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Nuclear Microprobe Studies of Elemental Distribution in Mycorrhizal and Nonmycorrhizal Roots of Ni-Hyperaccumulator *Berkheya coddii*

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Abstract. Micro-PIXE was used to assess the influence of arbuscular mycorrhiza on the elemental concentration and distribution in roots of *Berkheya coddii* and to evaluate the role of fungal hyphae in elemental uptake by this plant. Analysis revealed significant influence of mycorrhiza on the concentration and distribution of elements in roots of *Berkheya coddii*. Mycorrhizal roots had significantly higher concentration of P, Ca, Zn and Cu than the non-mycorrhizal ones. The mycorrhizal fungi also affected the distribution of elements (P, K, Cl, Zn, Ni) within the roots. The external fungal hyphae showed a high binding capacity of metals (Cu, Zn and Ni). Matrix corrections done on a basis of simultaneous use of proton backscattering technique were essential due to highly variable thickness of analyzed specimens.

Keywords: arbuscular mycorrhiza, elemental distribution, X-ray microanalysis, micro-PIXE

INTRODUCTION

Arbuscular mycorrhiza is the most common mutualistic symbiosis between fungi and plant roots. The principal function of mycorrhiza is an enhanced supply of essential nutrients from the soil by fungal mycelium [1, 2]. At the same time the mycelium could affect heavy metal transfer from the soil to plants [3, 4] and could be useful in phytoremediation techniques. The results on the influence of mycorrhiza on heavy metal uptake by plants are not consistent and could depend not only on the fungal species and isolates but also on plant species and cultivar [5]. Relatively little is known about the influence of mycorrhiza on elemental uptake, the distribution of elements within the roots and their translocation to the host tissues. More knowledge on the function of mycorrhiza is needed in developing phytoremediation methods.

In order to better understand a variety of phenomena in the field of fungal activity, the microanalytical techniques could be used. The localization of elements on the cellular and sub-cellular levels using one of such techniques gives important data on the distribution of elements and also provides a link between physiological and anatomical studies, which is especially important when studying responses of organisms to environmental extremes, such as presence of naturally occurring heavy metals or metals introduced by pollution [6].

A general advantage of microscale methods is low amount of material necessary for the analysis and possibility to control the physiological and developmental states at the ultrastructural level.

Recently, the presence of mycorrhizal symbiosis has been demonstrated in the Ni- hyperaccumulating plant, *Berkheya coddii* [7]. This South African endemic plant is growing on ultramafic soils and seems to be an excellent model to study phytoextraction and phytomining. The role of mycorrhiza in hyperaccumulating plants is poorly known.

The aim of this study was to assess the influence of arbuscular mycorrhiza on the elemental concentration and their distribution in roots of *Berkheya coddii* and to evaluate the role of fungal hyphae in element uptake by this plant.

MATERIALS AND METHODS

The seedlings of *B. coddii* plants were cultivated under laboratory conditions on sterilized ultramafic

soil and inoculated with mycorrhizal fungi or left noninoculated (non-mycorrhizal control). After three months, the plants were collected and washed with tap water. The pieces of roots and fungal hyphae were cryofixed by plunge-cooling in propane cooled by liquid nitrogen (Leica CPC) and freeze-dried (Leica CFD). Freeze-dried cross-sections of roots and hyphae were mounted between two layers of carbon-coated formvar film.

The elemental distribution in *B. coddii* roots and fungal hyphae (intermediate-thickness samples) was investigated with a nuclear microprobe at Materials Research Group, iThemba LABS, South Africa. Micro-PIXE (Particle Induced X-ray Emission) and BS (Backscattering Spectrometry) were simultaneously used. A proton beam of 3.0 MeV energy and current of 200 – 300 pA was focused to a 3 µm x 3 µm spot and scanned over selected areas. GeoPIXE II software package [8] was used for quantitative elemental mapping using *Dynamic Analysis* method complemented by data extracted from arbitrarily selected micro-areas of roots (whole root, epidermis, cortex, vascular tissues). Matrix corrections done on a basis of BS results were essential due to highly variable thickness of analyzed specimens.

Data were analyzed with the non-parametric Kruskal-Wallis test ($p<0.05$), Statistica 6.0, Statsoft.

RESULTS

The results revealed significant differences in elemental concentration between non-mycorrhizal and mycorrhizal roots (Fig. 1). The mycorrhizal roots were characterized by higher concentration of P, Ca, Zn and Cu. The concentration of P was 10 times higher in the mycorrhizal roots in comparison with the non-mycorrhizal ones, while the concentrations of Zn and Cu were 5 and 4 times higher, respectively.

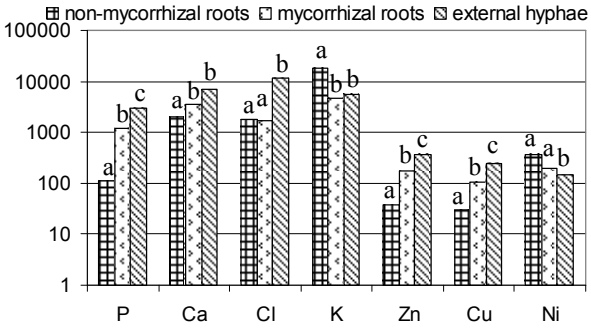

FIGURE 1. Elemental concentration in whole non-mycorrhizal and mycorrhizal roots and external fungal hyphae (averages in mg kg^{-1}); logarithmic scale. Different letters indicate statistically significant differences between external hyphae, non-mycorrhizal and mycorrhizal roots within each element.

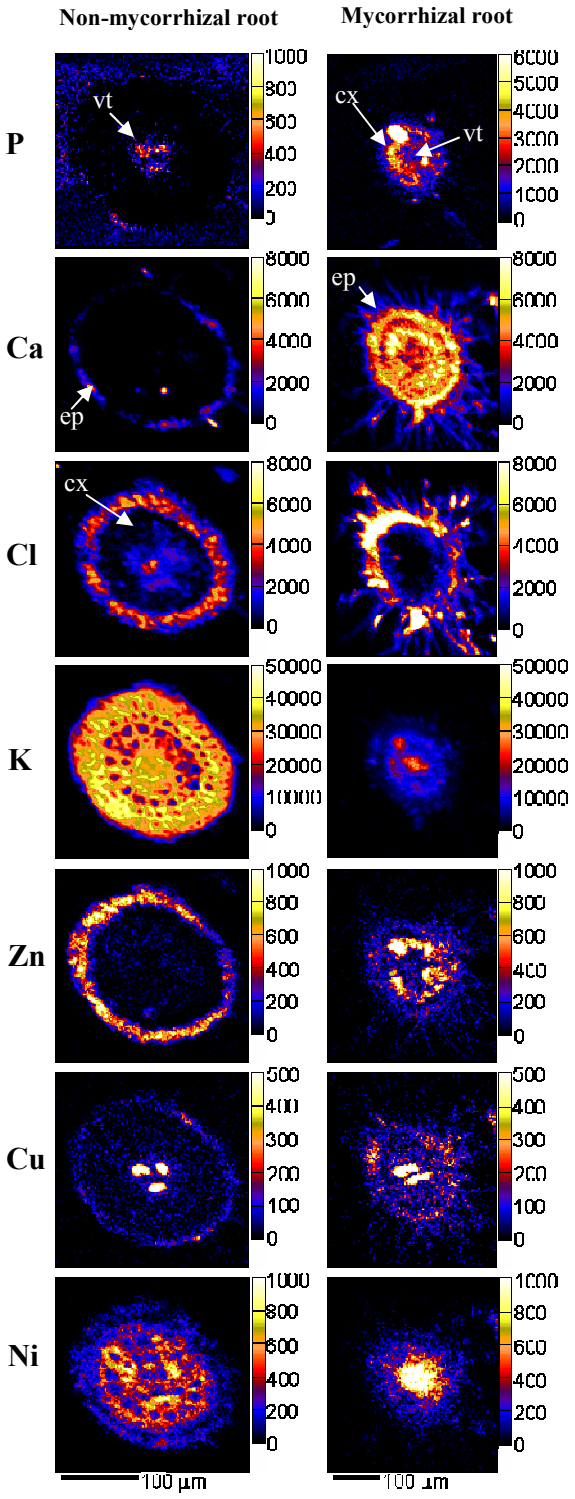

FIGURE 2. Elemental distribution in non-mycorrhizal and mycorrhizal roots. Concentrations reported in mg kg^{-1}; ep – epidermis, cx – cortex, vt – vascular tissues.

TABLE 1. Concentration of elements in selected non-mycorrhizal and mycorrhizal root regions. Concentrations reported in mg kg^{-1} ($\pm$ 1σ uncertainty) are based on PIXE and BS spectra extracted from respective regions.

elements	Non-mycorrhizal roots			Mycorrhizal roots		
	epidermis	*cortex*	*vascular tissue*	*epidermis*	*cortex*	*vascular tissue*
P	72±24	<7	560±30	1170±30	4289±97	3107±52
Ca	1970±20	1157±15	997±14	8580±90	4932±50	3869±31
Cl	2046±6	3629±15	2500±14	11380±180	2231±36	1041±34
K	18670±170	38910±210	29860±140	4200±40	6767±54	15750±130
Zn	369±9	150±6	30±2	243±9	343±7	104±8
Cu	46±1	29±1	242±12	148±11	89±3	1216±31
Ni	140±4	450±20	470±20	194±10	301±7	1127±34

Mycorrhiza did not influence the concentration of Cl and Ni in the roots, but negatively affected concentration of K (fig. 1). The results showed high concentration of elements within the external fungal hyphae. For all elements besides Ni, the values in hyphae were higher than those obtained in the mycorrhizal roots (fig. 1).

Elemental maps showed significant differences in the distribution of elements between mycorrhizal and non-mycorrhizal roots (table 1, fig. 2). The distribution of Ca and Cu in mycorrhizal and non-mycorrhizal roots was similar (table 1). In the case of Ca the highest concentration was found in epidermis. The highest concentration of Cu was found in the vascular tissue and very low concentration in the cortical layer. P was mostly concentrated in the cortex of mycorrhizal roots whereas in non-mycorrhizal roots the highest concentration was found in the vascular tissue (table 1, fig. 2). In the non-mycorrhizal roots the highest concentrations of K and Cl were observed in the cortex. In the mycorrhizal roots Cl was mainly distributed in epidermis whereas K had the highest concentration in vascular tissues (table 1) and cells containing the arbuscules (fig. 2).

The highest concentration of Zn in the non-mycorrhizal roots was found in the epidermis whereas in the mycorrhizal ones the highest concentration was present in the cortex (fig. 2). A common feature in the non-mycorrhizal and mycorrhizal roots was Ni depletion in the epidermis. For the mycorrhizal roots very high Ni concentration was observed in the vascular tissue. In the non-mycorrhizal roots there was no significant difference of Ni concentration between cortex and vascular tissue. The concentrations of P, Ca and Cu were higher in all regions of mycorrhizal roots than in the non-mycorrhizal ones.

DISCUSSION

The obtained results confirmed that mycorrhiza had influence on elemental uptake and distribution within plant roots and that arbuscular mycorrhizal fungi supply plants with essential nutrients from soil through uptake by extraradical hyphae. High concentration of nutrients in the fungal hyphae and higher concentration of P in the cortical layer of mycorrhizal roots as well as higher concentration of K, especially in cells containing the arbuscules, showed that the plant-fungi symbiosis functions properly under such extreme conditions. External hyphae showed high capacity of binding Zn, Cu and Ni, which could significantly influence the uptake of heavy metals by plants. Mycorrhizal plants were characterized by higher concentration of Cu and Zn than the non-mycorrhizal ones, but mycorrhiza had no effect on the total Ni content in roots of this Ni-hyperaccumulating plant. So far only few publications are available on heavy metal distribution at the cellular/sub-cellular level of plants growing on soil rich in heavy metals and being colonized by arbuscular mycorrhizal fungi [9-11]. All these reports, however, are related to plants that are not hyperaccumulators. Strong visible accumulation of Zn within the cortex of mycorrhizal roots, higher than in the vascular tissues, similarly as found by Kaldorf et al. [10] and Przybyłowicz et al. [11], suggests that mycorrhizal fungus was involved in Zn sequestration. This result is in accordance with data published by Tonin et al. [12] showing strong heavy metal binding capacity of the fungal mycelium colonizing the plant roots. Present results also show that mycorrhizal fungi significantly increased the concentration of Cu, but the highest concentration was found in the vascular tissues in which Ni content was also the highest (few times higher than in other root parts). Such distribution suggests that these two elements could be further delivered into the shoots. In case of *B. coddii* the role of mycorrhiza in increasing Ni uptake into shoots was already shown by pilot study based on AAS data [7].

In conclusion, in this Ni-hyperaccumulating plant mycorrhizal fungi play a filtering/sequestering role against elements such as Zn but not against Ni and Cu. At present it is not known what happens with heavy metals taken up by mycorrhiza and whether these elements could be transformed into non-toxic compounds. This also suggests the presence of additional mechanisms that may depend also on the plant enabling it to cope with metal transferred by the fungus.

Presented results confirmed usefulness of micro-PIXE in studies on microscale activity of mycorrhizal fungi.

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