Involvement of Pectin methylesterases in Arabidopsis pollen imbibitions and germination

Christelle Leroux, Arnaud Lehner, Marie-Christine Kiefer-Meyer, Jérôme Pelloux, Azeddine Driouich, Patrice Lerouge, Jean-Claude Mollet

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Leroux C[a], Lehner A[a], Kiefer-Meyer MC[a], Pelloux J[b], Driouich A[a], Lerouge P[a] & Mollet JC[a]

[a] *Laboratoire Glycobiologie et Matrice Extracellulaire Végétale, UPRES-EA 4358, PRIMACEN IBiSA, IRIB, Université de Rouen, 76821 Mont Saint-Aignan Cedex, France.*
[b] *Biologie des Plantes et contrôle des Insectes ravageurs, UPRES-EA 3900, Université de Picardie-Jules Verne, 80039 Amiens, France.*

❶ During sexual plant reproduction, pollen germination and pollen tube elongation in the pistil are essential for delivering sperm cells to the ovule (Fig. 1).
Pollen grain contains two sperm cells and a vegetative cell limited from the outside to the inside, by the exine, the intine and the plasma membrane. The water influx permit the imbibition (Fig. 2a), before a calcium influx (Fig. 2b) which is localized at the future site of emergence of the tube (Fig. 2c).

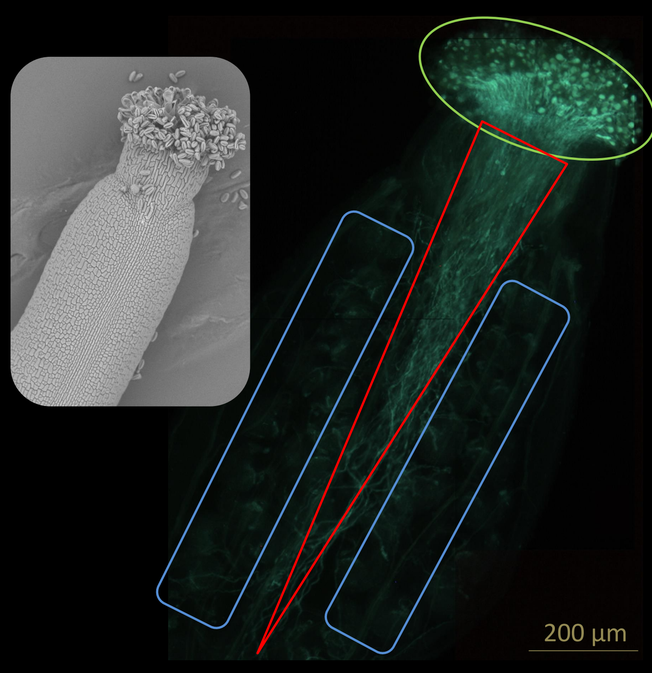


Figure 1: in-vivo pollen tube growth in the pistil stained by Aniline blue. Green zone: Pollen grains on stigma. Red zone: Pollen tubes in the transmitting tract. Blue zone: Ovary.

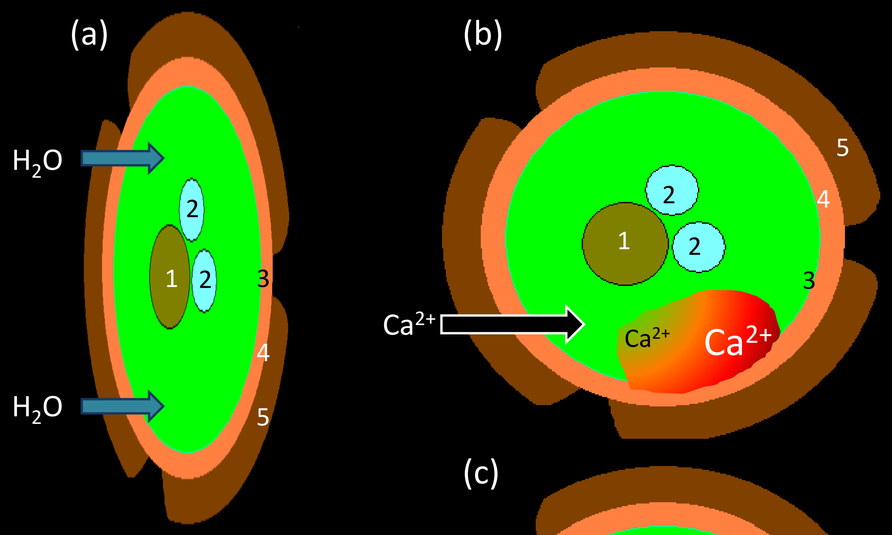


Figure 2: Schematic representation of dry (a), imbibed (b) and germinated (c) pollen grain.
1, Vegetative cell; 2, Sperm cells; 3, Plasma membrane; 4, Intine; 5, Exine
Blue arrow: H_20 influx; Black arrow: Calcium influx; (Konrad *et al.,* 2011) .

❷ The intine is composed of complex polysaccharides including homogalacturonans (HGs) which are secreted in the cell wall in a highly methyesterified form.
Demethylesterification of HGs is catalyzed in the cell wall by pectin methylesterases (PMEs). Interestingly, more than 20% of Arabidopsis PMEs (14 among 66) are specifically expressed in pollen grain and pollen tube. Their expression is gene- and time- dependent (Fig. 3).

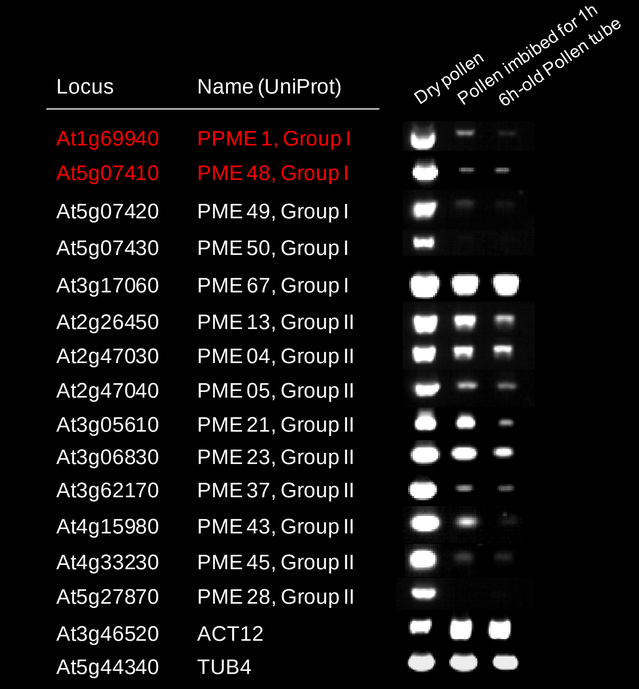


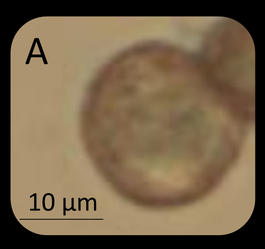
Figure 3: RT-PCR detection of PMEs transcripts in wild type pollen grains, imbibed pollen grains and pollen tubes. Gene identities of RT-PCR products are indicated on the left. An equal amount of cDNA was amplified in each sample. ACT12 and TUB4 have been used as positive controls.

❸Two knock-out mutants impaired in two homologous PME (*ppme1* and *pme48;* Fig. 3) present a strong delay in pollen germination (not shown) and a remarkable phenotype with multiple pollen tube tips emerging from the grain (Fig. 4D).

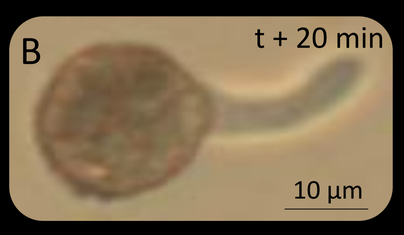
❹ The possible implication of PMEs, HGs, calcium ions and polygalacturonases during pollen germination is presented Figure 4. It is based on the phenotypes of WT (A, B) or mutant (C, D) pollen grains observed during imbibition and pollen tube growth.

i) Random action of PME : The partially de-methylesterified HGs may become a target for pectin-degrading enzymes, such as polygalacturonases.
-> Enzymatic degradation of the intine and mechanical rupture of the exine due to turgor pressure.

ii) Block-wize action of the PME : The blocks of de-methylesterified HGs can interact with Ca^{2+} promoting the formation of the “egg-box” structure and thus rigidifying the intine.
-> Mechanical rupture of the intine and exine due to turgor pressure.



WT: methylesterified HGs are hydrophobic. Upon the action of PME they become more hydrophilic.
->the imbibition is fast in WT (A).
-> PMEs are present, structure or mechanical properties of the intine are modified, the tube can emerge from the grain(B).



Mutants: methylesterified HGs are hydrophobic. De-methylesterification is perturbed.
->the imbibition is slow (C).
-> PME actions are reduced. Germination is perturbed : No germination (D, left grain) or abnormal germination with a first tube that fails to emerge and a second tube that takes over to ensure future fertilization (D, right grain)

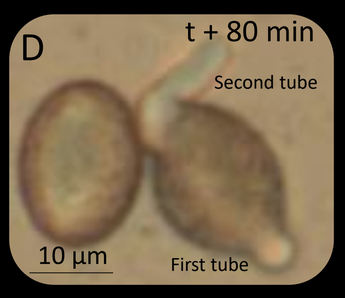
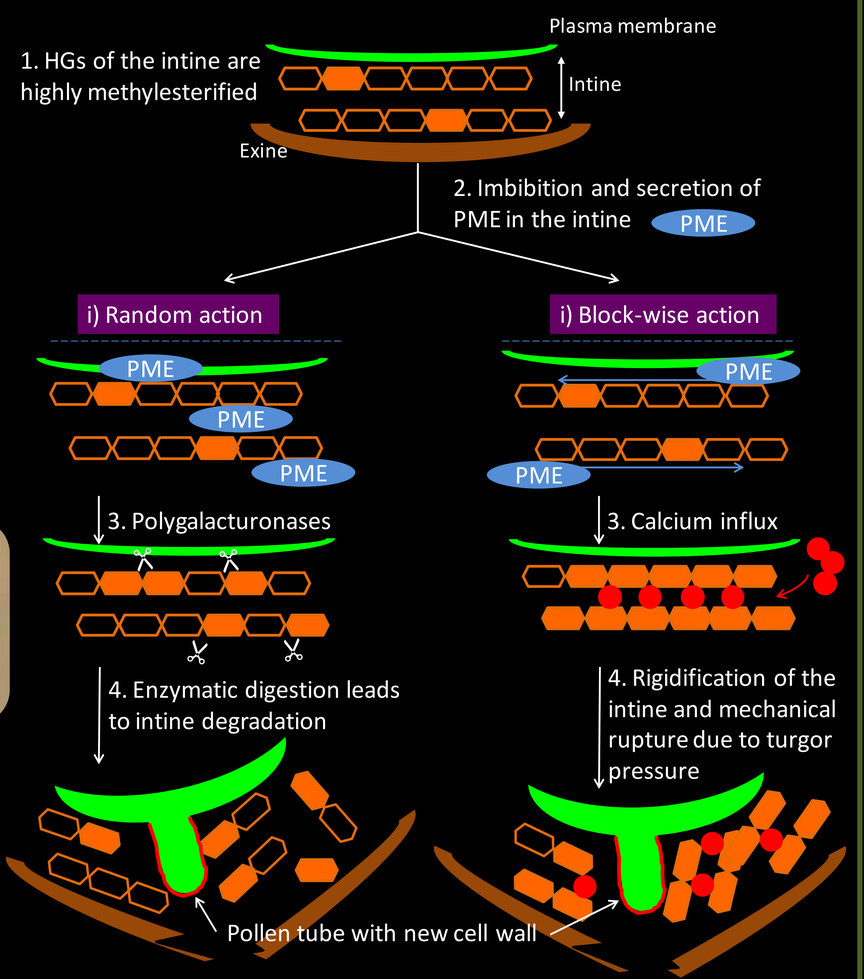


Fig 4: Theoretical model presenting the possible involvement of PME during pollen grain germination



Enzymatic activity of PME appears to be essential during pollen germination by facilitating the rupture of the pollen grain wall and then the emergence of the pollen tube

Konrad *et al*; (2011). Calcium regulation of tip growth: new genes for old mechanisms *Current opinion in plant biology* 14:1-10.