

APPI: a Versatile Platform for Whole Plant Imaging

B. M. ORLANDO MARCHESANO (1), G. TORTORA (2), S. BURATTI (1), A. CANDEO (2), L. LUONI (1), M. GRENZI (1), A. BASSI (2), A. COSTA (1)

(1) Department of Biosciences, University of Milano, 20133, Milano, Italy,

(2) Department of Physics, Politecnico di Milano, 20133, Milano, Italy

Unable to escape from unfavourable conditions, plants evolved complex mechanisms to perceive and respond to various environmental stimuli using signaling molecules and second messengers. *In vivo* imaging experiments on Arabidopsis plants expressing Genetically Encoded Fluorescent Indicators (GEFI) revealed that mechanical stimuli trigger long-distance apoplastic glutamate and cytosolic Ca^{2+} waves propagating from leaf-to-leaf or root-to-leaf.

These signals are essential for activating stress marker genes and hormone synthesis in systemic tissues. While Arabidopsis has been key in uncovering these processes, it remains unclear whether such mechanisms are conserved in larger plants. Thus, we investigated adult *Nicotiana benthamiana* plants expressing different GEIs to *in vivo* study Ca^{2+} and glutamate dynamics. To image *N. benthamiana* plants, we developed the Adult Plant Projective Imaging (APPI) platform, a custom-made large field-of-view fluorescence imaging setup, that allows the simultaneous orthogonal imaging of shoot and root of plants with sizes up to tens of centimetres. Thanks to the APPI system we were able to confirm the existence of local and systemic Ca^{2+} and glutamate signals propagation in the shoot in response to mechanical damages and insect chewing. By leveraging the orthogonal view and customized 3D-printed supports, we visualized shoot-to-root Ca^{2+} signals triggered by mechanical damage, revealing communication between above- and below-ground plant's organs.