Analysis of trace elemental distribution in plant specimens

Application Note

by Dr. Benjamin Stripe, Xiaolin Yang, Sylvia Lewis | Sigray. Inc

Abstract

In crop sciences, genetic modification of crops to improve uptake of trace elements such as Cu, Fe, and Zn is a major focus of research, because it has direct implications for increasing micronutrient content and crop yield [1]. This work is critical for addressing problems in regions of the world with poor soil nutrition. To determine the transportation mechanisms and signaling pathways to communicate demand for trace element uptake, it is necessary to localize such metals at the cellular level and at various growth stages of the plants. The AttoMap micro x-ray fluorescence (microXRF) system provides the femtogram-level sensitivity and sub-cellular resolution needed.

Introduction

Understanding the spatial distribution of inorganic content in plant specimens is of critical importance to a number of agricultural and environmental disciplines, including:

- New **phytoremediation techniques**, in which plants are designed to remove toxic contaminants and recover the expanding amount of polluted land;
- **Phytomining**, in which "hyperaccumulating" plants can harvest precious minerals in an economic and environmentally-friendly way; and
- **Agricultural studies**, in which plant uptake of metals is modified to improve crop growth, reduce the absorption of toxic elements, and increase the micronutrient value of the crop

Despite the importance of trace metals in plants, analysis is challenging and typically requires use of a synchrotron. These multi-hundred million dollar particle accelerator facilities produce brilliant beams of x-rays to enable the high resolution and sensitivity microXRF analysis required. However, synchrotron beamlines are oversubscribed, and access generally requires a peer-reviewed application process and, if granted, travel time and expenses.

Sigray AttoMap, a newly developed laboratory microXRF for agricultural research was used in this study for the uptake and partitioning of iron (Fe). This is one of the most challenging use cases for microXRFs due to extremely low (10-12 picogram- scale) Fe concentrations, which require parts per million (ppm) sensitivity to measure.

Iron is critical to the growth of plants and plays a major role in respiration and photosynthesis reactions; around 30% of the world's arable lands are considered iron-limited for plant growth [2]. The study results indicate that the transporter protein, OPT3 (Oligopetide Transporter 3), mediates Fe-loading into developing leaves which suggests OPT3 proteins regulate Fe demand signaling from shoots to roots.

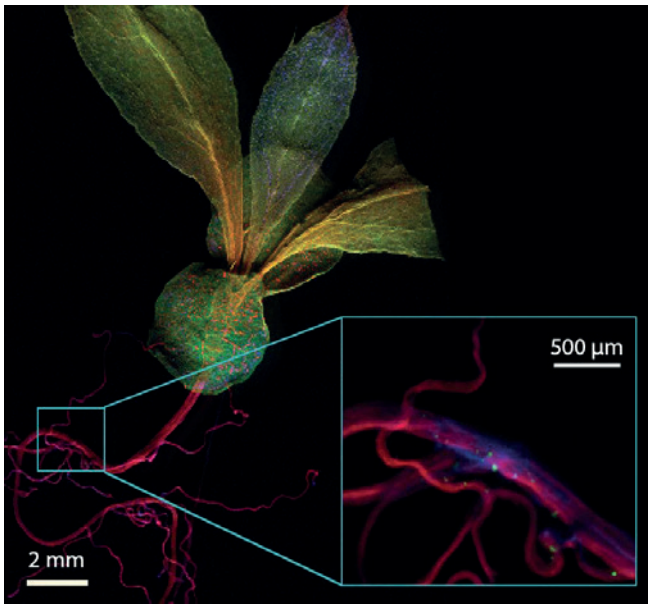

Figure 1. AttoMap Micro-XRF mapping of a hyperaccumulating seedling. Larger view is a tricolor composite of K (red), Ni (blue), and Cl (green). Zoom-in of roots shows trace uptake of Mn (green) . Courtesy of Dr. Antony van der Ent and Dr. Peter Erskine, University of Queensland, Australia.

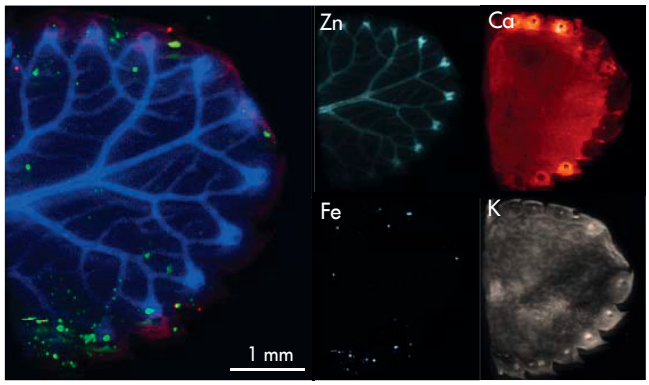

Figure 2. AttoMap Micro-XRF provides elemental imaging for multiple elements simultaneously. Left: tri-color composite of Zn (blue), Fe (green), and Ce (red). Right: individual channels for elements of interest. Courtesy of Cerege, CNRS, Aix-Marseille University.

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Method

This study analyzed leaves of a genetically modified arabidopsis knockout (OPT3-3), courtesy of Prof. Olena Vatamaniuk (Associate Professor of Soil and Crop Sciences, Cornell University), and a control (wild type) sample to determine the potential role of the OPT3 protein. Leaves from the plants were sampled at various stages of growth: one leaf was removed from the same plant at 16 days of growth and another at 19 days of growth. All elements were simultaneously analyzed with AttoMap microXRF, to characterize the distribution of important, plant growth-related minerals Ca, Zn, Mg, Fe, and K.

For the 16-day leaf, an area of 3.5 mm x 3.8 mm was mapped at a 10 µm spot size and a 10 µm step size. The x-ray source settings were configured to use a tungsten (W) target from the multi-target source, operated at 35 kV. Note that although tungsten (W) was selected because of the interest across the broad range of elements, a copper (Cu) target is optimal if Fe (6.4 keV) is of sole interest. Follow-up studies for even better Fe sensitivity can be uniquely achieved in the AttoMap using its patented multi-target x-ray source.

The 19-day leaf (Figure 4, next page) scan area was 4.0 mm x 8.3 mm at a spot size of 10 µm and a step size of 15 µm. Source settings were kept the same as the 16-day leaf.

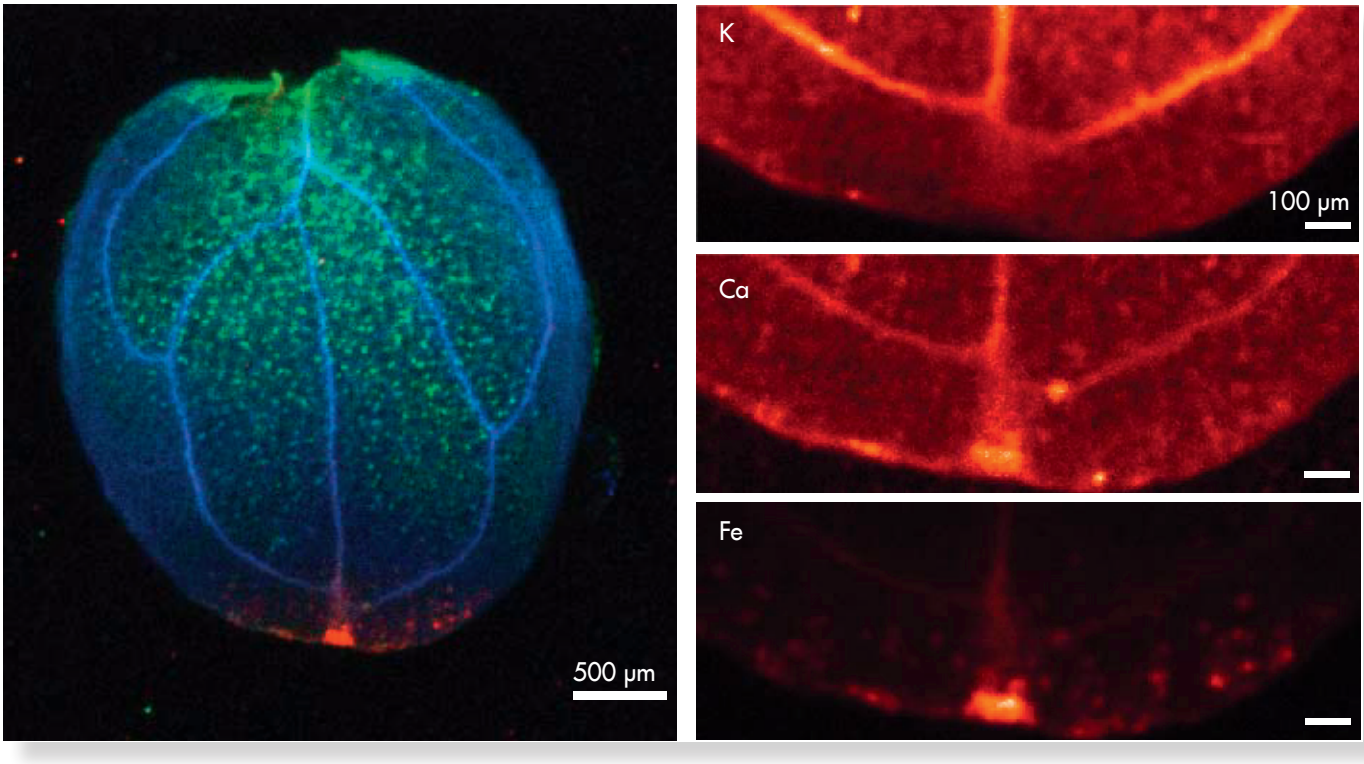

Figure 3. Left: tri-color composite of an opt3-3 mutant 16-day leaf: Fe (red), Ca (green), K (blue). Right: single-channel distribution maps of selected elements of interest. Samples courtesy of Dr. Olena Vatamaniuk, Cornell University

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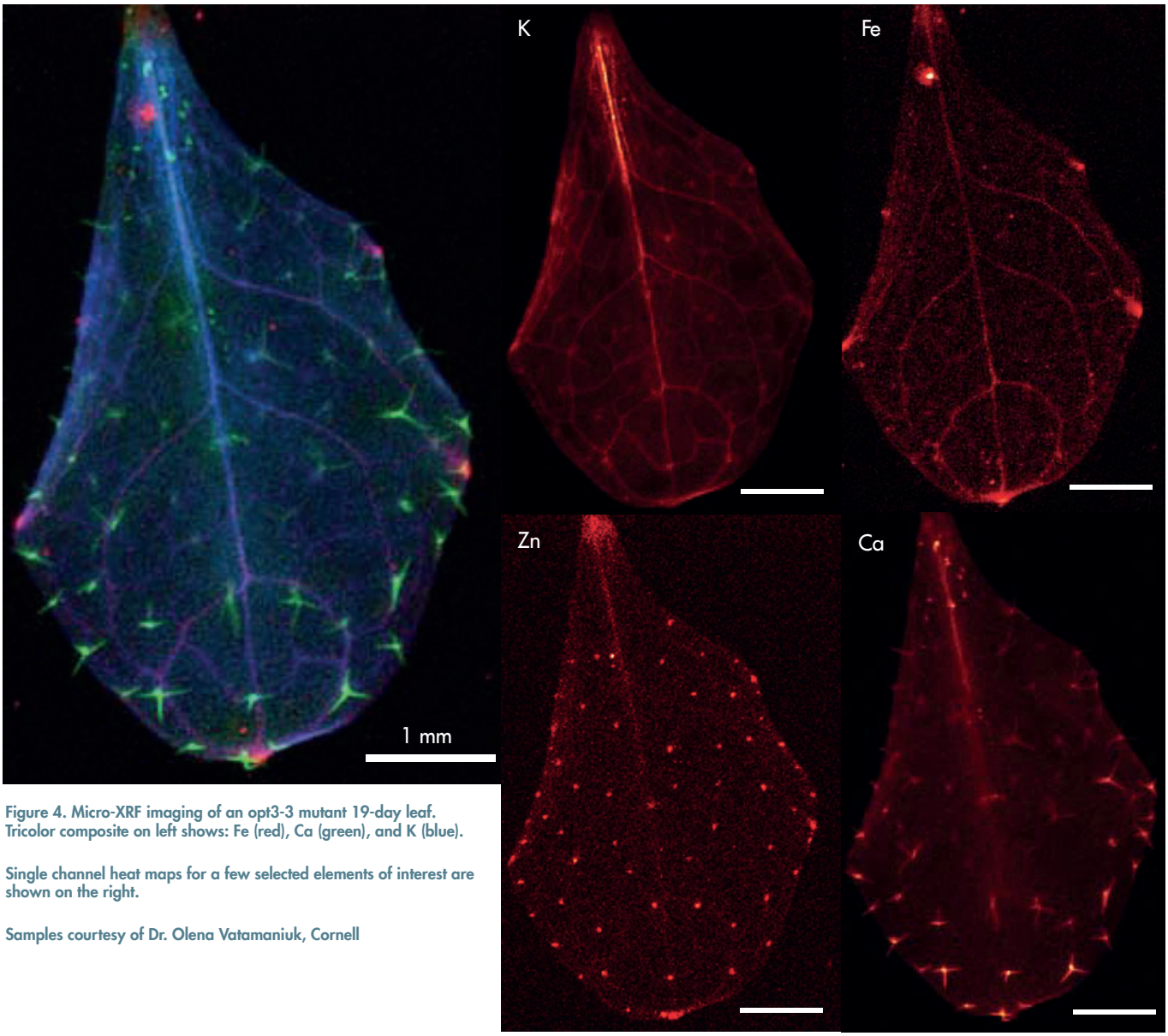

Figure 4. Micro-XRF imaging of an opt3-3 mutant 19-day leaf. Tricolor composite on left shows: Fe (red), Ca (green), and K (blue).

Single channel heat maps for a few selected elements of interest are shown on the right.

Samples courtesy of Dr. Olena Vatamaniuk, Cornell

Results and discussion

The results showed picogram-level anomalies in trace Fe distribution in the knockout opt3-3 plant. The Fe concentration in both the 16-day and 19-day leaves was found primarily at the minor veins of the leaves, located near the hydathodes (leaf pores) and toward the leaf blade periphery, with increased Fe accumulation in the older leaf's central minor veins. Because increased Fe was found in locations where OPT3 is preferentially expressed, the results indicate that OPT3 may be crucial for loading Fe back into the phloem, the vascular tissue that conducts sugars and nutrients from the leaves back downward to the stems to support plant development. In comparison, the wild type leaf showed significantly lower Fe distribution in the leaves, with accumulation seen only at a small outermost edge.

Studies by Prof. Olena Vatamaniuk of other elements involved in the transportation of water and solutes from leaves, such as potassium and calcium, did not show statistically significant differences in wild type versus opt3-3 distributions. Such findings indicate that overall loading and transportation of other nutrients does not appear to be affected, supporting the hypothesis that OPT3 affects Fe-specific pathways.

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Summary

This study demonstrates that with new developments in laboratory microXRF technology, plant-related trace (parts per million) element analysis is now possible outside of the synchrotron. In this study, the AttoMap provided picogram-scale measurements at sub-cellular (<10 μm) resolution. Interestingly, the AttoMap laboratory system appeared to have greater sensitivity for elements such as Ca (3.7 keV) and K (3.3 keV) compared to the synchrotron results previously obtained. This is likely due to the polychromatic beam of the AttoMap, which provides improved cross-sections than the 11 keV monochromatic synchrotron beam employed, and confirms previous studies that have suggested that "white light" beams are far preferred than standard synchrotron configurations for environmental samples [3]. Quantification of how much gain in signal for lower atomic number elements is achieved by using the AttoMap has been reserved for follow-on studies.

The AttoMap provides not only distribution imaging of elements, but can also be used to quantify the relative amounts of each element. Future exciting possibilities for the system include in-vivo studies, in which elements in growing and living plants or roots can be monitored. This is made possible by the large working distance (source-sample focusing distance) that allows for roots in soil and/or uneven surfaces, such as leaves, to be characterized.

References

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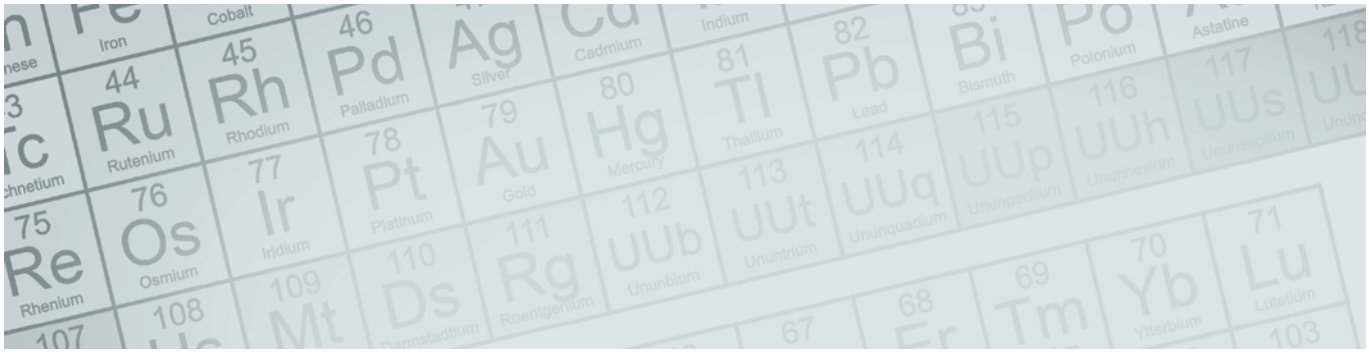
2. Z Zhai, et al. "OPT3 is a Phloem-specific iron transporter that is essential for systemic iron signaling and redistribution of iron and cadmium in arabidopsis." The Plant Cell 26 (2014): 2249-2264.

3. SR Barberie, et al. "Evaluation of different synchrotron beamline configurations for x-ray fluorescence analysis of environmental samples." Analytical Chemistry 86:16 (2014): 8253-8260.



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