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Christelle Leroux, Arnaud Lehner, Marie-Christine Kiefer-Meyer, Jérôme Pelloux, Azeddine Driouich, et al.. Involvement of Pectin methylesterases in Arabidopsis pollen imbibitions and germination. 1ere journée de l'Institut de Recherche et d'Innovation Biomédicale, Jun 2012, Rouen, France. hal-02082428

HAL Id: hal-02082428

<https://hal-normandie-univ.archives-ouvertes.fr/hal-02082428>

Submitted on 28 Mar 2019

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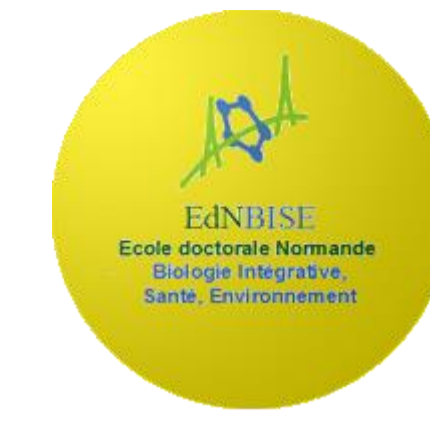
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Involvement of pectin methylesterases in *Arabidopsis* pollen imbibition and germination.

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1 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).

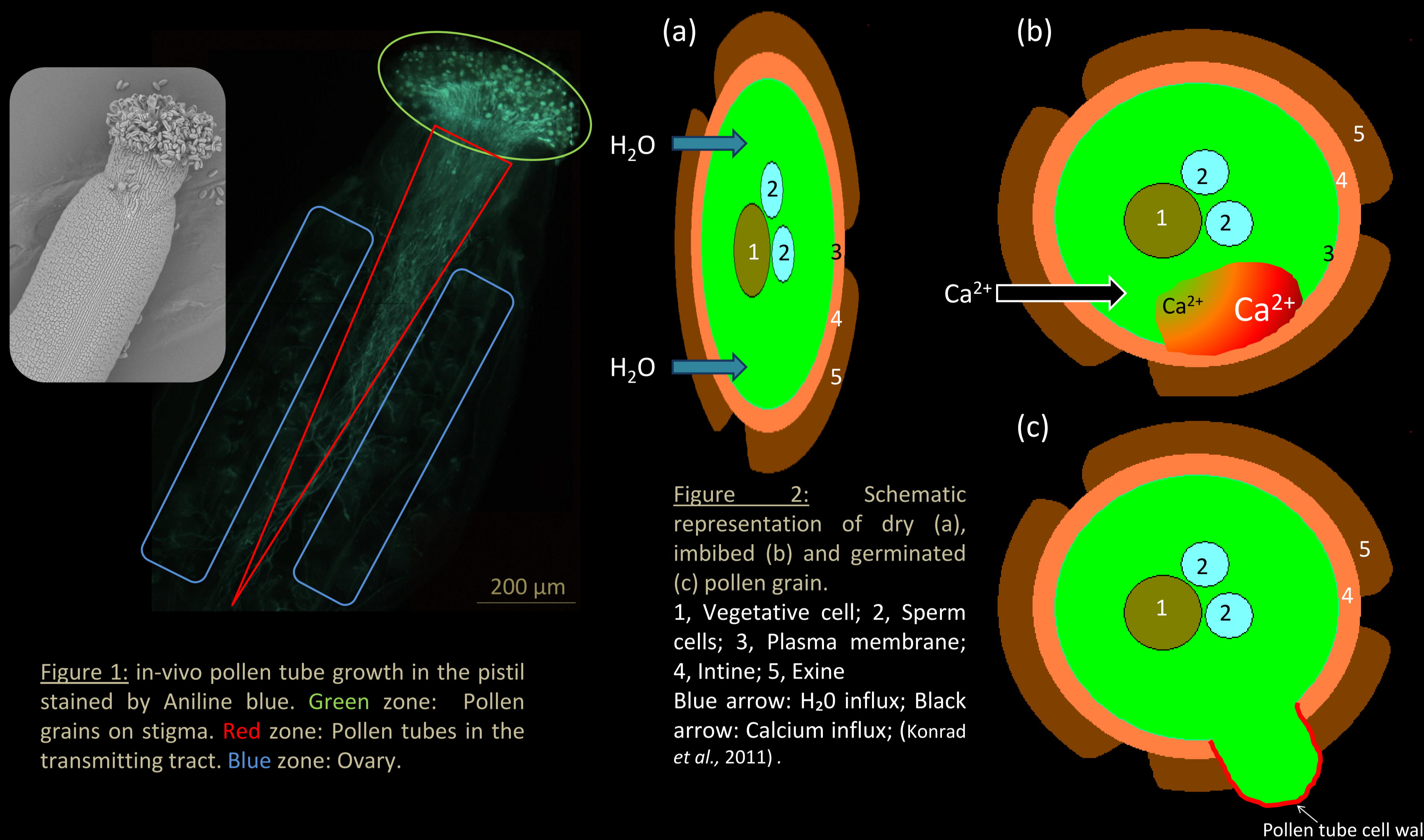


Figure 2: Schematic representation of dry (a), imbibed (b) and germinated (c) pollen grain. 1, Vegetative cell; 2, Sperm cells; 3, Plasma membrane; 4, Intine; 5, Exine. Blue arrow: H₂O influx; Black arrow: Calcium influx; (Konrad et al., 2011).

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.

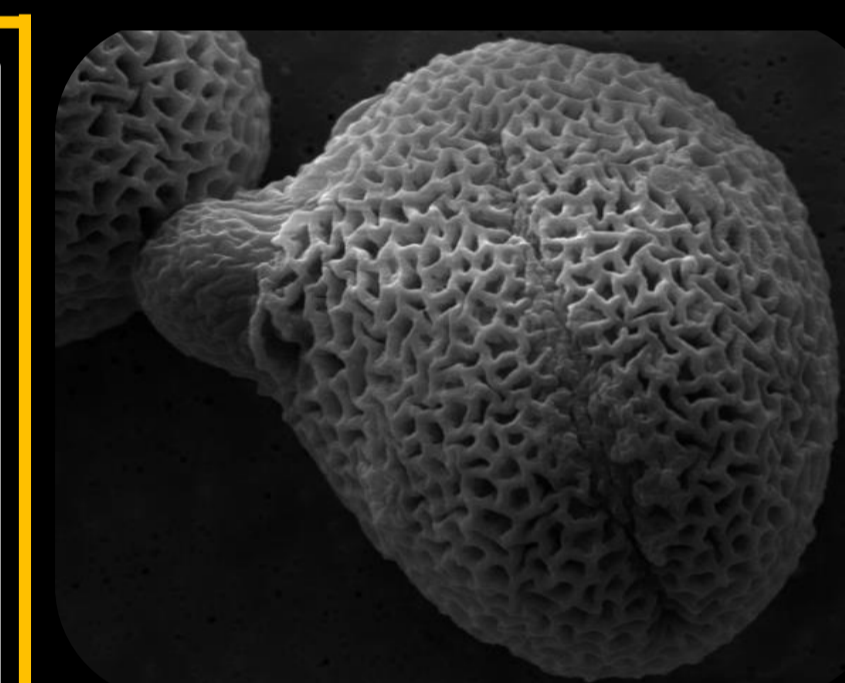
2 The intine is composed of complex polysaccharides including homogalacturonans (HGs) which are secreted in the cell wall in a highly methylesterified form.

Demethylesterification of HGs is catalyzed in the cell wall by pectin methylesterases (PMEs). 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).

| Locus | Name (UniProt) | Dry pollen | Pollen imbibed for 1h | 6h-old Pollen tube |
|-----------|------------------|------------|-----------------------|--------------------|
| At1g69940 | PPME 1, Group I | | | |
| At5g07410 | PME 48, Group I | | | |
| At5g07420 | PME 49, Group I | | | |
| At5g07430 | PME 50, Group I | | | |
| At3g17060 | PME 67, Group I | | | |
| At2g26450 | PME 13, Group II | | | |
| At2g47030 | PME 04, Group II | | | |
| At2g47040 | PME 05, Group II | | | |
| At3g05610 | PME 21, Group II | | | |
| At3g06830 | PME 23, Group II | | | |
| At3g62170 | PME 37, Group II | | | |
| At4g15980 | PME 43, Group II | | | |
| At4g33230 | PME 45, Group II | | | |
| At5g27870 | PME 28, Group II | | | |
| At3g46520 | ACT12 | | | |
| At5g44340 | TUB4 | | | |

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.

3 Two 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).



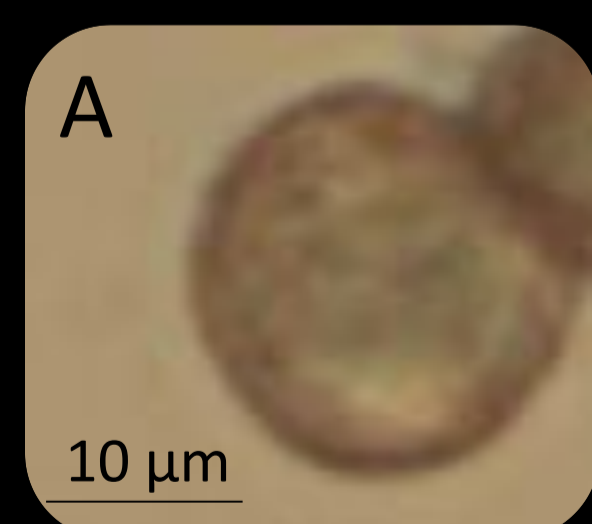
4 The possible implication of PMEs, HGs, calcium ions and polygalacturonases during pollen germination is presented Figure 4. It is based on the phenotypes of WT (A, B) or mutant (C, D) pollen grains observed during imbibition and pollen tube growth.

i) Random action of PME : The partially de-methylesterified HGs may become a target for pectin-degrading enzymes, such as polygalacturonases.

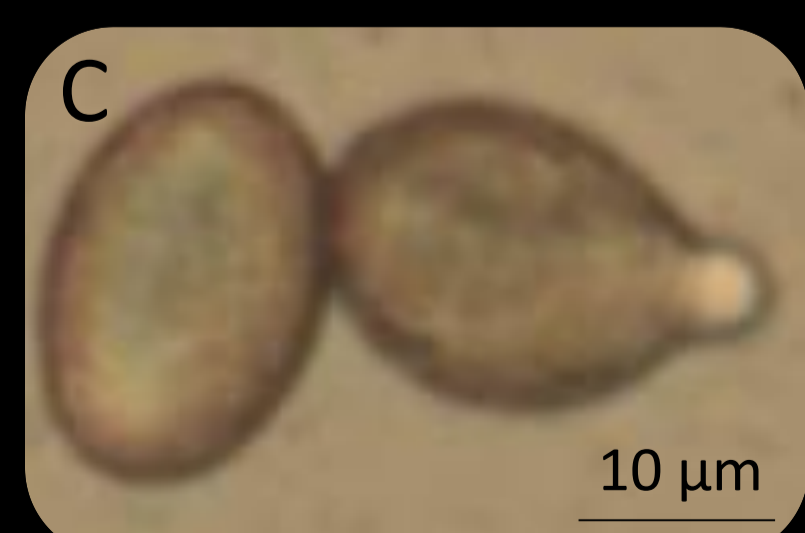
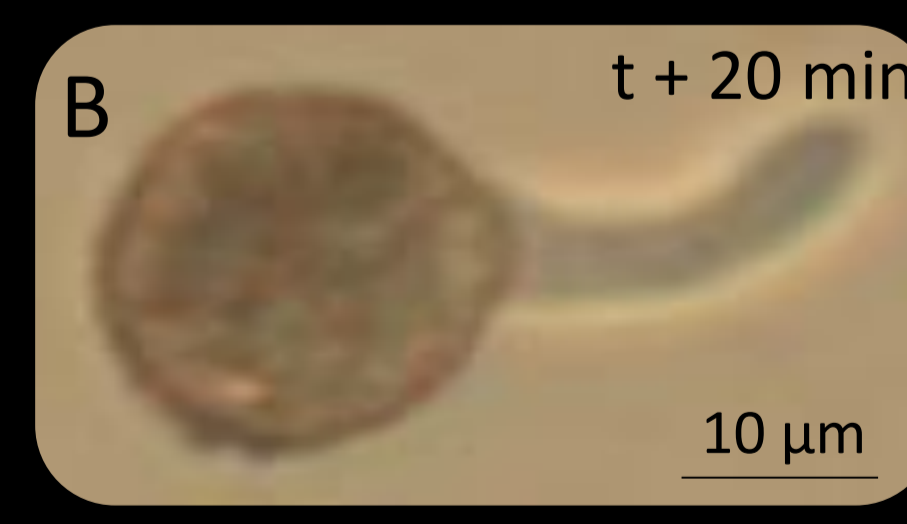
-> **Enzymatic degradation of the intine and mechanical rupture of the exine due to turgor pressure.**

ii) Block-wise action of the PME : The blocks of de-methylesterified HGs can interact with Ca²⁺ 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)

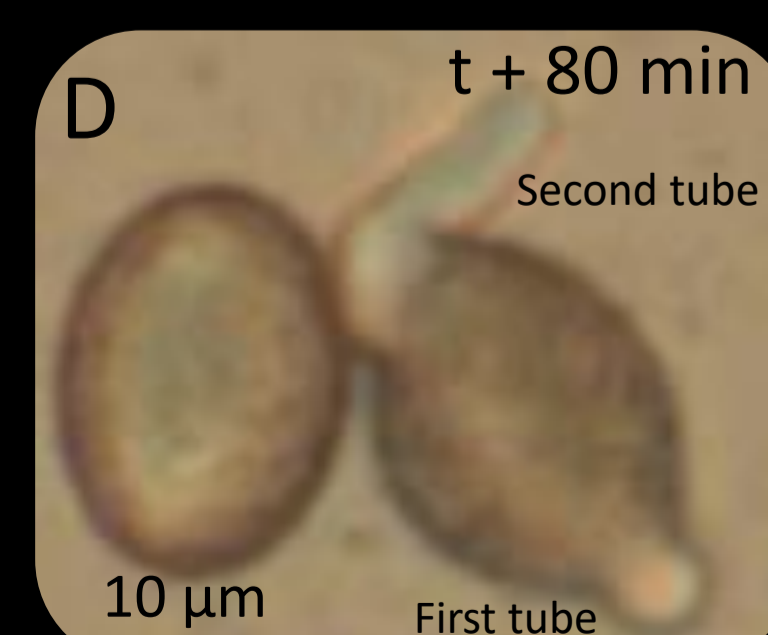
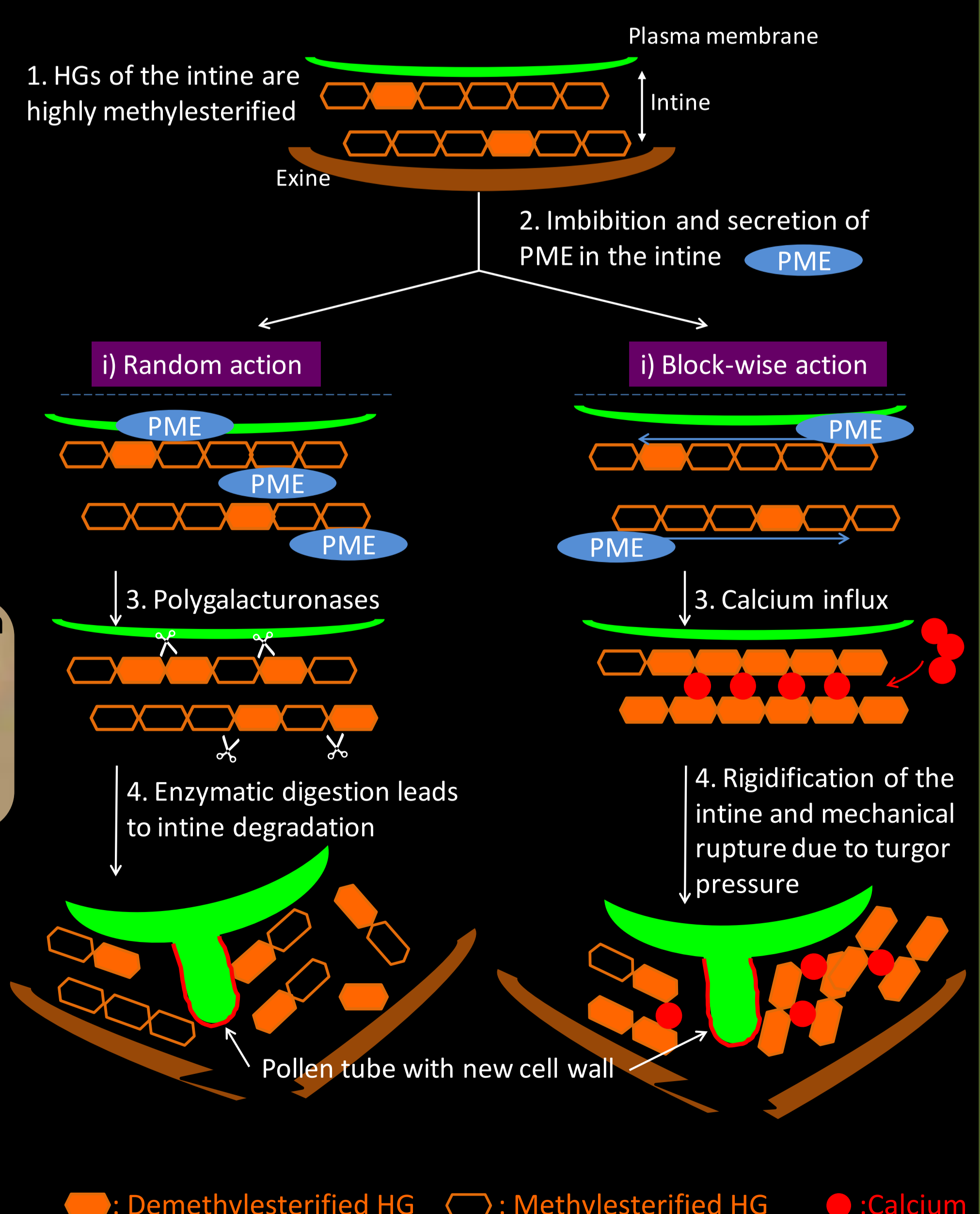


Fig 4: Theoretical model presenting the possible involvement of PME during pollen grain germination



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