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# Deciphering the links between ROS, $Ca^{2+}$ and cell wall remodeling during *Arabidopsis thaliana* pollen tube growth

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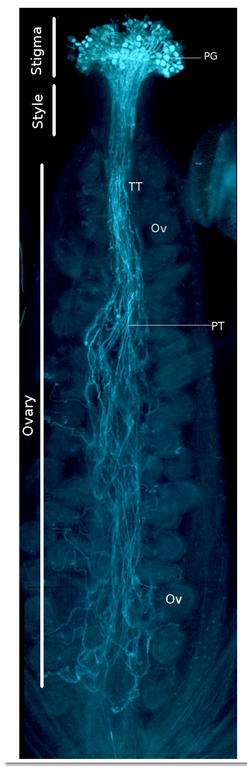
## Introduction

### 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.

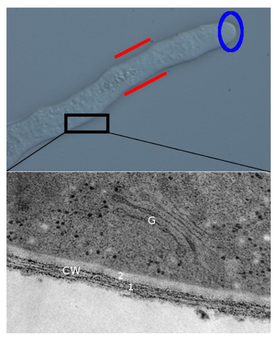


Figure 1 : Pollen tube morphology, with a single cell wall layer in the apicale zone (blue) and a bilayer wall on the shank (red) that ensure apical growth while keeping the cylinder shape of the tube. G : Golgi apparatus, CW : Cell Wall, 1 : Outer layer (pectins, XyG and cellulose), 2 : Inner layer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

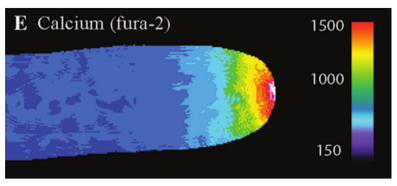



Figure 2 : Calcium is highly concentrated at the apex and oscillates in time to permit polarized growth (Cárdenas et al. 2008).

### Objectives :

1. Understand the signaling role of ROS and $Ca^{2+}$ during polarized growth
2. Characterize the cell wall modifications triggered by ROS and $Ca^{2+}$ oscillations

## Pharmacological study

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

| 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 | ✓ |

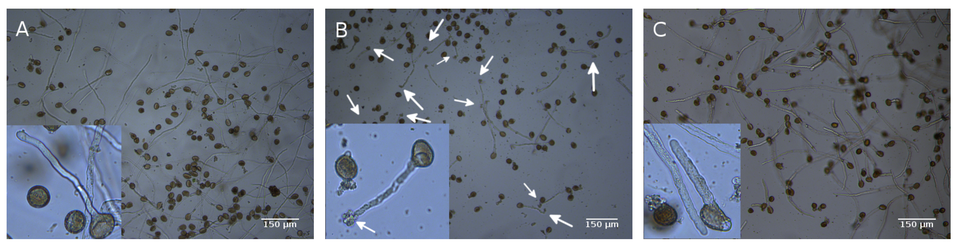



Figure 3 : Phenotype of pollen tubes after 6 hours of culture. A) Mock in liquid culture medium, B) DPI-supplemented medium (25$\mu$M), C) Lanthanum chloride-supplemented medium (50$\mu$M ). Arrows indicate burst of pollen tubes

### ROS

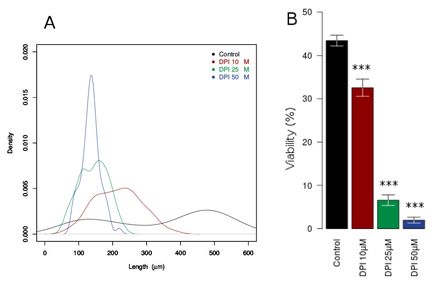



Figure 4 : Effects of DPI on pollen tube growth and viability. A) Distribution of pollen tubes length after 3h culture. B) Estimation of the percentage of viable pollen tube.

**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 :** $Ca^{2+}$ channel blocker

#### Results

- The **diameter and the surface of the tubes are increased** when $Ca^{2+}$ channels are blocked with lanthanum chloride,
- Pollen tubes are **shorter** upon LaCl treatment
- Pollen tubes treated with $Ca^{2+}$ channel blocker show **no burst** of tubes

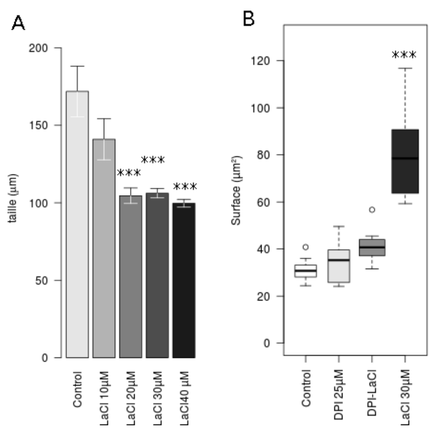



Figure 5 : Measure of the length (A) and surface (B) of pollen tubes after 3 hours of culture in culture medium suplemented with different concentrations of LaCl and/or DPI.

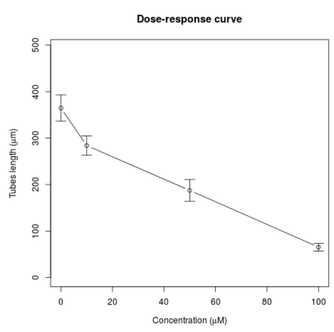



Figure 6 : Effects of sildenafil citrate on pollen tube growth.

**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).

## Cell wall charecterization

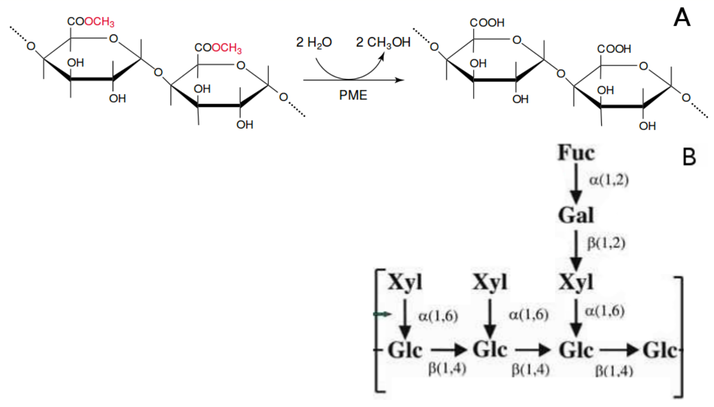



Figure 7 : Representation of the two mains cell wall components : (A) the pectin homogalacturonan with its demethylation process (from Micheli (2001)), and (B) xyloglucan (adapted from Del Bem & Vincentz (2010)).

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

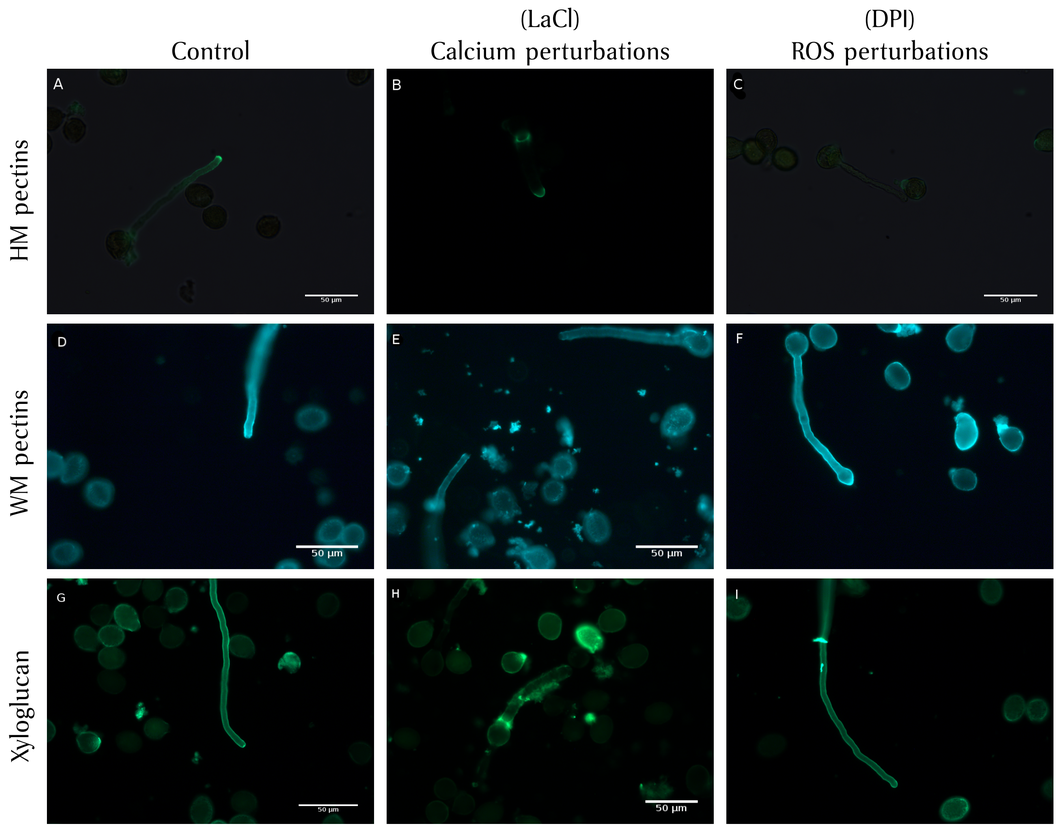



Figure 8 : Immuno-detection of cell wall associated epitopes. (A,B,C) LM20, (D,E,F) LM19, (G,H,I) LM25, under control conditions and DPI or LaCl treatment. (HM = Highly methylesterified, WM = Weakly methylesterified).

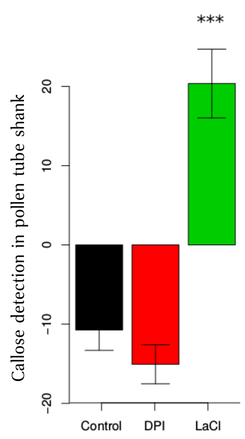



Figure 9 : Intensity of callose detection in the pollen shank for Mock, DPI-treated and LaCl-treated pollen tubes.

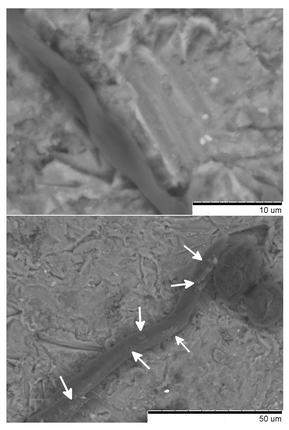


Figure 10 : SEM of LaCl-treated tubes shows small particles on the cell wall, similar to those seen in immunolabelling, which are absent on the Mock tube. These particles are supposed to be cell wall fragments which are peeling off the tube (indicated with arrows).

#### 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 $Ca^{2+}$ pathway

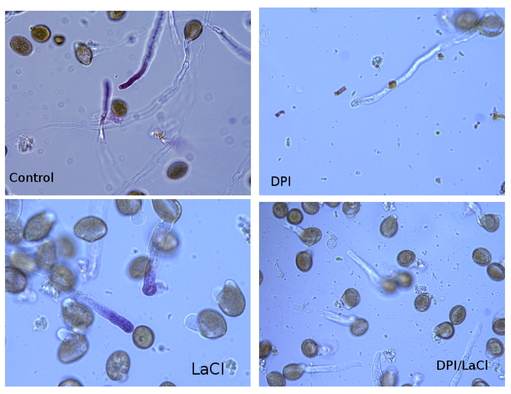



Figure 11 : NBT staining shows ROS at the apex of mock tubes (A) and LaCl treated pollen tube (C). The absence of ROS when treated with DPI (B) and cotreated with both DPI and LaCl (D) confirm that LaCl reversion of pollen tube phenotype does not involve a reactivation of NADPH oxidases by the alteration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

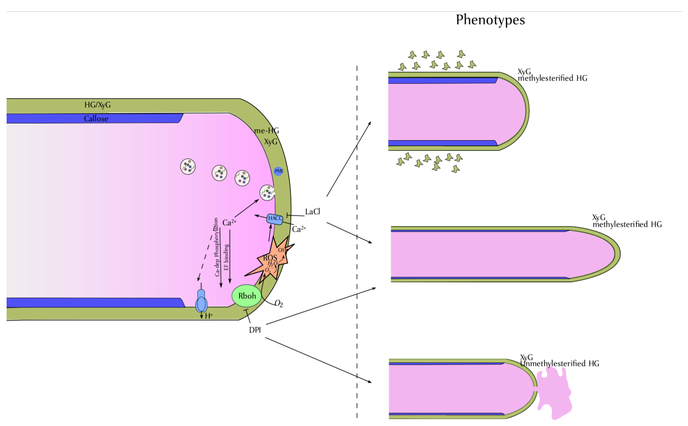



Figure 12 : Actions and interaction of ROS and Calcium and their impact on pollen tube growth.

## Perspectives

- Characterization of cell wall enzymes activity
- Characterization of possible changes in cell wall polysaccharidic composition
- Fine localization of $Ca^{2+}$ changes using Yellow Cameleon
- Analyses of transcriptional changes in cell wall remodelling enzymes

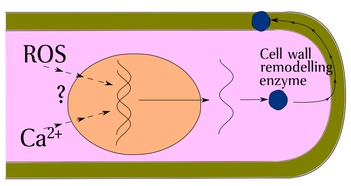



Figure 13 : Influence of ROS and $Ca^{2+}$ on transcriptional changes.

| 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 $Ca^{2+}$

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