regardless of the two lines and Si treatments, the uptake rate of arsenate was higher than that of arsenite by the excised roots with 50 μM As concentration during the short-term (30 min) experiment (Fig. 2, $p < 0.001$). The addition of different Si concentrations to the test solution had no significant effect on arsenite uptake of the excised roots of the wild and mutant rice (Fig. 2). However, arsenite uptake of the mutant roots was 26% lower than that of the wild-type roots during the short-term experiment (Fig. 2, $p < 0.001$). In contrast to arsenite, arsenate uptake of the mutant roots was 27% higher than that of the wild-type roots during the short-term experiment (Fig. 2, $p < 0.001$).

3.3 Arsenate uptake with different Si treatments

For the wild-type rice, the addition of 1 mM Si to the nutrient solution had no effect on shoot dry weight with no arsenate treatments, but significantly increased shoot dry weight with 10 and 20 μM –arsenate treatments (Tab. 1, $p = 0.002$). The addition of 1 mM Si had no significant effect on shoot dry weight of the mutant rice irrespective of arsenate treatments (Tab. 1). Root dry weights showed no significant difference between Si treatments (Tab. 1). The addition of 10 or 20 μM arsenate to the nutrient solution significantly decreased shoot

Figure 2: The As influx of excised roots of the wild and mutant rice treated with 50 μM arsenate ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) or arsenite (NaAsO_2) under different Si concentrations ($\text{K}_2\text{SiO}_3 \cdot n\text{H}_2\text{O}$). Bars present standard errors of the mean ($n = 4$). DW = dry weight.

dry weight of the wild-type rice as compared with the control (Tab. 1, $p < 0.001$). However, the addition of 10 μM arsenate to the nutrient solution had no effect on shoot dry weight of the mutant and only significantly decreased shoot dry weight of the mutant in the 20 μM –arsenate treatments (Tab. 1, $p < 0.001$). Root dry weights showed no significant difference between arsenate treatments (Tab. 1).

Table 1: Shoot and root dry weights of the wild and mutant rice with one of three As concentrations ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) and one of two Si concentrations ($\text{K}_2\text{SiO}_3 \cdot n\text{H}_2\text{O}$).

Line	Arsenate concentration / μM	Si concentration / mM	Shoot dry weight / g	Root dry weight / g
Wild	0	0	2.80 ± 0.12	0.74 ± 0.05
		1	3.01 ± 0.14	0.63 ± 0.06
	10	0	1.63 ± 0.16	0.57 ± 0.08
		1	2.27 ± 0.04	0.66 ± 0.04
	20	0	1.45 ± 0.03	0.63 ± 0.02
		1	2.06 ± 0.12	0.67 ± 0.06
Mutant	0	0	3.04 ± 0.19	0.80 ± 0.05
		1	3.21 ± 0.17	0.75 ± 0.03
	10	0	3.04 ± 0.09	0.90 ± 0.05
		1	3.10 ± 0.17	0.83 ± 0.07
	20	0	2.18 ± 0.22	0.74 ± 0.09
		1	2.18 ± 0.14	0.64 ± 0.03

Analysis of variance

Line (L)	$p < 0.001$	$p < 0.001$
Arsenate concentration (As(V))	$p < 0.001$	NS
Si concentration (Si)	$p = 0.002$	NS
L $\times$ As(V)	$p < 0.001$	$p = 0.031$
L $\times$ Si	$p = 0.020$	NS
As(V) $\times$ Si	NS	NS
L $\times$ As(V) $\times$ Si	NS	NS

error bars: standard errors ($n = 4$); NS = not significant.

Table 2: Shoot and root Si concentrations of the wild and mutant rice with one of three As concentrations ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) and one of two Si concentrations ($\text{K}_2\text{SiO}_3 \cdot n\text{H}_2\text{O}$).

Line	Arsenate concentration / μM	Si concentration / mM	Shoot Si concentration / mg g^{-1}	Root Si concentration / mg g^{-1}
Wild	0	0	1.01 ± 0.07	0.69 ± 0.15
		1	29.2 ± 2.68	2.15 ± 0.20
	10	0	1.08 ± 0.16	0.61 ± 0.14
		1	33.9 ± 1.13	2.39 ± 0.17
	20	0	1.06 ± 0.20	0.45 ± 0.06
		1	38.1 ± 2.03	2.13 ± 0.08
Mutant	0	0	0.54 ± 0.09	0.37 ± 0.03
		1	11.3 ± 0.16	1.69 ± 0.24
	10	0	0.76 ± 0.14	0.45 ± 0.01
		1	9.66 ± 0.38	1.27 ± 0.14
	20	0	0.83 ± 0.13	0.50 ± 0.06
		1	8.32 ± 0.92	1.27 ± 0.15
Analysis of variance				
Line (L)			$p < 0.001$	$p < 0.001$
Arsenate concentration (As(V))			NS	NS
Si concentration (Si)			$p < 0.001$	$p < 0.001$
L $\times$ As(V)			$p = 0.002$	NS
L $\times$ Si			$p < 0.001$	$p < 0.001$
As(V) $\times$ Si			NS	NS
L $\times$ As(V) $\times$ Si			$p = 0.001$	NS

Error bars: standard errors ($n = 4$); NS = not significant.

Irrespective of the lines and arsenate treatments, the addition of 1 mM Si to the nutrient solution significantly increased shoot and root Si concentrations compared with the control (Tab. 2, $p < 0.001$). Shoot Si concentrations of the wild-type rice were substantially higher than those of the mutant. Root Si concentrations of the wild-type rice were also higher than those of the mutant, but to a lesser extent (Tab. 2, $p < 0.001$).

In the present experiment, results showed that As accumulation in the mutant was significantly lower than that in the wild-type rice with 10 or 20 μM –arsenate and no Si treatments (Fig. 3, $p < 0.001$). For the wild-type rice, the addition of 1 mM Si to the nutrient solution containing 10 or 20 μM arsenate significantly decreased shoot and root As concentrations compared with no Si treatments (Fig. 3, $p < 0.001$). Shoot and root As concentrations of the wild type were reduced by 52.9%, 34.6%, 42.6%, and 26.7% in 10 or 20 μM arsenate with 1 mM–Si treatments, respectively. However, the addition of 1 mM Si to the nutrient solutions containing 10 or 20 μM arsenate had no significant effect on shoot and root As concentrations of the mutant rice (Fig. 3).

3.4 Effect of Si on As concentrations and As species in xylem sap

The Si concentration of the xylem sap of the wild-type rice was three-fold higher than that of the mutant rice with 1 mM–Si treatments (Fig. 4, $p < 0.001$). In both lines, the addition of

1 mM Si to the nutrient solution significantly increased Si concentrations in xylem sap compared with the control (Fig. 4, $p < 0.001$). There was no detectable Si in xylem sap of unamended control plants of both lines. The addition of 1 mM Si to the nutrient solution containing 15 μM arsenate significantly decreased As concentrations of the xylem sap of the wild-type rice compared with no-Si treatments (Fig. 4, $p < 0.001$), but had little effect on As concentrations in xylem sap of the mutant rice (Fig. 4). Arsenic concentrations of the xylem sap of the wild type were reduced by 51% with 15 μM –arsenate and 1 mM–Si treatments. With no Si addition, As concentrations in the xylem sap of the wild type were much higher than those of the mutant (Fig. 4, $p < 0.001$).

For the wild-type rice, the addition of 1 mM Si to the nutrient solution containing 15 μM arsenate significantly decreased As(III) concentration of xylem sap but had no effect on As(V) concentration (Fig. 5, $p < 0.001$). However, the Si addition had no effect on As(III) and As(V) concentrations in xylem sap of the mutant (Fig. 5). With no Si addition, As(III) concentrations in the xylem sap of the mutant were much lower than in the wild type (Fig. 5, $p < 0.001$).

4 Discussion

Ma et al. (2006) described the low-Si rice 1 (*Lsi1*) gene encoding a Si uptake transporter, which shows transport

Figure 3: Shoot and root As concentrations of the wild and mutant rice treated with one of two As concentrations (10 or 20 μ M As, $\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) and one of two Si concentrations (0 or 1 mM Si, $\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Different letters indicate significant differences by LSD test (0.05). Bars present standard errors of the mean ($n = 4$).

activity specific to silicon. They further confirmed that Lsi1 is permeable to arsenite in rice and is responsible for the primary uptake of arsenite from the external medium to the root cells (Ma et al., 2008). We firstly investigated the effect of internal and external Si on arsenite uptake by both lines. The Si uptake-defective mutant rice (*lsi1*) also showed a significant reduction in arsenite uptake (Fig. 1). These results are consistent with the reports of Ma et al. (2008). The presence of external Si restrained arsenite uptake and translocation by the wild type but not by the mutant (Fig. 1), which further confirms the results of Ma et al. (2008). The external Si-mediated reduction in arsenite uptake by rice is due to a direct competition in uptake between Si and arsenite because they share the same influx transporters Lsi1. Moreover, internal Si status also significantly affects arsenite uptake and translocation (Fig. 1). The effect of internal Si on arsenite uptake could appear in the translocation process from the root cells to the xylem.

Recently, an efflux transporter (Lsi2) for silicic acid has also been identified for rice roots (Ma et al., 2007). In contrast to Lsi1, Lsi2 mediates the efflux of silicic acid from the exodermis and the endodermis cells toward the direction of the stele. The following research indicated that Lsi2 is involved in the efflux of arsenite from the root cells to the xylem (Ma et al., 2008). The effect of internal Si on arsenite uptake is primarily explained by the involvement of Lsi2, and our results

Figure 4: The total of As and Si concentrations in xylem sap of the wild and mutant rice treated with 15 μ M As ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) with or without 1 mM Si ($\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Different letters indicate significant differences by LSD test (0.05). Bars present standard errors of the mean ($n = 4$).

further support the function of Lsi2. The internal Si-inhibited As(III) translocation from the root cells to the xylem occurs because of competition between Si and arsenite for Lsi2, which results in arsenite accumulation in the root cells and consequently a reduction in arsenite uptake by the wild-type rice (Fig. 1).

The presence of Si significantly decreased arsenate uptake by the wild-type rice (Fig. 3). These results are consistent with our former studies, which indicated that Si can reduce arsenate uptake by rice (Guo et al., 2005, 2007). Simultaneously, an interesting finding is that the Si uptake-defective mutant rice (*lsi1*) also showed a significant reduction in arsenate-uptake capacity (Fig. 3). Obviously, these results were not due to a direct competition in uptake between Si and arsenate because they do not share the same transporters (Meharg and Macnair, 1992; Ma et al., 2008). Duan et al. (2007) cloned and expressed both ACR2-like genes from the rice genome, which can catalyze arsenate reduction in rice. Although rice plants were supplied with arsenate, both As(III) and As(V) were detected in the xylem sap (Fig. 5), showing that As(V) can be reduced to As(III) and then transported to the xylem within rice. In addition, Xu et al. (2007) demonstrated that 92% of the As was arsenite from the PBS extracts of rice roots in the arsenate treatments for 1 d, indicating that most of the As in rice roots was in the form of

Figure 5: The As(III) and As(V) concentrations in xylem sap of the wild and mutant rice treated 15 μ M As ($\text{Na}_3\text{AsO}_4 \cdot 12\text{H}_2\text{O}$) with or without 1 mM Si ($\text{K}_2\text{SiO}_3 \cdot \text{nH}_2\text{O}$). Different letters indicate significant differences by LSD test (0.05). Bars present standard errors of the mean ($n = 4$).

arsenite when arsenate was initially supplied to the plants. Therefore, Si-inhibited arsenate uptake of rice may be explained by the involvement of Lsi2.

Arsenate taken up by rice-roots cells is immediately reduced to arsenite, which is transported toward the xylem sap *via* the Si/arsenite effluxer Lsi2 and is subject to a competitive inhibition from Si during the translocation process. Thus, arsenic loading to xylem sap showed the significant reduction in the wild-type rice with Si treatments (Fig. 4). Arsenic-species analysis further revealed that the decrease in As concentrations in xylem sap was mainly ascribed to the reduction in As(III) concentrations (Fig. 5), testifying that Si inhibited arsenate uptake by rice *via* the competition with the Si/arsenite effluxer Lsi2 during the translocation process. The *Lsi1* mutant is only defective in arsenite-uptake capacity from the external medium to the root cells but has the normal capacity of arsenite efflux from the root cells to the xylem (Ma et al., 2007). Therefore, the *Lsi1* mutant-reduced arsenate uptake cannot be explained in terms of involvement of Lsi2. Otherwise, rice roots rapidly reduce arsenate to arsenite, some of which is lost from the cells *via* efflux to the external medium (Xu et al., 2007). Xu et al. (2007) showed that arsenite efflux from roots is very rapid following arsenate uptake and arsenate in the nutrient solution was rapidly converted to arsenite. The *Lsi1* mutant showed a significant

reduction in arsenate-uptake capacity testifying the presence of an arsenite re-uptake process. Therefore, Si-inhibited arsenate uptake by rice may be explained with the involvement of Lsi1 when arsenite is present in the nutrient solution after transformation from arsenate. The re-uptake of arsenite effluxed from rice roots occurs *via* the transporter Lsi1 and is inhibited by Si, which results in a reduction in arsenate uptake by the wild-type rice.

5 Conclusion

We conclude that a high internal and external Si concentration can both inhibit arsenite and arsenate uptake by rice. External Si-mediated reduction in arsenite uptake by rice is due to a direct uptake competition between Si and arsenite as they share the same influx transporter Lsi1. Internal Si-mediated reduction in arsenite uptake by rice is probably due to the competition of the Si/arsenite efflux transporter Lsi2 during As(III) translocation from the root cells to the xylem. Thus, the Si-inhibited arsenate uptake by rice may involve two possible processes: The arsenate taken by rice roots is immediately reduced to arsenite, which is either pumped into the xylem *via* the Si/arsenite efflux transporter Lsi2 or exsuded to the external medium and taken up by the Si/arsenite influxer Lsi1. This arsenite re-uptake may be inhibited by Si.

Acknowledgments

We thank Prof. JianFeng Ma (Research Institute for Biore-sources, Okayama University, Japan) for providing rice seeds. This study was partly supported by the *Natural Science Foundation of China* (40861018) and the *Natural Science Foundation of the Inner Mongolia of China* (20080404MS0611).

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