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Soft X-ray Spectromicroscopy: A Versatile Tool to Probe Pristine Plant Cell Walls

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Plant cell wall and cuticular layer are the barriers to several biotic and abiotic stresses. For example, in the temperate zone, ice formation occurs in the extracellular space (apoplast) in plants [1]. Ice formation also causes dehydration stress due to subsequent water migration from the symplast to the apoplast along its vapour pressure deficit. Thus, the cell wall acts not only as a barrier to the mechanical stress of ice propagation, but also as a barrier to water loss [2] and may also have relevance to chilling injury [3]. It is known that cold temperature induces changes of the cell wall improving the ability of plants to resist extracellular freezing [4, 5]. In cell wall formation, calcium forms cross linkages to the free carboxyl groups of acidic pectin residues [6]. In addition, plants trigger the membrane resealing mechanism that involves calcium-dependent SYT1 function in freezing and thawing [7]. Therefore, calcium ions are largely involved in cold acclimation processes and freezing tolerance. We here present a novel synchrotron-based mapping technique to investigate calcium distribution in the apoplast and symplast of fresh *Arabidopsis* leaf tissues.

Traditionally, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) are used to study the presence of calcium ions [8] and calcium crystal formation [9] in *Arabidopsis* leaves at high spatial resolution. However, electron microscopy techniques require dehydration of the leaf samples through washing with alcohols or precipitation of calcium with chemicals, both these procedures affect the localization and quantitative information.

Soft X-ray spectroscopy coupled with nano-scale microscopy has been shown to have advantages to study plant biopolymers and their distribution at sub-cellular resolution (~25 nm) [10]. The soft X-ray Spectromicroscopy (SM) beamline at the Canadian Light Source (CLS) is equipped with a state-of-the-art Scanning Transmission X-ray Microscope (STXM) that combines high resolution microscopy (25 nm) with X-ray Absorption Spectroscopy (XAS) from 130 to 2700 eV, enabling unique capabilities to study plant cell wall components. However, most plant cell walls are tens of microns in size and therefore, too thick for soft X-ray spectromicroscopy at the carbon and calcium edges. Usually the samples are embedded in resins and cross-sectioned to 100 nm thick following similar protocols to electron microscopy.

In this study, we present a novel approach to study calcium ion localization and quantification in fresh *Arabidopsis* leaf cells using the STXM at 25 nm spatial resolution. To our knowledge, this is the first study to map calcium ions and biopolymers in the epidermal layers of Arabidopsis using STXM.

Epidermal layer of the leaves harvested from *Arabidopsis* plants grown in agar solution in a petri dish (Figure 1a) were peeled using forceps (both from the abaxial and adaxial sides) of the three wild types (Col-0, Fl-3 and Kas-1) used in the study. The epidermal layers were floated on water, transferred onto

the TEM grids (Figure 1b) and mounted on the STXM sample holder. The air inside STXM chamber was replaced by He as evacuating the air through the roughing pump ruptured the hydrated or partially air-dried cells on the TEM grids. A region of interest was chosen by selecting a single layer of intact cells (Figure 1c) and two images, one before (350.5 eV; Figure 2a) and one on the absorption peak ((352.4 eV; Figure 2b) of calcium were recorded at 100 nm resolution from 30 µm area per sample and two biological replicates were used in the study. The abaxial side was able to be peeled regardless of the accession and was suitable for comparative study. The results showed that there were no significant differences in the total calcium ion present among the control plants of the three accession (Figure 3).

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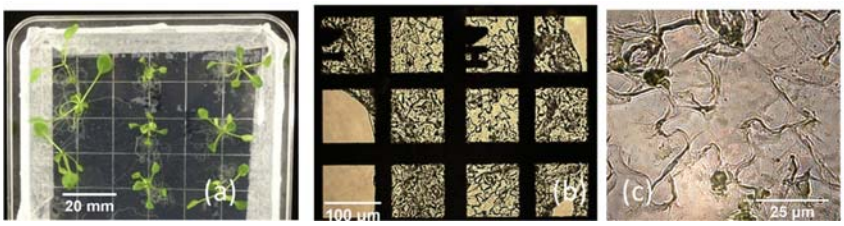

Figure 1. Materials and observation. (a) Arabidopsis accessions Fl-3, Col-0 and Kas-1 were grown in the petri dish with agar medium. (b) The epidermal cell layer was mounted on the TEM grids (SPI; 200 mesh). (c) Single cell layer region was chosen under the optical microscope for STXM analysis.

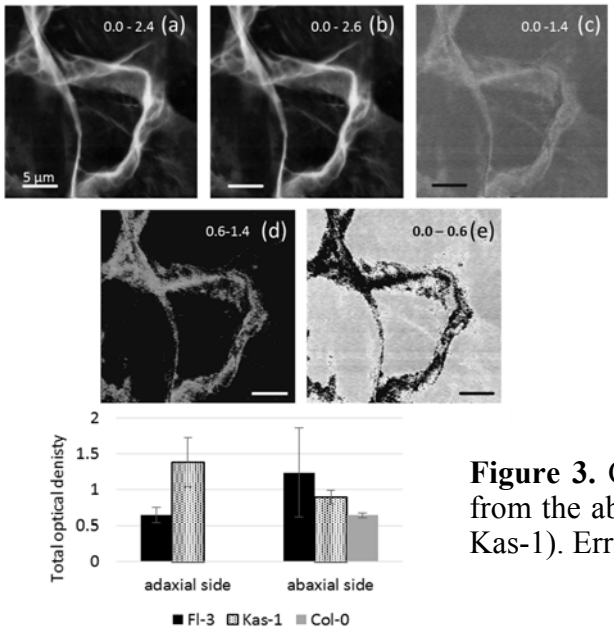

Figure 2. STXM image analysis. (a) The image before calcium absorption (350.5 eV). (b) The image at the absorption peak of calcium (352.4 eV). (c) The difference image between "at the" and "before" the absorption peaks of calcium. (d) The map of calcium ion on the cell wall. (e) The map of calcium ion in cytoplasm. The region of the cell wall and cytoplasm were separated by the "generate mask" function (aXis2000). Numbers in each image indicate the minimum and maximum values of the optical density.

Figure 3. Calcium ion density of cell wall in peeled epidermal cells from the abaxial and adaxial sides in three accession (Fl-3, Col-0 and Kas-1). Error bar indicates SE. n = 2.

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