BIOLOGIA PLANTARUM 52 (1): 80-86, 2008

Effect of Pb ions on superoxide dismutase and catalase activities in leaves of pea plants grown in high and low irradiance

E. ROMANOWSKA*, B. WRÓBLEWSKA, A. DROŻAK, M. ZIENKIEWICZ and M. SIEDLECKA

Department of Plant Physiology, Institute of Botany, University of Warsaw,
Miecznikowa 1, PL-02096 Warsaw, Poland

Abstract

The role of irradiance on the activity of antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT) was examined in the leaves of *Pisum sativum* L. plants grown under low (LL) or high (HL) irradiance (PPFD 50 or 600 µmol m^{-2} s^{-1}) and exposed after detachment to 5 mM Pb $(NO_3)_2$ for 24 h. The activities of both enzymes increased in response to LL compared with HL and no effect of Pb ions was observed. Photosystem (PS) 1 and PS 2 activities were also investigated in chloroplasts isolated from these leaves. LL lowered PS 1 electron transport rate and changes in photochemical activity of PS 1 induced by Pb^{2+} were visible only in the chloroplasts isolated from leaves of LL grown plants. PS 2 activity was influenced similarly by Pb ions at both PPFD. This study demonstrates that leaves of HL grown plants were less sensitive to lead toxicity than those from LL grown plants. Changes in electron transport rates were the main factors responsible for the generation of reactive oxygen species in the chloroplasts and as a consequence, in induction of antioxidant enzymes.

Additional key words: antioxidative enzymes, chloroplasts, lead, *Pisum sativum*, photosystem 1 and 2 activities.

Introduction

It is well established that photosynthetic apparatus responds to different irradiance quantity during growth by developing different forms (Anderson 1986). These photoadaptation events are accompanied by specific changes in composition, organization and function of the chloroplasts (Anderson *et al*. 1988). High irradiance (HL) grown plants possess fewer thylakoids per chloroplasts with a higher proportion of PS 1 pigment proteins and have higher level of electron carriers than chloroplasts of low irradiance (LL) grown plants (Lichtenthaler *et al*. 1982).

Plants acclimated HL use various mechanisms to protect the photosynthetic apparatus against the deleterious effects of excess light absorption. Oxygen free radicals (ROS) are produced in chloroplasts during irradiation (Grace and Logan 1996) and HL grown plants develop various antioxidants involved in their deactivation (Demmig-Adams and Adams 1992). Protection against ROS is provided by an integrate system of enzymatic (superoxide dismutase, catalase and peroxidase) and non-enzymatic (ascorbate, reduced glutathione, α-tocopherol and carotenoids) antioxidants located in the chloroplasts (Asada 1994, Alscher *et al.* 1997). Therefore, it is suggested that increased activities of these enzymes may be considered as circumstantial evidence for enhanced production of oxygen radicals (Fridovich 1986). When HL- or LL-exposed leaves experience other environmental stresses a pronounced and sustained increase in energy dissipation may be induced, which could largely account for the change in photochemical efficiency. It is unknown whether the growth irradiance can modify the response of plants to heavy metals by acting on the level/activity of antioxidant enzymes. It also remains to be determined whether a rate of electron transport in chloroplasts of HL or LL grown plants is involved in the changes in the antioxidant system.

Received 14 February 2006, *accepted* 23 September 2006.

Abbreviations: CAT - catalase, DBMIB - 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone, DCMU - 3-(3,4-dichlorophenyl)-1,1-dimethylurea, HL - high irradiance; LL - low irradiance; MV - methylviologen, NBT - nitroblue tetrazolium, OEC - oxygen evolving complex, PBQ - phenyl-*p*-benzoquinone, PPFD - photosynthetic photon flux density, PS 1 and PS 2 - photosystem 1 and 2, ROS - reactive oxygen species, SOD - superoxide dismutase , TEMED - tetramethylethylenediamine.

Acknowledgments: The work was supported by grant from the Polish Committee for Scientific Research. We would like to thank to Lida Hassani for technical help. We are grateful to Dr Joanna Kargul from Imperial College of London, Division of Molecular Biosciences for the English correction of the manuscript.

* Corresponding author; fax:(+48) 22 5543910, e-mail: romanela@biol.uw.edu.pl

It is well documented that lead has an inhibitory effect on the donor site of PS 2 (Becerill *et al.* 1988, Rashid and Popovic 1990, Rashid *et al.* 1994) and the degree of coupling in the electron transport chain decreases in response to the heavy metal treatment (Lucerno *et al.* 1976, Romanowska 2002). Mitochondria are known to be much more resistant to heavy metals than chloroplasts as they remain undisturbed even at high metal concentration (Ernst 1998, Romanowska *et al.* 2002). Moreover, the increase in dark respiration in response to mild metal stress is reported to occur in animal and plant mitochondria (Lamoreaux and Chaney 1978, Lee *et al.* 1976, Parys *et al.* 1998, Romanowska *et al.* 2002). Higher ATP synthesis in mitochondria may have protective effect against ROS by altering the synthesis of proteins involved in detoxifying active oxygen species. Because factors such as growth conditions may change the ways of heavy metals action we decided to study the impact of both, light and Pb ions on the activity of main antioxidant enzymes in respect to electron transport activity.

This study demonstrates that not only Pb toxicity increases with decreasing irradiance during growth but also activity of SOD and CAT is higher in LL grown plants.

Materials and methods

Plant material and growth conditions: Seeds of pea (*Pisum sativum* L. cv. Iłowiecki) were placed on moist filter paper in Petri dishes and kept at room temperature in darkness. Five days after germination the seedlings were transferred to the Knopp nutrient solution and kept in a growth chamber under a 14-h photoperiod and a day/night temperature of 25/20 ºC. Photosynthetic photon flux densities (PPFD) were 50 (LL) and 600 (HL) μmol m^{-2} s^{-1} supplied by halogen lamps (*LH-111*, Polam, Poland). The leaves of 3-week-old plants were used for experiments. Excised leaves of pea plants grown under HL or LL were detached immediately after the dark period and placed with their cut ends into a glass beaker containing either water (control) or 5 mM $Pb(NO_3)_2$. After 24 h of continuous exposure to weak irradiance (about 30 μmol m^{-2} s^{-1}) at room temperature and 80 % relative humidity the Pb concentration in pea leaves from HL and LL grown plants was similar [about 100 μg(Pb) mg^{-1}(Chl)] (Romanowska *et al.* 1998). These leaves were used for all measurements.

Determination of the PS 1 and PS 2 activities: Chloroplasts were isolated from the leaves according to the procedure of Romanowska and Albertsson (1994). Chlorophyll concentration was determined as described by Arnon (1949). Photosynthetic electron transport of isolated chloroplasts was measured using Clark oxygen electrode (*TriOximatic EO200*, *WTW*, Weilham, Germany). After 3 min adaptation of isolated chloroplasts in darkness, PS 2 and PS 1 activities were measured at 25 °C in 560 μmol m^{-2} s^{-1}. The reaction mixture of the total volume 2 cm^3 contained: 50 mM HEPES-KOH (pH 7.7), 100 mM sorbitol, 2 mM EDTA, 1mM KCl, 5 mM $MgCl_2$. PS 2 activity was measured as oxygen evolution with water as electron donor and 0.5 mM PBQ as an electron acceptor in the presence of 10 μM DBMIB as a PS 1 inhibitor. PS 1 activity was measured as oxygen uptake in a reaction using 0.1mM DCPIP reduced with 5 mM ascorbate as an electron donor and 0.1 mM MV as electron acceptor; 10 μM DCMU and 5 mM NaN_3 were used as PS 2 and catalase inhibitors, respectively. For electron transport measurements chloroplast suspension containing 30 μg chlorophyll was added to the reaction mixture.

SOD and CAT activities: Soluble proteins were extracted at 4 °C by grinding 1 g of pea leaves in 2.5 cm^3 of extraction buffer (0.1 M sodium phosphate buffer, pH 7.8) containing 3 mM EDTA, 3 mM $MgSO_4$, 1 mM DTT. The homogenate was centrifuged at 12 000 *g* for 10 min in 4 °C and supernatant was stored in separate aliquots at -80 °C to be used for CAT or SOD analyses. Protein was measured by the method of Bradford (1976).

Electrophoresis was carried out at 4 °C under native conditions in 10 or 6 % polyacrylamide mini-gels for SOD, (EC 1.15.1.1) and CAT (EC 1.11.1.6), respectively. The gels were than stained for SOD as described by Beauchamp and Fridovich (1971) and modified by Azevedo *et al.* (1998). The treatment of the gels in H_2O_2 and/or KCN allowed the classification of the SOD into isoenzymes. CAT activity was also assayed in the nondenaturating gels incubated for 10 min in darkness in the assay mixture like for SOD with 3 mM H_2O_2 and exposed to light for 10 min at room temperature (until colourless bands were visible). The concentration of proteins loaded onto the gel was 80 μg for SOD and 60 μg for CAT. Quantification of SOD and CAT bands was done by densitometric scanning of the gels. *Quantity One* software (*Bio-Rad,* USA) was used for semi-quantitative analysis of protein bands on stained gels.

Total SOD activity was assayed in the supernatant spectrophotometrically according to the method of Giannopolitis and Ries (1977). One unit SOD activity was defined as the amount of enzyme required for 50 % inhibition of the rate of nitroblue tetrazolium (NBT) reduction measured at 560 nm.

Results

Photosystem 1 and 2 activities: PS 1 activity in chloroplasts isolated from HL grown plants was about 20 % higher than in LL grown plants (Table 1). Furthermore, Pb ions had no effect on electron transport through PS 1 in the chloroplasts from HL grown plants, however in LL grown plants Pb^{2+} exposure resulted in a 34 % reduction of PS 1 activity. As results indicated, PS 2 activity of the chloroplasts from the leaves of plants grown under LL was about 40 % lower compared with HL treatment. Pb ions decreased PS 2 activity progressively during the Pb treatment and after 24 h it was inhibited in HL and LL up to 57 and 29 %, respectively. PS 2 activity after 6 h of a Pb treatment in chloroplasts from HL grown plants was the same as in control chloroplasts, but in LL Pb caused 25 % inhibition. 24 h after detachment PS 2 activity of the control chloroplasts from the leaves of plants grown under HL and LL was similar and Pb treatment caused about 30 % inhibition in both types of chloroplasts. It is the evidence that the heavy metal interfered with the functioning of PS 2 in LL grown plants to a much greater extent than that in HL grown plants.

Table 1. PS 1 (Asc/DCPIP → MV, DCMU) and PS 2 (H_2O → PBQ) activities [$mol(O_2)\ kg^{-1}(Chl)\ h^{-1}$] of chloroplasts isolated from the leaves of plants grown under HL or LL, control (C) or treated with $Pb(NO_3)_2$ (Pb) for 6 - 24 h. Values are the means of 4 - 5 separate experiments ± SE (3 replicates each).

Time [h]	PS 1				PS 2			
	HL		LL		HL		LL	
	C	Pb	C	Pb	C	Pb	C	Pb
6	110 ± 1	109 ± 1	87 ± 6	87 ± 6	78 ± 3	78 ± 4	60 ± 5	45 ± 8
14	107 ± 3	101 ± 5	88 ± 1	85 ± 5	74 ± 5	62 ± 5	45 ± 8	40 ± 11
24	103 ± 3	99 ± 5	81 ± 9	58 ± 3	71 ± 4	34 ± 5	50 ± 10	32 ± 8

Antioxidant enzymes: We examined the activity of antioxidant enzymes CAT and SOD in detached pea leaves treated with Pb ions in response to growth irradiance. When protein extracts from the leaves of HL and LL grown plants were separated by native electrophoresis, three clear SOD isozymes of varying amounts were observed for the control and Pb treated leaves (Fig. 1). Enzyme activity was estimated as a function of time of a Pb^{2+} treatment and we observed that lead had small effect on the activity of SOD isoenzymes. In general, activities of all SOD isoenzymes in the leaves from HL grown plants were lower (about 2-fold) than in the leaves of LL grown plants and none of the SOD isoenzymes showed major alteration in activity after Pb treatment. In addition, we found that control and Pb treated leaves of LL and HL grown plants contained cytosolic and chloroplast Cu/Zn SOD (designated as SOD 2 and SOD 1, respectively) at varying relative activities and they were higher than that of the mitochondrial form Mn SOD (SOD 3). The SOD 3 activity was much lower than SOD 2 and SOD 1 from LL grown plants and SOD 1 from HL grown plants for both, control and Pb treated leaves. LL increases the activity of both SOD 2 and SOD 1 as compared to HL. A respective densitometer scan revealed an increase in SOD 2 activity in control and Pb treated leaves twice and three times, respectively. SOD 1 activity was stimulated in LL similarly for control and Pb treated leaves, more than twice. Additionally Pb ions decreased slightly SOD 3 activity in the leaves of HL and LL grown plants, by about 15 and 5 %, respectively. The SOD 1 activity in the chloroplasts of HL grown plants was about 2-fold higher than SOD 2 in cytosol. In LL, SOD 1 activity was stimulated by about 40 % as compared to SOD 2.

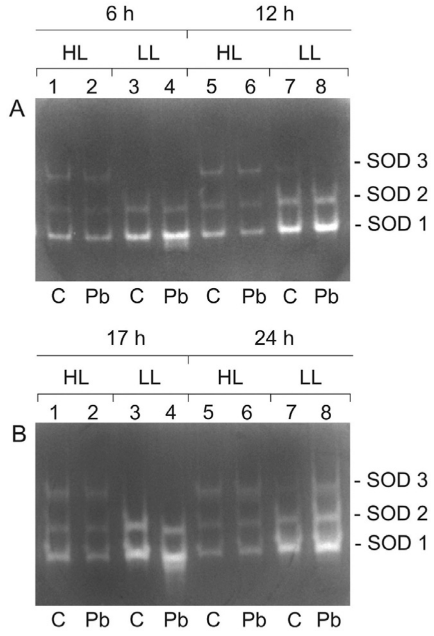

Fig. 1. SOD activity of pea leaves from plants grown at HL and LL for 6, 12 (*A*), 17 and 24 h (*B*), either control (C) or treated with 5 mM $Pb(NO_3)_2$ (Pb). Samples (70 µg of protein per well) were subjected to native-PAGE (10 % polyacrylamide gels) and SOD isoenzymes were localized in the gels as described in Materials and methods. SOD 3 was classified as mitochondrial Mn SOD, whereas the SOD 2 (cytosolic) and SOD 1 (chloroplast) were classified as Cu/Zn SOD, respectively.

Total SOD activity (Fig. 2) was higher in LL than in HL grown plants. Pb ions increased SODs activity as compared to control plants, higher in LL grown plants. The slight increase in the SOD activity was time-dependent only in Pb-treated LL grown plants.

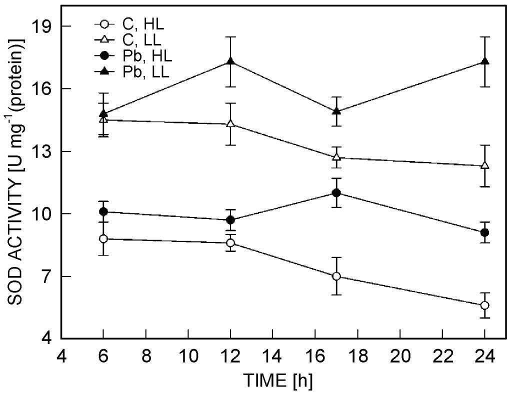

Fig. 2. Total SOD activity in the pea leaves of LL or HL grown plants and treated for 24 h with $Pb(NO_3)_2$ (Pb) or water (C). Means ± SE, *n* = 7.

The plant extracts used for SOD activity staining were also used for detection of CAT activity following PAGE (Fig. 3). Enzyme activity was estimated as a function of time of the Pb^{2+} treatment (2 - 24 h) after exposure of leaves detached from the HL or LL grown plants to 5 mM Pb $(NO_3)_2$. Only one widespread band of CAT activity was present following staining of leaf extracts. CAT activity in the leaves of HL and LL grown plants exhibited a similar pattern up to 12 h of Pb treatment and after that was an increase in activity up to 24 h in LL, as compared to HL. CAT activity showed a significant increase (by about 50 %) in the leaves of LL grown plants following 24 h treatment with Pb ions.

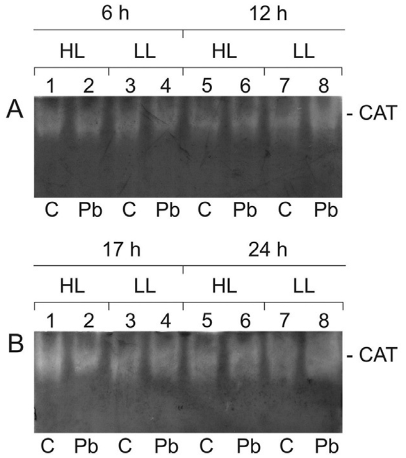

Fig. 3. Effect of $Pb(NO_3)_2$ on the catalase activity (CAT) of leaves from plants grown under HL or LL for 6, 12 (*A*), 17 and 24 h (*B*), either control (C) and treated with Pb ions. Proteins (60 µg of protein per well) were separated by native-PAGE (6 % polyacrylamide gels) and stained for catalase activity as described in Materials and methods.

Discussion

The effect of Pb ions on plants grown under HL or LL on SOD and CAT activities were investigated. The question is raised whether the affection of photochemical activity of photosystems by Pb ions can change oxidative stress and plant response to it according to irradiance during growth. Our results demonstrate that pea plants grown in the HL and LL differ significantly with respect to the tolerance to Pb ions suggesting a role of growth irradiance in the induction of the antioxidative system.

PS 1 and PS 2 activities were higher in HL than in LL grown plants (Table 1). These results are in agreement with other studies where electron transport rates also increased when the irradiance during growth was higher (Leong and Andersson 1984). Pb ions inhibited PS 2 activity in the chloroplasts isolated from the leaves of HL and LL grown plants with time of treatment, and after 24 h, the activity was the same in both types of chloroplasts. The effect of Pb^{2+} on PS activity is in agreement with the observations reported by Miles *et al.* (1972) and De Filippis *et al.* (1981). Interaction of irradiance and Pb ions in relation to oxidative stress has not been widely studied.

The main result of the present study is the stimulation of Cu/Zn SOD activity under LL. This result is somewhat unexpected. It is commonly accepted that exposure of plants to HL induces an increase in cellular antioxidant systems due to higher rates of O_2 photoreduction (Grace and Logan 1996, Perl-Treves and Galun 1991). Short-term exposure (1 h) of pea plants grown in moderate irradiance (200 µmol m^{-2} s^{-1}) to extremely high irradiance (4 000 µmol m^{-2} s^{-1}) caused an increase in ROS levels and accumulation of mRNAs encoding antioxidant enzymes (Hernández *at al.* 2006) differently in two pea cultivars. This result also confirms the earliest reports on the changes of antioxidant enzymes in response to HL stress.

To determine whether Pb-treatment was effective in creating conditions of oxidative stress with a limited CO_2 exchange, the activities of SOD and CAT were estimated. Three SOD isoenzymes bands were observed following the staining of native PAGE gels (Fig. 1). Studies with KCN and H_2O_2 indicate that the SOD 3 is Mn SOD, while SOD 2 and SOD 1 are classified as Cu/Zn SODs. Accumulation of SODs in the leaves was strongly regulated by irradiance during growth and under LL Cu/Zn SOD isoenzymes were more active than under HL.

Since Pb affected mostly chloroplasts (Romanowska *et al.* 2002, 2005) we expected that in the leaves from LL grown plants, the SOD 1 associated with these organelles may respond to Pb treatment. Surprisingly, we found that the chloroplast isoform was significantly increased in response to LL, but after Pb treatment no significant changes were noticed.

We observed previously (Romanowska *et al.* 2005, 2006) that Pb ions stimulated respiration rate in pea leaves only in HL grown plants, which was accompanied by the increase in ATP production in mitochondria. It now seems that Pb ions in LL conditions are not responsible for induction of the antioxidant defence system in mitochondria (Fig. 1), as we observed no stimulation of the Mn SOD activity. Different effects of Pb^{2+} or other heavy metals on SODs activity in plants were found, although the majority results demonstrated a decrease (Sandalio *et al.* 2001) or no change (Kampfenkel *et al.* 1995).

In present study, we show that LL during growth induced chloroplast and cytosolic SODs suggesting that electron flow to alternative acceptors such as O_2 may be occurring. Because the inhibition of photosynthesis by Pb ions was higher in LL than in HL (Romanowska *et al.* 2006), the dark reaction can slow down electron transport to $NADPH^+$ and as consequence, the electrons are absorbed by oxygen and production of ROS increases. It is known that the stomata opening is increased with irradiance. At LL opening of stomata is limited and there is a high probability that ROS will be generated (Aphalo and Jarvis 1993, Eckstein *et al.* 1996). Hence, irradiance during growth is responsible for changes in the antioxidant enzymes activities by affecting the rate of photosynthesis and related processes.

Because Pb ions and HL increased respiration rate (Romanowska *et al.* 2006) and did not cause changes in Mn SOD, as also observed by Kampfenkel *et al.* (1995) with iron ions, a simple correlation between the SOD content in mitochondria and stimulated respiration by metal stress does not seem to occur. In contrast to our observations, Bowler *et al.* (1989) showed that induction of Mn SOD was always accompanied by an increase in cytochrome oxidase activity, which suggests a specific protective role of Mn SOD when mitochondrial respiration is increased. These discrepancies are possibly partly due to differences in experimental design, especially that irradiances during growth of investigated plants were different. As the CO_2 uptake was higher in Pb treated leaves from HL compared to LL grown plants (Romanowska *et al.* 2006) and the photochemical efficiency of PS 2 (Table 1) was similar (after 24 h), we suggest that under LL conditions, chloroplast and cytosolic SOD isoenzymes have similar activity, thus oxygen evolved might also be reduced in the cytosolic compartment. Perl-Treves and Galun (1991) found that in the tomato seedlings very low irradiance was sufficient for induction of elevated SOD transcription and in mature plants exposed to strong irradiance this effect was not observed. This suggests that light could regulate not only SOD genes but also have influence on the enzyme proteins.

Previous studies have shown that heavy metals decrease CAT activity (Rucińska *et al.* 1999) although some reports demonstrate that this activity increases (Kampfenkel *et al.* 1995, Pereira *et al.* 2002) or is not altered significantly (Weckx and Clijsters 1997) by metal treatment. In the present study, growth in HL was not associated with an increase in the total CAT activity (Fig. 3). Earlier, Grace and Logan (1996) showed catalase inactivation in the leaves exposed to high irradiance. This may indicate, that in HL grown plants, enzymatic removal of H_2O_2 by the ascorbate peroxidase is the dominant pathway in the leaves. It is clearly seen in Fig. 3 that CAT activity was not influenced by irradiance during growth. Pb ions induced increase in the activity of CAT only in the LL grown plants after 12-h treatment. Increased activity of SOD isoforms in LL grown plants may enhance the CAT activity which is involved in hydrogen peroxide scavenging. Parallel increase in SOD and CAT activities in the leaves of LL grown plants, means that LL grown plants may accumulate less O^{2-} and H_2O_2 since there was the higher activity of both antioxidant enzymes. We observed lower level of H_2O_2 in LL as compared to HL grown plants (data not presented). If so, high level of antioxidant enzymes in LL can provide better protection of plants from the additional stress factor such as Pb ions.

These results suggest that irradiance during growth, not Pb ions, is a main factor for the induction of a LL-dependent accumulation of antioxidant enzymes in the leaves. Foyer and Harbinson (1994) have shown that under high irradiance coupled with limited capacity for electron transport a reversible down-regulation of PS 2 activity occurs. This is accompanied by an increase in O_2^- production and in the activity of enzymes that remove free radicals. Low irradiance and Pb ions act on electron transport through lowering the PS 2 activity accompanied by the stimulation of chloroplasts Cu/Zn SOD. Higher inhibition of PS 2 activity by Pb ions in the chloroplasts from the leaves of LL grown plants compared to HL is probably also parallel to the oxidative stress. However, further analysis of the antioxidative system is required. Our results may suggest that, although Pb may generate an oxidative stress, there is not a sufficient increase in superoxide to induce a change in SOD activity (Fig. 2). It seems possible that the oxidative stress induced by Pb ions is a secondary response different from the long time light-induced response. A direct evidence indicating for a strong connection between metal stress and oxidative stress is required such as induction of the SOD genes by Al treatments (Richards *et al.* 1998). It is likely that high irradiance induced fast activation or accumulation of quencher molecules is species and/or growth conditions-dependent. Previous studies have demonstrated (Sen Gupta *et al.* 1993) that in transgenic tobacco plants over-expressed chloroplast Cu/Zn SOD increase resistance to high irradiance and that in tomato chloroplasts Cu/Zn SOD is regulated in response to irradiance (Kardish *et al.* 1994). Therefore, our data indicated that irradiance during growth might largely modify the effect of Pb ions on SOD and CAT activities.

Additional experiments are required to elucidate whether long-term effects of high irradiance during growth can optimise the adaptation of plants to heavy metals.

References

Alscher R.G., Donahue J.L., Cramer C.L.: Reactive oxygen species and antioxidants: Relationships in green cells. Physiol. Plant. **100**: 224-233, 1997

Anderson, J.M.: Photoregulation of the composition, function, and structure of thylakoid membranes. - Annu. Rev. Plant Physiol. **37**: 93-136, 1986.

Anderson, J.M., Chow, W.S., Goodchild, D.J.: Thylakoid membrane organization in sun/shade acclimation. - Aust. J. Plant Physiol. **15**: 11-26, 1988.

Aphalo, P.J., Jarvis, P.G.: Separation of direct and indirect responses of stomata to light: results from leaf inversion experiment at constant intercellular CO_2 molar fraction. - J. exp. Bot. **44**: 791-800, 1993.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Asada, K.: Production and Action of Active Oxygen in Photosynthetic Tissue. - CRC Press, Boca Raton 1994.

Azevedo, R.A., Alas, R.M., Smith, R.J., Lea, P.J.: Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation in the leaves and roots of wild type and a catalase-deficient mutant of barley. - Physiol. Plant. **104**: 280-292, 1998.

Beauchamp, C., Fridovich, I.: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. - Anal. Biochem. **44**: 276-287, 1971.

Becerril, J.M., Gonzalez-Murua, C., Munoz-Rueda, A., De Felipe, M.R.: Changes induced by cadmium and lead in gas exchange and water relation of clover and lucerne. - Plant Physiol. Biochem. **27**: 913-918, 1988.

Bowler, C., Alliotte, T., De Loose, M., Van Montagu, M., Inzé, D.: The induction of manganese superoxide dismutase in response to stress in *Nicotiana plumbaginifolia*. - EMBO J. **8**: 31-38, 1989.

Bradford, M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Chugh, L.K., Sawhney, S.K.: Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. - Plant Physiol. Biochem. **37**: 297-303, 1999.

Cooke, D.T., Burden, R.S.: Lipid modulation of plasma membrane bound ATPases. - Physiol. Plant. **78**: 153-159, 1990.

De Filippis, L.F., Hampp, R., Zeigler, H.: The effect of zinc, cadmium and mercury on *Euglena*. II. Respiration, photosynthesis and photochemical activity. - Arch. Microbiol. **128**: 407-411, 1981.

Demmig-Adams, B. Adams, W.W. Photoprotection and other responses of plants to high- light stress. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Eckstein, J., Beyschlag, W., Mott, K.A., Ryel, R.J.: Changes in photon flux can induce stomata patchiness. - Plant Cell Environ. **19**: 1066-1074, 1996.

Ernst, W.H.O.: Effects of heavy metals in plants at the cellular and organismic level. - In: Schuurmann, G., Markert, B. (ed.): Ecotoxicology. Pp. 587-619. J Wiley & Sons, New York and Spektrum Akademischer Verlag, Heidelberg 1998.

Foyer, C.H., Harbinson, J.: Oxygen metabolism and the regulation of photosynthetic electron transport. - In: Foyer, C.H., Quick, W.P. (ed.): Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. Pp. 1-42. CRC Press, Boca Raton 1994.

Fridovich, I.: Biological effects of the superoxide radical. - Arch. Biochem. Biophys. **247**: 1-11, 1986.

Gardeström, P., Wigge, B.: Influence of photorespiration on ATP/ADP ratios in the chloroplasts, mitochondria and cytosol, studied by rapid fractionation of barley (*Hordeum vulgare*) protoplast. - Plant Physiol. **88**: 69-76, 1988.

Genty, B., Harbinson, J.: Regulation of light utilization for photosynthetic electron transport. - In: Baker, N.R. (ed.): Photosynthesis and the Environment. Pp. 69-99. Kluwer Academic Publishers, Amsterdam 1996.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutase. I. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Giardi, M.T., Masojídek, J., Godde, D.: Discussion on the stresses affecting the turnover of the D1 reaction center II protein. - Physiol. Plant. **101**: 635-642, 1997.

Gillham, D.J., Dodge, A.D.: Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: seasonal variations. - Plant Sci. **50**: 105-109, 1987.

Grace, S.C., Logan, B.A.: Acclimation of foliar antioxidant system to growth irradiance in three broad-leaved evergreen species. - Plant Physiol. **112**: 1631-1640, 1996.

Hernández, J.A., Escobar, C., Creissen, G., Mullineaux, P.M.: Antioxidant enzyme induction in pea plants under high irradiance. - Biol. Plant. **50**: 395-399, 2006.

Kampfenkel, K., Van Montagu, M., Inzé, D.: Effects of iron excess on *Nicotiana plumbaginifolia* plants. - Plant Physiol. **107**: 725-735, 1995.

Kardish, N., Magal, N., Aviv, D., Galun, E.: The tomato gene for the chloroplast Cu, Zn superoxide dismutase regulation of expression imposed in transgenic tobacco plants by a short promoter. - Plant mol. Biol. **25**: 887-897, 1994.

Lamoreaux, R.J., Chaney, W.R.: The effect of cadmium on net photosynthesis, transpiration and dark respiration of excised silver maple leaves. - Physiol. Plant. **43**: 231-236, 1978.

Lee, K.C., Cunningham, B.A., Poulsen, G.M., Liang, G.M., Moore, R.B.: Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. - Physiol. Plant. **36**: 4-6, 1976.

Leong, T.-Y., Anderson, J.M.: Adaptation of the thylakoid membranes of chloroplasts to light intensities. II. Regulation of electron transport capacities, electron carriers, coupling factor (CF1) activity and rates of photosynthesis. - Photosynth. Res. **5**: 117-128, 1984.

Lichtenthaler, H.K., Burstahler, R., Buschmann, C., Meier, D., Prenzel, U., Schonthal, A.: Effect of high light and high light stress on composition, function and structure of the photosynthetic apparatus. - In: Marcell, R., Clijsters, H., Van Poucke, M. (ed.): Effects of Stress on Photosynyhesis. Pp. 353-370. Martinus Nijhoff/Dr W. Junk Publ, The Hague - Boston - London 1982.

Lucerno, H.A., Carlos, S.A., Vallejos, R.H.: Sulphydryl groups in photosynthetic energy conservation III. Inhibition of photophosphorylation in spinach chloroplasts by $CdCl_2$. - Plant Sci. Lett. **6**: 309-313, 1976.

Miles, C.D., Brandle, J.R., Daniel, D.J., Chu-Der, O., Schnare, P.D., Uhlik, D.J.: Inhibition of photosystem II in isolated chloroplasts by lead. - Plant. Physiol. **49**: 820–825, 1972.

Parys, E., Romanowska, E., Siedlecka, M., Poskuta, J.: The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of *Pisum sativum*. - Acta Physiol. Plant. **20**: 313-322, 1998.

Pereira, G.J.G., Molina, S.M.G., Lea, P.J., Azevedo, R.A., Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. - Plant Soil **239**: 123-132, 2002.

Perl-Treves, R., Galun, E.: The tomato Cu, Zn superoxide dismutase genes and developmentally regulated and respond to light and stress. - Plant mol. Biol. **17**: 745-760, 1991.

Rashid, A., Popovic, R.: Protective role of $CaCl_2$ against Pb^{2+} inhibition in photosystem II. - FEBS Lett. **271**: 181-184, 1990.

Rashid, A., Camm, E.L., Ekramoddoullah, K.M.: Molecular mechanism of action of Pb^{2+} and Zn^{2+} on water oxidizing complex of photosystem II. - FEBS Lett **350**: 296-298, 1994.

Richards, K.D., Schott, E.J., Sharma, Y.K., Davis, K.R., Gardner, R.C.: Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. - Plant Physiol. **116**: 409-418, 1998.

Romanowska, E.: Gas exchange functions in heavy metal stressed plants. – In: Prasad, M.N.V. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 257-285. Kluwer Academic Publishers, Dordrecht 2002.

Romanowska, E., Albertsson, P.-A.: Isolation and characterization of the cytochrome *b*/*f* complex from whole thylakoids, grana and stroma lamellae vesicles from spinach chloroplasts. - Plant Cell Physiol. **35**: 557-568, 1994.

Romanowska, E., Igamberdiev, A., Parys, E., Gardeström, P.: Stimulation of respiration by Pb^{2+} ions in detached leaves and mitochondria of C_3 and C_4 plants. - Physiol. Plant. **116**: 148-154, 2002.

Romanowska, E., Parys, E., Słowik, D., Siedlecka, M., Piotrowski, T., Poskuta, J.: The toxicity of lead to photosynthesis and respiration in detached pea and maize leaves. - In Garab, G. (ed.): Photosynthesis: Mechanisms and Effects. Pp. 2665-2668. Kluwer Academic Publishers, Dordrecht 1998.

Romanowska, E., Pokorska, B., Siedlecka, M.: The effects of oligomycin on content of adenylates in mesophyll protoplasts, chloroplasts and mitochondria from Pb^{2+} treated pea or barley leaves. - Acta Physiol. Plant. **27**: 29-36, 2005.

Romanowska, E., Wróblewska, B., Drożak, A., Siedlecka, M.: High light intensity protects photosynthetic apparatus of pea plants against exposure to lead. - Plant Physiol. Biochem. **44**: 387-394, 2006.

Rucińska, R., Waplak, S., Gwóźdź, E.A.: Free radical formation and activity of antioxidant enzymes in lupine roots exposed to lead. - Plant Physiol. Biochem. **37**: 187-194, 1999.

Sandalio, L.M., Dalurzo, H.C., Gómez, M., Romero-Puertas, M.C., del Rio, L.A.: Cadmium-induced changes in the growth and oxidative metabolism of pea plants. - J. exp. Bot. **52**: 2115-2126, 2001.

Sen Gupta, A., Heinen, J.L., Holaday, A.S., Burke, J.J., Allen, R.D.: Increased resistance to oxidative stress in transgenic plants that overexpress chloroplast Cu/Zn superoxide dismutase. - Proc. nat. Acad. Sci. USA **90**: 1629-1633, 1993.

Weckx, J.E.J., Clijsters, H.M.M.: Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. - Plant Physiol. Biochem. **35**: 405-410, 1997.

Schekman, R., Goldstein L., Rossant, J. (ed.): **Annual Review of Cell and Developmental Biology. Vol. 23.** - Annual Reviews Inc., Palo Alto 2007. 731 pp. ISBN 978-0-8243-3128-8

The introductory chapter "Following the chromosome path to the garden of the genome." is written by M.-L. Pardue, an early leader in the investigation of the *Drosophila* polytene chromosome and the expression of ribosomal and heat shock genes in the fruitfly. Readers of Biologia Plantarum may find interesting the reviews: "SNARE-WARE: the role of SNARE-domain proteins in plant biology" (V. Lipka *et al.*) and "Embryonic pattering in *Arabidopsis thaliana*" (P.D. Jenik *et al.*). Other topics covered include: "Nonenveloped virus membrane penetration", "Heart field: from mesoderm to heart tube", "Transcription factors in wound healing", "Epithelial stratification", "Two families of chaperonin: physiology and mechanisms", "MicroRNA functions", "Cadherin and catenin mechanisms", "Semaphorin regulation of cellular morphology", "Wing vein pattering in *Drosophila*", "Peroxisome division and inheritance", "Cilia and developmental signaling", "Calcium signaling in neuronal motility", "Controls of germ cell fates in *Caenorhabditis elegans*", "Hyaluronan in tissue injury and repair", "Secretory mechanisms in cell mediated cytotoxicity", "Biogenesis and function of multivescular bodies", "Pattern coordination in the *Cerebellum*", "*Arf* family of G proteins", "AMPA receptor trafficking", and "Biology of cancer stem cells".

The reviews include extensive references to the literature and are written in such a way, as to be valuable both to advanced students and scientist. All articles were written by the foremost experts in the field. Annual Reviews is a nonprofit scientific publisher established to promote advancement of the sciences. Annual Review of Cell and Developmental Biology as well as other Annual Reviews are available online (htpp://arjournals. annualreviews.org).

T. GICHNER (*Prague*)