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Understanding Elemental Uptake in Plants Using High Resolution SIMS and Complementary Techniques

Katie Moore[1], Paola Tosi[2], Malcolm Hawkesford[2], Peter Shewry[2], Chris Grovenor[1], Fangjie Zhao[2]

[1.] Department of Materials, University of Oxford, Parks Road, Oxford, OX1 3PH, UK
[2.] Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Mapping the distribution of trace elements at the subcellular scale is analytically challenging but necessary in order to understand the mechanisms of uptake of toxic and beneficial elements into plants and crops which can affect the human diet. With subcellular localisation of the trace elements it is possible to determine the pathways by which these important elements are taken up by the plant and how they are stored and accumulated in edible tissues.

The irrigation of rice paddy fields in areas such as Bangladesh with water naturally contaminated with arsenic (As) has resulted in rice grain containing significant amounts of this toxic and carcinogenic element [1]. In order to minimise the amount of As consumed from rice, the mechanisms of uptake must be better understood and a detailed investigation of the As distribution in the grain, root and stems is required. Conversely trace elements such as iron, selenium and zinc are essential in human diets yet billions of people worldwide are deficient in at least one of these [2]. In this case it is not only important to understand their distribution in cereal grains, but also what elements they are co-localised with. Increasing the bioavailability, the proportion of the element consumed that is absorbed by the body, is as important as increasing the amount of the element consumed to improve the nutritional status.

In this presentation I will show how I have used high-resolution secondary ion mass spectrometry (NanoSIMS) to localise a range of trace elements in many different plant tissues. The NanoSIMS is a state-of-the-art microscope capable of high spatial resolution chemical imaging (down to 50 nm) and detecting very low elemental concentrations (ppm levels) making it ideally suited for subcellular trace element localisation in biological materials. It is possible to detect a wide range of elements in the periodic table from hydrogen to uranium allowing correlation of important lighter elements, such as silicon, sulphur and phosphorus, with the elements of interest which can indicate storage and uptake mechanisms. In combination with the NanoSIMS I have used other techniques such as synchrotron X-ray fluorescence (S-XRF) and antibody labelling to provide complementary information over different length scales.

Excellent sample preparation is vital for successful SIMS experiments due to the ultra-high vacuum in which the samples must be placed meaning that samples must be fully dehydrated. The sample preparation procedure must preserve both the morphology of the sample and the chemical distribution. This latter requirement is especially challenging when the elements are located to vacuoles (Fig. 1) and are highly diffusible. For the majority of samples in this presentation, sample preparation involved high pressure freezing, freeze substitution, resin embedding and sectioning with a microtome.

Combining NanoSIMS and S-XRF has proven to be a powerful methodology for investigating unknown and complex elemental distributions in rice stems. Excellent correlation was observed between the two techniques allowing information to be obtained across both the cellular and subcellular scales (Fig. 1) and has provided new insights into how As and other elements are stored and translocated in rice plants.

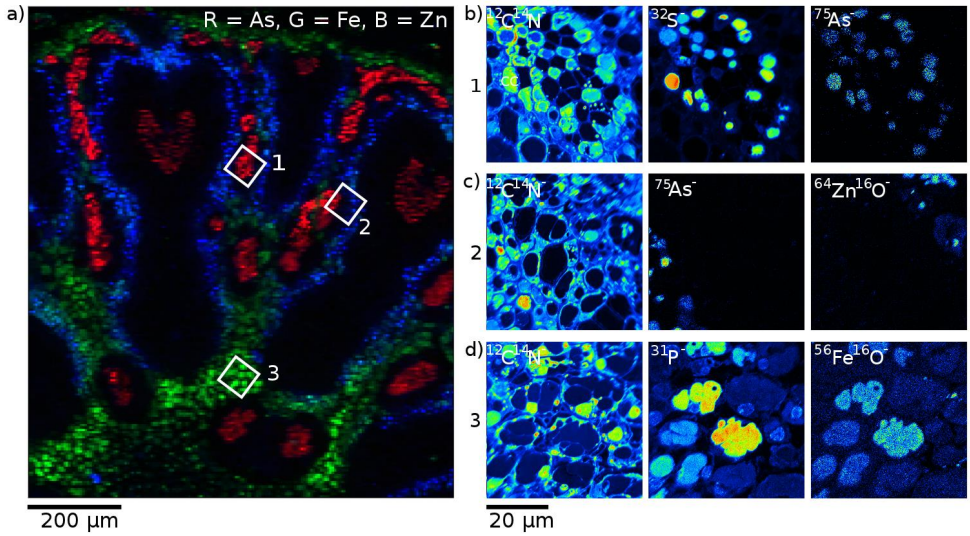

Figure 1: Synchrotron X-ray fluorescence (a) and NanoSIMS images (b-d) of a section of the stem of a rice plant. NanoSIMS images b), c) and d) were acquired from areas 1, 2 and 3 respectively.

An additional capability of the NanoSIMS is the detection of stable isotopes such as ^{2}D, ^{13}C, ^{15}N and ^{18}O. This allows the possibility of pulse-chase experiments to track the uptake of elements or molecules over time. I will show how this methodology, combined with antibody labelling, has been used to investigate protein deposition in wheat grain using ^{15}N labelled glutamine over several time points (Fig. 2). This research is important to improve the efficiency of nitrogen fertilizer application and gain a fundamental understanding of the growth of the wheat grain [3].

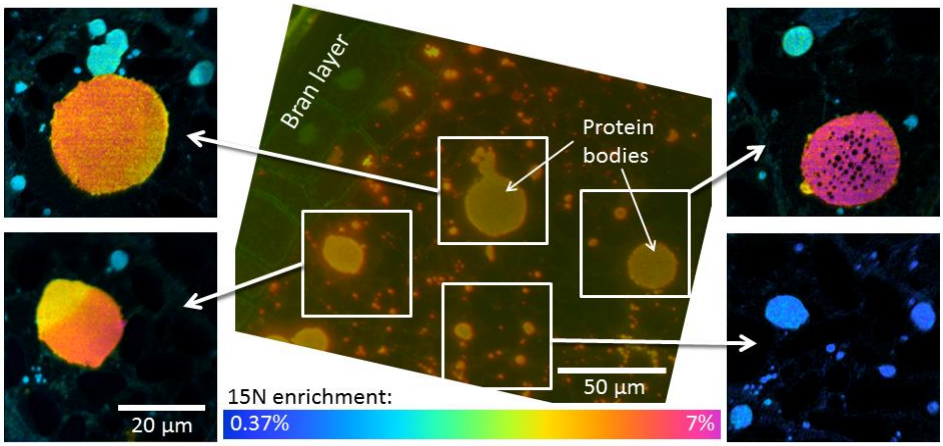

Figure 2: Complementary images of protein bodies in wheat grain of the antibody labelling (centre) and the corresponding NanoSIMS images (outside) which show the local enrichment of nitrogen after ^{15}N glutamine labelling. Note the large differences in the enrichment of ^{15}N in different protein bodies.

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