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## Time-resolved phase contrast µCT measurements of nanoparticle transport in living plants.

The transition to green agriculture faces several challenges. One major issue is the inefficient use of soil-based, conventional fertilizers. A solution to increase both the efficiency and flexibility of fertilization is to bypass the whole soil system and develop the concept of foliar fertilization. With the development of nanotechnology, new avenues have opened with the potential to truly revolutionize this concept. Recent observations suggest that mineral nutrients (P, Mn, and Zn) delivered as nanoparticles (NPs) can enter the plant through the leaves and be delivered to the target location inside the plant [1],[2]. However, several instances of the NPs pathways rely on hypotheses rather than experimental evidence. For example, the role of stomata is significant, yet remains poorly understood.

To shed light on how NP transports inside plants, we combine X-ray imaging, spectroscopy and scattering methods at synchrotrons and laboratory micro-tomography in the 3D imaging center at DTU. We will show results obtained with freeze-dried and living plants. In the latter case, perform longitudinal studies of different particle types (MnO, ZnO filled Mesoporous silica nano shells and hydroxyapatite) applied onto the leaves of Soy and barley plants. Synchrotron nano-tomography [Fig 1a shows Spring-8 data] reveals the 3D cellular ultrastructure and NP aggregates, while with SAXS (Small angle X ray scattering) and XRF we can identify non-aggregated nanoparticles in the plant tissue. The accumulated high density particles indicated by arrows in Fig 1c are perhaps the first direct visualization of foliar applied NP translocation in the vascular tissue of plants. We are currently performing validation studies for this claim.

Careful X-ray dose optimization allowed us to capture the 3D microstructure of living plants with applied NPs at multiple time frames. With the first commercial propagation based phase contrast tomograph installed at 3D imaging center at DTU we are investigating the NP transport in plants for up to 6 days from foliar application, while with synchrotron imaging we focus on sub-micrometer resolution 3D representation of cellular plant structures involved in NP transport.

- [1] Husted. et al., 2023. Trends in Plant Science. 8, (1), 90-105 https://doi.org/10.1016/j.tplants.2022.08.017
- [2] Burkhardt, J. (2010). Hygroscopic particles on leaves: nutrients or desiccants? Ecol. Monogr. 80, 369-399

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