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## Deciphering the links between ROS, Ca<sup>2+</sup> and cell wall remodeling during *Arabidopsis thaliana* pollen tube growth

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# Deciphering the links between ROS, Ca<sup>2+</sup> and cell wall remodeling during *Arabidopsis thaliana* pollen tube growth

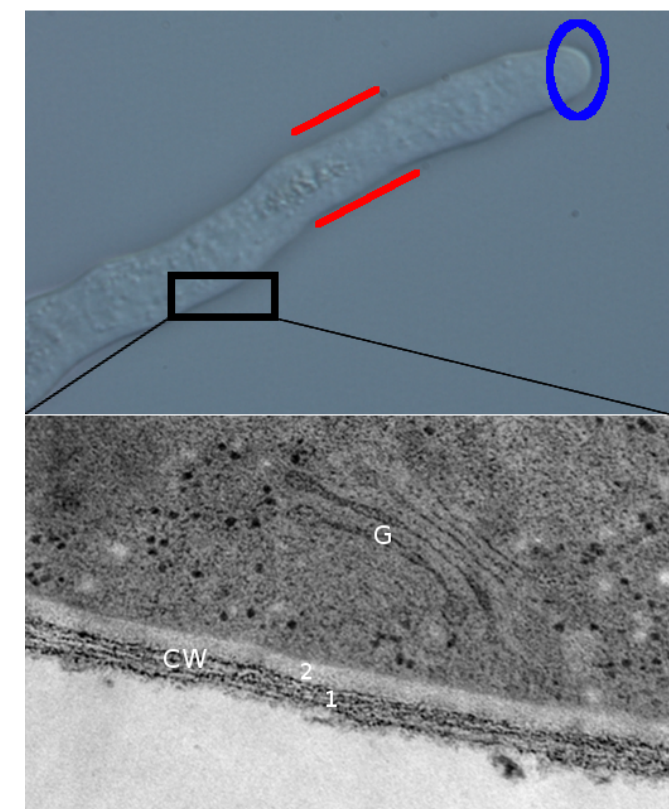
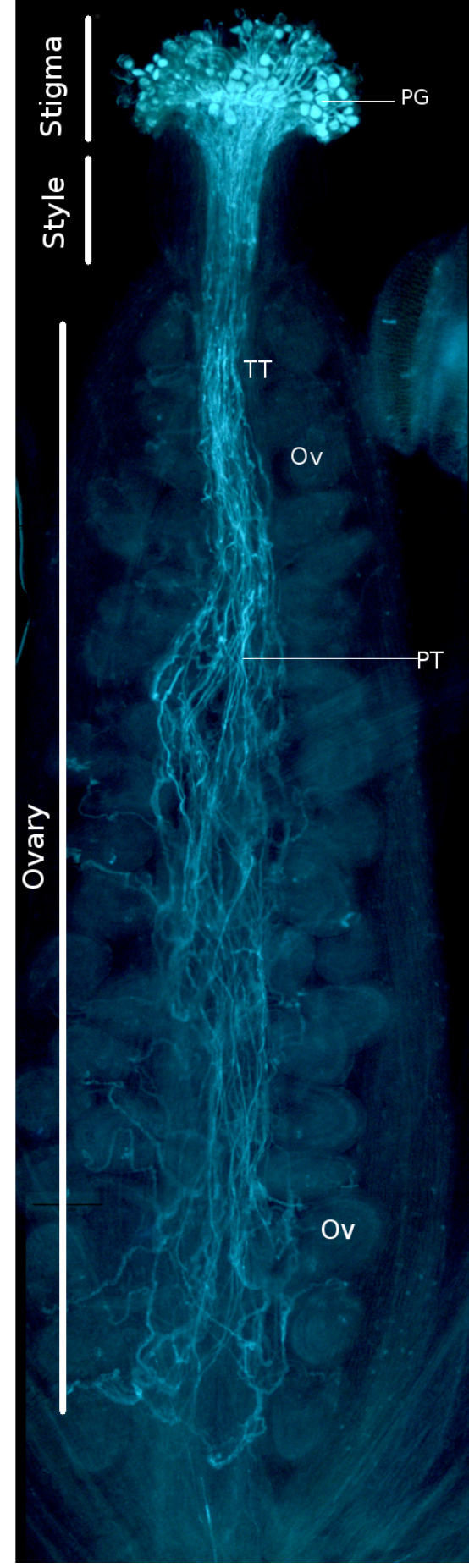
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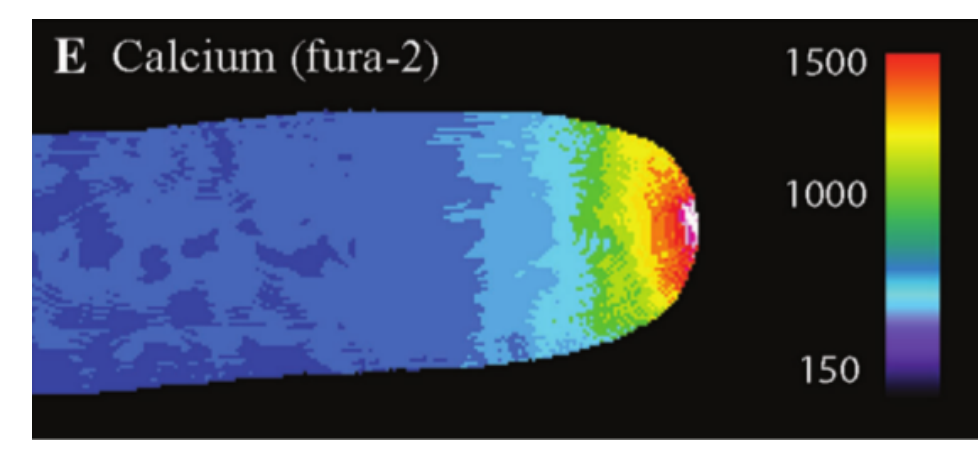
## Introduction

### Pollen tube : A model for cell growth and cell wall remodelling

Pollen cell wall must resist turgor pressure and allow fast and continuous growth, which involves fine tuning and remodelling of cell wall components.



**Figure 1 :** Pollen tube morphology, with a single cell wall layer in the apical zone (blue) and a bilayer wall on the shank (red) that ensure apical growth while keeping the cylinder shape of the tube. G : Golgi apparatus, CW : Cell Wall, 1 : Outer layer (pectins, XyG and cellulose), 2 : Inner layer (callose and cellulose). (Dardelle et al. 2010)



**Figure 2 :** Calcium is highly concentrated at the apex and oscillates in time to permit polarized growth (Cárdenas et al. 2008).

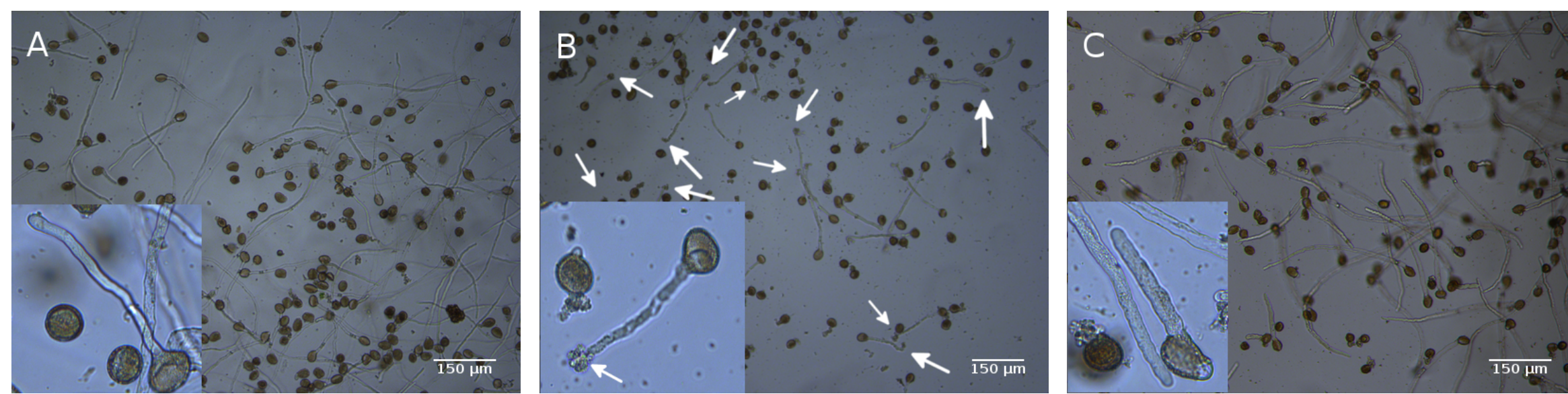
### Objectives :

- Understand the signaling role of ROS and Ca<sup>2+</sup> during polarized growth
- Characterize the cell wall modifications triggered by ROS and Ca<sup>2+</sup> oscillations

## Pharmacological study

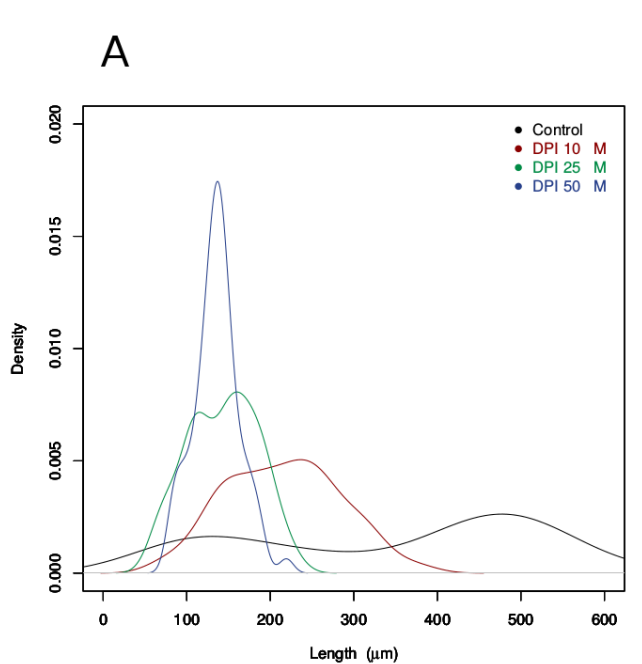
Chemical	Biological effect
Methylviologen	ROS inductor
DPI	NADPHox (ROS producing) Inhibitor
Nitroarginine	NOS (NO producing) inhibitor
Sodium benzoate	ROS scavenger
Lanthanum chloride	Calcium channels blocker
EGTA	Calcium chelator
Nitroprusside sodium	NO inductor
cPTIO	NO inhibitor
Sildenafil citrate	cGMP-specific phosphodiesterase inhibitor

**Table 1 :** List of the different chemicals used and their effects.



**Figure 3 :** Phenotype of pollen tubes after 6 hours of culture. A) Mock in liquid culture medium, B) DPI-supplemented medium (25 μM), C) Lanthanum chloride-supplemented medium (50 μM). Arrows indicate burst of pollen tubes

## ROS



**Figure 4 :** Effects of DPI on pollen tube growth and viability. A) Distribution of pollen tubes length after 3h culture. B) Estimation of the percentage of viable pollen tube.

### DPI : NADPHox Inhibitor

#### Results

Perturbation of ROS production shows various phenotypes :

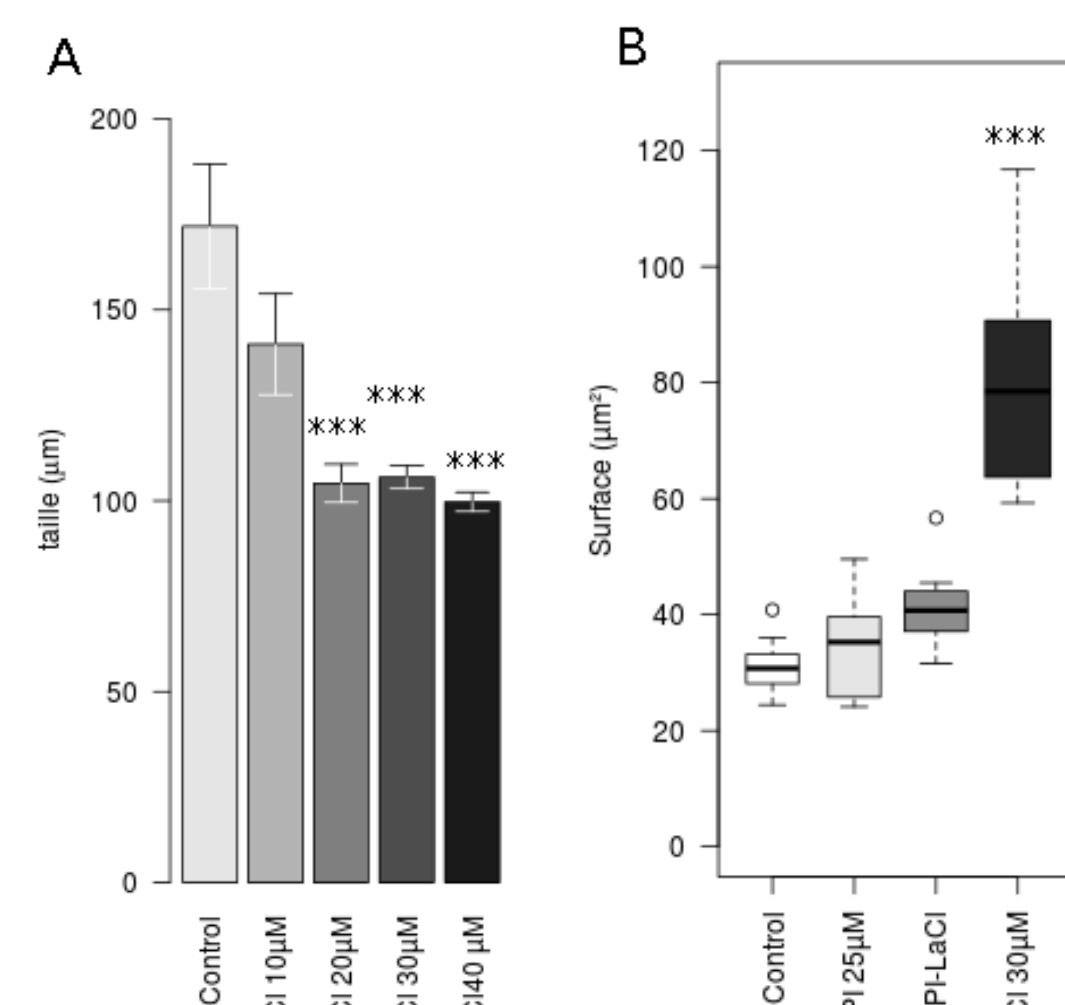
- Diminution of pollen tube length
- Increase of bursting at the tube tip
- DPI and cotreatment with LaCl show similar results concordant with *Rboh-H/Rboh-J* mutant treated with LaCl : Reversion of most of the phenotypes.

## Calcium

### LaCl : Ca<sup>2+</sup> channel blocker

#### Results

- The diameter and the surface of the tubes are increased when Ca<sup>2+</sup> channels are blocked with lanthanum chloride,
- Pollen tubes are shorter upon LaCl treatment
- Pollen tubes treated with Ca<sup>2+</sup> channel blocker show no burst of tubes

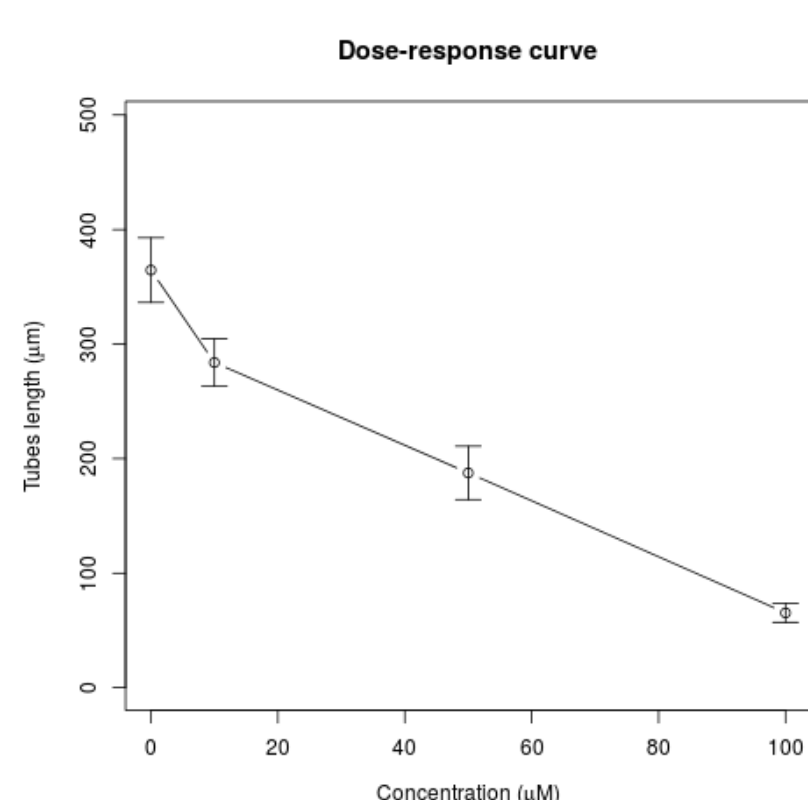


**Figure 5 :** Measure of the length (A) and surface (B) of pollen tubes after 3 hours of culture in culture medium supplemented with different concentrations of LaCl and/or DPI.

### Sildenafil citrate : cGMP phosphodiesterase inhibitor

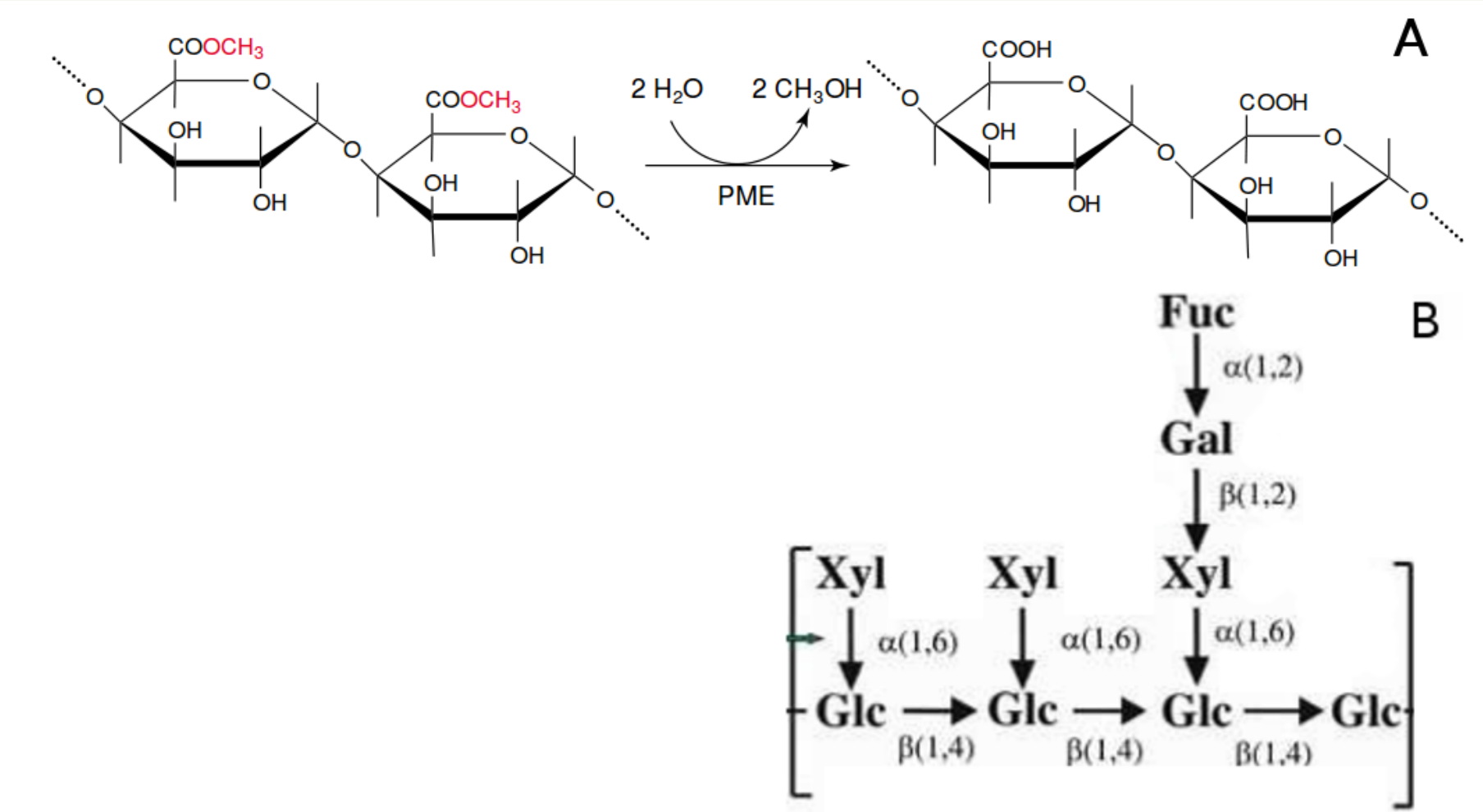
#### Results

- cGMP phosphodiesterase involved in NO signaling
- Decrease of pollen tube length
- Exposed tubes show no or few burst (called « Late burst »), but plenty of « early burst » (burst of pollen grain before germination).



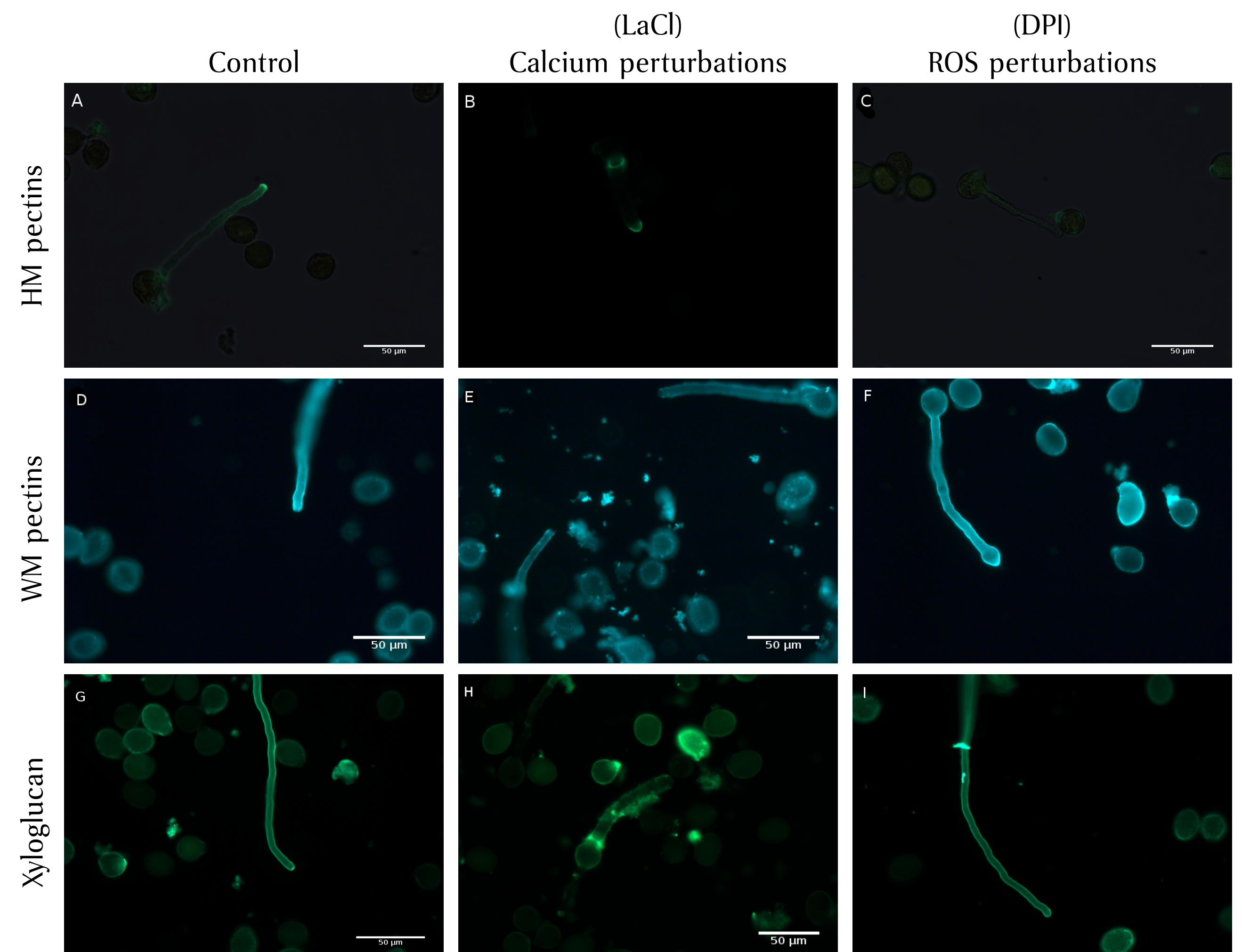
**Figure 6 :** Effects of sildenafil citrate on pollen tube growth.

## Cell wall characterization

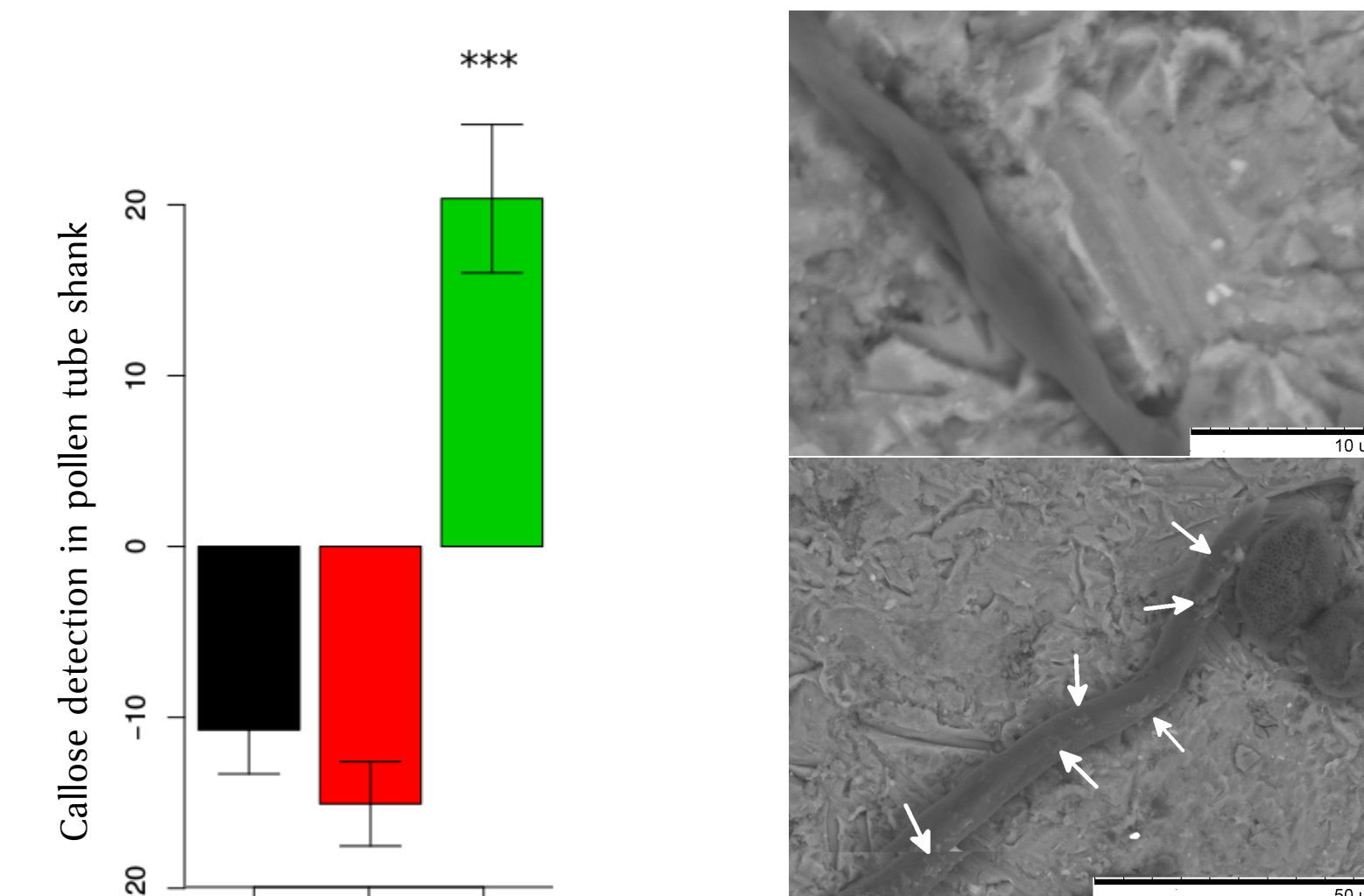


**Figure 7 :** Representation of the two main cell wall components : (A) the pectin homogalacturonan with its demethylation process (from Micheli (2001)), and (B) xyloglucan (adapted from Del Bem & Vincenz (2010)).

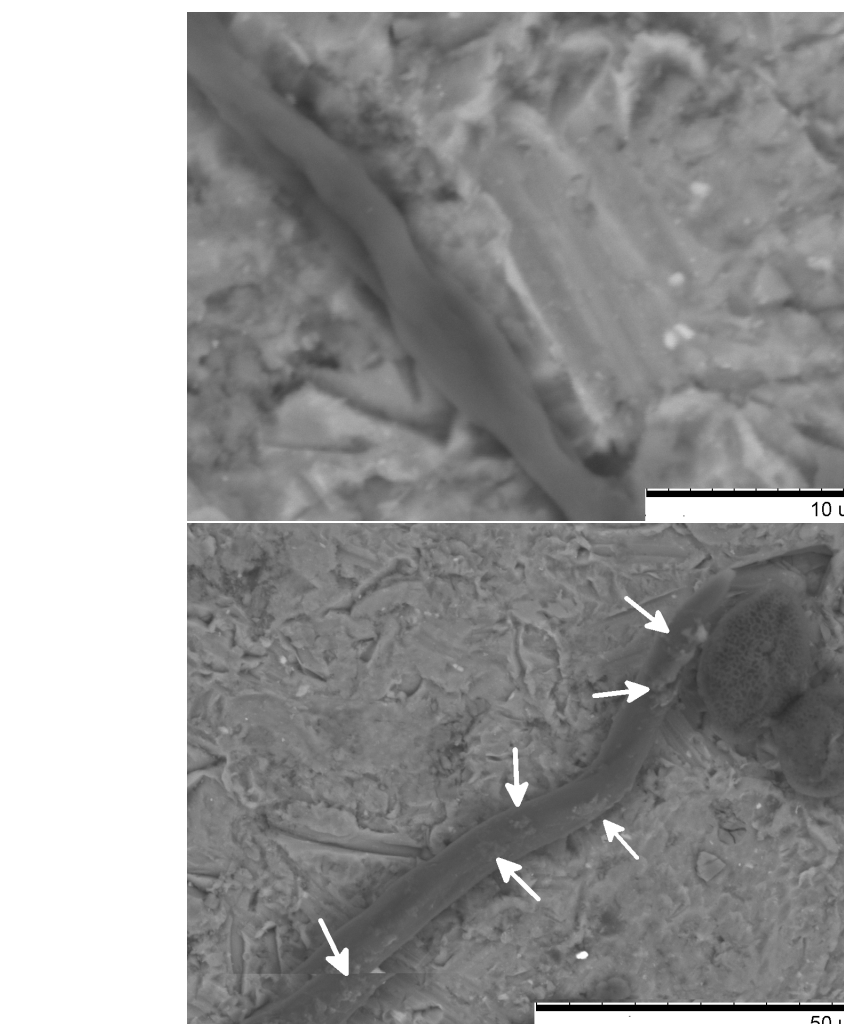
Demethylation of cell wall pectins (Figure 7A) has a huge impact on its properties, modulating the plasticity needed for anisotropic growth



**Figure 8 :** Immunodetection of cell wall associated epitopes. (A,B,C) LM20, (D,E,F) LM19, (G,H,I) LM25, under control conditions and DPI or LaCl treatment. (HM = Highly methylsterified, WM = Weakly methylsterified).



**Figure 9 :** Intensity of callose detection in the pollen shank for Mock, DPI-treated and LaCl-treated pollen tubes.

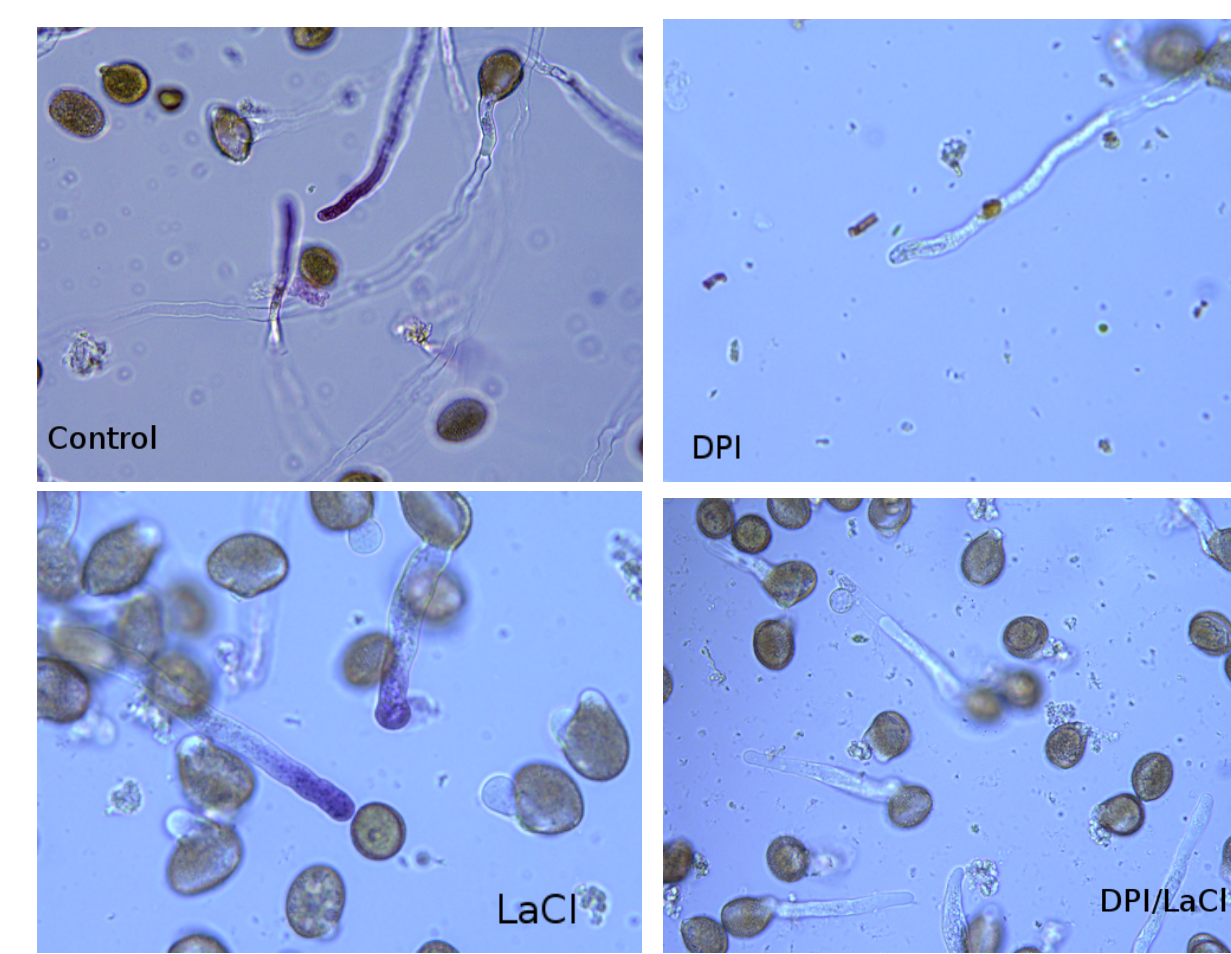


**Figure 10 :** SEM of LaCl-treated tubes shows small particles on the cell wall, similar to those seen in immunolabelling, which are absent on the Mock tube. These particles are supposed to be cell wall fragments which are peeling off the tube (indicated with arrows).

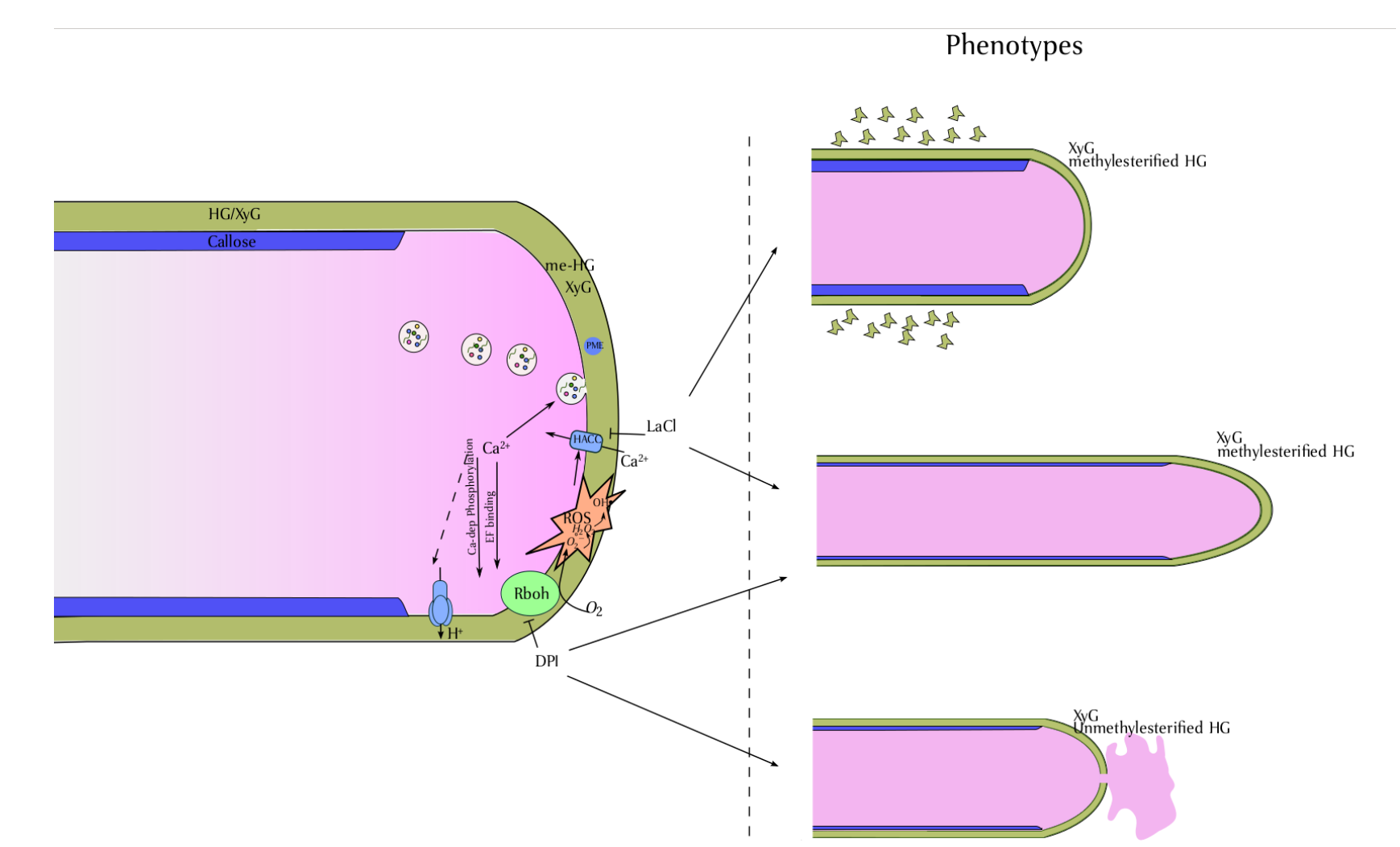
## Results

- Inhibition of ROS production leads to a decrease of the degree of methylesterification (DM) at the tip (Figure 8)
- LaCl leads to a degradation of the cell wall all along the tube, Fragments of pectin and xyloglucan are detectable in the culture medium (Figure 8 and 10)

## ROS and Ca<sup>2+</sup> pathway



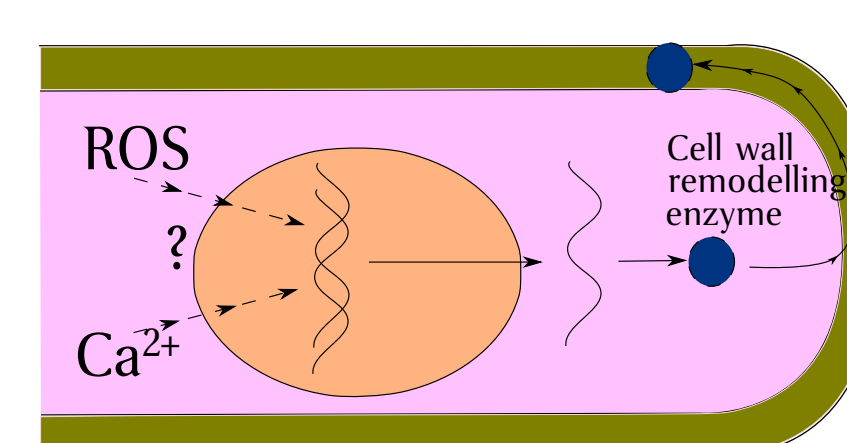
**Figure 11 :** NBT staining shows ROS at the apex of mock tubes (A) and LaCl treated pollen tube (C). The absence of ROS when treated with DPI (B) and cotreated with both DPI and LaCl (D) confirm that LaCl reversion of pollen tube phenotype does not involve a reactivation of NADPH oxidases by the alteration of calcium fluxes.



**Figure 12 :** Actions and interaction of ROS and Calcium and their impact on pollen tube growth.

## Perspectives

- Characterization of cell wall enzymes activity
- Characterization of possible changes in cell wall polysaccharidic composition
- Fine localization of Ca<sup>2+</sup> changes using Yellow Cameleon
- Analyses of transcriptional changes in cell wall remodelling enzymes



**Figure 13 :** Influence of ROS and Ca<sup>2+</sup> on transcriptional changes.

Family	Function	Number in pollen
PME	Pectin methyl esterase	14
PMEI	PME Inhibitor	10
RALF	Rapid alkalization factor	7
PG	Polygalacturonase	6
PL	Pectate lyase	4

**Figure 14 :** List of genes to be assessed if they are under the influence of ROS and/or Ca<sup>2+</sup>

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