Knocking Out OsPT4 Gene Decreases Arsenate Uptake by Rice Plants and Inorganic Arsenic Accumulation in Rice Grains

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ABSTRACT: Arsenic (As) accumulation in rice grains poses health risk to humans. Plants including rice take up arsenate (AsV) by phosphate transporters. In this study, rice phosphate transporter OsPT4 (OsPht1;4) was investigated based on two independent T-DNA insertion mutants of OsPT4 (M1 and M2), which displayed stronger AsV resistance than wild types WT1 and WT2. When cultivated in medium (+P or -P) with AsV, ospt4 mutants accumulated 16–32% lower As in plants, suggesting that OsPT4 mediates AsV uptake. Analysis of the xylem sap showed that AsV concentrations in ospt4 mutants was 20–40% lower than WT controls under -P condition, indicating OsPT4 may also mediate AsV translocation. Moreover, kinetics analysis showed that ospt4 mutants had lower AsV uptake rates than the WT controls, further proving that OsPT4 functions as an AsV transporter in rice. When grown in flooded soils with As, AsV concentrations in rice grains of ospt4 mutants decreased by 50–55%. More importantly, knocking out OsPT4 in M1 and M2 reduced inorganic As accumulation in rice grains by 20–44%, significant for controlling As exposure risk from rice. Taken together, our findings revealed a critical role of OsPT4 in AsV uptake and translocation in rice. Knocking out OsPT4 effectively decreased inorganic As accumulation in rice grains, shedding light on engineering low-As rice to enhance food safety.

INTRODUCTION

As the staple food for half of the world’s population, rice (Oryza sativa L.) is widely cultivated around the world, especially in Asia. However, due to mining and irrigation of contaminated water, agricultural soils are contaminated with arsenic (As).1,2 Compared to other cereals, rice is more efficient in As uptake.3,4 As a result, elevated As levels in rice grains pose great health risk to humans.5–7

Arsenic is mainly present as arsenate (AsV) and arsenite (AsIII) in soils. Due to biochemical processes, As can also be methylated in soils.8,9 Both AsV and AsIII are toxic but by different mechanisms. Being a phosphate analogue, AsV participates in phosphorylation reactions and interferes with energy and phosphate metabolism.10 By interfering with nucleic acid synthesis, AsV also exerts toxicity to nucleus.11 Different from AsV, AsIII affects the structures and functions of enzymes by binding to the sulphydryl (R–SH) groups of the cysteine residues in proteins.12 In plants, AsV can be reduced to AsIII inside plant cells readily,13–17 which is complexed and sequestered into the vacuoles or effluxed into external environment.18–22

Arsenite is predominant in flooded paddy soils where rice typically grows. However, due to oxygen release by rice roots and microbial oxidation, AsV is also present in rice rhizosphere.23,24 Besides, due to microbial transformation, methylated As species also exist in paddy soils including dimethylarsinic acid (DMA).5,6,8,25 Arsenite is absorbed by rice roots through the silicon transporters,26 whereas AsV is taken up via the P transporters.27–30 Rice is unable to methylate As in vivo so the methylated As in rice probably comes from the rhizosphere.31,32 It was reported that the AsIII transporter Lsi1 also mediates the uptake of methylated As in rice,33 but other transporters involved in this process are still unclear. In rice grains, As is present mainly as inorganic AsIII and AsV, and...
organic DMA.\textsuperscript{34} Considering inorganic As is more toxic than DMA,\textsuperscript{35} it is crucial to control both As(II) and As(V) uptake in rice.

Though As(V) accounts for only 5–20% of soluble As in flooded soils,\textsuperscript{36} As(V) is higher in the rhizosphere due to oxygen release by rice roots.\textsuperscript{17} Therefore, it is critical to understand As(V) uptake mechanism to limit As accumulation in rice. In rice genome, a total of 13 P transporter genes are present, that is, OsPT1-13, which mediate root P uptake.\textsuperscript{38} Among them, OsPT1, OsPT2, OsPT4, OsPT6, OsPT8, OsPT9, and OsPT10 all mediate P uptake and translocation in rice.\textsuperscript{39–41} Moreover, some P transporters also mediate As(V) uptake. For example, it was reported that knocking out OsPT1 decreases As accumulation by 60% in the shoots compared with wild-type (WT) plants, suggesting that OsPT1 involves in As(V) uptake and translocation.\textsuperscript{42} Besides, OsPT8 also plays an important role in As(V) uptake and translocation in rice.\textsuperscript{43,44}

In this study, P transporter OsPT4 was investigated. Reports showed that OsPT4 involves in P mobilization during vegetative growth, grain filling, and embryogenesis.\textsuperscript{45} Here, for the first time, we investigated its involvement in As(V) uptake and accumulation in rice. We found that As uptake and translocation significantly decreased in two independent T-DNA insertion mutants of OsPT4 under short-term hydroponic experiments. In addition, kinetics analysis showed that ospt4 mutants had lower As(V) uptake rates than the WT controls. Moreover, knocking out OsPT4 significantly reduced inorganic As accumulation in rice grains cultivated in flooded soils with As during its full life cycle. Our study demonstrated the important role of OsPT4 in controlling As(V) uptake and As accumulation in rice, providing insights into breeding low-As rice to enhance food safety and control As exposure from rice.

**MATERIALS AND METHODS**

**Growth of Rice Plants.** Rice seeds were surface sterilized for 30 min with diluted (1:3, v/v) NaClO (with 5.5% of efficient Cl), followed by thorough rinsing for 30 min with deionized water. Seeds were germinated in darkness at 25 °C for 3 days. The hydroponic experiments were conducted in a greenhouse with a 16-h-light (30 °C)/8-h-dark (22 °C) photoperiod and ∼70% relative humidity. Rice seedlings of 10-d old were transferred to complete nutrient solution containing 1.25 mM NH₄NO₃, 200 μM KH₂PO₄, 0.35 mM K₂SO₄, 1 mM CaCl₂, 1 mM MgSO₄, 20 μM Fe-EDTA, 20 μM H₂BO₃, 9 μM MnCl₂, 0.32 μM CuSO₄, 0.77 μM ZnSO₄, and 0.39 μM Na₂MoO₄. In hydroponic experiments, 20 μM As(V) with 0 or 300 μM KH₂PO₄ were used as P-deficient As treatment (−P +As) and P-sufficient As treatment (+P +As), respectively.

In addition, pot experiments were performed with six replications in a greenhouse using a soil collected from an experimental farm at Nanjing Agricultural University, which followed normal agricultural practices.\textsuperscript{46} Rice plants were grown in a pot containing 7 kg of air-dried soil, with 15 mg kg⁻¹ of As added. Each pot was provided with a guard column (Hamilton, UK). The mobile phase was a solution consisting of 8.0 mM (NH₄)₂HPO₄ and 8.0 mM NH₄NO₃ (pH 6.2) at a flow rate of 1.0 mL min⁻¹. Before flowing into chromatographic columns, it was sonicated and filtered through 0.22 μm filters. Quality assurance was obtained through blanks, standards, and spiked samples. Total As concentration and As(V) uptake were determined as described earlier.

**Arsenic Determination in Soil and Plant Samples.** Fresh plants were separated into the shoots and roots, lyophilized (FreeZone 12, LABCONCO) and stored at −80 °C for further analysis. For total As, freeze-dried plant samples (0.05 g) were digested with 50% HNO₃ at 105 °C following USEPA Method 3050 B and determined by inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 300X; detection limit at 0.1 ppb).\textsuperscript{47} For quality assurance and quality control during ICP–MS measurement, indium was used as internal standards and was added into samples, calibration standards, and blanks. In addition, blanks and certified reference material for plant samples (GBS 21, Chinese geological reference materials) were included for quality assurance, which were within expected values.

For As speciation, 0.05 g freeze-dried samples were ultrasonically extracted with 10 mL 1:1 methanol/water for 2 h and then centrifuged at 4000 rpm for 10 min. The supernatant was decanted into a 50 mL centrifuge tube. The procedure was repeated and the two extracts were combined. The residue was rinsed three times with 5 mL Milli-Q water and all supernatants were mixed and then diluted to 50 mL with Milli-Q water. After filtering through 0.22 μm filters, the samples were diluted to determine As speciation using high performance liquid chromatography (HPLC; Waters 2695) coupled with ICP-MS.\textsuperscript{48}

Specifically, inorganic As(III) and As(V) were separated by an anion exchange column (PRPX100, 10 mm, Hamilton, UK) fitted with a guard column (Hamilton, UK). The mobile phase was a solution consisting of 8.0 mM (NH₄)₂HPO₄ and 8.0 mM NH₄NO₃ (pH 6.2) at a flow rate of 1.0 mL min⁻¹. Before flowing into chromatographic columns, it was sonicated and filtered through 0.22 μm filters. Quality assurance was obtained through blanks, standards, and spiked samples. Total As concentration and As(V) uptake were determined using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) with deuterated standards.

**RT-PCR and qRT-PCR Analyses.** Total RNAs from various tissues were isolated using Trizol reagent (Invitrogen), and first-strand cDNA was synthesized with an oligo(dT)-18 primer, and HiScript II Reverse Transcriptase (Vazyme Biotech Co., Ltd). OsActin (accession no. AB047313) was used as an internal control for RT-PCR, and qRT-PCR analyses (Table S1 in Supporting Information (SI)). qRT-PCR analysis was performed using a SYBR green master mix (YIFEIXUE BIO TECH), and ABI StepOnePlus Sequence Detection System (Applied Biosystems).

**Measurement of P Concentrations in Plants.** Total P concentrations of plant samples were measured.\textsuperscript{46,47} Briefly, ∼0.05 g of crushed dry sample was digested with H₂SO₄-H₂O₂ at 300 °C. After cooling, the digested samples were diluted to 100 mL in distilled water. P concentration was analyzed by the molybdenum blue method based on dry weight.\textsuperscript{48}

**Arsenic Uptake Kinetics.** To determine As(V) uptake kinetics, 17-d old rice plants were transferred to growth media containing various As(V) concentrations (1, 5, 10, 25, and 50 μM) in the absence and presence of 100 mM P. After 30 min, the roots were rinsed briefly with deionized water, and desorbed of apoplastic As with an ice-cold desorption solution containing 1 mM K₂HPO₄, 0.5 mM CaCl₂, and 2 mM MES (pH 5.5) for 10 min. The shoots and roots were separated and dried, which were ground and digested with HNO₃ to determine As uptake concentration using ICP-MS. As uptake kinetics were fit to the Michaelis–Menten equation using two-
parameter hyperbola via nonlinear regression via software SigmaPlot (version 11.0) (Wang et al., 2002).

Statistical Analysis. Data are presented as the mean of four replicates with standard error. Analysis of variance was carried out by SPSS 20.0 software. Significant differences were determined with treatment means compared by Tukey’s mean group tests at $p < 0.05$.

■ RESULTS AND DISCUSSION

Ospt4 Mutants Displayed Enhanced Arsenate Resistance. In this study, two independent T-DNA insertion mutants of OsPT4, M1 (NE1260) and M2 (SHIP_ZSf6267), with Nipponbare (WT1) and Zhonghua11 (WT2) background were obtained. The T-DNA insertions in both M1 and M2 were within the exon of OsPT4 (Figure S1A). Using a second-round PCR analysis with a pair of T-DNA and OsPT4-specific primers, we isolated homozygous mutants for M1 and M2 (Figure S1B). The transcripts of OsPT4 were also investigated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis, which revealed that the OsPT4 transcripts were absent in ospt4 mutants (Figure S1C). These results confirmed that ospt4 mutants M1 and M2 were homozygous lines, which were used in our study.

To investigate whether OsPT4 gene played a role in As uptake and accumulation in rice, we first studied the phenotypic changes of M1 and M2 seedlings in response to AsV with WT1 and WT2 as controls. Rice seeds were grown in a hydroponic solution in a growth chamber for 3 d and germinated seeds were then transferred to complete nutrient solutions with 0 or 15 μM AsV to grow for 10 d. In the absence of AsV, growth traits of ospt4 mutant lines appeared similar to the WT plants (Figure 1A), indicating that, though P uptake may be impaired,42 insert mutation of OsPT4 caused no visible stunted growth in rice. This is probably because there are multiple P transporters, which are responsible for P uptake, so deletion of one P transporter may not impair the overall P uptake in rice. However, in the presence of 15 μM AsV, M1 and M2 exhibited significantly higher AsV resistance and better growth than WT1 and WT2 based on primary root lengths and plant heights (Figure 1B). With no As, root lengths and plant heights of ospt4 mutants M1 and M2 exhibited significantly higher AsV resistance and better growth than WT1 and WT2 based on primary root lengths and plant heights (Figure 1CE). However, under 15 μM AsV, ospt4 mutants exhibited 18–51% longer primary roots and 16–48% higher plant heights than WT1 and WT2 (Figure 1DF). In short, the results clearly demonstrated that knocking out OsPT4 in rice enhanced AsV resistance and promoted rice growth under AsV stress.

Ospt4 Mediated Arsenate Uptake in Rice. To further understand the function of OsPT4 gene, rice seedlings were cultivated hydroponically under P sufficient (+P) or P deficient (-P) conditions for 14 d, after which seedlings were exposed to 20 μM AsV for 3 d. Then As accumulation and As species in ospt4 mutants and WTs were analyzed in both +P treatment (Figure 2A-D) and -P+As treatment (Figure 2E-H).

Under +P condition, root As concentrations decreased by 16–32% in ospt4 mutants M1 and M2 compared with WT1 and WT2 (Figure 2A). Consistent with the root As...
concentrations, its shoot As concentrations also decreased by 21–23% in ospt4 mutants (Figure 2C). The results indicated that knocking out OsPT4 gene decreased AsV uptake and As accumulation in rice (Figure 2AC), consistent with their enhanced As tolerance (Figure 1).

In plants, after being taken up, AsV can be rapidly reduced to AsIII, making AsIII the dominant As form in plants. As such, the As species in rice seedlings were determined, which showed more AsIII than AsV in both ospt4 mutants and WTs (Figure 2BD). Moreover, under +P condition, AsV in the roots and shoots of ospt4 mutants decreased by 8.6–15% and 18–32% (Figure 2BD). Under -P condition, the As concentrations in ospt4 mutants were also lower than those of WTs. Though root As concentration of M1 showed little differences with WT1, the root As concentration of M2 were 27% lower than WT2 (Figure 2E). In addition, in the shoots, As concentrations in ospt4 mutants decreased by 18–22% (Figure 2G). Furthermore, As speciation analyses showed that the ratio of AsV to total As in the roots of mutants and WT plants were comparable (Figure 2F), but they decreased by 20–27% in the shoots (Figure 2H). Taken together, the results showed OsPT4 mutation not only decreased As accumulation but also decreased AsV proportions regardless the P levels. It is known that P transporters mediate AsV uptake in plants, so loss of OsPT4 gene decreased AsV uptake and leading to lower AsV uptake rate. Moreover, since knocking out OsPT4 gene led to larger decrease of Vmax in the roots and facilitates plant P acquisition, which is consistent with our data.

It is well-known that plants take up AsV via the P transporters including rice. Besides, this physiological process is also controlled by the P regulatory system. For example, PHF1 (phosphate transporter traffic facilitator1) encodes a protein that involves in proper trafficking of P transporters to the plasma membrane, so mutation of PHF1 impairs P transport in plants. As a result, knockout of OsPHF1 not only impairs P uptake, but also decreases AsV uptake and its translocation from the roots to shoots in rice, providing direct evidence that AsV is taken up via P transporters, which is controlled by the P regulatory system.

Moreover, using qRT-PCR analysis, we also found that the transcriptional expression of OsPT4 gene in WT1 and WT2 were not induced by AsV exposure (data not shown), different from the results in Kasalath cultivar. Thus, the transcriptional expression pattern of OsPT4 gene should be further characterized to understand its regulatory mechanisms.

Ospt4 Mutants Exhibited Decreased Arsenate uptake rate. To further determine AsV uptake of ospt4 mutants and wild-type plants, its uptake kinetics was investigated using 1–50 μM AsV concentration under +P (100 μM) and −P (0 μM) conditions. In the absence of P, the AsV uptake kinetics can be described by a Michaelis–Menten eq (Table S2). Under −P condition, maximum influx velocity (Vmax) of AsV uptake in ospt4 mutants M1 and M2 was 7.8 and 40% lower (Figure 3AB), further proving that OsPT4 mutation decreased AsV uptake and leading to lower AsV uptake rate. Moreover, since knocking out OsPT4 gene led to larger decrease of Vmax in

**Figure 2.** Arsenic concentrations and species in ospt4 mutants and wild types in short-term hydroponic experiments. As concentrations and species were analyzed in rice seedlings exposed to 20 μM AsV under P-sufficient conditions (A–D) or P-deficient conditions (E–H) for 3 d. Values are means ± SE (n = 4) and asterisk indicates that the values for ospt4 mutants differ significantly ( *P* < 0.05; **P** < 0.01) compared with wild-types. DW, Dry weight.
WT2 than in WT1, the data also suggested that the greater contribution of OsPT4 gene in AsV uptake in WT2 than in WT1. The Km values of AsV influx in M1 and M2 (8.4–8.5 μM) were comparable with that in WT1 and WT2 (6.4–8.5 μM), indicating that the affinities of the mutants for AsV was similar to that of the WTs. In the presence of P, AsV influx of ospt4 mutants and wild-type plants were comparable, which were linear over AsV concentrations of 1–50 μM, but the rates were markedly lower than that in the absence of P (Figure 3AB). The results indicated that, due to the chemical similarity between AsV and P, though OsPT4 had a great affinity for AsV, its affinity was much lower than that for P.

Like OsPT4, several P transporters also transport AsV with different affinity.30,45,50,51 In rice, OsPT8 gene contributed to AsV uptake under low AsV concentrations of 1–2 μM, suggesting its high affinity for AsV.30 More interestingly, PvPht1;3 gene, a P transporter from As-hyperaccumulator Pteris vittata, may have similar affinities for P and AsV.30 To further evaluate the contribution of OsPT4 in AsV uptake in rice, it is necessary to compare its affinity for AsV with other OsPTs.

OsPT4 Mutation Decreased Arsenate translocation. After exposing to 20 μM AsV for 1 h, xylem sap of ospt4 mutants and WT plants were analyzed. Under +P condition, the As concentration in xylem sap of M1 was 25% lower than that of WT1, while the As concentration in xylem sap of M2 showed little differences to WT2 (Figure 4A). Under −P condition, the As concentrations in xylem sap of both mutants and WT plants were generally higher than those under +P condition (Figure 4A). Remarkably, the xylem sap As concentrations in ospt4 mutants were 22–27% lower than that of WT1 and WT2 (Figure 4A). Analyses of As species in xylem sap showed that the percentage of AsV in the xylem sap of both mutants and WT plants were lower than that of AsIII (Figure 4B). Though the AsV proportions in xylem saps were similar in mutants and WT under +P condition, they were lower in mutants than that in WTs under −P condition (Figure 4B), indicating that OsPT4 mutation decreased AsV translocation under P starvation.

In combination, the decreased total As and AsV concentrations in xylem saps of ospt4 mutants suggested that OsPT4 gene mediated AsV xylem loading. Decreased As and AsV in the roots of ospt4 mutants may also attribute to decrease As and AsV in xylem; however, considering OsPT4 is an AsV transporter, and it also strongly expressed in endodermis and stelle cells of roots,42 we proposed that OsPT4 may mediate AsV translocation toward to xylem and AsV loading into xylem, which facilitates AsV translocation from the roots to shoots. As a result, the loss-function of OsPT4 gene impaired AsV translocation efficiency in rice.

To further verify the role of OsPT4, As translocation and distribution (total As in shoots/total As in roots) in ospt4 mutants and WT plants were analyzed. As shown in Figure S2, shoot/root As ratio of M1 and M2 were lower than that of WT plants under both +P and −P condition. Moreover, under −P condition, shoot/root As ratios of M1 and M2 were 27% and 20% lower than that of WT plants, further suggesting that knocking out OsPT4 gene decreased As translocation in rice.

OsPT4 gene facilitates acquisition and mobilization of P and is strongly expressed in cortex and stelle cells of the roots, independent of P supply conditions.42 Here, we also compared P accumulation between ospt4 mutants and their WTs. Like AsV, P concentration was decreased in the shoots of ospt4 mutants compared with WT plants (Figure S3). The results indicated that OsPT4 mutation in rice decreased the uptake and root-to-shoot translocation of both As and P.

Although it has been shown that P transporters are critical for AsV uptake, translocation and accumulation, the contribution of a given P transporter to AsV metabolism is still unclear. For example, overexpression of OsPT8 gene greatly increases AsV uptake and translocation in rice cultivar Nipponbare,42 whereas mutation of OsPT8 gene in Nipponbare and Kasalath cultivars significantly increases AsV tolerance and decreases AsV uptake (33–57%).43 OsPT1 is involved in uptake and root-to-shoot transfer of AsV in rice cultivars Nipponbare and Dongjin using T-DNA mutant and overexpression lines.44 However, OsPT1 mutation or overexpression conferred no significant impact on AsV tolerance in rice, so it is unclear if...
OsPT1 gene involves in As resistance in rice. In our study, using mutants of Nipponbare and Zhonghua11 cultivars, we showed that OsPT4 mutation significantly increased AsV tolerance in both cultivars, similar to the phenotypes of OsPT8 mutation. In addition, since M2 with Zhonghua11 background exhibited greater decrease of As accumulation than M1 with Nipponbare background (Figure 2), contribution of OsPT4 in As uptake and translocation in Zhonghua11 may be greater than that in Nipponbare. However, since the As uptake experiments were performed under different conditions using different materials in different studies, AsV uptake and translocation efficiency by different OsPT4 may not be comparable.

**Knocking Out OsPT4 Gene Decreased Inorganic Arsenic Accumulation in Rice Grains Growing in Flooded Soil.** Base on the fact that OsPT4 mediated AsV uptake and root-to-shoot translocation in rice under short-term hydroponic cultivation (Figure 2 and 3), knocking out OsPT4 gene may also reduce As accumulation in rice grains growing in flooded soils where AsV also exists. In flooded soils, microorganisms exist and large amount of As is bound to the soil and sediment, different from being soluble in nutrient solution. Moreover, soil cultivation provides suitable conditions for the full life cycle growth of rice.

We thus performed long-term experiments where ospt4 mutants and their WTs were grown until maturity in a flooded soil. The soil contained 15 mg kg\(^{-1}\) available P and supplied with 20 mg kg\(^{-1}\) As. After harvest, As concentrations in different rice tissues were determined, showing no significant difference in straw between ospt4 mutants and WT plants (data not shown). The As concentrations in the grain of ospt4 mutants and WT plants were also comparable (Figure 5A).

Arsenic speciation analyses showed that rice grains contained predominantly DMA and AsIII, with AsV being a minor species (Figure 5B). Knocking out OsPT4 gene in M1 and M2 decreased AsV concentration by 44–53%, compared to WT1 and WT2 (Figure 5B), suggesting OsPT4 may mediate AsV transport to rice grains thereby affecting their AsV accumulation. More importantly, both AsV and AsIII proportions in the grains of ospt4 mutants were lower than the WTs (Figure 5B), and knocking out OsPT4 gene in M1 and M2 cut down inorganic As accumulation in rice grains by 16–45% (Figure 5C), which is of significance to control inorganic As exposure from rice.

In rice, though P transporters confer As accumulation in hydroponic experiments, they may contribute little to As uptake and transport to rice grains under flooded soil conditions. For example, in hydroponic experiments, phf1 rice mutant lost much of the ability to take up AsV or transport it from the roots to shoots. However, under flooded soil conditions, similar effects on As accumulation and distribution were not observed, probably because the small proportion of AsV in both soils and plant tissues. Besides, OsPT1 is involved in AsV uptake from soil and knocking out OsPT1 gene decreases As accumulation in the leaves, but not in brown rice. It was reported that OsPT8 has a high affinity for AsV and its overexpression markedly increases its AsV uptake and translocation in rice. However, whether OsPT8 attributes to AsV accumulation in rice cultivated in flooded soils is still unclear. Different from these studies, we not only focused on As accumulation in rice under soil cultivation condition, but also emphasized the inorganic As accumulation in rice grains. This is because inorganic As is more closely related to AsV uptake and translocation, and also more relevant to human health risk.

In some Asian countries where rice is the national staple, As intake from rice impacts millions of people. For example, inorganic As in rice grain may be the largest contributor of As exposure through food chain for people in China. To reduce As risk from rice, much research focuses on selecting cultivars that biologically restrict As accumulation in the grains (Norton et al., 2012; Syu et al., 2015; Zhang et al., 2016). Moreover, some quantitative trait loci associated with As accumulation in rice have been mapped (Zhang et al., 2008; Norton et al., 2014). However, the candidate genes critical for As metabolism in plants including rice need to be identified to decrease As risk from the food chain. Thus, the investigation of OsPT4 gene in our study can be of significance as it helps to breed low-As rice to enhance food safety.

In soils, especially in the rhizosphere, As could be methylated by microorganisms. In our study, the proportions and concentrations of DMA in rice grains were higher in ospt4 mutants, this may be because OsPT4 mutation decreased AsV uptake in the roots, and increased inorganic As in soil solution, thereby enhancing microbial As methylation and leading to higher DMA accumulation in rice grains. This variation of As transformation is similar to the effects of P, which also leads to As release from soil solid phase, increasing inorganic As in soil solution and thereby enhancing microbial methylation and DMA accumulation in rice.

Taken together, our study showed that, phosphate transporter OsPT4 is involved in AsV uptake and translocation in rice. Mutation in OsPT4 gene significantly enhanced AsV tolerance in rice by decreasing As accumulation in rice seedlings in hydroponic experiments. Arsenic is present in rice grains mainly as inorganic As and DMA, but as a carcinogen, inorganic As is generally much more toxic than methylated As. Few studies showed that P pathways contribute to As accumulation in rice plants grown in flooded soil, and our study clearly showed that knocking out OsPT4 gene decreased inorganic As accumulation in rice grains, especially in Zhonghua11, which is of significance to control As exposure risk from rice. Better understanding of the functions of OsPT4 gene in As metabolism in rice also provides insight into breed low-As rice to enhance food safety.
ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03028.

Characterization of mutants of OsPT4 gene, As distribution (shoot/root) in ospt4 mutants and wild-types and total P concentration in ospt4 mutants and wild-types. (Figures S1–S3). Primers used for ospt4 mutants identification and qRT-PCR analysis and fitted parameters of arsenate uptake kinetics of ospt4 mutants and wild types (Table S1–S2) (PDF)

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