Experiment Report Form

ESRF	**Experiment title:** Fe homeostasis at the cell level in the model plant Arabidopsis thaliana	**Experiment number**: EC 713
Beamline: BM08 (GILDA)	**Date of experiment**: from: 24/11/2010 to: 29/11/2010	**Date of report**: 14/03/2011
Shifts: 15	**Local contact(s)**: Angela Trapananti	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Marie-Pierre Isaure*, LCABIE/IPREM UMR 5254, CNRS and Université de Pau et des Pays de l'Adour

Stéphane Mari*, Louis Grillet*, BPMP UMR 5004, INRA, CNRS, Université Montpellier II, SupAgro.

Report:

Introduction:

Iron (Fe) is an essential metal ion for plants, but the control of Fe distribution at organ and cell levels has been poorly understood. Preliminary histochemical studies on cells of embryos from two model plants, *Arabidopsis thaliana* and peas (*Pisum sativum*), indicated that beside the expected localization of Fe in plastids and cell walls, the nucleus contained high concentrations of Fe, which was quite unexpected. The objective of this proposal was to check this localization using a different sample preparation coupled to element detection to clarify the Fe homeostasis at the cell level. For that, chemical imaging with µ-XRF combined to XANES/ EXAFS is suited to localize the metal in cells and obtain information on its status redox and chemical forms. Thus, we applied for beamtime on ID21 beamline, dedicated to µ-XRF and µ-XANES experiments. Alternatively, we proposed to use ID22, which allows chemical imaging with a lateral resolution below the micrometer but does not allow spectroscopic measurements, combined to FAME or BM08 where XAS experiments are feasible on bulk samples. We only obtained beamtime on BM08, so we investigated bulk samples and Fe references compounds using XANES or EXAFS at the Fe K-edge. We expected to determine the redox status of Fe and its chemical forms in isolated compartments of peas embryos and *Arabidopsis thaliana*, as well as on mineral and organic model compounds.

Experimental:

Wild type and mutants of *pisum sativum* (dgl mutant where Fe is increased) and *A. thaliana* (double *ysl4ysl6* knock-out mutant in Arabidopsis impaired in chloroplastic nicotianamine and Fe transport) were germinated and grown in controlled laboratory. Peas embryos were collected, ground and prepared as frozen pressed pellets. Embryos nuclei were extracted by centrifugation and also prepared as pressed pellets while embryo liquid was collected and analyzed as liquid samples. Leaves of *A. thaliana* were prepared as frozen pressed pellets. Frozen samples were then transferred in the analysis chamber cooled at 150 K in frozen state, which was a first on Gilda beamline. Solid and liquid Fe model compounds were also measured.

EXAFS and XANES spectra were collected at Fe K-edge in transmission or fluorescence mode (using a 13 element solid state detector) depending on Fe concentration.

Results:

XAS spectra of embryo liquids collected of wild type and dgl mutant of peas show that the Fe chemical forms are similar in both species (Figure 1), and that the redox status of Fe is FeIII. Thus, although dgl mutant accumulates more Fe, the Fe chemical forms are the same than in the wild type. The identification of the Fe species is still in progress, but data suggest that Fe is present as organic acids and/or nicotianamine (carboxylic and amino groups). Fe-DNIC (DiNitrosyl Iron complexe) mentioned in living organisms in the bibliography is ruled out. More Fe references of organic compounds and particularly organic acids (malate, oxalate etc…) are necessary to progress in the identification of Fe species.

It was not possible to collect correct XAS spectra on whole peas embryos and extracted nuclei nor on *A. thaliana* due to the low Fe fluorescence counts. However, preliminary measurements performed on cross-sections of peas embryos by µ-XRF and µ-XANES (Lucia beamline at Soleil) showed that Fe in the nucleus was high and it was possible to collect µ-XANES spectra in this compartment: Fe is present in the nucleus as both Fe(II) and Fe(III) but the chemical forms are still unknown (Figure 2). Here, the measured signal was spoiled by metallic Fe diffused by the sample holder (probably screws). The extraction of nuclei has also to be improved.

As a conclusion, these first measurements allowed to collect EXAFS spectra of various Fe references, and of embryo liquids. To investigate the other isolated compartments, a beamline dedicated to diluted systems and equiped with a sensitive detection system is required.

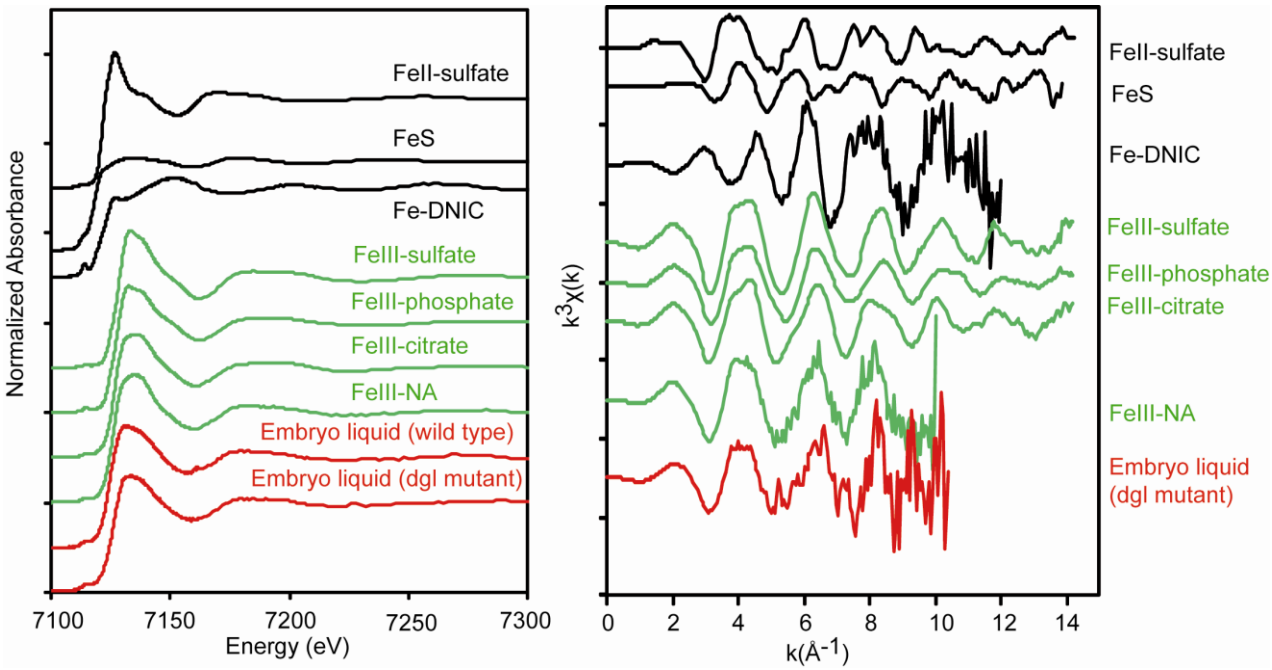

Figure 1: Fe K-edge normalized XAS spectra (Left) and EXAFS spectra (Right) of some mineral and organic Fe model compounds compared with embryo liquid of *pisum sativum* (wild type and dgl mutant).

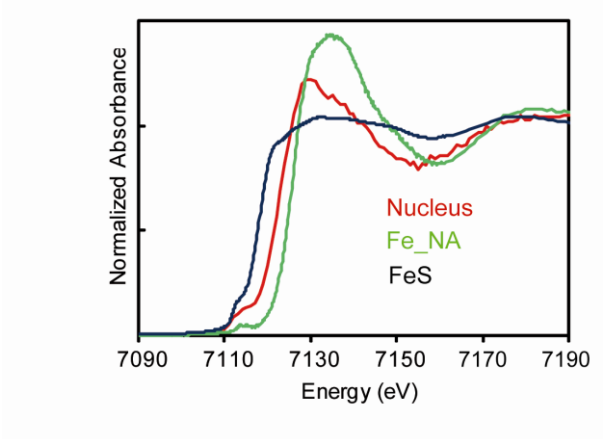

Figure 2: Fe K-edge µ-XANES spectrum collected on Fe-enriched nucleus compared to a Fe(II) model compound (FeS) and a Fe(III) one (FeIII-nicotianamine).