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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

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

Figure 1 : Pollen tube morphology, with a single cell wall layer in the apicale zone (blue) and a bilayer wall on

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







Pharmacological study

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)

Objectives :

- Understand the signaling role of ROS and Ca^{2+} during polarized growth
- ② Characterize the cell wall modifications triggered by ROS and Ca²⁺ oscillations

	Chemical	Biological effect	
Table 1: List of the dif- ferent chemicals used and their effects.	Methylviologen	ROS inductor	
	DPI	NADPHox (ROS producing) Inhibitor	\checkmark
	Nitroarginine	NOS (NO producing) inhibitor	
	Sodium benzoate	ROS scavenger	\checkmark
	Lanthanum chloride	Calcium channels blocker	\checkmark
	EGTA	Calcium chelator	
	Nitroprusside sodium	NO inductor	
	cPTIO	NO inhibitor	
	Sildenafil citrate	cGMP-specific phosphodiesterase inhibitor	\checkmark



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



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.

Calcium

LaCl : 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 LaCl treatment

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.





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

ROS and Ca²⁺ 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 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).

Phenotypes 4 4 4 4 44444

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

• 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)

cation (DM) at the tip (Figure 8)



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).

Perspectives

- Characterization of cell wall enzymes activity
- Characterization of possible changes in cell wall polysaccharidic composition
- Fine localization of Ca²⁺ changes using Yellow Cameleon

• Analyses of transcriptional changes in cell wall remodelling enzymes



Figure 13 : Influence of ROS and Ca²⁺ 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 Ca²⁺

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