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Inositol transporters AtINT2 and AtINT4 regulate arsenic accumulation in Arabidopsis seeds

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Abstract

Arsenic is a global environmental contaminant that threatens tens of millions people world-wide via food and water. Understanding how arsenic is accumulated in crop seeds is of critical importance. To date, membrane transport proteins catalyzing arsenic uptake by roots and translocation through xylem to shoots have been characterized. However, no transporters responsible for loading arsenic from xylem into phloem and further unloading into plant seeds have been identified. In this study we demonstrate that expressing the gene for either *Arabidopsis thaliana* inositol transporter *AtINT2* or *AtINT4* in *Saccharomyces cerevisiae* leads to increased arsenic accumulation and elevated sensitivity to arsenite [As(III)], and *Xenopus laevis* oocytes expressing AtINT2 import As(III). When *A. thaliana* plants with disruptions in either *AtINT2* or *AtINT4* were supplemented with As(III) through roots, there was a substantial decrease in both the

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Author Contributions

Y.G.Z., Z.J.L., B.P.R., and N.S. designed the research. G.L.D., Y. H., S.S., J.M., J.C and B.D. performed research and analyzed data. All authors were involved in extensive discussions and wrote the manuscript.

Competing financial interests

The authors declare that they have no competing financial interests.

arsenic content in the phloem extrude and in total arsenic accumulation in siliques and seeds. Similarly, when As(III) is fed through the leaves, there was a very large decrease in arsenic accumulation in siliques and seeds compared with wild-type plants. These results clearly demonstrate that inositol transporters are responsible for As(III) loading into phloem, the key step regulating arsenic accumulation in seeds.

Introduction

Arsenic is a Group-1 carcinogen[1]. This toxic metalloid is ubiquitous in soil and water due to weathering of minerals and to anthropogenic agricultural and industrial activities[2]. Arsenic in soil and water is taken up by plant roots and retained in edible tissues representing the major sources of dietary arsenic[3]. It is estimated that rice contributes up to 50% of the total dietary arsenic for West Bengal and Bangladesh populations and up to 60% for Chinese population[4–5]. Thus, reduction of arsenic in our food supply is essential for public health. A critical step in the accumulation of arsenic by plants is its transport across cellular membranes. Thus, the identification of the responsible genes and gene products can lead to new strategies to reduce the arsenic content of plants. The pathways of arsenic uptake by roots and translocation through the xylem to the shoots are known, but the key steps of loading arsenic from xylem into phloem and further unloading into seeds such as rice grains have not been understood until this study[6].

Plants, including *A. thaliana* and *Oryza sativa* (rice), take up pentavalent inorganic arsenate [As(V)] into roots by phosphate transporters (e.g. PHT1;1 and PHT1;4 in *A. thaliana*[7], and OsPTs in rice)[8–9]. Trivalent arsenite [As(III)], is taken up by cells of nearly every organism including plant root cells by aquaglyceroporins (AQPs)[10–13]. In rice the AQP channel Lsi1, which was first identified as a silicon influx transporter, also mediates As(III), MAs(V) and DMAs(V) uptake[12,14]. Once As(V) has been imported into the cytosol of root cells, it is rapidly reduced to As(III), part of which is sequestered in vacuole[15], and another part is translocated to the shoots via the xylem[16]. In rice, movement of As(III) into the xylem is mediated by the efflux carrier Lsi2, which is a transporter for Si(IV) and organoarsenicals as well[12,14]. In the straw of *Lsi2* mutants, arsenic accumulation was only 13 – 19% of the wild-type (WT), and in *Lsi2* grains 63% and 51% of the corresponding WT plant[12]. *Lsi1* and *Lsi2* are expressed only in roots[17] and determine the amount of arsenic loading into the xylem. However, xylem transport is directed mainly to the vegetative organs but not to the reproductive tissues such as grains[18]. This explains why *Lsi2* mutations result in a greater reduction of arsenic accumulation in rice straw than in grains. Phloem transport has been considered central for arsenic translocation to the grains, and approximately 90% of the As(III) in rice grains were transported via the phloem[19–23]. In addition, although the *Lsi2* mutation significantly reduced arsenic accumulation in rice grains, it also led to reduced silicon transport, which results in poorer plant growth and yield[12]. Therefore, it is of considerable importance to elucidate the pathways of arsenic loading into the phloem and from there into the seeds in terms of human exposure to arsenic.

Depending on the growth conditions, *S. cerevisiae* takes up about 20% of total As(III) by the AQP Fps1p and about 80% by hexose transporters[24]. Mammalian GLUT1 also transports

As(III) and MAs(V) [11,25]. Both the yeast hexose transporters and GLUT1 belong to the monosaccharide transporter-like (MST-like) superfamily. MST-like transporters mediate the uptake of a wide range of substrates, including pentoses, hexoses and inositols[26]. *A. thaliana* inositol transporters (INTs) represent a subgroup within the MST-like superfamily[27–28]. We, therefore, considered the possibility that As(III) might be a substrate of INTs. The INT family in *A. thaliana* includes three genes that encode AtINT1, AtINT2, AtINT4 and a pseudogene, *AtINT3*, that does not encode a functional protein[28]. While AtINT1 is a tonoplast protein[29], AtINT2 and AtINT4 are plasma membrane H^+-coupled transporters that mainly expressed in the companion cells of phloem and mediate inositol uptake into the phloem and deliver mesophyll-derived inositol to the reproductive tissues[28,30]. We hypothesize that AtINT2 and AtINT4 are involved in loading of arsenic into the phloem and are key transporters regulating arsenic accumulation in plant seeds. In this study, the arsenic transport properties of AtINT2 and AtINT4 were examined by expression in yeast, *X. leavis* oocytes and *A. thaliana*. Here we demonstrate that inositol transporters AtINT2 and AtINT4 are also functional arsenic transporters and required for the long-distance transport of arsenite through the phloem and into *A. thaliana* seeds. We propose that inositol transporters in crop plants such as rice may be the key to the introduction of arsenic into the food supply of the majority of the world's population.

Results

AtINT2 and AtINT4 catalyze arsenic uptake in yeast and X. laevis oocytes

AtINT2 and *AtINT4* were expressed in *S. cerevisiae* strain D458-1B[28–31]. This strain carries mutations in the *ITR1* gene, which encodes an AtINT ortholog, and in the *INO1* gene. Cells of yeast strain D458-1B expressing either *AtINT2* or *AtINT4* were more sensitive to As(III) than those with vector only (Fig. 1a). To further confirm the arsenic sensitive phenotype, the *AtINT2* and *AtINT4* cDNAs were expressed in *S. cerevisiae* strain MG100, which has a disruption of the *ACR3* gene that encodes an As(III) efflux transporter and is hypersensitive to As(III) [32]. MG100 expressing either *AtINT2* or *AtINT4* became even more sensitive to As(III) (Fig. 1b). These results indicated that either AtINT2 or AtINT4 expression elevated yeast sensitivity to As(III).

Yeast strains D458-1B expressing *AtINT2, AtINT4* or containing the empty vector were treated with 50, 100, 250 and 500 μM As(III) for 24 h, and accumulation of arsenic was measured. D458-1B expressing *AtINT2* or *AtINT4* accumulated more arsenic than those with the empty vector under the same As(III) treatment ($p<0.001$, Fig. 2a). In the 500-μM As(III) treatments, *AtINT2* and *AtINT4* expressing cells accumulated 2.2-fold and 2.5-fold, respectively, more arsenic than control. These results demonstrated that both AtINT2 and AtINT4 mediate the uptake of As(III). In this study, yeast strain D458-1B was used. This strain has a wild type *ACR3* gene, which encodes the primary arsenite efflux transporter. In this case, ACR3 would act in opposition to AtINTs, therefore, arsenic accumulation in D458-1B cells (Fig. 2a) was considerably lower than those in an *ACR3* deletion strain, such as the *Δacr3* strain that was used to express Lsi[12].

The transport properties of the AtINT2 for As(III) were further analyzed in *X. laevis* oocytes. Oocytes expressing *AtINT2* exhibited significantly higher transport activity of

As(III), which was approximately 2-fold higher compared with the control ($p<0.001$, Fig. 2b). These results clearly showed that As(III) is transported by AtINT2.

Myo-inositol inhibits As(III) uptake by AtINT2 and AtINT4

Yeast strains D458-1B expressing *AtINT2* or *AtINT4* were treated with 250 μM As(III) and various concentrations of *myo*-inositol for 24 h. The concentrations of arsenic in yeast cells expressing *AtINT2* or *AtINT4* decreased correlating with the increase of *myo*-inositol in the growth medium ($r^2 = 0.99$, $p<0.001$, Fig. 3). In contrast to that, arsenic concentrations in yeast cells containing the empty vector did not decrease significantly with increasing *myo*-inositol concentrations (Fig. 3). In D458-1B strain, the *AtINT* ortholog (*ITR1* gene) is mutated[28,30], so D458-1B transformed with vector could not accumulate arsenic through INT pathway, thus the accumulation of arsenic was not affected by *myo*-inositol in the growth medium. However, in D458-1B cells expressing *AtINT2* or *AtINT4,* As(III) uptake was facilitated by AtINT2 or AtINT4*,* which also transport *myo*-inositol. Therefore, substrate competition inhibited arsenic accumulation in D458-1B expressing *AtINT2* or *AtINT4*.

Kinetic parameters of AtINT2 and AtINT4

The Michaelis-Menten kinetics for As(III) uptake were investigated by treating yeast strains D458-1B expressing *AtINT2* or *AtINT4* with 2 μg mL^{-1} *myo*-inositol and various concentrations of As(III) for 30 min (Fig. 4). Kinetic constants were calculated using a SigmaPlot transformation. For AtINT2, the K_m for As(III) uptake was 219 μM As(III), and V_{max} was 10 μg g^{-1} yeast DW min^{-1} ($r = 0.999$, $p < 0.0001$). For AtINT4, the K_m for As(III) uptake was 174 μM As(III), and V_{max} was 8.2 μg g^{-1}yeast DW min^{-1} ($r = 0.995$, $p = 0.0012$). The K_m values for *myo*-inositol of AtINT2 and AtINT4 were 0.7–1.0 mM and 240 μM, respectively[28,30]. Compared to the physiological substrate *myo*-inositol, the K_m values for As(III) of both AtINT2 and AtINT4 were much lower, indicating that AtINT2 and AtINT4 have higher affinity to As(III) than to inositol.

AtINT2 and AtINT4 contribute to arsenic loading into phloem

To examine functions of AtINT proteins in uptake and distribution of arsenic in *A. thaliana*, plants with T-DNA insertions in the genomic sequence of either *AtINT2* or *AtINT4* were obtained from the *Arabidopsis* Biological Resource Center (ABRC) at Ohio State University. Homozygous disruptions were confirmed by PCR-based genotyping. *Atint2-1* (Salk_1264_A07) and *Atint2-2* (Salk_065862 C) were found to be homozygous lines, each with a T-DNA insertion in the second intron of *AtINT2* (Fig. S1a, b). *Atint4-1* (Salk_082659. 41. 45.X) and *Atint4-2* (WiscDsLox293-296invI7) were also shown to be homozygous lines, each with a T-DNA insertion in the second exon of *AtINT4* (Fig. S2a, b). No *AtINT2* or *AtINT4* mRNA was detected in the respective mutants, indicating that these T-DNA insertion mutants are null alleles (Figs. S1c and S2c). *Atint2 or Atint4* knockout mutants do not show alterations compared to wild type plants during their life cycle[28,30]. Usually, plants do not rely on inositol transport because they biosynthesize *myo*-inositol from glucose-6-phosphate[33].

To compare arsenic exuding from phloem, *A. thaliana* WT and mutant plants were grown in hydroponic MGRL solution[34], at the flowering stage, As(III) was added to the nutrient solution to final concentration of 50 μM, and the plants were treated with As(III) for one week. Phloem exudates were collected and arsenic content in the exudates was analyzed. Figure 5a shows that arsenic exuding from phloem of *Atint2* or *Atint4* mutants was significantly lower than that from WT plants, decreased about 27–35%. These results are consistent with our hypothesis that AtINT2 and AtINT4 are involved in arsenic loading into phloem.

Arsenic accumulation and distribution was compared in different organs of WT and mutant plants. To this end, plants were grown in hydroponic MGRL solution containing 5 μM As(III). After plant maturation, arsenic concentrations in different organs were determined. Arsenic was accumulated primarily in roots, with the concentration being approximately 12-fold higher than in shoots. The order of arsenic distribution was roots>shoots>empty siliques>seeds (Fig. 5b). The concentrations of arsenic in roots of mutants and WT were similar, while the concentrations in shoots, empty siliques and seeds were significantly lower in the mutants than in the corresponding organs of WT plants. Strikingly, *AtINT2* or *AtINT4* disruption resulted in a 45–64% reduction in arsenic accumulation in seeds (Fig. 5b). These results clearly demonstrate that AtINT2 and AtINT4 are necessary for arsenic accumulation in siliques and seeds of Arabidopsis. A similar situation has been described for the phloem-localized iron [Fe(II)]- and manganese [Mn(II)]-nicotianamine complex transporter OsYSL2 from rice. RNAi plants with a suppressed expression of *OsYSL2* exhibit a reduced Fe level within the shoots and seeds[35], comparable to our results for INT mutants and arsenite translocation.

To further demonstrate that AtINT2 and AtINT4 are involved in arsenic loading into phloem, leaf feeding experiments were conducted. One week prior to harvesting the seeds, rosette leaves were brushed daily with a solution containing 50μM As(III) and 0.1% Tween. The amounts of arsenic in shoots and seeds of *Atint2* or *Atint4* mutants decreased about 52–72% in seeds and 34–59% in shoots compared with WT plants, (Fig. 5c). These results are consistent with our hypothesis that AtINT2 and AtINT4 are involved in arsenic loading into phloem.

To demonstrate whether AtINT2 or AtINT4 mutation affects xylem arsenic loading, xylem sap was collected from plants treated with 5 μM As(III), and the arsenic concentration in xylem sap was determined. The results showed that arsenic concentration in xylem sap collected from mutant plants were similar to that from WT plants, except that in *Atint2-1* there was significantly higher than in WT (Fig. 5d). These results indicate that neither AtINT2 nor AtINT4 mutation affects xylem arsenic loading.

Discussion

It is becoming increasingly clearer that plant aquaglyceroporins of the NIP subgroup such as the rice AQP Lsi1 catalyze the uptake of As(III) into roots[12_13], and that the rice ArsB family member Lsi2 is responsible for the movement of As(III) from roots to shoots through the xylem[12]. The final piece of the puzzle is how arsenic is loaded from the shoots into the

seeds of plants[6]. In this study, we show that the *A. thaliana* inositol transporters AtINT2 and AtINT4 catalyze As(III) loading into phloem and are necessary for arsenic accumulation in the seeds of this model plant. We speculate that knowledge of the pathway of arsenic accumulation in *Arabidopsis* seeds will shed light on the corresponding mechanism in rice, the main source of dietary arsenic for the majority of the world's population. Understanding the loading mechanism of As(III) into rice grains, fruits or seeds of other crops is critical for enhancing food safety.

As(III) is the predominant arsenic species found in seeds, especially in rice grains [3,36]. A survey of arsenic speciation in Chinese rice showed that in market rice, 50–60% arsenic was present as As(III), and in rice collected from farmers' fields in mining areas, 60–70% was As(III) [35]. Approximately 90% of As(III) in rice grains is delivered via the phloem[19–23]. However, prior to the present study, little has been known about the mechanisms of arsenic loading and unloading during phloem transport[6]. Generally, solutes load into and unload from phloem through either the apoplastic or symplastic pathway. Apoplastic loading is driven thermodynamically via the proton motive force and conducted by plasma membrane transporters[37]. Symplastic loading is passive and conducted through plasmodesmata between adjacent cells[38–39]. In *A. thaliana*, AtINT2 and AtINT4 are located in the plasma membrane. Organ and tissue specificity of AtINT2 and AtINT4 expression showed that both *AtINT2* and *AtINT4* are strongly expressed in the vasculature, primarily in the companion cells of phloem, though there are also little expression in root tissue[28,30]. Functional analyses further demonstrated that AtINT2 and AtINT4 are H^+-coupled symporters that are responsible for loading of inositol into the phloem to supply the developing seeds. In the present study we demonstrate that AtINT2 and AtINT4 also transport As(III) (Figs. 1, 2). *Myo*-inositol in the growth medium inhibited the uptake of As(III) by AtINT2 and AtINT4 (Figs. 3). The disruption of *AtINT2* or *AtINT4* significantly decreased arsenic concentration in phloem exudates (Fig. 5a), and subsequently significantly decreased arsenic concentration in shoots, siliques and seeds (Figs. 5b, c). Most importantly, arsenic accumulation in siliques and seeds decreased by half (Fig. 5c), but the ratios of each arsenic species in plants tissues were similar between the mutants and WT (Fig. S4). Additionally, when plants were feed with arsenite through leaves, arsenic accumulation in shoots and siliques of mutants was significantly lower than those of WT (Fig. 5b). In contrast to that, arsenic concentrations in the xylem sap did not vary between WT and mutant plants (Fig. 5d). These results clearly demonstrate that inositol transporters AtINT2 and AtINT4 are responsible for arsenite loading into phloem, and essential for arsenite accumulation in *A. thaliana* seeds.

We conclude that AtINT2 and AtINT4 are responsible for the loading of arsenic from the apoplast into the phloem (Fig. S5). Our results are consistent with the tissue- and cell-specificity of expression[28,30]. Nevertheless, single mutation of *AtINT2* or *AtINT4* did not totally suppress translocation of arsenic into seeds (Fig. 5). This could be contributed by *AtINT4* in *Atint2* or *AtINT2* in *Atint4,* or other transporters may be also involved in arsenic loading to phloem. *AtINT2* and *AtINT4* are not expressed in young sink leaves[28,30], so it was anticipated that neither *AtINT2* nor *AtINT4* mutations would affect arsenic accumulation in seedlings treated with As(III) (Fig. S3). Once entry into the companion cells of the phloem, arsenic passively diffuses through the plasmodesmata into the sieve elements and is finally released into the sink cells of seeds (Fig. S5). As is the case for nutrients,

unloading of arsenic from the phloem into the sink cells of plant seeds is likely to be mediated by specific transport proteins[40], and identification of these transporters should be a priority of future research.

In summary, we demonstrate here that inositol transporters AtINT2 and AtINT4 adventitiously catalyze loading of As(III) into the phloem, a possible pivotal step of arsenic translocation to the seeds of higher plants. To our knowledge, this is the first identification of transporters responsible for arsenic loading into phloem. If these findings prove to be applicable to rice, then inositol transporters may be candidates for future genetic modification to reduce the arsenic content in rice grain. If so, this discovery will enable development of new cultivars that accumulate lower amounts of arsenic in their grain without affecting yield production, a major advance toward mitigation of health risks posed by arsenic in rice.

Materials and Methods

Yeast constructs and *arsenite sensitivity* analysis

AtINT2 and *AtINT4* were cloned into the yeast/*E. coli* shuttle vectors NEV-N-Leu[30] (*AtINT2*) or NEV-E-Leu[41] (*AtINT4*), respectively; the constructs and the empty vectors were used to transform *S. cerevisiae* strain D458-1B (Schneider et al., 2006; 2007)[28,30]. In this study, *AtINT2* and *AtINT4* constructed plasmids were also transformed into *S. cerevisiae* strain MG100 (*acr3Δ*) (U.S. patent US 20050260739 A1). Arsenite sensitivity phenotypic studies were performed as reported[42], the cell growth was determined by light absorbance at 600 nm.

For As(III) uptake assay, yeast strains D458-1B expressing *AtINT2*, *AtINT4* or with empty vector were grown in 5 ml liquid SD-Leu medium supplemented with 2 μg mL^{-1} *myo*-inositol until mid-exponential phase. The cells in the cultures were harvested by centrifuge and re-suspended in 50 ml of fresh SD-Leu medium containing 2 μg mL^{-1} *myo*-inositol and different concentrations of As (50, 100, 250 and 500 μM). After 24 h incubation (30°C, 170 rpm), yeast cells were harvested for arsenic concentration determination. For substrate competition, mid-exponential phase yeast cells were treated with 250 μM As(III) and different concentrations of *myo*-inositol (0, 2, 4 and 8 μg mL^{-1}) for 24 h incubation (30°C, 170 rpm). For kinetic Assays, mid-exponential phase yeast cells were treated with 2 μg mL-1 myo-inositol and various concentrations of As(III) (50, 100, 250 and 500 μM). After 30 min incubation (30°C, 170 rpm), yeast cells were harvested for arsenic concentration determination.

Expression of AtINTs in X. laevis oocytes and arsenite uptake

AtINT2 were cloned into plasmid pL-5 in the BglII/KpnI. The primer sequences for constructions of different genes are as follows: forward primer 5′-GCAGATCTATGGAGGGAGGAATAATAC-3′ (*BglII* site underlined) and reverse primer 5′-GCGGTACCTCATGCACTCTGGTTTTG-3′ (*KpnI* site underlined). The plasmids were linearized by NotI digestion, and the capped cRNA of NaPi-IIb1 was transcribed *in vitro* using an mMessage mMachine T7 ultra kit (Ambion Co., Austin, TX, USA). Stage V-VI *X.*

laevis oocytes were isolated and treated with 0.2% collagenase A (Roche, Indianapolis, IN, USA) for 2 h. Defoliated oocytes were injected with 25 ng (25 nL volume) of cRNA. Oocytes were then incubated in ND96 complete buffer (96-mM NaCl, 2-mM KCl, 1-mM $MgCl_2$, 1.8-mM $CaCl_2$, 5-mM Hepes, pH5.5, supplemented with 1 mg/ml Gentamicin) for 3 d at 16°C [43].

Accumulation of arsenicals in oocytes was assayed by incubation of the oocytes with 1mM As(III) dissolved in ND96 buffer (pH 7.4) at room temperature for 30 min. After incubation, the oocytes were washed, dissolved in 70% nitric acid at 70°C for 2 h, and then arsenic concentration was analyzed.

Plant treatments

To assay arsenic in phloem exudates, uniform homozygote and WT seedlings (10 d) were transferred from plates to hydroponic pots containing 5 L of MGRL nutrient solution. At flowering stage, As(III) was added to the nutrient solution to a final concentration of 50-μM. On the 3rd day of As(III) treatment, nutrient solution was renewed with As(III), and, after one hour, rosette leaves were harvested and weighed. Phloem exudates were rapidly collected by an EDTA-facilitated method[44]. After 8 h of collection, phloem exudates solutions were passed through a filter of 0.22 μm, and stored at −4 °C until arsenic concentration determination.

To analyze total arsenic in mature *A. thaliana* tissues, from flowering stage to harvest, As(III) was added to the nutrient solution to a final concentration of 5 μM. After harvesting, plants were separated into roots, shoots, empty siliques and seeds. Samples were washed, dried for arsenic determination.

To conduct leaf feeding experiments, one week before seed harvesting, both sides of rosette leaves were brushed with a solution containing 50 μM As(III) and 0.1% Tween using a painting brush[45]. Each plant was brushed with a 2 ml solution daily. After harvesting, the plants were separated into shoots (including upper stem and leaves that had not been brushed) and siliques (including seeds). Samples were washed, dried for arsenic determination.

For determination of the concentration of arsenic in the xylem sap of WT and mutants, soil-grown *A. thaliana* plants at flowering stage were used. Treatment with 5 μM As(III) was performed for three days before xylem sap collection. Xylem sap collection was performed as described[46] except that plants were not irrigated with NaCl. Xylem sap of two plants was pooled for each sample. Collected samples were stabilized by adding phosphoric acid to a final concentration of 10 mM, passed through a 0.22 μm filter and stored at +4 °C until determination of arsenic concentration.

Total arsenic analysis

For total arsenic analysis, yeast and plant subsamples were weighed and digested with 2.5 ml of concentrated nitric acid in a microwave oven (CEM Mars 5, CEM Corp, Matthews, NC). Arsenic concentrations were determined by ICP-MS (Agilent Technologies 7500, USA).

Statistical analysis

Experiments using *X. laevis* oocytes adopt n=4–6, and experiments in yeast and plant tissues adopted n=4. Mean and standard errors were derived using SigmaPlot. Statistical differences were assessed by the Student pair-wise t test. Data were presented as mean ± SD. All p values < 0.05 were regarded as statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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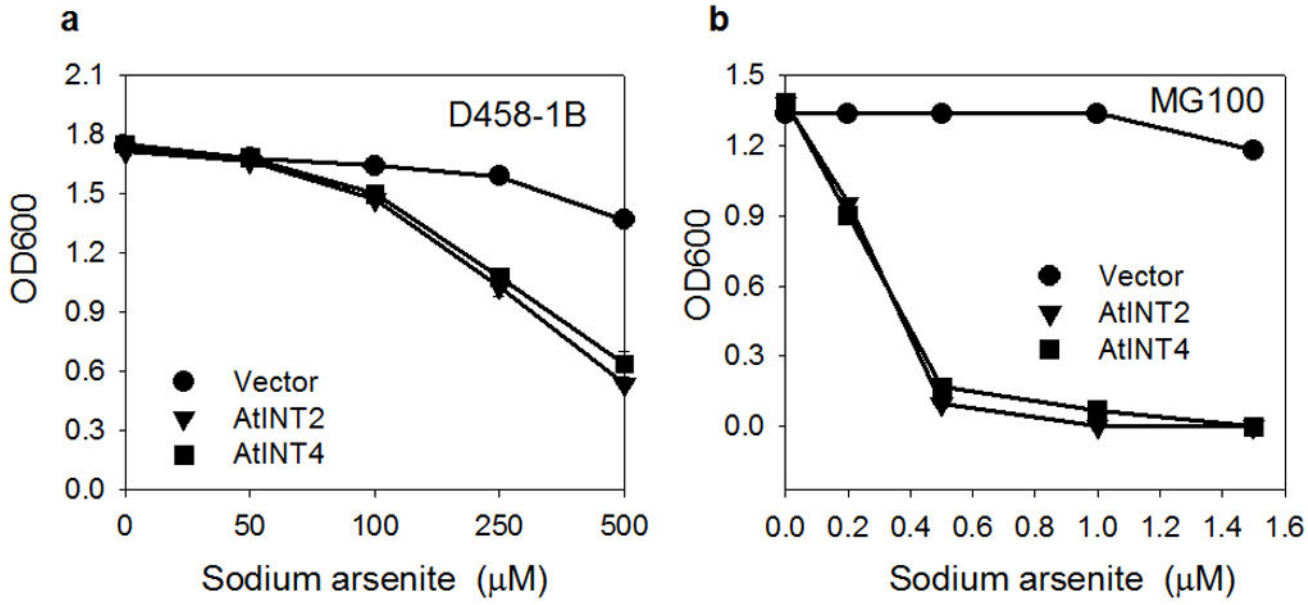

Figure 1. *AtINT2* and *AtINT4* expression elevated *S. cerevisiae* sensitivity to arsenite

Growth curve of yeast strains D458-1B **(a)** and MG100 **(b)** expressing *AtINT2* or *AtINT4* and containing empty vector. Overnight grown cells were diluted 100-fold with liquid SD medium containing the indicated concentrations of As(III). After overnight incubation at 30 °C, cell growth was determined by light absorbance at 600 nm. Averages and standard errors are shown; $n = 4$.

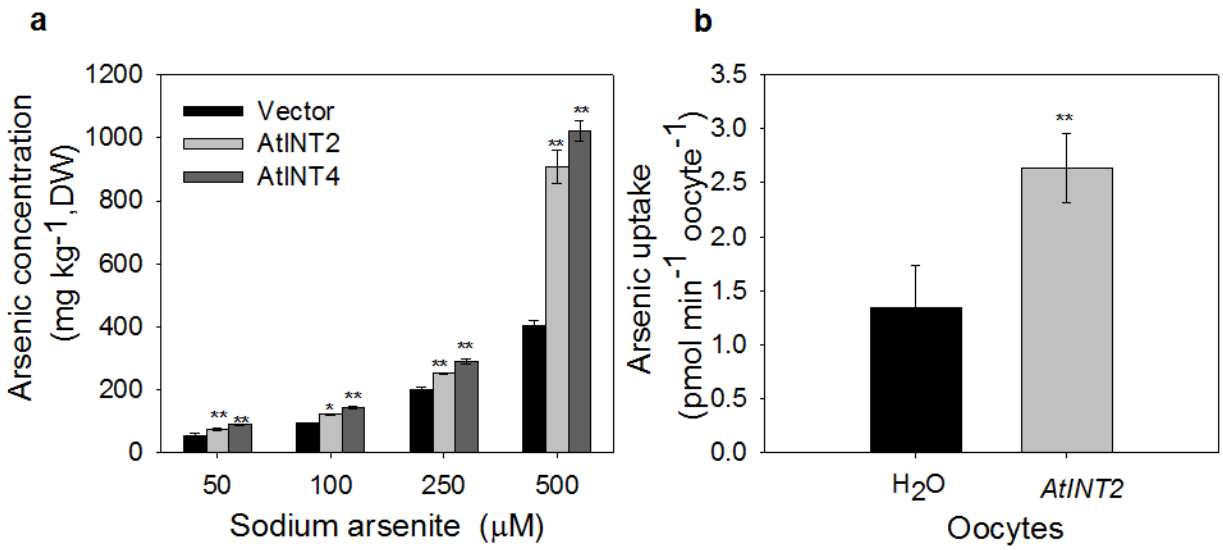

Figure 2. Arsenite transportation with expression of *AtINT2* and *AtINT4* in yeast and oocytes

a: Arsenic concentration in yeast cells D458-1B after grown at 30°C for 24 h in liquid minimal medium supplemented with 2 μg mL^{-1} *myo*-inositol and different concentrations of As(III).

b: Oocytes from *X. laevis* were incubated in ND96 complete buffer supplemented with 1 mM of sodium As(III) at room temperature for 30 min. Oocytes injected with water were used as controls.

Asterisk indicates significance at $P<0.05$, and double asterisk indicates significance at $P<0.01$ compared to controls. Averages and standard errors are shown; $n = 4$.

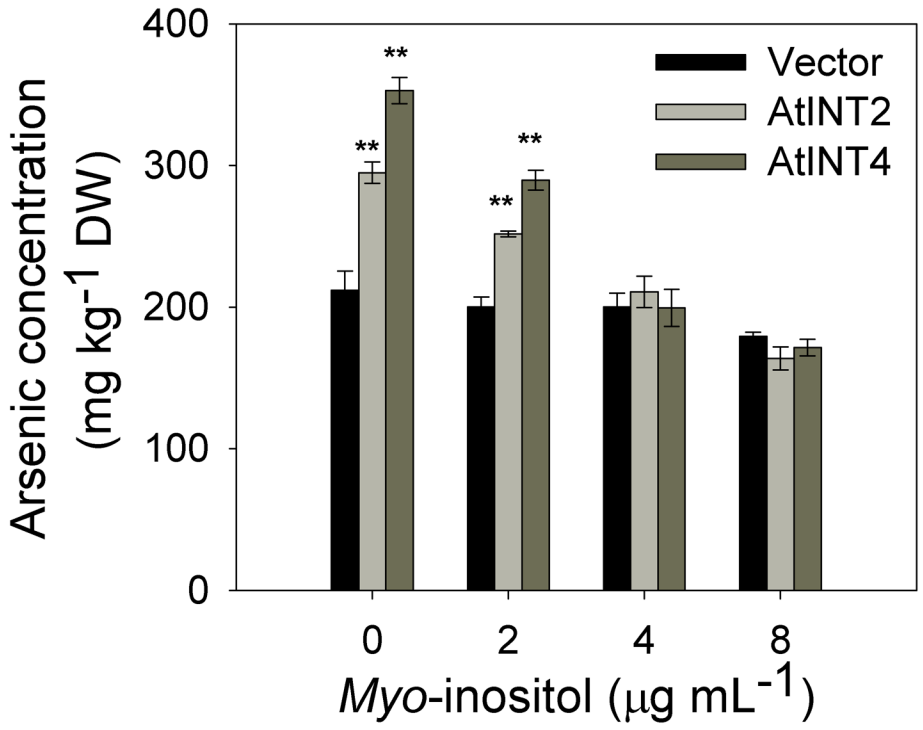

Figure 3. Arsenite uptake inhibition by *Myo*-inositol

Arsenic concentration in yeast cells D458-1B expressing *AtINT2* or *AtINT4* and containing empty vector after grown at 30 °C for 24 h in liquid minimal medium supplemented with 250 μM As(III) and the indicated concentration of *myo*-inositol. Double asterisk indicates significance at $P<0.01$ compared to cells with vector only. Averages and standard errors are shown; $n = 4$.

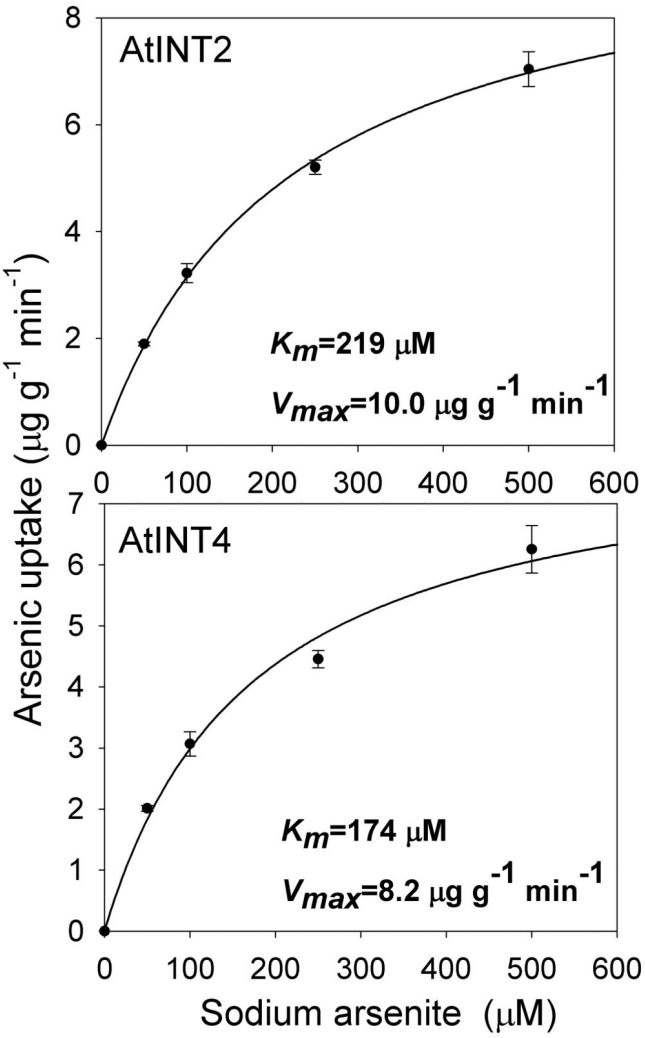

Figure 4. Kinetic properties of *AtINT2* and *AtINT4* for As(III)

Cultures of yeast strain D458-1B expressing either *AtINT2* or *AtINT4* were incubated in liquid minimal medium supplemented with 2 μg mL^{-1} *myo*-inositol and the indicated concentration of As(III) for 30 min. Kinetic data were fitted using a least-squares analysis with SigmaPlot 12.0. Averages and standard errors are shown; $n = 4$.

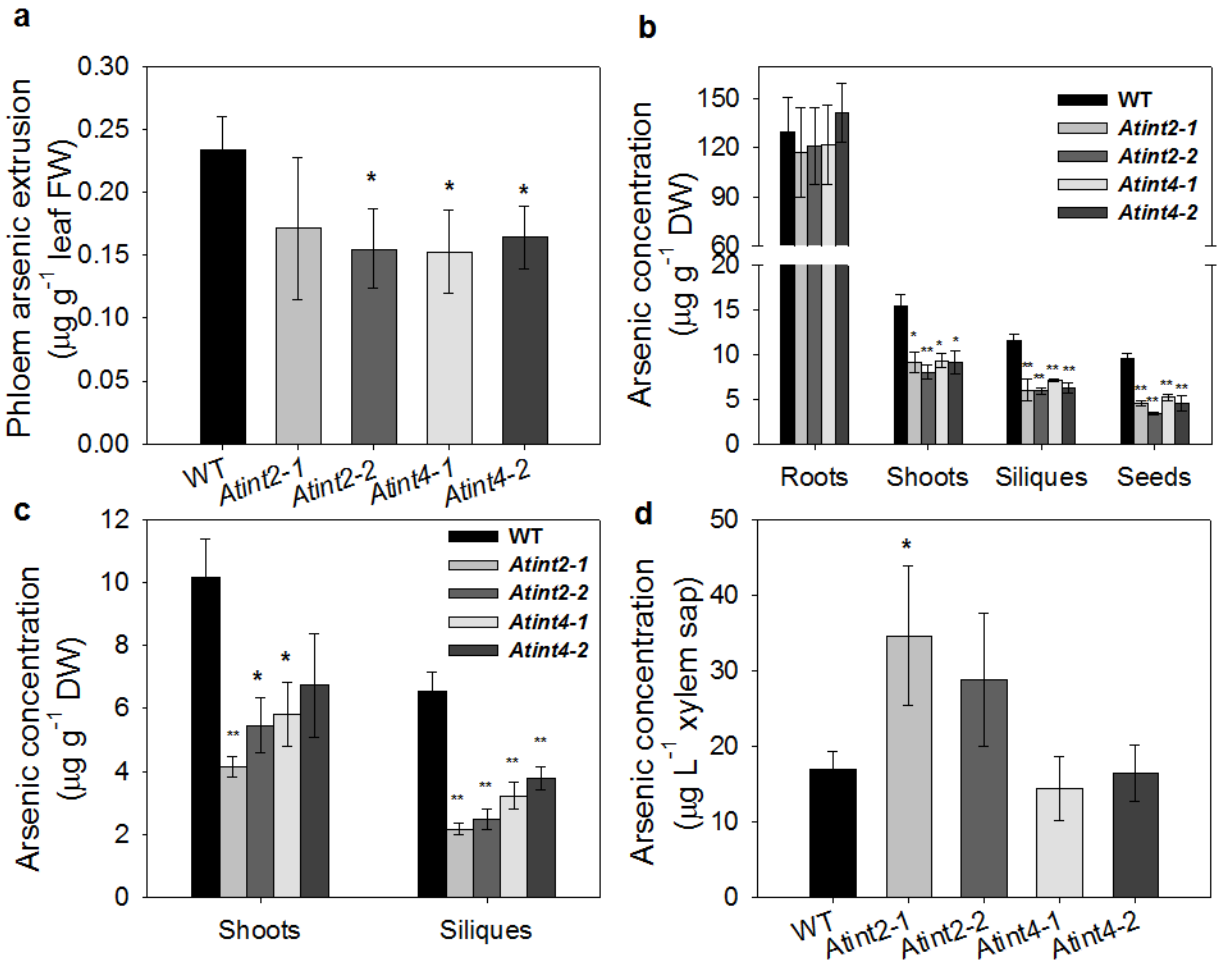

Figure 5. Arsenic concentration in phloem exudates, xylem sap and plant tissues

a: Plants were grown in hydroponic solution, after 3 d treatment with 50-μM As(III), rosette leaves were harvested and phloem exudates were rapidly collected by an EDTA-facilitated method.

b: Plants were grown in hydroponic solution, one week before harvesting, rosette leaves were brushed with a solution containing 50 μM As(III) and 0.1% Tween using a painting brush. Each plant was brushed with a 2 ml solution daily. After harvesting, the plants were separated into shoots (including upper stem and leaves that had not been brushed) and siliques (including seeds).

c: Plants were grown in hydroponic solution, from flowering stage to harvest, As(III) was added to the nutrient solution to a final concentration of 5 μM. After harvesting, plants were separated into roots, shoots, empty siliques and seeds.

d: Plants were grown in soil, after 3 d treatment with 5-μM As(III), xylem sap was collected.

* indicates significance at $P<0.05$, and ** indicates significance at $P<0.01$ compared to WT. Data are shown as average ± SE; $n = 4$.