ESRF	**Experiment title:** Selenium speciation in wheat tissues by XAS	**Experiment number**: CH 3101
Beamline: BM25	**Date of experiment**: from: 16/04/2010 to: 20/04/2010	**Date of report**: 25/02/2011
Shifts: 15	**Local contact(s)**: Jon Ander Gallastegui	*Received at ESRF:*

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Report:

We are working on a target system that includes a quantitative speciation analysis of selenium species and their distribution through different wheat tissues after a hydroponic culture with a selenite and selenate biofortification process as well as the monitorization of the consequent bioassimilation process.[1] The aim of the study concerns with a strategy to determine the best practice to anthropogenically enrich wheat. The information of the speciation of selenium in wheat will be of key importance to understand the role of selenium on the observed health benefits of functional foods beyond basic nutrition. Nowadays it is well-recognized that the particular physico-chemical form in which an element is present in a sample will determine the toxicity, the biological activity, the bioavailability and the environmental impact of the element. For that reason the topic "speciation" has raised an unusual interest in the last years in areas so diverse such as toxicology, nutrition, agricultural, medical, biochemical and environmental sciences.[2]

The main objective of the study is to distinguish between the different selenium species that can be found in selenium enriched wheat by applying XANES, in particular in different parts of the plant, in order to establish an appropriate selenium enrichment strategy concerning wheat biofortification process after agreement with direct speciation analysis. This is the second experiment at ESRF to the study of selenium

speciation in order to improve the results reported the first time in the Spline BM25 beamline, which were basically qualitative.

Enrichment treatments were done by adding selenium to the nutrient solution cultures in the form of sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) and sodium selenate (Na_2SeO_4) separately at two different selenium concentrations: 1µM and 10µM Se. Additionally, the same selenium total concentrations levels were reached by mixing both selenium species at equal quantities. After 5 days of incubation in selenium enriched media, plants were harvested and roots desorbed with $CaCl_2$ solution to remove selenium in root apoplast. After desorption, plants were rinsed with distilled water, divided into shoots and roots, frozen into liquid nitrogen and then lyophilized. Finally, root and shoot samples were homogenized in a mortar and converted into pellets by hydraulic pressure to be analyzed at the experimental station of the synchrotron facility.

The XANES experiments were carried out at the beamline BM25. The photon absorption of selenium was recorded at the edge energy for its K line at 12658eV, and its Kα1 11224eV and Kα2 12497eV fluorescent line intensities were measured in fluorescence mode. All XANES spectra were collected at room temperature. Pure reference compounds diluted in cellulose were analyzed in transmittance mode, while fluorescence detection mode was used for the analysis of selenium diluted wheat root and shoot samples. The XAS data were averaged (3-5 scans), normalized and background subtracted using Sixpack software package.[3] Quantitative selenium speciation data were obtained by principal component analysis and linear least-squares fitting of the spectra from reference compounds, including sodium selenite and sodium selenate as inorganic selenium compounds, whilst Selenomethionine (SeMet) and Selenocystine (SeCy) were modeled as organic selenoaminoacids (see Fig.1). Nowadays, the least-squares fitting is widely accepted as a valid method for the speciation of complex biological samples.[4] To examine the sample composition as a linear combination of standard components various fittings of selenium reference compounds were calculated repeating the process until no more significant components could be identified and the sum of all components was equal to 100% (±15%). The relative quality of the fit was quantified by the residual value, a measure of how close the fit is to the data based on a sum of squares determination of the fractional misfit.

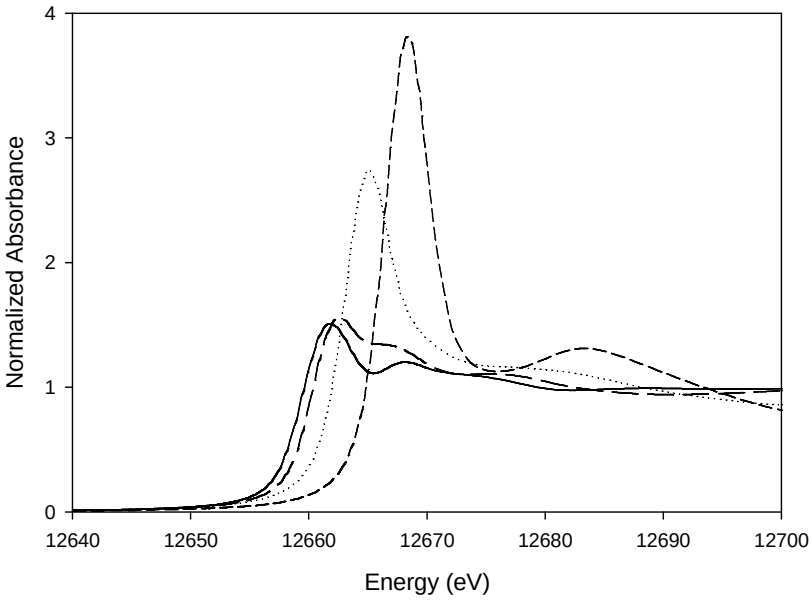
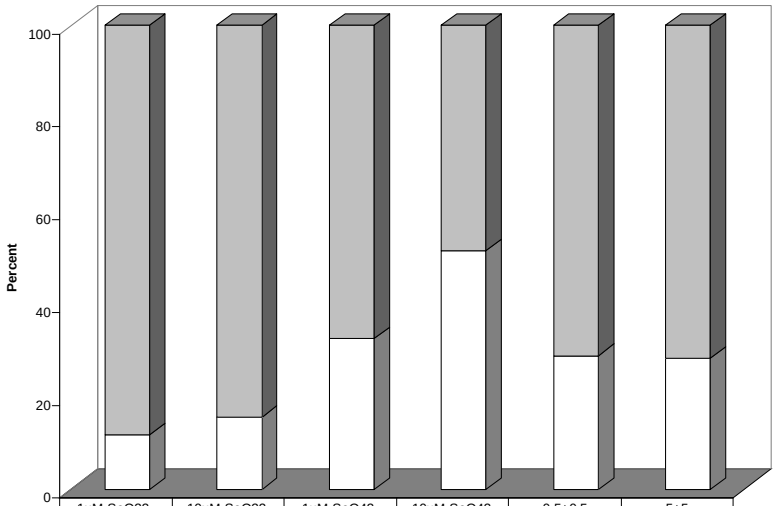


Figure 1. Se XANES spectra for reference compounds (solid line SeCy, long dashed line SeMet, dotted line SeO_3^{2-} and short dashed line SeO_4^{2-}).

Concerning selenium biofortification, it is noteworthy that we are looking for selenium enrichment procedures that mainly accumulate organic selenium forms in shoots, so shoots are the first stage of wheat

growth and they will determine the selenium wheat content that is going to be used for the manufacture of selenium enriched products. Comparing all selenium enrichment treatments (Fig.2), enriching with selenate we obtain the lower selenium organic content in shoots, on the other hand, the selenite one accumulates a higher organic selenium content in shoots but previous studies have shown toxicity symptoms when wheat is exposed to that treatment. In mixture treatments the selenium total organic content in shoots is higher that in selenate though slightly lower than in selenite, however, it is proved in previous studies that mixtures attenuate selenite toxicity. To conclude, results have shown that the best way to anthropogenically enrich selenium in order to obtain a higher organic selenium content in shoots and no toxicity symptoms is mixing both selenite and selenate species at equal concentrations (5µM Se as selenite and 5µM Se as selenate). However, it should be emphasized that wheat was grown in hydroponic culture, but in soil conditions, selenium behaviour could be different due to the presence of other compounds and the metal-soil interactions, so further studies will be necessary to elucidate wheat response under real soil growth conditions.



	1µM SeO32-	10µM SeO32-	1µM SeO42-	10µM SeO42-	0,5+0,5	5+5
Org Se	88	84	68	49	71	72
Inorg Se	12	16	32	51	29	28

Figure 2. Total organic and inorganic Se distribution in shoots normalized to percentage.

References

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