Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat

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Abstract

Effects of different concentrations of arsenite and arsenate (0–16 mg/l) on seed germination, relative root length and shoot height, arsenic accumulation in young seedlings, α -amylase, β -amylase and total amylolytic activity in wheat were investigated in order to elucidate the toxicity of arsenic in the early developmental stage. Germination percentages of different wheat varieties had different responses to arsenic species and decreased significantly with increasing arsenic concentrations except Duokang 1. Relative root length (RRL) and relative shoot height (RSH) of wheat seedlings decreased with increasing concentrations of arsenite and arsenate. The relative root lengths were correlated with the relative shoot heights for arsenite ($r^2 = 0.79$) and arsenate ($r^2 = 0.77$). Arsenic uptake by seedlings increased with the increasing concentrations of arsenite or arsenate and followed the Michaelis–Menten kinetics function. The average total amylolytic activity and β -amylase activity had no significant difference comparable to that of controls at the concentration ≤ 2 mg/l arsenite or arsenate, but decreased apparently when the concentration was higher than 2 mg/l. Whereas the α -amylase activity decreased with increasing concentrations of arsenite or arsenate over the whole concentration range. Arsenite decreased all the endpoints more remarkably than arsenate. In comparison, shoot height and root length were more sensitive to arsenic than other endpoints and might be used as indicators for arsenic toxicity. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Arsenite; Arsenate; Wheat; Germination; Relative root length; Relative shoot height; Amylolytic activity

1. Introduction

Arsenic is one of the ubiquitous environmental contaminants with heavy pollution from mining, industrial and agricultural activities (Huysmans and Frankenberger, 1990; Phillips, 1990). Groundwater contamination with arsenic is reported from many regions in the world,

and this problem is also serious in China (WHO, 2001). Arsenic contaminated groundwater is not only used as a source of drinking water but also extensively used for irrigation in some regions.

Wheat is the main crop in China and covers about 22% of the total arable lands. Long-term use of arsenic contaminated water for irrigation has resulted in elevated arsenic level in agricultural soils (Ullah, 1998; Alam and Sattar, 2000). The presence of arsenic in irrigation water or in soil at an elevated level could hamper normal growth of plants with the toxicity symptoms such as biomass reduction in root and shoot

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(Tang and Miller, 1991; Carbonell-Barrachina et al., 1998), wilting and necrosis of leaf blades (Odanaka et al., 1987; Frans et al., 1988), reduction in leaf area and photosynthesis (Marin et al., 1993a; Knauer et al., 1999), and lower fruit and grain yield (Tsutsumi, 1980; Frans et al., 1988; Carbonell-Barrachina et al., 1995). Metal sensitivity and toxicity to plants are influenced by not only the concentration and the toxicant types, but also by the life-stage or biological process (germination, seedling survival, vegetative growth). In comparison seed germination and the early seedling growth are more sensitive to metal pollution because some of the defense mechanisms have not developed, and hence are important considerations in toxicity assessment. In addition, starch is the corresponding form of storage polysaccharide for energy of seeds. Evidence indicates that in germinating seeds starch degrade predominantly via the amylolytic pathway (Swain and Dekker, 1966a,b). So a participation of the α -amylase and β -amylase in hydrolytic pathway for starch degradation must be considered. It is unknown whether arsenic affects the amylolytic activity sensitively.

Arsenate is the predominant arsenic species in aerobic soils, whereas arsenite dominates under anaerobic conditions (Smith et al., 1998). Arsenate and arsenite are interconvertible dependent on the redox condition of the soil (Masscheleyn et al., 1991; Onken and Hossner, 1995, 1996; Rochette et al., 1998). The toxicity effect of arsenic is species dependent. However, there are discrepancies concerning the toxicity of different arsenic species (Breed et al., 1996; Carbonell-Barrachina et al., 1998, 1999; Knauer et al., 1999). Therefore, it is necessary to understand the different toxicity effects of the arsenic species on different wheat varieties.

In this study the apparent toxic symptoms of arsenate and arsenite including seed germination, early seedling growth of six wheat varieties, total amylolytic activity, α -amylase activity and β -amylase activity were investigated. The sensitive symptoms, which could be used as indicators for arsenic toxicity, were evaluated.

2. Materials and methods

2.1. Germination assay

Effects of different concentrations of arsenite and arsenate on the seed germination of different wheat varieties were evaluated. The concentrations of arsenate and arsenite were 0, 0.5, 1, 2, 4, 8 and 16 mg/l and were prepared freshly as $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ and NaAsO_2 . The pH was adjusted to 5.8 by 2 mM Mes–Tris.

Six varieties of wheat (*Triticum aestivum* L., obtained from Chinese Academy of Agricultural Sciences, Beijing, China), Duokang 1, Jing 9428, Jing 411, Jingdong 8, Zhongmai 8 and Jingdong 11, were chosen for the test.

Prior to germination, all the seeds were surface-sterilized in 3% H_2O_2 and then rinsed with distilled water. Seed germination was tested on moist filter paper. A piece of filter paper was placed on a Petri plate and moistened with 5.0 ml aqueous solution of arsenite or arsenate. Controls were maintained by moistening the filter paper with 5 ml deionized water. Thirty seeds of each variety were placed in each plate, covered by lid, and incubated in dark at 23 °C. Germinated seeds were counted 4 days after initiation, and the results were expressed as percentage over control. Seeds were considered germinated when both the plumule and radicle were extended to more than 2 mm from their junction. Each treatment was replicated three times.

2.2. Seedling toxicity test

Seeds germinated for 4 days were transferred to a vitreous pot containing 50 ml of test solution of 0–16 mg As/l with arsenite or arsenate. Each treatment was replicated three times. The pots were then placed randomly in a growth cabinet to ensure normal growing conditions. Temperature was maintained at 25 °C. The test solutions were renewed every day to avoid the change of arsenic concentration and speciation.

2.3. Determination of relative root length and relative shoot height

After 2-day growth, shoot height was measured from culms base to the tip of the longest leaf and root length was measured from the root-shoot junction to the tip of the longest root. Root length and shoot height grown in the test solution containing arsenic were expressed as relative root length (RRL) and relative shoot height (RSH) compared to that of the control.

2.4. Determination of arsenic

In the process of metal uptake by roots, the metal ions were first adsorbed onto the root surface and then adsorbed by cell wall, and then cross membrane into the cytoplasm. Principally, the extracellular ions cannot be considered as the uptake fraction by plants. Therefore, removal of metal ions from the root surface is essential to the measurement of metal uptake by plants. In this work, desorption of the arsenic on root surface was by immersing the root in ice-distilled water for 2 min as suggested by Meharg and Jardine (2003). For the determination of arsenic, three uniform seedlings 6 days after initiation were rinsed with deionized water thoroughly, blotted with tissue paper and the fresh weights were recorded, then transferred to digestion tubes and digested with ultra pure concentrated HNO_3 (5 ml) and 30% w/v H_2O_2 (5 ml) using a closed-vessel microwave digestion protocol (CEM Mars V, Matthews,

NC, USA). Arsenic was determined by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-610A model atomic fluorescence spectrophotometer, Beijing BRAIC Instrumental Company, Beijing, China). All measurements were repeated in triplicate.

2.5. Determination of amylase

Fifteen viable and uniform seedlings 6 days after initiation were selected. The seedlings were rinsed with deionized water thoroughly, blotted and weighed. Total amylase preparations were made by grinding 6-day germinated seeds in a mortar and pestle with 0.05 M acetate buffer (pH = 6.0). All operations were performed at 4 °C unless indicated otherwise. The resulting homogenate was strained through cheesecloth and then centrifuged at 18000*g for 10 min and filtered. α -amylase preparations were made same as total amylase except heating at 70 $\pm$ 0.5 °C for 15 min after filter. The substrate for amylase is a 1% solution of soluble starch in 0.1 M acetate buffer (pH = 5.6). The enzyme prepared to be tested was diluted to 1 ml with water and then 1.0 ml of the substrate solution was added. After 1 h incubation at 25 °C, 2 ml of a 3,5-dinitrosalicylic acid was added and the tubes were placed in a boiling water bath for 5 min (Swain and Dekker, 1966a) and then cooled to room temperature and diluted with 20 ml of water. The absorbance values of the resulting colored solutions were determined by a spectrophotometer. The results are the average of three replicates $\pm$ SE. β -amylase was calculated from the difference between α -amylase and the total contents of amylase.

3. Results

3.1. Quality control for arsenic determination

Quality assurance was carried out by the determination of total arsenic in certified reference materials of tea (GBW 08505) and peach leaf (GBW 08501). Seven replicated analyses were made for each sample. The results obtained were 0.21 $\pm$ 0.02 μ g/g and 0.32 $\pm$ 0.03 μ g/g for GBW 08505 and GBW 08501, respectively. The certified values are 0.19 $\pm$ 0.03 μ g/g and 0.34 $\pm$ 0.03 μ g/g for GBW 08505 and GBW 08501, respectively. Good agreements were achieved between the data obtained by the present method and the certified values.

3.2. Effect of arsenite and arsenate on germination

The mean (average of all 6 varieties) germination percentage over control decreased significantly ($p < 0.01$) with increasing concentration of arsenite and arsenate (Fig. 1). Arsenite had more restrictive to wheat seed germination than arsenate. Germination decreased by

Fig. 1. Seed germination percentage compared to control at different concentrations of arsenite (■) and arsenate (◇). Each data point is the mean of six wheat varieties.

4.8%, 11.6%, 14.6%, 20.1%, 25.6% and 38.0% at 0.5, 1, 2, 4, 8 and 16 mg/l, respectively, for arsenite, while the decrease was by 4.2%, 8.4%, 10.6%, 13.3%, 16.3% and 24.2% at 0.5, 1, 2, 4, 8 and 16 mg/l, respectively, for arsenate.

There were differences in germination with increasing concentrations of arsenite and arsenate for different wheat varieties. All the varieties, except Duokang 1, showed poor germination at the highest concentration of both As species (Table 1). The germination percentage of Jing 9428 and Zhongmai 8 decreased significantly by 19.5% and 11.1% for arsenate, 28.9% and 21.0% for arsenite, respectively, when the concentrations of arsenite and arsenate reached 1 mg/l. Germination for arsenite was significantly lower than that of arsenate in all varieties. Arsenate had no significant effect on seed germination of Jingdong 11 until the concentration reached 8 mg/l, however, arsenite had significant adverse effect on Jingdong 11 seed germination when the concentration of arsenate in solution only reached 2 mg/l. Duokang 1 was the only variety, which showed a considerable resistance to the highest concentration of arsenate and arsenite (16 mg/l). Jingdong 8 had a little decrease with the increase of arsenate concentration, while it had more decrease with the increasing of arsenite concentration.

Germination rates for Jing 9428, Duokang 1, Jingdong 11, Jing 411, Jingdong 8 and Zhongmai 8 were 49.4%, 95.4%, 52.3%, 60.2%, 64.0% and 50.6%, respectively, (Table 1) at the highest arsenite concentration (16 mg/l). The corresponding values for arsenate were 55.8%, 96.6%, 84.9%, 78.3%, 83.7% and 55.6%, respectively (Table 1). More reduction in germination percentage with arsenite than with arsenate was due to the much higher toxicity of arsenite to plants than arsenate (Wauchope, 1983).

Table 1

Seed germination percentage (%) compared to the control at different arsenic concentration ($n = 3$)

As concentration (mg/l)	Jing 9428	Duokang 1	Jingdong 11	Jing 411	Jingdong 8	Zhongmai 8
<i>Arsenate</i>						
0.0	100.0 ± 8.1	100.0 ± 2.0	100.0 ± 2.0	100.0 ± 2.1	100.0 ± 2.0	100.0 ± 3.7
0.5	87.0 ± 2.3	98.9 ± 3.4	101.2 ± 1.9	98.8 ± 2.1	97.7 ± 3.5	90.1 ± 8.6
1.0	80.5 ± 2.3	97.7 ± 3.9	100.0 ± 4.4	92.8 ± 2.1	89.5 ± 2.0	88.9 ± 3.7
2.0	80.5 ± 6.0	93.2 ± 7.1	98.8 ± 2.0	90.4 ± 6.3	91.9 ± 4.0	81.5 ± 3.7
4.0	76.6 ± 8.1	94.3 ± 7.9	98.8 ± 4.0	85.5 ± 2.1	89.5 ± 2.0	75.3 ± 5.7
8.0	64.9 ± 13.7	100.0 ± 3.9	98.8 ± 2.0	83.1 ± 3.6	87.2 ± 3.5	67.9 ± 7.7
16.0	55.8 ± 2.3	96.6 ± 3.9	84.9 ± 2.0	78.3 ± 5.5	83.7 ± 3.5	55.6 ± 3.7
<i>Arsenite</i>						
0.0	100.0 ± 8.1	100.0 ± 3.3	100.0 ± 2.0	100.0 ± 2.1	100.0 ± 1.9	100.0 ± 3.3
0.5	85.7 ± 3.9	97.7 ± 5.1	103.5 ± 2.0	97.6 ± 0	96.5 ± 1.9	90.1 ± 6.9
1.0	70.1 ± 3.9	100.0 ± 1.4	98.8 ± 7.3	92.8 ± 4.2	89.5 ± 3.9	79.0 ± 6.9
2.0	68.8 ± 6.0	96.6 ± 3.3	90.7 ± 7.0	92.8 ± 7.5	88.4 ± 1.9	75.3 ± 5.1
4.0	67.5 ± 8.1	90.8 ± 6.9	87.2 ± 3.5	81.9 ± 8.4	81.4 ± 1.9	70.4 ± 5.8
8.0	61.0 ± 4.5	96.6 ± 0	76.7 ± 3.5	75.9 ± 7.2	76.7 ± 3.3	59.3 ± 3.3
16.0	49.4 ± 4.5	95.4 ± 1.9	52.3 ± 2.5	60.2 ± 5.5	64.0 ± 5.1	50.6 ± 5.1

3.3. Effect of arsenite and arsenate on the early development of root and shoot

Effects of arsenite and arsenate on relative root length (RRL) are presented in Fig. 2. RRL for all varieties decreased significantly with the increase in arsenite and arsenate concentrations (Table 2). Such reduction was more prominent with arsenite than arsenate ($p < 0.05$). RRL reduced by 5.2%, 13.6%, 22.3%, 30.6%, 43.7% and 67.1% at 0.5, 1, 2, 4, 8 and 16 mg/l treatments respectively for arsenite, while it decreased by 3.9%, 14.5%, 20.4%, 27.6%, 35.0% and 52.6% at 0.5, 1, 2, 4, 8 and 16 mg/l treatments respectively for arsenate. This

Fig. 2. Relative root length of wheat at different concentrations of arsenite (■) and arsenate (◇). Each data point is the mean of six wheat varieties.

result indicated that arsenite caused greater root reduction than arsenate, especially at the higher concentration range (4–16 mg/l). Analysis of variance showed that arsenic species, concentrations and wheat varieties had significant effects ($p < 0.05$ for species, variety and concentration) on RRL of wheat.

Differential root length responses to arsenite and arsenate were noted at different concentrations among the wheat varieties (Table 2) ($p < 0.05$). RRL decreased significantly ($p < 0.05$) when the concentrations of arsenate and arsenite were more than 2 mg/l for all the wheat varieties. Though no variety showed particular resistance to arsenic in terms of root growth, differential responses were observed among the wheat varieties ($p < 0.05$). RRL of all the varieties varied insignificantly comparing arsenate and arsenite treatment at low concentrations (0–4 mg/l), while it decreased much more for arsenite treatment than for arsenate treatment at higher concentrations (8–16 mg/l).

Effects of arsenite and arsenate on wheat relative shoot height (RSH) are presented in Fig. 3. Analysis of variance indicates that arsenic species, arsenic concentrations and different wheat varieties had significant effect on RSH of wheat ($p < 0.05$). Significant reduction of RSH was observed with the increase of arsenic concentration. Mean relative shoot height reduced by 7.6%, 24.3%, 33.2%, 38.5%, 45.1% and 58.5% at 0.5, 1, 2, 4, 8 and 16 mg/l treatments respectively for arsenite and by 8.6%, 22.0%, 29.6%, 34.3%, 39.3% and 50.5% for arsenate.

Wheat relative shoot height reduced prominently when the concentration of arsenic in solution was more than 1 mg/l (Table 3). No resistance to arsenic treatment was observed for shoot growth. Relative shoot height values for Jing 9428, Duokang 1, Jingdong 11, Jing

Table 2
Relative root length (%) of wheat at different arsenic concentration ($n = 3$)

As concentration (mg/l)	Jing 9428	Duokang 1	Jingdong 11	Jing 411	Jingdong 8	Zhongmai 8
<i>Arsenate</i>						
0.0	100.0 $\pm$ 7.0	100.0 $\pm$ 7.5	100.0 $\pm$ 7.1	100.0 $\pm$ 8.6	100.0 $\pm$ 4.1	100.0 $\pm$ 8.6
0.5	98.9 $\pm$ 8.9	99.1 $\pm$ 9.9	86.9 $\pm$ 7.7	88.7 $\pm$ 4.9	105.3 $\pm$ 4.9	100.6 $\pm$ 2.8
1.0	95.8 $\pm$ 14.5	94.4 $\pm$ 11.5	76.2 $\pm$ 0.6	89.6 $\pm$ 13.4	87.5 $\pm$ 13.4	91.5 $\pm$ 3.7
2.0	86.6 $\pm$ 2.9	94.4 $\pm$ 2.5	72.8 $\pm$ 3.7	73.7 $\pm$ 9.9	83.9 $\pm$ 13.1	85.5 $\pm$ 4.2
4.0	75.6 $\pm$ 9.5	87.7 $\pm$ 3.3	61.5 $\pm$ 3.5	51.2 $\pm$ 7.7	76.5 $\pm$ 5.0	81.9 $\pm$ 6.2
8.0	69.3 $\pm$ 3.3	85.3 $\pm$ 9.9	52.4 $\pm$ 7.7	48.2 $\pm$ 11.4	56.4 $\pm$ 2.5	76.2 $\pm$ 6.3
16.0	45.6 $\pm$ 5.1	67.9 $\pm$ 3.6	29.9 $\pm$ 5.3	26.9 $\pm$ 4.5	48.2 $\pm$ 9.5	49.9 $\pm$ 4.1
<i>Arsenite</i>						
0.0	100.0 $\pm$ 7.0	100.0 $\pm$ 7.5	100.0 $\pm$ 7.1	100.0 $\pm$ 8.6	100.0 $\pm$ 4.1	100.0 $\pm$ 8.6
0.5	95.6 $\pm$ 10.8	94.3 $\pm$ 5.6	91.5 $\pm$ 6.2	97.3 $\pm$ 10.5	93.3 $\pm$ 5.6	92.2 $\pm$ 12.5
1.0	94.8 $\pm$ 7.6	93.3 $\pm$ 3.3	76.9 $\pm$ 0.9	73.4 $\pm$ 5.4	84.0 $\pm$ 8.9	95.9 $\pm$ 4.0
2.0	77.3 $\pm$ 2.4	93.2 $\pm$ 6.8	68.7 $\pm$ 2.1	68.2 $\pm$ 10.8	77.5 $\pm$ 4.3	81.3 $\pm$ 4.7
4.0	68.3 $\pm$ 2.5	87.9 $\pm$ 3.7	59.8 $\pm$ 3.8	49.5 $\pm$ 4.7	73.2 $\pm$ 1.9	77.4 $\pm$ 2.1
8.0	53.0 $\pm$ 1.0	78.1 $\pm$ 6.5	47.0 $\pm$ 2.5	45.3 $\pm$ 0.7	51.7 $\pm$ 3.8	62.4 $\pm$ 3.7
16.0	40.3 $\pm$ 5.4	52.8 $\pm$ 6.6	14.0 $\pm$ 1.9	15.7 $\pm$ 2.3	35.4 $\pm$ 2.2	39.0 $\pm$ 4.5

Fig. 3. Relative shoot height of wheat at different concentrations of arsenite (■) and arsenate (◇). Each data point is the mean of six wheat varieties.

411, Jingdong 8 and Zhongmai 8 were 51.4%, 68.9%, 43.0%, 43.2%, 39.2% and 51.4% respectively at the highest arsenate concentration (16 mg/l). The corresponding values for arsenite were 46.3%, 48.6%, 35.1%, 39.6%, 34.7% and 44.7%, respectively. More reduction of RSH was caused by arsenite than that of arsenate, and some differential responses to arsenite and arsenate for RSH at the highest concentration were observed among the wheat varieties ($p < 0.05$).

A positive correlation ($r^2 = 0.79$ for arsenite and $r^2 = 0.77$ for arsenate, $p < 0.001$) existed between relative root length and relative shoot height when wheat seeds

were exposed to different concentrations of arsenite or arsenate (Fig. 4), showed that reduction of shoot growth strongly depended on the reduction of root growth.

3.4. Arsenic concentration in wheat seedlings

The concentrations of arsenic in seedlings of six wheat varieties are presented in Fig. 5 and the concentration of arsenic increased with increasing concentrations of arsenite or arsenate in solution. Comparable accumulations were observed for both arsenic species at low concentrations (0–2 mg/l) and much higher accumulation for arsenite at the concentrations range of 4–16 mg/l than that observed for rice by [Abedin and Meharg \(2002\)](#). The arsenic uptake in the concentration range could be fitted by the Michaelis–Menten equation (Fig. 5 and Table 4). Similar Michaelis constant values (K_m) for these two As species (Table 4) were obtained, but the maximum influx rate (V_{max}) for arsenite was higher than for arsenate, indicating more arsenite influx into the root symplasm than arsenate.

3.5. Effect on amylases

The average total amylolytic activity of six wheat varieties of seeds germinated in arsenite or arsenate solution is presented in Fig. 6a. The average total amylolytic activity in seeds kept in 0.5, 1 and 2 mg/l arsenite or arsenate solutions was comparable to that of controls, but it was progressively depressed with increasing concentration of arsenite or arsenate (4–16 mg/l). The average total amylolytic activity of wheat seeds germinated in arsenate or arsenite solution (0.5, 1 and 2 mg/l) was even more than that of controls. While total amylolytic activity of wheat seeds germinated in arsenic solution reduced

Table 3
Relative shoot height (%) of wheat at different arsenic concentration ($n = 3$)

As concentration (mg/l)	Jing 9428	Duokang 1	Jingdong 11	Jing 411	Jingdong 8	Zhongmai 8
<i>Arsenate</i>						
0.0	100.0 ± 8.3	100.0 ± 3.2	100.0 ± 5.6	100.0 ± 7.7	100.0 ± 3.8	100.0 ± 15.9
0.5	96.1 ± 9.2	102.6 ± 10.9	73.5 ± 8.5	85.8 ± 6.5	91.2 ± 3.6	99.0 ± 6.7
1.0	84.1 ± 1.0	95.9 ± 12.8	58.3 ± 3.4	67.8 ± 12.0	78.4 ± 3.8	83.8 ± 2.8
2.0	70.7 ± 2.3	89.4 ± 4.2	56.4 ± 0.6	54.4 ± 2.1	72.8 ± 3.9	78.6 ± 3.4
4.0	65.4 ± 1.8	90.5 ± 6.0	49.6 ± 3.9	51.3 ± 2.0	65.7 ± 2.7	71.6 ± 5.8
8.0	62.9 ± 3.8	84.0 ± 3.7	49.0 ± 2.4	50.6 ± 1.9	52.8 ± 1.0	64.8 ± 3.1
16.0	51.4 ± 3.6	68.9 ± 6.9	43.0 ± 3.6	43.2 ± 4.1	39.2 ± 4.4	51.5 ± 3.5
<i>Arsenite</i>						
0.0	100.0 ± 8.3	100.0 ± 3.2	100.0 ± 5.6	100.0 ± 7.7	100.0 ± 3.8	100.0 ± 15.9
0.5	91.9 ± 3.5	100.0 ± 13.6	78.6 ± 15.9	85.5 ± 7.0	97.5 ± 10.9	101.1 ± 4.2
1.0	83.7 ± 11.9	92.8 ± 9.7	56.6 ± 4.5	62.4 ± 1.7	76.6 ± 6.7	81.9 ± 3.6
2.0	60.0 ± 12.2	95.6 ± 1.3	49.8 ± 2.5	55.1 ± 3.9	64.9 ± 4.0	75.4 ± 7.0
4.0	59.0 ± 3.7	85.7 ± 0.7	46.1 ± 5.9	51.4 ± 2.9	58.7 ± 3.0	68.3 ± 6.1
8.0	54.2 ± 2.2	77.8 ± 4.0	43.8 ± 3.7	48.6 ± 2.3	48.5 ± 0.4	56.8 ± 2.1
16.0	46.3 ± 0.5	48.6 ± 4.4	35.1 ± 0.6	39.6 ± 2.0	34.7 ± 5.7	44.7 ± 6.1

Fig. 4. Relationship between relative shoot height and relative root length for arsenate (a) and arsenite (b).

Fig. 5. Concentrations of As in wheat seeds. Each data point is the mean of six wheat varieties.

Table 4

Kinetic parameters calculated from mean As influx ($n = 3$) into six wheat varieties using Michaelis–Menten function for arsenate and arsenite treatment

	R^2	p	K_m (mM)	V_{max} (nmol g ⁻¹ FW h ⁻¹)
Arsenate	0.98	<0.01	0.11	345.69
Arsenite	0.99	<0.01	0.13	419.76

by 29.3%, 36.6% and 52.7% at 4, 8 and 16 mg/l treatments respectively for arsenite, and it decreased by 15.1%, 24.0% and 40.2% for arsenate, respectively. Arsenite caused greater total amylolytic activity reduction than arsenate. Similar response curves were observed for β -amylase activity (Fig. 6b).

Different from the total amylolytic activity and β -amylase activity, α -amylase activity reduced apparently

Fig. 6. Total amylolytic activity (a), β -amylase activity (b) and α -amylase activity (c) in germinating wheat seeds at different concentrations of arsenite (■) and arsenate (◇). Each data point is the mean of six wheat varieties.

with the increase of arsenite or arsenate even at low concentration. The average α -amylase activity of six wheat varieties seeds germinated in arsenic solution decreased by 4.2%, 9.2%, 18.3%, 36.0%, 48.5% and 67.6% at 0.5%, 1%, 2%, 4%, 8% and 16 mg/l treatment, respectively, for arsenite, and by 7.4%, 7.1%, 13.9%, 22.6%, 30.2% and 53.0% for arsenate. The α -amylase activity

for arsenite was significantly lower than that of arsenate in these six wheat varieties (Fig. 6c).

4. Discussion

The results showed that germination of wheat seeds was affected considerably by both arsenite and arsenate. Duokang 1 showed a high tolerance in germination to arsenic even at 16 mg/l of arsenite and arsenate. High resistance to arsenic could be achieved by (1) complexation of arsenic by such as peptides with SH-groups (Maitani et al., 1996; Sneller et al., 1999; Schmöger et al., 2000), (2) reduction of arsenic influx by suppressing phosphate/arsenate uptake systems (Meharg and Macnair, 1992; Meharg, 1994), and (3) enhanced production of antioxidants that detoxify free reactive oxygen species (ROS) produced in response to arsenite and arsenate (Mylona et al., 1998; Hartley-Whitaker et al., 2001).

Germination of some varieties decreased significantly when the concentrations of As reach 1 mg/l. Therefore, under normal field conditions, application of contaminated irrigation water may have adverse effect on seed germination. Sure, we cannot make a generalized conclusion, as there are significant variations among the varieties in tolerance to arsenite and arsenate. Furthermore, most of the added arsenic will be adsorbed on soil particle surfaces, leaving a relatively lower concentration in the soil solution (Onken and Hossner, 1995, 1996). Soil factors can significantly influence solubility, mobility and toxicity of arsenic in soil (Masscheleyn et al., 1991; Marin et al., 1993b; Onken and Hossner, 1995).

Shoot height and root length reduced significantly with the increase in arsenic concentration, even for Duokang 1 whose germination percentage did not reduce in response to arsenic exposure. Significant reduction in root length with increasing concentrations of arsenic was due to the fact that plant roots were the first point of contact for these toxic arsenic species in the growth media. It was found that arsenite was more toxic than arsenate in term of root length and shoot height. These results are in agreement with the reported results (Coddington, 1986).

Seed germination relies almost exclusively on the seed reserves for the supply of metabolites for respiration, as well as other anabolic reactions. Starch is quantitatively the most abundant storage material in all seeds, and available evidence indicates that in germinating seeds starch is degraded predominantly via the amylolytic pathway (Swain and Dekker, 1966b; Juliano and Varner, 1969; Dua and Sawhney, 1991). It was found in this work that the average total amylolytic activity, β -amylase activity and α -amylase activity were significantly depressed by higher arsenic concentrations (4–16 mg/l), which is consistent with the observation of the stress

Table 5

Relative toxicity of arsenate and arsenite on germination, early seedling growth and amylolytic activity of wheat

As concentration (mg/l)	0.0	0.5	1.0	2.0	4.0	8.0	16.0
<i>Arsenate</i>							
Germination percent (%)	100.0	95.8	91.6	89.4	86.7	83.7	75.8
Relative root length (%)	100.0	96.1	85.5	79.6	72.4	65.0	47.5
Relative shoot height (%)	100.0	91.4	78.0	70.4	65.7	60.7	49.5
Relative total amylolytic activity	100.0	99.9	101.7	96.2	84.9	75.9	59.8
Relative β -amylase activity	100.0	101.8	103.9	98.2	86.9	77.6	63.1
Relative α -amylase activity	100.0	92.9	92.9	86.3	77.3	69.7	46.9
<i>Arsenite</i>							
Germination percent	100.0	95.2	88.4	85.4	79.9	74.4	62.0
Relative root length	100.0	94.8	86.4	77.7	69.4	56.3	32.9
Relative shoot height	100.0	92.4	75.7	66.8	61.5	54.9	41.5
Relative total amylolytic activity	100.0	96.8	92.2	88.6	70.7	63.4	47.3
Relative β -amylase activity	100.0	97.0	92.5	90.2	72.3	66.2	50.8
Relative α -amylase activity	100.0	95.5	90.5	81.5	64.0	51.5	32.5

of high concentration of Cu^{2+} , Cd^{2+} and Hg^{2+} for rice germination (Ge et al., 2002).

Many different vegetative response endpoints such as germination percentage, root length, shoot height, root biomass, shoot biomass and total biomass are used to indicate metal resistance to plants (Karataglis, 1980; Abedin and Meharg, 2002). Karataglis (1980) described germination as one of the best indicators for the development of a plant on a certain soil type. However, Kapustka et al. (1995) described germination as the least effective technique and placed it at the end of sensitivity order for vegetative response endpoints. The proposed vegetative response endpoint follows the order of sensitivity: root length > root mass > shoot length > total mass (root plus shoot) > shoot mass > germination. In this study, relationship between the concentration of As in wheat seedlings and germination percentage, relative root length, relative shoot height exhibited significant negative correlation by linear regression analysis ($p < 0.05$). With the increase in arsenic concentrations in the growth media shoot height and root length reduced more significantly than the germination rate, total amylolytic activity, β -amylase activity and α -amylase activity (Table 5) and the decrease followed the order: $\text{RSH} \geq \text{RRL} > \alpha\text{-amylase activity} > \beta\text{-amylase activity} \geq \text{total amylolytic activity} > \text{germination percent}$. Furthermore, root length and shoot height were more sensitive to relatively low levels of arsenic. Therefore, they might be used as indicators for arsenic toxicity.

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