	Experiment title: Zn and Fe homeostasis in *Arabidopsis thaliana*: novel molecular factors and their influence on metal speciation and localization	**Experiment number:** EV57
Beamline: ID21	**Date of experiment**: from: 29 Jan 2014 to: 03 Feb 2014	**Date of report:** 24/02/2014
Shifts: 15	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Report:

This experiment was designed to obtain additional insight into the underlying mechanisms of Fe homeostasis in *Arabidopsis thaliana* model plant by comparing wild type (WT) seedlings and mutants created in the department of Plant Physiology in Bochum University. These mutants were first phenotypically characterized in the lab and the most interesting mutant (*roz1*) was studied during this experiment. The *roz1* mutant was recovered during an EMS mutant screen to identify plants lacking the appropriate transcriptional response to high Zn conditions. It is now well-known that Fe and Zn homeostasis are closely linked. Preliminary ICP-AES data demonstrated that this mutant accumulates higher concentrations of Fe and Zn in both root and shoot and a lower concentration of Mn in shoot compared to WT.

For this experiment, seedlings were grown in plates on high Fe (50 µM) for 15 days. After exposure, leaves and roots were embedded in OCT resin and flash-frozen in isopentane cooled with liquid nitrogen (LN). Cross-sections (35 µm) were performed on ID21 cryo-microtome just before analysis. Whole plant tissues were also analyzed. Mapping was performed in cryo-conditions using a vibration-free cryo-stage in fluorescence mode using a Silicon Drift Detector. The beam size was 0.3 x 1 µm. µXRF maps were recorded at 7.2 keV and µXANES spectra between 7.1 and 7.25 keV (0.5 eV step) on Fe-rich regions. Fe model compound spectra were recorded during a previous experiment (EV32). µXRF data were processed using PyMCA software to extract elemental maps, and µXANES spectra were analyzed by linear combination fits of standard spectra using Athena software.

In addition, preliminary results have been obtained on another promising mutant with disrupted Ca distribution (*dez*) when growing on Zn rich medium. Both µXRF maps (on frozen hydrated tissue) and FTIR spectra (on freeze-dried cross-sections) were recorded.

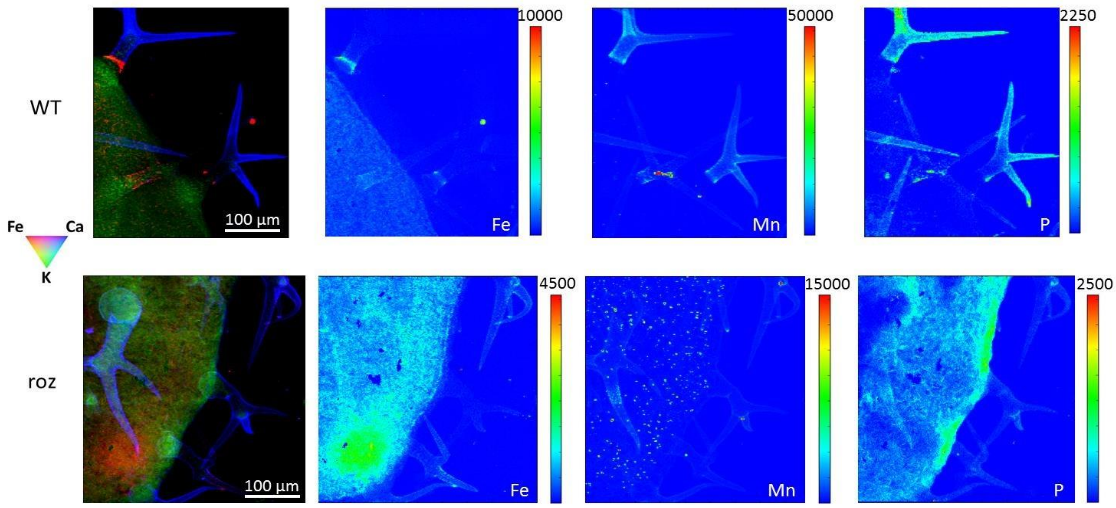

Figure 1. Whole leaf analysis by µXRF at the Fe-K edge for the WT top panel and the mutant (*roz1*) bottom panel.

Whilst in roots, elemental distribution does not seem to be modified (data not shown), in leaves Fe, Mn and P distributions are dramatically altered in *roz1* (Figure 1).

In the WT, trichomes present high concentrations of Mn and P and a ring of Fe at the trichome base (Figure 1 top panel). In the mutant, grown in the same conditions, Fe was more concentrated in leaf tissue and no rings were observed at the base of the trichomes. Mn was detected in high intensity spots in the leaf tissue. Likewise, P fluorescence was higher in the leaf rather than in trichomes.

Not only was Fe distribution changed, but also its speciation (Figure 2). The predominant form of Fe in the WT as fitted was Fe(II) (around 65%) whereas in the mutant Fe(III) was the main chemical form of Fe (more than 70%).

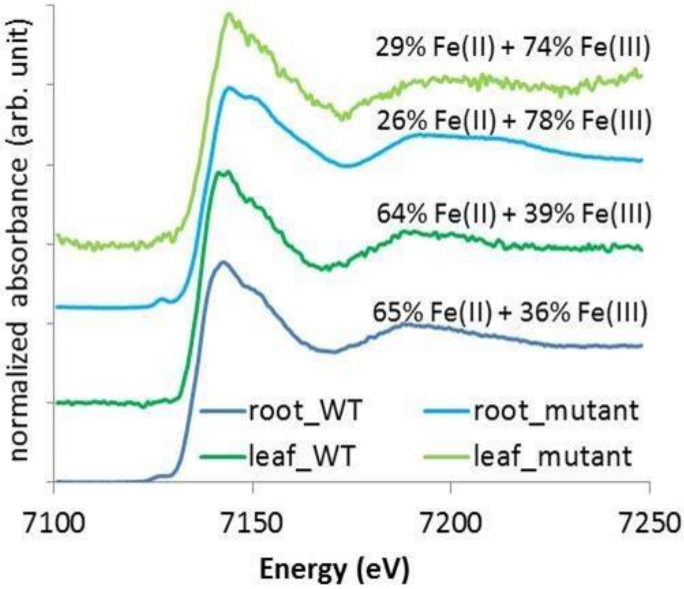

Figure 2. µXANES spectra recorded on roots and leaves of WT and mutant (*roz1*) with corresponding linear fitting combination

This beamtime was also used to study the *dez* mutant that has a photomorphogenic phenotype when grown in the dark on Zn-rich medium. µXRF maps were acquired showing a drastic difference in Ca distribution between WT and *dez* seedlings. Preliminary results suggested cell wall modifications in mutants grown on high Zn. To investigate this hypothesis WT and *dez* stem cross-sections were analyzed by µFTIR. First results show clear increase of cell wall components (Figure 3), in particular in the carbohydrate region (800 to 1200 cm^{-1}) with cellulose, pectin, rhamnogalacturonan and xyloglucan peaks. Next, we intend to treat the data for partial least squares for discriminant analysis using the variables selected by the Bayesian information criteria for dimension reduction.

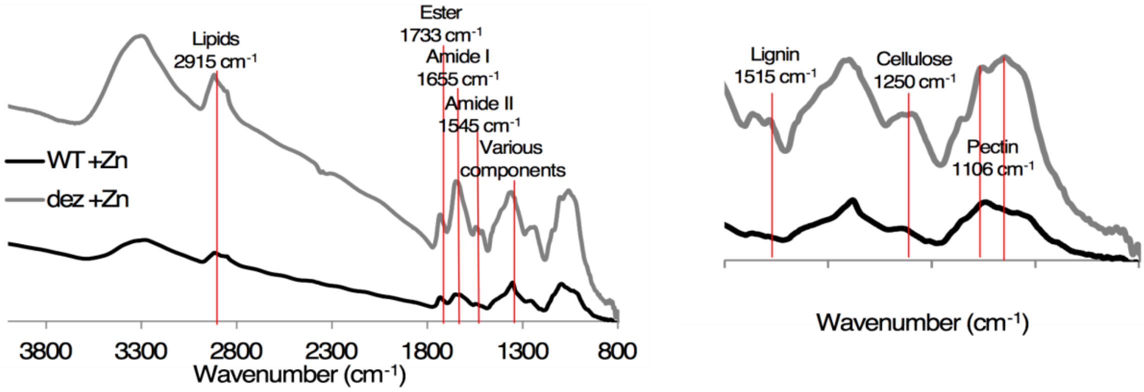

Figure 3. Raw comparison between spectra acquired on WT and *dez* hypocotyl cross-sections for plants grown in high Zn medium.

Scientific production related to this experiment

Results obtained during this beamtime are very promising and investigation of Zn distribution would be needed to have a complete understanding of these two mutants. Fe chemical environment would also need to be studied more deeply by EXAFS. These results are part of two different projects at the host institute and will be published in two articles (one is already under redaction).