New clearing protocol for tannic roots optical imaging

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Trends in Plant Science | Technology of the Month

New clearing protocol for tannic roots optical imaging

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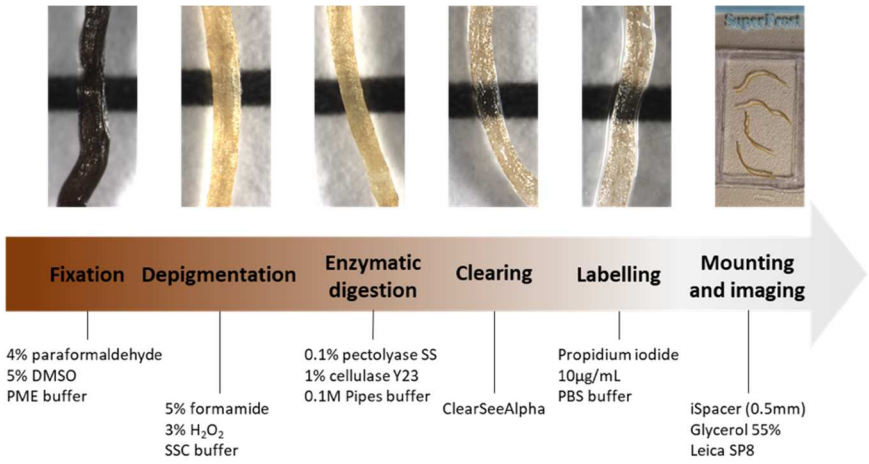
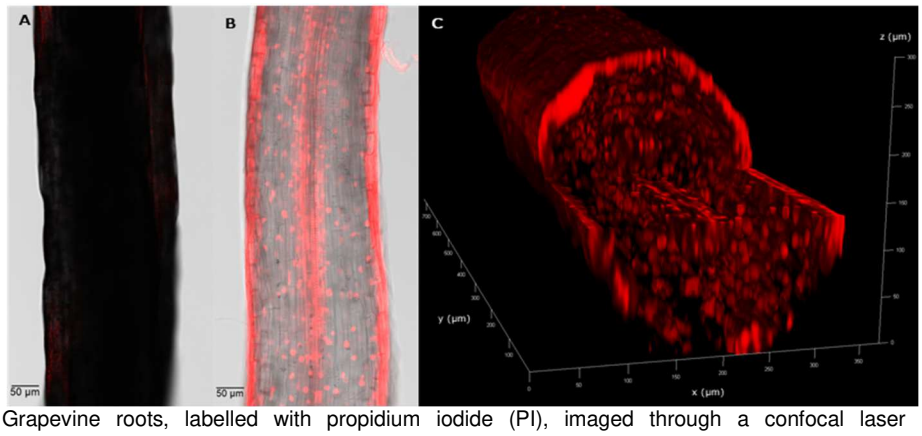


The understanding of plant physiological complexity requires 3D imaging of entire organ with subcellular resolution. However, most plant organs are highly opaque to light, and their study under optical sectioning microscopes is difficult. By gathering latest developments in the field of optical clearing of pigmented plant tissues Clearing tannic roots (CTR) protocol drastically reduces light absorption by a depigmentation step, and light scattering by the homogenization of refractive indices through entire grapevine roots.



Grapevine roots, labelled with propidium iodide (PI), imaged through a confocal laser fluorescent microscope (Leica SP8, x16, immersion objective glycerol 55%, wavelength excitation: 552nm, emission: 589 – 653nm). Overlay of brightfield and PI signal of an uncleared root (A), cleared root (B) at a depth of 130µm in z axis. 3D reconstruction of PI signal (C) corresponds to root B. The nuclear staining by PI is clearly recorded on the entire root thickness (250µm).

ADVANTAGES:
Clearing tannic roots (CTR) provides access to 3D anatomical features of perennial plant tissues without physical sectioning.
CTR is efficient on lignified roots larger than 250µm of two different rootstocks (Riparia and SO4).
ClearSeeAlpha clearing process preserves fluorescence signal and is suitable with endogenous probes (*e.g.* mClover).
CTR can be applied to roots colonized by bacteria and/or fungi. Promising new anatomical and physiological insights concerning parasitism and symbiosis mechanisms at the organ and entire plant scale can be obtained.

CHALLENGES:
The sample preparation is time consuming (2 weeks minimum are needed). Each step has to be adapted according to the age of the sample and growing conditions (*e.g.* substrates). This method must be tested on older tissues, species and field samples.
Chemical and enzymatic treatments can lead to a loss of tissue component (*e.g.* lipids and oligosaccharide, respectively).
Imaging in depth requires a specific mounting procedure (spacers between glace and coverslip) and requires specific equipment (long working distance and immersion objectives).
By using CTR and a performant microscope rather than a confocal microscope concerning depth imaging, the limitation of 250µm in depth should be overcome (*e.g.* light sheet fluorescent microscope).

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