ESRF	Experiment title: **Studies of localization and speciation of iron in vegetative and seed tissues of rice and wheat to enhance biofortification and improve crop yields**	**Experiment number**: LS-2225
Beamline: ID21	**Date of experiment**: from: 1.3.2013 to: 5.3.2013	**Date of report**: 31.08.2013
Shifts: 12	**Local contact(s)**: Hiram Castillo Michel	*Received at ESRF:*

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Report:

Iron is an essential element for all higher organisms assuming many important biological roles [1]. Although Fe is abundant in soils, this element is not readily accessible to the plants. According to the World Health Organization, Fe deficiency is one of the most common nutritional disorders in the world, affecting nearly 30% of the world's population. Delivery of Fe via micronutrient rich bio-fortified cereal grains would offer a sustainable and food-based approach for addressing Fe malnutrition. In order to develop Fe rich wheat and rice cultivars, knowledge of basic mechanisms of Fe uptake, transport and accumulation is needed [2].

Rice is cultivated in waterlogged soils. Since the majority of soils are rich in Fe, in waterlogged conditions reduction of insoluble Fe^{3+} into soluble Fe^{2+} occurs due to both anaerobic and low pH conditions. This increases Fe biodisponibility to plants and may induce Fe toxicity, often leading to yield losses [3]. This project focused on the resolving of underlying mechanisms of ion uptake, translocation and compartmentation in rice and wheat through techniques of Fe distribution imaging, speciation and complexation in order to enhance biofortification technologies and improve crop yields.

Rice plants were grown in hydroponics in basic nutrient solution with short (3 days) and a long term (3 weeks) exposure to Fe (0, 125 and 250 mg l^{-1}), while wheat plants were cultivated in a substrate with equal Fe supply until seeding. After harvest, different rice organs (imbibed grains, roots and shoots) and imbibed wheat grains, were rapidly frozen in liquid propane cooled with liquid nitrogen and cryo-sectioned at -35 °C [4]. The sections (2-3 per cultivar/ treatment) were mounted on holders and kept at -196 °C till the measurements, which were performed in frozen-hydrated state in order not to alter element distribution patterns and Fe speciation. The 2D elemental mapping was performed at the ID 21 beamline using SXM set-up equipped with a vibration free cryo-stage passively cooled by LN_2. The X-ray beam delivered by the undulator was monochromatized by means of Si double crystal monochromator and focused to a submicron probe (0.3 x 0.7 μm^2) using Kirkpatrick-Baez focusing. The fluorescence emission of the sample was collected by a large Bruker SDD detector. The excitation energy for the scan was set to 7.2 keV (i.e above the Fe-K edge) to record maps of Fe, Mn, Ca, K, Cl, S and P. Finally the Fe-K edge XANES spectra were

recorded in different regions of plant tissues and cells, depending on local Fe concentrations, to determine Fe speciation. Fe-K XANES spectra of standard reference materials were also measured.

For rice plants treated with 125 mg l^{-1} of Fe for three days in hydroponic solution, Fe was mainly accumulated in root epidermis, where strongly colocalized with phosphorus as denoted by pink colour in Fig. 1. Sulphur was mainly accumulated in root cortex and P was seen also in central cylinder (Fig. 1). In the leaves of plants treated with 125 mg l^{-1} of Fe for three days in hydroponic solution Fe was mainly seen in veins, where also partly colocalized with P (Fig. 1). In the seeds, Fe was mainly localized in the husk with trace concentrations found in embryonic tissues (Fig. 1). Fe K-edge micro XANES spectra were also recorded in the tissues with high Fe concentrations (Fig. 2). The analysis of those spectra is in progress.

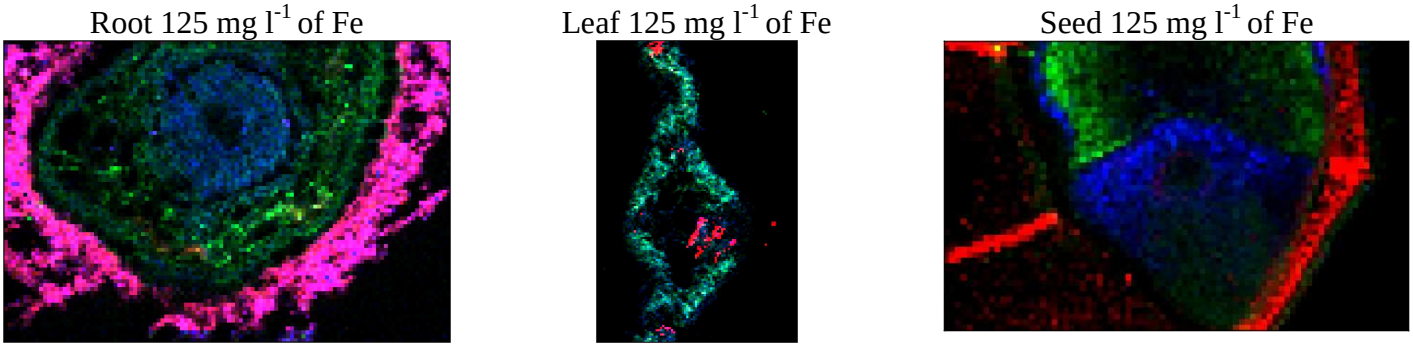

Figure 1. Element distribution maps (Fe-red, S-green, P-blue) in rice roots, leaves and seeds treated with 125 mg l^{-1} of Fe. Roots and leaves belong to the plants treated for 3 days, while the seeds belongs to the plants treated for 3 months.

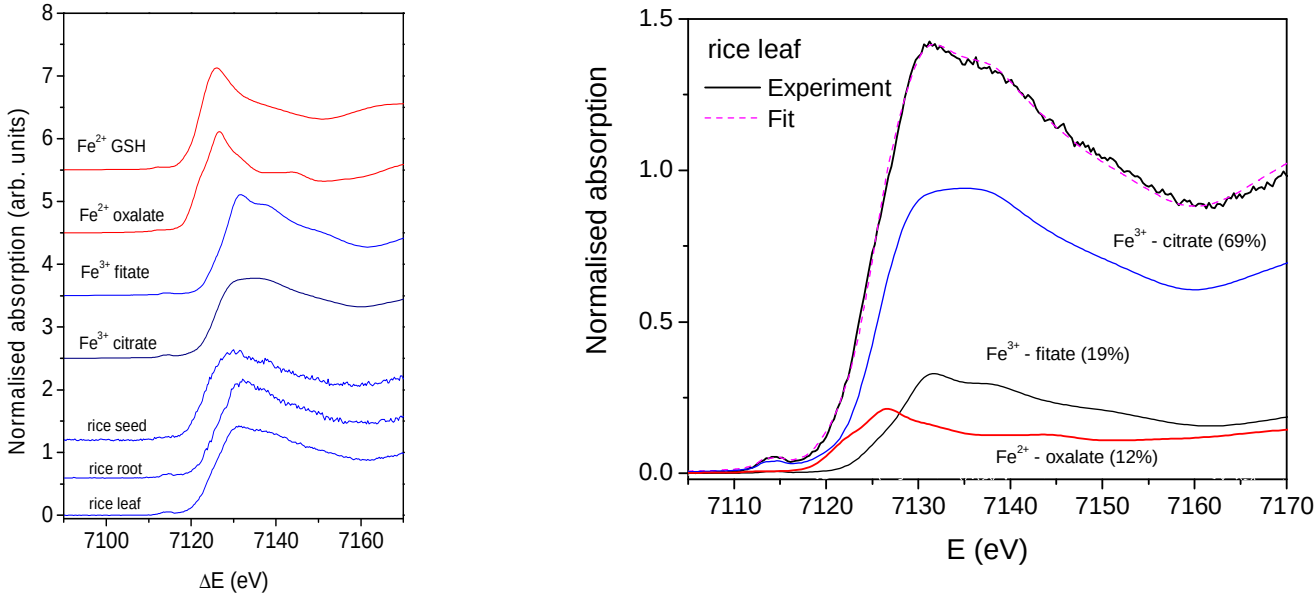

Figure 2. Left panel: Fe K-edge micro-XANES spectra recorded in the selected regions of rice leaf, root and seed, together with standard reference compounds Fe^{2+} glutathione, Fe^{2+} oxalate, Fe^{3+} citrate and Fe^{3+} phytate. Right panel: Linear combination fit of Fe K-edge XANES measured on rice leaf with XANES spectra of reference Fe complexes Fe^{3+} citrate, Fe^{3+} phytate and Fe^{2+} oxalate.

For wheat three different genotypes (IITR26 - high Fe genotype, WL-711 and WH-293) and *Aegilops kotschyi*, all obtained from India (dr. Sudhir Singh, National Agri-food Biotechnology Institute, Department of Biotechnology, Mohali, India) were scanned for element distribution. Preliminary results show that Fe was mainly accumulated in aleurone in all the genotypes, however there was a distinct difference in Fe distribution between the genotypes as already observed [5]. In *A. kotschyii* Fe was retained in pigment

strand, while in wheat genotypes Fe distribution was shifted to maternal tissues (Fig. 3) - in IITR 26 the highest concentration was seen in nucellar projection and in WH-293 and WL-711 in the crease aleurone. In the pigment strand Fe was colocalized with S (Fig. 3 indicated by orange colour), so as in nucellar projection, while in the crease aleurone colocalization with P prevailed (Fig. 3 indicated by pink colour). Micro-XANES on Fe K-edge was also recorded in high Fe regions (Fig. 3). Publication of these results is in preparation.

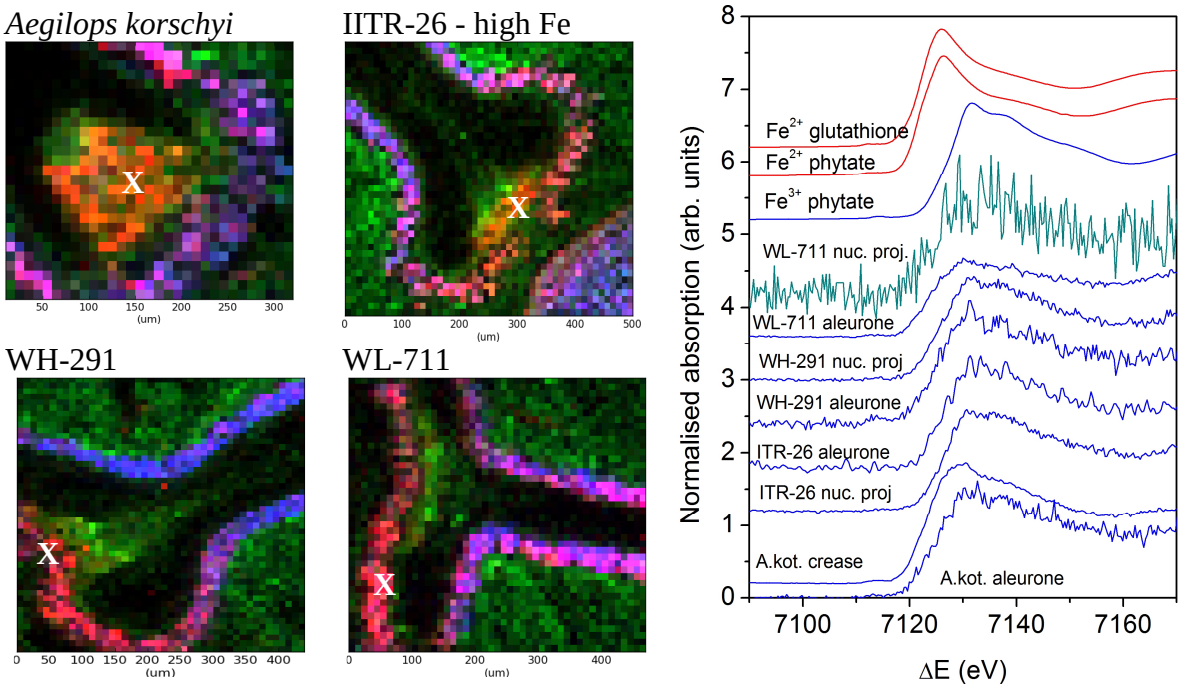

Figure 3. Left panel: Element distribution maps (Fe-red, S-green, P-blue) in *Aegilops kotschyi* and three wheat genotypes. X and Y denote the regions where Fe K-edge micro-XANES spectra were recorded (Y - aleurone, X - pigment strand in *A. kotschyi* and nucellar projection in the other genotypes. Right panel: Fe K-edge micro-XANES spectra recorded in the selected regions of wheat genotypes, together with standard reference compounds Fe^{2+} glutathione, Fe^{2+} phytate and Fe^{3+} phytate.

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