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Effects of arsenic on seed germination and physiological activities of wheat seedlings

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Abstract

The effects of arsenic (As) were investigated on seed germination, root and shoot length and their biomass and some other factors to elucidate the toxicity of As. The results showed low concentrations of As (0–1 mg/kg) stimulated seed germination and the growth of root and shoot, however, these factors all decreased gradually at high concentrations of As (5–20 mg/kg). The contents of $O_2^{\bullet -}$, MDA, soluble protein and peroxidase (POD) activity all increased with increasing As concentrations. Soluble sugar content, ascorbate peroxidase (APX), and superoxide dismutase (SOD) activities decreased at low concentrations of As, and increased at high concentrations of As. While acetylsalicylic acid (ASA) and chlorophyll contents, catalase (CAT) activity displayed increasing trend when the concentrations of As was lower than 1 mg/kg, and then decreasing trend. By polyacrylamide gel electrophoresis (PAGE), As induced the expression of POD isozymes of wheat seedlings. As induced the expression of CAT isozymes but inhibited the expression of SOD isozymes of wheat seedlings at concentrations lower than 1 mg/kg. However, As inhibited the expression of CAT isozymes but induced the expression of SOD isozymes at concentrations higher than 5 mg/kg. The results indicated As could exert harmfulness in the early development stage of wheat at inappropriate concentrations.

Key words: arsenic; wheat; germination; physiological activities

Introduction

Arsenic (As) contamination in soil and groundwater is a worldwide problem, resulting from natural geologic activity and manmade sources such as mining, heavy industry, semiconductor manufacturing, forest products, landfill leachates, fertilisers, pesticides and sewage (Francisco *et al*., 2002). It is very difficult to eliminate arsenic contamination in the environment. Arsenic contamination of soil, streams, and underground water causes a major environmental and human health risk. The threat that arsenic poses to human and animal health is aggravated by their long-term persistence in the environment (Mercedes *et al*., 2002). Groundwater contaminations with arsenic are also serious problems in China. As-contaminated groundwater is used for irrigation as well as for drinking. The World Health Organization provisional guideline value for drinking water is 0.01 mg/kg. There are concerns that arsenic may be absorbed by plants, particularly cereals, entering the grains and thus the food chain. Therefore arsenic poisoning events of the human beings and livestock occur frequently. The serious arsenic harm has become one of the problems causing the attention of researchers in the world (IPCS, 2001; Smith *et al*., 1998; Sadiq, 1997; Luo *et al*., 1997). Large numbers of studies indicated that low concentrations of arsenic stimulated the growth of plants; but excessive arsenic did harm to plants (Han *et al*., 2002). Mohammad *et al*. (2005) found that protein content and superoxide dismutase (SOD) activity were diminished, but malondialdehyde (MDA) content, peroxidase (POD) and catalase (CAT) activities all increased in *Phalaenopsis* under temperature stress. Kim *et al*. (2005) found that under salt stress, the activities of SOD, CAT, POD and ascorbate peroxidase (APX) all increased in barley shoots. Milone *et al*. (2003) found that root length and SOD activity decreased proportionally with increasing Cd concentrations. The activities of CAT, POD and APX all increased in wheat leaves. Many studies were regarding arsenic accumulation, uptake, distribution, binding forms and content in plants (Mercedes *et al*., 2002; Alam *et al*., 2003; He *et al*., 2000; Manomita *et al*., 2000). But there were few reports about effects of As on physiological activity of the wheat seedling. Wheat is one of the main grains in China. Its quality directly affects people's life and food security. It is of practical significance to study the effects of arsenic stress on wheat. Experiments of water culture have been done with Zhengzhou-9023 as the material. The effects of As stress were evaluated on germination energy, germination percentage, germination

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index, vitality index, root and shoot length and their biomass of wheat and resistant physiological indices in the leaves of wheat seedlings, which could be used as indictors.

1 Materials and methods

1.1 Germination assay

The concentrations of As were 0, 0.5, 1, 5, 15, 20 mg/kg and were prepared freshly as $NaAsO_2$ (A.R.). One type of wheat (*Triticum aestivum* L.), Zhengzhou-9023, was chosen for the test. Prior to germination, seeds were surface-sterilized with 0.1% mercuric chloride ($HgCl_2$) for 6 min and rinsed in distilled water. Seed germination was tested on wet filter paper. A piece of filter paper was placed on a petri dish and moistened with 3.0 ml aqueous solution of As. Controls were set up by moistening the filter paper with 3.0 ml deionized water. One hundred seeds were placed in each petri dish, covered by lid and incubated in normal conditions at 15–20°C. Germinated seeds were counted 7 d after initiation. Seeds were considered germinated when the shoot extends to half of seed length and the radical extends to the seed length. Each treatment was replicated three times.

1.2 Determination

1.2.1 Germination energy, germination percentage, germination and, vitality index, length and biomass of root and shoot

The numbers of germinated seeds on the day 3 and 7 after initiation were germination energy and germination percentage, respectively. After 7-d growth, shoot length 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. The fresh plant samples were oven-dried at 70°C and the dry matters of shoots and roots were weighed, respectively. Germination index (GI) and vitality index (VI) were calculated following the equations:

$$\mathrm{GI} = \sum (G_t/D_t) \quad (1)$$

$$\mathrm{VI} = \sum (G_t/D_t)\times \mathrm{S} \quad (2)$$

where, G_t means germination rate at day t, D_t means day t, S means shoot length.

1.2.2 Contents of $O_2^{\cdot-}$, MDA, ASA, soluble sugar and protein, Chl assays

The content of superoxide anion free radical ($O_2^{\bullet-}$), MDA and acetylsalicylic acid (ASA) were measured by the method of reference (Shanghai Institute for Plant Physiology, 1999). The content of soluble sugar, soluble protein, chlorophyll (Chl) were measured by the method of DNS technique, Bradford and acetone, non-water ethanol technique, respectively. The absorbency value measurements were carried out with a model UVmini-1240 spectrophotometer (Shimadzu, Japan).

1.2.3 Enzymes assays

APX activity was measured by the method of Dalton *et al*. (1987). SOD and POD activities were measured by the method of reference (Shanghai Institute for Plant Physiology, 1999). CAT activity was measured by the method of Chance and Machly (1955). One unit of SOD activity was the amount of enzyme required to cause 50% inhibition of the rate of pyrogallo self-oxidation. One unit of the activities of CAT, POD and APX was taken to be equal to the change of absorbance values of per min per gram fresh weight material at 240 nm, 470 nm and 290 nm, respectively. SOD, CAT and POD isozymes were measured by polyacrylamide gel electrophoresis with a model DYY-6C electrophoresis instrument (Wode Life Sciences Instrument Company, Beijing).

1.2.4 Electrophoretic SOD, CAT, POD separation

SOD isozymes were resolved on nondenaturing polyacrylamide gels (10% acrylamide, 3% bis-acrylamide) using Tris-Gly buffer 5 mmol/L, pH 8.3. The gels were run at 80 V for 60 min, and then 200 V for 120 min or so. To visualize the band patterns the gels were incubated in the dark for 20 min in the aqueous solution of 2.45 mmol/L nitro blue tetrazolium, then were incubated in the dark for 15 min in 0.036 mol/L phosphate buffer (pH 7.8) containing 0.028 mmol/L ovoflavin, 0.028 mol/L tetramethylethylenediamine, and then were incubated in 0.05 mol/L phosphate buffer (pH 7.8) containing 0.1 mmol/L ethylenediamine tetraacetic acid in illumination incubator for 40 min at 25°C. Gels were densitometryically scanned.

CAT and POD isozymes were detected on nondenaturing polyacrylamide gels (7% acrylamide, 3% bis-acrylamide) using Tris-Gly buffer 5 mmol/L, pH 8.3. The gels were run at 100 V for 60 min, and then 200 V for 120 min or so. To visualize the catalase band patterns the gels were incubated for 15 min at room temperature in aqueous solution A containing 3% hydrogen peroxide (H_2O_2), 0.1 mol/L phosphate buffer (pH 7.0), 0.06 mol/L sodium thiosulfate ($Na_2S_2O_3$), and then rinsed in distilled water followed by an incubation with aqueous solution B containing 0.09 mol/L potassium iodide (KI) and 99.5% acetic acid glacial. Gels were densitometryically scanned. For peroxidese, the gels were incubated in solution of benzdine-acetic acid containing H_2O_2 for 1–15 min. Gels were densitometryically scanned.

Data from the measurements and analyses were evaluated statistically by analyses of variance. LSD test was performed to allow separation of means. Relation between As concentrations and various parameters were analyzed by regression methods. The significance was determined by using the F-test.

2 Results

2.1 Effects of As on seed germination and the growth of wheat seedlings

2.1.1 Germination energy and percentage, germination and vitality index of wheat seeds

The percentage of germination may reflect the reaction rate of plant seeds to their living environment. Germination

Table 1 Changes of germination energy, germination percentage, GI, VI of wheat seeds at different As concentrations

As concentrations (mg/kg)	Germination energy	Germination percentage	GI	VI
0	88.5 (100%) ab A	99 (100%) a A	133.22 (100%)ab AB	1154.87 (100%) a A
0.5	92.33 (104.33%) a A	98.67 (99.66%) a A	135.85(101.97%)a A	1187.29(102.81%)b AB
1.0	88 (99.44%) ab A	98 (98.99%) a AB	129.4 (97.13%)bc B	1230.59 (106.56%)b B
5.0	87.5 (98.87%) ab A	97.5 (98.48%) a AB	128.93 (96.78%)c B	990.04 (85.73%) c C
15	85 (96.05%) bc AB	97.33 (98.32%) a AB	124.69(93.60%)d BC	720.82 (62.42%) d D
20	80 (90.40%) c B	95 (95.96%) b B	118.92 (89.27%) e C	616.61(53.39%) e E

Digitals in the brackets represent percentage compared to the control (%); different small and capital letters indicate significant difference at $p<0.05$ and $p<0.01$, respectively; LSD test. GI: germination index; VI: vitality index.

index (GI) and vitality index (VI) are two important parameters that reflect the seed quality. From Table 1, it can be seen that germination energy, GI and VI firstly increased, then decreased with the increase of As concentrations. Germination percentage displayed decreasing trend. When As concentration was 0.5 mg/kg, the germination energy and GI increased to 104.33% and 101.97%, respectively. They showed poor germination at high concentrations of As. VI increased by about 6.56% at As concentration of 1 mg/kg. All of the four factors reduced significantly by 9.60%, 4.04%, 10.73% and 46.61%, respectively at the highest As concentration (20 mg/kg). According to the statistical analysis, germination energy, germination percentage, GI, VI at the highest As concentration had significant difference with those at other concentrations.

2.1.2 Length and biomass of root and shoot

Fig.1 shows the length and biomass of root and shoot first increased, then decreased with As treatment. When the concentration of As was 1 mg/kg, they increased to 107.75%, 109.70%, 108.82% or 117.53%, respectively. After that they decreased. The length and biomass of root and shoot reduced by 56.53%, 40.19%, 25% or 20.62%, respectively, at the highest As concentration (20 mg/kg). According to the statistic analysis (Table 2), As had a significant adverse effect on root length, shoot length ($p<0.01$) and root biomass, shoot biomass ($p<0.05$) of wheat seedlings. These results indicate that the wheat seedling was sensitive to As, and the growth of root and shoot was all inhibited by high concentration of As (5–20 mg/kg).

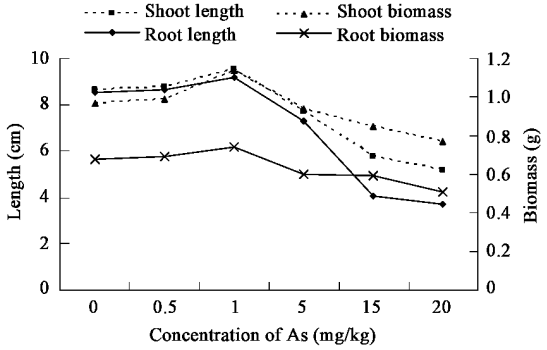

Fig. 1 Length and total dry biomass of shoot and root in wheat treated with external As, after 7 d of growth in hydroponics.

Table 2 Regression analysis of the length and biomass of root and shoot of wheat seedlings

Index	Regression equation	Correlation coefficient
Root length	$y=8.9969-0.4093x+0.0068x^2$	$r=0.9869^{**}$
Shoot length	$y=9.0682-0.2764x+0.004x^2$	$r=0.9762^{**}$
Root biomass	$y=0.6961-0.0088x$	$r=-0.9017^{*}$
Shoot biomass	$y=1.0319-0.0128x$	$r=-0.862^{*}$

* and ** represent significance at $p<0.05$ and $p<0.01$, respectively.

2.2 Effects of As on physiological activities in leaves of wheat seedling

2.2.1 Contents of $O_2^{\cdot-}$ and MDA

$O_2^{\bullet-}$ content in leaves of wheat seedlings increased with increasing concentrations of As (Fig.2), it increased by 3.16%, 28.47%, 41.83%, 49.91%, 56.94% at 0.5, 1, 5, 15, 20 mg/kg, respectively. According to the statistic analysis, the regression equation between $O_2^{\bullet-}$ content (y) and concentrations of As (x) was:

$$y = 0.2405 + 0.15x - 0.0005x^2 \quad (r = 0.9187^{*}) \qquad (3)$$

MDA is the product of membrane lipid peroxidation, whose content can reflect the damage degree of cell membrane poisoned by the oxygen free radical. Fig.2 shows MDA content increased with increasing concentrations of As. MDA content increased slightly when the concentration was lower than 1 mg/kg, but increased dramatically at high concentrations (5–20 mg/kg). When As concentration reached 20 mg/kg, MDA content increased by 22.68%. According to the statistic analysis, the regression equation between MDA content (y) and concentrations of As (x)

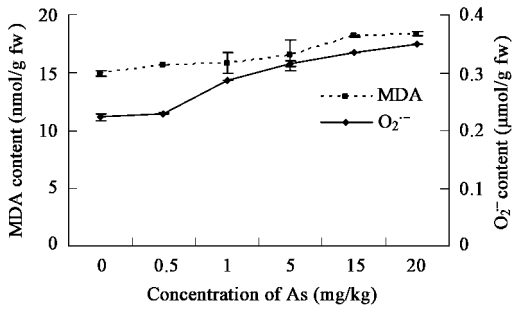

Fig. 2 MDA and $O_2^{\cdot-}$ contents in wheat treated with external As, after 7 d of growth in hydroponics. Error bars represents standard errors (SE). Bars represent standard errors of replicates.

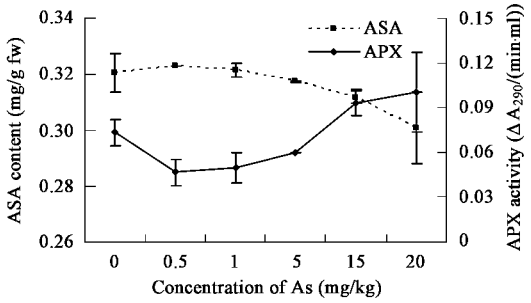

Fig. 3 ASA content and APX activity in wheat treated with external As, after 7 d of growth in hydroponics. Error bars represents standard errors (SE). Bars represent standard errors of replicates.

was:

$$y = 15.3234 + 0.2968x - 0.0073x^2 \quad (r = 0.9864^{**}) \qquad (4)$$

2.2.2 ASA content and APX activity

Fig.3 shows the changes of ASA content with the increase of As concentrations. ASA content increased slightly at lower concentrations of As (0–1 mg/kg), then decreased at As concentration higher than 5 mg/kg. ASA content was reduced by about 6.16% when the concentration of As reached 20 mg/kg. According to the statistic analysis, the regression equation between ASA content (y) and the concentration of As (x) was:

$$y = 0.3213 - 0.0001x - 0.00004x^2 \quad (r = 0.9818^{**}) \qquad (5)$$

APX activity decreased slightly at low concentrations of As (0–5 mg/kg), and then increased with the increase of concentrations of As (Fig.3). When the concentration of As was 20 mg/kg, APX activity increased significantly by 36.36%. As (15–20 mg/kg) had significant effect on APX activity ($P<0.01$). The statistic analysis indicates that the regression equation between APX activity (y) and concentrations of As (x) was:

$$y = 0.0545 + 0.0023x \quad (r = 0.886^{*}) \qquad (6)$$

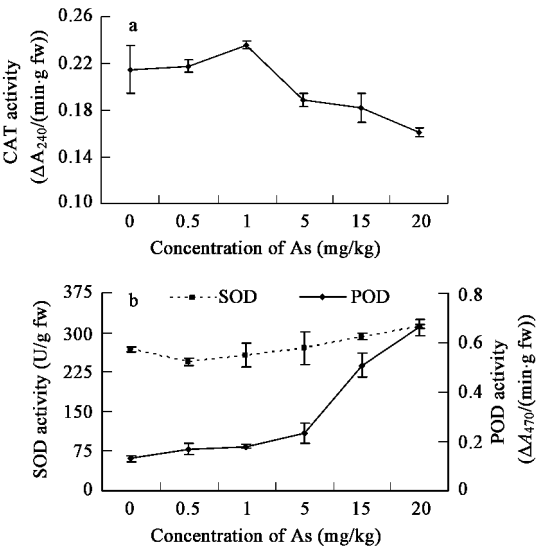

Fig. 4 The activities of CAT (a), SOD and POD (b) in wheat treated with external As, after 7 d of growth in hydroponics. Error bars represents standard errors (SE). Bars represent standard errors of replicates.

2.2.3 Activities of CAT, SOD and POD

Effects of As on CAT activity are presented in Fig.4a. CAT activity displayed increasing trend at lower concentration range (0–1 mg/kg), and then decreasing trend with increasing concentrations of As (5–20 mg/kg). When the concentration of As was 1 mg/kg, CAT activity increased to 109.66%. However, it reduced to 74.92% at the highest concentration (20 mg/kg). According to the statistic analysis, the linear regression equation between CAT activity (y) and concentrations of As (x) was:

$$y = 0.2200 - 0.0029x \quad (r = -0.9055^{*}) \qquad (7)$$

It can be seen in Fig.4b that SOD activity decreased first, and then increased under As treatment. When the concentration of As was 0.5 mg/kg or 1 mg/kg, SOD activity reduced to 91.25% or 95.83%, respectively. But it increased gradually at high concentration range (5–20 mg/kg). SOD activity increased by 16.25% at As concentration of 20 mg/kg. The linear regression equation between SOD activity (y) and concentrations of As (x) was:

$$y = 255.514 + 2.6944x \quad (r = 0.9434^{**}) \qquad (8)$$

The effects of As on POD activity are presented in Fig.4b. POD activity increased significantly with the increase of As concentrations. When the concentration of As only reached 0.5 mg/kg, POD activity increased by 30.58%. And POD activity increased by 5-fold at the highest concentration of As (20 mg/kg) than that of control (0 mg/kg). Analysis of variance showed that POD activity and As concentrations had significant effects ($p<0.01$). The regression equation between POD activity (y) and the concentration of As (x) was:

$$y = 0.1474 + 0.0173x + 0.0004x^2 \quad (r = 0.9985^{**}) \qquad (9)$$

2.2.4 Contents of soluble sugar and soluble protein

Environmental stress can make carbohydrate metabolism disorder. In this study, the changes of soluble sugar content with the concentration of As are shown in Fig.5. Soluble sugar content reduced at lower concentrations of As (0–1 mg/kg). Soluble sugar content reduced to 71.58% at As concentration of 1 mg/kg, then it increased progressively with increasing concentrations of As (5–20 mg/kg), and increased by about 82.52% as the As concentration reached 20 mg/kg. According to the

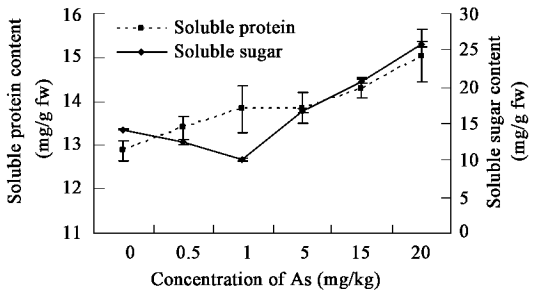

Fig. 5 The contents of soluble protein and soluble sugar in wheat treated with external As, after 7 d of growth in hydroponics. Error bars represents standard errors (SE). Bars represent standard errors of replicates.

statistical analysis, the linear regression equation between soluble sugar content (y) and the concentration of As (x) was:

$$y = 12.1851 + 0.6469x \quad (r = 0.955^{**}) \tag{10}$$

Soluble protein content displayed increasing trend with the increase of the concentration of As (Fig.5). Soluble protein content increased by 4.05%, 7.35%, 7.54%, 11.08%, 16.74% at 0.5, 1, 5, 15, 20 mg/kg of As, respectively. The statistical analysis indicates that the linear regression equation between soluble protein content (y) and the concentration of As (x) was:

$$y = 13.3299 + 0.0794x \quad (r = 0.9138^{*}) \tag{11}$$

2.2.5 Chlorophyll content

The contents of Chl-a, Chl-b and total Chl all first displayed increasing trend, and then decreasing trend with the increase of As concentration (0–20 mg/kg) (Table 3). They increased by 18.46%, 20.98% and 19.20%, respectively, when the concentration was 0.5 mg/kg. However, they were reduced by 31.70%, 34.66% and 32.56%, respectively, at the highest concentration (20 mg/kg). A negative correlation existed between As concentrations and Chl-*a*, Chl-*b* and total Chl content. The linear regression equation between Chl-*a* content ($y_{\text{Chl-}a}$), Chl-*b* content ($y_{\text{Chl-}b}$) and the total Chl content (y_{Chl}) and the concentration of As(x) was:

$$y_{\text{Chl-}a} = 0.9162 - 0.0183x \quad (r = -0.9209^{**}) \tag{12}$$

$$y_{\text{Chl-}b} = 0.3813 - 0.0083x \quad (r = -0.9203^{**}) \tag{13}$$

$$y_{\text{Total Chl}} = 1.2975 - 0.0266x \quad (r = -0.9209^{**}) \tag{14}$$

2.3 Effects of As on CAT, SOD and POD isozymes of wheat seedlings

Fig.6a shows that two kinds of CAT isozyme bands were detected in wheat seedling leaves. They were C1 and C2. C1 was weaker than C2 that was separated at the beginning. CAT isozymes bands were strong at lower concentrations, and then became fainter with the increase of the concentration of As. The expression of CAT isozymes were induced by low concentrations of As (0.5–1.0 mg/kg) but inhibited significantly by As at concentrations higher than 5 mg/kg. From Fig.6, As did not induce expression of new CAT isozymes.

In Fig.6b, SOD displayed six isozyme bands in the wheat seedling leaves, they were s1, s2, s3, s4, s5, and s6 whose R_f value was 0.98, 0.96, 0.9, 0.8, 0.6, 0.48, respectively. s5 and s6 were detected first. They were the brightest, belonging to strong band. s3 and s4 were detected at last, belonging to weak band. SOD isozymes especially s1, s3, s4 were inhibited by low concentrations of As (0.5, 1 mg/kg). The expression of SOD isozymes were induced significantly by As at concentrations higher than 5 mg/kg, but no expression of new SOD isozymes was induced.

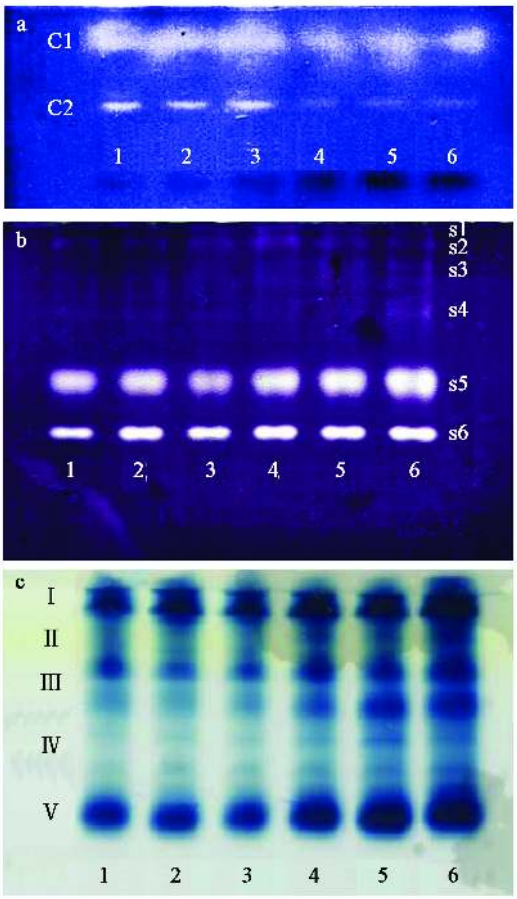

Fig. 6 The responses of CAT (a), SOD (b) and POD (c) isozymes in wheat treated with As, after 7 d of growth in hydroponics. CAT and POD isozymes were detected on nondenaturing polyacrylamide gels (7% acrylamide, 3% bis-acrylamide). SOD isozymes were detected on nondenaturing polyacrylamide gels (10% acrylamide, 3% bis-acrylamide). 1, 2, 3, 4, 5, 6 are As treatment with the concentrations at 0, 0.5, 1, 5, 15 or 20 mg/kg respectively.

Fig.6c shows that, at normal conditions (controls), POD isozymes were resolved into five sections: I (including at least two bands, the R_f value was 0.93–0.98), II (including at least three bands, the R_f value was 0.83–0.90), III (including two bands, the R_f value was 0.80 and 0.71, respectively), IV (including two bands, the R_f value was

Table 3 Chlorophyll contents and percentage at different As concentrations in wheat seedlings

Concentration of As (mg/kg)	Chl-*a*		Chl-*b*		Total Chl	
	Content (mg/g fw)	Percentage (%)	Content (mg/g fw)	Percentage (%)	Content (mg/g fw)	Percentage (%)
0	0.8642	100	0.3559	100	1.2200	100
0.5	1.0237	118.46	0.4305	120.98	1.4543	119.20
1.0	0.8757	101.33	0.3640	102.28	1.2396	101.61
5.0	0.7848	90.82	0.3217	90.41	1.1065	90.70
15	0.5994	69.36	0.2387	67.06	0.8380	68.69
20	0.5902	68.30	0.2325	65.34	0.8228	67.44

0.59 and 0.51, respectively), V (including one band, the R_f value was 0.41). I and V were strong bands sections, while II and IV were weak band sections. There was a strong band whose R_f value was 0.80 and a subaltern strong band whose R_f value was 0.71 of III section. The expression of POD isozymes of II, III, IV and V was induced by As treatment, but no new POD isozymes was induced by As.

3 Discussion

Seed germination is the first physiological process affected by metals (Shanker *et al*., 2005). An (2004) found that the presence of Cd decreased the wheat seedling growth. In the present study, we showed that germination energy and germination index increased at low concentrations of As (0–0.5 mg/kg). Vitality index, length and biomass of root and shoot were all increased at low concentrations of As (0–1 mg/kg). Our results indicate that low concentration of As could stimulate the growth of root and shoot of wheat seedlings (Ma and Hong, 1998). Carbonell-Barrachina *et al*. (1998) showed that the plant biomass with low concentrations of As treatment was slightly higher than that of the control in the conditions of nutrition cultivating. While the growth of root and shoot was inhibited by high concentrations of As (5–20mg/kg) treatment, germination energy, germination percentage, germination index, vitality index, length and biomass of root and shoot all displayed decreasing trend with increasing concentrations of As. Reduced root length growth in response to arsenic exposure has been reported by a number of investigators in other plants (Hartley-Whitaker *et al*., 2001; Kapustka *et al*., 1995; Sneller *et al*., 2000). Abedin and Meharg (2002) reported that germination and early seedling growth of rice decreased significantly with increasing concentrations of As. The inhibition was stronger in the root than in the shoot when treated with As (Wang *et al*., 2002). When uptake of nutrition was inhibited in roots, the growth of the whole plant was constrained, and the plant biomass decreased finally (Mitchell and Barr, 1995). The reason is that plant roots were the first point of contact for these toxic arsenic species in the nutrient media (Abedin and Meharg, 2002).

Active oxygen free radical was produced unavoidably in non-polluted wheat, which was adjusted normally in order not to accumulate excessively (Barcelo, 1998; Ushimaru *et al*., 1999). However, the balance could be broken by environmental stress. According to many studies, excessive heavy metal in grains caused the accumulation of active oxygen free radical (Gupta *et al*., 1999). Our study results showed that active oxygen species (including $O_2^{\bullet -}$) was more and more in wheat seedling leaf cells with the increase of As concentrations, so $O_2^{\bullet -}$ content was higher and higher, which caused the producing speed of active oxygen free radical surpass the elimination ability of the active oxygen free radical in plants. Therefore, the balance between production and elimination of the active oxygen free radical was damaged in cells in stress condition, so the wheat organism cells were poisoned (Fridovich, 1995). At the same time, active oxygen free radical could induce chain-like peroxidation that the unsaturated fat acid in the membrane was produced, which could make the fat acid degenerate and product lipid peroxide, and MDA was one of the major products (Chris *et al*., 1992). Our experimental results indicate that MDA content increased gradually with the increase of stress concentration. On the other hand, ASA could eliminate the oxygen free radical that damaged membrane and the enzyme molecular structure, and inhibited membrane lipid peroxidation, and played an important protective role to plants. The growth of wheat seedling was stimulated and ASA content increased under low concentrations of As. With the increase of the concentration of As the oxygen free radical increased, and ASA that eliminated the oxygen free radical needed more, so ASA content decreased in wheat seedling leaves.

Antioxidant enzyme system including APX, SOD, CAT, POD is some of the major metal detoxification mechanisms in plants (Shanker *et al*., 2003). Both increase and decrease in the activity of many antioxidant enzymes have been observed in Cd-treated plants (Ali *et al*., 2002; Gallego *et al*., 1996; Patra and Panda, 1998; Sandalio *et al*., 2001). According to our experimental result, APX and SOD activities first displayed decreasing and then increasing trend, POD activity displayed increasing trend, while CAT activity first displayed increasing and then decreasing trend. Gwozdz *et al*. (1997) also found that at lower heavy metal concentrations, activity of antioxidant enzymes increased, whereas at higher concentrations, CAT activity decreased in plants. Sgherri *et al*. (2001) already reported that Cu toxicity also enhances peroxidases in leaves of wheat seedlings. Milone *et al*. (2003) found Cd could increase POD and APX activities in wheat seedling leaves. The increase in APX, SOD, POD activities could represent an appropriate protection against overproduction of peroxides when As accumulated in wheat (Murzaeva, 2004). H_2O_2 elimination was blocked because APX and SOD activities were inhibited firstly, H_2O_2 was eliminated by POD and CAT. CAT activity was decreased with the increase of As concentration, and the excessive H_2O_2 in wheat seedling leaves was eliminated by POD mainly.

Isozyme charts are molecular level types after gene expression. The enzyme bands color can reflect relative quantity of the isozyme activity (Milone *et al*., 2003). According to SOD isozyme chart, SOD isozyme bands were darker with lower concentrations of As treatment, and it meant that the activity was weak. With the increase of As, SOD isozyme bands became strong, and it meant that the activity was enhanced, which coincided with the measured results of SOD activity. According to CAT isozyme chart, CAT isozyme bands were strong with low concentrations of As, and it meant that the activity was enhanced. With the increase of As, CAT isozyme bands became dark, and it meant that the activity decreased, which coincided with the measured results of CAT activity. According to POD isozyme chart, with the increase of the concentration of As, POD isozyme bands color was deeper and deeper, and it meant that the activity was increased gradually, which coincided with the measured results of POD activity. Our results show that SOD, CAT and POD isozyme pattern in wheat seedling leaves did not changed

under As treatment. Milone *et al*. (2003) found POD isozyme pattern in wheat seedling leaves were changed by Cd stress. The changes in POD isozyme pattern in leaves confirm that the plants exposed to stress induced by Cd toxicity initiate a series of acclimative responses that may provide increased protection against more severe stress.

The change of soluble carbohydrate is the most significant response in polluted plants. It is the base of the plant metabolism (Foy and White, 1978). In the same way, the matters of wheat cells changed under As stress. Our experimental results indicate that with the increase of concentrations of As, soluble sugar content first displayed decreasing and then increasing trend. The possible reasons were as follows: on the one hand, soluble sugar was the respiration substrate. When plants were polluted, the respiration was strengthened and needed more substrate to provide more energy for organism. At the same time, soluble sugar was one of the cell seepage adjustment main materials. The increase of soluble sugar content with high concentration of As treatment was advantageous in holding water of cell or organization, and preventing the dehydration, which caused that the macro-molecule carbohydrate decomposition was strengthened but synthesizes was inhibited in the cells. And it turned directly low molecular weight and soluble sucrose, glucose, fructose, galactose and so on, which caused soluble sugar content rise finally. On the other hand, the growth of plants was inhibited by As treatment. Therefore, the protein that was not used in cells was accumulated, which caused soluble protein content increase (Yu *et al*., 1995). As for chlorophyll, Chl content decreased by high concentration of As treatment, for As affected Chl synthesis and decomposition. Li *et al*. (2005) also reported at high concentration of Cu and Zn treatment Chl content depressed. Van and Clijsters (1990) believed that the reason was that protochlorophyllide reductase activity was inhibited by heavy metal. Chl content decreased by As treatment, because heavy metal entered in the leaves and accumulated excessively in some parts and combined with -SH base of protein or substituted for Fe^{2+}, Zn^{2+}, Mg^{2+} and so on, and then destroyed the structure and function of chloroplast.

4 Conclusions

In this study, low concentrations of As stimulated seed germination and growth of root and shoot. But they were inhibited at high concentrations of As treatment. The inhibition was stronger in the root than in the shoot.

With the increase of As concentrations, the contents of $O_2^{\bullet -}$, MDA, soluble proteins increased. Soluble sugars content displayed decreasing and then increasing trend. The contents of ASA and Chl displayed increasing and then decreasing trend.

Effects of As on the activities of APX, CAT, SOD and POD were different. CAT activity displayed increasing and then decreasing trend. The activities of APX and SOD displayed decreasing and then increasing trend. POD activity increased under the stress of As.

Expressions of CAT, SOD and POD isozymes changed under the stress of As. The expression of CAT isozymes were induced by low concentration of As but inhibited significantly by high concentration of As. SOD isozymes were contrary with CAT. The expression of POD isozymes was induced by As. But no new CAT, SOD and POD isozymes were induced by As.

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