Review

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Imaging element distribution and speciation in plant cells

Fang-Jie Zhao[1,2], Katie L. Moore[3], Enzo Lombi[4], and Yong-Guan Zhu[5,6]

[1] National Key Laboratory of Crop Genetics and Germplasm Enhancement and Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
[2] Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[3] Department of Materials, University of Oxford, Oxford OX1 3PH, UK
[4] Centre for Environmental Risk Assessment and Remediation, University of South Australia, Building X, Mawson Lakes Campus, Mawson Lakes, South Australia SA-5095, Australia
[5] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China
[6] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

To maintain cellular homeostasis, concentrations, chemical speciation, and localization of mineral nutrients and toxic trace elements need to be regulated. Imaging the cellular and subcellular localization of elements and measuring their *in situ* chemical speciation are challenging tasks that can be undertaken using synchrotron-based techniques, such as X-ray fluorescence and X-ray absorption spectrometry, and mass spectrometry-based techniques, such as secondary ion mass spectrometry and laser-ablation inductively coupled plasma mass spectrometry. We review the advantages and limitations of these techniques, and discuss examples of their applications, which have revealed highly heterogeneous distribution patterns of elements in different cell types, often varying in chemical speciation. Combining these techniques with molecular genetic approaches can unravel functions of genes involved in element homeostasis.

Spatial and chemical information of mineral element homeostasis

Plants take up a range of mineral elements from the soil, some of which are essential for growth, whereas others are non-essential [1]. Deficiencies of essential elements are a major limiting factor for crop production in many areas worldwide, whereas excessive accumulation of both essential and non-essential elements can lead to phytotoxicity [1]. Accumulation of some elements, such as cadmium (Cd) [2] and arsenic (As) [3], in the edible parts of crops may pose a significant risk to human health well before phytotoxicity occurs. By contrast, there is a need to increase essential micronutrients, such as iron (Fe) and zinc (Zn), in plant-based foods to alleviate their deficiencies in humans [4]. Plant nutrition research aims to understand how minerals are acquired, transported, distributed, stored, and used in plants. This knowledge is important not only for sustainable agricultural production but also for ensuring the nutritional quality and safety of agricultural products [5].

Analyses of total elemental concentrations can now be performed using high-throughput platforms to reveal the ionomic profile (see Glossary) of plant tissues [6]. Although the total concentrations of minerals can provide information about the capacity for uptake and translocation, it is well recognized that minerals are distributed heterogeneously across different cell types [7]. Not only may the total concentrations vary at the tissue, cellular, and subcellular scales but also the chemical speciation of minerals may vary. This spatial information is crucial for understanding the homeostasis of minerals, particularly how different cell types and, fundamentally, different genes function in controlling the distribution, complexation, and storage of minerals, and how these processes vary among diverse plant species in the ecophysiological context.

A range of techniques are available for mapping element distribution at various spatial scales. Traditional methods, such as energy-dispersive X-ray microanalysis (EDX) and proton (particle)-induced X-ray emission (PIXE), have been useful in mapping the cellular distributions of macronutrients or metals and metalloids that accumulate to high concentrations in hyperaccumulating plants (e.g., [8,9]). Visualizing the spatial distribution of micronutrients or toxic trace elements in non-hyperaccumulating plant species is much more challenging because of their low concentrations. Obtaining reliable *in situ* information about the chemical speciation of mineral elements presents an even greater challenge. These tasks have been greatly facilitated by the novel uses of imaging or analytical techniques that offer greater sensitivity, spatial resolution, or capability for chemical speciation, such as synchrotron-based X-ray absorption or fluorescence and mass spectrometry-based techniques. Here, we review the advantages and limitations of these techniques and discuss examples

Corresponding authors: Zhao, F.-J. (Fangjie.Zhao@njau.edu.cn); Zhu, Y.-G. (ygzhu@rcees.ac.cn).
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Glossary

Absorption edge: a sharp discontinuity in the graph of the absorption coefficient of a substance plotted against the wavelength of X-rays being absorbed. It represents the minimum energy necessary to free electrons from particular shells of the atoms of interest.
Beamline: the instrumentation that generates and transports synchrotron radiation to an experimental end station where the appropriate radiation is selected, focused, and directed on a sample mounted on a stage. It also includes appropriate detection systems for the signals generated from the sample.
Chemical speciation: the distribution of an element among defined chemical species in a system. Chemical species refer to a specific form of an element defined as electronic or oxidation state, and/or complex or molecular structure.
Complexation: formation of a coordination entity consisting of a metal or metalloid center and its ligands.
Ecophysiological: the interrelationship between the physiology of an organism and its environment.
Edge positions: the energy of the incident X-ray where the absorption edge is observed.
Electronegativity: the tendency of an atom or a functional group to attract electrons towards itself.
Energy-dispersive X-ray microanalysis (EDX): a microanalytical technique that uses the characteristic spectrum of X-rays emitted by the specimen after excitation by high-energy electrons to obtain information about its elemental composition.
Fast fluorescence detector technologies: devices that are able to detect the fluorescence signal originating from a sample efficiently.
Fourier transformation: a mathematical transformation employed to transform signals between time or spatial domain and frequency domain.
Freeze substitution: the process of replacing ice in a frozen sample with alcohol or another solvent at sub-zero temperatures.
High-pressure freezing: the rapid freezing of a sample under the application of pressure, which allows samples 200–300 μm in size to be frozen with minimal formation of damaging ice crystals.
Hyperaccumulating plants (or hyperaccumulators): refer to plant species that are able to accumulate and tolerate large concentrations of metals or metalloids in their aboveground parts. The concentration thresholds used to define hyperaccumulation vary among metals or metalloids, but usually are more than two orders of magnitude higher than those attained by normal non-hyperaccumulating plant species growing on uncontaminated soils.
Image stacks: a dataset where maps of the same areas are collected at different incident energies (e.g., across the absorption edge of an element).
Incident beam: a wave or particle beam which intercepts a sample.
Ionomic profile: the feature of the mineral nutrient and trace element composition of an organism.
K_α line: refers to the emission line when an electron transitions from a 2p orbital of the second or 'L' shell to the innermost 'K' shell.
K-edge: the absorption edge of the K shell electrons.
Laser ablation system: instrumentation for removing material from a solid surface by irradiating it with a laser beam.
Laser microdissection (LMD) instrument: instrumentation for isolating specific cells of interest from microscopic regions of tissue or organisms using laser beam.
Lateral resolution: the ability of a system to distinguish two points in the direction perpendicular to the direction of an incident beam.
Metalloids: a chemical element that has properties bordering those of metals and non-metals (e.g., arsenic and selenium).
Non-hyperaccumulating plants: plant species that are not hyperaccumulating plants (see above).
Proton (particle)-induced X-ray emission (PIXE): emission of X-rays specific to an element in a specimen when it is irradiated with an ion (proton or α particle) beam, used as a technique to obtain information about the elemental composition of a sample.
Rastering: scanning of a sample in a defined pattern.
Secondary ion mass spectrometry (SIMS): emission of ions from the surface of a sample after bombardment with a high-energy primary ion beam. Secondary ions from the sample are analyzed in a mass spectrometer to build up a chemical map of the sample surface. Isotopes as well as molecular species can be detected.
Spatial resolution: the ability of a system to distinguish the position of two points in a 3D space.
Synchrotron: a particular type of particle accelerator where electrons are accelerated to almost the speed of light to produce electromagnetic radiation (i.e., synchrotron radiation).
Synchrotron-based X-ray absorption: a technique based on the absorption profile of a sample as function of the energy of an incident X-ray beam generated at a synchrotron.
Synchrotron X-ray fluorescence (S-XRF): a mapping technique based on the detection of the fluorescence emitted by a sample while it is rastered through an X-ray beam generated at a synchrotron.
Tomographic techniques: techniques that allow the reconstruction of virtual sections within a specimen.

of their uses in imaging the distributions of minerals, particularly micronutrients or trace elements, and for probing their chemical speciation in plant cells.

Synchrotron-based techniques

Synchrotron facilities provide high-intensity photon sources that are >10 orders of magnitude brighter than those generated by conventional X-ray tubes [10,11]. Owing to this characteristic, synchrotron-based techniques are highly sensitive and can be used to detect a wide range of elements with a high spatial or lateral resolution. The sensitivity increases with the atomic number, meaning that the techniques are ideally suited to investigations on trace elements and heavy metals or metalloids. Furthermore, these techniques require minimal sample preparation and can be used to probe hydrated plant samples and to investigate element speciation *in situ*.

There are two main types of synchrotron-based techniques that can be used to analyze plant samples in the context of this review: X-ray fluorescence (XRF, or S-XRF for synchrotron XRF) for imaging elemental distribution and X-ray absorption spectrometry (XAS) for analyzing chemical speciation of elements (Figure 1). Both techniques involve irradiating the sample with X-rays of a definite energy, which excite and eject the core electrons of atoms with a binding energy lower than the incident X-ray. As a result, a vacancy is created in a core orbital, which is then filled by an electron from a higher energy orbital, releasing the excess energy in the form of fluorescence. The energy of this fluorescence signal is characteristic for each element. By rastering a sample through an X-ray beam, fluorescence spectra can be recorded at each position and used to generate XRF elemental maps. A step-by-step guide on the use of S-XRF for elemental mapping in plants is provided in [12]. In an XAS experiment, the energy of the incident X-ray beam is progressively increased while keeping the sample in the same position and the fluorescence signal is collected incrementally. As the energy approaches the binding energy of the core electrons of the element of interest, absorption of the incident beam progressively increases and so does the emitted fluorescence. XAS spectra are therefore generated by detecting and recording the absorption or fluorescence at each energy point. An XAS spectrum is conventionally divided into two parts, the X-ray absorption near-edge structure (XANES) and the extended X-ray absorption fine structure (EXAFS) (Figure 1), covering the energy range from approximately –50 to +200 eV of the absorption edge and from the absorption edge to approximately +800 eV, respectively [10]. XANES is particularly sensitive to the oxidation states of elements and the electronegativity of the ligands, whereas EXAFS can provide information about the coordination chemistry such as the identity and number of the coordinating atoms and the interatomic distance.

In general, there are two types of beamlines used for analyzing elements in plant samples: one uses a millimeter X-ray beam to assess the average speciation of the element of interest in the sample (usually homogenized) and the other uses a micro-focused X-ray beam (tens of nm to a few μm) to collect spatial information of intact specimens at the cellular or subcellular scales (Figure 1).