



POSTER #12

**PLANTS AND LIGHT MANIPULATION:
THE INTEGRATIVE MINERAL SYSTEM IN OKRA LEAVES**

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Leaves are primarily organs for light utilization and carbon fixation. Even though minerals are often an integral part of different leaf tissues, they are not generally thought to be involved in optimizing the light absorption process. The documented exceptions are *Ficus microcarpa* and pecan leaves [1]. Leaves deposit various types of minerals: the most widespread are silica and calcium oxalate, and to a lesser extent calcium carbonate. Until now calcium oxalate and silica functions in leaves were poorly understood, with the most common hypotheses being that calcium oxalate crystals and silica phytoliths help control calcium concentration and defend against herbivores respectively. These hypotheses do not satisfactorily explain the fine regulation of mineral morphology and distribution. Here we investigate calcium oxalate and silica mineral functions in okra (*Abelmoschus esculentus*) leaves. We introduce a correlative approach to visualize druses, silica, chloroplasts and leaf soft tissue in 3D without affecting the minerals or the organic components. We use micro-Computed Tomography (microCT) to image the leaf soft tissue and the distribution mainly of calcium oxalates. MicroCT however cannot distinguish between silica phytoliths and soft tissue unless the phytoliths are large and thick. A different tool for silica examination was developed. We infiltrate the leaf with the PDMPO fluorophore to study the 3D distribution of silica deposits with confocal microscopy, which also allows mapping chloroplasts. The correlative combination of the two techniques shows that silica and calcium oxalates, together with chloroplasts, form a complex system with a highly regulated relative distribution. This distribution points to a significant role of both oxalates and silica in light manipulation. We demonstrate that in okra leaves calcium oxalates increase the illumination level into the underlying tissue by scattering the incoming light, and silica reduces UV radiation damage. This new correlative method opens the way to addressing the roles of different minerals in leaves in general.

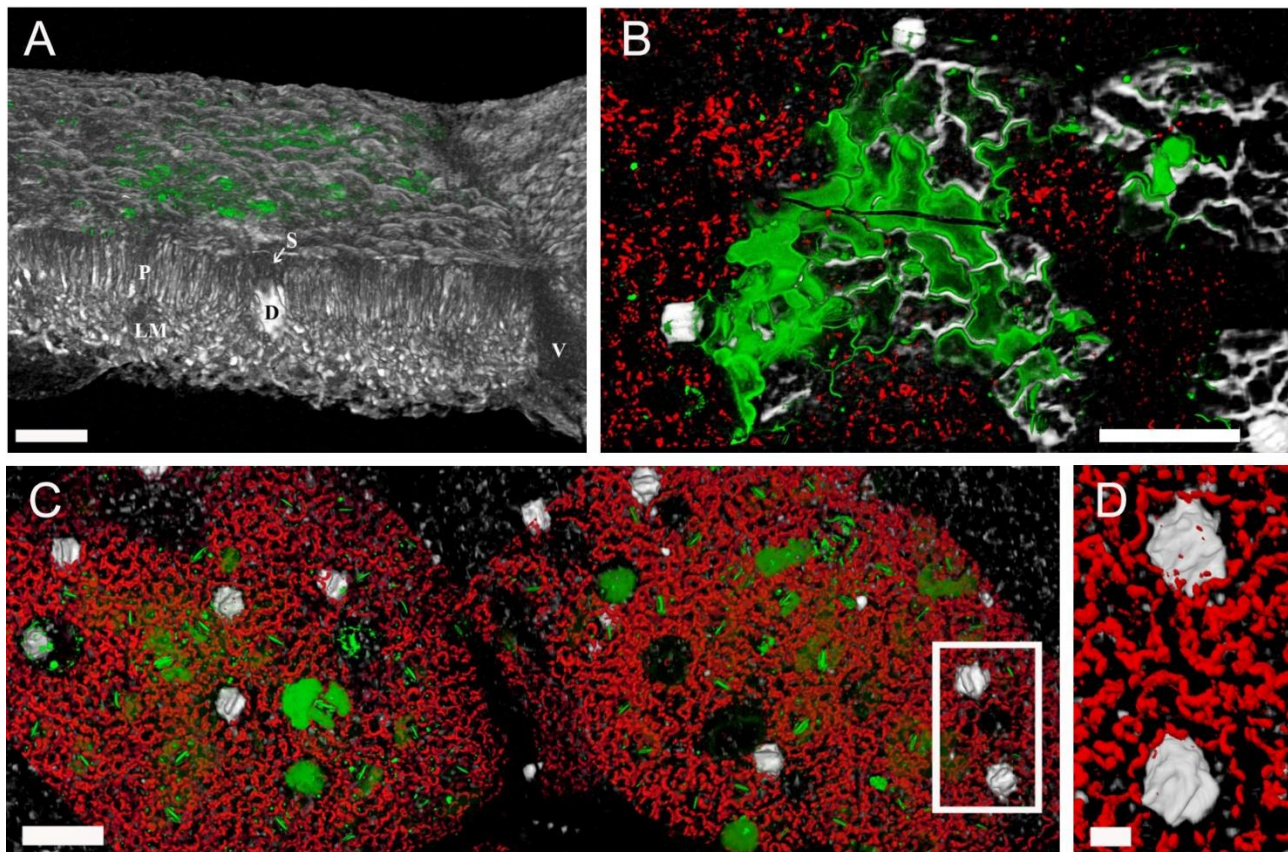


Figure 1 overlay of microCT and confocal microscope volumes. Druses are white, phytoliths green, chloroplasts red: A) The surface and cross section of the leaf. A druse (D) extends from the palisade layer (P) cells to the lower mesophyll (LM) layer. In the druse-containing cell free space (S) is left between crystals and upper epidermis. A vein (V) runs perpendicular to the leaf section. Phytoliths are located in the upper epidermis. Scale bar 100 μ m. B) The upper surface of a leaf showing the limitations of the two techniques. When silica deposition in the cell walls is still very thin or only spherical micrometric size particles are deposited, phytoliths are visible only by confocal microscopy. The microCT shows areas in which phytoliths are out of the confocal scan focus, they are only slightly visible even if the silica deposition is thick. Scale bar 150 μ m. C-D) Relative distribution of oxalates, silica and chloroplasts in the leaf. C) Reciprocal distribution of druses, phytoliths and chloroplasts. Calcium oxalates almost never overlap with either silica or chloroplasts. Scale bar 100 μ m. D) Magnification of the area delimited by the rectangle in C. Chloroplasts are never located above druses and create a crown around the minerals. Scale bar 10 μ m.

References:

1. Gal A., Brumfeld V., Weiner S., Addadi L., Oron D. Certain Biominerals in Leaves Function as Light Scatters, *Adv. Mater.* 2012, 24, OP77-OP83
2. Pierantoni M., Tenne R., Brumfeld V., Kiss V., Oron D., Addadi L., Weiner S., *Plants and Light Manipulation: the Integrative Mineral System in Okra Leaves*, *Advanced Science*, 2017: p. 1600416-n/a.