EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **The role of phytochelatins in Cd tolerance and hyperaccumulation in hyperaccumulator A. halleri**	**Experiment number**: LS-2403
Beamline: ID21	**Date of experiment**: from: 24/6/15 to: 30/6/15	**Date of report**: 18/10/17
Shifts: 18	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Stephan Clemens, Department of Plant Physiology, Universitaet Bayreuth Universitaetsstrasse 30, DE-95440 BAYREUTH

***Stephan Höreth,** Department of Plant Physiology, Universitaet Bayreuth Universitaetsstrasse 30, DE-95440 BAYREUTH

***Paula Pongrac**, Department of Plant Physiology, Universitaet Bayreuth, Universitaetsstrasse 30, DE-95440 BAYREUTH, current address: The James Hutton Institute, Invergowrie, Dundee, UK

***Katarina Vogel-Mikuš**, Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna Pot 111, SI-1000 LJUBLJANA

***Iztok Arčon**, University of Nova Gorica, Vipavska 13, PO Box 301, SI-5000 NOVA GORICA

Report:

INTRODUCTION – Under conditions of metal excess the phytochelatin (PC) pathway represents a major metal tolerance mechanism in plants. To plants, cadmium (Cd) is toxic even at low concentrations (<10 μg g^{-1} dry weight). Some plants are able to tolerate and accumulate >100 μg Cd g^{-1} in their shoots. The role of PC in these plants remains unresolved, therefore phytochelatin synthase (PCS) knockdown lines (PCS-RNAi lines) with more than 90% (strong lines) or more than 60% (intermediate lines) reduction of PCS transcript were generated for a Cd hyperaccumulating plant, *Arabidopsis halleri.* These lines had significantly decreased shoot biomass when grown in 10 μM Cd for 6 weeks in hydroponics indicating a role of AhPCS in Cd hypertolerance. The aim was to study Cd distribution and Cd local chemical environment in these lines and in non-transgenic control plants to conclude on the role of PC in Cd tolerance in this plant.

EXPERIMENTAL – Plants (non-transgenic control line (CL), strong (2.5RNAi) and weak (3.5RNAi) PCS RNAi lines) were grown in hydroponics without or with 10 μM Cd (as Cd sulphate). Fresh plant material was frozen in propane cooled with liquid nitrogen and cryo-sectioned at -25 °C. The measurements were performed using the SXM set-up equipped with cryostat. The excitation energy for the scan was first set to 3.55 keV (i.e. above the Cd- LIII edge) to record maps of Cd, P, S and Cl simultaneously below the potassium K edge in order to avoid the strong K signal. Finally, the Cd LIII – edge XANES spectra were recorded in different plant tissues and cells, depending on local Cd concentrations, to determine Cd chemical environment. CdLIII–edge XANES spectra of standard reference materials were also measured.

RESULTS – Two dimensional maps of Cd distributions in roots, petioles and leaves were generated at E=3.55 keV (e.g. **Fig. 1A,** distribution of Cd in red, S in green and P in blue) and revealed homogeneous distribution of Cd in petioles and leaves, while in roots, Cd was located in cortex. Cd-LIII edge XANES spectra were recorded on selected hotspots in different tissues (in control line 14 spectra in roots, 18 in petioles and 24 in leaves and in 2.5RNAi line 13 spectra in roots, 16 in petioles, 27 in leaves); selection of spectra is shown in **Fig 1B**). All XANES spectra measured on plant tissues can be described as linar combination of three reference XANES spectra, measured on standard reference Cd compounds, namely Cd-

glutathione (representing Cd-S complex), Cd-pectin and Cd-oxalate (both representing Cd-O complex). Plant organs and tissues differed in relative amounts of Cd ligands, from intermediate amount of Cd-S ligands in roots (**Fig. 1C)**, to predominant Cd-S ligands in leaves (**Fig. 1D**) and only minute amounts of Cd-S ligands in trichomes (**Fig. 1G**). In general, roots and petioles of 2.5RNAi had smaller amounts of Cd-S ligands than the control line, while in leaves the 2.5RNAi line had greater amounts of Cd-S than the control line. Analysis of weak PCS RNAi line 3.5 was performed only in bulk (whole leaves and roots). No differences in Cd-S contribution between the weak PCS RNAi line 3.5 and the control line were observed, therefore detailed analyses were not pursued.

Figure 1: Cadmium localisation and ligand environemnt in tissues of *Arabidopsis halleri* control line and strong PCS-RNAi line (2.5RNAi). A) Co-localisation of Cd, P and S in cross-section of a petiole of control line, B) examples of Cd-LIII edge XANES on roots, petioles, leaves, trichomes and the reference compounds used for linear combination fits: C) Cd-LIII edge XANES on root of 2.5RNAi line, D) Cd-LIII edge XANES on leaf vein of control line, and G) Cd-LIII edge XANES on trichome of 2.5RNAi line. The relative amount of each component in the linear combination fit is given in parentheses.