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Plant autotrophy for mineral nutrition is a key feature for life. Despite this important issue, the mechanisms involved in ion sensing remain unknown in plants. Phosphate homeostasis is a key process in plants and it is highly regulated depending on the phosphate availability in the medium, the metabolic demand of the plant and the phosphate content in tissus or cells of the plant. Free phosphate in the cells triggers the gene response depending on its concentration and on the cell layer of the root. Taking into account the fact that inorganic phosphate can represent a large portion of total P in the cell, our aim is to visualize separately phosphorus (P) and free phosphate in the different cell layers of the root (based on 2D-imaging of root sections). The ultimate goal is to correlate the phosphate content and the gene expression.

1- Discrimination of free phosphate and organic phosphate :
The first round of μ-XRF and μ-XANES experiments performed at ESRF (ec554 on ID21, 2-8 February 2010) with the aim of measuring free phosphate and comparing it to total P revealed several difficulties concerning the analysis itself and the sample preparation :
all the pure compounds tested showed a similar peak at 2.153 keV in the P K-edge XANES spectra with distinct secondary structures (Fig 1A). Nevertheless, the spectra seem also to be modified by the physical state and the concentration of the compounds. However, XANES measurements on biological materials did not allow us to differentiate free phosphate and organic P (containing phosphate radicals) in cryofixed root samples (Fig1B). The overlap of signals corresponding to multiple phosphate-containing molecules may likely mask the signal due to free phosphate. The analysis of the signals at distinct places on the root section (inside *vs* on the boarder of the cells) gave similar results with variable intensities but without any distinguishable pattern.

2- Tests with phosphite, an analog of phosphate :
Phosphate analogs like phosphite and arsenate could be more easily distinguished from the global P content of the cell. In fact, we obtained very encouraging results regarding the detection of phosphite (H_3PO_3, a reduced form of phosphate) and their comparison to free phosphate (Fig 1C) and total P contents. Phosphate and phosphite could be identified thanks to the shift of their white line in the P K-edge XANES spectra (respectively at 2.153 and 2.151 keV, Fig 1C). Considering that these phosphate analogs can mimic the presence of free phosphate and trigger similar responses in the plant, their study by μ-XANES in replacement for the study of free-Pi would meet our scientific challenge. To pursue this strategy, we are requesting the opportunity to bring new samples at ESRF, corresponding to plants containing phosphate analogs. Beside phosphite (analysed on ID21), we will test arsenate, another phosphate analog, on ID22.

3- Sample preparation :
Arabidopsis root sections have been prepared according to 2 techniques based on cryofixation (high pressure fixation and cryosubstitution in an Epon resin or in isopentane in tisssutek and freeze-drying). Sections in Epon resin are much easier to locate and their structure looks better preserved but the cell content seems very poor and the P signal is weak. Besides, sections in tissutek are very fragile and many of them are lost. Nevertheless, their preparation is much easier and more rapid and gives a measurable P signal. We will continue with this technique, trying to improve it. We will also analyse the root section after fixation without freeze-drying. For that, it will be necessary to operate at low temperature to maintain the sample in a frozen state.

Figure 1 : (A) μ-XANES spectra of free phosphate (H2PO4-) and organic phosphate (ATP, glucose-6P), (B) P-imaging of a root section, (C) μ-XANES spectra of free phosphate (H2PO4-) and phosphite (H3PO3).