New state-of-the-art imaging tools to study how crops adapt to environmental changes: *Lycopersicum esculentum* as key study

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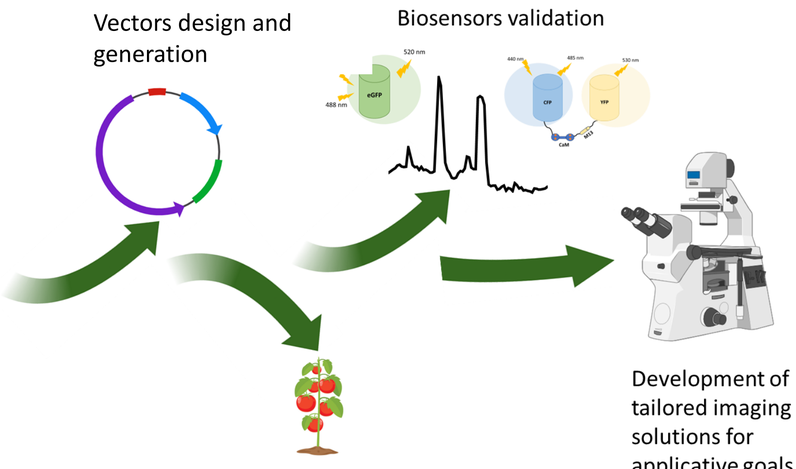
Being sessile organisms, plants are subjected to environmental challenges during their entire life. Both abiotic and biotic stresses, such as water deficiency, salt stress and pathogen attack, could significantly influence plant growth as well as crop yield. Therefore, understanding plant response to external challenges is of vital importance.

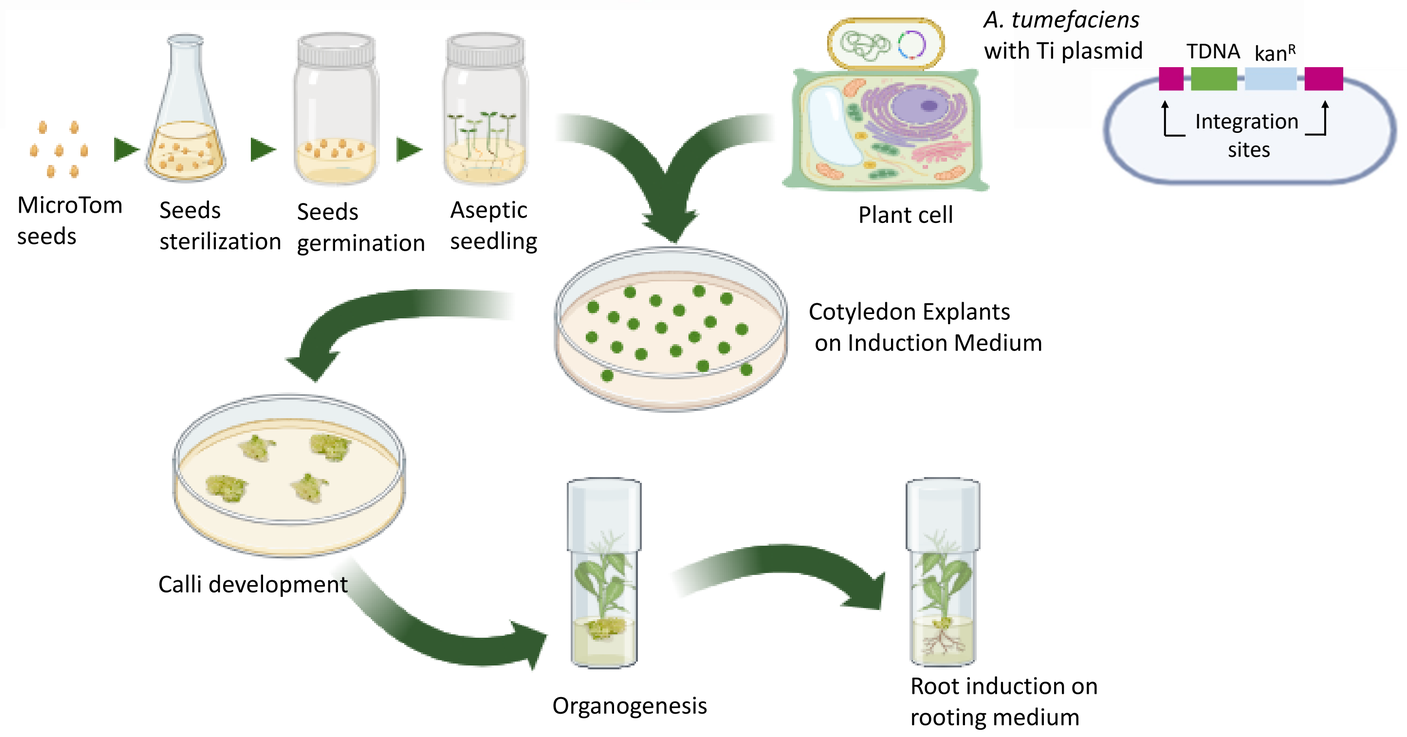
In the last decades, molecular signalling from the stimulus perception to plant physiological response was largely investigated. However, most of the available knowledges on plant signalling derive from studies performed on *Arabidopsis thaliana* model plant.

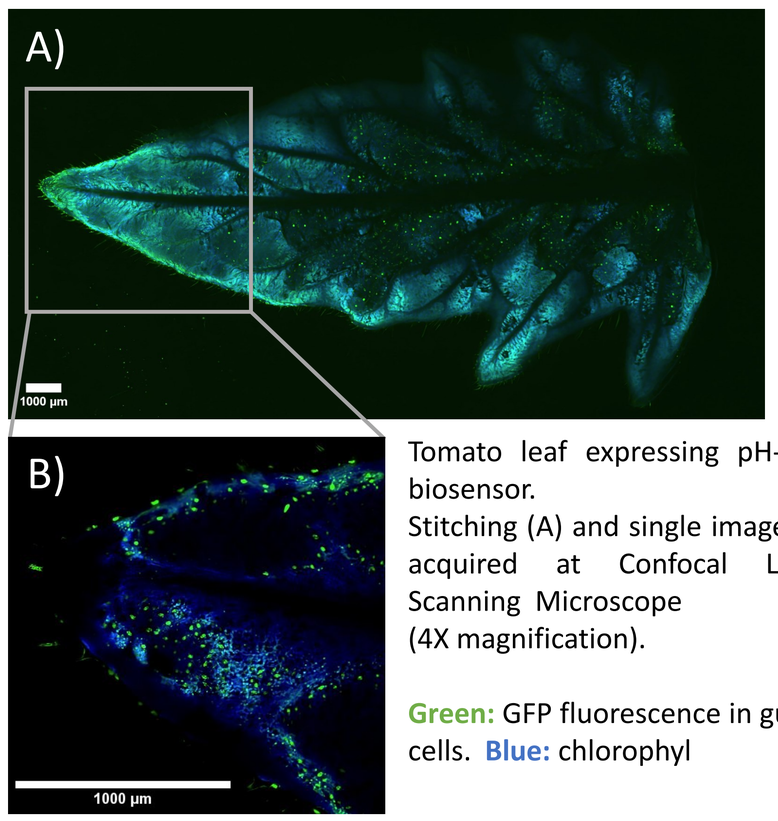
To investigate how crops deal with environmental stimuli, we decided to focus our attention on *Lycopersicum esculentum.* Tomato represents a promising model plant since it is a worldwide economically important crop and its growth as well as its productivity are sensitive to inappropriate external conditions. To better understand crop signalling, we aimed at combining development of a battery of tomato lines expressing Genetically Encoded Fluorescent Indicators (GEFIs) with tailored *in-vivo* imaging approaches.

So far, we set up an efficient transformation protocol by employing an *ad hoc* vector. We succeeded in obtaining MicroTom lines expressing Ca^{2+} and pH biosensors which will allow us to study their cellular dynamics in response to external stimuli or during plant development. Preliminary experiments carried out with the two sensor lines will be presented.



MicroTom Transformation and Regeneration



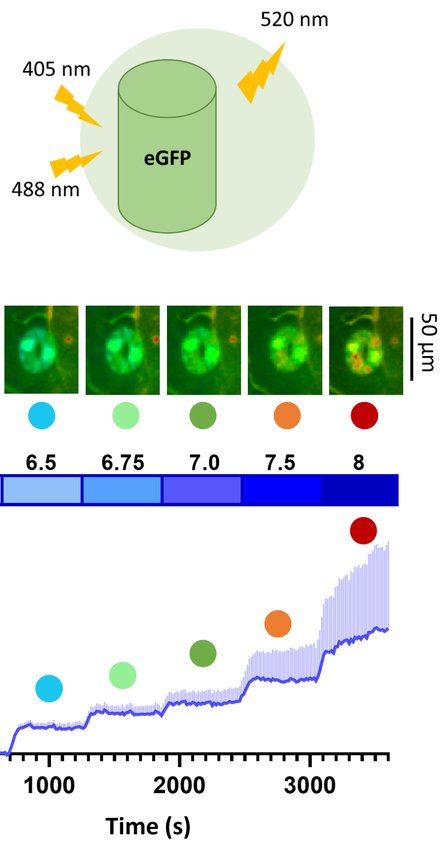


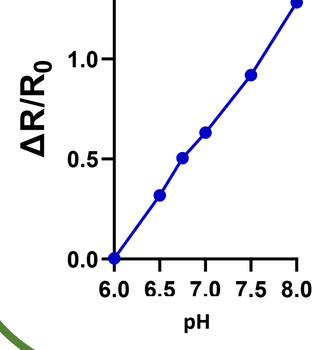
Tomato leaf expressing pH-GFP biosensor.
Stitching (A) and single image (B) acquired at Confocal Laser Scanning Microscope (4X magnification).

Green: GFP fluorescence in guard cells. **Blue:** chlorophyl

pH-GFP

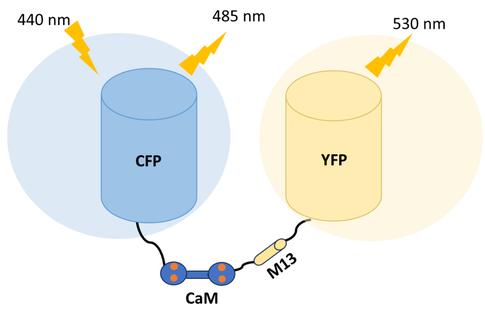
To validate pH-GFP MicroTom lines, we tested the *in vivo* responsiveness of the biosensor. Gradual increase in pH values of exogenous administrated $[H^+]$ buffer solutions generates subsequent cytosolic basification [1].
$\Delta R/R_0$: normalized ratiometric response

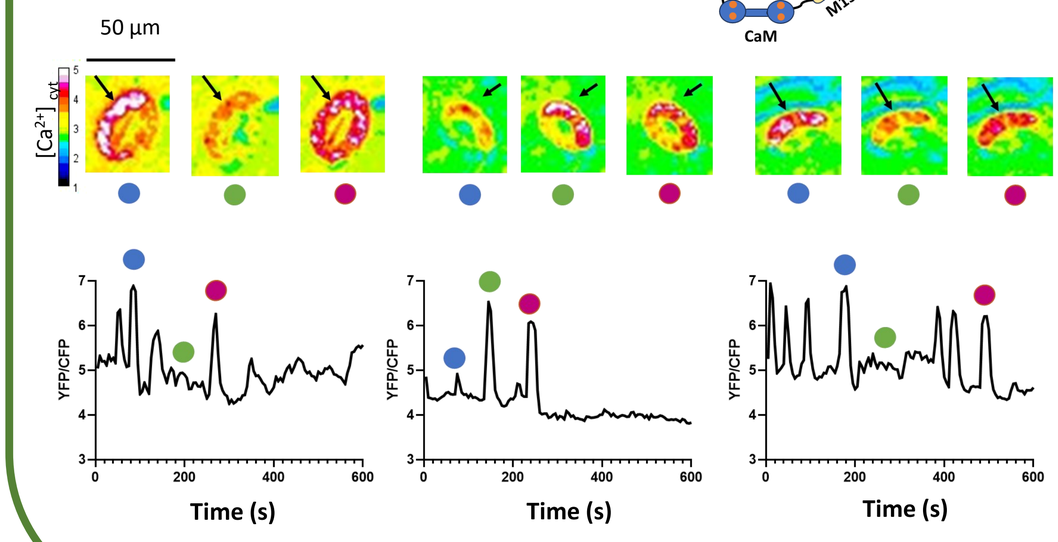




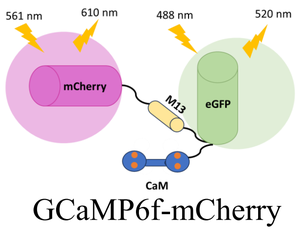
NES YC3.6

To validate MicroTom lines expressing NES-Yellow Cameleon 3.6 sensor, we monitored guard cell spontaneous Ca^{2+} oscillations.

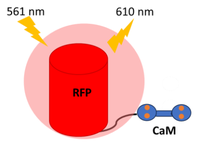




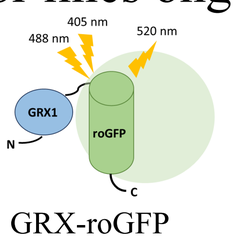
New MicroTom biosensor lines ongoing



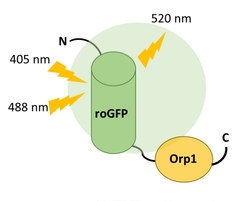
GCaMP6f-mCherry



RGECO1



GRX-roGFP



roGFP-Orp1

References

[1] Behera et al. 2018 “Cellular Ca^{2+} Signals Generate Defined pH Signatures in Plants” The Plant Cell

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