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The role of the rice aquaporin Lsi1 in arsenite efflux from roots

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Summary

- When supplied with arsenate (As(V)), plant roots extrude a substantial amount of arsenite (As(III)) to the external medium through as yet unidentified pathways. The rice (*Oryza sativa*) silicon transporter Lsi1 (OsNIP2;1, an aquaporin channel) is the major entry route of arsenite into rice roots. Whether Lsi1 also mediates arsenite efflux was investigated.
- Expression of *Lsi1* in *Xenopus laevis* oocytes enhanced arsenite efflux, indicating that Lsi1 facilitates arsenite transport bidirectionally.
- Arsenite was the predominant arsenic species in arsenate-exposed rice plants. During 24-h exposure to 5 μM arsenate, rice roots extruded arsenite to the external medium rapidly, accounting for 60–90% of the arsenate uptake. A rice mutant defective in Lsi1 (*lsi1*) extruded significantly less arsenite than the wild-type rice and, as a result, accumulated more arsenite in the roots. By contrast, *Lsi2* mutation had little effect on arsenite efflux to the external medium.
- We conclude that Lsi1 plays a role in arsenite efflux in rice roots exposed to arsenate. However, this pathway accounts for only 15–20% of the total efflux, suggesting the existence of other efflux transporters.

Introduction

Arsenic (As) is a nonessential and toxic metalloid to plants. Plants have evolved different resistance or tolerance mechanisms to deal with excess As in the environment (Meharg & Hartley-Whitaker, 2002; Zhao *et al.*, 2009). Arsenic is also highly toxic to animals and humans. Human intake of inorganic As, a nonthreshold carcinogen, comes mainly from drinking water and foods (Schoof *et al.*, 1999; Kile *et al.*, 2007). Recent studies have shown that paddy rice (*Oryza sativa*) is particularly efficient in As accumulation (Williams *et al.*, 2007) because of a combination of increased As bioavailability in submerged paddy soil (Xu *et al.*, 2008) and arsenite (As(III)) sharing the pathway which mediates strong uptake of silicon (Si) in rice (Ma *et al.*, 2008). Consumption of rice constitutes a major exposure route of inorganic As in populations relying on rice as the staple food (Ohno *et al.*, 2007; Mondal & Polya, 2008; Meharg *et al.*, 2009). It is therefore imperative that the mechanisms of As uptake and metabolism be understood so as to develop counter measures to reduce this widespread food-chain contamination.

Arsenate (As(V)) is taken up by plant roots via phosphate transporters (for a review, see Zhao *et al.*, 2009). Plants can also absorb arsenite when it is present in the medium (Meharg & Jardine, 2003). Recent studies have shown that a number of plant aquaporins belonging to the NIP (nodulin 26-like intrinsic protein) subfamily are permeable to arsenite, which is present predominantly as the undissociated neutral molecule (arsenous acid) at pH < 8 (Bienert *et al.*, 2008; Isayenkov & Maathuis, 2008; Ma *et al.*, 2008; Kamiya *et al.*, 2009). In rice, two silicon (Si) transporters, Lsi1 (OsNIP2;1 aquaporin) and Lsi2 (an efflux transporter), play a major role in the uptake of arsenite (Ma *et al.*, 2008). Lsi1 is localized to the distal side of the plasma membranes in the exodermal and endodermal cells responsible for the influx of silicic acid and arsenite (Ma *et al.*, 2006, 2008), whereas Lsi2 is localized to the proximal sides of the same root cells responsible for the efflux of silicic acid and arsenite towards the stele for xylem loading (Ma *et al.*, 2007,

2008). Interestingly, Lsi1 also contributes to the uptake of methylated As species, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Li *et al.*, 2009). In *Arabidopsis thaliana*, AtNIP1;1 and AtNIP7;1 have been shown to mediate arsenite uptake (Isayenkov & Maathuis, 2008; Kamiya *et al.*, 2009).

Recently, Xu *et al.* (2007) showed that following arsenate uptake and its rapid reduction to arsenite in the root cells of rice and tomato (*Lycopersicon esculentum*), a large proportion of arsenite is released to the external medium. This phenomenon was observed in a number of other plant species grown in hydroponic cultures (Logoteta *et al.*, 2009; Su *et al.*, 2009; Zhang *et al.*, 2009), suggesting that arsenite efflux may be a constitutive mechanism of As detoxification in plants. Accumulation of arsenite in the rhizosphere of sunflower (*Helianthus annuus*) and maize (*Zea mays*) grown in aerobic soil or sand/goethite treated with arsenate (Ultra *et al.*, 2007; Vetterlein *et al.*, 2007) provides further evidence that a cycle of arsenate uptake, reduction and arsenite efflux also occurs in soil-grown plants. This cycle is reminiscent of that found in microorganisms which extrude arsenite from the cells as a major detoxification mechanism (reviewed by Bhattacharjee & Rosen, 2007). Arsenite is extruded by the membrane transporters ArsB or ArsAB pump in *Escherichia coli* (Dey *et al.*, 1994; Kuroda *et al.*, 1997) and by Acr3p in yeast (*Saccharomyces cerevisiae*) (Wysocki *et al.*, 1997), both of which are active mechanisms coupled to the proton motive force (in the case of ArsB or Acr3p), or to an ATPase (in the case of ArsAB). Another pathway of arsenite efflux through the aquaglyceroporin aqpS was found to operate in the legume symbiont *Sinorhizobium meliloti* (Yang *et al.*, 2005). This pathway is different from that in *E. coli* and yeast in that arsenite efflux is a passive diffusion down the concentration gradient across the membrane. The mechanism(s) responsible for arsenite efflux to the external medium have not yet been elucidated in plants. Expression in yeast of several plant genes encoding NIP aquaporins, including rice *OsNIP2;1*, was found to enhance the tolerance to arsenate, suggesting that these aquaporins may be involved in As detoxification through arsenite efflux (Bienert *et al.*, 2008; Isayenkov & Maathuis, 2008), similar to that found in *S. meliloti* (Yang *et al.*, 2005). The studies of Bienert *et al.* (2008) and Isayenkov & Maathuis (2008) suggest that some NIP aquaporins may mediate bidirectional transport of arsenite. However, the *in planta* function of aquaporins in arsenite efflux has not been established.

The objective of this study was to investigate the role of the rice silicon transporter Lsi1 in arsenite efflux to the external medium. The ability of Lsi1 to mediate arsenite efflux was determined in a heterologous assay with *Xenopus laevis* oocytes. Arsenite efflux, As accumulation and speciation in rice plants were characterized in mutants defective in either Lsi1 or Lsi2 and their corresponding wild types exposed to arsenate.

Materials and Methods

Arsenite efflux assay in *X. laevis* oocytes

Arsenite efflux mediated by rice Lsi1 was assayed using *X. laevis* oocytes. Oocytes were isolated from adult female *X. laevis* frogs, and placed in a modified Barth's saline (MBS) solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 15 mM Tris-HCl pH 7.6, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 10 μg ml^{-1} sodium penicillin, 10 μg ml^{-1} streptomycin sulfate). Oocytes were then treated with 0.1% collagenase type B (Roche Diagnostics) in a calcium (Ca) free MBS for 1.5 h to remove follicular cell layers, washed five times with MBS free of collagenase and selected according to the size and development stage. Selected oocytes were incubated for 1 d in MBS at 18°C. The open reading frame of the *Lsi1* gene was amplified and cloned as described previously (Mitani *et al.*, 2008) and the cRNA with cap analogue was synthesized with mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit (Ambion) according to the manufacture's instructions. Fifty nanoliters of cRNA (1 ng nl^{-1}) were injected into the selected oocytes using a Nanoject II automatic injector (Drummond Scientific, Broomall, PA, USA). As a negative control, 50 nl of RNase-free water were injected. Each treatment was replicated three times, with 15 oocytes per replicate. One day after injection of cRNA or water, 50 nl of arsenite (0.1 mM or 1 mM) were injected to each oocyte. The oocytes were then placed in a fresh MBS buffer solution. At 30 min and 60 min, the solution was collected for quantification of As efflux. At 60 min, oocytes were harvested for the determination of the amount of remaining arsenite.

Effects of Lsi1 or Lsi2 mutation on arsenite efflux and arsenic accumulation in rice

The rice (*Oryza sativa* L.) *lsi1* mutant (Ma *et al.*, 2002) and its wild-type (WT1) cv. Oochikara, and *lsi2* (Ma *et al.*, 2007) and its wild type (WT2) cv. T-65 were used. Seeds were surface-sterilized with 0.5% NaOCl for 15 min, rinsed thoroughly and soaked in deionized water overnight. Seeds were placed on a nylon net floating on a 0.5 mM $CaCl_2$ solution. After germination, seedlings were transferred to a 30-l container filled with the half strength Kimura solution and precultured for 12–20 d. The nutrient composition was as follows: 0.091 mM KNO_3, 0.183 mM $Ca(NO_3)_2$, 0.274 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.183 mM $(NH_4)_2SO_4$, 0.5 μM $MnCl_2$, 3 μM H_3BO_3, 0.1 μM $(NH_4)_6Mo_7O_{24}$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 40 μM NaFe(III)-EDTA, 2 mM MES (2-morpholinoethanesulfonic acid; pH adjusted to 5.5 with NaOH). Nutrient solution was renewed every 3 d. The growth conditions were 16 h photoperiod with a light intensity of 500 μmol

m^{-2} s^{-1}, 30°C/25°C day/night temperatures and 70% relative humidity.

Twenty-day-old seedlings of WT1 and *lsi1* were transferred to 1-l pots (four plants per pot) containing 0.9 l nutrient solution of the same composition as described earlier, except that 0.1 mM KH_2PO_4 was replaced with KCl to allow a faster uptake of arsenate. Arsenate, in the form of Na_2HAsO_4, was added to each pot at 5 μM. Each rice line was replicated in four pots. At 2, 6 and 24 h, an aliquot of nutrient solution (0.5 ml) was collected from each pot for As speciation analysis. Water loss through transpiration was recorded. At 24 h, roots were rinsed briefly in an ice-cold desorption solution containing 1 mM K_2HPO_4, 0.5 mM $Ca(NO_3)_2$ and 5 mM MES (pH 5.5), and immersed in 0.9 l of the same solution for 10 min to remove apoplastic As. Roots and shoots were separated, blotted dry, weighed and frozen in liquid nitrogen for analysis of As species. A similar experiment was performed to compare arsenate uptake and arsenite efflux between WT2 and *lsi2*.

In a further experiment, 20-d-old seedlings of WT1 and *lsi1* were exposed to 5 μM arsenate (in a phosphate-free nutrient solution) for 4 h as described earlier. Aliquots of the exposure solutions were taken at 4 h for As speciation analysis. Roots were rinsed briefly with deionized water, followed by 10 min desorption of apoplastic As with 1 mM K_2HPO_4, 0.5 mM $Ca(NO_3)_2$ and 5 mM MES (pH 5.5) at the normal temperature (30°C). Plants were then transferred to 0.9 l fresh nutrient solution (no phosphate) for 2 h to collect arsenite effluxed from the roots. Arsenic speciation in both the exposure and efflux solutions were determined as described later.

Effects of mercury on arsenate uptake and arsenite efflux

This experiment was carried out to investigate the effect of mercury (Hg^{2+}), which is often used as an aquaporin channel blocker (Maurel, 1997), on arsenate uptake and arsenite efflux. Twelve-day-old seedlings of WT1 and *lsi1* were transferred to 0.38-l pots (three plants per pot) containing 0.35 l nutrient solution without phosphorus (P) as described above. Arsenate was added to each pot at 5 μM. Fifty micromoles of $HgCl_2$ was added to four pots of each rice line, with another four pots receiving no $HgCl_2$. Aliquots (0.5 ml) of the nutrient solutions were taken for As speciation at 2, 6 and 24 h. Water loss through transpiration was also recorded.

Analytical methods

For the analysis of As speciation in rice roots and shoots, 0.2–0.5 g frozen fresh materials were ground in liquid nitrogen to fine powder and were extracted with 20 ml phosphate buffer solution (PBS: 2 mM NaH_2PO_4, 0.2 mM Na_2-EDTA, pH 6.0) for 1 h under sonication. The extracts were filtered through a filter paper and then through 0.45-μm filters. Arsenic speciation in nutrient solutions and plant extracts was determined using high-performance liquid chromatography (HPLC)-inductively coupled plasma (ICP)-MS (Agilent LC1100 series and Agilent ICP-MS 7500ce; Agilent Technologies, Santa Clara, CA, USA), as described previously (Xu *et al.*, 2007) with modification. Arsenic species (arsenite, arsenate, DMA and MMA) were separated by an anion-exchange column (Hamilton PRP X-100; Reno, NV, USA). The mobile phase contained 6.6 mM $NH_4H_2PO_4$, 6.6 mM NH_4NO_3 and 0.2 mM EDTA (pH 6.3), and was run isocratically at 0.65 ml min^{-1}. The outlet of the separation column was connected to a concentric nebulizer and a water-jacketed cyclonic spray chamber of the ICP-MS. An internal standard (germanium (Ge)) was mixed continuously with the post-column solution through a peristaltic pump. Signals at m/z 75 (As), 72 (Ge) and 35 (chlorine (Cl)) were collected with a dwell time of 0.5 s for As and Ge and 0.2 s for Cl. Possible polyatomic interference of $^{40}Ar^{35}Cl$ on *m/z* 75 was removed by the Agilent Octopole Reaction System operating in the helium gas mode. The As signal was normalized by the Ge signal to correct any signal drift during the analysis. Arsenic species in the samples were quantified by external calibration curves with peak areas. Only arsenite and arsenate were detected in the samples.

Statistical analysis

The significance of the difference between treatments or between rice lines was determined by ANOVA.

Results

Expression of *Lsi1* in *X. laevis* oocytes enhances arsenite efflux

To investigate whether the rice Lsi1 channel allows arsenite efflux, the *Lsi1* gene from the wild-type rice was expressed in *X. laevis* oocytes. Arsenite (0.1 mM or 1 mM) was then injected into oocytes, and the amount of arsenite remaining in the oocytes 1 h after injection was determined. Compared with the control, *Lsi1* expression decreased the amount of arsenite remaining in oocytes by 29% ($P = 0.058$) in the 0.1 mM arsenite treatment, and by 43% in the 1 mM arsenite treatment ($P < 0.001$; Fig. 1). The amount of arsenite extruded into the external solution could not be determined reliably in the 0.1 mM arsenite treatment because of the very small amount of arsenite injected into the oocytes. In the 1 mM arsenite treatment, the amount of arsenite extruded by the *Lsi1*-expressing oocytes (expressed as a percentage of the arsenite injected) was more than 10 times higher than that in the control at both 30 min and

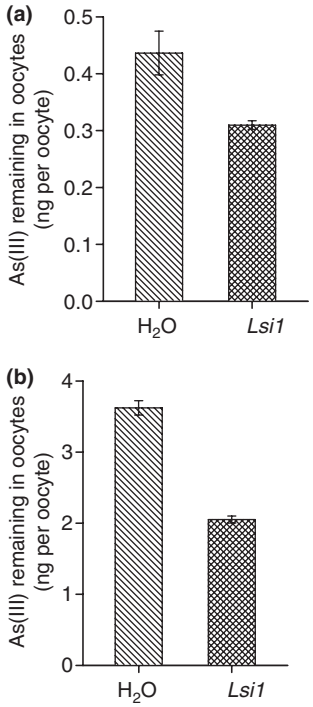

Fig. 1 Functional assay of arsenite efflux in *Xenopus laevis* oocytes expressing the rice *Lsi1* gene. The amount of arsenite remaining in oocytes 1 h after arsenite (As(III)) injection was determined: (a) injection of 0.1 μM arsenite, (b) injection of 1 μM arsenite; H_2O serves as a control. Data are means ± SE (n = 3).

60 min ($P < 0.001$ and $P < 0.05$, respectively; see the Supporting Information, Fig. S1). These results indicate that *Lsi1* expression enhanced arsenite efflux from oocytes.

Arsenate uptake and arsenite efflux by the *lsi1* mutant and wild-type rice

To investigate the role of Lsi1 in arsenite efflux from rice roots, the *lsi1* mutant and its wild-type rice (WT1) were exposed to 5 μM arsenate in the nutrient solution. During the 24-h time-course, arsenate in the nutrient solution was depleted, accompanied by the appearance of arsenite. Arsenate uptake and arsenite efflux were calculated from the changes in the arsenate and arsenite concentrations in the nutrient solution over the 24 h period, corrected for water loss owing to transpiration and normalized by root biomass (Fig. 2a,b). Arsenate uptake was not significantly different between *lsi1* and WT1 at 2 h and 24 h, but was significantly ($P < 0.001$) higher in WT1 at 6 h. Arsenite efflux was not significantly different between *lsi1* and WT1 at 2 h, but was 42% and 23% lower in *lsi1* than WT1 at 6 h and 24 h, respectively ($P < 0.001$ and $P = 0.06$, respectively). Fig. 2(c) shows arsenite efflux as a percentage of arsenate uptake; this gives a better measure of arsenite efflux because it takes into account the variation in arsenate uptake. A large proportion (65–88%) of arsenate uptake was extruded to the external solution as arsenite. The relatively large error bars at 2 h were a result of small uptake and efflux at this time-point. At both 6 h and 24 h, proportionally less arsenite was extruded from *lsi1* roots than WT1 roots; the difference was 13% at both time-points ($P < 0.001$). On a relative scale, arsenite efflux from *lsi1* roots was 15–20% lower than that from WT1. After 24 h exposure to arsenate, As uptake and speciation in roots and shoots were determined (Fig. 3). On average 96% of the As in roots was arsenite, with no significant difference between *lsi1* and WT1 in As speciation. However, *lsi1* roots accumulated 43% more arsenite than WT1 ($P < 0.001$), while no difference was found in the arsenate concentration (Fig. 3a). Only arsenite was detected in the shoots and its concentration was similar between *lsi1* and WT1 ($P = 0.14$). In contrast to *lsi1*, *lsi2* behaved similarly to WT2 with regard to arsenate uptake and arsenite efflux (Fig. S2).

In a further experiment, arsenite efflux from *lsi1* and WT1 roots was measured both during the initial 4-h period of arsenate exposure and in the subsequent 2-h period without arsenate. During the initial arsenate exposure phase, arsenite efflux from *lsi1* accounted for 57% of the arsenate

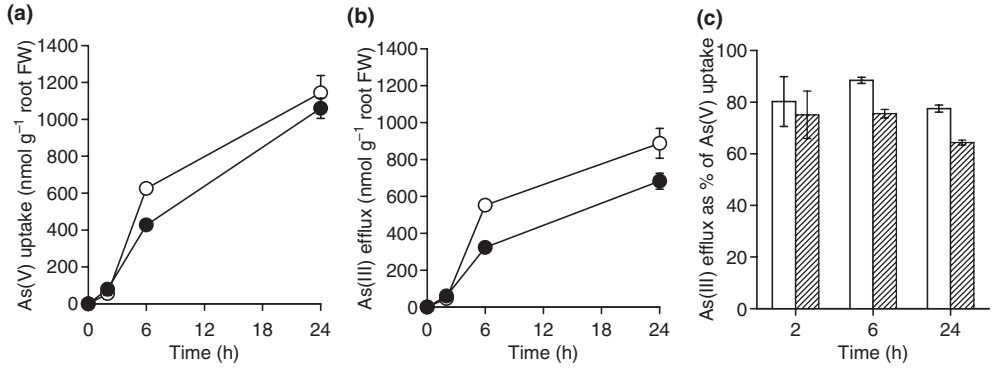

Fig. 2 Arsenate (As(V)) uptake (a) and arsenite (As(III)) efflux (b) of the rice *lsi1* mutant and its wild-type (WT1) after exposure to 5 μM arsenate for 24 h. (c) Arsenite efflux as a percentage of arsenate uptake. Open circles/bars, WT1; closed circles/hatched bars, *lsi1*. Data are means ± SE (n = 4).

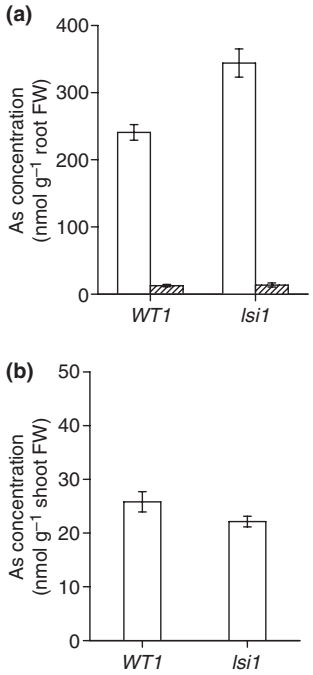

Fig. 3 Arsenic (As) speciation in (a) roots and (b) shoots of the rice *lsi1* mutant and its wild type (WT1) after exposure to 5 μM arsenate for 24 h. Open bars, arsenite (As(III)); hatched bars, arsenate (As(V)). Data are means ± SE (n = 4).

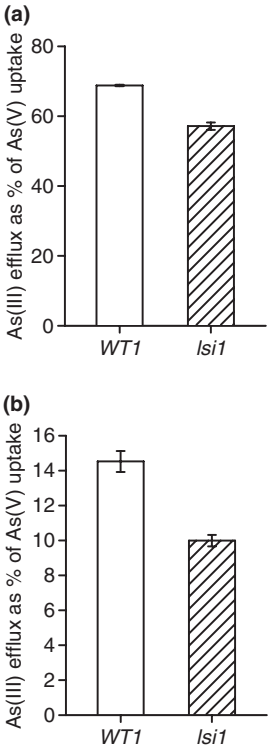

Fig. 4 Arsenite (As(III)) efflux as a percentage of arsenate uptake of the rice *lsi1* mutant and its wild type (WT1) during (a) the initial 2-h phase of arsenate (As(V)) exposure, (b) the subsequent 2-h period without arsenate exposure. Data are means ± SE (n = 4).

uptake; this was significantly ($P < 0.001$) smaller than WT1 (69% of the arsenate uptake; Fig. 4a). During the subsequent 2-h period, *lsi1* also extruded less arsenite than WT1 (10% and 14% of the As accumulated in the roots of *lsi1* and WT1, respectively, $P < 0.001$; Fig. 4b).

Effect of Hg^{2+} on arsenate uptake and arsenite efflux

The effect of Hg^{2+} on arsenate uptake and arsenite efflux was investigated. The addition of 50 μM $HgCl_2$ inhibited arsenate uptake markedly. In the treatment without Hg^{2+}, arsenate in the nutrient solution was depleted by 72–86% by *lsi1* and WT1 at 24 h; this compares with only *c.* 2% in both lines of rice in the presence of Hg^{2+} (Fig. S3). In the absence of Hg^{2+}, considerable amounts of arsenite were produced in the nutrient solution as a result of efflux; by contrast, no arsenite was detected in the $+Hg^{2+}$ treatment.

In the absence of Hg^{2+}, there was no significant difference between WT1 and *lsi1* in arsenate uptake ($P > 0.05$ for all three time-points), but the latter had less arsenite efflux (significant at $P < 0.01$ at all three time-points, Fig. S4). At 6 h and 24 h, arsenite efflux as a percentage of arsenate uptake was 10% smaller in *lsi1* than in WT1 ($P < 0.001$; Fig. S4). These differences were similar to those observed in the previous experiment (Fig. 3), although the absolute values of arsenate uptake and arsenite efflux by both WT1 and *lsi1* were larger in this experiment, probably owing to smaller plants and hence larger solution volume to root biomass ratio. The addition of Hg^{2+} reduced arsenate uptake by 84–92% in both WT1 and *lsi1* (Fig. S4). Furthermore, no arsenite efflux could be detected in the $+Hg^{2+}$ treatment and, because of this, arsenite efflux as a percentage of arsenate uptake was zero. Our results are in agreement with those of Meharg & Jardine (2003), who found that Hg^{2+} inhibited the uptake of both arsenite and arsenate by rice to a similar extent. Therefore, it appears that Hg^{2+} is not a specific inhibitor of aquaporin channels.

Water loss through transpiration was also decreased significantly by Hg^{2+} in both *lsi1* and WT1 ($P < 0.05$), but there was no significant difference between the two rice lines (data not shown; also see Tamai & Ma, 2003).

Discussion

As has been reported previously in a number of plant species (Xu *et al.*, 2007; Logoteta *et al.*, 2009; Su *et al.*, 2009; Zhang *et al.*, 2009), uptake of arsenate by rice roots was accompanied by a rapid efflux of arsenite to the external solution. Arsenate taken up by rice roots was efficiently reduced to arsenite (Fig. 3); this reaction may be catalysed by arsenate reductase(s) such as the yeast ACR2 homologue in plants (Bleeker *et al.*, 2006; Dhankher *et al.*, 2006; Duan

et al., 2007). A large proportion of arsenite (*c.* 60–90% of the arsenate uptake) was extruded to the external medium during the 24-h period of arsenate exposure (Figs 2,4; Fig. S4). When roots pre-exposed to arsenate were transferred to arsenate-free solution, there was further efflux of arsenite, although not as much as during the arsenate exposure period (Fig. 4). This decreased efflux with time may be caused by increased arsenite–phytochelatin complexation in roots (Raab *et al.*, 2005).

The question specifically addressed by this study was whether the aquaporin Lsi1 plays a role in arsenite efflux from rice roots. A previous study by Ma *et al.* (2008) had established the function of Lsi1 in mediating arsenite uptake into both *Xenopus* oocytes and rice roots. In the present study, when arsenite was injected into oocytes, expression of *Lsi1* clearly enhanced arsenite efflux to the external medium (Fig. 1; Fig. S1), indicating that Lsi1 is permeable to arsenite from both directions, with the direction of flux depending on the concentration gradient. In the plant experiments (Figs 2,4; Fig. S4), arsenite efflux, measured as a percentage of arsenate uptake, was significantly lower in the *lsi1* mutant than the wild-type, with the mutant losing *c.* 15–20% of the efflux capacity of the wild-type. As a result, considerably more (43%) arsenite accumulated in the roots of the mutant initially exposed to arsenate (Fig. 3). These results indicate that Lsi1 plays a part in arsenite efflux from rice roots when the concentration of cellular arsenite is larger than that in the external medium. This would be the case during the initial phase of arsenate exposure when there was no, or very little, arsenite in the nutrient solution. Whether the arsenite gradient still existed at the end of arsenate exposure would depend on the extent of arsenite complexation by thiol compounds. If by 24 h, 60% of the cellular arsenite was complexed (Raab *et al.*, 2005), the cellular arsenite concentration would be *c.* 30–50 times higher than the external arsenite (estimated from the data in Figs 2 and 3). However, if the extent of arsenite complexation reached 99% (Pickering *et al.*, 2000), cellular and external arsenite concentrations would be more or less in equilibrium and passive efflux of arsenite would stop. The conclusion that Lsi1 allows arsenite efflux from rice roots is also consistent with an enhanced arsenate tolerance in yeast expressing *Lsi1* and several other NIP genes, which are likely to facilitate arsenite efflux and decrease the cellular burden of As (Bienert *et al.*, 2008; Isayenkov & Maathuis, 2008).

Interestingly, Lsi2 mutation had no effect on arsenite efflux to the external solution (Fig. S2), even though Lsi2 is an efflux transporter for both silicic acid and arsenite (Ma *et al.*, 2007, 2008). This is not surprising because of its localization to the proximal side of the plasma membranes in the exodermis and endodermis cells, mediating active efflux of silicic acid and arsenite toward the stele rather than toward the external medium (Ma *et al.*, 2007, 2008).

The nature of bidirectional permeability of Lsi1 means that its mutation can affect both the uptake and the efflux of arsenite. This can lead to either decreased As accumulation in roots when plants are supplied with arsenite (Ma *et al.*, 2008), or increased As accumulation when plants are exposed to arsenate (as in the present study). In submerged paddy soil, although arsenite is the dominant species of As (Takahashi *et al.*, 2004; Xu *et al.*, 2008), arsenate may also be present especially in the rice rhizosphere, which is made more oxic than the bulk soil because of the release of oxygen from the root aerenchyma. In a situation where both arsenate and arsenite are taken up by rice roots, the overall effect of Lsi1 mutation on net As accumulation may be small. This was indeed observed when the *lsi1* mutant was grown to maturity in a paddy field (Ma *et al.*, 2008).

The rice Lsi1 aquaporin channel provides the main entry route of silicic acid into root cells (Ma *et al.*, 2006). Lsi1 is also permeable to silicic acid from both directions (Mitani *et al.*, 2008). However, Lsi1 mutation has a profound effect on Si accumulation in rice in both short- and long-term experiments (Ma *et al.*, 2004, 2006). The difference between Si and As can be explained by the highly efficient pumping of Si toward xylem in rice (Mitani & Ma, 2005). This process results in a relatively low symplasmic concentration of Si in root cortical cells and thus minimizes the chance of Si diffusion out of the cells to the external medium. In comparison, root to shoot transport of As is restricted in most plant species with the exception of As hyperaccumulators (Zhao *et al.*, 2009). An important reason is the complexation of arsenite with phytochelatins in root cells, which appears to restrict As mobility to the shoots (Raab *et al.*, 2005). Furthermore, uncomplexed arsenite appears to be much more easily released to the external medium than loaded into the xylem in nonhyperaccumulator plants, such as rice. This behavior is different from that of Si, even though both Si transporters Lsi1 and Lsi2 also transport arsenite (Ma *et al.*, 2008). The reasons for this difference remain unknown.

Although Lsi1 plays a role in arsenite efflux from rice roots, this pathway accounts for a relatively small proportion (15–20%) of the total efflux. It is possible that other NIP aquaporins may also contribute to arsenite efflux (Bienert *et al.*, 2008). In *E. coli*, arsenite is actively extruded from the cell by the ArsB or ArsAB pump and in yeast by Acr3p (Bhattacharjee & Rosen, 2007). The sensitivity of arsenite efflux from tomato roots to the protonophore carbonylcyanide *m*-chlorophenylhydrazone (CCCP) suggests the possibility of an active efflux mechanism in plants (Xu *et al.*, 2007) in addition to the passive efflux through aquaporins, although direct evidence for this mechanism has yet to be obtained.

Future research should aim to identify other transporters involved in arsenite efflux from plant roots, as enhanced

arsenite efflux may be a strategy to increase tolerance to As and to decrease As accumulation in food crops.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Effect of *Lsi1* expression on arsenite efflux from the *Xenopus laevis* oocytes.

Fig. S2 Arsenate uptake and arsenite efflux of the rice *lsi2* mutant and its wild-type (WT2) after exposure to 5 μM arsenate for 24 h.

Fig. S3 Changes in arsenic (As) speciation in the nutrient solution during the 24 h time-course as influenced by the addition of 50 μM $HgCl_2$.

Fig. S4 Effect of $HgCl_2$ on arsenate uptake and arsenite efflux of the rice *lsi1* mutant and its wild-type (WT1) after exposure to 5 μM arsenate for 24 h.

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