AtPME48 encodes a pectin methylesterase involved in Arabidopsis pollen grain germination

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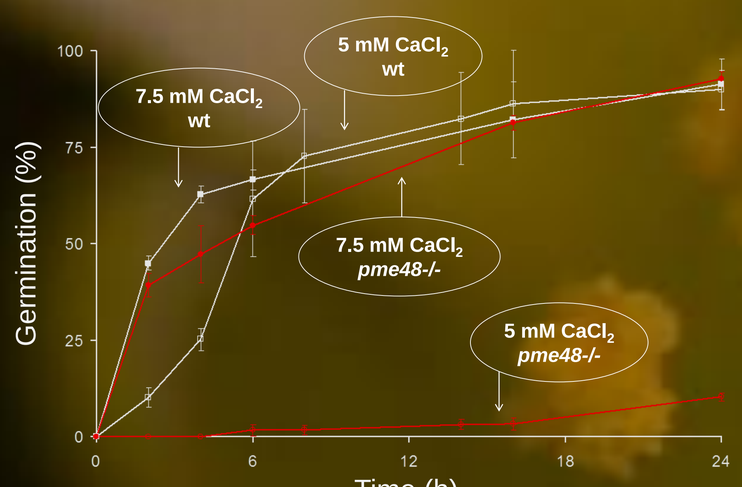
ABSTRACT

PME48 encodes for a pollen-specific PME. Homozygous mutant lines (*pme48-/-*) were isolated and the pollen grains were analysed. Germination was strongly delayed in *pme48 -/-* and might be related to a slower imbibition. Interestingly, when calcium was added to the germination medium, the imbibition and the germination were fully restored in *pme48-/-*. Moreover, we showed that the intine wall of the mutant contained more highly methylesterified homogalacturonan (HG) than in the wild type. A model summarizing the role of PME48 and calcium during germination of the pollen grain is presented .

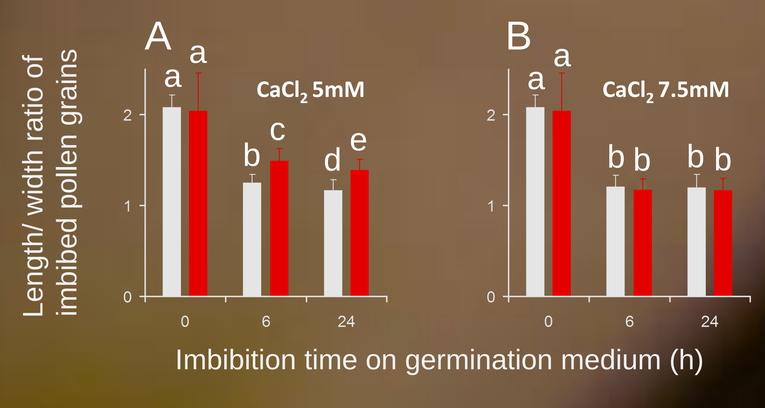
1. pollen grain germination



In standard germination medium (5 mM $CaCl_2$), pollen grain germination is strongly delayed in *pme48-/-* compared to the wild type. The percentages of germination reached 10 and 90 % after 24h for *pme48-/-* and for the wild type, respectively .

When calcium concentration is increased in the germination medium (7.5 mM), pollen grains from *pme48-/-* germinated as fast as the wild type and reached 90% after 24h.

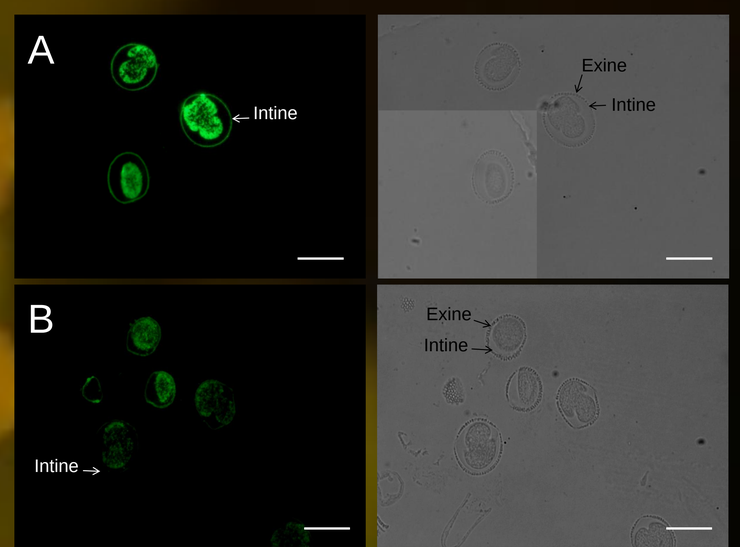
2. Measurement of the imbibition rates



As pollen grains became spherical during rehydration, the rate of imbibition was estimated by measuring the ratio length/width of the pollen grains. The imbibition of *pme48-/-* was never fully achieved in 5 mM $CaCl_2$ (A). Interestingly the imbibition was fully restored for *pme48-/-* in the presence of 7.5 mM $CaCl_2$ (B).

Letters indicate statistically significant differences among the wild-type and *pme48-/-* lines, as determined by Student's t-test (P<0.0001)

3. Immuno-localization of highly methylesterified HG



Scale bars = 20 µm.

Immuno-localisation of the highly methylesterified epitopes of HG was performed on dry pollen grains from *pme48-/-* and wild type plants using the monoclonal antibody JIM7. the intine wall was strongly labelled in *pme48-/-* (A) compared to the wild type (B) suggesting that the intine wall of the mutant is more methylesterified.

As methyl groups are hydrophobic, the higher level of methylesterified HG in the mutant compared to the wild type may explain the difficulty of the pollen grains to rehydrate properly in *pme48-/-*.

4. What may be the role of PME48 in pollen grains?

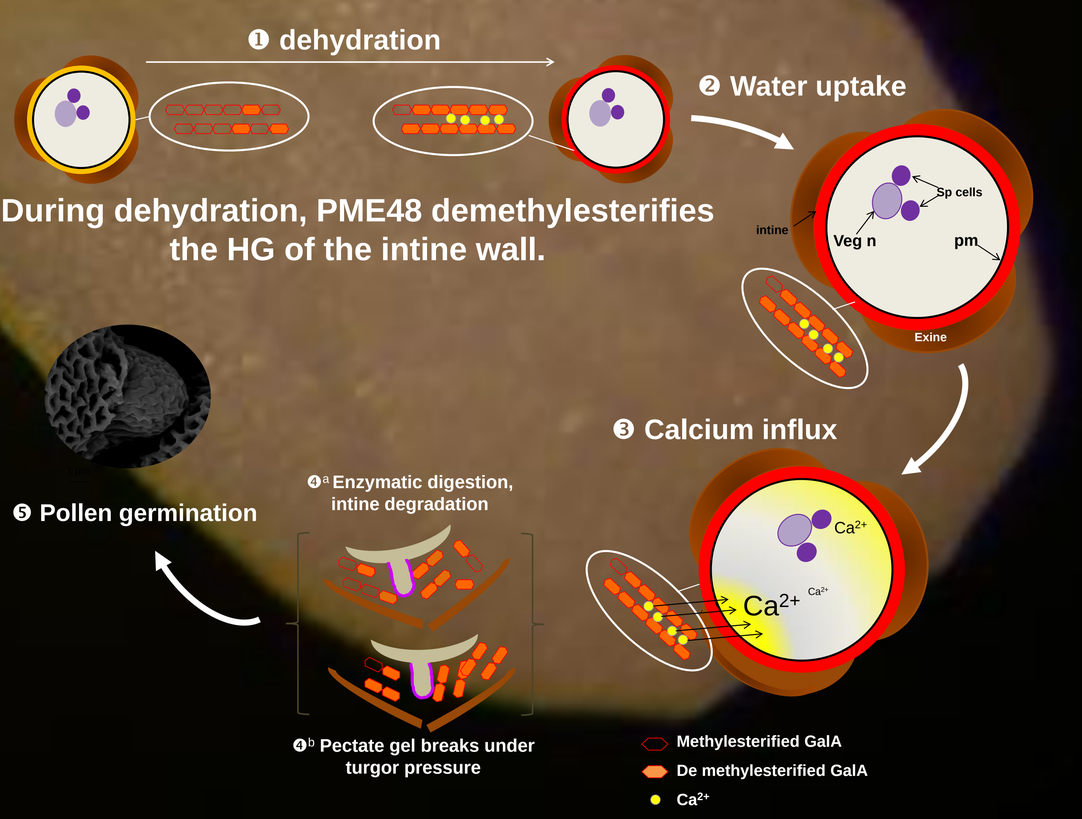
❶, PME48 may be secreted in the intine wall during the dehydration and starts to demethylesterify the HG. In wild type dehydrated pollen grains, intine is weakly methylesterified compared to the mutant *pme48-/-*.

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 thus weakening the mechanical properties of the intine that may break under turgor pressure (❹[b]).

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

❺, Pollen tube germination.



GalA, galacturonic acid; pm, plasma membrane; Sp cells, sperm cells; Veg n, vegetative nucleus.