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Deciphering the links between ROS, Ca²⁺ 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 distances in the female tissue (Figure 1, 2) to perform the fertilization allowing the production of numerous and healthy seeds.

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

shown that a perturbation of Ca²⁺ 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.

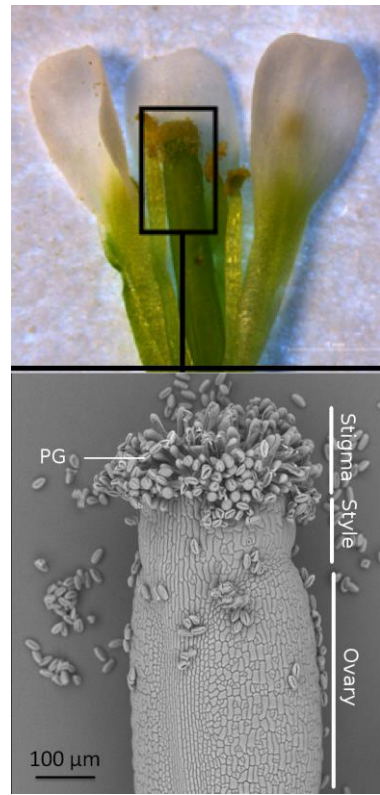


Figure 1 :flower of *Arabidopsis thaliana* (upper panel) and isolated pistil covered up of pollen grain (lower panel, Scanning Electron Microscopy). PG : Pollen grain, S : Style, St : Stamens

Pollen tubes are fast growing tip-polarized cells (Figure 3). To sustain this fast growth, the pollen 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 different cell wall polymers (Figure 3).

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

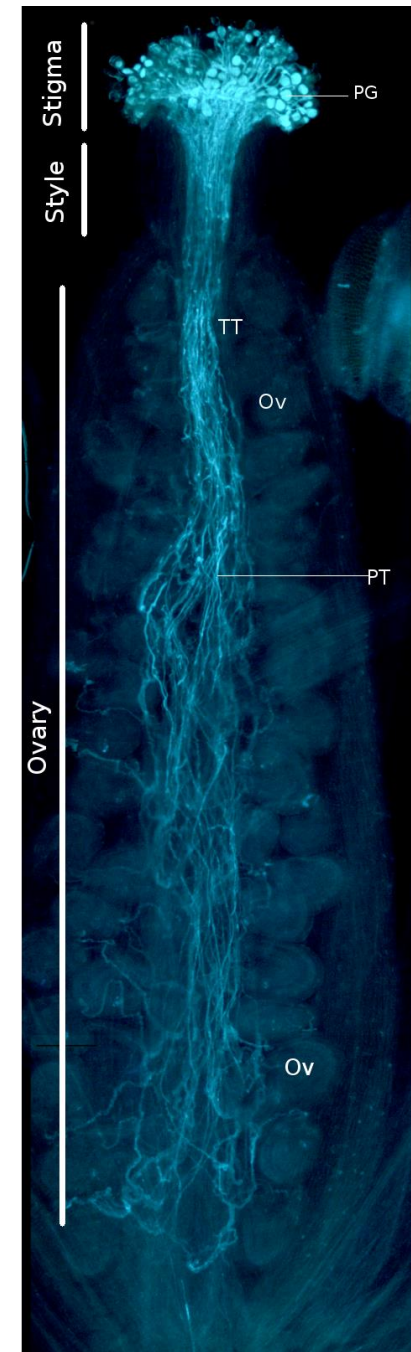


Figure 2 :Pollen tubes of *Arabidopsis thaliana* growing in female tissues, from the stigma to the ovules, over distances reaching 100 times the initial pollen size. Aniline blue staining reveals callose in the pollen tube cell wall. PG : Pollen grain, S : Style, TT : Transmitting tract, Ov : Ovary,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

and hemicellulose fuse at the tip to sustain growth. This process is fine-tuned by reactive oxygen species (ROS) and Ca²⁺ (Figure 4). Several studies have

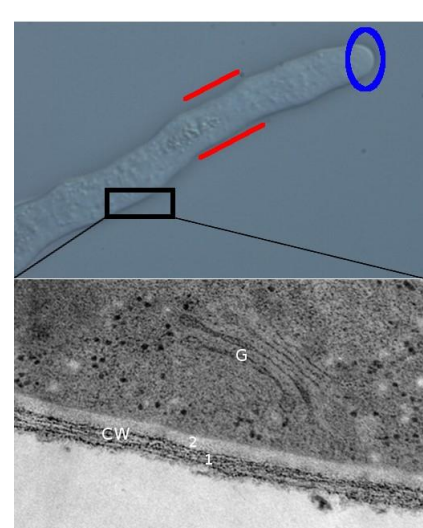


Figure 3 :Pollen tube morphology, with a single cell wall layer in the apical zone (blue) and a bilayer wall on the shank (red) providing a flexibility that ensure apical growth while keeping the cylinder shape of the tube. G: Golgi apparatus, CW: Cell Wall, 1: Outer layer, 2: Inner layer

Modifications of vesicle secretion and cell wall remodeling might affect the growth and integrity of the whole pollen tube. Ca²⁺ and ROS are suspected to influence the synthesis and changes of the cell wall.

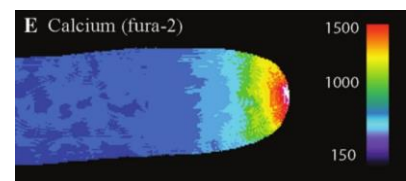


Figure 4:Calcium is highly concentrated at the apex and oscillates in time to permit polarized growth (Cárdenas et al. 2008).

Objectives :

Understand the role of ROS and Ca²⁺ during polarized growth
Characterize the cell wall modifications triggered by ROS and Ca²⁺ oscillation

Pharmacological study

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

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

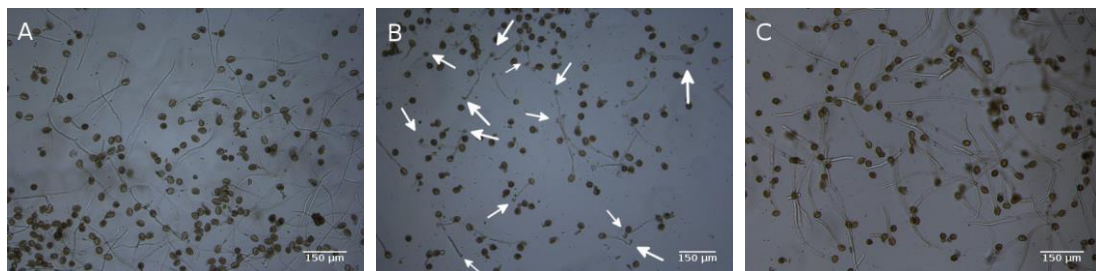


Figure 5:Phenotype of pollen tubes after 6 hours of culture. A) Mock in liquid culture medium, B) DPI-supplemented medium (25µM), C) Lanthanum chloride-supplemented medium (50µM). Arrows indicate burst of pollen tubes

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Chemical screening

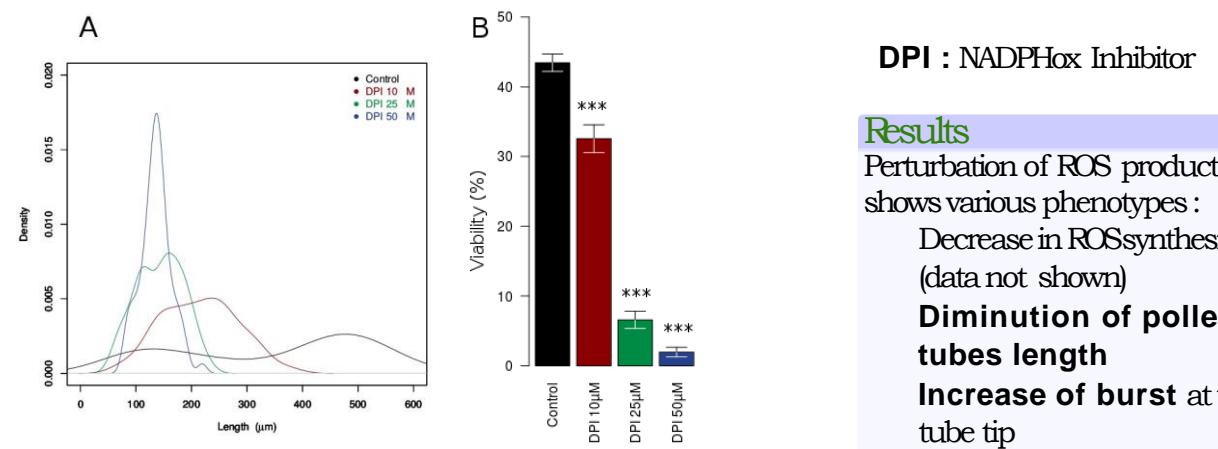


Figure 6 :Exposure of pollen tube with various concentrations of DPI. A) Distribution of pollen tubes length after 3h culture. B) Estimation of the percentage of viable pollen tube.

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

LaCl₃ : Ca²⁺ channel blocker

Results

The diameter and the surface of the tubes are increased when Ca²⁺ channels are blocked with lanthanum chloride, Pollen tubes are shorter upon LaCl₃ treatment
Pollen tubes treated with Ca²⁺ channel blocker show no burst of tubes

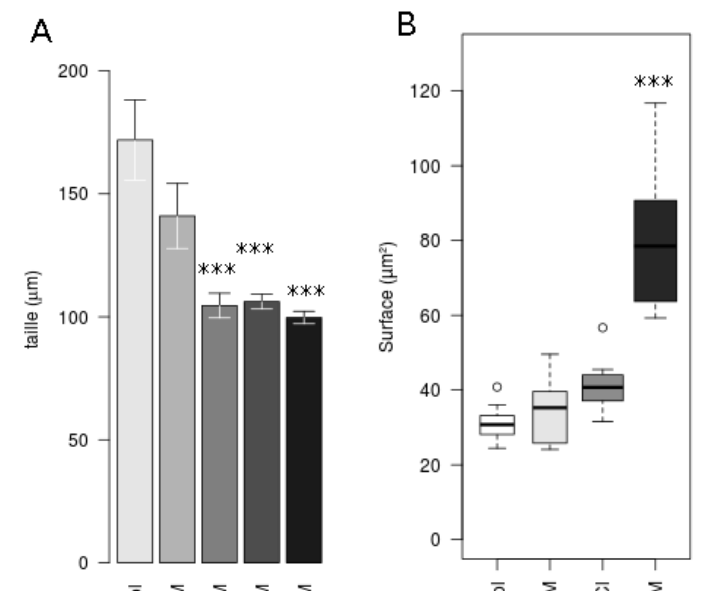


Figure 7:Measure of the length (A) and surface (B) of pollen tubes after 3 hours of culture in culture medium supplemented with different concentrations of LaCl₃ and/or DPI.

Immunolabelling of cell wall epitopes

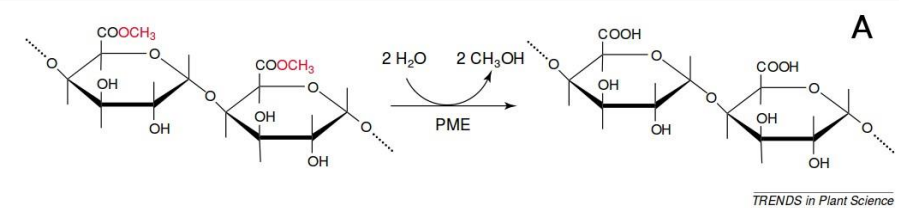
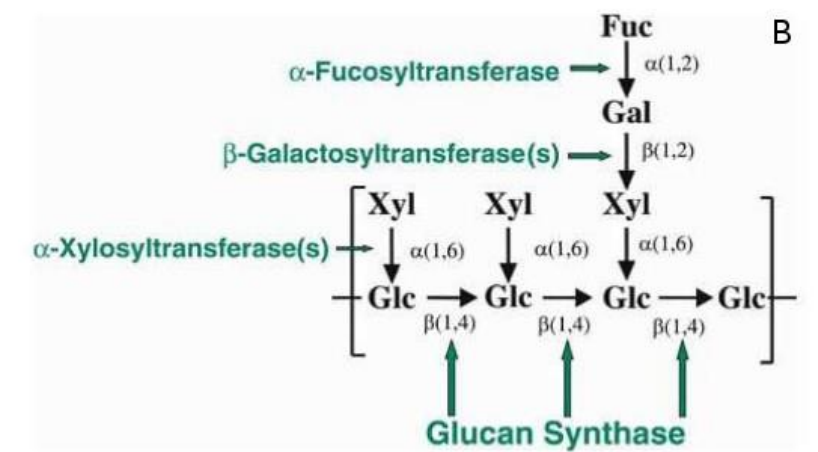


Figure 8:Structure of two of the main cell wall components, the pectin homogalacturonan (A) (from Micheli (2001)), and xyloglucan, the major cell wall hemicellulose (B) (adapted from Del Bem & Vincenz (2010)).

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)



Results

Inhibition of ROS production leads to a decrease of the degree of methylesterification (DM) at the tip (Figure 9B)

LaCl₃ 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)

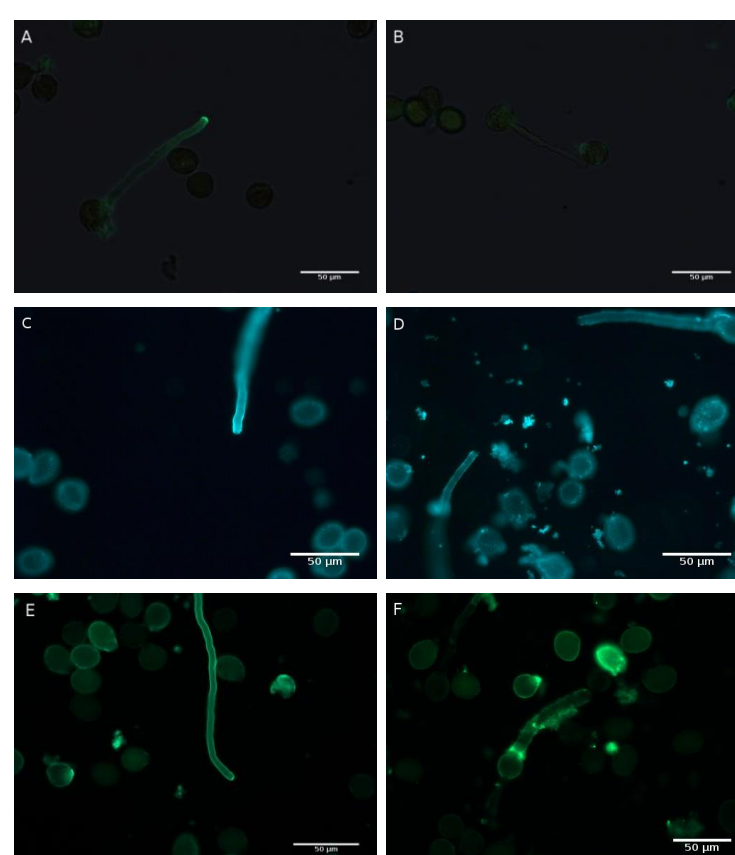


Figure 9:Immunodetection of cell wall epitopes. (A,B) LM20, (C,D) LM19, (E,F) LM25, under control conditions (A,C,E) DPI supplementation (B) or LaCl₃ (D,F).

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

Ca²⁺

Modification in Ca²⁺ influx is accompanied by an increase of the diameter and the degradation of the cell wall