



**HAL**  
open science

## New clearing protocol for tannic roots optical imaging

Mathilde Hériché, Christine Arnould, Daniel Wipf, Pierre-Emmanuel Courty

► **To cite this version:**

Mathilde Hériché, Christine Arnould, Daniel Wipf, Pierre-Emmanuel Courty. New clearing protocol for tannic roots optical imaging. *Trends in Plant Science*, 2022, 27 (6), pp.616-617. 10.1016/j.tplants.2021.08.015 . hal-03716441

**HAL Id: hal-03716441**

**<https://hal.inrae.fr/hal-03716441v1>**

Submitted on 22 Jul 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

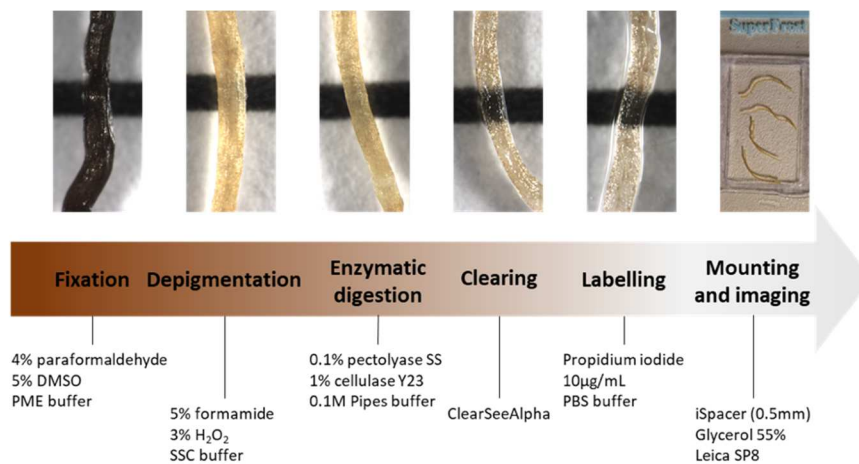


Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

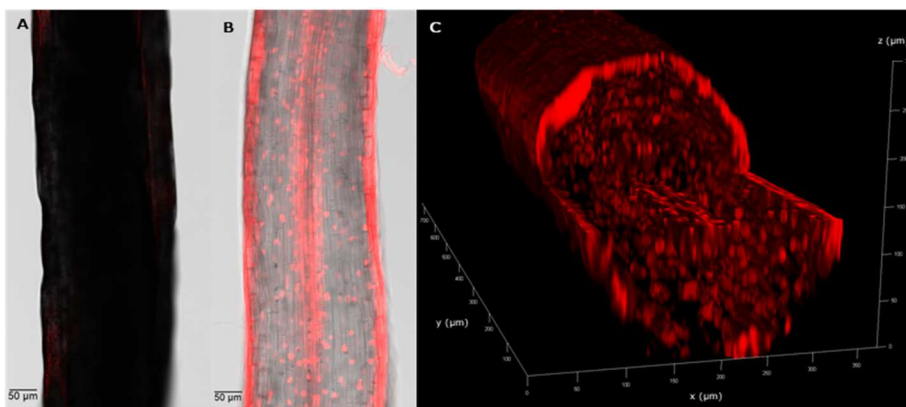
# New clearing protocol for tannic roots optical imaging

Hériché Mathilde<sup>1</sup>, Arnould Christine<sup>1</sup>, Wipf Daniel<sup>1</sup> and Pierre-Emmanuel Courty<sup>1,\*</sup>

<sup>1</sup>Agroécologie, AgroSup Dijon, CNRS, Univ. Bourgogne, INRAE, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France



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 uncolored 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 glass 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).

\*Correspondence:  
emmanuel.courty@inrae.fr

pierre-emmanuel.courty@inrae.fr  
(P.-E.Courty)

## Acknowledgments

The authors thank the Burgundy Franche Comté Regional Council. MH receives a doctoral contract accredited by the French Ministry of Higher Education, Research and Innovation. This work has benefited from the facilities of the Centre de Microscopie INRAE Dijon/Université de Bourgogne, Plateforme DImaCell (INRAE, Agroécologie, Plateforme DImaCell, Centre de Microscopie INRAE/Université de Bourgogne, Dijon, France).

## Literature

- 1 Affaticati, P. *et al.* (2018) X-FaCT: Xenopus-Fast Clearing Technique. In *Xenopus 1865* (Vleminckx, K., ed), pp. 233–241, Springer New York
- 2 Nagaki, K. *et al.* (2017) ePro-ClearSee: a simple immunohistochemical method that does not require sectioning of plant samples. *Scientific Reports* 7, 42203
- 3 Kurihara, D. *et al.* (2021) ClearSeeAlpha: Advanced Optical Clearing for Whole-Plant Imaging. *Plant and Cell Physiology* DOI: 10.1093/pcp/pcab033
- 4 Hériché, M. *et al.* (2021) Optical imaging of cleared plant organs. Submitted to *Trends in Plant Science*
- 5 Warner, C.A. *et al.* (2014) An Optical Clearing Technique for Plant Tissues Allowing Deep Imaging and Compatible with Fluorescence Microscopy1[W][OPEN]. *Plant Physiol* 166, 1684–1687
- 6 Kurihara, D. *et al.* (2015) ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging. *Development* 142, 4168–4179
- 7 Imoto, A. *et al.* (2021) A ClearSee-Based Clearing Protocol for 3D Visualization of Arabidopsis thaliana Embryos. *Plants* 10, 190
- 8 Li, Y. *et al.* (2016) Optically Transparent Wood from a Nanoporous Cellulosic Template: Combining Functional and Structural Performance. *Biomacromolecules* 17, 1358–1364
- 9 Rae, A.E. *et al.* (2021) New methods for confocal imaging of infection threads in crop and model legumes. *Plant Methods* 17, 24
- 10 Moukarzel, R. *et al.* (2020) An improved clearing and staining protocol for evaluation of arbuscular mycorrhizal colonisation in darkly pigmented woody roots. *NZPP* 73, 33–39
- 11 Yu, T. *et al.* (2021) Physical and chemical mechanisms of tissue optical clearing. *iScience* 24, 102178