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Effects of arsenic, cadmium and lead on growth and respiratory enzymes activity in wheat seedlings

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The toxic effects of heavy metals, including arsenic (As), cadmium (Cd) and lead (Pb), on length and biomass of shoots and roots, their respiratory rate, the gene expression levels of cytochrome oxidase (COD), isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH) isoenzymes were studied in the germination stage of wheat (var. ZhengZhou-9023). The results showed that both length and total dry biomass of wheat shoot and root increased at lower As concentrations (1 mg·L\(^{-1}\)) but decreased gradually at higher As concentrations (5 to 25 mg·L\(^{-1}\)). Similarly, the increase in the concentration of Pb, increased shoot length and biomass initially but later decreased gradually. Decline of root and shoot’s biomass was observed with increasing concentrations of Cd yet. The respiratory rate of root displayed an increasing trend at As concentrations lower than 1 mg·L\(^{-1}\), but a decreasing trend was observed at higher concentrations in the root respiratory rate, while the respiratory rate of shoot increased gradually. Respiratory rates of shoot and root increased at lower concentrations of Cd or Pb but decreased at higher concentrations overall. The levels of COD, IDH and MDH isoenzymes in shoot and root were induced mainly with increasing concentrations of As. Interestingly, their levels were induced at lower concentrations of Cd and Pb, but could not be measured at higher concentrations of them. However, expression of a new IDH or a new MDH isoenzyme homologue in the root was induced at higher concentrations of Cd or Pb. Therefore, the presence of heavy metals could change the expression of some important enzymes in respiratory process such as COD, IDH or MDH isoenzymes, thereby affecting respiration in wheat, eventually leading to physiological changes in Wheat.

Key words: Wheat, arsenic, cadmium, lead, respiration, isoenzymes.

INTRODUCTION

Heavy metals pollution is widespread, due to rapid industrialization and current agricultural practices. These pollutants persist in the environment for a longer period of time, as they are not easily degraded by soil microorganisms and therefore, can easily be absorbed by plants (Raghunath et al., 1999; Gallego et al., 2002). Plants that have absorbed heavy metals show inhibiting growth and development, increasing senescence, which leads to decreasing crop yield. Moreover, they can be accumulated in the crops affecting human health upon consumption (Zhang et al., 2007; Cui et al., 2005).

In China, arsenic (As) ranks the 5th as a major soil pollutant with sullage irrigation and ranks the 6th as a major water pollutant (Chen and Liu, 1993). As is known to have a strong capacity of accumulating and bioenrichment in plants. Plants can absorb a certain quantity of As actively or passively from the external environment during growth (Song et al., 1999). A number of studies indicate that lower concentrations of As stimulate growth of plants. However, excessive As is harmful to plants (Han et al., 2002), as it affects transpiration rate, inhibits root activity, and blocks uptake and transport of water, nitrogen (N), potassium (K), phosphorus (P), etc. These aforementioned effects result in abscission in plant leaves, severe inhibition of plant growth, and sharp reduction of biomass. Particularly, in recent years, As contamination has been reported to inhibit wheat growth and reduce its yield remarkably.

Cadmium (Cd) is already considered one of the most toxic heavy metals that are harmful to mankind because of high ambulation (Huang et al., 2004). Cd inhibits growth of plant root (Belimov et al., 2003), uptake of water and nutrients (Boussama et al., 1999), photosynthesis...
(Krupa and Miniak, 1998) and respiratory vigor (Bazzaz et al., 1974), affecting carbohydrate metabolism and other physiological aspects, and thereby reducing yield (Verma and Dubey, 2001; Sandalio et al., 2001). These effects could get worse with increasing Cd concentration.

Lead (Pb) can also be accumulated in roots, shoots and leaves after being absorbed by plants (Morelli and Scarano, 2001). Root growth in Indian mustard is inhibited by Pb(NO$_3$)$_2$ in a concentration and duration dependent manner (Begonia et al., 1998). Liu (2002) reported that Pb at low concentrations could promote normal physiological and metabolic activities in plants such as the activities of nitrate reductase (NR), the contents of soluble sugar and chlorophyll of stems and leaves. However, Pb at higher concentrations severely affect normal physiological and metabolic activities in plants, resulting in the symptoms of leaf etiolation and withering of stems and leaves.

Moreover, As, Cd, Pb etc. have been known to affect the respiration in plants. Pb inhibited the respiration strength remarkably during seed germination in rice in a concentration dependent manner (Yang et al., 1986). Seed germination and respiratory rate in soybean are also observed to be repressed by the pollution of Cu or As (Liu and Wang, 2002). Another study indicated that the structure of cell membrane is affected seriously and the mitochondrion crista disappeared with the Cd stress (Li, 2000).

There are very few reports about the effects of As, Cd or Pb stresses on wheat respiration now. So it is of practical significance to study the toxicity of these stresses on wheat. And the physiological mechanism of respiration in wheat with As, Cd or Pb stresses is not well known yet. Additionally, being the third dominant crop only next to rice and corn in China, wheat’s yield and quality are directly related to improving civil dietetic level and ensuring food safety. And respiratory metabolite is one of the most important physical metabolites providing necessary energy for plant activity, and its interim production can be translated into organic substances, such as protein, nucleic acid, fat etc.

Therefore, respiration can be considered as the metabolic center of the plant. This study reports the effects of different concentrations of As, Cd and Pb on growth and biomass of shoots and roots, their respiratory rate, the enzyme levels of COD, IDH and MDH isoenzymes to elucidate the physiological mechanisms in wheat seedlings.

**MATERIALS AND METHODS**

**Germination assay**

A variety of wheat (*Triticum aestivum* L.), obtained from Henan normal university, Xinxiang, China), Zhengzhou-9023, was chosen for this study. Prior to germination, seeds were surface-sterilized in 0.1% mercuric chloride (HgCl$_2$) for 6 min and rinsed with distilled water. Seed germination was tested on wet filter paper placed on a petri dish and moistened with aqueous solutions of As, Cd and Pb at different concentrations, with 3.0 ml deionized water as control. One hundred seeds were placed in each petri dish, covered by lid and incubated at 15 to 20°C. Germinated seeds were counted 7 days 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 for reliability.

The range of concentrations for As: 1, 5, 15, 25 mg·L$^{-1}$, Cd: 25, 50, 75, 100 mg·L$^{-1}$, and Pb: 50, 100, 200, 400 mg·L$^{-1}$, were prepared freshly as Na$_2$AsO$_3$, CdCl$_2$·2.5H$_2$O, Pb(CH$_3$COO)$_2$·3H$_2$O, respectively.

**Determination of root length, shoot length, root biomass and shoot biomass**

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 after 7 days of germination. The fresh plant samples were oven-dried at 70°C and the dry matters of shoots and roots were weighed individually.

**Determination of respiratory rate**

The respiratory rate was measured by following the infrared gas analysis technique (Li, 2000). In short, wheat seedlings were separated into root and shoot, and then the respiration rate was quantified using portable infrared CO$_2$ analyzer (Model: GHX-305, Manufacturer: Beijing analysis instrument plant) in an aerometry room. The respiration rate is calculated using the formula:

\[
R_r = \frac{C \times V \times 273 \times P}{(\Delta t \times S \times 22.4 \times (273 + t)) \times 0.1013}
\]

Where: \( R_r \) = respiration rate (µmol·g$^{-1}$·s$^{-1}$); \( \Delta C \) = CO$_2$ concentration difference (µmol·L$^{-1}$); \( \Delta t \) = minute (s); \( S \) = shoot’s weight (g); \( V \) = aerometry room’s cubage (L); \( t \) = aerometry room’s temperature(°C); \( P \) = barometric (MPa). Each treatment was replicated three times.

**Determination of enzyme levels of COD, IDH, MDH isoenzymes**

About 0.2 g sample (leaves or roots) was homogenized with a mortar and pestle in 1.2 ml cold extraction buffer (sucrose 11.98 g, tris 0.6 g, sodium ascorbate 0.088 g, cysteine 0.03 g, magnesium chloride 0.02 g in 100 ml), then centrifuged at 12000 g for 20 min at 4°C. The supernatant was used in the enzyme assays of COD, IDH, MDH isoenzymes.

Electrophoretic resolution of COD, IDH, MDH: COD, IDH, MDH isoenzymes were performed on nondenaturating polyacrylamide gels (7% acrylamide, 3% bis-acrylamide) using Tris-Gly buffer 5 mmol·L$^{-1}$, pH 8.3. The gels were run at 100 V for 60 min, and then at 200 V for 120 min.

To visualize the patterns of cytochrome oxidase bands, the gels were incubated in the dark for 20 min at 37°C in an aqueous solution before scanning using densitometer. The aqueous solution contained 1% N,N-dimethyl p-phenylenediamine 1.5 ml, 1% α-naphthol (dissolved in 40% ethanol) 1.5 ml, 0.1 mol·L$^{-1}$ phosphate buffer (pH 7.4) 25 ml.

For detecting isocitrate dehydrogenase, the gels were incubated in the dark for 1 to 2 h at 37°C in a solution containing 3% DL-trisodium-iso-citrate 1.9 ml, 0.5% nicotinamide adenine dinucleotide phosphate (NADP) 0.5 ml, 1% nitro blue tetrazolium (NBT) 0.5 ml, 1% phenyl methane sulfonyl fluoride (PMS) 120 µl, 10% magnesium chloride (MgCl$_2$) 286 µl, 0.05 mol·L$^{-1}$ tris hydrochloride (Tris-HCl buffer) (pH 8.0) 28.6 ml. Gels were then densitometrically
Table 1. Germination energy, germination rate, germination index, vitality index of wheat seeds at different As, Cd and Pb concentrations.

<table>
<thead>
<tr>
<th>Concentration (mg·L(^{-1}))</th>
<th>Length of shoot</th>
<th>Length of root</th>
<th>Weight of shoot</th>
<th>Weight of root</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.63 (100%)</td>
<td>7.10 (100%)</td>
<td>0.66 (100%)</td>
<td>0.68 (100%)</td>
</tr>
<tr>
<td>1</td>
<td>5.96 (105.8%)</td>
<td>8.09 (113.8%)</td>
<td>0.74 (112.1%)</td>
<td>0.70 (102.9%)</td>
</tr>
<tr>
<td>5</td>
<td>5.77 (102.4%)</td>
<td>7.31 (102.8%)</td>
<td>0.70 (106.1%)</td>
<td>0.66 (97.1%)</td>
</tr>
<tr>
<td>15</td>
<td>4.38 (77.8%)</td>
<td>1.66 (23.3%)</td>
<td>0.68 (103%)</td>
<td>0.42 (61.8%)</td>
</tr>
<tr>
<td>25</td>
<td>4.33 (76.9%)</td>
<td>1.40 (19.7%)</td>
<td>0.64 (97%)</td>
<td>0.40 (58.8%)</td>
</tr>
<tr>
<td>25</td>
<td>5.06 (89.8%)</td>
<td>3.53 (49.7%)</td>
<td>0.64 (97%)</td>
<td>0.52 (76.5%)</td>
</tr>
<tr>
<td>50</td>
<td>4.53 (80.3%)</td>
<td>2.25 (31.7%)</td>
<td>0.62 (93.9%)</td>
<td>0.44 (64.7%)</td>
</tr>
<tr>
<td>75</td>
<td>4.20 (74.5%)</td>
<td>1.15 (16.2%)</td>
<td>0.58 (87.9%)</td>
<td>0.42 (61.8%)</td>
</tr>
<tr>
<td>100</td>
<td>3.97 (70.4%)</td>
<td>0.65 (9.1%)</td>
<td>0.54 (81.8%)</td>
<td>0.38 (55.9%)</td>
</tr>
<tr>
<td>25</td>
<td>6.15 (109.1%)</td>
<td>6.39 (89.9%)</td>
<td>0.70 (106.1%)</td>
<td>0.62 (91.2%)</td>
</tr>
<tr>
<td>50</td>
<td>6.24 (110.7%)</td>
<td>5.93 (83.4%)</td>
<td>0.82 (124.2%)</td>
<td>0.60 (88.2%)</td>
</tr>
<tr>
<td>200</td>
<td>6.12 (108.7%)</td>
<td>3.90 (55%)</td>
<td>0.80 (121.2%)</td>
<td>0.54 (79.4%)</td>
</tr>
<tr>
<td>400</td>
<td>5.13 (91.1%)</td>
<td>1.86 (26.2%)</td>
<td>0.74 (112.1%)</td>
<td>0.48 (70.6%)</td>
</tr>
</tbody>
</table>

Digits in the brackets represent percentage compared to the control (%).

To visualize the patterns of malate dehydrogenase, the gels were incubated in the dark for 2 to 4 h in an aqueous solution of 2 mol·L\(^{-1}\) DL-malic acid (pH 8.0) 3 ml, 1% nicotinamide adenine dinucleotide (NAD) 1.5 ml, 1% NBT 1 ml, 1% PMS 120 \(\mu\)l, 0.2 mol·L\(^{-1}\) Tris-HCl buffer (pH 8.0) 16 ml and were then densitometrically scanned.

Regression relations of heavy metal concentration and growth index (root length, shoot length, root biomass and shoot biomass) of wheat seedlings were analysed (Table 2). As had significant effects on root and shoot lengths and root biomass \((P < 0.05)\) compared to the control. Cd had significant effects on root length and biomass \((P < 0.05)\), and very significant effect on shoot length and biomass \((P < 0.01)\). Pb had very significant effects on root length and root biomass \((P < 0.01)\).

These results indicated that wheat seedlings were sensitive to As, Cd and Pb, and the growth of both root and shoot was inhibited by high concentrations of treatments. Furthermore, root growth was more sensitive to these heavy metals compared to the shoot growth. Similarly, root biomass was more sensitive than the shoot about biomass.

RESULTS

Effects of As, Cd and Pb on root length, shoot length, root biomass and shoot biomass

Length and biomass measurements of both roots and shoots displayed an increasing trend at first and then decreased with As treatment (Table 1). When the concentration of As was 1 mg·L\(^{-1}\), all the parameters under investigation reached maximum. Root length, shoot length, root biomass and shoot biomass decreased gradually upon increasing the concentration of Cd. Increasing the concentration of Pb led to an initial increase in the shoot length and its dry biomass but decreased gradually later, while the root length and dry biomass of roots decreased continuously.

Regression relations of heavy metal concentration and growth index (root length, shoot length, root biomass and shoot biomass) of wheat seedlings were analysed (Table 2). As had significant effects on root and shoot lengths and root biomass \((P < 0.05)\) compared to the control. Cd had significant effects on root length and biomass \((P < 0.05)\), and very significant effect on shoot length and biomass \((P < 0.01)\). Pb had very significant effects on root length and root biomass \((P < 0.01)\).

These results indicated that wheat seedlings were sensitive to As, Cd and Pb, and the growth of both root and shoot was inhibited by high concentrations of treatments. Furthermore, root growth was more sensitive to these heavy metals compared to the shoot growth. Similarly, root biomass was more sensitive than the shoot about biomass.

Effects of As, Cd and Pb on respiratory rate of leaves and roots of wheat seedlings

The respiratory rate of leaves of wheat seedlings displayed increasing trend at lower concentrations of As, Cd and Pb, but decreasing trend at higher concentrations of them, reaching maximum of 115.16, 111.74, 101.19 \(\mu\)mol·g\(^{-1}\)·s\(^{-1}\) at 1 mg·L\(^{-1}\) of As, 75 mg·L\(^{-1}\) of Cd, 100 mg·L\(^{-1}\) of Pb, respectively (Figure 1). This implied that the respiratory rate of roots was induced when As, Cd and Pb at lower concentrations but was inhibited at higher concentrations.

Respiratory rate in the roots displayed a tendency of increasing in a whole, increasing slightly at lower concentrations of As (0 to 1 mg·L\(^{-1}\)), but decreasing at 1 to 5 mg·L\(^{-1}\), and then increasing again to the top of 186.82 \(\mu\)mol·g\(^{-1}\)·s\(^{-1}\) at 25 mg·L\(^{-1}\) of As, which was 179.14% increase compared to the control. Respiratory rate first displayed an increasing trend and then a decreasing trend with increasing concentrations of Cd and Pb. When the concentration of Cd at 75 mg·L\(^{-1}\) or Pb at 200 mg·L\(^{-1}\),
Table 2. Regression analysis of heavy metals concentration and growth index (root length, shoot length, root biomass and shoot biomass) of wheat seedlings.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Index</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Shoot length</td>
<td>$y = 5.8483 - 0.0686x$</td>
<td>$r = -0.849^*$</td>
</tr>
<tr>
<td></td>
<td>Root length</td>
<td>$y = 7.7646 - 0.2885x$</td>
<td>$r = -0.869^*$</td>
</tr>
<tr>
<td></td>
<td>Shoot biomass</td>
<td>$y = 0.7048 - 0.0023x$</td>
<td>$r = -0.391$</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>$y = 0.6942 - 0.0133x$</td>
<td>$r = -0.902^*$</td>
</tr>
<tr>
<td>Cd</td>
<td>Shoot length</td>
<td>$y = 5.5160 - 0.0168x$</td>
<td>$r = -0.968^{**}$</td>
</tr>
<tr>
<td></td>
<td>Root length</td>
<td>$y = 5.9944 - 0.0611x$</td>
<td>$r = -0.878^*$</td>
</tr>
<tr>
<td></td>
<td>Shoot biomass</td>
<td>$y = 0.6680 - 0.0012x$</td>
<td>$r = -0.970^{**}$</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>$y = 0.6280 - 0.0028x$</td>
<td>$r = -0.868^*$</td>
</tr>
<tr>
<td>Pb</td>
<td>Shoot length</td>
<td>$y = 6.1342 - 0.0019x$</td>
<td>$r = -0.399$</td>
</tr>
<tr>
<td></td>
<td>Root length</td>
<td>$y = 7.0471 - 0.0134x$</td>
<td>$r = -0.984^{**}$</td>
</tr>
<tr>
<td></td>
<td>shoot biomass</td>
<td>$y = 0.7215 + 0.0002x$</td>
<td>$r = 0.126$</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>$y = 0.6545 - 0.0005x$</td>
<td>$r = -0.939^{**}$</td>
</tr>
</tbody>
</table>

* or ** represent significant at $p < 0.05$ or $p < 0.01$, respectively.

The concentration of As, Cd, Pb (mg·L$^{-1}$)

Figure 1. Respiratory rates of leaves and roots of wheat seedlings treated with 1, 5, 15, 25 mg·L$^{-1}$ external As, 25, 50, 75, 100 mg·L$^{-1}$ external Cd, 50, 100, 200, 400 mg·L$^{-1}$ external Pb, after 7 days of germination in hydroponics, 0: control. Bars represent standard errors (SE) of replicates.

Effects of As, Cd and Pb on COD isoenzymes of wheat seedlings

Cytochrome oxidase (COD) is a terminal oxidase in the respiratory chain of eukaryotic cells. It transfers electrons between cytochrome aa$_3$ and oxygen.

COD displayed nine isoenzymes in the leaves, example, C1, C2, C4, C6, C9, C10, C11, C12 and C13, with relative migration values at 0.1, 0.18, 0.28, 0.4, 0.6, 0.66, 0.76, 0.82 and 0.9 respectively (Figure 2A). These bands were initially expressed in lower levels and later in higher levels with increasing concentrations of As, implying that the expression of these isoenzyme bands were inhibited by As at lower concentrations but induced at higher concentrations. However, the bands C9 to C12 all exhibited a similar trend with increasing concentrations

the respiratory rate reached a maximum of 138.19 or 129.24 µmol·g$^{-1}$·s$^{-1}$, respectively.
of Cd and Pb. These bands were strong at 75 mg·L\(^{-1}\) of Cd or 100 mg·L\(^{-1}\) of Pb respectively, implying that the expression of these four isoenzyme bands were induced by Cd or Pb at lower concentrations, but inhibited at higher concentrations.

A total of 13 COD isoenzyme bands were detected in the roots, example, C1 to C13, with relative migration values at 0.1, 0.18, 0.22, 0.28, 0.34, 0.4, 0.48, 0.56, 0.6, 0.66, 0.76, 0.82 and 0.9, respectively (Figure 2B). Of these, C1, C2, C9, C10, C11 and C12 were strong bands while C3, C4, C5, C6, C7, C8 and C13 were weak bands. The expressions of these isoenzymes displayed a decreasing trend with increasing concentrations of As. All the isoenzymes exhibited increased levels at lower concentrations, and then decreased levels with increasing concentrations of Cd or Pb. These bands were strong at 25 mg·L\(^{-1}\) of Cd and 50 mg·L\(^{-1}\) of Pb, respectively, indicating that the expression of COD isoenzymes in roots were induced by lower concentrations but inhibited at higher concentrations of Cd or Pb. In addition, no new COD isoenzymes was induced by As, Cd or Pb at all.

**Effects of As, Cd and Pb on IDH isoenzymes of wheat seedlings**

Isocitrate dehydrogenase (IDH) participates in the tricarboxylic acid cycle, catalyzing the conversion of isocitrate to \(\alpha\)-ketoglutarate through oxidative decarboxylation and reducing oxidative NAD to be NADH, it is considered to be a rate-limiting enzyme in tricarboxylic acid cycle. Thus IDH activity has a tremendous influence on the organism’s metabolism.

Two IDH isoenzymes were detected as two bands in the leaves; I1 and I5, with relative migration values at 0.17 and 0.48, respectively (Figure 3A). The expression levels of two isoenzymes were different in response to the heavy metals. The I1 isoenzyme displayed no significant change with increasing concentrations of As. Contrarily, with increasing concentrations of Cd or Pb, the expression levels increased initially and then weakened. I5 band expression level was high at 25 mg·L\(^{-1}\) of As, but higher than the control under Cd or Pb treatment, indicating that the expression of I5 isoenzyme band was induced variably in response to the treatment with As, Cd or Pb.

Two IDH isoenzymes were detected as two bands in roots, when cultured in deionized water, I3 and I4, with relative migration values at 0.31 and 0.45, respectively (Figure 3B). The two bands were enhanced gradually with the increase in the concentrations of As. The expression of I3 and I4 isoenzymes were induced significantly by Cd at lower concentrations (0 to 50 mg·L\(^{-1}\)) but inhibited by Cd at concentrations higher than 50 mg·L\(^{-1}\). Moreover, the expression of I3 isoenzyme band was so severely repressed that no activity was detected at 50 mg·L\(^{-1}\) of Cd. However, Cd at the concentration of 50 mg·L\(^{-1}\) induced the expression of two new isoenzyme bands I2 and I6, whose relative migration values were 0.27 and 0.57 respectively, although the inducible effects became weaker with the increase in the concentrations of Cd. The expression of I6 and I2 isoenzyme bands was induced by Pb at 50 mg·L\(^{-1}\) and 200 mg·L\(^{-1}\) respectively.

**Effects of As, Cd, Pb on MDH isoenzymes of wheat seedlings**

Malate dehydrogenase (MDH) plays a very important role in the tricarboxylic acid cycle, catalyzing the transformation of DL-malic acid to oxaloacetate.
MDH displayed three isoenzyme bands in the leaves: M1, M3 and M5 with relative migration values at 0.32, 0.37 and 0.49 respectively (Figure 4A). The expression levels of these bands were lower initially but increased later with the increasing concentrations of As. M1 and M3 became weaker gradually while M5 was enhanced at lower concentrations, and then became less detectable with the increased concentrations of Cd or Pb.

At normal conditions, just as control, three MDH isoenzyme bands were detected in the roots: M2, M3 and M5 with relative migration values 0.33, 0.37 and 0.49 respectively (Figure 4B). The expression levels of these isoenzymes showed an increasing trend with increasing concentrations of As. Besides, a new isoenzyme band M1 was induced by As at concentrations higher than 5 mg·L\(^{-1}\). The expression of M4 (relative migration 0.45) as well as M1 were induced by Cd, but the expression levels decreased gradually with the increase in the Cd concentrations. M1 expressed when treated with Pb, and the expression levels became weaker with increase in the concentration of Pb.

**DISCUSSION**

The growth of wheat seedlings requires a great deal of energy, which is supplied primarily by respiration. Respiration not only provides large quantities of adenosine triphosphate (ATP) and other metabolites required for seed germination, formation of new tissues and organs, but also contributes to other physiological and biochemical processes (Wang et al., 2001). A series of changes in the physiology and biochemistry of seed germination process would be influenced once respiration is disturbed. It has been demonstrated that heavy metals such as Pb have remarkable effects on respiration in
plants (Yang et al., 1986). Wang et al. (2001) reported an enhance in the respiration was due to the production of energy via stimulation of respiratory enzymes and tricarboxylic acid cycle at lower concentration of Cd. However, the activities of respiratory enzymes were inhibited and respiration rate decreased with the increasing concentration of Cd (Allan and Jarrel, 1989). In our study, the respiration rate in leaves and roots of wheat seedlings enhanced at lower concentrations at first, but then declined with the increase in concentrations of Cd or Pb. However, the respiration rate in roots displayed an increasing trend with the increase in the concentrations of As. These results indicated that there was a stimulatory effect in the respiration rate in leaves and roots of wheat seedling at lower concentrations of As, Cd and Pb, at the same time a suppressive effect at higher concentrations of them. Additionally, plants have the capacity to self-adjust when subjected to stress such as heavy metals. So a series of protective mechanisms initiate in the cells to enhance their resistance and adaptation to the environmental changes. However, the protective systems (involving several enzymes systems) would severely affect the normal physiological activities when the concentrations of heavy metals beyond a certain limit.

As we all know, isoenzyme profiles could be the reporters of a cell state at the molecular level of gene expression. The strength of the isoenzyme bands can reflect relative quantity and the activity of the isoenzyme (Milone et al., 2003). Our results indicated that the expression of COD, MDH and IDH isoenzyme bands in leaves and roots of wheat seedling were induced mostly during the increase of As; the activities of these isoenzymes were enhanced at low concentrations of Cd or Pb, but reduced at higher concentrations of As, Cd and Pb. These tendency of changes described correlate largely with the results of respiration rates, establishing a link between the change in the respiration rate and the expression of COD, MDH and IDH isoenzymes. It is therefore likely that heavy metals usually affect the respiration rate by influencing the expression of isoenzymes involved in respiratory mechanism.

Meanwhile, these data also indicated that the length and biomass of roots and shoots increased at low concentrations of As (1 mg·L\(^{-1}\)). The growth of roots and shoots was inhibited by high concentrations of As (5 to 25 mg·L\(^{-1}\)), length and biomass of both roots and shoots displayed a decreasing trend with increasing concentrations of As. These indexes represented a decreasing trend with the increase in concentrations of Cd. Plants treated with Pb led to increase in the length and biomass of shoots initially and then decreased, while length and biomass of roots declined continuously. These results indicated that roots and shoots were sensitive to As, Cd and Pb, and the inhibition was stronger in roots than in shoots. This conclusion is in agreement with views that plant roots are the first point of contact to heavy metals in the nutrient media (Abedin and Meharg, 2002).

All in all, heavy metals could change the expression of important enzymes in the respiratory process such as COD, IDH and MDH isoenzymes, thus influencing respiration in wheat, and further affecting the syntheses of metabolites and energy, eventually resulting in morphological changes in wheat.

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REFERENCES


