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BRIEF COMMUNICATION

Growth and endogenous cytokinins of juniper shoots as affected by high metal concentrations

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Abstract

The growth and the content of endogenous cytokinins (CKs) of current-year-old shoots from juniper plants (*Juniperus communis* L.) growing over and off ore site were compared. The juniper shoots from ore site (M plants) had higher metal content and exhibited delayed growth. Less bases and nucleosides of Z- and iP- type CK and more iP-conjugates were present in the M shoots. These changes were probably due to inhibited CK export from the roots and/or altered CK metabolism forming less biologically active CKs.

Additional key words: immunoaffinity chromatography, indirect competitive ELISA, N^6-(Δ^2-isopentenyl)adenine, N^6-(Δ^2-isopentenyl)adenosine, thin-layer chromatography, zeatin, zeatin riboside.

Natural forest stands growing over shallow ore depositions are often rich of metal ions, which are considered to be toxic for the plants (Foy *et al.* 1978). Mostly, high metal availability in the root settled zone limits the water uptake, thus decreasing the water content in the whole plant (Barceló and Poschenrieder 1990). The high metal availability and limited water supply inhibit the growth and cell differentiation of the available vegetation. It is well known that the intensity of these processes correlates with the content and physiological activity of cytokinins (Hare *et al.* 1997, Pospíšilová *et al.* 2000). The knowledge on changes in the cytokinin metabolism in natural woody species caused by the influence of the mentioned unfavourable environmental conditions is still scarce. The aim of the current study was to elucidate the relation between the contents of metal ions, water, and endogenous cytokinins (CKs) in current-year-old shoots of juniper (*Juniperus communis* L.) growing over rocks with polymetal deposition - a source of Fe, Ni, Pb, Co, Ag, Cu, Zn and V.

Current-year-old shoots from more than 30-year-old juniper plants growing on and off ore site were collected in the summer (June - July, 1998 - 2000). For evaluation of the shoot growth, their length, fresh and dry masses were determined. The mineral content was analyzed in samples (1 g) of oven-dried and milled plant material using emission spectroscopy. For CK analysis the samples (10 g) were frozen in liquid nitrogen, ground to powder and extracted twice with cold 80 % ethanol. The extracts were reduced *in vacuo* at 40 °C and aliquots were incubated in 0.1 M HCl at 100 °C for 1 h (Köhler 1988) to hydrolyze CK nucleotides and glucosides into bases and nucleosides. Extracts before and after acid hydrolysis were purified by *DEAE*-cellulose column running into *Sepharose 4B*-column with linked antibodies against *trans*-zeatin riboside (ZR) and N^6-(Δ^2-isopentenyl) adenosine (iPR) (Hansen *et al.* 1989). The eluates were applied on silica *60F*$_{254}$ plates and developed with *n*-butanol:water:NH_4OH (6:2:1, v/v/v) for separation of *trans*-zeatin (Z) and N^6-isopentenyladenine (iP) and their nucleosides (ZR and iPR). The zones corresponding to UV_{254} absorbing areas of CK standards ZR, iPR, Z and iP were scrapped off and eluted with 80 % ethanol. The eluates were evaporated and tested by indirect competitive anti-ZR- and anti-iPR-ELISA which measured ZR and iPR as well as Z and iP due to the

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Abbreviations: CK(s) - cytokinin(s); ELISA - enzyme-linked-immunosorbent assay; iP - N^6-(Δ^2-isopentenyl)adenine; iPR - N^6-(Δ^2-isopentenyl)adenosine; M plants - plants with increased metal content; Z - *trans*-zeatin; ZR - *trans*-zeatin riboside.

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cross-activity of CK-specific antibodies to CK nucleoside and base. The results are presented as means of three ELISA determinations from two experiments. The recovery of known quantities of CK standards (Z, ZR, iP and iPR) was controlled during all analytical steps. The estimates of CKs were corrected for a recovery of 50 - 70 % for Z and ZR and 35 - 50 % for iP and iPR.

The length, fresh and dry biomass of the current-year-old shoots of juniper plants from ore region, referred as M shoots, were considerably lower compared to control shoots grown off the site (Table 1). In the M shoots a considerable increase in the concentrations of some mineral elements, particularly of Fe, Al, Cu, Zn and Pb was found (Table 1). The content of dry biomass calculated per g fresh mass was higher in M shoots which was associated with their lower water content (Table 1). The higher dry mass and the lower water content of M shoots indicate reduced water supply and/or denser tissue structure. Such alterations, evaluated as xeromorphic, were considered as adaptive changes in plant tissues surviving water deficiency stress (Barceló and Poschenrieder 1990). Thus, the observed increase in the dry mass of M shoots may be interpreted as manifestation of xeromorphism and supports the suggestion that plants adapted to high metal content may also develop some tolerance to drought (Barceló and Poschenrieder 1990). The high metal availability as well as impaired water relations (water deficit) in the plant induced suppression of cell division and elongation and obstructions in the formation of secondary cell wall thickenings that disturbed vascular element differentiation and conductivity (Aloni 1987, Barceló *et al.* 1988). These changes decreased root to shoot CK delivery patterns (Hare *et al.* 1997, Pospíšilová *et al.* 2000).

The analysis of endogenous CKs in control and M shoots showed significant differences in their type and content (Fig. 1). The M shoots contained low contents of bases and nucleosides of Z- and iP-type and more conjugated metabolites of iP-type (determined as bases and nucleosides after acid treatment). On the contrary, in the control shoots pronounced levels of ZR and iPR and a lot of metabolites of Z-type (transformed to bases and nucleosides by acid hydrolysis) were found. The low contents of physiologically active bases and ribosides, particularly of Z-type, and the presence of iP conjugates in the M shoots may signify altered CK metabolism in order to diminish the content of the growth-promoting hormone.

The iP-type cytokinins are considered to be precursors in the synthesis of zeatins (Mok and Mok 2001). The hydroxylation of iPR to Z increased its physiological activity (Letham and Palni 1983) and this conversion was suggested to be necessary for normal shoot and bud growth (King and Van Staden 1988, Mok 1994). The enzyme responsible for the hydroxylation of iP to Z was localized predominately in roots and less in stems of woody species. Thus, the rate of conversion of iP to Z in stems would be lower (Einset and Silverstone 1987, King and Van Staden 1988). The higher amounts of iP-metabolites found in M juniper shoots may be due to a reduced capacity for hydroxylation to Z-type cytokinin. The juniper plants of ore region were smaller, their stems were shorter which might be related to a reduction of the tissues, involved in the conversion of iP to Z. Similar results were reported by Schwartzenberg *et al.* (1995), who found that the metabolism of iPR was delayed in roots of spruce seedlings subjected to Al stress.

Even though the shoots have limited capacity for CK

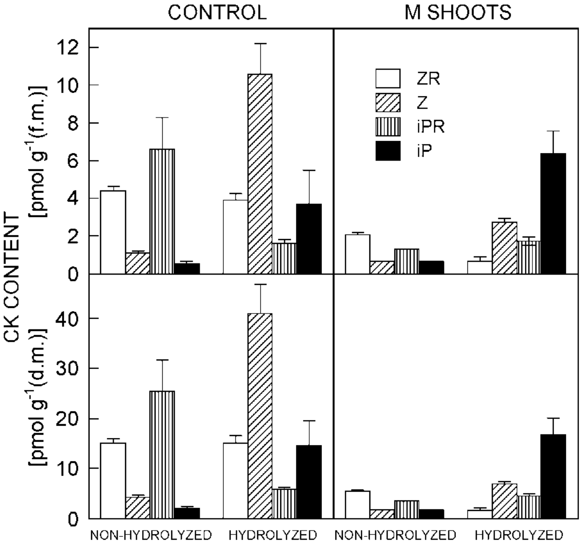

Fig. 1. Quantitative profiles of cytokinin contents in current-year-old control and M shoots of juniper plants. The cytokinin contents before (non-hydrolyzed) and after acid treatment (hydrolyzed) were calculated per fresh (*upper part*) or dry mass (*lower part*) unit.

Table 1. Comparison of mineral element contents, growth parameters and water content of shoots from control and M shoots of juniper plants.

	Control shoots	M shoots
Pb [μg g $^{-1}$(d. m.)]	6.40 ± 0.30	19.40 ± 0.20
Cu [μg g $^{-1}$(d. m.)]	3.80 ± 0.20	19.40 ± 0.30
Zn [μg g $^{-1}$(d. m.)]	19.00 ± 7.00	97.40 ± 4.50
Ni [μg g $^{-1}$(d. m.)]	0.03 ± 0.004	2.90 ± 0.40
Al [μg g $^{-1}$(d. m.)]	19.00 ± 0.50	97.40 ± 6.50
Fe [μg g $^{-1}$(d. m.)]	38.10 ± 1.70	292.00 ± 14.00
Mn [μg g $^{-1}$(d. m.)]	127.00 ± 6.30	293.00 ± 12.00
Length [cm shoot^{-1}]	9.66 ± 2.65	2.35 ± 0.25
Fresh mass [g shoot^{-1}]	0.93 ± 0.25	0.10 ± 0.00
Dry mass [g shoot^{-1}]	0.24 ± 0.80	0.04 ± 0.00
Dry mass [g g^{-1}(f. m.)]	0.26 ± 0.17	0.38 ± 0.00
Water content [g(H_2O) g^{-1}(f.m.)]	2.84 ± 0.06	1.63 ± 0.04

biosynthesis, the roots are widely accepted as the main site of CK production (Letham 1994). The exposure of roots to unfavourable environment reduces the CK supply from roots to the shoots by disturbing the transpiration rate, thus negatively affecting the shoot development (Letham 1994, Pospíšilová *et al.* 2000). Other mechanisms for maintenance of CK homeostasis at unfavourable conditions are CK interconversions (Hare *et al.* 1997). Changes in the ratio between the different forms of CKs, in particular the accumulation of biologically less active forms, should affect also shoot growth and development. In common our results support the understanding that environmental factors affecting root growth decrease the levels of CKs in plant tissues. The comparison of CK status of junipers growing on and off ore site shows that CKs with lower biological activity (less bases and nucleosides of Z- type and more conjugates of iP-type) are available in the shoots of plants from ore site. The observed changes might be due to reduced CK supply from roots to shoots resulting from disturbances in the vascular tissues or/and changed CK metabolism in the whole plant. The CK status of M shoots correlates with their delayed growth as well as with altered water relations and reduced photosynthetic activity of M needles found previously (Andonova and Popov 1984, Popov and Andonova 1998). Obviously, the juniper plants growing over ore site have evolved adaptations which would enable them to survive in an unfavourable environment.

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