BIOLOGIA PLANTARUM 53 (4): 728-732, 2009

BRIEF COMMUNICATION

Low concentration of exogenous abscisic acid increases lead tolerance in rice seedlings

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Abstract

The effects of exogenous abscisic acid (ABA) on lead tolerance in rice (*Oryza sativa* L.) seedlings were investigated. Pre-treatment with 0.1 g m^3 ABA for 2 d restricted amount of Pb translocated from roots to shoots, decreased malondialdehyde and H_2O_2 contents in leaves, and alleviated Pb-induced decrease in plant growth and leaf chlorophyll content. Further, ABA pre-treatment adjusted leaf antioxidative enzyme activities (increased ascorbate peroxidase and catalase activities while decreased superoxide dismutase activity) and so alleviated oxidative stress.

Additional key words: APX, CAT, MDA, *Oryza sativa* L., SOD, transpiration rate.

Lead is one of the most significant heavy metal environmental pollutants (Salt *et al.* 1998), although it is not an essential element, it is absorbed and accumulated in plants, and then channeled into the food chain (Wierzbicka *et al.* 2007). Not only disturbs the growth of plant and causes sharp decrease in crop productivity (Hall 2002), Pb accumulation in the human body damages the brain, kidney, the reproductive and nervous systems (Body *et al.* 1991, Rensing *et al.* 1998, Eun *et al.* 2000).

In the seedlings of rice, Pb affects activities of many enzymes, mineral nutrition, water balance, hormonal status and membrane structure and permeability (Sharma and Dubey 2005). Pb can induce oxidative stress due to enhanced production of reactive oxygen species (Chaitanya and Naithani 1994). Although some of the ROS may function as important signaling molecules altering gene expression and modulating activity of specific defense proteins, all ROS can be extremely harmful at high concentrations (Kunsch and Medford 1999, Zaninotto *et al.* 2006). A wide series of enzymes exist in plants that serve to remove ROS, such as peroxidase, superoxide dismutases, ascorbate peroxidase, catalase, *etc.* (Verma and Dubey 2003).

Abscisic acid is ubiquitous in higher plants and influences several physiological and developmental events of plant (Creelman 1989), including seed development, dormancy, germination, and plant stress responses (Sharp *et al.* 2000, Finkelstein *et al.* 1998, Xiong and Zhu 2003, Zhou and Guo 2009). Increased internal ABA content under stress induces changes of gene expression (Zeevaart and Creelman 1988, Xiong *et al.* 1999, Fediuc *et al.* 2005) and so increase stress tolerance. Accumulation of ABA and a significant decrease in cytokinin content also have been reported in the case of plants treated with toxic metals (Prasad 1995). Hsu and Kao (2003, 2004) proved that the increase of endogenous ABA content is closely related to Cd tolerance of rice seedlings. They suggested that ABA-induced decrease in transpiration rate reduced the translocation of Cd to the shoots (Hsu and Kao 2003). Here we have studied the effects of different concentrations of ABA on the Pb tolerance in rice seedlings with the aim to determine if low concentration of exogenous ABA can ameliorate Pb toxicity in rice seedlings.

Received 27 September 2007, *accepted* 14 May 2008.

Abbreviations: ABA - abscisic acid; APX - acorbate peroxidase; AsA - ascorbic acid; CAT - catalase; DTT - dithiothreitol; EDTA - ethylenediamine tetraacetic acid; GSH - glutathione; MDA - malondialdehyde; PBS - phosphate buffer solution; ROS - reactive oxygen species; SOD -superoxide dismutase.

Acknowledgements: The first two authors contributed equally to this paper, the research is supported by the Project of National Key Basic Research and Development (2007CB109305) and National Nature Science Foundation (No. 30671255) of China.

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Uniform rice (*Oryza sativa* L. cv. Nanjing 40) 28-d-old seedlings were grown in vessels with nutrient solution supplemented with 0, 0.1, 1 or 10 mg dm^{-3} ABA for 2 d. Then the seedlings were washed with distilled water carefully to remove the extra ABA and transferred to fresh nutrient solution containing 0.25 mM $Pb(NO_3)_2$ for 7 d. As a control, another 4 pots of ABA pre-treated seedlings were cultured in nutrient solution without Pb. For enzyme measurements, samples were frozen in liquid nitrogen and stored at -80 °C.

Chlorophyll content in leaves was determined according to Arnon *et al.* (1949). Briefly, 0.1 g leaves were homogenized with 5 cm^3 80 % acetone with some $CaCO_3$ (Geebelen *et al.* 2002), the homogenate was centrifuged at 3 000 *g* for 5 min and the supernatant was used for chlorophyll assay. The amounts of chlorophyll were determined spectrophotometrically (*UV-2550*, *Shimadzu*, Kyoto, Japan), by reading the absorbance at 663 nm and 645 nm. H_2O_2 content was determined according to the method of Jana and Choudhuri (1981). Briefly, 0.1 g leaves were ground into fine powder and homogenized with 6 cm^3 pre-chilled 50 M PBS buffer solution (pH 6.5), the homogenate was centrifuged at 6 000 *g* for 25 min, supernatant was collected and mixed with 1 cm^3 0.1 % titanium dichloride, then the mixture solution was centrifuged at 6 000 *g* for 15 min, the absorbance was assayed spectrophotometrically at 410 nm. MDA, as an indicator of lipid peroxidation, was extracted with 20 % (m/v) thiobarbituric acid and determined according to the method of Metwally (2003).

Rice seedlings were rinsed with 1 mM citrate for 30 min to remove surface Pb and then oven dried. The roots and shoots were ground into powder and then digested with mixture of HNO_3 and $HClO_4$. The Pb concentration was determined with a flame atomic absorption spectrometry.

For enzyme assay, samples were ground into fine powder and homogenized in chilled extraction buffer, the homogenate was centrifuged at 12 000 *g* for 20 min and the supernatant was used for the assays, all operations were carried out at 4 °C. SOD activity was determined at 560 nm according to the method of Cakmak and Horst (1991) with some modifications. CAT activity was determined by measuring the decrease in absorbance of H_2O_2 at 240 nm according to the method of Aebi (1984). APX was determined according to the method of Hossain and Asada (1984) by measuring the decrease in absorbance of the oxidized ascorbate at 290 nm.

All the experiments were repeated three times. In each treatment at least five replicates were used. The statistical analyses were conducted with *Excel* and *OriginPro* version *7.5G*.

In the present study, the effects of exogenous ABA pre-treatment on some physiological and enzymatic parameters symptomatic for oxidative stress of rice seedlings under Pb stress were assessed. Compared with the control group, all seedlings treated with 0.25 mM $Pb(NO_3)_2$ became chlorotic to some extent, and the growth of roots and shoots was inhibited. Under the same Pb concentration, the seedlings pre-treated with 0.1 mg dm^{-3} ABA grew better than the seedlings without ABA. However, with the increasing of the ABA concentrations, the amelioration of Pb toxicity in rice seedlings was not enhanced, in contrast, the growth of shoots and roots was inhibited by Pb even more seriously (data not presented).

Under 0.25 mM $Pb(NO_3)_2$, the chlorophyll (Chl) content in leaves decreased significantly compared with the control, and Chl contents of the seedlings pre-treated with 0.1 and 1.0 mg dm^{-3} ABA increased by 8.96 and 8.42 %, respectively, compared with the seedlings not pre-treated with ABA (Fig. 1*A*). However, when the concentration of ABA was raised to 10.0 mg dm^{-3}, Chl content did not significantly increased (only 0.4 %). In the seedlings pre-treated with different concentrations of ABA and then cultured without Pb for 7 d, Chl content also decreased with the increasing ABA concentration. An enhancement of chlorophyll degradation may occur in Pb treated plants due to increased chlorophyllase activity (Drazkiewicz 1994). Pb also inhibits chlorophyll synthesis due to decreased uptake of essential elements such as Mg and Fe (Burzynski 1987). Our results proved that low concentration of ABA ameliorates Pb toxicity to leaf chlorophyll content and plant growth.

Another phytotoxic effect of Pb on plants appears to be induction of oxidative stress. H_2O_2 and MDA contents in leaves were chosen as indicators of cellular redox status under Pb stress. The H_2O_2 content increased under Pb treatment also after ABA pre-treatment, but at the 0.1 mg dm^{-3} ABA decreased the H_2O_2 content by 9.52 % compared with 0 mg dm^{-3} ABA. However, in the seedlings cultured without Pb, the H_2O_2 contents increased with increasing ABA concentrations (Fig. 1*C*). We also observed that Pb application increased MDA content obviously and only pre-treatment with 0.1 mg dm^{-3} ABA, but not with higher ABA concentrations, decreased MDA content (Fig. 1*B*). Verma and Dubey (2003) proved increased lipid peroxidation in rice seedlings grown in sand culture under Pb stress. We obtained similar results in present study, and our results also show that 0.1 mg dm^{-3} ABA pre-treatment for 2 d alleviates oxidative damage under Pb stress.

The role of antioxidative enzymes such as SOD, APX and CAT play crucial roles in scavenging ROS in plants (Xu *et al.* 2007). Not only the pre-treatment with ABA but also the stress of 0.25 mM Pb increased SOD activity in the leaves of rice seedlings. However, SOD activity in the leaves of seedlings pre-treated with 0.1 mg dm^{-3} ABA was lower than with other concentrations of ABA under Pb stress (Fig. 1*D*). APX reduces H_2O_2 content in the ascorbate-glutathione cycle. APX activities increased significantly under Pb stress, and ABA at concentration 0.1 but not 1 and 10 mg dm^{-3} further increased APX activity (Fig. 1*E*). Our results accord with previous researches that under Pb stress SOD and APX activities increased (Lee *et al.* 1976, Lozano *et al.* 1996). CAT alleviates oxidative stress by converting H_2O_2 into water and oxygen (Mandhania *et al.* 2006). In the not pre-treated seedlings, CAT activity decreased significantly under the

Pb stress. Pre-treatment with 0.1 and 1.0 mg dm^{-3} ABA recovered CAT activity to the same levels compared with control group. When the ABA concentration was 10 mg dm^{-3}, CAT activity began to decrease (Fig 1*F*). Pre-treatment of ABA affected activities of all the three enzymes, low concentrations of ABA increased APX and CAT activities

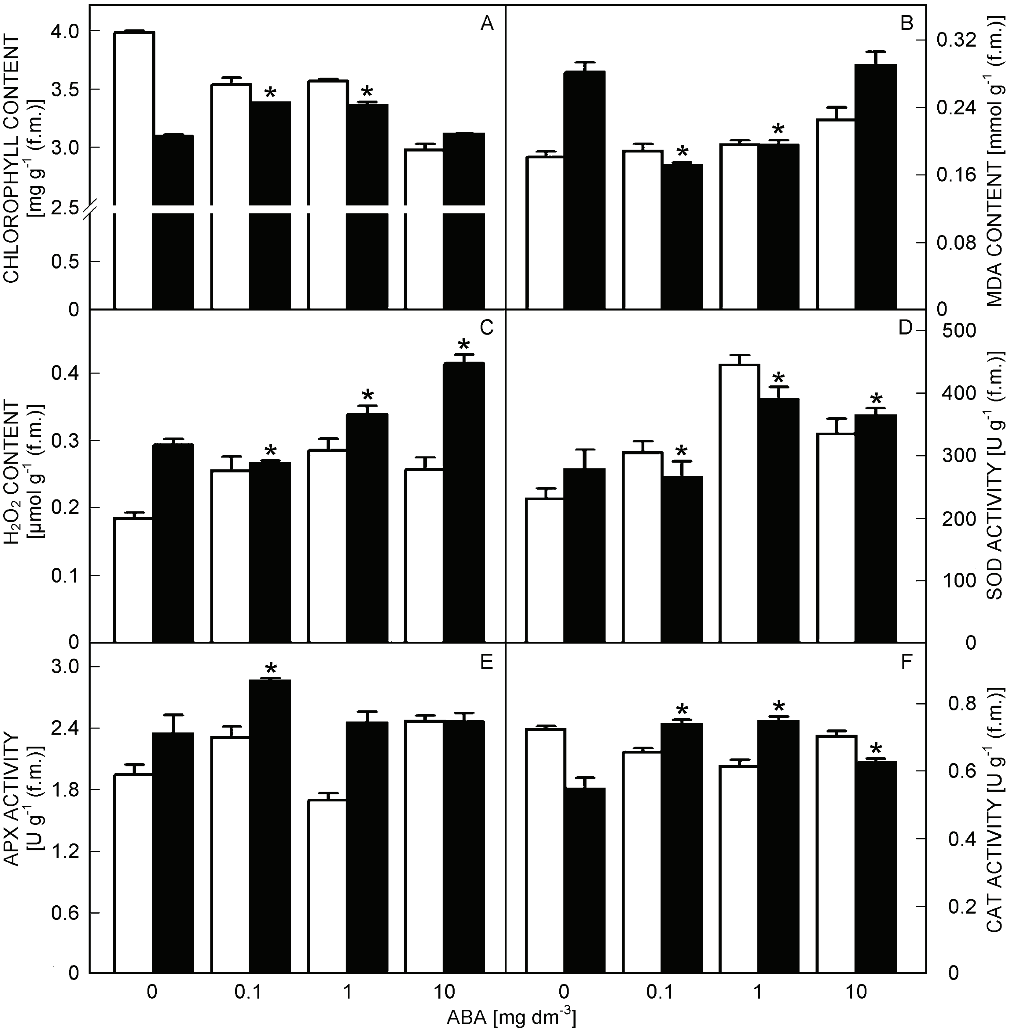

Fig. 1. Effects of 0.1, 1.0 and 10 mg dm^{-3} ABA pre-treatment on chlorophyll, H_2O_2, MDA contents and SOD, APX, CAT activities in leaves of rice seedlings under the absence (*empty bars*) and presence (*filled bars*) of 0.25 mM $Pb(NO_3)_2$ in nutrient solution. Means ± SE were calculated from at least 5 replicates, * - significantly different at 0.05 level compared with not pre-treated plants under Pb stress.

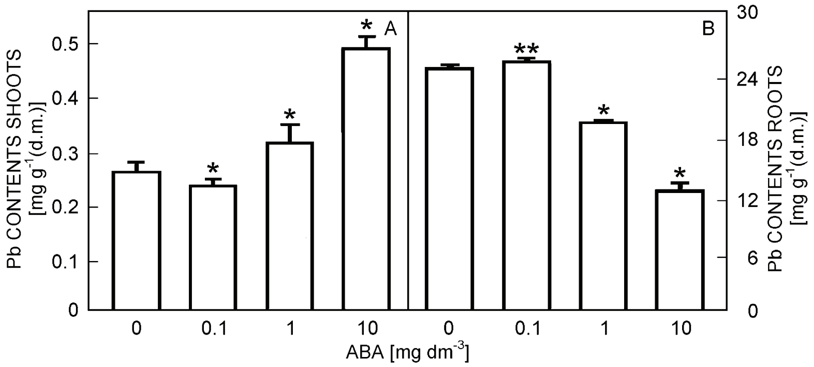

Fig. 2. Effects of 0.1, 1.0 and 10 mg dm^{-3} ABA pre-treatment on Pb contents in shoots and roots of rice seedlings under the presence of 0.25 mM $Pb(NO_3)_2$ in nutrient solution. Means ± SE were calculated from at least 5 replicates, * or ** - significantly different at 0.05 or 0.01 level compared with not pre-treated plants under Pb stress.

and decreased SOD activities under Pb stress. Thus it is possible that low concentrations of exogenous ABAreduce H_2O_2 content to the normal level. In contrast, high concentration of ABA is not able to reduce Pb toxicity in rice seedlings.

One potential mechanism of tolerance to heavy metals is avoiding the high heavy metal concentrations in the sensitive sites within the cell and thus preventing the damaging effects (Xiong *et al*. 2009). The metal contents are generally greater in roots than in the shoots (Ramos *et al*. 2002). In the not pre-treated seedlings, the Pb accumulated in the roots almost 100 folds more than in the shoots. In the seedlings pre-treated with 0.1 mg dm^{-3} ABA, Pb content increased by 2.84 % in the roots while decreased by 11.62 % in the shoots. This proved that 0.1 mg dm^{-3} ABA pre-treatment decreased the Pb stress in shoots which are more sensitive to Pb toxicity. In contrast, after pre-treatment with 1 and 10 mg dm^{-3} ABA, the Pb content increased in the shoots while decreased in the roots (Fig. 2). Heavy metal translocation to shoot has been suggested to be driven by transpiration (Hsu and Kao 2003, Salt *et al*. 1995), and exogenous ABA could reduce the transpiration rate of plants, so low concentration of ABA may slow down the transpiration rate and reduce the translocation of Pb to the shoots. As a crucial plant hormone, ABA not only reduces the transpiration rate but also affects a series of physiological and developmental events in plants. The elucidation of the reason why high concentration of ABA decreases the Pb content in roots but increases in shoots needs further experiments.

In conclusion, all of the results proved that 0.1 mg dm^{-3} ABA pre-treatment for 2 d alleviated Pb toxicity to rice seedlings, application of 0.1 mg dm^{-3} ABA not only decreased Pb translocation from roots to shoots but also adjusted antioxidative enzyme activities to alleviate oxidative stress. It was hoped that the study would provide basis for studying ABA functions in heavy metal resistance in crops and developing methods for reducing the risks of Pb toxicity to human beings.

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