ESRF	**Experiment title:** Understanding of the mechanisms of TiO_2-NP uptake	**Experiment number**: EV 138
Beamline: ID21	**Date of experiment**: from: 28/10/2015 to: 03/11/2015	**Date of report**: 25/02/2016
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Report:

The goal of the experiment was to try to uncover the fine mechanisms implied in NP uptake by plants using the model plant *Arabidopsis thaliana* and its mutants. In the proposal few mutants were suggested dealing with the density of root hair, cell wall mutations and number of stomata on leaf surface. Between the writing of the proposal and the beamtime, preliminary tests were performed in the lab as forecasted. Though the mutants from the proposal gave very interesting results, we chose to reset the priority of mutants to analyse. So finally our experiment focused on wild type plants (Col 0), plants lacking trichomes on leaf surface (*glabra*) and plants with impaired trichome anatomy (*exo70h*). Since the mutations of interest were linked with leaf morphology we decided to expose the plants in contaminated soils which is a more environmentally relevant condition than hydroponics as we first planned (to study root mutation).

A preliminary experiment was performed to chose the type of soil to grow plants. 4 different soils with different texture from Lufa Speyer were used for a first approach. The soil allowing the highest NP translocation (Ti concentration in leaves) was chosen to grow plants for synchrotron analyses.

Soil was artificially contaminated by adding 15 ml of a TiO_2-NP suspension into 15 g of soil to reach a final concentration of 500 mg TiO_2-NPs.kg^{-1} soil. The mixture was agitated for 2 hours and the water in excess was filtrated. Control soil was prepared in the same way but only with deionized water. Seeds of the three genotypes were then sown and grown under controlled conitions (long days, 22°C day/20°C night and 60% humidity). After 4 weeks plants were harvested, leaves washed with deionized water and embedded in OCT resin for cross-sectionning or analyzed as whole leaves.

Mapping was performed in cryogenic conditions using a vibration-free cryo-stage in fluorescence mode using a Silicon Drift Detector. The beam size was 0.48 x 0.85 µm. µXRF maps were recorded at 5.1 keV and µXANES spectra between 4.92 and 5.1 keV (0.5 eV step). Ti model compound spectra were recorded during previous experiments. 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.

Just as a reminder, previous experiments have been carried out on ID21 beamline dealing with the distribution of TiO_2 NPs after both exposure by roots in hydroponics and by deposit on leaf surface on different plant species (wheat, rapeseed, lettuce). Results obtained demonstrated the uptake and translocation of NPs throughout the plants with no particular distribution.

Analyses of *A. thaliana* Col0 seedlings exposed in soil showed a different distribution pattern: accumulation of Ti in the leaf epidermis (mainly in the abaxial side) and in trichomes *ie*. in the non-metabolically active cells of leaves (Figure 1A-E). This type of compartmentalization is a classic detoxification mechanism in plants to protect the photosynthetic cells. It implies that active mechanisms (like transporters) are involved in NP trafficking in plants which is a completely new result. Ti also seems to localize in particular in round structure on trichome surface (papillae) which can be interpreted as an excretion mechanism. On top of this, it is important to notice that this distribution was not seen in plants exposed in hydroponics, highlighting the importance of the exposure medium (though plant species might also be the reason of this difference). One of the other goal of our study was to investigate if the ratio anatase/rutile in the leaf was different than the one of the nanopowder used for exposure. We did not detect a clear change in that ratio however, we detected a different form of Ti that we had never seen before in the plants we analyzed (Figure 1F, XANES 3): a Ti atom in a tetrahedral configuration. This result suggests that Ti underwent some modifications during its transfer from the soil to the plant leaves which is quite surprising since TiO_2 is known for its very low dissolution.
Since TiO_2 accumulated in trichomes (ring and papillae: Figure 1D, E) we were curious to see how a plant without trichomes will deal with Ti. To investigate that question we analyzed the *glabra* mutant of *A. thaliana* which has no trichomes. In this mutant we detected a higher accumulation of Ti in the epidermis (on both sides of the leaf) and also some hot spots in the epidermis of about 30 µm. We can hypothesize that these highly concentrated hot spots should have been the cells supposed to evolve as a trichome without the mutation. But a closer investigation is needed to conclude.

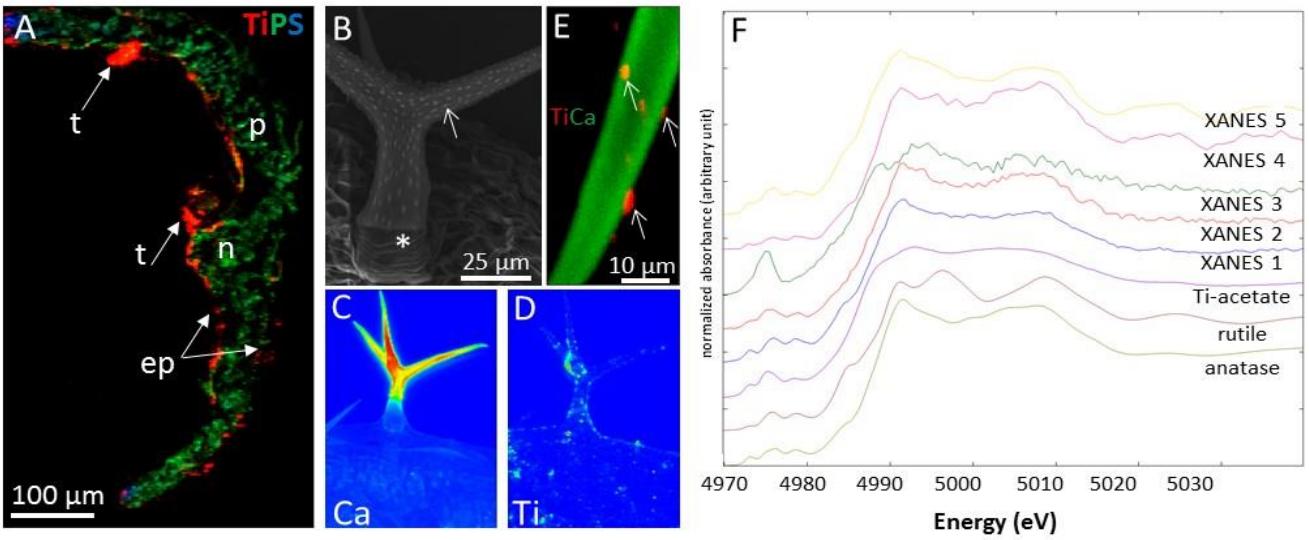

Figure 1: A. µXRF map showing the distribution of Ti (red), phosphorus (green) et sulfur (blue) in a A. thaliana leaf cross-section (ep : epidermis, n : vein, p : parenchyma, t : trichome), B. SEM picture of a trichome showing papillae (arrows) and ring (*). C and D. µXRF maps of Ca and Ti distribution respectively. E. µXRF map focused on a branch of a trichome with Ca in green and Ti in red. F. In situ µXANES analysis of Ti speciation in comparison with reference compounds (anatase, rutile, acetate).

Trichomes have a special structure at their base which is called Ortmannian ring and made of callose. During previous experiments with heavy metals (EV 57) and in the literature, overaccumulation of metals has been noticed in this structure. Ti has also been detected associated with this structure in the *Col0* ecotype. To investigate the role of this structure we grew and analyzed a mutant called *exo70h* lacking this structure. Ti was not detected in the *exo70h* trichomes (nor in the ring neither in the papillae). However, we did not reach a conclusion on that topic because the formation of the callose ring is dependent on leaf and trichome age which was difficult to evaluate.
FTIR acquisitions were first scheduled but some technical issues with the IR microscope forced us to delay. This experiment has been rescheduled later with in house beamtime.

Scientific production related to this experiment
Results obtained during this beamtime are very exciting and need some more experiments before publishing in particular investigation of Ti form and size in the storage cells identified during this beamtime.