Deep-tissue live-imaging in plant organs with multi-photon microscopy

A.C.J. Timmers[1], C. Gough[2], S. Bensmihen[2]

[1] Central Microscopy, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany; [2] Laboratory of Plant-Microbe Interactions and Environment (LIPME), University Toulouse III, INRAE, CNRS, 31326 Castanet-Tolosan Cedex, France.

Abstract

The use of fluorescent proteins in plant research allows the study of cellular processes in living plant tissues and has increased our knowledge of cellular functioning enormously in the last 2 decades. The fluorescent markers are localised and analysed by sophisticated fluorescent microscopy systems of which point scanning confocal microscopy is most widely used. Other systems are light sheet microscopy, spinning disk confocal microscopy and rescan confocal microscopy. All these microscopes are optical sectioning systems using laser light for excitation. This guarantees precise specific excitation of fluorescent markers within plant organs. Unfortunately, in most living plant organs, fluorescent signals can only be registered from cell layers that are on the periphery of the organ, and deeper cell layers are not accessible. Only in thin roots, like those of Arabidopsis thaliana with only one cortical layer, can inner tissues be studied. In other plant species with thicker roots and in shoots of all plant species, only the peripheral cell layers are amenable to imaging with fluorescence microscopes. Even if the laser light reaches the inner layers the emitted fluorescent signal is diffracted and reflected by the plant cell wall which results in very poor image quality from the inner cell layers of living plant organs. Recently developed clearing protocols of whole plant organs overcome the problem of poor image quality from deep cell layers in almost all plant organs from many plant species. Clearing requires chemical fixation prior to clearing and thus, excludes live cell imaging. For a complete picture of the early cortical steps of the symbiotic interaction between Medicago truncatula and its microbial symbiont, Sinorhizobium meliloti, and to study cellular processes inside later stage nodules, live cell imaging is required. We developed a protocol combining the expression of strong plasma membrane and cell wall located fluorescent markers with multi-photon fluorescence microscopy for the study of cellular processes deep within living plant organs. We tested several strong promoters and a number of bright fluorescent proteins and imaged the localisation with multi-photon confocal microscopy. We obtained high quality images from all cell layers of living roots and deep inside living nodules. In addition, long-term live imaging was possible with very little photobleaching.