Decrease in PME48 activity leads to abnormal pollen germination

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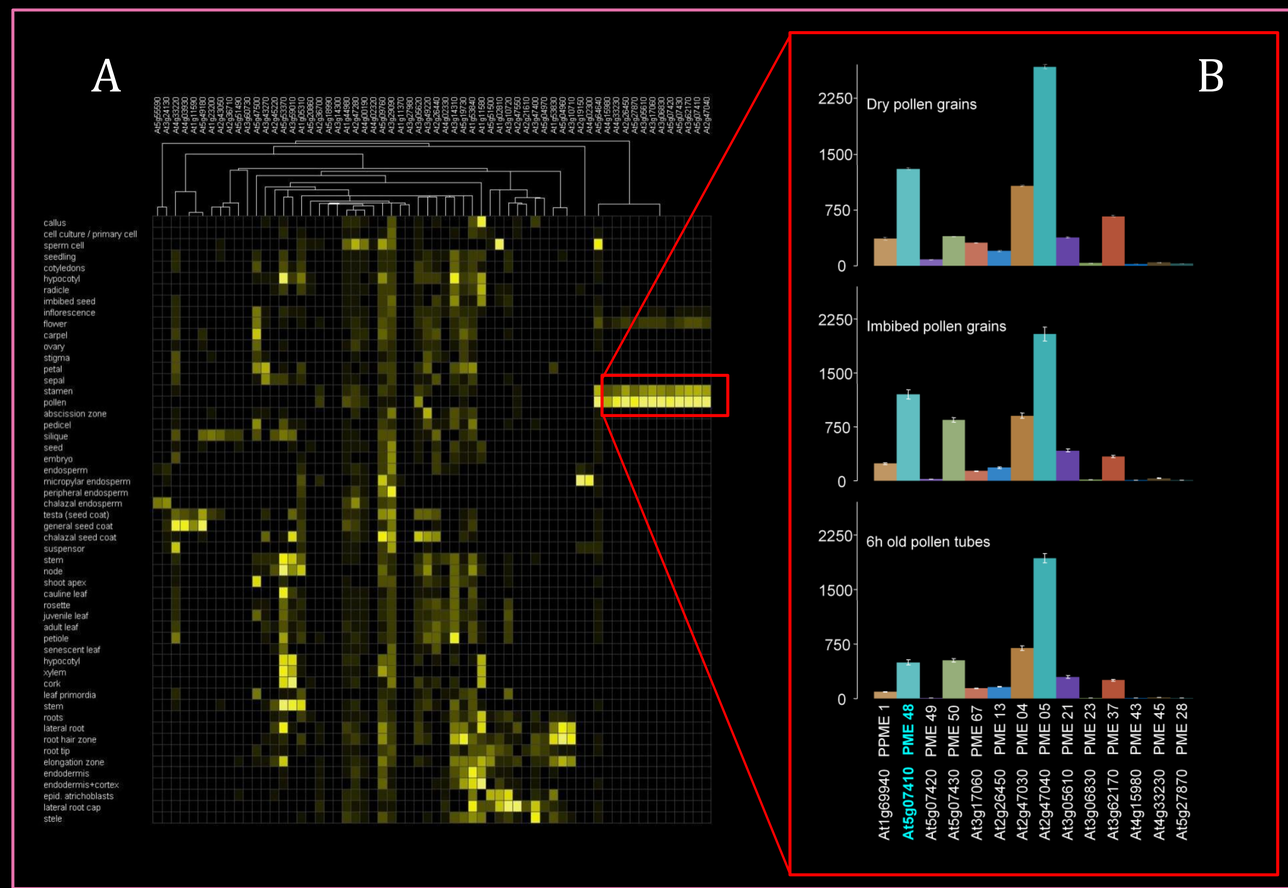
INTRODUCTION

Germination of the pollen grain on the stigma is the first step of the reproduction process. It needs a complete and rapid rehydration of the pollen that may be controlled by the variation of the degree of methylesterification of pectic homogalacturonans (HGs). Methylester groups are hydrophobic and they can be removed from the HGs by the action of pectin methylesterases (PME)[(1)]. The expression of a gene coding for a pollen specific PME : At*PME48* has been analyzed by RT-qPCR and a *PRO::PME48-sYFP* line. Moreover, we have investigated the role of reduced PME activity on the pollen grain germination using a knock-down mutant for *PME48*.



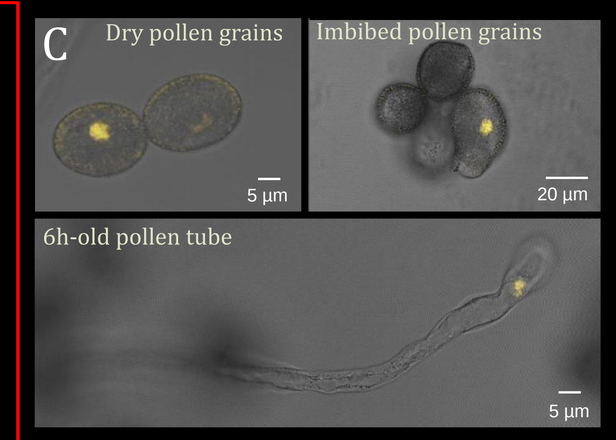


Figure 1:
A, Genevestigator[(2)] heat map of the 66 *PME* genes showing the specific expression of 14 *PME* in pollen (red box). 12 genes are presented as At*PPME1* and At*PME48* as well as At2g47030 and At2g47040 are too similar to be distinguished. **B,** qPCR expression of the 14 pollen specific *PME* genes in wild-type pollen grains and during germination. (n=3 biological replicates). **C**, Expression of the promoter of *PME48* using PRO_{PME48}*::YFP* construction by confocal microscopy in dry pollen grains, imbibed pollen grains for 1 h and during pollen tube growth. Images are the overlay of the brightfield and fluorescent pictures.

1. At*PME48* expression profile

- 14 *PMEs* are pollen specific (Fig. 1A)
- *AtPME48* is strongly expressed in wild-type dry pollen grains, imbibed pollen grains and 6h-old pollen tubes grown *in vitro* (Fig. 1B).
- The promoter of *PME48 (PRO::PME48)* is strongly expressed in dry pollen grains, imbibed pollen grains and in 6h-old pollen tube (Fig. 1C).

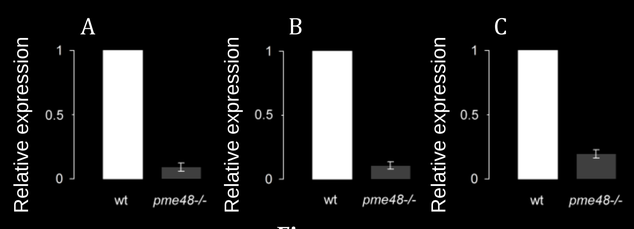


Figure 2:
A-C, qPCR analyzes of the relative expression of *AtPME48* in wild-type (white bars) and in *pme48-/-* (grey bars) dry pollen grains (**A**), imbibed pollen grains (**B**) and in 6h-old pollen tubes (**C**). (n=3 biological replicates),

2. Effect of the loss of the PME48

- In *pme48-/-*, the expression of *AtPME48* is reduced by 90% in dry and imbibed pollen grains (Fig. 2A, B) and by 80% in pollen tubes (Fig. 2C).
- PME activity corresponding to PME48 is lost in the mutant (Fig. 3A, blue circle). Note that PME28 does not appear on the zymogram as its pI is too acidic.

The total PME activity is reduced of 50% in the mutant (Fig. 3B).

- FT-IR analyzes on pectin extracts from dry pollen grains show a significant 2-fold higher level of the degree of methylesterification of the HG in *pme48-/-* (Fig. 3C-D).
- The higher level of degree of methylesterification of the HG in *pme48-/-* pollen grains is correlated with a strong delay in pollen germination. Only 10% of the mutant grains were germinated after 24 h (Fig. 3E).

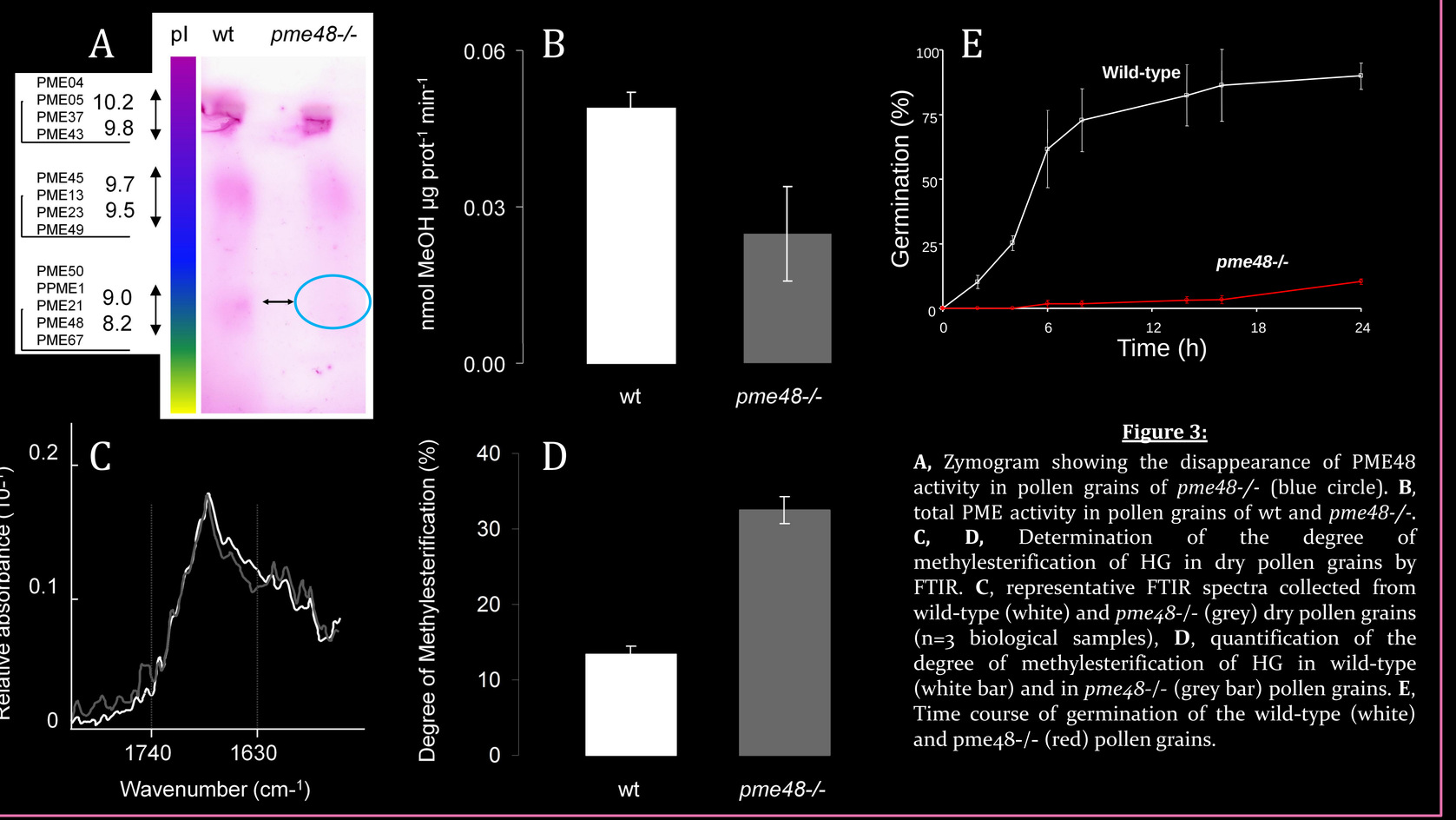


Figure 3:
A, Zymogram showing the disappearance of PME48 activity in pollen grains of *pme48-/-* (blue circle). **B**, total PME activity in pollen grains of wt and *pme48-/-*. **C, D,** Determination of the degree of methylesterification of HG in dry pollen grains by FTIR. **C**, representative FTIR spectra collected from wild-type (white) and *pme48-/-* (grey) dry pollen grains (n=3 biological samples), **D**, quantification of the degree of methylesterification of HG in wild-type (white bar) and in *pme48-/-* (grey bar) pollen grains. **E**, Time course of germination of the wild-type (white) and pme48-/- (red) pollen grains.

CONCLUSIONS

The loss of PME48 activity leads to a higher degree of methylesterification of the HG in the intine of the pollen grain resulting in a strong delay of pollen germination.

PME48 participates in the removal of the hydrophobic methylester groups of the HG during maturation of the pollen grain promoting its proper germination.

References: 1. Micheli *et al.,* 2001; 2. Hruz *et al.,* 2008;

http://glycomev.univ-rouen.fr/