BIOLOGIA PLANTARUM 55 (4): 771-774, 2011

BRIEF COMMUNICATION

Adventitious root system reduces lead uptake and oxidative stress in sunflower seedlings

J. STRUBIŃSKA[1] and A. HANAKA[2]*

Department of Cell Biology, M. Curie-Skłodowska University, Akademicka 19, PL-20033 Lublin, Poland[1]
Department of Plant Physiology, M. Curie-Skłodowska University, Akademicka 19, PL-20033 Lublin, Poland[2]

Abstract

In this work, the effect of lead on sunflower seedlings with two root system types: primary - formed from embryonic tissues and adventitious - originating from hypocotyl after cutting off primary roots was investigated. The seedlings were subjected to $Pb(NO_3)_2$ in doses: 0, 0.5, 2.5, 5 and 20 mg(Pb) dm^{-3} for a week. Lead accumulation, seedling length and mass as well as selected parameters representative of oxidative damage (malondialdehyde) and protection (superoxide dismutase and glutathione) were used to compare stress response of plants. The comparison showed significant differences between plants with different root systems in almost all the parameters and the plants with adventitious root were more tolerant to lead.

Additional key words: antioxidants, *Helianthus annuus*, lipid peroxidation.

Lead is one of the most hazardous heavy metals which can easily penetrate and accumulate in plant tissues and it leads to stress manifested by inhibition of growth, photosynthesis, respiration, formation of reactive oxygen species and degeneration of the main cell organelles (Rucińska *et al.* 1999, Gratão *et al.* 2005). Though the effect of lead induced oxidative stress on plants is well studied, it is not so with respect to different reaction to stress of plants differing in the root system origin. The aim of present work is to show that the different root system can modify lead uptake and stress response in sunflower seedlings.

Sunflower (*Helianthus annuus* L.) cv. Frankasol seeds were germinated at 25 °C in half-strength Hoagland solution (Hoagland and Arnon 1959) in the dark for 5 d. After that, the seedlings were divided into two groups. In the first (PRS - primary root seedlings), the primary root system formed from embryonic tissues was saved and in the second one (ARS - adventitious root seedlings) primary roots were cut off about 1 cm above the main root base. During the experiment these plants regenerated adventitious roots from the pericycle tissue from about 2.5 cm long hypocotyl fragment dipped in the medium. All the plants were transferred into modified half-strength Hoagland nutrient solution (Michalak and Wierzbicka 1998), lead was added to the solution in doses: 0, 0.5, 2.5, 5 and 20 mg dm^{-3} as $Pb(NO_3)_2$ and pH was adjusted to 5.0 ± 0.2. The plants were grown under 16-h photoperiod (irradiance of 150 μmol m^{-2} s^{-1}) at day/night temperature of 25/20 °C and air humidity 70 % for 7 d and then 12-d-old seedlings were analysed.

The total plant root system length was calculated as a sum of the root length in every individual root system. As an equivalent of adventitious roots in the primary roots system the length of lateral roots was taken. Lead content in dry plant material was estimated using atomic absorption spectrometer *SpectrAA 880* and *880Z* (*Varian,* Australia). The level of lipid peroxidation products was determined by measuring the content of malondialdehyde (MDA) using colorimetric assay (*Bioxytech*® *LPO-586*™ (*OxisResearch*, Portland, USA). The activity of superoxie dismutase (SOD) was measured using a spectrophotometric assay (*Bioxytech*® *SOD*-525™, *OxisResearch*). The protein content was determined by the *Bio-Rad* (Hercules, USA) micro assay procedure using bovine serum albumine as a standard. The content of glutathione

Received 5 May 2010, *accepted* 23 September 2010.
Abbreviations: ARS - adventitious root seedlings; GSH - glutathione; MDA - malondialdehyde; PRS - primary root seedlings; ROS - reactive oxygen species; SOD - superoxide dismutase.
* Corresponding author; fax: (+48) 815375901, e-mail: agnieszka.hanaka@umcs.pl

(GSH) was estimated by HPLC method according to Wójcik and Tukiendorf (2004).

Statistical analysis was carried out using two-way *ANOVA* for determination interaction significance ($P < 0.05$). The data for growth parameters were obtained from 5 independent replications (about 20 plants in each) and for SOD and MDA from 3 replications (3 plants in each). Analyses of lead and GSH content were performed in triplicate (10 and 5 plants in each, respectively).

Lead application caused a decrease in the length and fresh mass of roots both in PRS and ARS with the exception of 0.5 mg(Pb) dm^{-3} (Table 1). The significant root length inhibition was found in the PRS root system at 2.5 mg(Pb) dm^{-3}. At 5 and 20 mg(Pb) dm^{-3}, both PRS and ARS root systems showed a significant length and fresh mass reduction, but PRS roots appeared more sensitive. The total root length per plant at the control and 0.5 mg(Pb) dm^{-3} was greater in the PRS than in the ARS root system. In both, 0.5 and 2.5 mg(Pb) dm^{-3} increased shoot length in comparison with the control plants. Shoot length was reduced only at 5 and 20 mg(Pb) dm^{-3}. A significant shoot fresh mass reduction was found at 5 and 20 mg(Pb) dm^{-3} and was greater in the PRS than in the ARS.

The amount of lead absorbed in both roots and shoots of PRS and ARS increased with an increasing lead dose in the growth medium (Table 1). Lead content was from 13 to 86-fold greater in the roots than in the shoots. All PRS contained more metal in their tissues than ARS did. These differences ranged from 20 to 96 % in the shoots and from 160 to 530 % in the roots.

In the control as well as under lead stress, the content of MDA in PRS roots was significantly higher than that of ARS. In PRS shoots, an increase in MDA content was observed at 2.5, 5 and 20 mg(Pb) dm^{-3} (Table 2). In ARS roots, the MDA content decreased at 0.5 and 2.5 mg(Pb) dm^{-3} by about 20 % with respect to the control and increased only at the highest lead dose. The content of MDA in the shoots of both control and lead treated plants was higher than in the roots. However, in the shoots of PRS, the MDA content increased with an increase in the lead dose, while in the shoots of ARS, lipid peroxidation was markedly raised only at 5 and 20 mg(Pb) dm^{-3}.

Lead treatment resulted in a significant decrease in the GSH content in the roots (Table 2). In the PRS roots, the GSH reduction was about 60 % at 0.5 mg(Pb) dm^{-3} similarly as at the higher lead doses used. In the ARS root system, a progressive decrease in the GSH content was found, with the exception of 20 mg(Pb) dm^{-3}. In contrast, in the PRS and ARS shoots of lead treated plants, no significant changes in the GSH content were observed compared to the corresponding controls, with the exception of PRS exposed to 5 mg Pb dm^{-3} (about 20 % reduction *versus* control). In the all investigated plants, higher content of GSH was found in the shoots than in the roots.

SOD activity differed significantly between PRS and ARS. In ARS roots, significantly higher SOD activity was observed in the control and in the 0.5 and 20 mg(Pb) dm^{-3}, compared to PRS (Table 2). PRS roots were characterized by increase in SOD activity up to the maximum at 5 mg(Pb) dm^{-3} and decrease at the highest lead dose by about 20 %. In ARS roots, a relatively high control level of SOD activity further increased at higher Pb doses by about 20 - 40 %, with the exception of 2.5 mg(Pb) dm^{-3}. In all the seedlings not exposed to lead, the shoots maintained higher SOD activity than the roots. However, in some treatments (from 2.5 to 20 mg(Pb) dm^{-3} in PRS and 20 mg(Pb) dm^{-3} in ARS) higher SOD activity was detected in the roots. The shoots of PRS showed a

Table 1. Fresh mass, length of roots and shoots and Pb content in 12-d-old sunflower seedlings with primary root systems (PRS) and adventitious root systems (ARS) as influenced by different Pd concentrations. Results are means ± SD of 5 independent replications (at least 15 plants in each) for fresh mass and length and of 5 independent replications (about 20 plants in each) and 3 replications (10 plants in each) for total roots length and lead content, respectively. Different letters in the same column and between primary and adventitious root plants represent a significant difference ($P < 0.05$) for shoots and roots separately.

Lead doses [mg dm^{-3}]		0	0.5	2.5	5	20
Fresh mass [% of control]	shoots PRS	100 ± 28ab	113 ± 35a	85 ± 17bc	61 ± 14c	33 ± 10e
	shoots ARS	100 ± 36ab	113 ± 36a	85 ± 23b	79 ± 18d	52 ± 11e
	roots PRS	100 ± 36a	85 ± 15a	82 ± 27a	49 ± 14b	35 ± 10d
	roots ARS	100 ± 33b	96 ± 27b	87 ± 42b	79 ± 19c	50 ± 13d
Length [% of control]	shoots PRS	100 ± 21a	149 ± 31c	127 ± 21c	90 ± 24a	57 ± 22d
	shoots ARS	100 ± 26b	149 ± 34a	116 ± 35a	89 ± 38b	70 ± 33d
	roots PRS	100 ± 29a	93 ± 29a	54 ± 15b	19 ± 5d	3 ± 0.8f
	roots ARS	100 ± 29b	109 ± 17c	87 ± 9b	63 ± 13e	5 ± 1f
Total roots length [mm]	roots PRS	218.9 ± 82.2a	242.5 ± 79.7a	135.4 ± 34.2b	49.5 ± 21.6c	4.2 ± 4.3d
	roots ARS	163.9 ± 68.9b	183.6 ± 45.9b	129.9 ± 39.2b	97.0 ± 29.8e	5.1 ± 4.2d
Lead content [mg kg^{-1}(d.m.)]	shoots PRS	21 ± 1a	48 ± 2b	167 ± 33c	445 ± 53d	783 ± 49e
	shoots ARS	24 ± 2a	40 ± 5f	95 ± 2g	227 ± 4h	400 ± 15d
	rootsPRS	86 ± 9a	3232 ± 189b	13920 ± 2171c	23830 ± 282d	67130 ± 138e
	rootsARS	82 ± 14a	513 ± 21f	4623 ± 168g	9200 ± 612h	24600 ± 1384d

Table 2. MDA content, GSH content and SOD activity in the control and lead-treated 12-d-old sunflower seedlings with primary (PRS) and adventitious (ARS) root systems. Results are means ± SD of 3 independent replications (3 plants in each) for MDA concentration and SOD activity and 3 replications (5 plants in each) for GSH content. Different letters represent a significant difference ($P < 0.05$) for shoots and roots separately.

	Lead doses [mg dm^{-3}]	MDA [mM] PRS	MDA [mM] ARS	SOD [U mg protein^{-1}] PRS	SOD [U mg protein^{-1}] ARS	GSH [nmol g^{-1}(f.m.)] PRS	GSH [nmol g^{-1}(f.m.)] ARS
Shoots	0	10.2 ± 2.1a	13 ± 1.4b	2846 ± 95a	3397 ± 276bc	191 ± 18a	199 ± 5.3a
	0.5	10.8 ± 2.9a	12.4 ± 3.4ab	3456 ± 222b	3078 ± 22c	192 ± 32a	191 ± 20a
	2.5	14.6 ± 1.8b	10.9 ± 1.6a	2328 ± 51d	3455 ± 162b	175 ± 20ab	178 ± 23ab
	5	18.3 ± 1.8c	17.1 ± 1.5c	1920 ± 122e	3327 ± 135b	149 ± 3.7b	200 ± 11a
	20	35.8 ± 5.6d	26.3 ± 1.6e	989 ± 22f	2768 ± 74a	193 ± 1.1a	181 ± 33ab
Roots	0	9.3 ± 3.3ag	6.2 ± 1.4b	1520 ± 69a	2591 ± 27b	166 ± 24a	155 ± 12a
	0.5	8 ± 1.7ae	5 ± 0.7c	1670 ± 27c	3094 ± 99d	64 ± 0.1b	124 ± 2.8c
	2.5	9.2 ± 1.9a	4.8 ± 0.8c	2474 ± 25e	2580 ± 20b	59 ± 3.9bg	87 ± 5d
	5	13.7 ± 1.1d	7.1 ± 0.9be	3505 ± 14f	3157 ± 89d	54 ± 2.3e	69 ± 0.3f
	20	19.8 ± 1.7f	11.8 ± 0.8g	2747 ± 3g	3568 ± 6h	61 ± 0.1g	74 ± 8.5fb

significant increase in SOD activity at the lowest lead dose. Only the highest lead dose used caused a significant decrease in the SOD activity in the shoots of ARS.

The presented results indicate the dose dependent influence of lead on sunflower seedlings. At 5 and 20 mg(Pb) dm^{-3}, a significant decrease in vigour was found and lead from 2.5 (shoots) to 5 mg(Pb) dm^{-3} (roots) induced oxidative stress (measured as MDA content). As described in literature the toxic effect of lead on plant growth is associated with the increasing oxidative stress and lipid peroxidation in plant tissues (Qureshi *et al.* 2005, 2007, Zhao *et al.* 2009). The present results show that in sunflower seedlings (both PRS and ARS) the lead accumulation was greater in the roots than in the shoots, whereas some toxicity symptoms were more pronounced in the shoots than in the roots.

In the lead treated shoots, in comparison with the roots, a relatively higher MDA content was found. It was correlated with a gradual SOD activity decrease. Similar results were obtained for copper treatment in tomato (Mazhoudi *et al.* 1997) and for nickel treatment in wheat (Gajewska *et al.* 2006). This may be due to the capability of lead to generate in shoots greater amounts of ROS which can react with the polyunsaturated fatty acids of the chloroplast membranes (Qureshi *et al.* 2005) or to the main potential of tolerance mechanisms operating in root cells (Mazhoudi *et al.* 1997). The decreased SOD activity observed in this study was probably due to harmful effects of overproduction of H_2O_2 or its poisonous ROS derivates, which is confirmed by the very high MDA content. However, despite of all described disturbances, growth indexes for the shoots were as good (for mass) or better (for length) than for the roots.

In the roots, despite the higher lead content, a lower MDA content accompanied by increasing of SOD activity was found. As described by Rucińska and Gwóźdź (2005) in lupine roots, lipid peroxidation decreased after lead application. The results are inconsistent with the data presented by Mishra and Choudhuri (1999) where the increase in MDA content was indicated. An increase in SOD activity, which is commonly taken as an indicator of an increased ROS level in roots, has also been reported as a consequence of lead application, *e.g.*, in lupine (Rucińska *et al.* 1999). Contrary to SOD activity, the lead induced decrease in GSH content in the roots was observed. Diminution of the GSH content in sunflower roots was also observed after treatment with iron, copper and cadmium (Gallego *et al.* 1996). High GSH content is characteristic for lead hyperaccumulating plants, *e.g.*, *Sesbania drummondii* and can be important for ability of plants to withstand metal-induced oxidative stress (Gratão *et al.* 2005). As described by Aravind and Prasad (2005) for cadmium treatment, the decrease in the GSH content can be connected with disturbances of GSH biosynthesis. It can be also related to its oxidation in ROS scavenging cycles, *e.g.*, GSH-ascorbic acid cycle. The obtained results suggest that both SOD activity and MDA content are not exclusively correlated with the shoot and root lead content or growth disturbance but demonstrated rather different sensitivities of these organs. However, GSH content seems to be more directly dependent on the lead tissue concentration and strongly connected with the root growth reduction.

This study was performed to compare the lead accumulation potential as well as the cell response of sunflower seedlings with primary and adventitious root systems, what is only marginally mentioned in literature. Michalak and Wierzbicka (1998) compared tolerance of *Allium cepa* plants grown from seeds (with primary roots) and bulbs (with adventitious root system). They determined the lower tolerance index of seedling primary roots at the average level of about 24 %. In the present study, the ARS was also found to be more tolerant to lead than the PRS. Significant differences in the lead-induced vigour decrease and MDA content increase were found between seedlings with two different root systems. In *Allium cepa*, a higher lead content was observed in plants developed from seeds than from bulbs (Michalak and

Wierzbicka 1998). The reason might be the ability of bulb to accumulate large amount of Pb and to protect plants against its toxicity, different developmental phases and a marked difference in seedling and bulb roots morphology. As shown in this study, the ARS had about three times lower tissue lead content than the PRS. However, anatomical, morphological or size differ-rentiation of plants cannot be suspected to be responsible for dissimilarity. In all the experiments, plants at the same age and developmental stage were used. Furthermore, no significant differences in size and morphology were found in the lateral and adventitious roots (main components of the root systems). No reasons can also be found in the different primary and adventitious root systems size or plant water balance. The only difference between the seedlings used in the experiment could be a different age of the roots in primary and adventitious systems. However, for that reason, adventitious root system, being younger, should be not more but less tolerant to lead. The possibility of reducing metal uptake in consequence of changing plasma membrane composition and modification of its permeability includes changes in unsaturated lipid and steroid content, surface charge, redox potential or membrane architecture (Siedlecka *et al.* 2001). However, the explanation of such mechanisms in sunflower adventitious roots requires further investigations.

Another expression of different stress responses of sunflower plants with different root systems is higher antioxidant potential of ARS. The above results suggest that higher SOD activity and GSH content in ARS cannot be taken as a simple consequence of a lower lead content, which can result in its lower disturbing potential. Comparison of the results obtained for seedlings with similar Pb tissue content in most cases showed significantly higher GSH content and SOD activity in the ARS. Besides, the ARS control plants were characterized by significantly higher SOD activity than the PRS.

All these results suggest that in ARS some additional mechanisms must act to protect them against lead penetration and lead-induced oxidative stress. The origin of the changes leading to limited lead accumulation and elevated antioxidant response in sunflower ARS are still unconfirmed. As described by Capiati *et al.* (2006), wounding increased salt tolerance in tomato plants due to activation of some stress-response pathways. It is possible that similar mechanism of enhancing Pb tolerance by wounding (cutting off primary roots) might work. There is a lot of evidence that the response of plants to a combination of two different abiotic stresses cannot be directly extrapolated from the response of plants to each of the different stresses applied individually and that different stress factors might exaggerate or extenuate the effects. The possibility of interaction between wounding and lead stress should be the focus of future research.

References

Aravind, P., Prasad, N.M.V.: Modulation of cadmium-induced oxidative stress in *Ceratophyllum demersum* by zinc involves ascorbate-glutathione cycle and glutathione metabolism. - Plant Physiol. Biochem. **43**: 107-116, 2005.

Capiati, D.A., País, S.M., Téllez-Iñón, M.T.: Wounding increases salt tolerance in tomato plants: evidence on participation of calmodulin-like activities in cross-tolerance signalling. - J. exp. Bot. **57**: 2391-2400, 2006.

Gajewska, E., Skłodowska, M., Słaba, M., Mazur, J.: Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. - Biol. Plant. **50**: 653-659, 2006.

Gallego, S.M., Benavides, M.P., Tomaro, M.L.: Effect of heavy metal ion excess on sunflower leaves: evidence for involve-ment of oxidative stress. - Plant Sci. **121**: 151-159, 1996.

Gratão, P.L., Polle, A., Lea, P.J., Azevedo, R.A.: Making the life of heavy metal-stressed plants a little easier. - Funct. Plant Biol. **32**: 481-494, 2005.

Hoagland, D.R., Arnon, D.J.: The water-culture method of growing plants without soil. - Calif. Agr. Exp. Sta. Circ. **347**: 26-29, 1959.

Mazhoudi, S., Chaoui, A., Ghorbal, M.H., Ferjani, E.E.: Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill.). - Plant Sci. **127**: 129-137, 1997.

Michalak, E., Wierzbicka, M.: Differences in lead tolerance between *Allium cepa* plants developing from seeds and bulbs. - Plant Soil **199**: 251-260, 1998.

Mishra, A., Choudhuri, M.A.: Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. - Biol. Plant. **42**: 409-415, 1999.

Qureshi, M.I., Israr, M., Abdin, M.Z., Iqbal, M.: Responses of *Artemisia annua* L. to lead salt-induced oxidative stress. - Environ. exp. Bot. **53**: 185-193, 2005.

Qureshi, M.I., Abdin, M.Z., Qadir, S., Iqbal, M.: Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. - Biol. Plant. **51**: 121-128, 2007.

Rucińska, R., Gwóźdź E.A.: Influence of lead on membrane permeability and lipoxygenase activity in lupine roots. - Biol. Plant. **49**: 617-619, 2005.

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

Siedlecka, A., Tukendorf, A., Skórzyńska-Polit, E., Maksymiec, W., Wójcik, M., Baszyński, T., Krupa, Z.: Angiosperms. - In: Prasad M.N.V. (ed.): Metals in the Environment. Analysis by Biodiversity. Pp. 171-217. Marcel Dekker, New York - Hyderabad 2001.

Wójcik, M., Tukiendorf, A.: Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. - Plant Growth Regul. **44**: 71-80, 2004.

Zhao, L., Xiong, J., Li, L.P, Zhu, C.: Low concentration of exogenous abscisic acid increases lead tolerance in rice seedlings. - Biol. Plant. **53**: 728-732, 2009.