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BRIEF COMMUNICATION

Amelioration of Pb and Mn toxicity to arbuscular mycorrhizal fungus *Glomus intraradices* by maize root exudates

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Abstract

The effect of maize root exudates on the toxicity of lead and manganese to arbuscular mycorrhizal fungus (AMF) *Glomus intraradices* was studied *in vitro* by observation of intraradical hyphae regrowth from colonised root segments. Higher heavy metal (HM) concentrations strongly reduced the hyphal growth, however, the inhibitory effect was to a large extent eliminated by the addition of maize root exudates to the media. However, the capacity of exudates to ameliorate HM toxicity was limited and did not operate when a threshold HM concentration was reached.

Additional key words: heavy metals, hyphal growth, *Zea mays*.

A role of arbuscular mycorrhizal fungi (AMF) in the interaction of host plants with heavy metals (HM) has not been elucidated yet and conflicting results have been published (Leyval *et al.* 1997). Some studies gave evidence about a protective role of AMF for the plants growing in HM-contaminated soil and lower HM uptake was reported for mycorrhizal plants (*e.g.* Dueck *et al.* 1986, Dehn and Schüepp 1989, Hildebrandt *et al.* 1999). However, little attention has been paid to the question whether also the plant can protect the fungus from HM toxicity. Firstly, the roots act for the fungus as a niche with lower HM concentration in comparison to adjacent soil. HM toxicity to AMF, particularly to an extraradical phase, could be also decreased via chelating HM by root exudates that play a major role in the interactions between roots, microorganisms and soil. For plants, HM-induced excretion of organic acids to the rhizosphere resulting in an alleviation of metal toxicity has been reported (Saber *et al.* 1999, Kochian *et al.* 2002). The aim of the present study was to elucidate the effect of root exudates produced by a host plant on Pb and Mn toxicity to AMF.

Root exudates were obtained from axenic maize plants (*Zea mays* L. cv. CE240) cultivated in sterile assemblies of glass tube (diameter 35 mm, length 300 mm) immersed into 500-cm^3 Erlenmayer flask initially containing 50 cm^3 distilled water. Each tube contained 8 surface sterilised (0.1 % $HgCl_2$, 20 min) pregerminated seeds and was closed by a cotton-wool plug at the top and by a layer of gauze at the bottom to keep the seeds inside. The vertical position of the tube was adjusted so that the gauze layer with seeds was just in the contact with the level of water. After 3 weeks, the liquid was collected and lyophilised. A quantity of 250 mg dry exudate was obtained from the total volume of 670 cm^3 of the liquid. The lyophilised material contained 38.3 % of the total C, had C/N ratio equal to 30.4 and contained 793 mg kg^{-1} of the total phosphorus.

The effect of root exudates on HM toxicity was studied using observation of intraradical hyphae regrowth from mycorrhizal root segments (hyphal proliferation). Three experiments were conducted where root exudates were added into the incubation media containing different Mn or Pb concentrations. Experiment 1 included the combinations of the two factors: *1*) Pb concentration

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Abbreviations: AMF - arbuscular mycorrhizal fungus; HM - heavy metal.
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(0, 0.05, 0.1 mM) and *2*) addition of root exudates. In Experiment 2, the interaction of exudates with higher Pb concentrations (0, 0.1, 0.5 mM) was tested. Experiment 3 comprised six treatments resulting from the combination of the following two factors: *1*) Mn concentration (0, 0.05, 0.1 mM) and *2*) addition of root exudates. The root segments were collected from 5-week-old maize plants inoculated with *Glomus intraradices* Schenck & Smith, isolate PH5. The isolate originates from Pb-contaminated waste disposal site near lead smelter Příbram (Czech Republic) and the lineage used was sub-cultured in metal-free substrate. The preparation of root segments for the experiments followed the method described by Gryndler *et al.* (1998). Pb and Mn were added to the media in the form of nitrate and sulphate, respectively. Root exudates were applied at the amount of 10 mg of lyophilised material per 50 cm^3 of media. The dissolution of exudates was supported by 2-min sonification followed by 5-min shaking. Then, incubation media containing root exudates were filtrated through a paper filter to remove insoluble compounds. 1 mM BIS-TRIS was used as a buffering substance in the incubation solutions and pH was adjusted to 6.3. The segments were incubated in 0.03-cm^3 drops of filter-sterilised solutions on the inside of lids of polystyrene Petri dishes. The dishes, each with 16 hanging drops of the medium with root segments, were incubated in the dark for 5 d at 25 °C. The proliferation of hyphae was observed under a microscope (magnification ×63). Each treatment involved 7 Petri dishes as replicates and one Petri dish represented an experimental unit. The length of proliferating hyphae per root segment was estimated using a grid-line intersect method and mean percentage of segments with proliferating hyphae was calculated. The results of experiments were analysed using two-way analysis of variance and comparisons between the means were carried out using the Duncan's multiple range test at $P < 0.05$.

Table 1. Effects of lead and root exudates on the percentage of root segments with proliferating hyphae and on the hyphal growth of the arbuscular mycorrhizal fungus *Glomus intraradices* PH5. Means followed by the same letters are not significantly different according to Duncan's multiple range test at $P < 0.05$, $n = 7$. *F*-values were significant at * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

	Pb [mM]	Root exudates	Proliferation of hyphae [% root segments]	Mean length of hyphae [mm root segment^{-1}]
Exp. 1	0	–	73 ab	5.1 a
	0	+	79 a	5.3 a
	0.05	–	56 b	3.4 bc
	0.05	+	80 a	5.6 a
	0.1	–	19 c	2.0 c
	0.1	+	84 a	4.5 ab
F-values	Pb		8.3 **	3.9 *
	exudates		39.2 ***	10.7 **
	Pb × exu.		11.6 ***	5.4 **
Exp. 2	0	–	83 a	9.2 ab
	0	+	75 a	12.1 a
	0.1	–	21 c	0.5 c
	0.1	+	60 b	6.8 b
	0.5	–	5 d	0.0 d
	0.5	+	6 d	0.1 d
F-values	Pb		151.0 ***	134.7 ***
	exudates		10.2 **	21.7 ***
	Pb × exu.		16.7 ***	12.9 ***

Table 2. Effects of manganese and root exudates on the percentage of root segments with proliferating hyphae and on the hyphal growth of the arbuscular mycorrhizal fungus *Glomus intraradices* PH5. Means followed by the same letters are not significantly different according to Duncan's multiple range test at $P < 0.05$, $n = 7$. *F*-values were significant at * - $P < 0.05$, *** - $P < 0.001$.

Mn [mM]	Root exudates	Proliferation of hyphae [% root segments]	Mean length of hyphae [mm root segment^{-1}]
0	–	88 a	9.9 a
0	+	95 a	11.8 a
0.05	–	50 c	2.9 c
0.05	+	87 a	9.7 a
0.1	–	5 d	0.9 c
0.1	+	70 b	5.9 b
F-values	Mn	56.7/***	32.6/***
	exudates	75.1/***	36.5/***
	Mn × exu.	16.3/***	3.5/*

Pb concentration and root exudates, as well as their interaction, influenced significantly the percentage of root segments bearing proliferating hyphae. The concentrations 0.05 and 0.1 mM Pb reduced this parameter by 24 and 74 %, respectively, in comparison to the control (Table 1). For the length of proliferating hyphae, the drop at 0.05 and 0.1 mM Pb was about 33 and 61 %, respectively. These results correspond with our earlier study where an inhibition of *G. intraradices* hyphal growth was observed at 0.01 mM Pb and only negligible growth was recorded at 0.5 mM Pb (unpublished data). In contrast, no inhibition of hyphal growth was observed when root exudates were added to the media. However, Pb concentration of 0.5 mM almost suppressed the growth of hyphae regardless of the exudates addition. The media containing 0.05 and 0.1 mM Mn decreased the percentage of root segments with proliferating hyphae to about 57 and 95 %, respectively (Table 2). Similarly,

inhibitory effect of Mn concentrations higher than 0.05 mM was shown for another AMF species, *G. claroideum* (Malcová *et al.* 2002). The addition of root exudates to the media with higher Mn concentrations resulted in a significant improvement of fungal development, but the effect of root exudates on Mn toxicity differed between the Mn concentrations tested. Mn toxicity was to a large extent eliminated at 0.05 mM Mn, whereas only partial amelioration was observed at 0.1 mM. In all experiments, root exudates did not display any stimulation of hyphal growth when root segments were incubated in the media without HM-stress. Similarly, the addition of the root exudates did not affect the proliferation of *G. claroideum* BEG23 (unpublished data). Likewise, Bécard and Piché (1989) did not observed any effect of root exudates alone on hyphal proliferation, but together with root volatiles a strong stimulation was demonstrated. In contrast, a stimulation of hyphal growth and branching caused by root exudates of mycotrophic plants has been reported (*e.g.* Elias and Safir 1987, Tawaraya *et al.* 1996).

The observed protective ability of root exudates may be ascribed to metal chelatation by organic compounds and also to metal precipitation with phosphate. However, the concentration of phosphorus in root exudates was so low in our study that it could not eliminate a significant part of metal, even in the case if all the phosphorus would be present in the form of dissolved phosphate ions. In conclusion, although it is difficult to relate the concentration of exudates in the incubation media to their levels in the rhizosphere, the results of the present study show the potential of root exudates to protect AMF from HM toxicity and demonstrate the mutual benefit of both partners from the symbiosis under HM stress.

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