ESRF	**Experiment title:** **Transfer and accumulation of cesium by *Arabidopsis thaliana* vegetal cells and plants**	**Experiment number**: SC 1351
Beamline: ID 21	**Date of experiment**: from: October 30, 2003 to: November 04, 2003	**Date of report**: July 29th, 2004
Shifts: 15	**Local contact(s)**: Barbara FAYARD	*Received at ESRF:*

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Report:

Introduction

Cesium (Cs) was largely released in the environment during the Chernobyl accident in April 1986, and as a result, is detected in many European soils as a diffuse pollution. The use of higher plants to extract metals accumulated in soils (phytoextraction) might represent a low cost alternative strategy. However, a better understanding of the mechanisms responsible for metal tolerance and accumulation is needed to improve the efficiency of this technology. We study the capacity of *Arabidopsis thaliana*, chosen as a model organism because of the complete knowledge of its genome, to accumulate Cs.

The aims of our synchrotron radiation experiments were (i) to identify the cellular distribution of Cs in both *A. thaliana* individual cells and plant tissues, and its evolution with time (ii) to compare Cs distribution with chemical elements entering the composition of the cells and tissues to evidence chemical associations and/or competitive effects, and (iii) to identify the Cs coordination environment (speciation).

Samples and experimental setup

Suspensions of *A. thaliana* cells were cultured in controlled conditions in a nutritive MS (Murashige and Skoog) medium containing 20mM K or 0mM K and contaminated with 1mM CsCl. After four days of treatments, cells were sampled, rinsed with deionised water and deposited on an ultralene film. They were then cryofixed in isopentane chilled with liquid nitrogen and freeze dried at –36°C. *A. thaliana* seeds were sown on MS agar in Petri dishes and grown in controlled conditions. After 7 days, they were transferred onto the same cultured media containing normal (20mM K) and depleted K media (0mM K) contaminated with 1mM CsCl. Plants were collected after 6h, 24h and 4 days of treatments, rinsed in deionised water, frozen in liquid nitrogen, and freeze dried at –36°C. Cell and plant samples were disposed between two ultralene films for X-ray measurements.

Measurements were performed on ID21 using the Scanning X-ray Microscope (SXM) in fluorescence mode and under vacuum. Elemental maps were obtained for P, S, K, Ca and Cs by recording the X-ray fluorescence with a Ge solid state detector and scanning the samples under a 5.80 keV monochromatic beam, and a beam size on the sample of H=0.7µm x V=0.3 µm.

Cs LIII-edge (5.02 keV) µ-XANES spectra were collected on points-of-interest selected from the elemental maps and on 'bulk' powdered samples. Measurements were performed in fluorescence-yield detection mode using the same Ge-detector.

Results

Elementals maps recorded on vegetal cells treated with 1mM Cs, 20mM K showed that Cs and K have the same distribution, and are mainly located on grains of the cells, probably plastes (Fig. 1). Measurements performed on cells treated with 1mM Cs, 0mM K showed a similar elemental distribution (data not shown) but the ratio Cs/S increased in the carenced K medium, indicating that the proportion of Cs intering the cell increased.

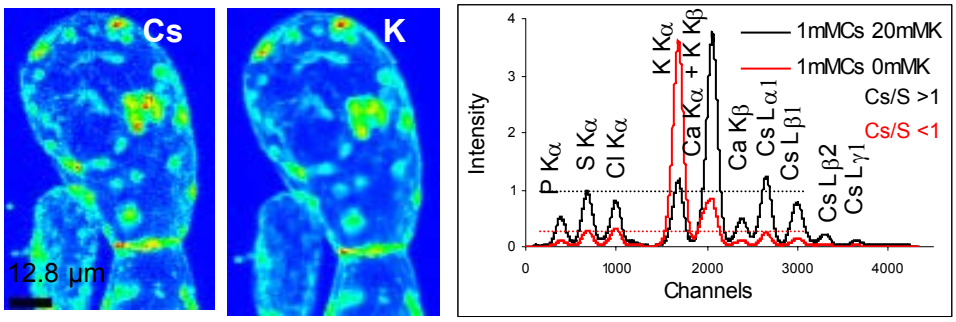

Fig 1 : Cs and K maps for *A. thaliana* cells treated with 1mMCs, 20mMK, and X-ray fluorescence spectra for 20 mMK and 0mMK. Beam size: 0.7 x 0.3 µm. Energy : 5.80 keV. Step size: 0.6 µm, Dwell time: 500 ms.

In plants, Cs and K also follow a similar localization, and are found in canals in roots and stems. In leaves, they are mainly located at the base of trichomes, epidermial hair on leaves (Fig. 2). Experiments performed on shorter time of treatment (6h and 48h) indicated lower concentrations of Cs in plants, but an identical elemental distribution (data not shown). Results obtained on plants grown on K-depleted media showed that the proportion of cesium entering the plant increased, attesting the competition between Cs and K.

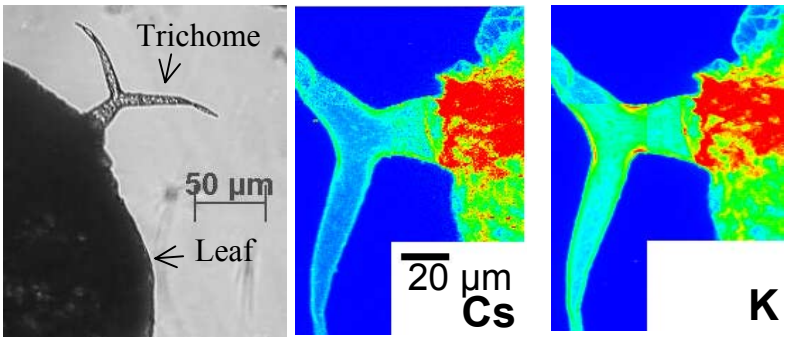

Fig 2 : Optical image of trichome and corresponding Cs and K fluorescence maps for *A. thaliana* plant treated with 1mMCs and 20mMK. Beam size: 0.7µm x 0.3µm. Energy : 5.80 keV. Step size: 0.6 µm. Dwell time: 300 ms.

Cs LIII-edge µ-XANES measurements collected on bulk powdered cells and selected areas of plant treated with 1mMCs, 20mMK (Fig. 3) showed that spectra have similar structure whether they were collected on cells, total leave, vein, trichome, stem and root, suggesting that the chemical form of cesium, probably Cs^+, is unique in the plant and cell.

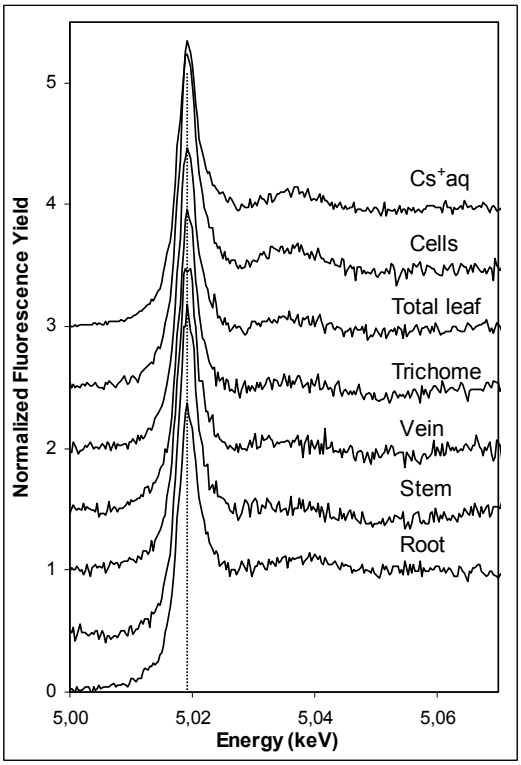

Fig 3 : Normalized Cs LIII-edge µ-XANES spectra from cells and different tissues of *A. thaliana* samples treated with 1mMCs and 20mMK, and from reference Cs^+.

Reference related to this work:

Isaure MP, Le Lay P, Fayard B, Barthès V, Bourguignon J. 2003. Accumulation du césium chez *Arabidopsis thaliana* : étude macroscopique et microscopique par µSXRF et µXANES. 3ème Journées Scientifiques de l'Institut des Métaux en Biologie de Grenoble, Autrans, France, 8-9 Décembre (Poster) Volume des résumés, p.51.