

Development of the Adult Plant Projective Imaging (APPI) System: Democratizing Imaging for Non-Model Plants

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Through their lifespan, plants face various abiotic and biotic stresses. Unable to escape from unfavourable conditions, they have evolved complex mechanisms to perceive and respond to various stimuli employing a battery of signaling molecules and second messengers. *In vivo* imaging experiments with *Arabidopsis thaliana* plants expressing Genetically Encoded Fluorescent Indicators (GEFI) revealed that mechanical stimuli trigger long-distance apoplastic glutamate and cytosolic Ca²⁺ waves propagating from leaf-to-leaf or root-to-leaf. Importantly, these signals are needed to induce the upregulation of stress marker genes and the synthesis of stress hormones in systemic tissues. While *Arabidopsis* has been instrumental in uncovering these processes, it remains unclear whether such mechanisms are conserved in larger species.

To answer this question, we focused on adult *Nicotiana benthamiana* plants expressing different GEFls to study *in vivo* the dynamics of Ca²⁺ and glutamate. To perform imaging experiments with *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 glutamate and Ca²⁺ signals propagation in the shoot in response to mechanical damages. By leveraging the orthogonal view, and the design of custom-made rhizotrons, we could also visualize root-to-shoot Ca²⁺ waves triggered in response to salt stress.

Altogether, APPI represents a revolutionary imaging system for understanding how big plants perceive and face to current environmental stresses.