



Department of Science
PhD project - XXXIX cycle - 2023/2024

The role of ROS production and perception in root xylem phenotypic plasticity and stomatal closure under abiotic stress in *Arabidopsis thaliana*

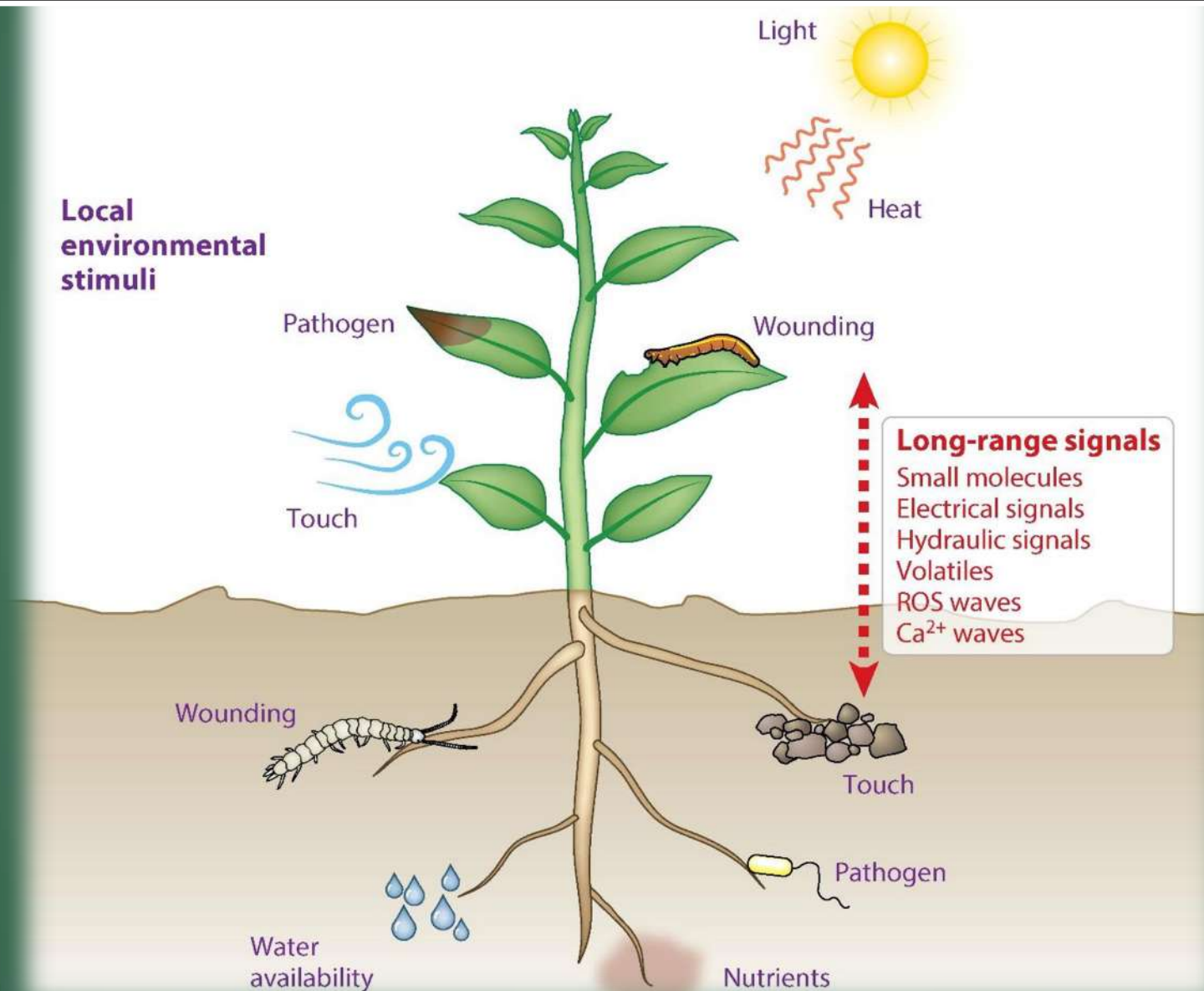


Tutor: Prof.ssa Alessandra Cona
Co-tutor: Dott.ssa Ilaria Fraudentali

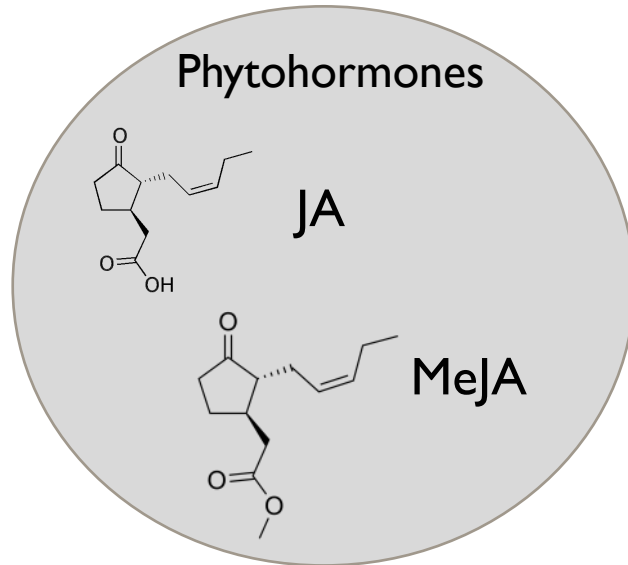
Adriana Furlani



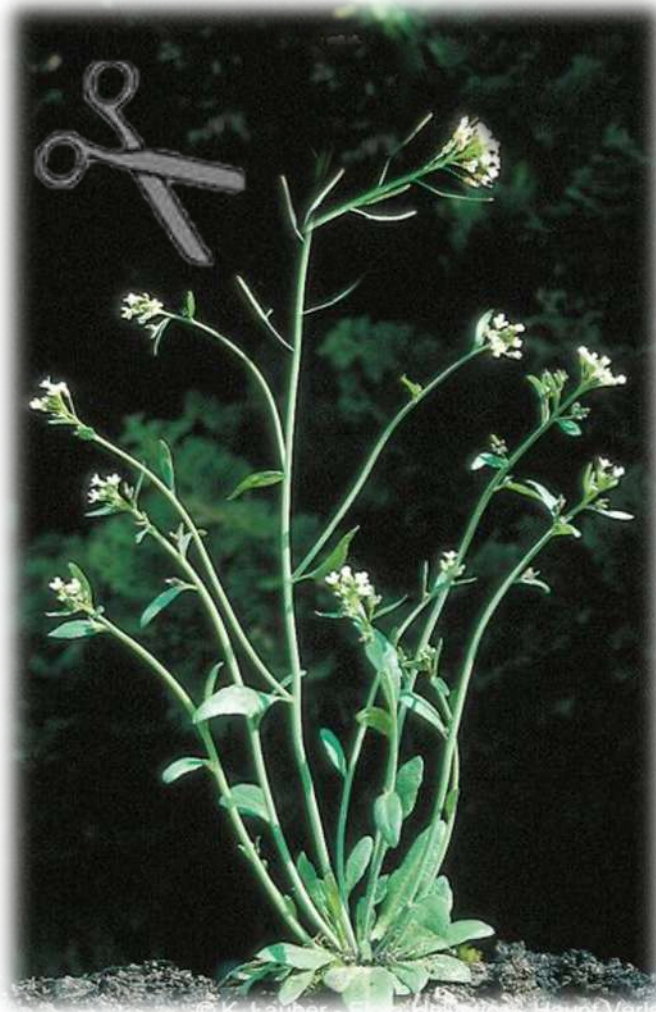
PLANTS AND ENVIRONMENTAL STRESS



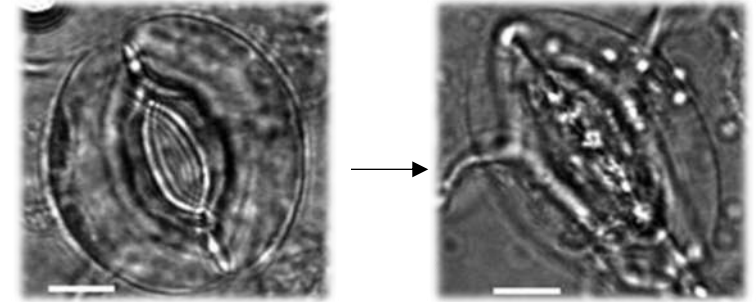
ABIOTIC STRESS: WOUNDING



A. thaliana

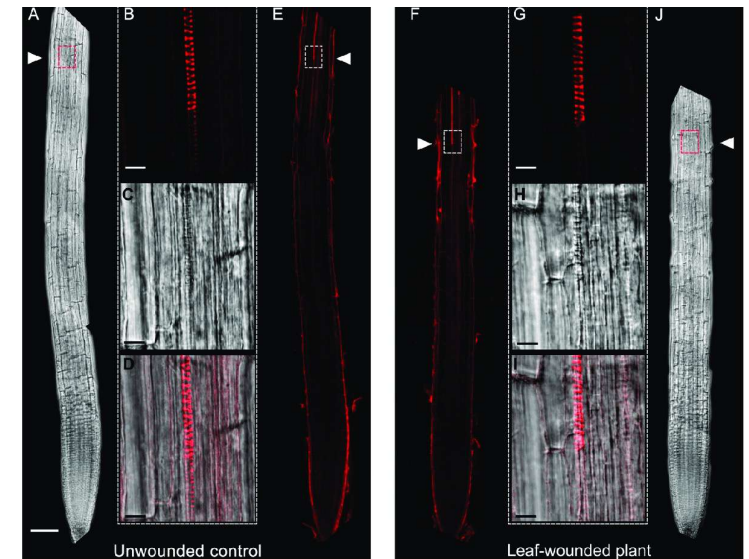


STOMATAL CLOSURE



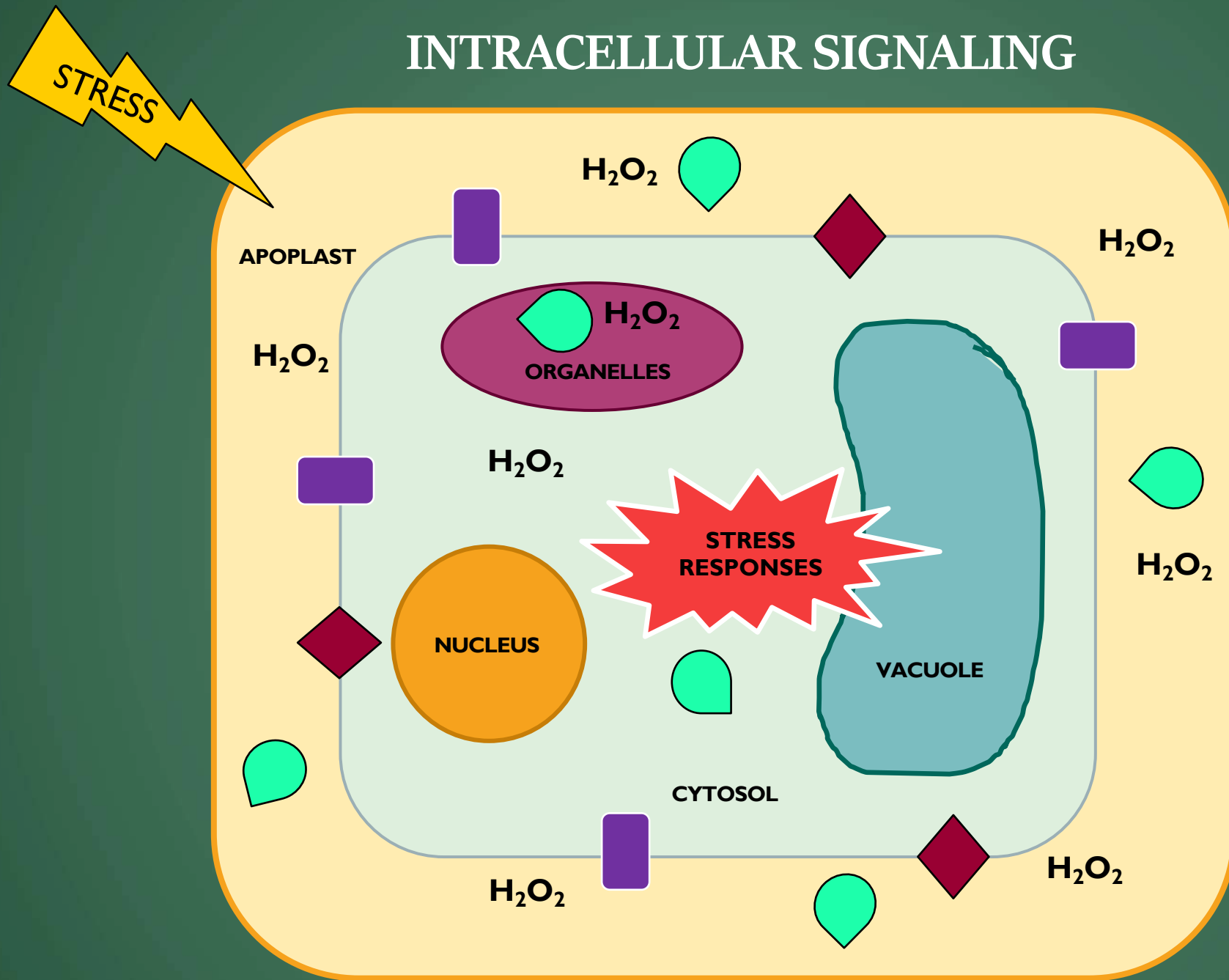
Mirasole et al., 2023

EARLY ROOT PROTOXYLEM DIFFERENTIATION



Fraudentali et al., 2018

INTRACELLULAR SIGNALING

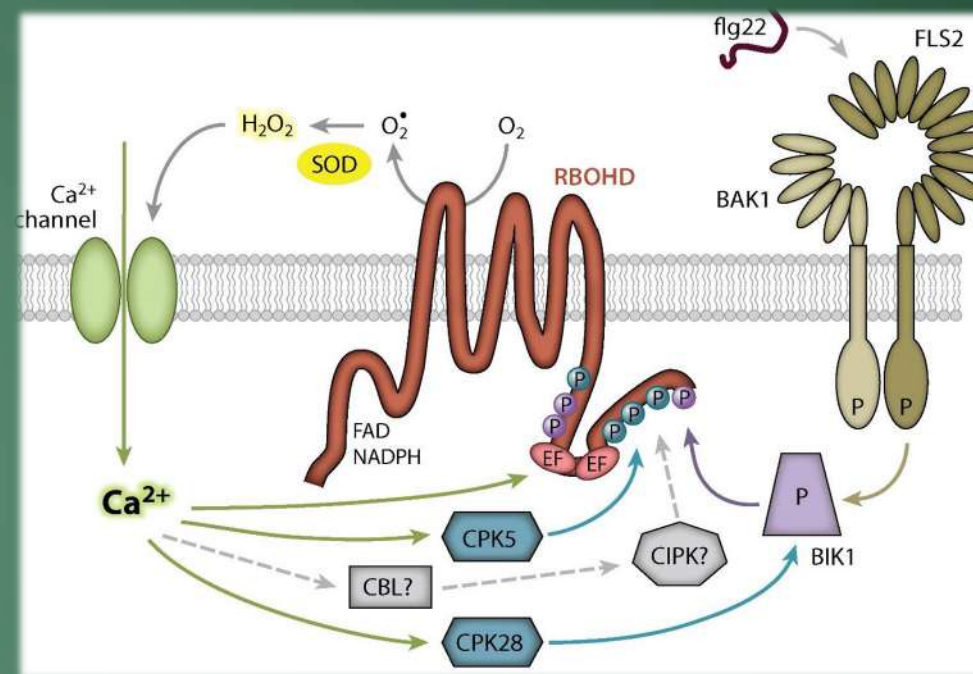


-  Amine oxidases (AOs)
-  RBOH proteins
-  HPCA1 proteins



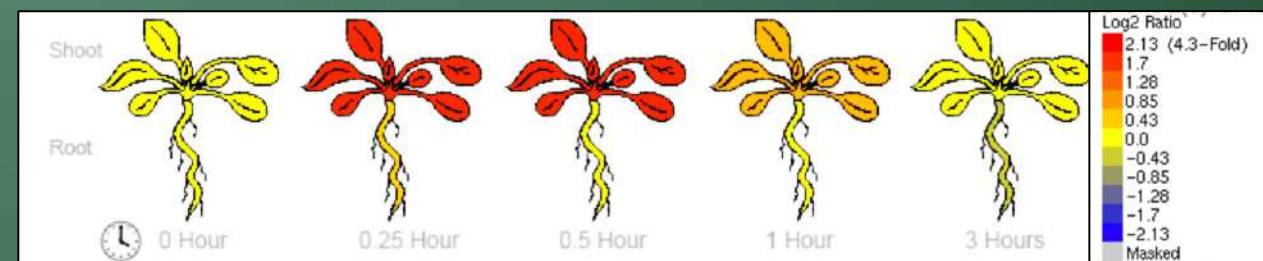
RBOH proteins

Plant NADPH oxidases (RBOHs) are plasma membrane proteins that catalyze the formation of superoxide anion ($O_2^{\cdot -}$), which converts, spontaneously or thanks to the superoxide dismutase activity, into H_2O_2 , which **accumulates in the apoplast**.

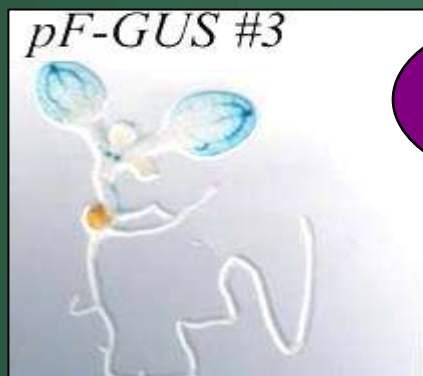


Choi et al., 2016

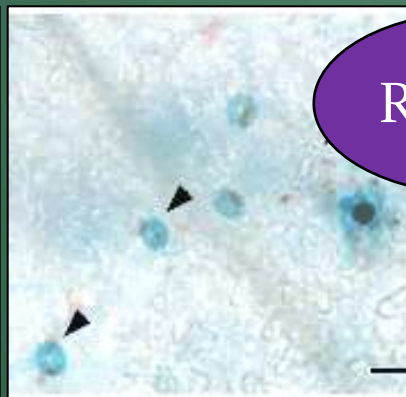
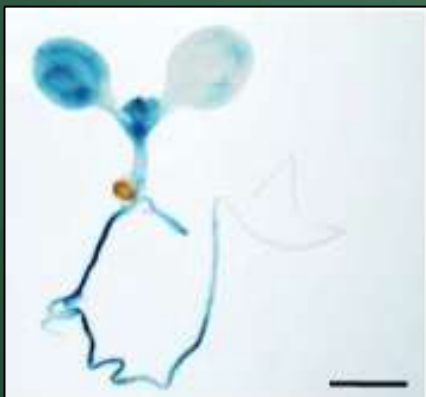
WOUNDING



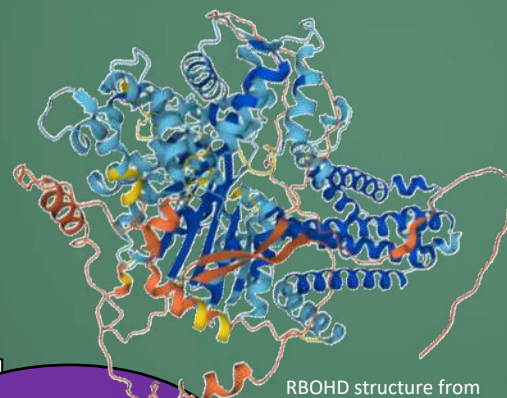
https://bar.utoronto.ca/efp2/Arabidopsis/Arabidopsis_eFPBrowser2.html



RBOHF



RBOHD



RBOHD structure from <https://www.uniprot.org>

Modified from Morales et al., 2016

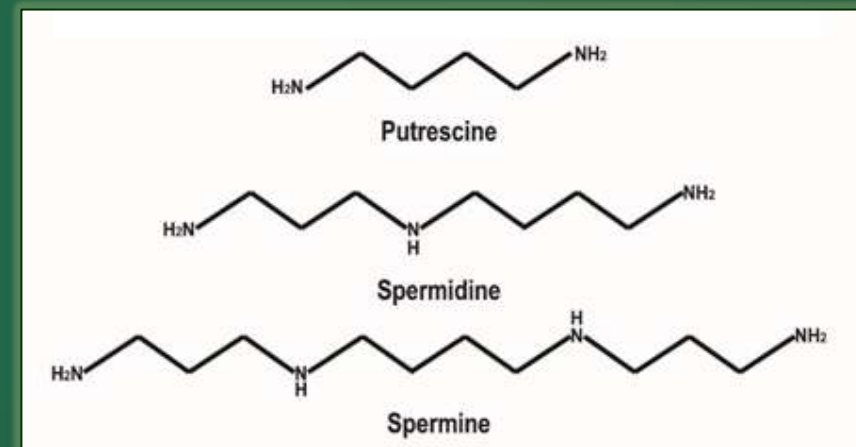


AtCuAOs



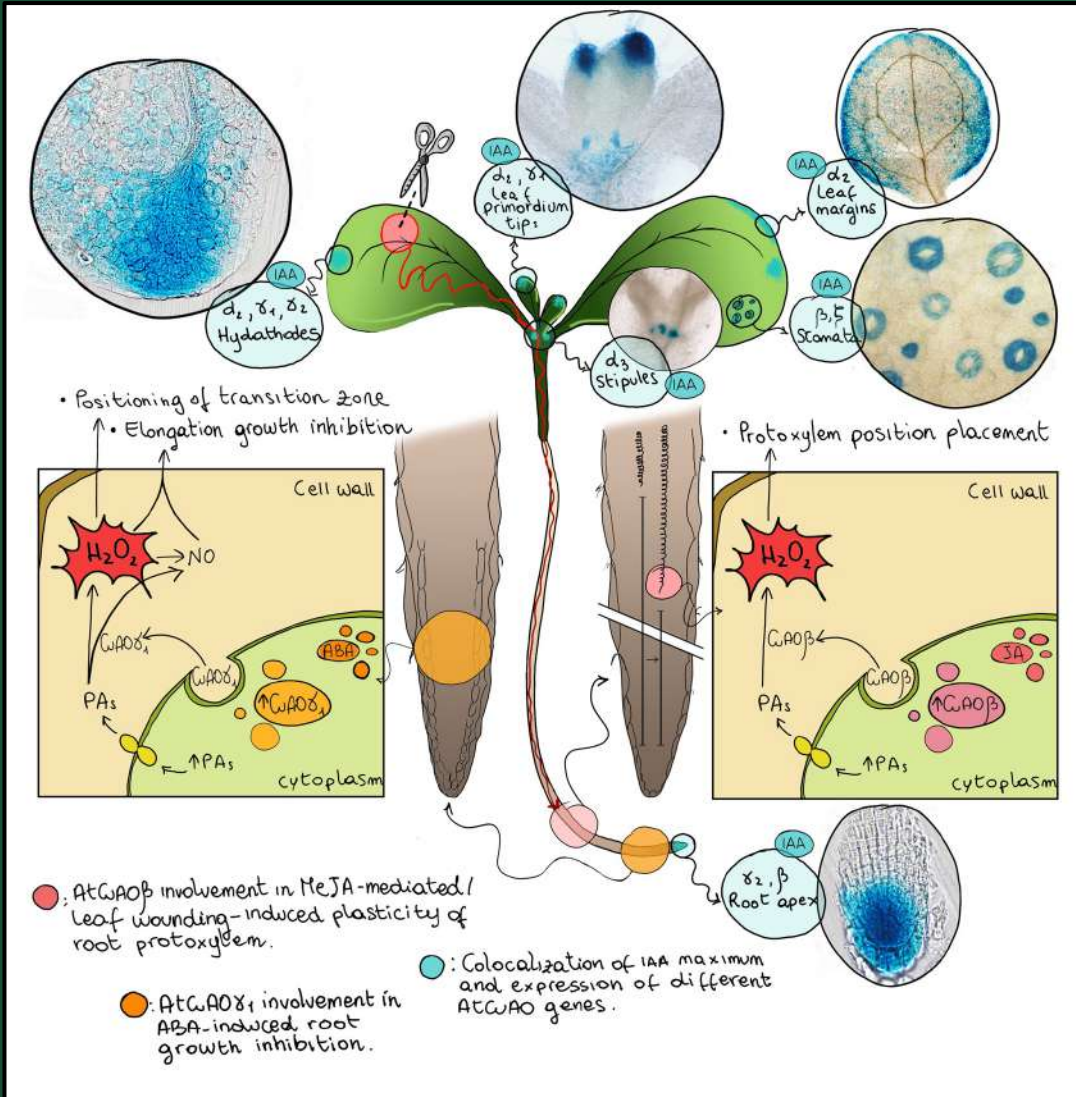
AOs include two distinct enzyme classes, copper amine oxidases (CuAOs) and flavin adenine dinucleotide (FAD)- dependent polyamine oxidases (PAOs).

POLIAMINES



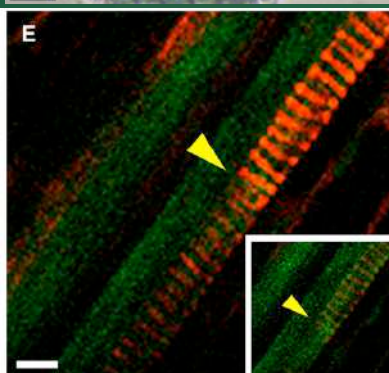
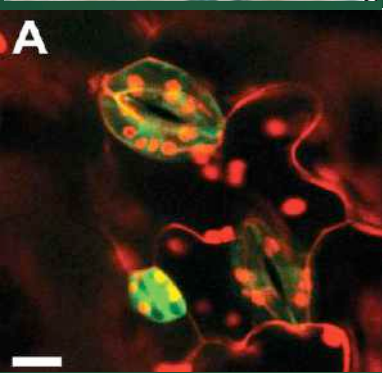
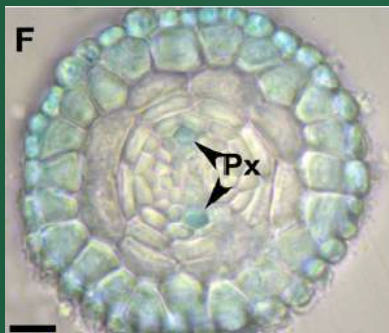
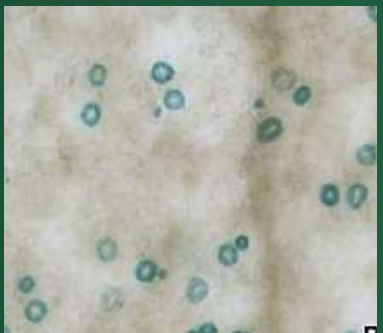
AtCuAOs

- regulate the homeostasis of PAs
- catabolize PAs, producing aminoaldehyde, aminobutanal and



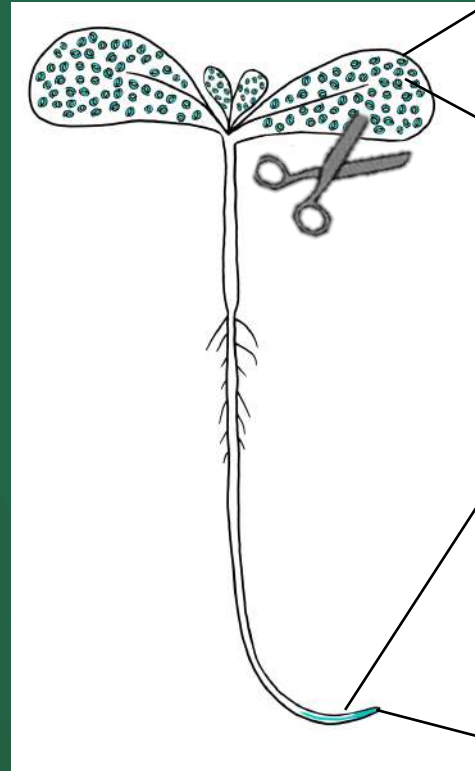
Arabidopsis thaliana CuAO β

CONSTITUTIVE ESPRESSION

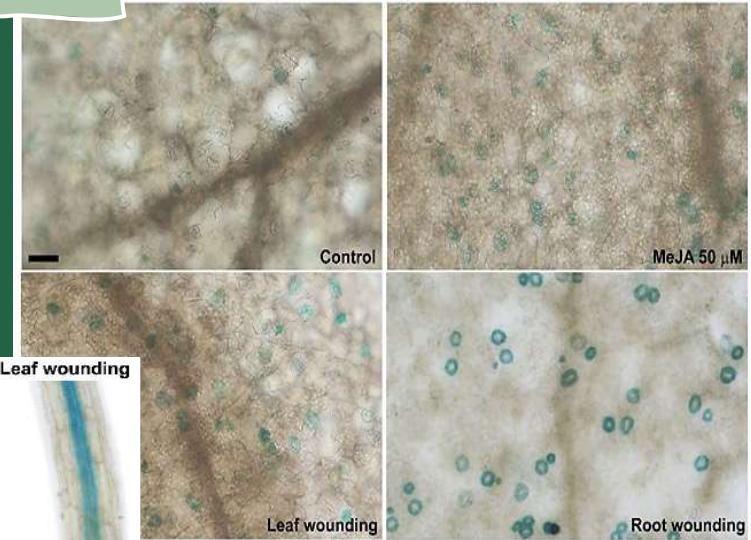


LEAF/ROOT WOUNDING AND MeJA TREATMENT INDUCE *AtCuAOβ* TISSUE SPECIFIC EXPRESSION

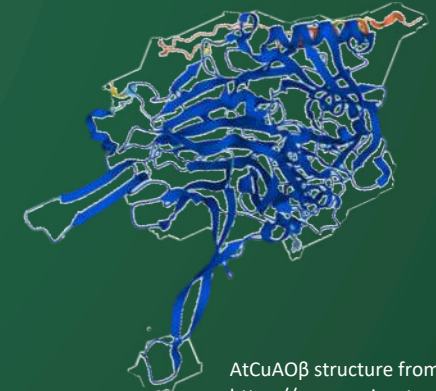
AtCuAOβ-promoter::YFP
AtCuAOβ-promoter::GFP-GUS



INDUCIBLE ESPRESSION



Fraudentali et al., 2020

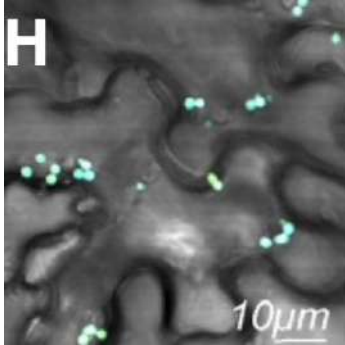


AtCuAO β structure from
<https://www.uniprot.org>

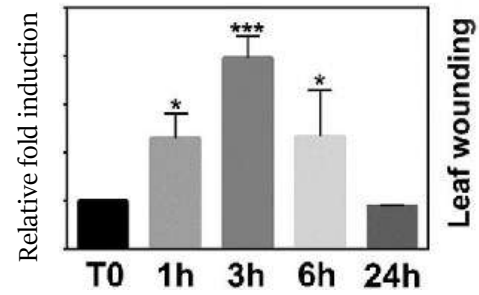
Other AtCuAOs localized in the root

AtCuAOα3

Peroxisomal localization

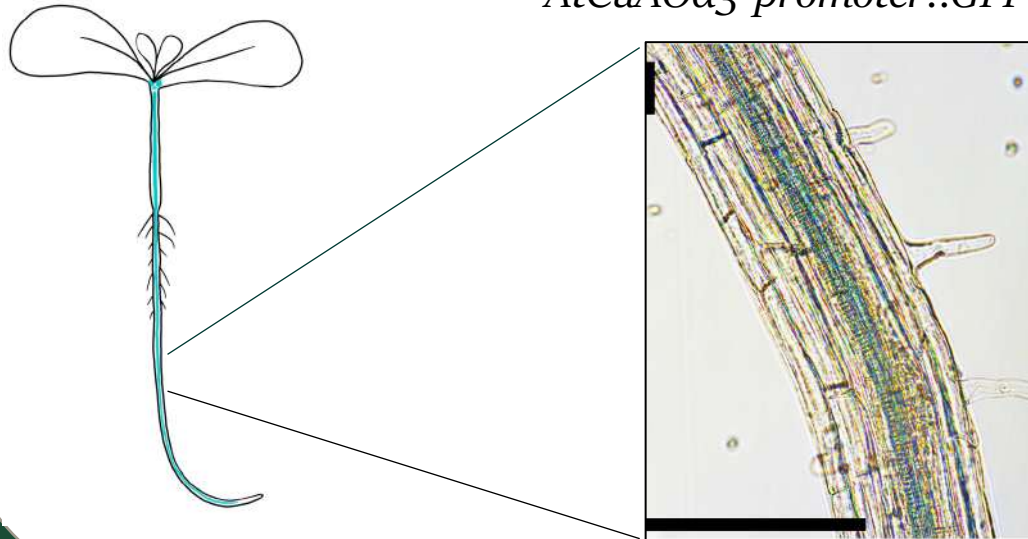


RT-qPCR analysis



Leaf wounding

AtCuAOα3-promoter::GFP-GUS



Fraudentali et al., 2020

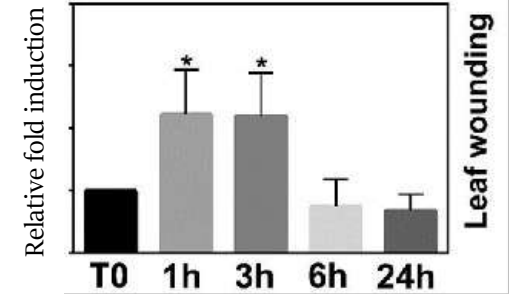


AtCuAOγ2

Localization

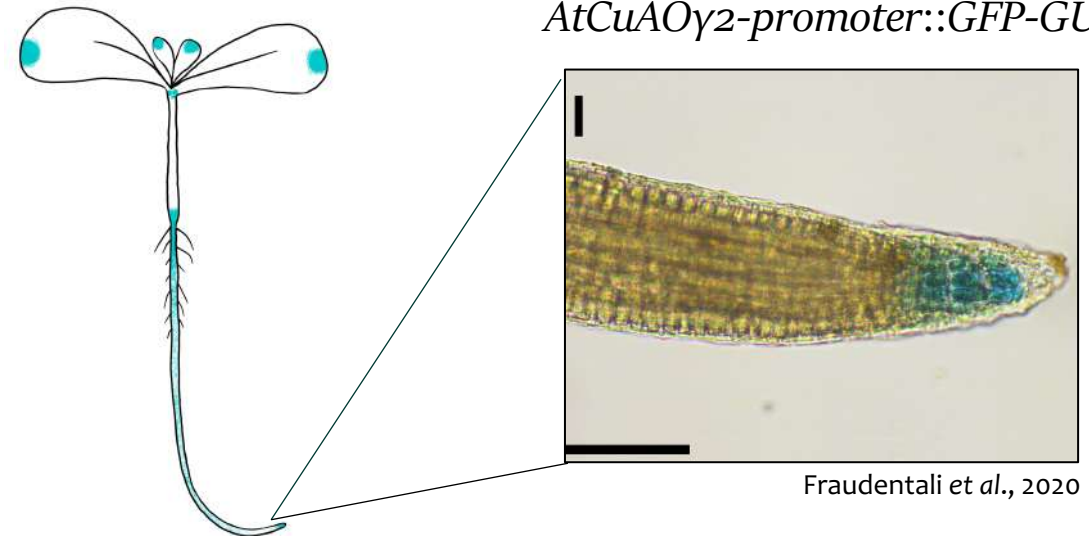
Putative apoplastic?

RT-qPCR analysis



Leaf wounding

AtCuAOγ2-promoter::GFP-GUS



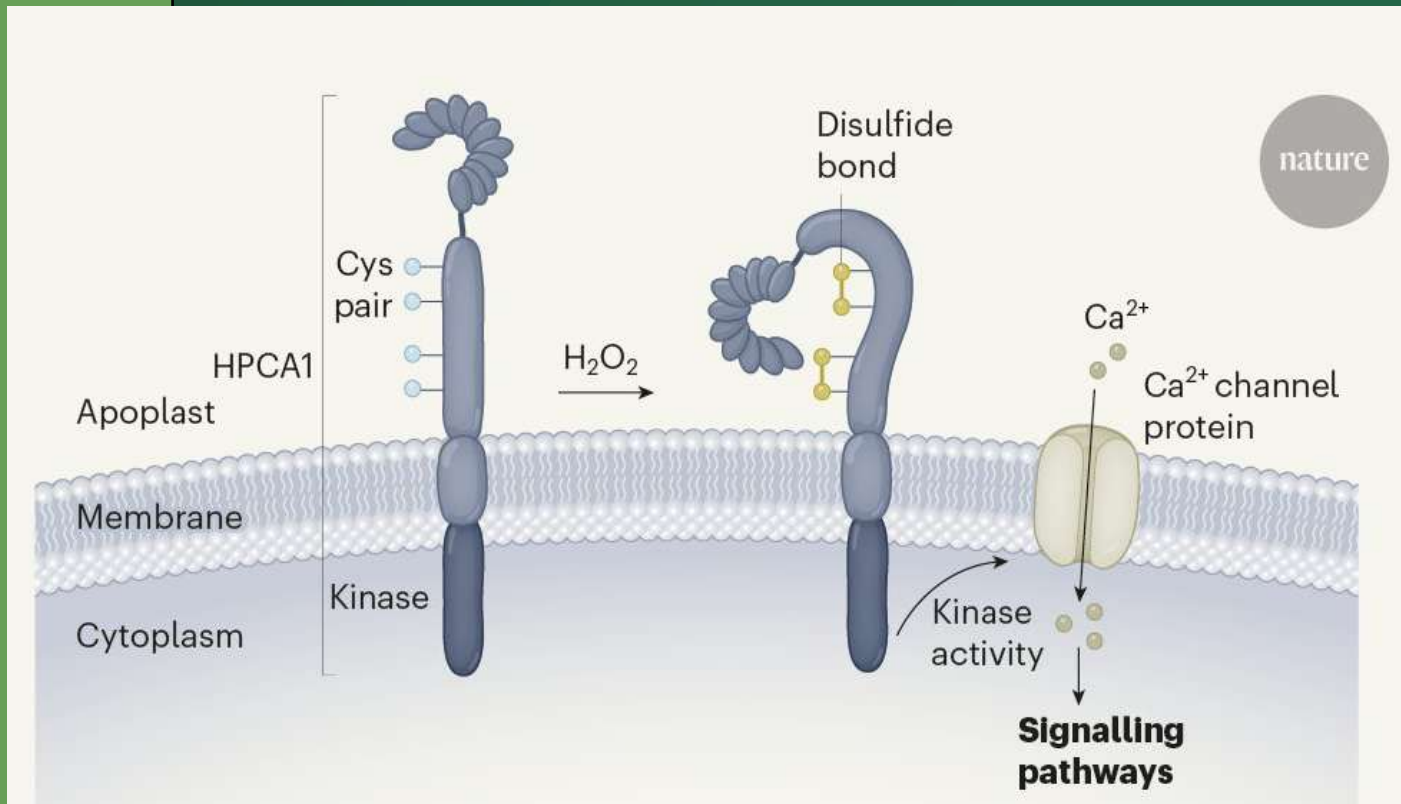
Fraudentali et al., 2020



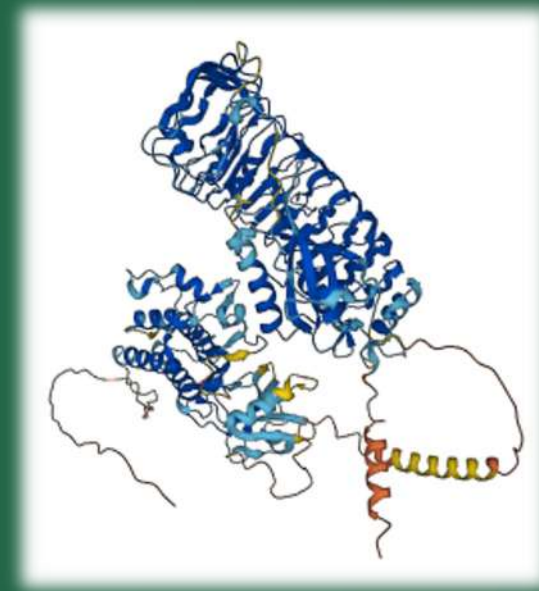


HPCA1 protein

The *Arabidopsis thaliana* leucine-rich-repeat receptor-like kinase H₂O₂-INDUCED Ca²⁺ INCREASES 1 (**HPCA1**) acts as a H₂O₂ receptor required for the propagation of cell-to-cell ROS signals triggered by ROS accumulation in the apoplast after stress

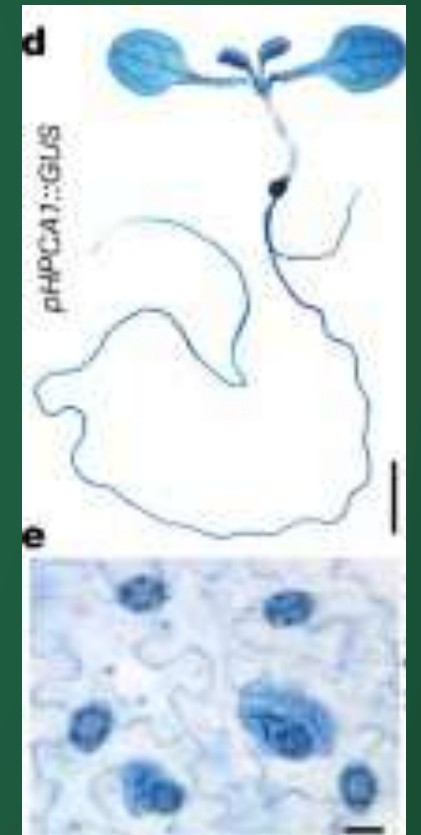


Foyer, 2020



<https://alphafold.ebi.ac.uk/entry/AoA178UIE9>

Constitutive expression



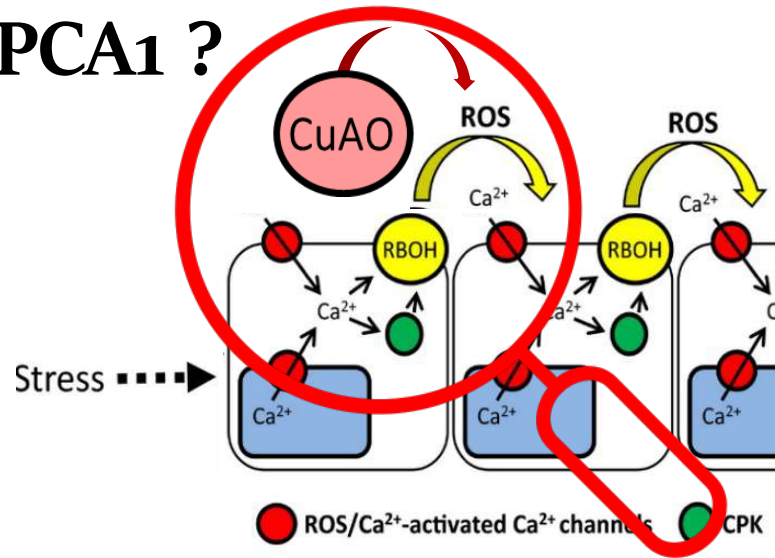
Wu et al., 2020



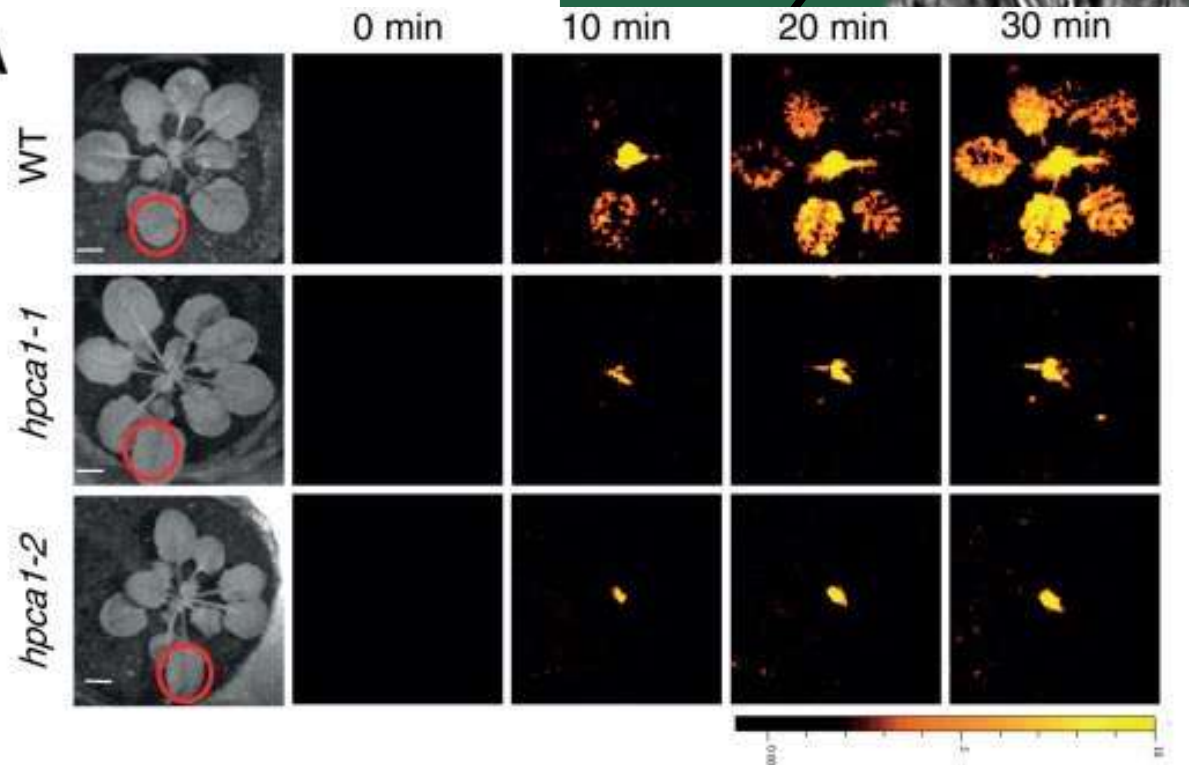
RBOHs, AtCuAOs and HPCA1: ROS AND Ca^{2+} cooperation

HPCA1 mediates H_2O_2 -induced activation of Ca^{2+} channels in guard cells and is required for stomatal closure and, likely, for early root protoxylem differentiation

HPCA1 ?



A



THE AIM OF THE PROJECT

✓ AtCuAO β

AtCuAO α 3

AtCuAO γ 2

RBOHD

HPCA1

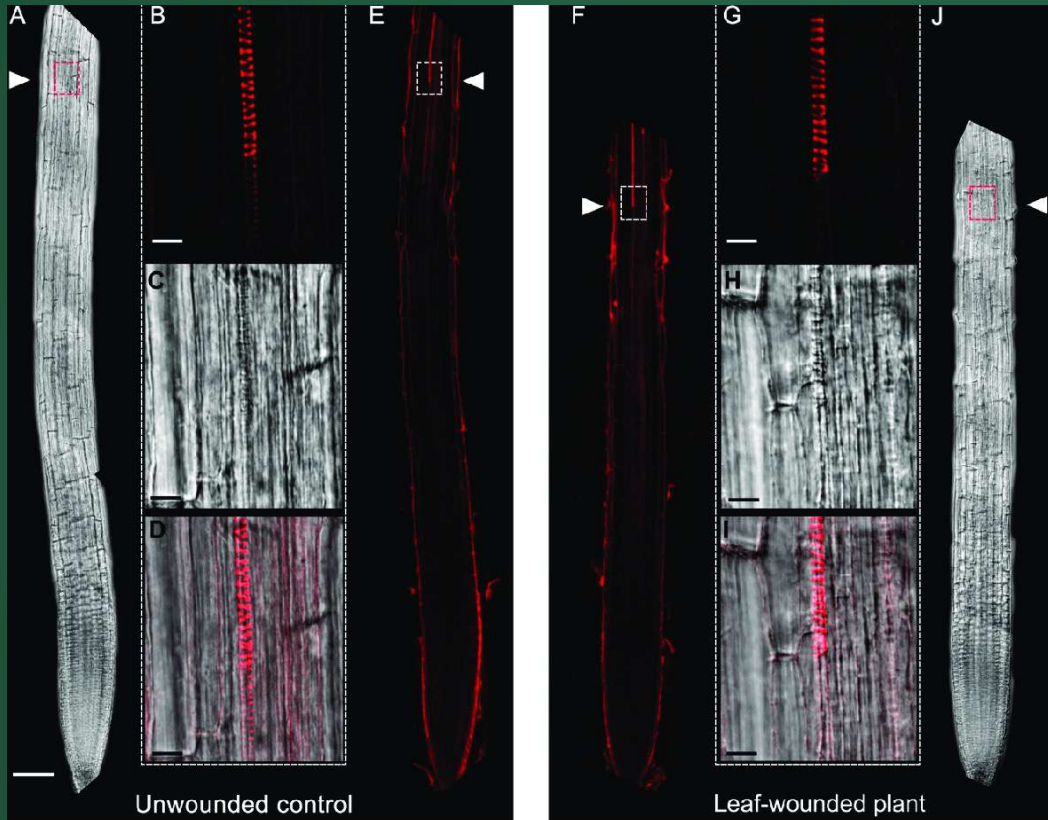
?



- To investigate the possible involvement of the other ROS sources expressed in root **AtCuAO α 3**, **AtCuAO γ 2**, **RBOHD** and **HPCA1** in local and systemic signaling after wounding, as well as their possible interactions and cooperation.

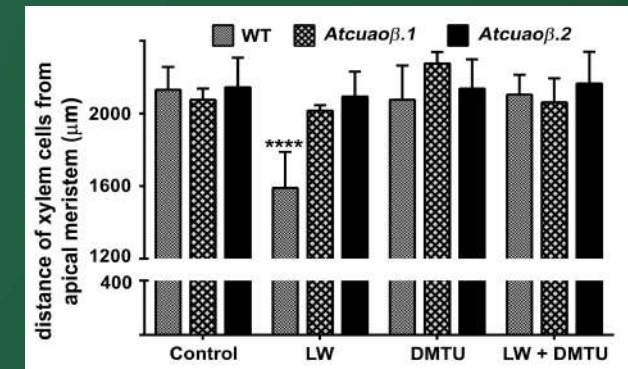
AtCuAO β is involved in systemic leaf-to-root signaling leading to early root phenotypic plasticity

SYSTEMIC LEAF-TO-ROOT SIGNALING



Fraudentali et al., 2018

In AtCuAO β gene is fundamental for **early root protoxylem differentiation** and apoplastic H₂O₂ accumulation after leaf wounding



Fraudentali et al., 2018

OBJECTIVE 1



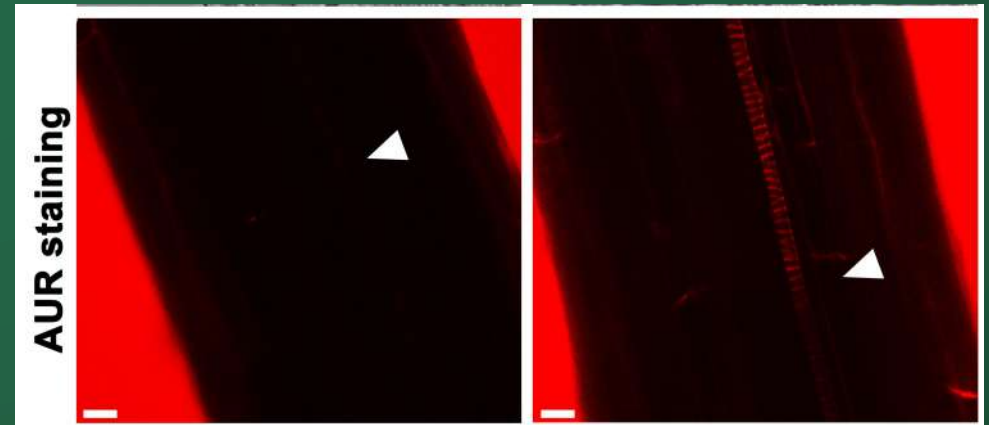
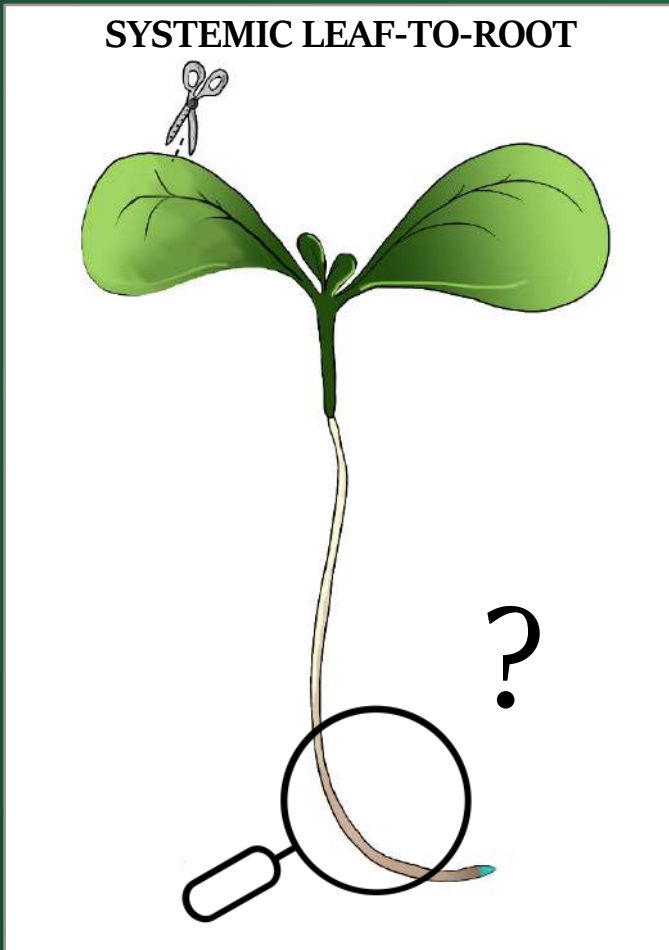
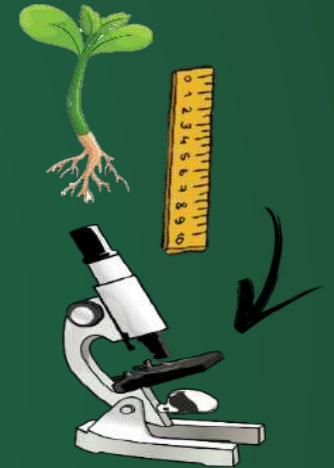
Analysis of **RBOHD** and the AOs **AtCuAO α 3** and **AtCuAO γ 2** possible involvement in **systemic leaf-to-root signaling** induced by leaf wounding

*rboh*d, *atcuao α 3* and *atcuao γ 2* mutants

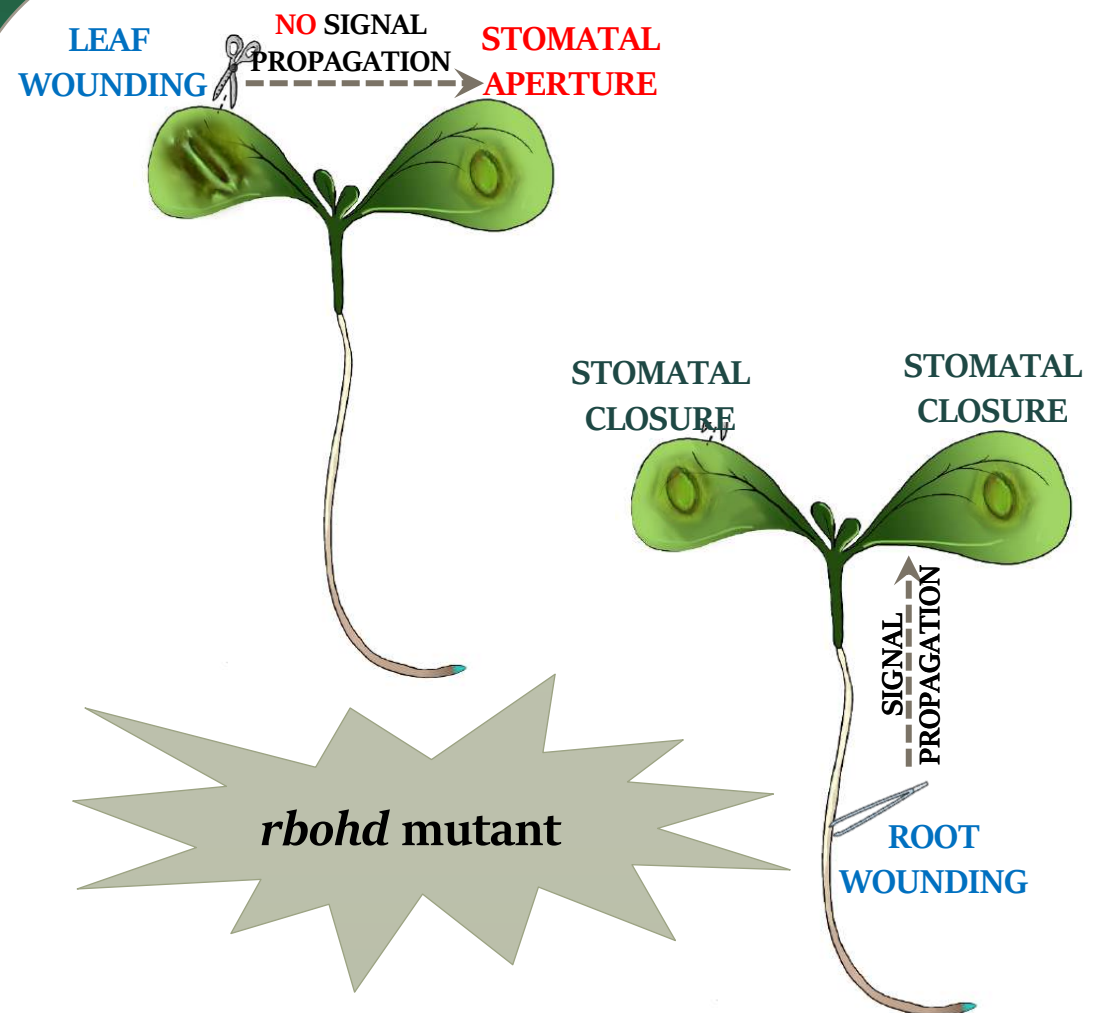
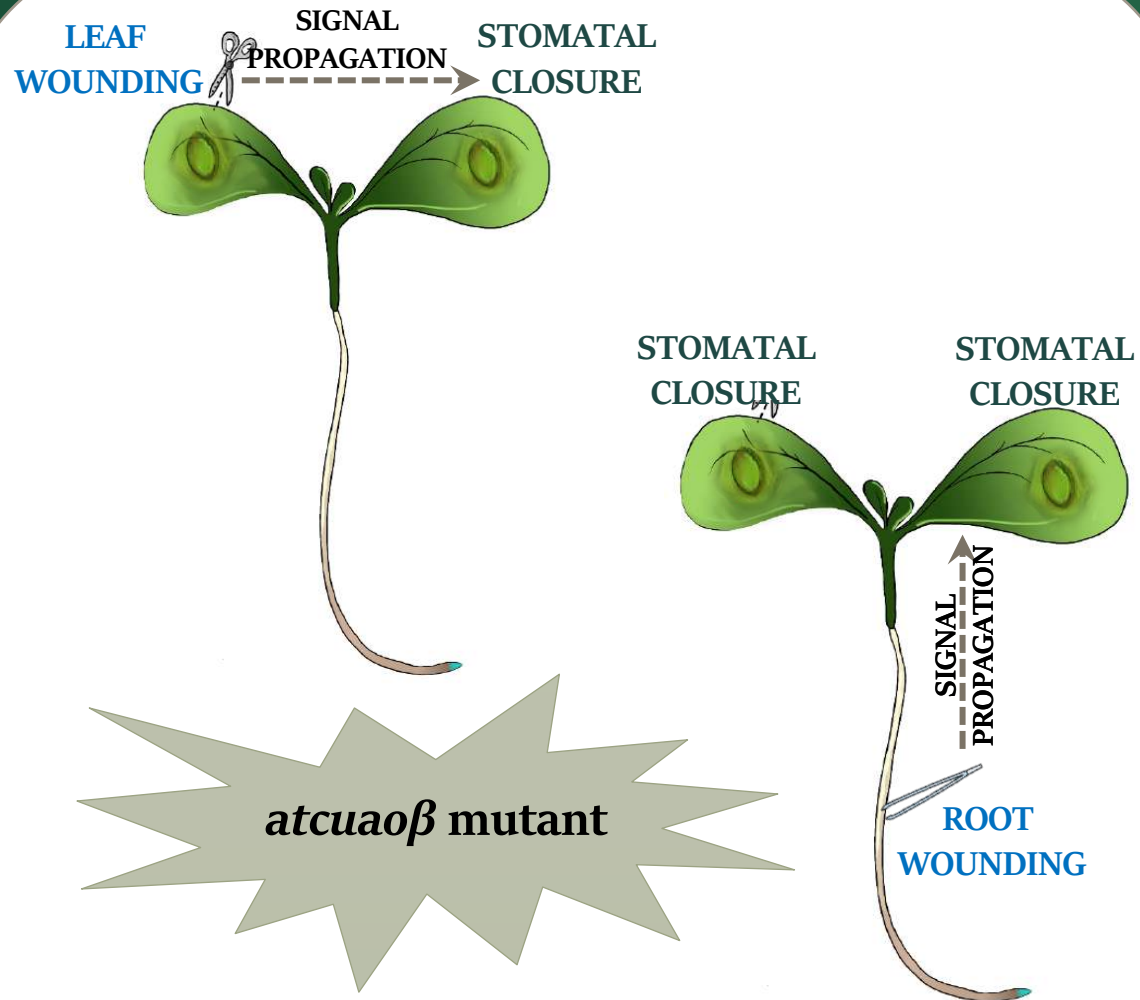
LEAF WOUNDING

Early protoxylem differentiation?

- Confocal microscopy analysis of the the **protoxylem position** with **PI staining** (propidium iodide)
- Analysis of ROS accumulation in roots of WT, *atcuao α 3*, *atcuao γ 2*, and *rboh*d seedlings (AUR)



AtCuAO β and RBOHD cooperation in wound-induced systemic stomatal closure



OBJECTIVE 2



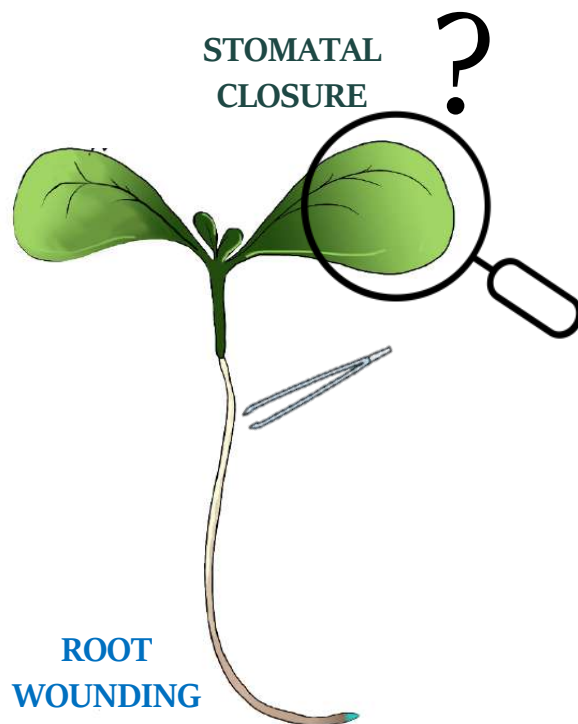
Investigate **AtCuAO α 3** and **AtCuAO γ 2** possible involvement in systemic root-to-leaf and root-to-root signaling triggered by root wounding

ROOT WOUNDING

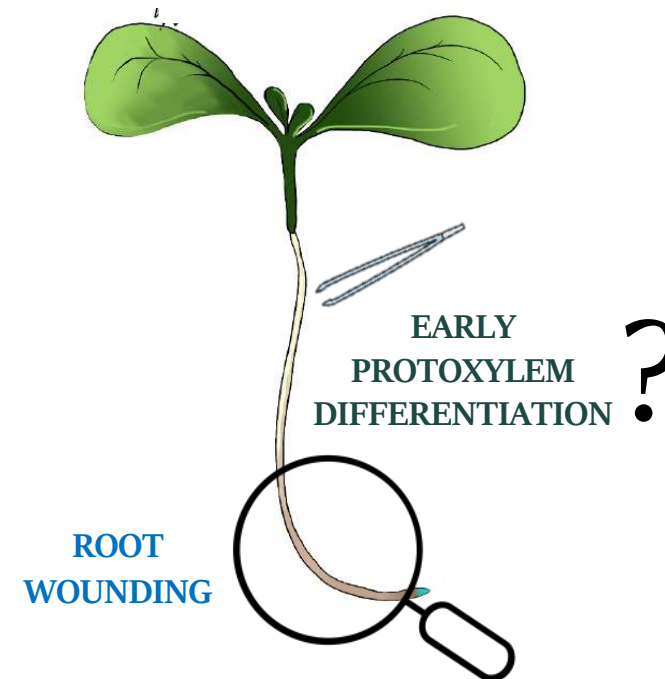
atcuao α 3 and
atcuao γ 2 mutants

- Analysis of stomatal movements
- Confocal microscopy analysis of the **protoxylem position** with **PI (propidium iodide) staining**

SYSTEMIC ROOT-TO-LEAF



SYSTEMIC ROOT-TO-ROOT

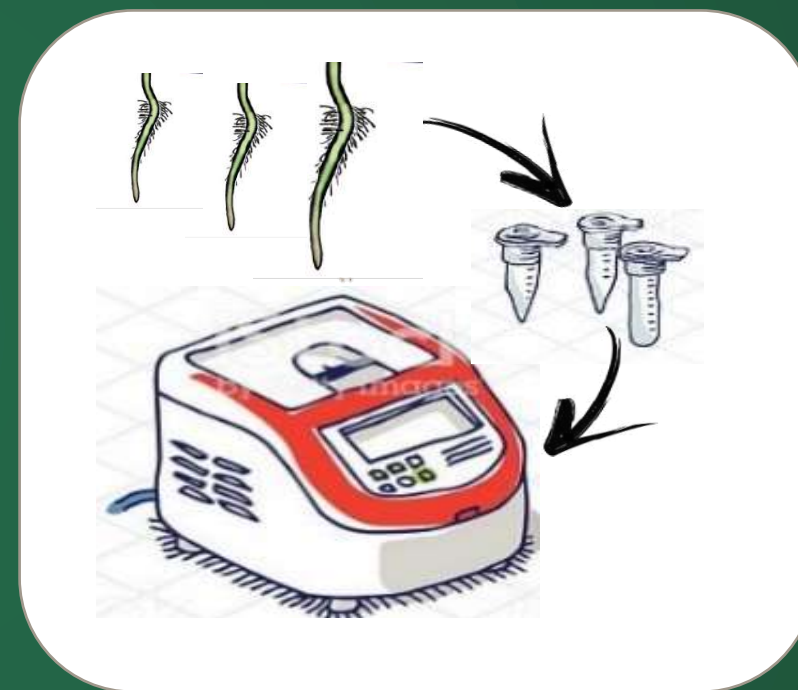


OBJECTIVE 2



Analysis of the possible cooperation between **RBOHD** protein, **AtCuAO β** , **AtCuAO α 3** and **AtCuAO γ 2** in wound-induced systemic or local signaling

1. RT-qPCR for *AtCuAO β* , *AtCuAO α 3*, *AtCuAO γ 2*, and *RBOHD* genes in roots of **WT seedlings**, unwounded (control) or upon cotyledonary-leaf or root wounding
2. RT-qPCR of *AtCuAO β* , *AtCuAO α 3*, *AtCuAO γ 2*, and *RBOHD* in roots of ***atcua β*** , ***atcua α 3***, ***atcua γ 2***, and ***rbohd* seedlings**, unwounded (control) or upon cotyledonary-leaf or root wounding

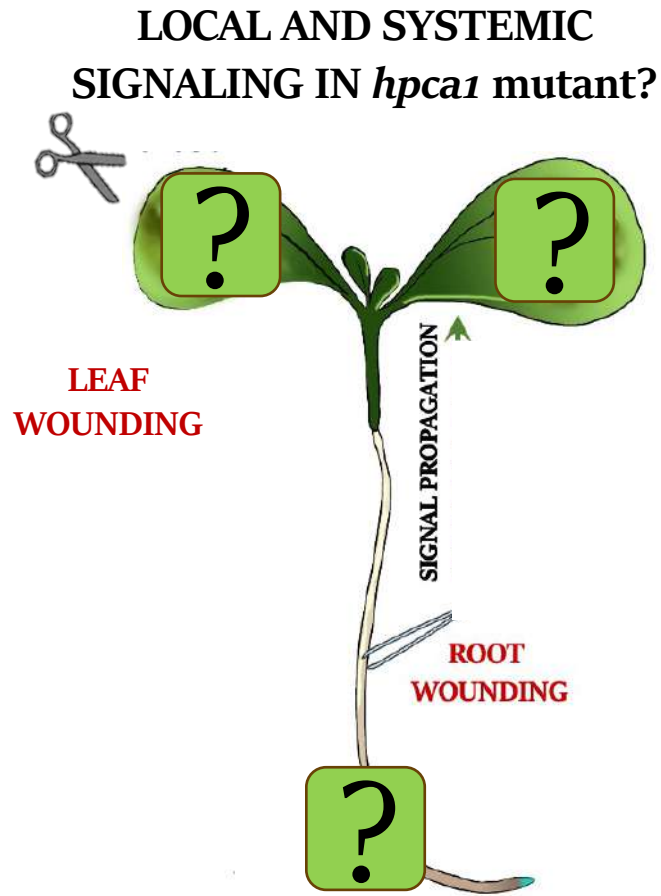


RT-quantitative PCR (RT-qPCR)

OBJECTIVE 3



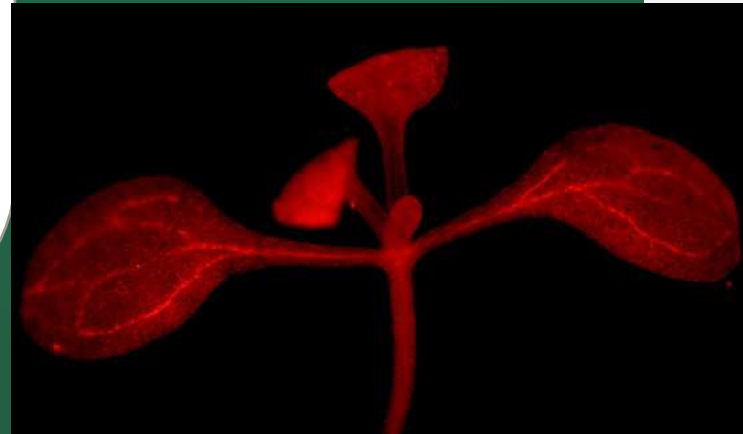
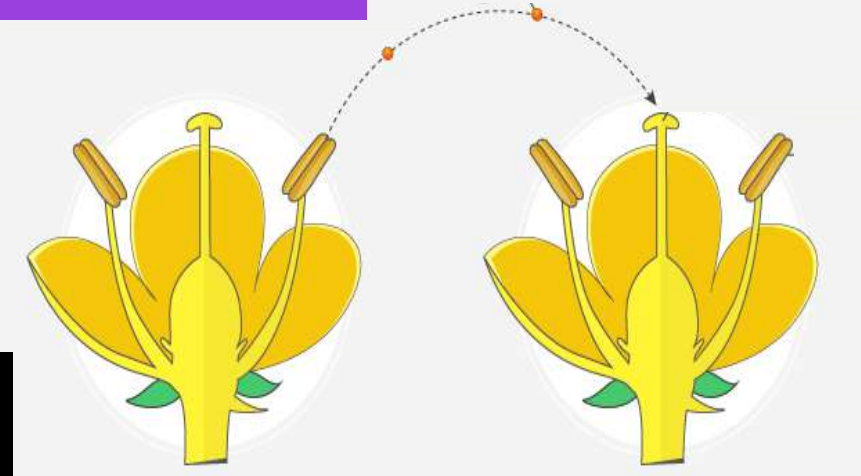
1) Analysis of HPCA1 activity in wound-induced systemic or local signaling



2) To study the possible cooperation between HPCA1 the AtCuAOs and RBOHD in inducing ROS Wave leading to protoxylem differentiation

hpca1 x *atcuaoα3*
hpca1 x *atcuaoγ2*
hpca1 x *rboh*d

CROSS POLLINATION



cellular Ca^{2+} influx

hpca1 x R-GECO1

A close-up photograph of several small white flowers with yellow centers, likely from a mustard plant, set against a dark, blurred background. The flowers are in sharp focus, showing the delicate structure of the petals and the vibrant yellow of the stamens. A green rectangular box with a white border is centered over the middle of the image, containing the text "THANKS FOR YOUR ATTENTION!".

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