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TECHNICAL ARTICLE

Inferring the Geometry of Fourth-Period Metallic Elements in *Arabidopsis thaliana* Seeds using Synchrotron-Based Multi-Angle X-ray Fluorescence Mapping

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• *Background* Improving our knowledge of plant metal metabolism is facilitated by the use of analytical techniques to map the distribution of elements in tissues. One such technique is X-ray fluorescence (XRF), which has been used previously to map metal distribution in both two and three dimensions. One of the difficulties of mapping metal distribution in two dimensions is that it can be difficult to normalize for tissue thickness. When mapping metal distribution in three dimensions, the time required to collect the data can become a major constraint. In this article a compromise is suggested between two- and three-dimensional mapping using multi-angle XRF imaging.
• *Methods* A synchrotron-based XRF microprobe was used to map the distribution of K, Ca, Mn, Fe, Ni, Cu and Zn in whole *Arabidopsis thaliana* seeds. Relative concentrations of each element were determined by measuring fluorescence emitted from a 10 μm excitation beam at 13 keV. XRF spectra were collected from an array of points with 25 or 30 μm steps. Maps were recorded at 0 and 90°, or at 0, 60 and 120° for each seed. Using these data, circular or ellipsoidal cross-sections were modelled, and from these an apparent pathlength for the excitation beam was calculated to normalize the data. Elemental distribution was mapped in seeds from ecotype Columbia-4 plants, as well as the metal accumulation mutants *manganese accumulator 1* (*man1*) and *nicotianamine synthetase* (*nasx*).
• *Conclusions* Multi-angle XRF imaging will be useful for mapping elemental distribution in plant tissues. It offers a compromise between two- and three-dimensional XRF mapping, as far as collection times, image resolution and ease of visualization. It is also complementary to other metal-mapping techniques. Mn, Fe and Cu had tissue-specific accumulation patterns. Metal accumulation patterns were different between seeds of the Col-4, *man1* and *nasx* genotypes.

Key words: X-ray fluorescence mapping, metal distribution, *Arabidopsis thaliana* seeds.

INTRODUCTION

Elements of the fourth period of the periodic table, such as K, Ca, Mn, Fe, Ni, Cu, Zn and Se, are important for plant metabolism and physiology. In plants, the concentration of these elements is partially regulated by gene expression (Delhaize, 1996; Hall and Williams, 2003; Jokoby *et al.*, 2004; Kim *et al.*, 2006*b*). Seeds from plants with single-gene mutations, or from different ecotypes, may exhibit large differences in the accumulation of certain elements. For example, *manganese accumulator 1* (*man1*) mutant plants have higher Mn and Cu concentrations in their tissues (Delhaize, 1996), while *nicotianamine synthetase* (*nas*) mutants accumulate more of Fe in vegetative tissues (Yoshimura *et al.*, 2000; Takahashi *et al.*, 2003), but lower amounts in the siliques (Y. Wei, pers. comm.). In both mutants the altered phenotype is due to altered metal transport within the plant. Other recent reports discuss the function of genes involved in metal metabolism (Cobbett, 2003; Kim *et al.*, 2006*a*; Loscos *et al.*, 2006).

A variety of different techniques have been used to observe the distribution and determine the relative amount of metals in intact plant tissues, such as energy-dispersive X-ray spectroscopy (EDXS; Lott and West, 2001; Bhatia *et al.*, 2003), micro-proton-induced X-ray emission spectrometry (Bhatia *et al.*, 2003), X-ray fluorescence (XRF) spectroscopy (Pickering *et al.*, 2000, 2003) and XRF- or absorption-computed microtomography (CMT; McNear *et al.*, 2005; Kim *et al.*, 2006*b*). Understanding the role of gene activity or environment on the distribution and accumulation of metals in plants requires extensive use of the chosen analytical techniques.

XRF spectroscopy is a useful tool for examining the relative concentration and distribution of fourth period elements in intact samples. The distribution and concentration of elements in single bacterial cells was determined using synchrotron-based XRF (Kemner *et al.*, 2004). XRF was used to determine the concentrations of 16 elements (from K to Pb) in powdered aquatic plants (Sokolovskaya *et al.*, 2000; Kipriyanova *et al.*, 2001) as well as to map the distribution of Se in the leaves and stems of

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