ESRF	**Experiment title:** Zinc tolerance and accumulation traits in the hyperaccumulating plant *Arabidopsis halleri*	**Experiment number**: 30-02-806
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Report:

Introduction

Metal hyperaccumulation by higher plants is a rare and scientifically interesting phenomenon. Metal hyperaccumulators are able to accumulate metal(s) in the aboveground parts to concentrations two to three orders higher than normal plant species. A great effort is being done to better understand the genetic and physiological implications of metal tolerance and hyperaccumulation, in the perspective to use these plants in phytoremediation. *Arabidopsis halleri* is a Zn and Cd hyperaccumulator present in contaminated and non-contaminated soils of Europe. One of its major interests is that its genome is close to *A. thaliana*, the well known genetic model for higher plants, so the molecular tools developed for *A. thaliana* (genetic map, gene ships, putative genes involved in metals homeostasis…) can be used for *A. halleri*. Recent results (Willems et al., accepted) showed that Zn tolerance and accumulation are governed by at least 3 and 5 genes, respectively, one of them being common to the two traits.

In this experiment, we have studied the chemical form of Zn accumulated in a crossing between the tolerant and hyperaccumulating species *A. halleri* and the non tolerant non hyperaccumulating species *A. lyrata*. Plants of the second one (F2) present a variety of phenotypes where tolerance and accumulation are segregated. The purpose of this experiment was to link the chemical form of Zn with genetic information on tolerance and hyperaccumulation traits.

Materials and Methods

Parent plants (*A. lyrata and A. halleri*) and F1 and F2 progeny plants were grown on Zn-contaminated compost for 5 weeks in controlled conditions at the Laboratory of Plant Physiology & Molecular Genetics (Brussels). After harvesting, leaves were frozen in liquid N2 and prepared as frozen hydrated pellets using liquid N2 for the grinding and pressing. Zn K-edge EXAFS spectra were recorded at 5-10°K using a Helium cryostat available on FAME beamline, in fluorescence mode using a Canberra 30-element detector. Depending on Zn content, 5 to 12 spectra of 40 min each were averaged. Data were treated by linear combination fits and FEFF simulations for the first and second shell.

Results

Figure 1 B shows the EXAFS spectra for the plant leaves and their linear combination fits using 3 components. The spectra for *A. halleri* in freeze-dried and frozen hydrated state are clearly different. The former was simulated with 100% Zn malate in solid state, and the latter with Zn-malate in solution as major species, and another Zn-organic acid complex in solution and Zn phosphate. Thus, the dehydration treatment modifies Zn speciation. All other spectra were simulated with a combination of Zn-organic acid complexes in solution, Zn-cell wall complexes and Zn phosphate.

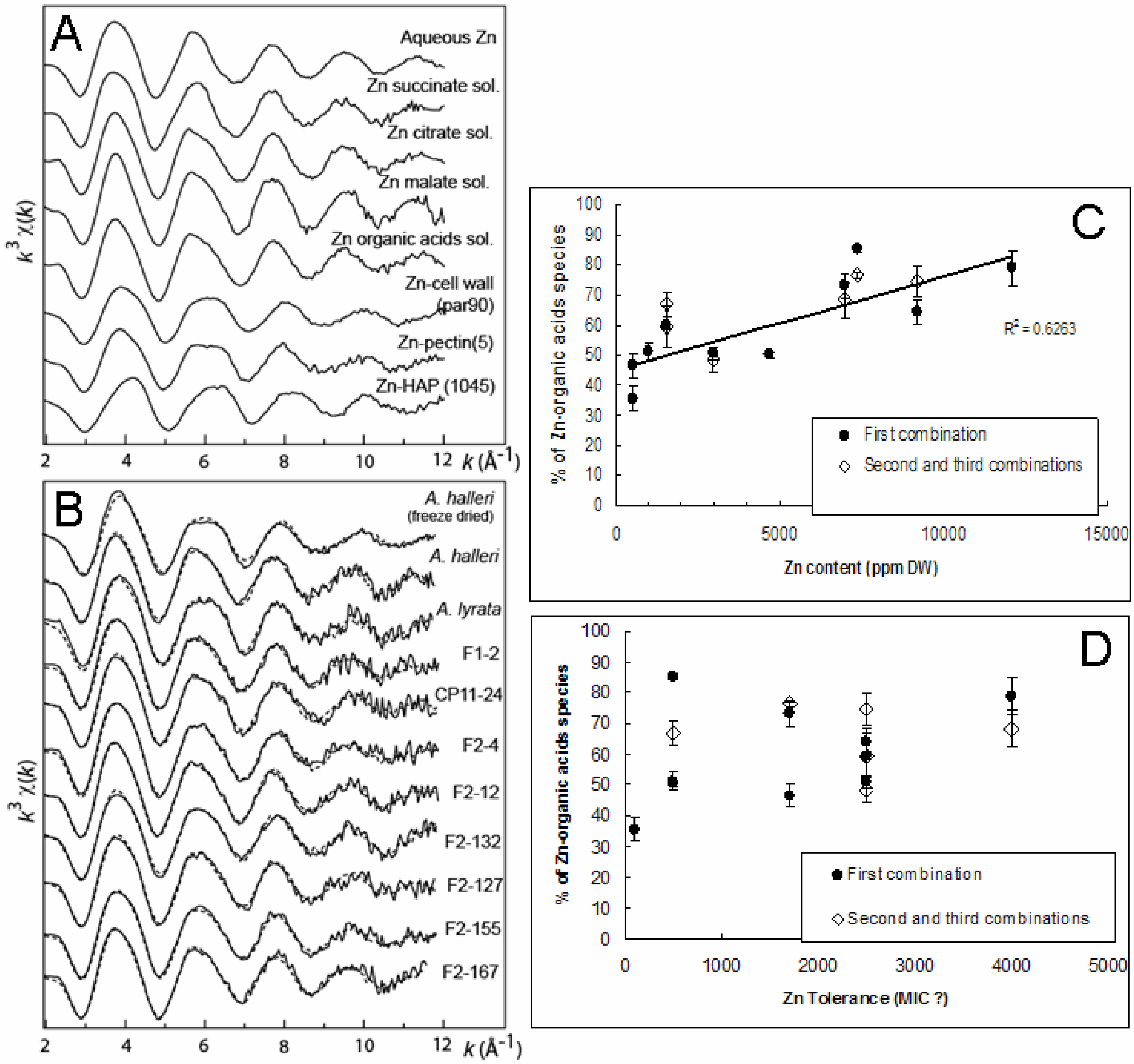

Figure 1: A, Zn K-edge EXAFS spectra of selected Zn species used in the linear combination fits. B. Zn K-edge EXAFS spectra of the leaves (plain lines) and best linear combination fits (dashed lines). All plant samples were analyzed in frozen hydrated state except, one spectrum of *A. halleri* recorded freeze-dried (sample A. h-C, 250 µM in Sarret et al., 2002). C and D: relationship between the proportion of Zn-organic acid complexes determined by LCF and the accumulation and tolerance traits of each plant.

The proportion of Zn-organic acid complexes is correlated with Zn accumulation in the shoots (Fig. 1C), but not with Zn tolerance (Fig. 1D). This is consistent with a storage of Zn in the vacuoles for the most accumulating plants following active thansport through the vacuole membrane, as suggested by molecular genetics studies (Clemens et al., 2006). No correlation was found between the proportion of Zn phosphate or Zn-cell wall and the accumulation or tolerance traits.

Scientific production related to this experiment

Huguet S., Bert V., Laboudigue A., **Isaure** MP., **Sarret** G., Cd localization and speciation in a contaminated sediment and in the Zn, Cd hyperaccumulating plant Arabidopsis halleri, 9*th International Conference on the Biogeochemistry of Trace Elements (ICOBTE*), 15-19 July 2007, Beijing, China.

Sarret et al., Are Zn localization and speciation in plant leaves related to Zn tolerance and accumulation traits? An interspecific study, manuscript in prep.

Straczek A, **Sarret** G., Manceau A., Hinsinger P, **Geoffroy** N., Jaillard B, Zinc distribution and speciation in roots of various genotypes of tobacco exposed to Zn, Environmental Experimental Botany 63, 80-90 (2008).

References

Clemens S., 2006, Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* **88**(11), 1707-1719.

Sarret G., Saumitou-Laprade P., Bert V., Proux O., Hazemann J. L., Traverse A., Marcus M. A. and Manceau A., 2002, Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol*., 130, 1815-1826.