Cross-validation of XAFS with non-synchrotron radiation-based methods to evidence the implication of amino acids in copper complexation on plant roots

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ABSTRACT: Recent results suggest that the minimum rhizotoxicity of metal cations is mainly due to a non-specific mechanism related to the binding strength of metal cations to hard ligands in the root cell walls, especially to the carboxyl groups located within the pectic matrix. However, among metal cations, copper (Cu) belongs to the four metal cations that exhibit a rhizotoxicity higher than that estimated from the binding strength to carboxyl groups. Reexamining this theory involving hard ligands, we investigated the speciation and the sorption of Cu in root apoplast to evaluate whether amine groups significantly contribute to Cu binding in addition to carboxyl groups.

As it is difficult to ascertain the distinction between oxygen (O) and nitrogen (N) ligands using X-ray absorption fine structure spectroscopy (XAFS), this method was coupled with two non-synchrotron radiation (SR)-based methods, namely: (i) the modelling of proton and Cu sorption isotherms and (ii) ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. These three methods were used to investigate the sorption and speciation of Cu on the isolated cell walls and the whole roots of a monocotyledon (*Triticum aestivum* L.) and a dicotyledon (*Solanum lycopersicum* L.).

XAFS revealed a homogeneous speciation of Cu in the root apoplast of both species. N/O ligands presumably associated to both carboxyl and amine groups of amino acids were identified in slightly higher proportion than single O ligand assimilated to carboxyl groups of polysaccharides. In agreement, the modelling of proton and Cu sorption isotherms enabled to distinguish two types of binding sites: (i) low-pKa (< 7.5) and low-affinity (logK_{Cu} = 2.1-3.9) sites representing at 40 % of total sites and presumably corresponding to carboxyl groups and (ii) high-pKa and high-affinity (logK_{Cu} = 5.9-7) sites presumably corresponding to amine groups. A semi-quantitative analysis of the ^{13}C-NMR spectra of whole roots exposed to increasing Cu concentration revealed that the surface area of the peak around 172 ppm progressively diminished by 10 to 30 %, thus demonstrating the contribution of carboxyl groups in Cu binding. In addition, a progressive decrease in the surface area of the peak around 62 ppm in wheat roots likely traduced the contribution of amino acids in Cu binding.

By coupling SR and non SR-based methods, we confirmed the dual local environment of Cu in the apoplast of plant roots. If cell wall pectins contribute to Cu binding as usually expected, Cu speciation was (co)-dominated by amino acids embedded in root apoplast. The high affinity of amino acids for Cu leads us to further infer that their contribution may help to explain the unexpectedly high rhizotoxicity exhibited by Cu.