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Uptake and Translocation of Arsenic and Phosphorus in \textit{pho2} Mutant and Wild Type of \textit{Arabidopsis thaliana}

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ABSTRACT

A pot experiment was conducted in a growth chamber to investigate the difference in arsenic (As) uptake and translocation by the \textit{pho2} mutant and wild type (WT) of \textit{Arabidopsis thaliana} (L.) Heynh. The \textit{pho2} mutant had higher shoot phosphorus (P) and As concentrations than did WT, but had lower shoot P uptake and similar total shoot As uptake relative to WT because of its smaller shoot biomass. Similarly, root P uptake and As uptake were lower in \textit{pho2}, but \textit{pho2} had similar root P and slightly higher As concentrations than those of WT. Furthermore, specific P and As uptake and P and As translocation from roots to shoots in \textit{pho2} were higher than in WT. The results indicate that a small portion of As may have been co-translocated with P from roots to shoots. Addition of 10 mg As kg\(^{-1}\) enhanced the growth of WT without P addition. Addition of 10 mg P kg\(^{-1}\) promoted plant yield and As uptake, and further addition up to 40 mg P kg\(^{-1}\) reduced shoot and root As concentrations. The results indicate that the overall effect of phosphate on As accumulation in the plants may be related to competition between P and As on the surfaces of soil particles and plant roots.

Keywords: uptake, \textit{Arabidopsis}, arsenic, \textit{pho2}, phosphate, translocation

INTRODUCTION

Arsenic (As) is ubiquitous in the environment and As in soils is derived from both natural and anthropogenic sources (Juhasz et al., 2003; Xie et al., 1997).
Although As is an essential element for animals, both arsenate and arsenite are highly toxic to plants, animals, and microbes (Rodriguez et al., 2003; Ghosh et al., 2004). The toxicity of arsenate to plants is mediated by the disruption of phosphorylation and the inhibition of phosphate (P) uptake, and the toxicity of arsenite is due to its reaction with sulfhydryl (-SH) groups in enzymes and tissue proteins (Meharg and Hartley-Whitaker, 2002). Phytoremediation of As-contaminated soils has been proposed as a cost-effective cleanup method (McGrath et al., 2002), but successful phytoremediation would depend greatly on plant growth (biomass production) and on As uptake and distribution within the plant. Certain species of fern have been identified as As hyperaccumulators (Ma et al., 2001; Zhao et al., 2002).

In all plants tested so far, it has been shown that arsenate (the dominant form of As in aerated conditions) is taken up via the phosphate transport systems because of the chemical similarity between arsenate and phosphate (Meharg and Macnair, 1992; Wang et al., 2002; Dixon, 1997). It has been demonstrated that arsenate inhibits phosphate uptake by yeast, phytoplankton, the As hyperaccumulator Chinese brake fern *Pteris vittata*, and *A. thaliana* (Wang et al., 2002; Rothstein and Donovan, 1963; Blum, 1966; Clark et al., 2003). Similarly, arsenate uptake is suppressed by phosphate in phytoplankton, rice (*Oryza sativa* L.), the As-tolerant grass *Holcus lanatus*, and the As hyperaccumulator *P. vittata* (Meharg and Macnair, 1992; Wang et al., 2002; Planas and Healey, 1978; Abedin et al., 2002; Tu and Ma, 2003).

However, the mechanisms of As translocation to shoots have yet to be elucidated. To understand the translocation of As from roots to shoots, it would be helpful to use plant genotypes with different patterns of P translocation from roots to shoots. The *pho2* mutant of *Apidopsis thaliana* (L.) Heynh. was reported to accumulate excessive P (inorganic phosphate) in shoots compared with wild type (WT) (Delhaize and Randall, 1995). Dong et al. (Dong et al., 1998) suggested that the high accumulation of P in shoots of the *pho2* genotype might result from a partial defect in P transport in the phloem between shoots and roots or from an inability of shoot cells to regulate internal P concentrations. Because of the similarity between arsenate and phosphate, we might expect *pho2* to accumulate more As and P than WT. The present study, therefore, aimed to investigate differences in As uptake and allocation by *pho2* and WT and interactions between P and As in the soil-plant system.

**MATERIALS AND METHODS**

**Soil Characterization**

The growth medium used in this experiment was a soil/vermiculite mixture (1:1 v/v). A low- P soil was collected from Luancheng Experimental Station, Chinese Academy of Sciences (lat 37° 50’ N, long 114° 40’ E). Soil chemical
properties were measured using methods recommended by the Chinese Society of Soil Science (Lu, 1999). Soil pH was measured in a 1:1 soil/water suspension with a combination electrode and available P was extracted using 0.5 M NaHCO₃. Soil total As was measured by digestion in aqua regia (1:3 HNO₃/HCl) and HClO₄ at 160°C. Selected chemical properties of the soil were as follows: pH 7.74, OM 17.5 mg g⁻¹, Olsen-P 4.55 mg kg⁻¹, and total As 10.1 mg kg⁻¹.

**Experimental Design**

The experiment was set up in a completely randomized design with three factors: two As addition rates (0 and 10 mg As kg⁻¹ as Na₃AsO₄·12H₂O), three P addition rates (0, 10, and 40 mg P kg⁻¹ as KH₂PO₄), and two genotypes (WT and pho2). The growth medium was uniformly amended with nitrogen (N) and potassium (K) fertilizers (200 mg N kg⁻¹ as NH₄NO₃ and 150 mg K kg⁻¹ as K₂SO₄) and the experimental P and As treatments. The soil/vermiculite mixture was placed in plastic pots, each of which contained 300 g of the growth medium.

**Plant Culture**

Seeds of WT A. thaliana (L.) Heynh. (ecotype ‘Columbia’) and the pho2 mutant (Columbia genetic background, backcrossed at least three times to WT) were provided by Dr. E. Delhaize, CSIRO, Australia. Seeds of the pho2 mutant and WT were sterilized in 10% (v/v) H₂O₂ for 10 min followed by thorough washing in de-ionized water, and were transferred onto the soil surface by toothpick, with six seeds per pot. Ten days later, the seedlings were thinned to four per pot. Each treatment had four replicates. The seedlings were grown in a growth chamber with a 14/10 h light/dark cycle and a light intensity of about 280 µmol m⁻² s⁻¹. The pots were re-randomized daily during the growth period. Soil moisture content was regularly adjusted to 30% by weight with de-ionized water.

**Plant Analysis**

Thirty-seven days after emergence, plants were harvested by cutting off the shoots and floating the whole soil/vermiculite mixture in each pot in a beaker containing deionized water. Once in the water, most of the soil/vermiculite mixture fell off the roots and sank to the bottom of the beaker. Any soil/vermiculite remaining on the roots was gently removed with tweezers. The roots and soil/vermiculite were inspected after washing and care was taken to ensure complete recovery of each root system using a sieve. The plant material was oven-dried at 70°C for 48 h and the dry weights of shoots and roots were determined. Dried shoots and roots were finely ground in a stainless-steel mill and
sub-samples (about 0.25 g) were digested in 5 mL of high-purity nitric acid, first at 80°C for 2 h and then at 120°C for 20 h. Arsenic and P concentrations in the digest were determined using a Perkin Elmer Optima 2000DV inductively coupled plasma-optical emission spectrometer (ICP-OES). Arsenic concentrations in shoots were generally lower than those in roots and were therefore determined using an AF-610A atomic fluorescence spectrometer (Beijing Ruili Analytical Instrument Co., Beijing, China). Tealeaf samples (obtained from the Chinese Center for Standard Materials and with P and As concentrations of 2840 ± 60 and 0.28 ± 0.03 mg kg⁻¹, respectively) were used as a standard reference material for quality assurance during digestion and analysis by ICP-OES and atomic fluorescence spectrometry.

**Data Analysis**

Specific P or As uptake (SPU or SAsU) was calculated as the ratio of total P or total As uptake in each pot to the root dry weight in that pot. Phosphorus and As translocation from roots to shoots was calculated as the ratio of shoot P or As uptake to root uptake.

All data were subjected to analysis of variance performed using Genstat for Windows (VSN International Ltd, Hemel Hempstead, UK). When neither P nor As was added to the pots, WT grew poorly because of low Olsen-P (4.55 mg kg⁻¹) in soil; the results of this treatment are not presented. When no As was added, As concentrations in the plants were below the detection limit.

**RESULTS**

**Plant Biomass**

Phosphorus additions markedly increased the yield of both genotypes (Figure 1, \( P < 0.001 \)). Arsenic also slightly increased the biomass of the wild type but had no effect on that of *pho2* except for some toxicity symptoms at the P addition rate of 40 mg kg⁻¹. When P additions were 10 and 40 mg kg⁻¹, *pho2* had significantly lower biomass than WT (\( P < 0.001 \)). Root biomass in both genotypes was much smaller than shoot biomass, irrespective of P and As additions (\( P < 0.001 \)).

**Phosphorus and Arsenic Concentrations in Shoots and Roots**

With increasing P additions, shoot P concentrations of both genotypes increased significantly (Figure 2, \( P < 0.001 \)), but As additions had no effect on shoot P concentrations in either genotype. At the same P and As addition rates, *pho2*
As and P Uptake by Arabidopsis

Figure 1. Shoot and root biomass of pho2 and WT of A. thaliana. The error bars represent one SE of the mean from four replicates. By analysis of covariance, genotype (G), P level (P), and the two-way interaction (G × P), were highly significant (P < 0.001) for both shoot and root biomass. In addition, G × As (P = 0.003) and P × As (P = 0.024) were significant for root biomass.

had higher P concentration in the shoots than did WT (Figure 2, P < 0.001). Phosphorus and As additions did not result in significant differences in root P concentrations between the two genotypes (Figure 2). Furthermore, total P in shoots and roots was lower in pho2 than that in WT (Table 1).

In both genotypes, addition of P at the rate of 10 mg kg⁻¹ did not affect shoot As concentrations, but increased root As concentrations compared with the zero-P controls (Figure 3). Further addition of P from 10 to 40 mg kg⁻¹ significantly
Figure 2. P concentrations in the shoots and roots of pho2 and WT in A. thaliana. The error bars represent one SE of the mean from four replicates. By analysis of covariance, genotype and P addition were both highly significant ($P < 0.001$) and the $G \times P \times As$ interaction was significant ($P = 0.004$) for shoot P concentration.

Reduced As concentrations in both shoots and roots of both genotypes ($P < 0.001$). The pho2 genotype had higher As concentrations in roots and shoots than did WT, except for root As concentration at zero-P addition (Figure 3, $P < 0.001$). When P was added at both 10 and 40 mg kg$^{-1}$, total As in roots was lower in pho2 than in WT and there was little difference in total shoot As between the two genotypes across all P treatments (Table 1).
Table 1
Uptake of P and As in *pho2* and WT in *A. thaliana*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>P addition (mg kg$^{-1}$)</th>
<th>As addition (mg kg$^{-1}$)</th>
<th>P uptake (µg pot$^{-1}$)</th>
<th>As uptake (µg pot$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td>Roots</td>
</tr>
<tr>
<td>WT</td>
<td>0</td>
<td>10</td>
<td>32 ± 3</td>
<td>246 ± 22</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>83 ± 2</td>
<td>1020 ± 48</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>72 ± 2</td>
<td>1123 ± 30</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>111 ± 14</td>
<td>3320 ± 91</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>125 ± 6</td>
<td>3458 ± 95</td>
</tr>
<tr>
<td><em>pho2</em></td>
<td>0</td>
<td>0</td>
<td>16 ± 1</td>
<td>203 ± 16</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>15 ± 3</td>
<td>190 ± 35</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>33 ± 0.4</td>
<td>781 ± 47</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>29 ± 2</td>
<td>842 ± 11</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>63 ± 3</td>
<td>2650 ± 37</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>55 ± 4</td>
<td>2352 ± 187</td>
</tr>
</tbody>
</table>

Analysis of covariance

- P addition (P): <0.001 <0.001 <0.001 <0.001
- Genotype (G): <0.001 <0.001 =0.003 NS*
- P × G: <0.001 <0.001 NS NS
- As × G: =0.023 =0.011 NS NS

*NS, not significant.
Values are the means of four replicates ± one standard error.

**Specific Phosphorus Uptake (SPU) and Specific Arsenic Uptake (SAsU)**

With increasing P addition, SPU increased significantly in both genotypes (Figure 4a, *P* < 0.001). However, P addition at 10 mg kg$^{-1}$ increased the SAsU of both genotypes (Figure 4b) and the further increase in P addition to 40 mg kg$^{-1}$ significantly reduced the SAsU of both genotypes (*P* < 0.001). Unlike P addition, As addition had little effect on SPU in both genotypes except that when P was added at 10 mg kg$^{-1}$, As addition increased SPU in *pho2*. The *pho2* plants always had higher SPU and SAsU than did WT at the same addition levels of P and As (Figure 4a,b).

**Phosphorus and Arsenic Translocation from Roots to Shoots**

In both genotypes, P additions significantly increased P translocation from roots to shoots but had no significant effect on As translocation (Figure 5). Only at the P addition of 10 mg kg$^{-1}$ did As additions increase P translocation. The *pho2* mutant showed higher P and As translocation from roots to shoots than did WT at the same addition rates of P and As.
Figure 3. As concentrations in the shoots and roots of pho2 and WT in *A. thaliana*. The error bars represent one SE of the mean from four replicates. By analysis of covariance, genotype (\( P < 0.001 \) for shoots and \( P = 0.013 \) for roots), and P addition (\( P < 0.001 \) for both roots and shoots) had significant effects on plant As concentrations.

The roots had a higher ratio of As concentration to P concentration than did the shoots (Table 2). With increasing rate of P addition, the As/P ratio increased at first and then decreased in the roots, whereas in the shoots the ratio decreased markedly. The two genotypes showed little difference in As:P ratio in either the roots or the shoots.

**DISCUSSION**

Although it was found that pho2 had higher P concentrations in shoots than did the WT (Figure 2), the highest P concentration in pho2 was 5 mg g\(^{-1}\), far less than the 20 mg g\(^{-1}\) reported by Delahaize and Randall (1995), but similar to the 4 mg g\(^{-1}\) reported by Quaghebeur and Rengel (2004), due perhaps to low transpiration rates in the culture condition. The pho2 phenotype had higher P
Fig 4. Specific P (a) and As (b) uptake in A. thaliana. The error bars represent the SE of the mean from four replicates. By analysis of covariance, SPU was highly significantly affected ($P < 0.001$) by genotype, P addition, As addition, all three two-way interactions, and the $G \times P \times As$ three-way interaction. SAsU was significantly affected by $G$ and $P$ (both $P < 0.001$) and by $G \times P$ ($P = 0.033$).

and As concentrations in the shoots than did WT, but had lower total shoot P and total shoot As similar to WT because of the smaller shoot biomass. For the same reason, P and As uptake in the roots were less for $pho2$, although the roots of $pho2$ had similar P concentrations and slightly higher As concentrations than those of WT (Table 1, Figure 1).

The WT grew poorly when there was no addition of As or P because of low Olsen-P (4.55 mg kg$^{-1}$) in the soil, but grew well with the addition
Figure 5. P and As translocation from roots to shoots in *A. thaliana*. The error bars represent the SE of the mean from four replicates. By analysis of covariance, P translocation was significantly affected by all three factors and interactions (all $P < 0.001$ except As at $P = 0.004$). Arsenic translocation was affected by genotype only ($P = 0.017$).

of As (Figure 1), possibly because of improved P nutrition through arsenate-induced phosphate release from the soil (Lambkin and Alloway, 2003). For both genotypes, P addition of 10 mg kg$^{-1}$ increased As concentrations in roots and uptake of As by roots and shoots and did not reduce shoot As concentrations (Figure 3, Table 1) because P addition promoted plant growth and induced As desorption from soil particles, and thus increased As uptake. However, a further increase in P addition rate up to 40 mg kg$^{-1}$ resulted in a significant reduction in As concentration in shoots and roots (Figure 3) because P competed with arsenate for the same absorption site in the root plasma membrane. Therefore,
two types of interaction between P and As may occur at two different sites: adsorption-desorption processes on soil particles and competition for the same absorption sites on the root cell membrane, which is controlled by different plant species and cultivars. The overall effect of As and P interactions on As and P accumulation in plants may thus depend on the balance between these two processes at two different sites in the soil-plant system.

In the present study, pho2 had higher shoot As and P concentrations than did WT at the same P and As addition rates (Figures 2 and 3), indicating that pho2 had slightly higher As translocation from roots to shoots as well as P translocation. We also found that wheat cultivars with higher shoot P concentrations had higher shoot As concentrations (data not shown). Moreover, P additions increased As translocation from roots to shoots in wheat and rice (data not shown), but this did not occur in Arabidopsis (Figure 5). Meharg and Macnair (1991) also found that in an arsenate-tolerant genotype of Holcus lanatus, increasing phosphate concentration in nutrient solution increased As transport to the shoots and As stress inhibited transport of As to the shoots. Quaghebeur and Rengel (2004) recently examined As uptake and translocation in pho1 and pho2 mutants, and found that in solution culture pho2 accumulated more As in shoots than did WT; however, this did not occur in a pot experiment in which the plants grew in soil. Whether or not (or to what extent) As is co-transported with P within plants may depend on the speciation of As in the xylem sap, and only arsenate would be translocated via the P pathway to shoots. In Indian mustard (Brassica juncea), both arsenate and arsenite (41% and 51%, respectively) were detected in the xylem sap and were not coordinated by sulfur (S) (Pickering et al., 2000). In H. lanatus, when grown in a higher P (100 µM) solution, the plants contained a higher proportion of arsenate (with regard to total As) in both roots and shoots than did plants supplied with lower P (10 µM) (Quaghebeau

<table>
<thead>
<tr>
<th>Genotype</th>
<th>P addition (mg kg(^{-1}))</th>
<th>Roots</th>
<th>Shoots ((× 10^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0</td>
<td>0.05</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.11</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.07</td>
<td>0.69</td>
</tr>
<tr>
<td>pho2</td>
<td>0</td>
<td>0.06</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.15</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.09</td>
<td>0.84</td>
</tr>
</tbody>
</table>
These results indicate that a small fraction of As might be translocated to shoots as arsenate in the xylem in Arabidopsis.

Although pho2 had higher shoot As concentrations than WT, the transfer of As from soil to plant was minimal, for several possible reasons: (1) low bioavailability of As in soil; (2) restricted uptake by plant roots; (3) limited translocation of As from roots to shoots; and (4) As phytotoxicity at relatively low concentrations in plant tissues (Wang et al., 2002). It was found that the shoot-to-root ratio of As concentrations varied from 0.03 to 0.07, with no difference between the two genotypes (data not shown). Other workers have also found shoot-to-root ratios of As concentrations of <0.02 in tomato (Lycopersicon esculentum), <0.1 in Brassica juncea, and <0.2 in rice when arsenate was supplied (Pickering et al., 2000; Burló et al., 1999; Marin et al., 1992), far lower than P translocation.

Although arsenate is supposed to be taken up by P transport systems, the uptake system was highly selective for phosphate (Meharg et al., 1994). In the present study, at the highest ratio of P to As in soil was 4:1; the ratios in roots were 16:1 and 11:1 for WT and pho2, respectively, indicating that P transporters on root cell membranes were selective for phosphate. Furthermore, under the same conditions the ratios in shoots were 1491:1 and 1198:1 for WT and pho2, respectively, implying that the translocation system was even more selective for P than for As, which may serve as an internal detoxification mechanism in plants. It is not clear if a certain protein controlling As translocation from roots to shoots exists. Recently, a gene (ARAth;Pht2;1) involved in P translocation within plants has been cloned (Daram et al., 1999). However, further research is warranted for the elucidation of the mechanisms involved in As translocation within plants.

ACKNOWLEDGMENTS

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