Arsenate (As) uptake by and distribution in two cultivars of winter wheat (*Triticum aestivum* L.)

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Abstract

Two cultivars of winter wheat (*Triticum aestivum* L.) (Jing 411 and Lovrin 10) were used to investigate arsenate (As) uptake and distribution in plants grown in hydroponic culture and in the soil. Results showed that without As addition, Lovrin 10 had higher biomass than Jing 411 in the soil pot experiment; in the hydroponic experiment Lovrin 10 had similar root biomass to and lower shoot biomass than Jing 411. Increasing P supply from 32 to 161 μM resulted in lower tissue As concentrations, and increasing As supply from 0 to 2000 μM resulted in lower tissue P concentrations. Increasing P supply tended to increase shoot-to-root ratios of As concentrations, and increasing As supply tended to decrease shoot-to-root ratios of As concentrations. Both cultivars invested more in root production under P deficient conditions than under P sufficient conditions. Lovrin 10 invested more biomass production to roots than Jing 411, which might be partly responsible for higher shoot P and As concentrations and higher shoot-to-root ratios of As concentrations. Moreover, Lovrin 10 allocated less As to roots than Jing 411 and the difference disappeared with decreasing P supply.

Keywords: *Triticum aestivum*; Arsenic (As); P efficiency; As uptake; As distribution

1. Introduction

Arsenic (As) may play an essential role in animal nutrition (Uthus, 1992, 1994), possibly in methionine metabolism, but it is generally considered as an element highly toxic to plants and animals (National Research Council, 1977). Indeed, arsenic toxicity in humans has recently received increasing attention due to large scale contamination in regions such as Bangladesh (Dhar et al., 1997) and northwest China (L.F. Wang et al., 2002). Arsenic can be derived from both geogenic (Juhasz et al., 2003) and anthropogenic sources, such as mining and agricultural activities (Xie et al., 1997; Wang et al., 1999). As a result of this environmental contamination, tens of millions of people are exposed to elevated levels of As in drinking water and diets. It is reported that in the vicinity of an As mine in Hunan, China, as high as 35% of the local population had severe arsenism, and that the percentage increased with age (Wang et al., 1999). Epidemiological studies further demonstrated that there was a significant correlation between As concentrations...
in human hairs and those in local rice (Oryza sativa L.), wheat (Triticum aestivum L.) and soils (Lin et al., 2001). It is generally accepted that soil–plant transfer of As is one of the principal pathways for human exposure to As.

Inorganic arsenic (arsenate and arsenite) is highly toxic to plants because it uncouples phosphorylation and inhibits phosphate uptake. At higher concentrations, arsenic interferes with plant metabolic processes and can inhibit growth, and under severe conditions may lead to plant death. For example, biomass production and yields of a variety of crops were reduced significantly at elevated As concentrations (Carbonell-Barrechina et al., 1997). The concentration of 50 mg As kg\(^{-1}\) in soil significantly decreased the yields of barley (Hordeum vulgare L.) and ryegrass (Lolium perenne L.) (Jiang and Singh, 1994). Arsenic, the dominant form of As in aerated conditions, is taken up by plants via the phosphate (Pi) transport systems because of the chemical similarity between arsenate and Pi (Dixon, 1997). It has been demonstrated that arsenate inhibits Pi uptake by yeast (Rothstein and Donovan, 1963), phytoplankton (Blum, 1966), Arabidopsis thaliana (Clark et al., 2003) and the As hyperaccumulator, Chinese brake fern Pteris vittata (J.R. Wang et al., 2002). Similarly, Pi suppresses arsenate uptake by phytoplankton (Planas and Healey, 1978), rice (Abedin et al., 2002), Lupinus albus (Esteban et al., 2003), the As tolerant plants Holcus lanatus and Cytisus striatus (Meharg and Macnair, 1992; Bleeker et al., 2003), and P. vittata (J.R. Wang et al., 2002; Tu and Ma, 2003).

Under field conditions, the wheat cultivar Lovrin 10 has been identified as more efficient than cultivar Jing 411 in P uptake and utilization (Davies et al., 2002). In wheat, several Pi transporters have been identified and the expression pattern of phosphate transporters differs between these two cultivars (Davies et al., 2002). Since arsenate was taken up by phosphate transporters, these two cultivars may also show different patterns of As uptake and translocation. Thus the objectives of this study were to investigate the difference in As uptake and translocation between cultivars Jing 411 and Lovrin 10, grown in solution and soil.

2. Materials and methods

2.1. Plant culture in hydroponics

Seeds of two winter wheat cultivars Jing 411 and Lovrin 10 were sterilized in 10% H\(_2\)O\(_2\) (w/w) for 10 min followed by thorough washing in de-ionized water, and then germinated on moist filter paper for 2 days. Germinated seeds were transferred to moist perlite and cultivated for 10 days. The seedlings were then removed from the perlite and were washed carefully under tap water to remove any adhering particles. Seedlings were then transferred to PVC pots containing 500 ml modified Hoagland’s solution containing (in mM): KNO\(_3\), 2.0; Ca(NO\(_3\))\(_2\), 2.0; MgSO\(_4\), 0.7; and (in \(\mu\)M), FeEDTA, 50; ZnSO\(_4\), 0.5; CuSO\(_4\), 0.5; MnSO\(_4\), 2.5; H\(_2\)BO\(_3\), 5; Na\(_2\)MoO\(_4\), 0.25; CoSO\(_4\), 0.20; NaCl, 50. The solution was supplemented with two P concentrations (32 or 161 \(\mu\)M) as KH\(_2\)PO\(_4\). Ten days later the solutions were amended with three As concentrations (0, 3.3 and 6.7 \(\mu\)M) as Na\(_2\)AsO\(_4\)·12H\(_2\)O and the corresponding P concentrations for another 13 days. Altogether there were six treatments of P and As levels with four replicates for each treatment. The seedlings were grown in a growth chamber with 14/10 h light/dark cycles, temperature was kept at 28 °C during the day and 20 °C during the night. Light intensity was around 280 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). The nutrient solution was rene-wed twice a week and aerated continuously. Pots were randomly arranged every day during the growth period.

2.2. Plant culture in the soil pot experiment

A low P soil was taken from the Luancheng Experimental Station, Chinese Academy of Sciences (37°50′N, 114°40′E). Soil chemical properties were measured using methods recommended by the Chinese Society of Soil Science (Lu, 1999). The selected chemical properties of the soil are as follows: pH 7.74, OM 1.75%, Olsen-P 4.55 mg kg\(^{-1}\), and total As 10.1 mg kg\(^{-1}\). Uniform fertilizers were amended as follows: 14.3 mM N as NH\(_4\)NO\(_3\), 1 mM P as KH\(_2\)PO\(_4\) and 3.8 mM K as K\(_2\)SO\(_4\). Three As concentrations (0, 667 and 2000 \(\mu\)M) were also added into the soil. Seven days later, the soil was packed into plastic pots, each pot contained 500 g soil. Seeds of Jing 411 and Lovrin 10 were sterilized and germinated as in hydroponics and germinated seeds were transferred to plastic pots. Ten days later the seedlings were thinned to two per pot. Each treatment had four replicates. The seedlings were grown in the greenhouse for 70 days with a 12/12 h light/dark cycle and a light intensity of about 280 \(\mu\)mol 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.

2.3. Plant analysis

At harvest, plants were divided into roots and shoots. Plant materials were then oven dried at 70 °C for 48 h and the dry weights of shoots and roots were recorded. Dried shoots and roots were finely ground in a stainless steel miller. The powdered dry materials were digested in 5 ml of high-purity concentrated nitric acid, first at 80 °C for 2 h and then 120 °C for 20 h. The As and P concentrations of the solution were determined by ICP-OES (inductively coupled plasma optical emission spectrometer, Optima 2000 DV, Perkin–Elmer, USA). The As concentrations for each treatment. The seedlings were grown in a growth chamber with 14/10 h light/dark cycles, temperature was kept at 28 °C during the day and 20 °C during the night. Light intensity was around 280 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). The nutrient solution was rene-wed twice a week and aerated continuously. Pots were randomly arranged every day during the growth period. Soil moisture content was regularly adjusted to 30% by weight with de-ionized water.
concentrations in shoots were generally lower than those in roots, and were determined by an AF-610A atomic fluorescence spectrometry (Beijing Ruili Analytical Instrument Co., Beijing, China). Standard material (tea leaves, obtained from China Standard Material Center) was used to ensure the accuracy of digestion and analysis. No As was detected in wheat with no As addition.

2.4. Data analysis

P and As distribution between roots and shoots were calculated as the percentages of P or As uptake to roots with respect to total P or As uptake (root + shoot), respectively.

All data were subjected to analysis of variance (ANOVA) performed on the Windows-based Genstat package (sixth ed., NAG Ltd., England).

3. Results

3.1. Plant growth response

In the hydroponic experiment, arsenic supply slightly reduced both root and shoot biomass (Table 1, $P = 0.012$ and $0.008$, respectively). For both cultivars P supply slightly reduced root dry weights, but markedly increased shoot dry weights ($P = 0.007$ and $P < 0.001$, respectively). Increasing external P concentrations resulted in a reduction in the percentages of biomass allocated to roots ($P < 0.001$). At the same P and As supply, Jing 411 had higher shoot biomass, but lower percentages of biomass allocated to roots than Lovrin 10 ($P < 0.001$).

In the soil pot experiment, without As addition, Lovrin 10 had higher root and shoot biomass than Jing 411 (Table 2, $P < 0.001$). At As addition of 667 μM, Lovrin 10 had lower shoot biomass than Jing 411. Lovrin 10 always invested more in root biomass than Jing 411 irrespective of As addition.

3.2. As concentrations in roots and shoots

In the hydroponic experiment, As supply markedly increased root and shoot As concentrations, but P supply markedly decreased As concentrations in roots and shoots (Fig. 1, $P < 0.001$). At the same P and As supply, the two cultivars had similar root As concentrations, but Lovrin 10 higher shoot As concentrations than Jing 411 ($P < 0.001$).

### Table 1

<table>
<thead>
<tr>
<th>P supply (μM)</th>
<th>As supply (μM)</th>
<th>Jing 411</th>
<th>Lovrin 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root biomass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0.0</td>
<td>0.38 ± 0.02</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>0.29 ± 0.02</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>0.32 ± 0.02</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>0.32 ± 0.03</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>0.27 ± 0.02</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>0.27 ± 0.02</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>32</td>
<td>0.79 ± 0.03</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>0.62 ± 0.03</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>0.69 ± 0.04</td>
<td>0.50 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>1.07 ± 0.12</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>0.89 ± 0.06</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>0.85 ± 0.04</td>
<td>0.80 ± 0.13</td>
</tr>
<tr>
<td>Root biomass (%)</td>
<td>32</td>
<td>32.6 ± 1.1</td>
<td>38.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>31.8 ± 0.2</td>
<td>37.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>31.6 ± 0.4</td>
<td>37.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>161</td>
<td>23.3 ± 0.3</td>
<td>26.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>23.6 ± 0.7</td>
<td>26.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>24.2 ± 0.4</td>
<td>26.4 ± 1.2</td>
</tr>
</tbody>
</table>

### Analysis of variance

<table>
<thead>
<tr>
<th></th>
<th>Root biomass</th>
<th>Shoot biomass</th>
<th>Root biomass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P supply (P)</td>
<td>$P = 0.007$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>As supply (As)</td>
<td>$P = 0.012$</td>
<td>$P = 0.008$</td>
<td>NS</td>
</tr>
<tr>
<td>Cultivars (C)</td>
<td>NS</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
</tr>
<tr>
<td>C × P</td>
<td>NS</td>
<td>NS</td>
<td>$P = 0.003$</td>
</tr>
</tbody>
</table>

NS indicates not significant, $P > 0.05$. 

P addition tended to increase and As addition tended to decrease shoot-to-root ratios of As concentrations (Table 3, $P < 0.001$). At the same P and As supply, Lovrin 10 had higher shoot-to-root ratios of As concentrations than Jing 411 ($P < 0.001$).

In the soil pot experiment, at the As addition of 2000 µM, Lovrin 10 had higher tissue As concentrations than Jing 411. At As addition of 667 µM, Lovrin 10 had lower shoot As concentrations than and similar root As concentrations to Jing 411 (Fig. 2, $P < 0.001$).

### 3.3. P concentrations in roots and shoots

P concentrations in roots were not shown due to their similar pattern to shoot P concentrations. In hydroponics, increasing external P concentrations significantly enhanced P concentrations in roots and shoots irrespective of cultivars and external As concentrations. Arsenic addition had little effect on root P concentrations, but slightly increased shoot P concentrations ($P < 0.001$). At the same P and As supply, Lovrin 10 had higher shoot P concentrations than Jing 411 ($P < 0.001$), but there was no difference in root P concentrations between the two cultivars.

In the soil pot experiment, arsenic addition to the soil resulted in lower tissue P concentrations (Fig. 3(b), $P < 0.001$). With no As addition, Lovrin 10 had lower tissue P concentrations, and with As addition the difference in shoot P concentrations between the cultivars tended to disappear.

### 3.4. As and P distribution between roots and shoots

In the hydroponic experiment, for both cultivars, P supply resulted in significantly lower P and As allocation to roots (Fig. 4, $P < 0.001$). At 32 µM P, there was little difference in As allocation to roots between the two cultivars at both As concentrations. However, at 161 µM P, Lovrin 10 had lower As allocation to roots than Jing 411 at both As concentrations ($P < 0.001$).

### 4. Discussion

Since plant uptake of arsenate is mainly via phosphate (Pi) transporters (e.g. Clark et al., 2003), it has been well documented that Pi can effectively reduce plant uptake of...
arsenate, and arsenate can also inhibit plant uptake of Pi (Abedin et al., 2002; Esteban et al., 2003). Under hydroponic conditions, our results confirmed that increasing Pi concentrations in the external solution significantly hindered arsenate uptake by winter wheat (Fig. 1). Although P inhibited both root and shoot As concentrations, the reduction in roots seemed greater than that in shoots (Fig. 1). Moreover, although both root and shoot As concentrations increased with As additions, root As concentrations increased more rapidly than shoot As (Fig. 1). These data suggested that roots were more sensitive to P and As supply than shoots. J.R. Wang et al. (2002) also found that the inhibitory effect of P on As was more apparent in roots than in shoots.

However, increasing arsenate concentration in the growth solution did not affect P concentrations in plant roots, and marginally increased shoot P concentrations (albeit statistically significant). In the soil experiment, the addition of arsenate caused substantial reduction in P concentrations in both roots and shoots, this could be due to the much higher dose of arsenate freshly spiked into the soil used in the this experiment (Fig. 3). High efficiency of P uptake by plants growing in soil can mainly be ascribed to (1) the mobilization of sparingly soluble P in the rhizosphere; (2) root architecture and the density of Pi transporters on root cell membranes; (3) symbiotic association with mycorrhizal (especially arbuscular mycorrhizal) fungi (Föhse and Jungk, 1983; Bates and Lynch, 1996; Neumann et al., 2000; Zhu and Smith, 2001). However, in solution culture high P uptake efficiency can only depend on root architecture and the density of Pi transporters. Under field conditions, Lovrin 10 has been identified as more efficient than Jing 411 in P uptake and utilization (Davies et al., 2002). In the present hydroponic study, irrespec-

<table>
<thead>
<tr>
<th>P supply (µM)</th>
<th>As supply (µM)</th>
<th>Jing 411</th>
<th>Lovrin 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>3.3</td>
<td>43 ± 7</td>
<td>64 ± 4</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>27 ± 4</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>161</td>
<td>3.3</td>
<td>46 ± 5</td>
<td>92 ± 28</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>40 ± 5</td>
<td>68 ± 11</td>
</tr>
</tbody>
</table>

**Table 3** Shoot-to-root ratios of As concentrations in two wheat cultivars (Jing 411 and Lovrin 10) pretreated with 32 and 161 µM P for 10 d and then exposed to three As concentrations (0, 3.3, 6.7 µM) and the corresponding P concentrations (32 and 161 µM) for another 13 d in hydroponics (×10^{-3}, mean ± SE, n = 4).

**Analysis of variance**

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P supply (P)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>As supply (As)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cultivars (C)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P × As</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P × C</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>As × C</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P × As × C</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Root and shoot As concentrations in two wheat cultivars (Jing 411 and Lovrin 10) exposed to two As concentrations (667 and 2000 µM) and 1 mM P in the soil for 70 d. J and L refer to two wheat cultivars Jing 411 and Lovrin 10, respectively. Error bars represent standard errors of means from four replicates.
411, this could be due to a “dilution effect” since shoot biomass of Lovrin 10 was higher than that of Jing 411 in soil pot.

Compared to higher P supply (161 µM), lower P supply (32 µM) resulted in more biomass allocated to roots (higher root/shoot ratios, Table 1), which is a standard plant response to P deficiency (Chapin and Bieleski, 1982). This pattern also coincided with more absorbed As allocated into roots under 32 µM P supply than under 161 µM P supply (Fig. 4 (b)). In hydroponics, higher biomass allocation to roots by Lovrin 10 may contribute to relatively higher shoot P and As concentrations. The hydroponic experiment also demonstrated that there was genotypic difference in As allocation to the aboveground with Lovrin 10 being higher than Jing 411 (Fig. 4(b)).

The shoot-to-root ratios of As concentrations varied from 0.03 to 0.09 (Table 3), indicating that As allocation to the aboveground is relatively low. At the same P and As additions, Lovrin 10 had higher shoot-to-root ratios of As concentrations than Jing 411. In Arabidopsis, the shoot-to-root ratios of As concentrations also varied from 0.03 to 0.07, but there was little difference between pho2 (a mutant with higher shoot P concentrations than wild type) and wild type (Geng et al., in press). Other workers have also found similar shoot-to-root ratios of As concentrations, e.g. <0.02 in tomato (Lycopersicum esculentum), <0.1 in Brassica juncea, and <0.2 in rice when arsenate was supplied (Marin et al., 1992; Burló et al., 1999; Pickering et al., 2000; Geng et al., in press). Arsenic allocation to the above ground portion of cereal crops is generally undesirable, as it will reduce the quality of the grains. Our results indicated P supply may result in relatively higher As allocation to the above-ground (Fig. 4(b)), but absolute shoot As concentrations were inhibited by P supply, which has practical application in soil-crop systems. Arsenic concentration (3.3 and 6.7 µM) used in hydroponics commonly exists in soil solution. For example, arsenate concentration in irrigation water was 106 µM, arsenic concentration in soil solution was 4.3 µM in areas with arsenic contamination...
from groundwater and mining activities (Abedin et al., 2002). In such case, P application to the soil may reduce As uptake by crops and hence improve the quality of the grains.

Acknowledgements

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