A new model depicting the role of PME during dehydration and germination of pollen grain

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I. During sexual plant reproduction, pollen germination and pollen tube elongation in the pistil are essential for delivering sperm cells to the ovule (Fig. 1, a-e).

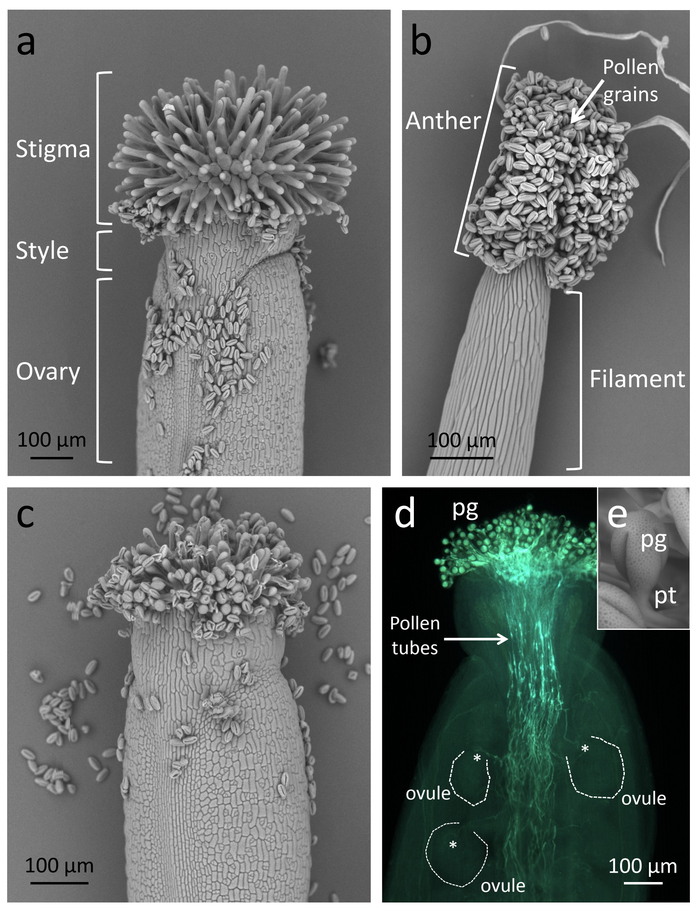


Figure 1. Scanning Electron Micrographs of unpollinated pistil (a), stamen (b) and pollinated pistil (c) of the model plant *Arabidopsis thaliana*. d, Aniline blue staining of pollen tubes growing through the female tissue. Pollen tubes that have reached the ovule are marked with an *. e, Scanning Electron Micrograph of a germinated pollen grain on the stigma. f, Schematic representation of a transversal section of a pollen grain. Pg, pollen grain, pt, pollen tube, sp cell, sperm cell, veg n, vegetative nucleus.

Pollen grain contains two sperm cells and a vegetative cell limited by the exine, the intine and the plasma membrane (Fig.1, f). The intine is composed of complex polysaccharides including homogalacturonans (HGs) which are secreted in the cell wall in a highly methylesterified form. Demethylesterification of HGs is catalyzed in the cell wall by pectin methylesterases (PMEs).

II. Two *PME*, *PPME1* and *PME48* are strongly expressed in dry pollen grain (PG) and slightly expressed in imbibed PG and pollen tube (Fig.2)

Locus	Name (UniProt)
At1g69940	PPME 1, Group I
At5g07410	PME 48, Group I
At3g46520	ACT12
At5g44340	TUB4

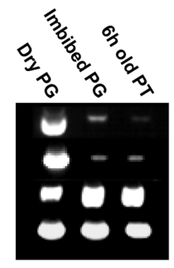
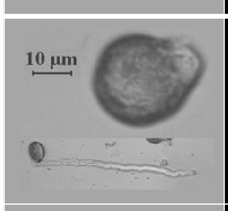
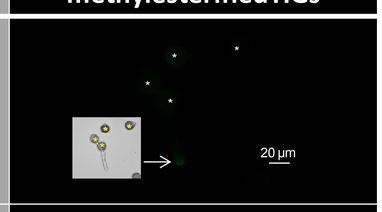
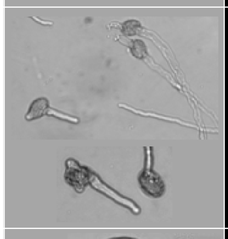
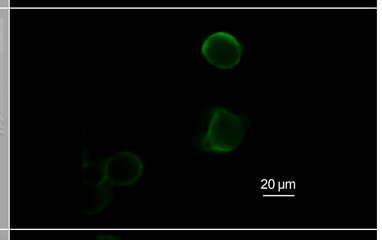
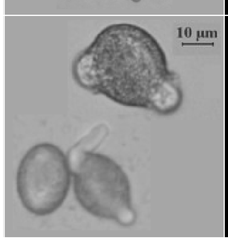
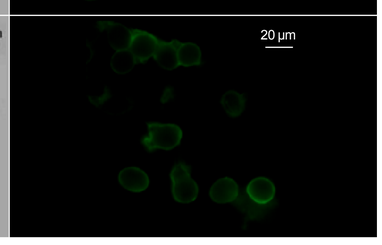


Figure 2. RT-PCR amplification of *PPME1* and *PME48* transcripts in wild type pollen grains, imbibed pollen grains and pollen tubes. *ACT12* and *TUB4* have been used as positive controls.

III. Mutants for *PME48* and *PPME1* germinated very slowly, 13h to reach 50% of germination (T_{50}) compared to 2h for the wild type. Mutants presented phenotypes with two tubes emerging from the grain.

IV. Immunodetection of highly methylesterified HG showed that intine was more methylesterified in the mutants than in the wild type.

line	T_{50} (h)	phenotype	Immunodetection of highly methylesterified HGs
Wild type	2		
pme48 -/- KO for PME48	13		
ppme1-/- KO for PPME1	13		

What are the roles of PMEs during pollen dehydration, imbibition and germination?

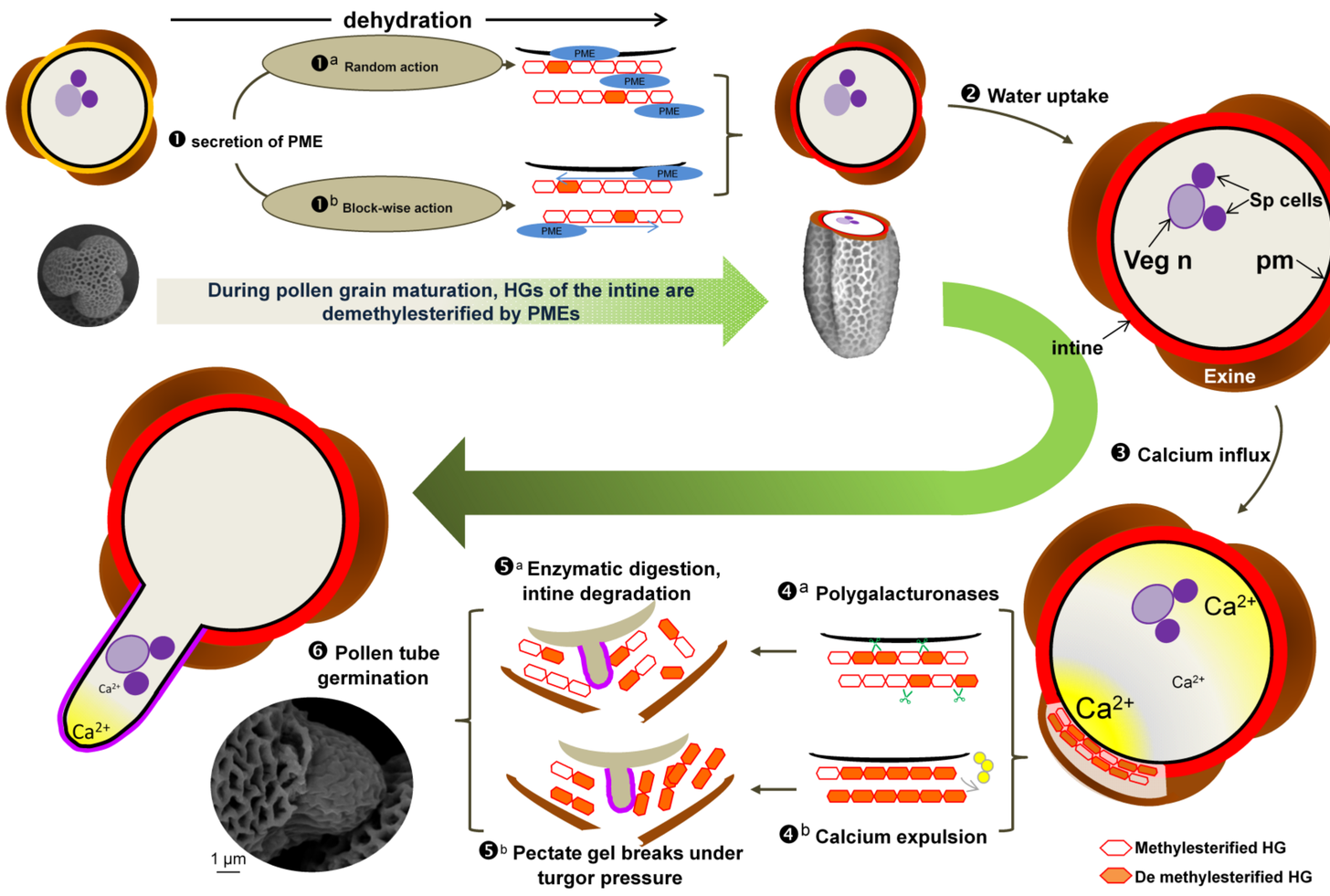
❶, PME may be secreted in the intine during the dehydration and start demethylesterifying HG, randomly (❶[a]) or upon block wise actions (❶[b]). In dry grain, intine is weakly methylesterified.

❷, imbibition of pollen grain is enhanced by the hydrophilic weakly methylesterified HG.

❸, Calcium influx in the pollen grain may originate from the expulsion of Ca^{2+} from the pectate gel (❹[b]) thus weakening the mechanical properties of the intine that may break under turgor pressure (❺[b]).

Alternatively, the randomly demethylesterified HG of intine may be degraded by polygalacturonases (❹[a]) leading to the degradation of the intine wall (❺[a]).

❻, Pollen tube germination.



HG, homogalacturonans, pm, plasma membrane, Sp cells, sperm cells, Veg n, vegetative nucleus