Phyton (Austria) Special issue: "Plant Physiology"	Vol. 39	Fasc. 3	(155)-(159)	30. 11. 1999

Lead Phytotoxicity in Norway Spruce (*Picea abies* (L.) Karst.): The Effect of Pb and Zeatin-Riboside on Root Respiratory Potential

By

Dominik VODNIK[1], Alenka GABERŠČIK[2] & Nada GOGALA[1]

Key words: Norway spruce, heavy metal stress, lead, cytokinins, zeatin-riboside, root respiratory potential.

Summary

VODNIK D., GABERŠČIK A. & GOGALA N. 1999. Lead phytotoxicity in Norway spruce (*Picea abies* (L.) Karst.): The effect of Pb and zeatin-riboside on root respiratory potential. - Phyton (Horn, Austria) 39 (3): (155) - (159).

Hydroponically grown *Picea abies* (L.) Karst. seedlings were exposed to lead (5 μM) and zeatin-riboside (ZR) treatments (1 μM, periodically) in a factorial experiment. The influence of Pb and ZR on the seedlings was examined studying photochemical efficiency of PSII in green parts and terminal electron transport system (ETS) activity of the root tissue. Lead and cytokinin, separately or in combination, did not influence the light use efficiency. The lead exerted an influence mainly on the root system. In Pb treated seedlings ETS activity of the root tissue was lowered by app. 25% in comparison to the control. Zeatin-riboside mitigates the lead induced inhibition of root growth and the ETS activity is even higher than in control plants. Higher potential respiration increases the ability to overcome stress conditions. The presence of cytokinin in the rhizosphere could be an important factor affecting Pb toxicity.

Introduction

Rhizosphere organisms, both free living and symbiotic can alter hormonal status in the rhizosphere and influence hormonal concentrations in plants (GOGALA 1991, KAPULNIK 1996). This can have consequences in physiological processes and also in the plant response to the environmental stress. It has been suggested that plant hormones may be involved in delaying the senescence and

[1] Department of Biology, BF, University Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia.

[2] National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia.

chlorosis in plants treated with heavy metals (GODBOLD 1994). The role of cytokinins in plant responses to adverse environmental conditions has been recently reviewed by HARE & al. 1997. However, information on the possible effects of cytokinins on heavy metal (HM) toxicity and plant tolerance to HM is still very scarce.

Lead is one of the stress factors contributing to the forest decline in Europe and North America. The major effect of Pb is the inhibition of root growth (GODBOLD 1994). The aim of present study was to test the effect of cytokinin on Pb toxicity in Norway spruce (*Picea abies* (L.) Karst.). In order to simulate rhizosphere processes that moderate hormonal concentrations the zeatin-riboside (ZR) was applied to the roots of hydroponically grown seedlings at concentration of 1 μM. This is a concentration close to that found in soil extracts (VAN STADEN & DIMALLA 1976) and in vitro cultures of mycorrhizal fungi (WULLSCHLEGER & REID 1990, KOVAČ & ŽEL 1995). The toxic effect of Pb was monitored by studying primary electron transport in green parts, i.e. the fluorescence of chlorophyll a, and terminal electron transport system (ETS) activity in underground parts of the spruce seedlings treated with lead.

Material and Methods

Plant material and growth conditions

Seeds of *Picea abies* (L.) Karst. were surface-sterilized in 30% H_2O_2 and germinated for 3 weeks on filter paper watered with distilled water. The seedlings were transferred to non-sterilized nutrient solutions containing (μM): NH_4NO_3 (300), Na_2SO_4 (50), K_2SO_4 (100), KH_2PO_4 (30), $MgSO_4$ (60), $CaSO_4$ (130), $FeCl_3$ (5), H_3BO_3 (5), Na_2MoO_4 (0.1), $ZnSO_4$ (0.1), $CuSO_4$ (0.1), HCl (120). The pH was 4.0. The solutions were constantly aerated and renewed every 5 days to prevent depletion of nutrients. The 20-25 seedlings per treatment were grown in pot containing 5 l of nutrient solution. Plants were grown under 22/20°C day/night temperatures, 75±5 % relative humidity, 300 μmol $m^{-2}s^{-1}$ photon flux density and a 16h photoperiod. After four weeks one half of the seedlings was exposed to 5μM Pb. Lead was added to the solution in a form of $PbCl_2$. Seven days after the beginning of lead treatment cytokinin treatment started. The root systems of one half of lead-treated and control seedlings were submerged into the 1μM solution of zeatin-riboside in the basic culture solution for 5 hours and thereafter transferred back to the hydroculture. Plants were exposed to the cytokinin treatment once a week for the following three. In the fourth week seedlings were harvested.

Growth measurements

Root length was determined using line intersecting method. Dry mass of shoots and roots was determined after 1-day oven drying at 120 °C.

Chlorophyll fluorescence measurements and pigment analysis

The fast chlorophyll fluorescence kinetics was measured in vivo using fluorometer PSM (Plant Stress Meter, Biomonitor, Sweden). Measurements were carried out in 10 parallel samples, on fully developed needles in one week intervals. Photosynthetic pigments were determined according to LICHTENTHALER 1987.

Terminal electron transport system (ETS) activity

The terminal electron transport (ETS) activity was determined on the one-centimetre segment of the root tips using a method described by KENNER & AHMED 1975. Analyses were performed on 9 parallel samples, after the experiment was accomplished.

Statistical evaluation

Results were subjected to two-way analysis of variance (ANOVA) and LSD comparison of means, $p < 0.05$, using Statistica™ software (Statsoft™).

Results and Discussion

After 4 weeks of Pb treatment no visible symptoms of lead toxicity were observed in spruce shoots. The shoot dry weight was similar in all four groups of seedlings: control (C), control-ZR treated (C-ZR), Pb treated-control (Pb-C) and Pb plus ZR treated (Pb-ZR) ranging between 178.5 and 209.4 mg. Due to high variability, there was no significant difference in root dry weight among the groups of seedlings ranging between 44.0 mg and 55.8 mg. The smallest dry weight was observed in Pb-C seedlings. The shoot to root ratio was similar in all groups of seedlings (3.75 - 4.79). Lead slightly reduced the concentrations of clorophylls and carotenoids in the needles (Table 1), ZR partly ameliorated this negative effect. Similar effects were found for kinetin in Pb-treated barley (WOZNY & al. 1995). Exogenous ZR alone did not affect photosynthetic pigments.

Table 1. Photosynthetic pigments in the needles of *Picea abies* seedlings exposed to the different treatments with Pb (5μM) and zeatin-riboside. Means ± SE are presented, N=7. Values followed by the same letter do not differ (Tukey test, $p < 0.05$). Probability levels for 2-way ANOVA: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. Chlorophyll = chl.

	Pigments, mg g^{-1} DW				ANOVA		
	C-C	C-ZR	Pb-C	Pb-ZR	Pb	ZR	PbxZR
chl a	0.86 ± 0.05 a	0.74 ± 0.04 ac	0.57 ± 0.03 b	0.64 ± 0.03 bc	****	ns	*
chl b	0.38 ± 0.03 a	0.31 ± 0.02 ab	0.24 ± 0.01 b	0.28 ± 0.01 b	***	ns	**
carotenoids	0.30 ± 0.01 a	0.25 ± 0.01 ab	0.20 ± 0.01 b	0.23 ± 0.01 b	***	ns	*

The average values of Fv/Fm (ratio of variable and maximal fluorescence) (Fig.1) showed only slight decrease in light use efficiency in lead treated plants, due to great variability among measured values which was increasing during spruce growth. The addition of ZR in the growth medium did not mitigate this effect. When comparing half raise times ($T_{1/2}$) no significant influence of the treatments was observed what indicates the same pool size of the electron acceptors in PS II. The analyses of the root system revealed obvious influence of lead. Significant differences were obtained when the lengths of roots were measured (Fig. 2a). Lead treated roots were significantly shorter than the control ones, even though the weights of the root system were very close to each other (data not shown). The beneficial effect of the hormone added in the root zone of the lead treated plants was outstanding. Root system was well developed, roots were longer and thinner. These results are also supported by the measurements of the potential respiration of the root system (Fig. 2b). The ETS-activity of the roots which was fairly suppressed by the presence of the lead in the growth medium, was even stimulated in plants treated with zeatin-riboside when compared with a control group.

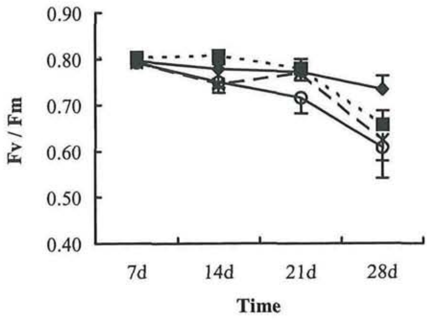

Fig. 1. Photochemical efficiency of PSII, Fv/Fm ratio in Norway spruce seedlings subjected to the following treatments: ◆ control, ■ zeatin-riboside, O lead, x lead/zeatin-riboside. Vertical bars = SE, n = 10.

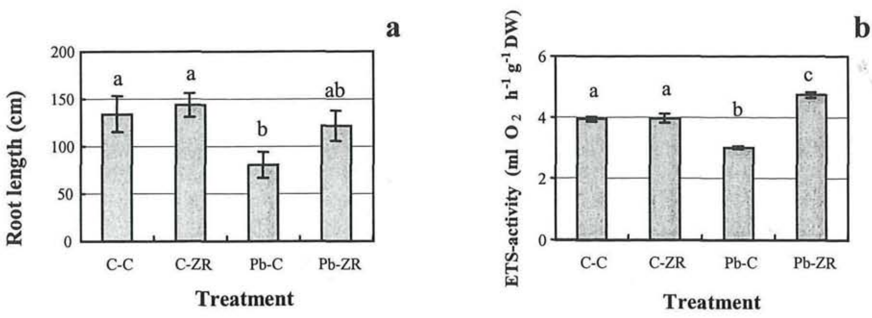

Fig. 2. Root length (a) and termina electron transport (ETS) activity (b) of the root tissue of Norway spruce seedlings subjected to the different treatments: For abreviations see Table 1, n = 9-12.

Our investigation confirms that the primary target of Pb toxicity is the root and not the shoot. Negative effect of Pb on the level of cytokinins produced by the roots is not excluded. The addition of the zeatin-riboside diminish effects of Pb. The vitality of the root system seems to be even better than in the control plants. Higher potential respiration of certain tissue or organism increase the ability to overcome stress conditions. In our previous studies of lead partitioning in spruce (VODNIK 1998, Thesis) it was shown that zeatin-riboside strongly influenced also lead uptake and translocation. Both results suggest that the presence of cytokinins in the rhizosphere may influence HM toxicity and tolerance.

Acknowledgements

This study was financed by Ministry of Science and Technology, Slovenia, grant J1-7391-0487-97 and by U.S.-Slovene Science and Technology Joint Found in cooperation with U.S.D.A., grant 95-FS-1.

References

GODBOLD D.L. 1994. Aluminium and heavy metal stress: From the rhizosphere to the whole plant. - In: GODBOLD D.L. & HÜTTERMAN A. (Eds.), Effects of acid rain on forest processes, pp. 232-264. - Wiley-Liss, New York.

GOGALA N. 1991. Regulation of mycorrhizal infection by hormonal factors produced by host and fungi. - Experientia 47: 331-340.

HARE P.D., CRESS W.A. & VAN STADEN J. 1997. The involvement of cytokinins in plant responses to environmental stress. - Plant Growth Regulation 23: 79-103.

KAPULNIK Y. 1996. Plant growth promotion by rhizosphere bacteria. - In: WAISEL Y., ESHEL A. & KAFKAFI U. (Eds.), Plant roots - the hidden half. Sec. ed., pp. 769-781. - Marcel Deker, New York. ISBN 0-8247-9685-3.

KENNER A.A. & AHMED S.I. 1975. Measurements of electron transport activities in marine phytoplankton. - Mar. Biol. 33: 119-127.

KOVAČ M. & ŽEL J. 1995. The effect of aluminium on cytokinins in the mycelia of *Amanita muscaria*. - J. Plant Growth Regul. 14: 117-120.

LICHTENTHALER H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. - Methods in Enzymology 148: 350-382.

VAN STADEN J. & DIMALLA G.G. 1976. Cytokinins from soils. - Planta 130: 85-87.

VODNIK D. 1998. The role of mycorrhizae in lead phytotoxicity. - Ph.D. Thesis, University of Ljubljana, 146 pp.

WOZNY A., SCNEIDER J. & GWOZDZ E.A. 1995. The effects of lead and kinetin on greening barley leaves. - Biolog. Plant. 37: 541-552.

WULLSCHLEGER S.D. & REID C.P.P 1990. Implication of ectomycorrhizal fungi in the cytokinin relations of loblolly pine (*Pinus taeda* L.). - New Phytol. 116: 681-688.