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MICROBIOLOGICAL IMAGE

The microPIXE technique to understand the distribution of heavy metals in arbuscular mycorrhizal symbiosis

Uso de la técnica de microPIXE para comprender la distribución de metales pesados en la simbiosis micorrícica arbuscular

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Soil colloids tend to retain heavy metals (HMs), potentially reaching toxic concentrations. Phytoremediation, a decontamination technique, involves plants and their associated microorganisms to mitigate the toxicity of HMs in the environment. Most plant species employed in phytoremediation establish symbiotic associations with arbuscular mycorrhizal fungi (AMF). AMF can adsorb or uptake HMs through their hyphal network, translocating them towards the host plant[1]. Particle-induced X-ray emission with a micro-focused beam (microPIXE) enables the micrometric mapping of elements in a sample[2], helping to determine the concentration and distribution of HMs. In this work, we used microPIXE to analyze the distribution and accumulation of copper (Cu) in structures of the AMF *Rhizophagus intraradices* GC3 strain associated to Ri T-DNA carrot roots under *in vitro* condition (*Banco de Glomeromycota in vitro*)[3]. This would enable us to understand the role of AMF in HM-polluted soils. An *in vitro* assay was performed using bi-compartmentalized Petri plates. The root compartment (RC) contained solid minimal medium (MM) with transformed carrot roots colonized by GC3. The hyphal compartment (HC), where only AMF developed, contained sterile soil artificially contaminated with Cu (300 mg/kg)[4]. After three months, roots and intraradical vesicles from RC and extraradical mycelium from HC were sampled for microPIXE analysis. The map of Ca, a major element in biological systems, helped us to visualize the biological structures and identify other elements; P and Mn (a MM compound) were selected as AMF structure indicators. P, Mn and Cu, were mainly detected in vesicles, and to a lesser extent in colonized roots (considering the dimensions of the structures), while P and Mn were mostly localized in the extraradical mycelium (Fig. 1). These results showed significant Cu accumulation in intraradical fungal structures but not in root cells, suggesting a potential mech-

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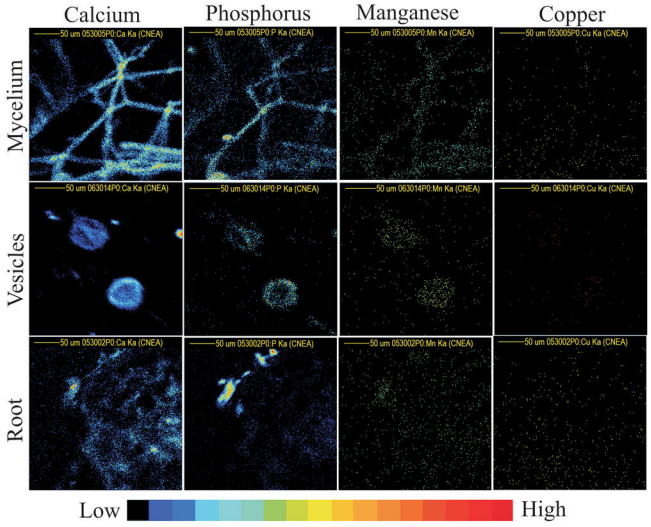

Figure 1 2D X-ray emission distribution maps of Ca, P, Mn and Cu from the extraradical mycelium and vesicles of the arbuscular mycorrhizal fungus *Rhizophagus intraradices* GC3 strain and a cross section of a mycorrhized carrot root. MicroPIXE conditions: 50 MeV $^{16}O^{5+}$ beam scan size 250 μm × 250 μm and 200 μm × 200 μm, respectively. Spot size 3 μm × 3 μm. The colour scale, from blue to red, represents the increase in the concentration of the chemical elements.

anism of toxicity alleviation in the host plant, and laying the foundation for future studies focusing on Mn.

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Conflict of interest

Authors have no conflict of interest.

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