Deciphering the links between ROS, Ca 2+ and cell wall remodeling during Arabidopsis thaliana pollen tube growth

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Introduction

During sexual reproduction, the male gametophyte carrying the two sperm cells must travel long distancies in the female tissue (Figure 1, 2) to perform the fertilization allowing the production of numerous and healthy seeds.

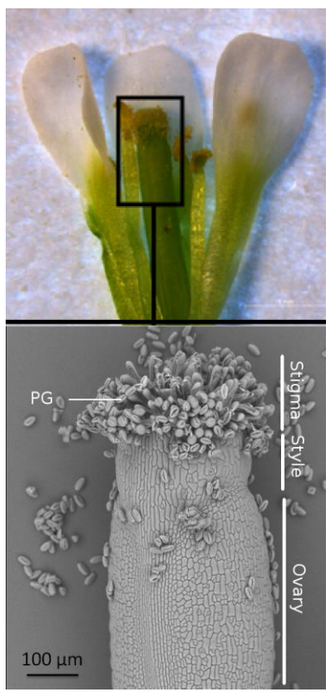

Figure 1 :flower of *Arabidopsis thaliana* (upper panel) and isolated pis- til covered up of pollen grain (lower panel, Scanning Electron Microscopy). PG : Pollen grain, S : Style, St : Stamens

Pollen tubes are fast growing tip-polarized cells (Figure 3). To sustain this fast growth, the pol- len tube cell wall must be sufficiently plastic at the tip to promote growth, and rigid at the shank to resist internal turgor pressure. The cell is therefore able to adapt its structure by modifying the cell wall mechanical properties. To this purpose, a rigid cell wall composed of two layers is found in the shank with diWerents cell wall polymers (Figure 3).

- Inner layer
  - Callose (mostly)
  - Cellulose
- Outer layer
  - Cellulose
  - Hemicellulose
  - s Pectins

At the tip, only one layer is found with a composition similar to the outer layer of the shank (Dardelle et al. 2010). During pol- len tube growth, massive Golgi-derived vesicles containing cell wall polymers such as pectins

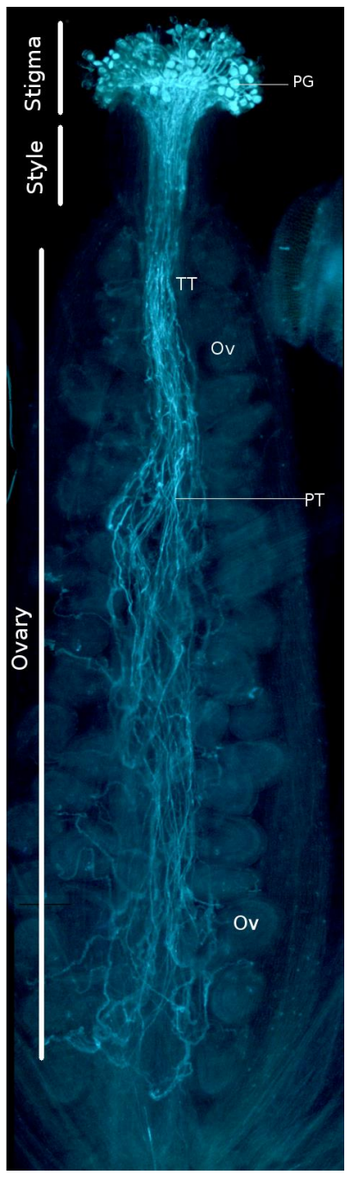

Figure 2 :Pollen tubes of *Arabidop- sis thaliana* growing in female tissues, from the stigma to the ovules, over distances reaching 100 times the initial pollen size. Aniline blue staining reveals callose in the pollen tube cell wall. PG : Pollen grain, S : Style, TT : Transmitting tract, Ov : Ovary, PT : Pollen tube.

and hemicellulose fuse at the tip to sustain growth. This pro- cess is fine-tuned by reactive oxygen species (ROS) and Ca^{2+} (Figure 4). Several studies have shown that a perturbation of Ca^{2+} pulses at the apex leads to a growth arrest (Pierson et al. 1996). Moreover, inhibition of ROS production decreases the pollen tube length and increase pollen tube burst.

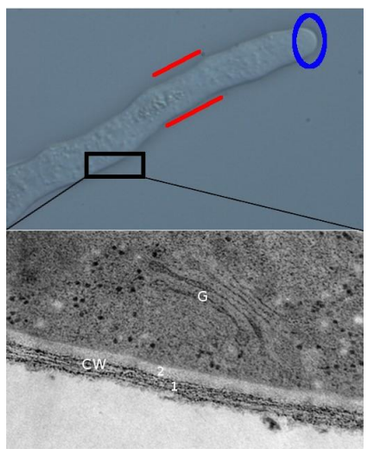

Figure 3 :Pollen tube morphology, with a single cell wall layer in the apicale zone (blue) and a bilayer wall on the shank (red) providing a flexibility that ensure apical growth while keeping the cylinder shape of the tube. G: Golgi apparatus, CW: Cell Wall, 1: Outer layer, 2: Inner layer

Modifications of vesicle secretion and cell wall remodeling might aWect the growth and integrity of the whole pollen tube. Ca^{2+} and ROS are suspected to influence the synthesis and changes of the cell wall.

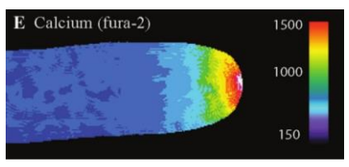

Figure 4:Calcium is highly concentrated at the apex and oscillates in time to permit polarized growth (Cárdenas et al. 2008).

Objectives :

Understand the role of ROS and Ca^{2+} during polarized growth

Characterize the cell wall modifications triggered by ROS and Ca^{2+} oscillation

Pharmacological study

Table 1 :List of the diWerent chemicals used and their eWects.

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

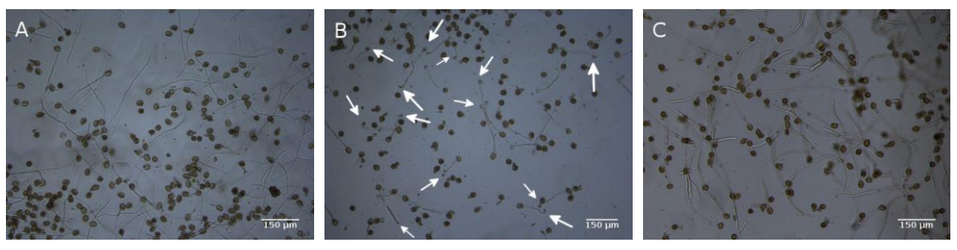

Figure 5:Phenotype of pollen tubes after 6 hours of culture. A) Mock in liquid culture medium, B) DPI-supplemented medium (25µM), C) Lanthanum chloride-supplemented medium (50µM). Arrows indicate burst of pollen tubes

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Chemical screening

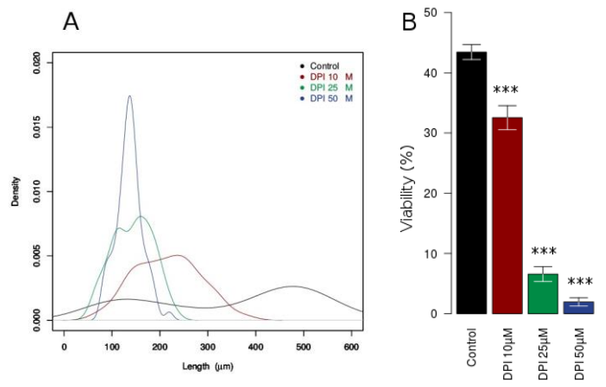


Figure 6 :Exposure of pollen tube with various concentrations of DPI. A) Distribution of pollen tubes length after 3h culture. B) Estimation of the percentage of viable pollen tube.

DPI : NADPHox Inhibitor

Results

Perturbation of ROS production shows various phenotypes :

- Decrease in ROS synthesis (data not shown)
- **Diminution of pollen tubes length**
- **Increase of burst** at the tube tip

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

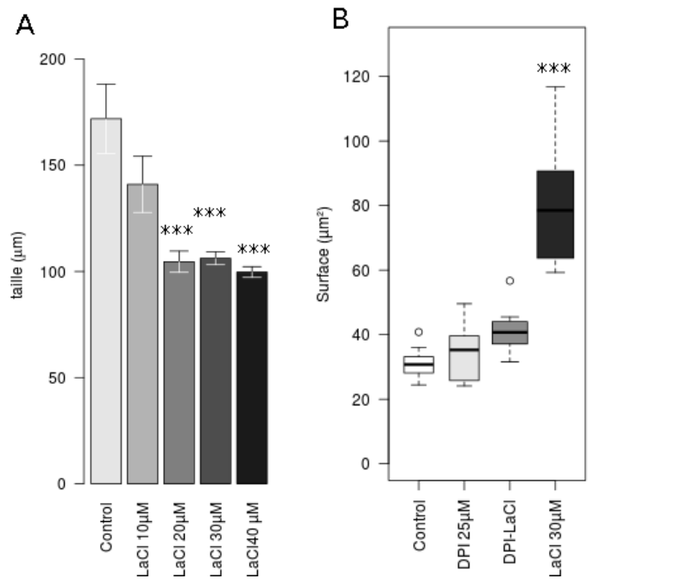


Figure 7:Measure of the length (A) and surface (B) of pollen tubes after 3 hours of culture in culture medium suplemented with diWerent concentrations of LaCl and/or DPI.

Immunolabelling of cell wall epitopes

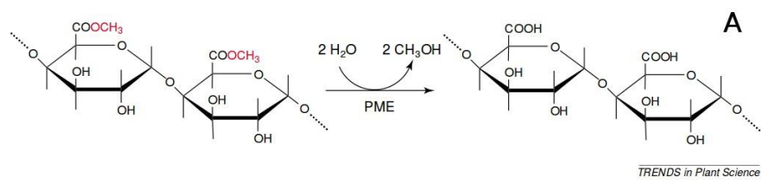
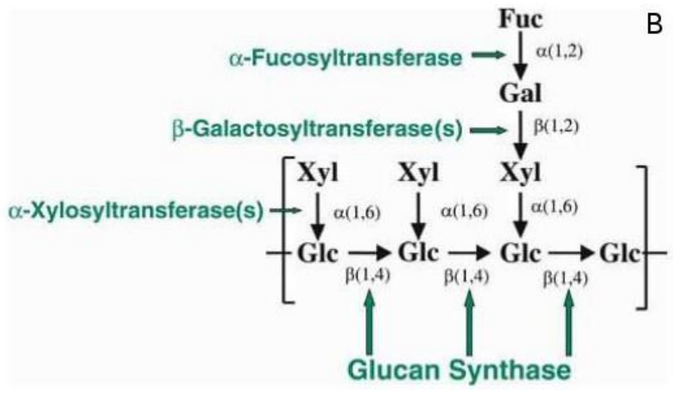


Figure 8 :Structure of two of the mains cell wall components, the pectin homogalacturonan (A) (from Micheli (2001)), and xyloglucan, the major cell wall hemicellulose (B) (adapted from Del Bem &Vincentz (2010)).



Cell wall

- Pectins are secreted at the tip with a high degree of methylesterification and are demethylesterified by Pectin Methyl Esterases (Figure 8A) in the subapical region.
- Xyloglucan (the major hemicellulose) is synthesized in the golgi apparatus and made of a β-glucan backbone substituted by xylose, xylose-galactose, or xylose-galactose-fucose (Figure 8B)

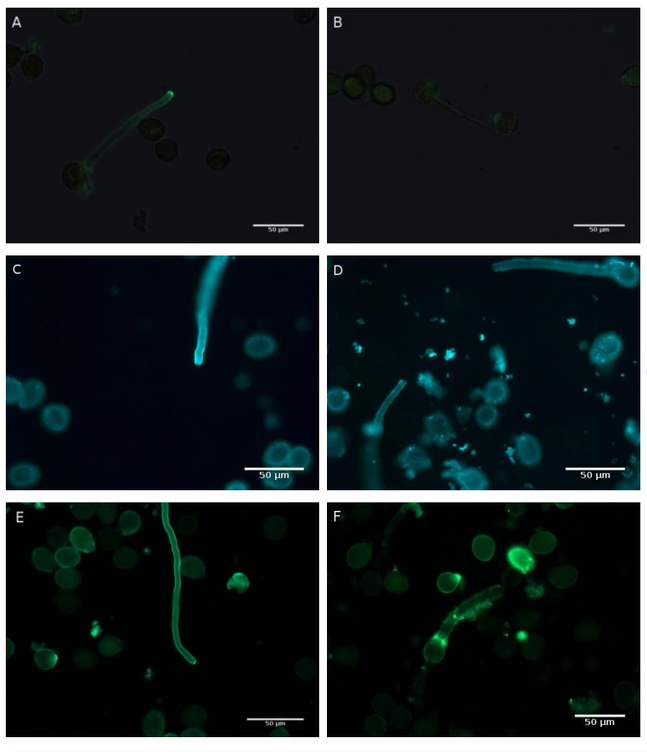


Figure 9 :Immuno-detection of cell wall epitops. (A,B) LM20, (C,D) LM19, (E,F) LM25, under control conditions (A,C,E) DPI supplementation (B) or LaCl (D,F).

Results

- Inhibition of ROS production leads to a decrease of the degree of methlesterification (DM) at the tip (Figure 9B)
- LaCl leads to a degradation of the wall all along the tube, Fragments of pectin and xyloglucans are detectable in the culture medium (Figure 9D,F)

Conclusions

ROS

- Inhibition of ROS is accompanied by a change in the DM of pectin associated with an increase of the burst of the tube

Ca^{2+}

- Modification in Ca^{2+} influx is accompanied by an increase of the diameter and the degradation of the cell wall