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RESEARCH ARTICLE

Effect of exogenous abscisic acid on the level of antioxidants in *Atractylodes macrocephala* Koidz under lead stress

Jinchuang Wang · Juan Chen · Kaiwen Pan

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Abstract This study hypothesized that the positive or negative effects of exogenous abscisic acid (ABA) on oxidative stress caused by lead were dose dependent. The effects of different levels of ABA (2.5, 5, and 10 mg L^{-1}) on lead toxicity in the leaves of *Atractylodes macrocephala* were studied by investigating plant growth, soluble sugars, proteins, lipid peroxidation, and antioxidative enzymes. Excess Pb inhibited root dry weight, root length, and the number of lateral roots, but increased shoot growth. In addition, lead stress significantly decreased the levels of chlorophyll pigments, protein, and activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD). Different levels of ABA significantly increased SOD, CAT, POD, and APX activities, but decreased the level of hydrogen peroxide and malondialdehyde in nonstressed plants. Exogenous application of 2.5 mg L^{-1} ABA detoxified the stress-generated damages caused by Pb and also enhanced plant growth, soluble sugars, proteins, and all four antioxidant enzyme activities but reduced Pb uptake of lead-stressed plant compared to lead treatment alone. However, the toxic effects of Pb were further increased by the applications of 5 and 10 mg L^{-1} ABA. The levels of antioxidants caused by a low concentration of exogenous ABA might be responsible for minimizing the Pb-induced toxicity in *A. macrocephala*.

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J. Wang
MOE Laboratory of Plant–Soil Interaction and College of Resources and Environmental Sciences,
China Agricultural University,
Beijing 100193, China

J. Wang (✉) · J. Chen · K. Pan
Chengdu Institute of Biology, Chinese Academy of Sciences,
Chengdu 610041, China
e-mail: jinchuangwang@yahoo.com

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Introduction

Lead is considered to be one of the most important pollutants of the air and is also a very significant pollutant of agricultural soils. Pb-contaminated soils cause sharp decreases in crop productivity and thus pose a serious problem for agriculture. Its increasing levels in soil environment inhibit seed germination and root growth of maize seedlings (Eun et al. 2000). In addition, it is known to induce oxidative stress that cause damage to the biomolecules such as chloroplasts, soluble protein, proline, ascorbate, enzymes, etc (Verma and Dubey 2003; Qureshi et al. 2007; Zhao et al. 2009; Shu et al. 2012).

Abscisic acid (ABA) is considered a plant stress hormone. It controls plant development and growth, including embryo development, seed dormancy, inhibition of lateral root formation, transpiration, synthesis of proteins, and photosynthesis. It has been documented that ABA can induce the expression of antioxidant genes and enhance the capacity of antioxidant defense systems, including enzymatic and nonenzymatic constituents (Jiang and Zhang 2001, 2002). Meanwhile, since ABA causes oxidative stress in plants, a high concentration of ABA (1,000 μm) induces an excessive generation of active oxygen species (AOS) and leads to an oxidative damage in plant cells (Jiang and Zhang 2001; Li et al. 2010). Besides this, ABA is also recognized to have an important role in plants subjected to various environmental stresses such as cold, salt, and drought (Gong et al. 1998; Jiang and Zhang 2002; Li et al. 2004; Zhou et al. 2006). Pretreatment with abscisic acid has been reported to significantly increase antioxidant enzymes activities and antioxidants in maize seedlings exposed to water stress (Jiang and

Zhang 2002). Under Cd stress, exogenous application of ABA reduced transpiration rate and photosynthesis and enhanced Cd tolerance of rice seedlings (Hsu and Kao 2003). However, less is known about the role of ABA in the regulation of antioxidant defense and oxidative stress in heavy metal tolerance of plants, especially in lead stress.

The rhizome of *Atractylodes macrocephala* Koidz is an important ingredient of several Chinese herbal prescriptions. In China, Pb content in the A-horizon of natural soils varies from 0.68 to 1,143 mg kg^{-1} (Chen 2004). Thus, there is an urgent need to study the mechanism of Pb tolerance of *A. macrocephala* plants. In addition, it is also of great necessity and significance to increase *A. macrocephala* productivity through taking all sorts of effective measures to promote its lead tolerance. In recent years, a low price (S)-ABA was biosynthetically produced in China, which allows its potential application for protection of crops against abiotic stresses. However, different results have been reported regarding the effect of applied ABA showing an inhibitory or enhanced effect of ABA on plant growth (Jiang and Zhang 2001; Li et al. 2010); therefore, it is important to evaluate whether the Pb tolerance of *A. macrocephala* will be enhanced or weakened under different concentrations of exogenous ABA.

The purpose of the present study was to examine the role of ABA in the regulation of antioxidant defense and oxidative stress in *A. macrocephala* under lead stress. In this study, the effects of different concentrations of ABA on plant growth, antioxidant enzyme activities, soluble protein, soluble sugars, chlorophyll, and lipid peroxidation in *A. macrocephala* under lead stress were systematically studied in detail. We postulated the following hypothesis that the positive or negative effects of exogenous ABA on oxidative stress caused by lead were dose dependent. This information could lead to improved techniques enhancing lead tolerance and productivity of *A. macrocephala.*

Materials and methods

Plant material and experimental design

Seeds of *A. macrocephala* were surface-sterilized with 0.5 % sodium hypochlorite and were sown in plastic pots (15 cm diameter) containing a mixture of peat and perlite (3:1, *v*/*v*) for germination. When the second leaf emerged, seedlings of uniform size were transferred to hydroponics pots (1 L, PVC, six plants per pot) in a growth chamber. Each pot contained 1 L of full-strength Hoagland nutrient solution, which was aerated 20 min daily and renewed every other day. The pots were randomly arranged daily during the growth period. Seedlings grew under the conditions of 18–25 °C, photosynthetic photon flux density of 350 mmol m^{-2} s^{-1} and photoperiod of 14/10 h (day/night), and 70 % relative humidity for 14 days. Then, the seedlings were sprayed uniformly with 0, 2.5, 5, and 10 mg L^{-1} of (S-)ABA (90 % power, Lomon BioTechnology Co, Ltd., Sichuan Province, China), until the canopy was completely wetted. These concentrations of ABA were based on earlier studies, and these concentrations have been shown to be adequate as modulator of the response of plant to environmental stress (Jiang and Zhang 2001; Zhou et al. 2005, 2006). Two days after hormone treatment, seedlings were supplied with 0 or 300 μmol of lead in the form of $Pb(NO_3)_2$, with nutrient solution. The choice of 300 μmol Pb represents the concentrations mimicking polluted soils. The following eight treatments with four replicates each were established, including control (neither addition of Pb nor ABA), 300 μmol Pb, 2.5 mg L^{-1} ABA, 5 mg L^{-1} ABA, 10 mg L^{-1} ABA, 300 μmol Pb + 2.5 mg L^{-1} ABA, 300 μmol Pb + 5 mg L^{-1} ABA, and 300 μmol Pb + 10 mg L^{-1} ABA. Six days after Pb treatment, plants were sampled with leaves to assess the parameters discussed below.

Estimation of growth parameters

The number of lateral roots and root length were measured after harvesting. Shoot and root were collected separately, washed, and then dried in an oven at 70 °C for 72 h for biomass assay.

Chlorophyll content

Leaf chlorophyll content was determined according to Arnon (1949). Briefly, 0.1 g leaves were homogenized with 5 mL 80 % acetone with some $CaCO_3$. The homogenate was centrifuged at 20,000 × *g* for 20 min. The absorbance of the clear supernatant was read spectrophotometrically at 663 and 645 nm. The values were placed in the following formula to calculate chlorophyll content: $[(A645 \times 28.2) + (A663 \times 8.3)] \times [(V/1,000) \times W]$.

Determination of Pb content in seedlings

The concentrations of Pb in shoots and roots of seedlings were determined according to the method described previously (Zhao et al. 2009). Seedlings were rinsed with 1 mM citrate for 30 min to remove surface Pb. The harvested root and shoot samples were oven-dried at 105 °C for 20 min and then kept at 80 °C for 72 h. These dried materials were ground into powder. Of the powdered sample, 0.2 g was digested with 10 mL of 10:1 HNO_3–$HClO_4$ solution. The Pb concentration was determined with a flame atomic absorption spectrometer.

Assay of antioxidative enzymes

Leaf tissue (0.5 g) was ground into a fine powder and homogenized in 5 mL of 50 mmol phosphate buffer (pH

7.0) containing 1 % insoluble polyvinylpyrrolidone. The homogenate was centrifuged at 15,000 × *g* for 20 min. The supernatant was stored at 4 °C for analysis of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities.

SOD activity was assayed using the procedure described by Giannopolitis and Ries (1977). The reaction mixture (3.0 mL) contained 79.2 mM Tris–HCl buffer (pH 6.8), containing 0.12 mM EDTA and 10.8 mM tetraethylene diamine, bovine serum albumin (0.0033 %), 6 mM nitroblue tetrazolium (NBT), 600 mM riboflavin in 5 mM KOH, and 0.05 mL of enzyme extract. The reaction mixture, which was not exposed to light, did not develop color and served as control. One unit SOD activity was defined as the amount of enzyme required to result in 50 % inhibition of the reduction of NBT.

POD activity was assayed according to Beffa et al. (1990). Briefly, the assay mixture contained 2.95 mL 100 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 6.0), 2 mM hydrogen peroxide (H_2O_2), 9 mM guaiacol, and 0.05 mL enzyme extract in a total volume of 3.0 mL. The changes in absorbance at 460 nm during polymerization of guaiacol were recorded for calculating POD activity.

CAT activity was determined by the method of Aebi (1984). The reaction mixture comprised 1.8 mL 50 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 7.0), 1.0 mL 10 mM H_2O_2, and 0.2 mL of enzyme extract. The activity was determined by following the consumption of H_2O_2 at 240 nm for 3 min.

APX activity was estimated by the method of Nakano and Asada (1981). APX activity was determined by recording the decrease in 290 nm as ascorbates were oxidized. The reaction mixture contained 1.1 mL 50 mM potassium phosphate buffer (pH 7.0), 1.5 mL 1 mM AsA, 0.3 mL 1 mM H_2O_2, and 0.2 mL of enzyme extract. The time interval was 10 min. The reaction was started by adding H_2O_2, and the contents of protein were measured at the same time.

Estimation of soluble sugar content

Total soluble sugar was extracted following the method of Chow and Landhäusser (2004). Fresh ground samples (0.5 g) were extracted by boiling in 80 % neutral aqueous ethanol for 6 h. The extract was filtered through Whatman filter paper no. 1. After filtration, the clear solution was made up to a known volume with ethanol solution. An aliquot ethanol extract (10 ml) was transferred to a clean, dry beaker and heated to dryness in a water bath. The residue was then dissolved in water and quantitatively transferred to a 25-mL volumetric flask and made up to the mark with distilled water. One milliliter water extract, 1 mL phenol solution (5 %), and 5 mL sulfuric acid (96 %) were added. The absorbance was measured at 490 nm using a Unicam spectrophotometer. Graphic plot of the O.D. values against various standard solutions of different concentrations of glucose was used as a standard curve.

Determination of soluble protein concentration

Soluble protein concentration was measured following the method described by Bradford (1976), with standard curve prepared using bovine serum albumin. The assay is based on the stable dye–albumin complex, which can be quantified spectrophotometrically at 590 nm. The protein–dye reagent consisted of 3 mL of 0.01 % (*w*/*v*) Coomassie Brilliant Blue G-250, 4.7 % (*w*/*v*) ethanol, and 8.5 % (*w*/*v*) phosphoric acid.

Concentration of malondialdehyde and H_2O_2 in *A. macrocephala* leaves

The lipid peroxidation products were estimated by the formation of thiobarbaturic acid-reactive substances and quantified in terms of malondialdehyde (MDA). Briefly, fresh tissues (0.5 g) were homogenized in 3 mL 10 % trichloroacetic acid solution. The homogenate was centrifuged at 2,500 × *g* for 10 min, and the supernatant was assayed for MDA concentration using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as micromole per gram fresh weight, following the method of Heath and Packer (1968). H_2O_2 levels were determined by reading the absorbance at 390 nm according to Velikova et al. (2000).

Statistical analysis

All data were subject to analysis of variance and expressed as means ± standard errors of four replicates. Statistical significance of the means was compared by Duncan's new multiple range test at the 5 % probability level using SPSS software.

Results

Growth parameters and Pb uptake

Shoot dry mass of plant exposed to lead was increased by 40 %, but root dry mass was reduced by 30 %, as compared to the control. Meanwhile, lead treatment had clearly detrimental effects on root length and the number of lateral roots. Root-to-shoot ratio (*R*/*S*) was also reduced by lead treatment (Table 1).

The effects of ABA application on plant growth were concentration dependent; 2.5 mg L^{-1} ABA applications significantly reduced root length and *R*/*S*, but increased shoot dry mass and had no significant effects on root dry mass as compared to control treatment. *R*/*S* was decreased

Table 1 Effect of different concentrations of ABA on shoot and root dry mass, root length, number of lateral roots, and contents of Pb of *Atractylodes macrocephala* Kiodz under lead stress

Treatments	Shoot dry mass (mg)	Root dry mass (mg)	Root length (mm)	Root-to-shoot ratio (*R*/*S*)	The number of lateral root	Shoot Pb (mg g^{-1} DM)	Root Pb (mg g^{-1} DM)
0	19.9±1.2 b	14.8±1.6 b	7.5±0.8 b	0.74±0.03 c	12±1.73 c	Not detected	Not detected
Pb	23.2±1.8 a	10.3±1.0 c	6.5±0.9 b	0.46±0.08 f	9.7±0.53 d	0.26±0.0.3 c	20.35±1.22 c
2.5 mg L^{-1} ABA	24.1±1.9 a	14.5±1.6 ab	6.0±0.8 b	0.60±0.01 d	17.7±1.83 b	Not detected	Not detected
Pb + 2.5 mg L^{-1} ABA	23.2±1.9 a	12.2±1.2 b	6.6±0.4 bc	0.53±0.02 d	18.3±1.53 ab	0.20±0.00 d	18.11±1.21 d
5 mg L^{-1} ABA	16.8±1.1 c	15.3±3.9 ab	6.9±0.9 b	0.94±0.03 b	15.7±1.89 ab	Not detected	Not detected
Pb + 5 mg L^{-1} ABA	15.3±3.6 d	7.7±0.8 d	5.0±0.4 d	0.51±0.01 e	11.3±1.53 c	0.34±0.01 b	23.88±1.41 b
10 mg L^{-1} ABA	15.4±2.9 d	18.6±1.8 a	5.5±0.6 a	1.21±0.02 a	20.3±2.63 a	Not detected	Not detected
Pb + 10 mg L^{-1} ABA	10.4±1.6 e	4.7±0.3 e	4.1±0.2 e	0.45±0.02 f	8.3±1.53 d	0.41±0.02 a	31.29±2.38 a

Date are means ± SE, *n*=4. Means followed by different letters within the same column are significantly different at $P<0.05$ according to Duncan's new multiple range test at the 5 % probability level

by 5 mg L^{-1}ABA, but the number of lateral root was increased; 10 mg L^{-1} ABA application significantly increased root dry mass, *R*/*S*, and the number of lateral root and significantly reduced shoot dry mass (Table 1). Addition of 2.5 mg L^{-1} ABA to Pb treatment significantly increased root dry mass, *R*/*S*, and the number of lateral roots, but decreased Pb contents in shoots and roots compared with Pb treatment alone. However, the addition of 5 mg L^{-1} ABA to Pb treatment significantly reduced shoot dry mass, root dry mass, and root length, but increased Pb content in shoots and roots compared with Pb treatment alone. The inhibition effects were further enhanced by 10 mg L^{-1} ABA plus Pb (Table 1).

Total chlorophyll content

Lead stress caused a significant reduction in total chlorophyll content in the leaves of *A. macrocephala*. Exogenous application of ABA had no effect on total chlorophyll content in lead-stressed and nonstressed plants (Fig. 1).

Soluble sugar content

There was no significant effect on soluble sugars in the leaves of *A. macrocephala* when exposed to lead stress. Accumulation of soluble sugars increased with an increase in exogenous level of ABA, i.e., 10 mg L^{-1} ABA application caused a greater accumulation of soluble sugars in the leaves than did 2.5 or 5 mg L^{-1} (Fig. 2). Different levels of ABA application also had a significant effect on the soluble sugars in the leaves of *A. macrocephala* under lead conditions; 2.5 mg L^{-1} ABA caused a significant increase in the accumulation of soluble sugars, whereas 5 or 10 mg L^{-1} ABA markedly reduced it. Ten milligram per liter ABA was more effective in decreasing soluble sugars in the leaves than 5 mg L^{-1} ABA (Fig. 2).

Soluble protein

Lead stress decreased soluble protein in the leaves of *A. macrocephala* compared with control. Application of different levels of ABA significantly increased the soluble protein in nonstressed plants, particularly at higher levels (5 or 10 mg L^{-1} ABA). In contrast, 2.5 mg L^{-1} ABA improved soluble protein of lead-stressed plants, whereas higher levels (5 or 10 mg L^{-1} ABA) did not change it (Fig. 3).

H_2O_2 and MDA

Lead stress caused a significant accumulation in H_2O_2 content of *A. macrocephala* compared with control. Exogenous application of 10 mg L^{-1} ABA was 37 and 40 % more effective in reducing the H_2O_2 content of nonstressed plants than 2.5 and

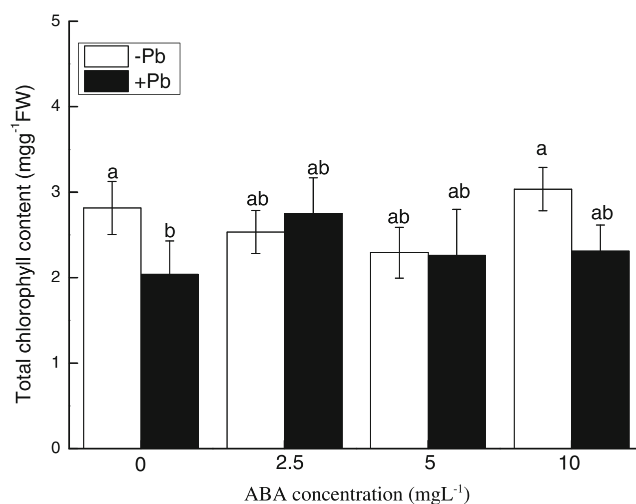

Fig. 1 Effects of different concentrations of ABA on total chlorophyll content of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

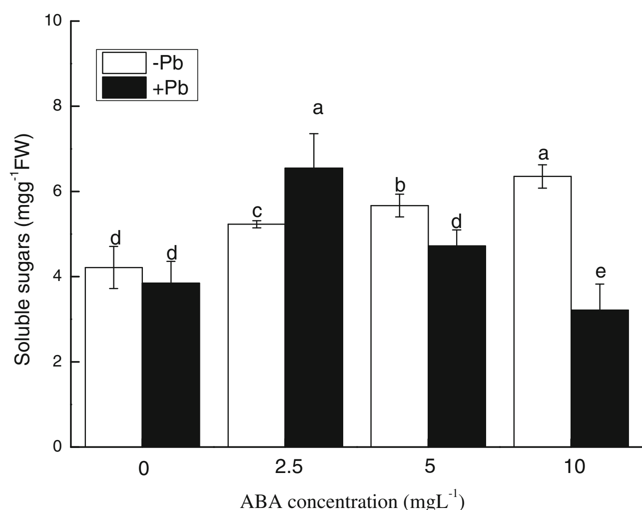

Fig. 2 Effects of different concentrations of ABA on soluble sugar content of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

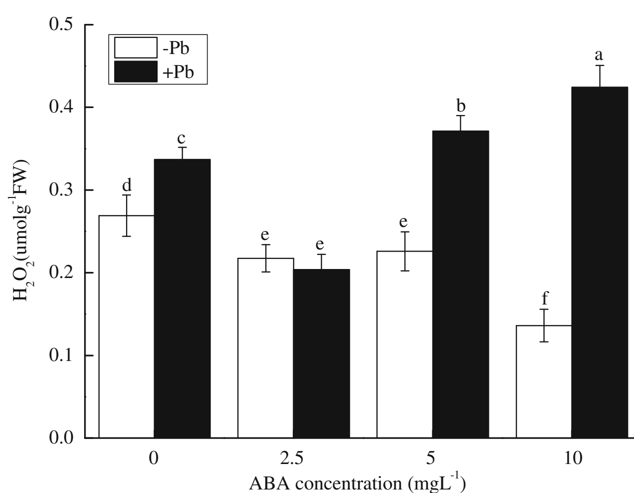

Fig. 4 Effects of different concentrations of ABA on H_2O_2 of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

5 mg L^{-1} ABA, respectively (Fig. 4); 2.5 mg L^{-1} ABA caused 40 % reduction, whereas 5 and 10 mg L^{-1} ABA showed 10 and 26 % increase in H_2O_2 content of lead-stressed plants (Fig. 4). Similarly, excessive amounts of lead in the growth medium also significantly increased MDA content in the leaves of *A. macrocephala*. Application of ABA at different levels had a significant effect on MDA content. Overall, higher levels of ABA were more effective in reducing MDA content in nonstressed plants. Under lead stress, 2.5 mg L^{-1} ABA significantly decreased MDA content by 26 % as compared to lead treatment. However, 10 mg L^{-1} ABA markedly increased MDA content in the leaves of *A. macrocephala* under lead conditions (Fig. 5).

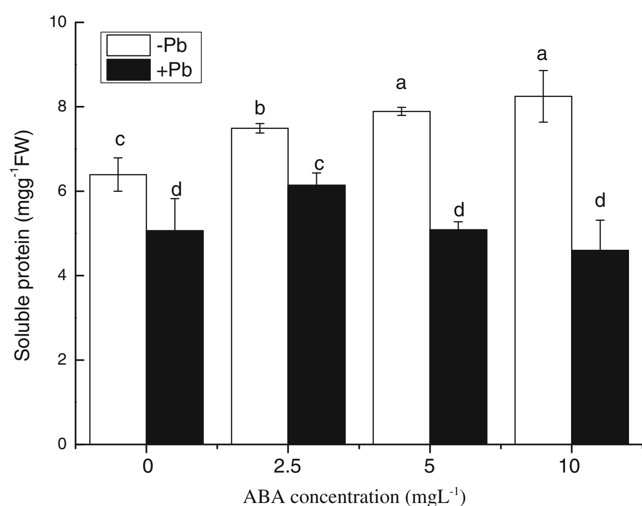

Fig. 3 Effects of different concentrations of ABA on soluble protein content of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

Antioxidant enzyme activities

In this study, lead stress caused the reduction in SOD, POD, CAT, and APX activities of *A. macrocephala* (Figs. 6, 7, 8, and 9), but the exogenous application of all ABA treatments alone enhanced their activity particularly when ABA was applied at a high level. In contrast, under lead conditions, both 2.5 and 5 mg L^{-1} ABA were equally effective in increasing SOD and APX activities in plants, whereas 10 mg L^{-1} ABA did not change SOD activity but increased APX activity as compared to lead treatment. Exogenous application of 2.5 mg L^{-1} ABA was 23 % more effective in enhancing the POD activity of lead-stressed plants than 5 mg L^{-1} ABA (Fig. 8).Compared to lead treatment, 10 mg L^{-1} ABA did not change POD activity of plants

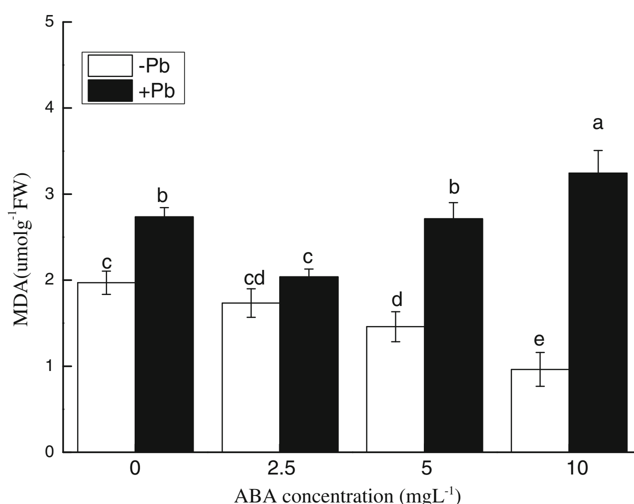

Fig. 5 Effects of different concentrations of ABA on MDA of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

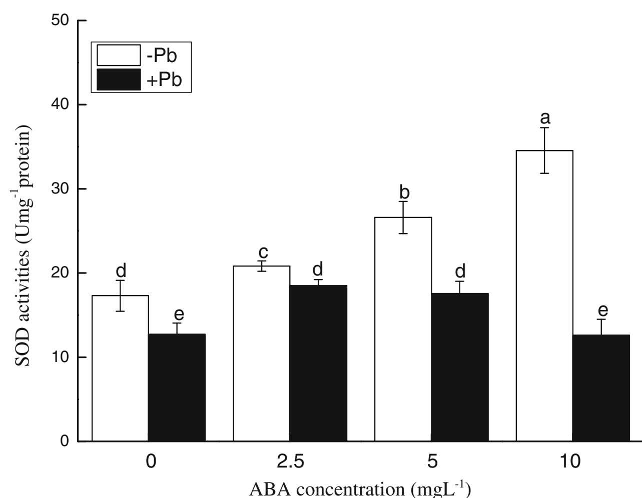

Fig. 6 Effects of different concentrations of ABA on SOD activity of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

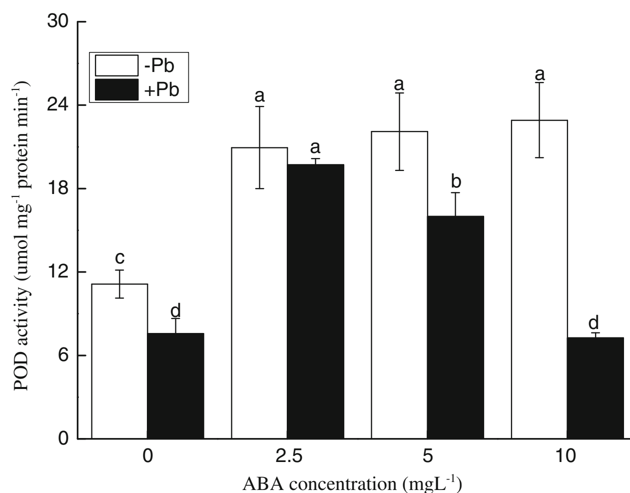

Fig. 8 Effects of different concentrations of ABA on POD activity of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

under lead stress. Similarly, 2.5 mg L^{-1} ABA caused 59 % increase, whereas 10 mg L^{-1} ABA showed 19 % reduction in CAT activity of lead-stressed plants as compared to lead treatment.

Discussion

It has been shown that Pb can inhibit plant growth by affecting the metabolic and biochemical processes associated with normal growth and development of the plant (Verma and Dubey 2003; Strubinskai and Hanaka 2011). In our study, however, Pb improved shoot dry mass, although it reduced root growth and *R*/*S* (Table 1), indicating that the toxic effects of Pb on root growth are greater than for shoot growth. Although a similar study has already been reported for wheat under Ni stress (Gajewska et al. 2006), the underlying physiological reasons for such an improvement remain unknown. It appears to be possible due to less translocation of Pb to shoot since accumulation of heavy metals in the roots and less metal translocation to shoot are quite common (Zhao et al. 2009).

Under our experimental conditions, exogenous 2.5 mg L^{-1} ABA treatment led to an increase in shoot dry weight and the number of lateral roots of the nonstressed seedlings. Root dry weight did not change. In addition, *R*/*S* and root length decreased significantly after ABA treatment. This result suggests that the increase in lateral root number mainly contributed to root growth and that 2.5 mg L^{-1} ABA mediated root maintenance and shoot growth accumulation. Five milligram per liter

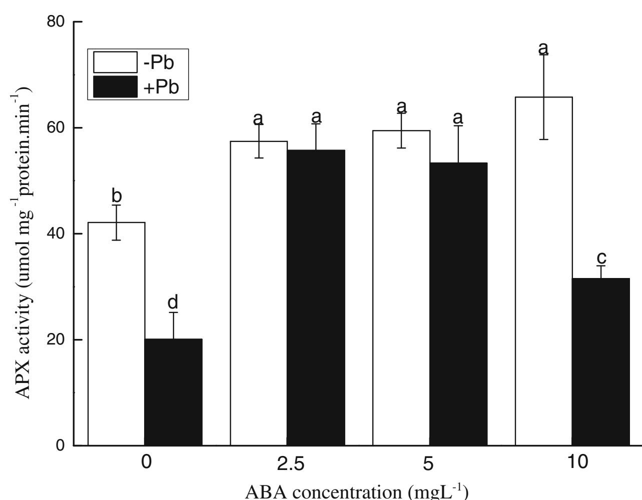

Fig. 7 Effects of different concentrations of ABA on APX activity of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

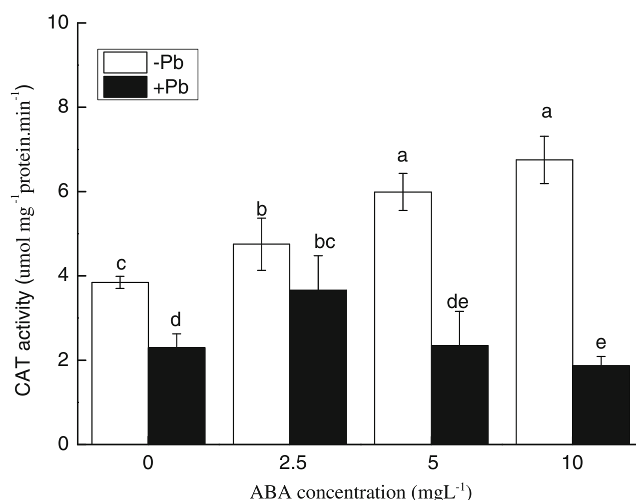

Fig. 9 Effects of different concentrations of ABA on CAT activity of *A. macrocephala* under Pb stress. *Bars* represent SD of means. *Different letters* indicate values that differ significantly from the control at $P<0.05$

ABA inhibited shoot growth and had no effect on root growth with an increase of lateral root number in the absence of Pb, whereas 10 mg L^{-1} ABA treatment promoted root and inhibited shoot growth. Previous studies have shown that exogenous ABA usually inhibits shoot growth, but maintains or even promotes root growth when applied to nonstressed rice (Chen et al. 2006) and *Cynanchum komarovii* (Yang et al. 2007) seedlings, but a few studies report that exogenous ABA can increase shoot growth. The stimulatory effects of ABA on shoot growth might be the result of a combination of improved cell extensibility and stimulated cell division attributable to arrest at the G1 phase of the cell cycle since cell development in the G1 phase is closely linked to shoot growth (Finkelstein et al. 2002). The inhibited root length may be because ABA produces more ethylene (Chen et al. 2003). Exogenous ABA increased the formation of lateral roots. This may be due to the fact that ABA may transmit a Ca^{2+} signal to cells in the epidermis and vascular cylinder either directly or indirectly, to alter cell fates (Chen et al. 2006).

Under lead stress, 2.5 mg L^{-1} ABA significantly increased root dry mass, *R*/*S*, and the number of lateral roots compared with Pb treatments alone, indicating that 2.5 mg L^{-1} ABA application partially counteracted the inhibitory effects of Pb on plant growth (Table 1). The increase in the number of lateral roots may reduce Pb uptake, thereby resulting in less oxidative stress (Strubinskai and Hanaka 2011). However, the toxic effects of Pb were further increased by the applications of 5 and 10 mg L^{-1} ABA (Table 1). The increased toxic effects were due to increases in oxidative damage induced by a higher concentration of ABA with Pb, as exemplified by enhancement of H_2O_2 and MDA concentrations in *A. macrocephala* (Figs. 4 and 5). At the same time, 2.5 mg L^{-1} ABA resulted in less Pb contents in the shoot and root of Pb-treated seedlings. In contrast, after pretreatment with 5 and 10 mg L^{-1} ABA, the Pb contents were increased in the shoot and root. The differences in Pb uptake are a consequence of the morphological differentiation of roots, change in plasma membrane composition, and modification of its permeability (Siedlecka et al. 2001; Strubinskai and Hanaka 2011). Thus, we provide evidence that a low concentration of ABA is able to alleviate Pb-induced toxicity in the leaves of *A. macrocephala* while high concentrations of ABA improve Pb-induced toxicity.

Pb is known to induce oxidative damage to higher plants (Qureshi et al. 2007). In this study, the enhancement of H_2O_2 and MDA concentrations in *A. macrocephala* showed that the oxidative stress and the peroxidation of membrane lipids were induced by Pb treatment (Figs. 4 and 5). Accumulation of H_2O_2 in plants under stress conditions may be a consequence of disturbing the balance between H_2O_2 and scavenging (Zhang et al. 2009). Activities of the major antioxidant enzymes (SOD, POD, CAT, and APX) in the leaves of *A. macrocephala* were reduced by Pb stress. These decreased antioxidative enzymes were probably due to the harmful effects of overproduction of H_2O_2 or its poisonous AOS derivates, indicating that these antioxidant enzyme activities under Pb stress were insufficient to cope with an increased concentration of H_2O_2, and the plant presented a negative endogenous protective effect. This was demonstrated in previous studies (Zhao et al. 2009; Strubinskai and Hanaka 2011). The reduction in antioxidant enzyme activities under stressful conditions has been attributed to the inactivation of enzyme protein due to AOS (Verma and Dubey 2003), decrease in enzyme synthesis, or change in assembly of enzyme subunits (Hertwig et al. 1992; Verma and Dubey 2003).

ABA is a signal molecule and regulates many aspects of plant development and growth (Chen et al. 2006). In most cases, the ABA-mediated responses in plants are associated with generation of H_2O_2 (Jiang and Zhang 2002; Zhao et al. 2009). For example, treatments of rice with exogenous ABA induced H_2O_2 accumulation (Zhao et al. 2009). Recent studies have shown that H_2O_2 at moderate levels may act as a second messenger for stress signaling and lead to activation of the antioxidative defense system (Jiang and Zhang 2002; Zhao et al. 2009). According to Gong et al. (1998) and Jiang and Zhang (2002), exogenous ABA can result in an enhancement of H_2O_2 and enhance the activities of antioxidative enzymes such as SOD, CAT, POD, and APX (Gong et al. 1998; Agarwal et al. 2005; Jiang and Zhang 2002). These suggest that ABA can cause an oxidative stress in plants. However, our results have shown that ABA enhanced the activities of antioxidative enzymes which caused a decrease in oxidative stress (Figs. 6, 7, 8, and 9). One possible model is that the generation of ABA-induced H_2O_2 may trigger the response of the whole antioxidative defense systems against oxidative stress. Such an enhancement in the antioxidant defenses is able to scavenge this increased H_2O_2, and the oxidative damage expressed as lipid peroxidation (MDA) was reduced. The positive effect of ABA was further manifested by an increase in plant growth. Similarly, a lower MDA content accompanied by increasing of SOD activity was found in sunflower and lupine roots under lead stress (Rucińska and Gwóźdź 2005; Strubinskai and Hanaka 2011).

ABA has been reported to induce tolerance to different abiotic stresses including drought, heat, and chilling (Li et al. 2004; Zhou et al. 2005; Gong et al. 2005). However, a few studies have shown that ABA caused a more detrimental effect on plant under heavy metal stresses. In the present study, compared with lead stress, low concentrations of ABA increased the activities of all the four enzymes under Pb stress. Thus, it is possible that low concentrations of exogenous ABA reduce H_2O_2 and MDA content to the normal level and improve plant growth. A similar role of

ABA in mediating antioxidant defense system has been confirmed in the chilling-stressed *Stylosanthes guianensis* (Zhou et al. 2006), heat-stressed wheat (Gong et al. 1998), and drought-stressed maize (Jiang and Zhang 2002). Therefore, ABA-enhanced antioxidant defense capacity appears to be a universal mechanism for ABA-enhanced tolerance to various forms of abiotic stress in plants. In contrast, high concentration of ABA (10 mg L^{-1}) had no changes in SOD and POD activity, but declined the activity of CAT and increased APX activity under Pb stress. CAT is thought to contribute more strongly than APX to the elimination of hydrogen peroxide in some green alga (Yoshida et al. 2003). Moreover, the former activity was more strongly decreased by ABA plus Pb treatment compared with the latter. These results suggest that hydrogen peroxide produced by the dismutation of superoxide radical in *A. macrocephala* is mainly eliminated by CAT. Therefore, the combination of a high concentration of ABA and Pb may lead to a more abundant generation of H_2O_2, which induced a more severe oxidative stress. This oxidative stress cannot be controlled by the antioxidant defense systems since an enhancement in the capacity of antioxidant defense systems induced by ABA is limited (Jiang and Zhang 2001). This result indicates that alleviation of Pb stress in *A. macrocephala* by exogenous abscisic acid supply is dose dependent.

Our data indicated that a low concentration of ABA elevated the levels of soluble sugar and soluble protein under Pb stress (Figs. 2 and 3). A high level of soluble sugar is essential to maintain osmotic adjustment under drought stress (Yang et al. 2007). Since Pb stress induces secondary water stress and oxidative damages to cellular structure (Verma and Dubey 2003), the ability of ABA to synthesize those osmoprotectants may be involved in their ability to cope with Pb stress. The accumulation of soluble protein in plant cells is generally related to the synthesis of antioxidative enzymes and osmotic adjustment (Verma and Dubey 2003; Yang et al. 2007). Our results suggested that ABA modulated the accumulation of soluble sugar, soluble protein, and conferred *A. macrocephala* tolerance to Pb stress. Chlorosis is the common symptom of toxicity of heavy metals, including Pb (Joseph et al. 2002; Gajewska et al. 2006; Zhao et al. 2009). In the present study, the additions of ABA to Pb treatment have no effect (Fig. 1). This result indicated that total chlorophyll content might not contribute to *A. macrocephala* tolerance to Pb stress. Similar to our observation, Li et al. (2010) have reported that exogenous ABA did not alter total chlorophyll and chlorophyll *a* in green lettuce, but increased chlorophyll *b* and total carotenoid content.

In conclusion, ABA is able to regulate Pb-induced oxidative stress in the leaves of *A. macrocephala*. Moreover, the positive or negative effect of exogenous ABA on lead-treated plants was dose dependent. Low concentration ABA-mediated reduction of Pb-induced toxicity to the *A. macrocephala* leaves is also associated with the increase of antioxidative defense systems and increase in accumulation of soluble sugars, soluble protein, and chl. On the contrary, a higher concentration of ABA further increased oxidative damage, thereby decreasing the growth of Pb-stressed *A. macrocephala* plants.

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