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Pectin methylesterases and Arabidopsis pollen tube growth : case study of a pollen-specific PME mutant

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Introduction

Little is known about the molecular mechanisms involved in the spatial and temporal controlled growth of the pollen tube through the female tissues. Variation of cell wall stiffness of the tube has been proposed to participate in tube growth. Such a variation in cell wall properties is likely to be related to the level of methylesterification of pectic homogalacturonans (HG). Modulation of the degree of HG methylester is regulated in the cell wall by the action of pectin methylesterases (PMEs). In Arabidopsis, putative PME proteins are encoded by a 66-member gene family. Among them, 14 PME transcripts are specifically expressed in pollen grain or pollen tubes (Fig. 1A), indicating that more than 20% of the Arabidopsis PMEs are dedicated to the male gametophyte. Using a pollen-specific PME (Fig. 1B) knock-out mutant (*At3g06830*), we investigated *in vitro* pollen tube growth and morphology, as well as the impact of this mutation on 1) the distribution of weakly and highly methylesterified HG epitopes in pollen tube cell wall and 2) on plant fertilization. Moreover, expression of GUS protein, under the control of At3g06830 promoter, during tube growth is also presented.

14 genes coding for PME among 66 are pollen specific

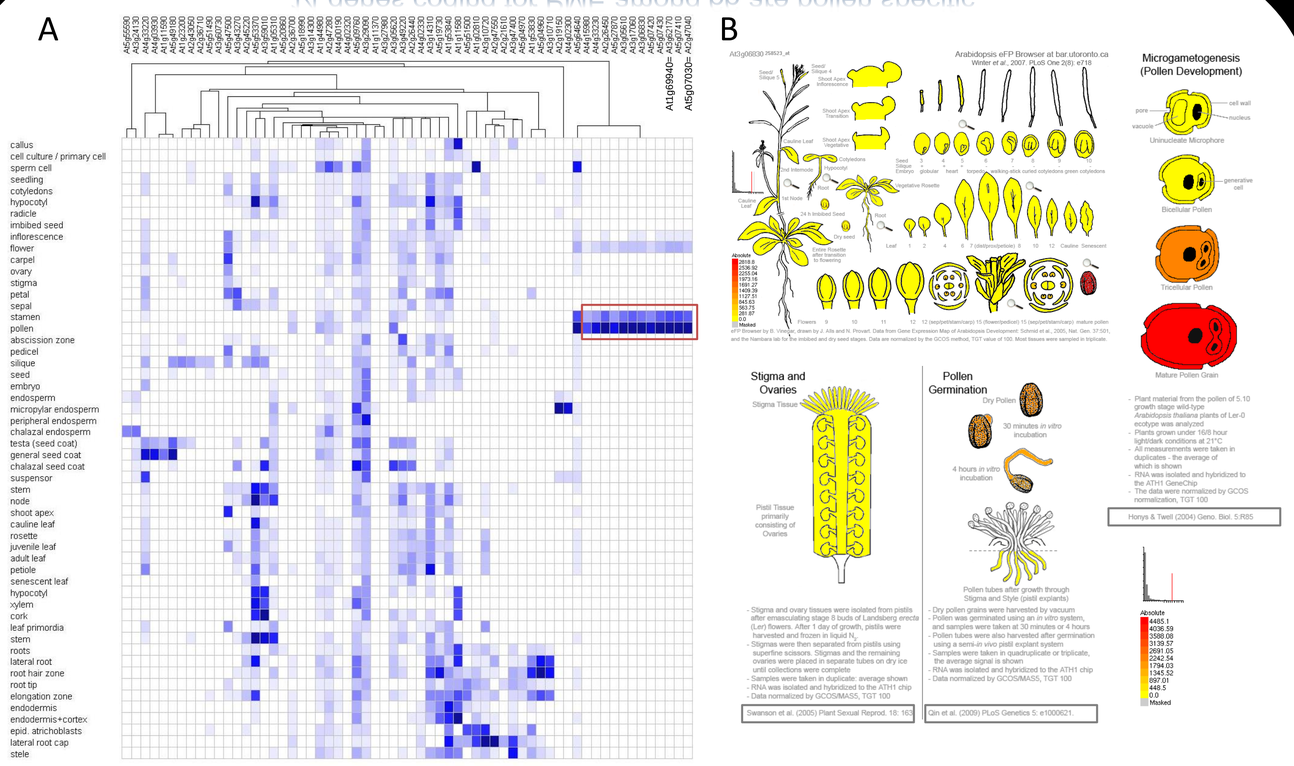

Figure 1. Transcriptomic data. A, specific expression of the 14 PME genes in pollen (boxed in red). 12 genes are presented as At1g69940 and At5g07410 as well as At2g47030 and At2g47040 are duplicated in the genome of Arabidopsis. therefore, their distinctive expression levels are not distinguishable. B, Organ specific expression of At3g06830 encoding for a pollen-specific PME. Data from genevestigator and EFP browser (Hruz et al., 2008; Winter et al., 2007).

At3g06830-ko pollen is impaired in hydration and germination *in vitro*

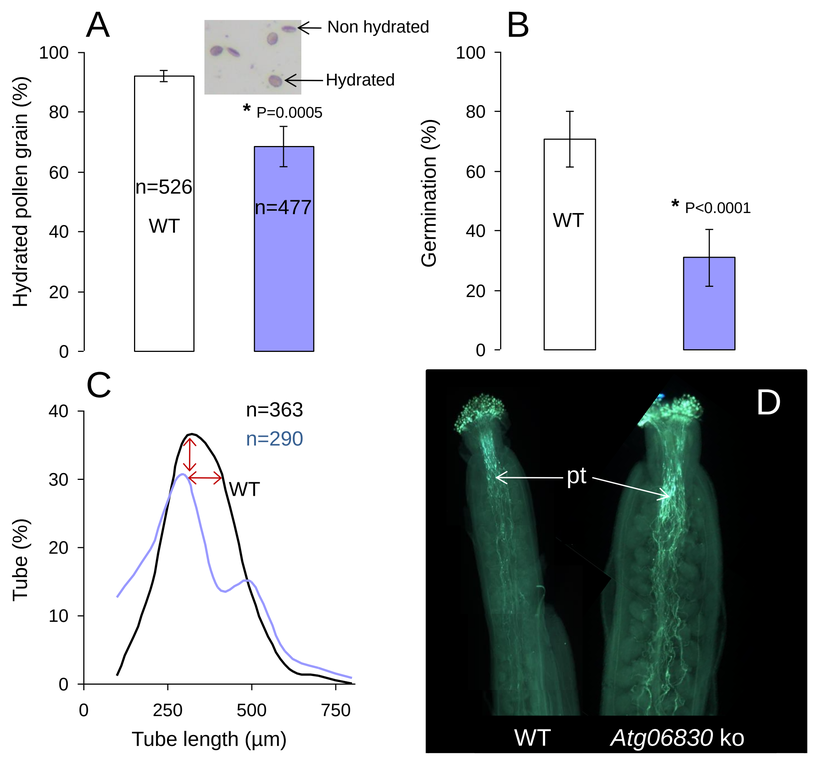

Figure 2. Percentage of hydrated (A) and germinated pollen grains (B) after 6h of culture in liquid germination medium. C, Distribution of the length of the pollen tubes 6h after rehydration (data are expressed as percentages). D, Aniline blue staining of *in vivo* self pollinated pistils. n, number of measurements; pt, pollen tubes.

Results

Hydration and germination rates: 92% of the pollen grain harvested from the wild type flowers have rehydrated after 6h of culture, whereas 68 % of the pollen grains of the *At3g06830*-ko mutant were rehydrated (Fig. 2A). This difference of pollen grain hydration may explain the reduction of pollen germination rate in the *At3g06830*-ko mutant (Fig. 2B).

Pollen tube length and fertilization : *In vitro* pollen tubes of the mutant are shorter compare to the WT (Fig. 2C). *In vivo*, WT and *At3g06830*-ko pollen tubes were able to fertilize all the ovules (Fig. 2D).

Dapi, JIM5 and JIM7 staining: The two sperm nuclei and the vegetative nucleus were stained in both WT and *At3g06830*-ko mutant pollen grain. Weakly methylesterified HG were labeled back from the tip in WT pollen tube. In contrast, labeling with JIM5 was very strong at the tip of the mutant pollen tube (Fig. 3). JIM7 labeling may extend further back from the tip in the mutant compare to the WT.

GUS staining : The expression of At3g06830 has been localized using GUS construction (Fig. 3). GUS staining showed a pollen grain- and pollen tube- specific expression. Expression was not found in any other part of the plant.

Conclusions

- Pollen grains of *At3g06830*-ko are clearly affected in their capacity to rehydrate *in vitro.*
- The distribution of Highly methylesterified HG seems to be more widely extended in the mutant suggesting that the mutation may have reduced the demethylesterification process.
- At3g06830 is specifically expressed in pollen grain and in pollen tube (*in vitro and in vivo*).

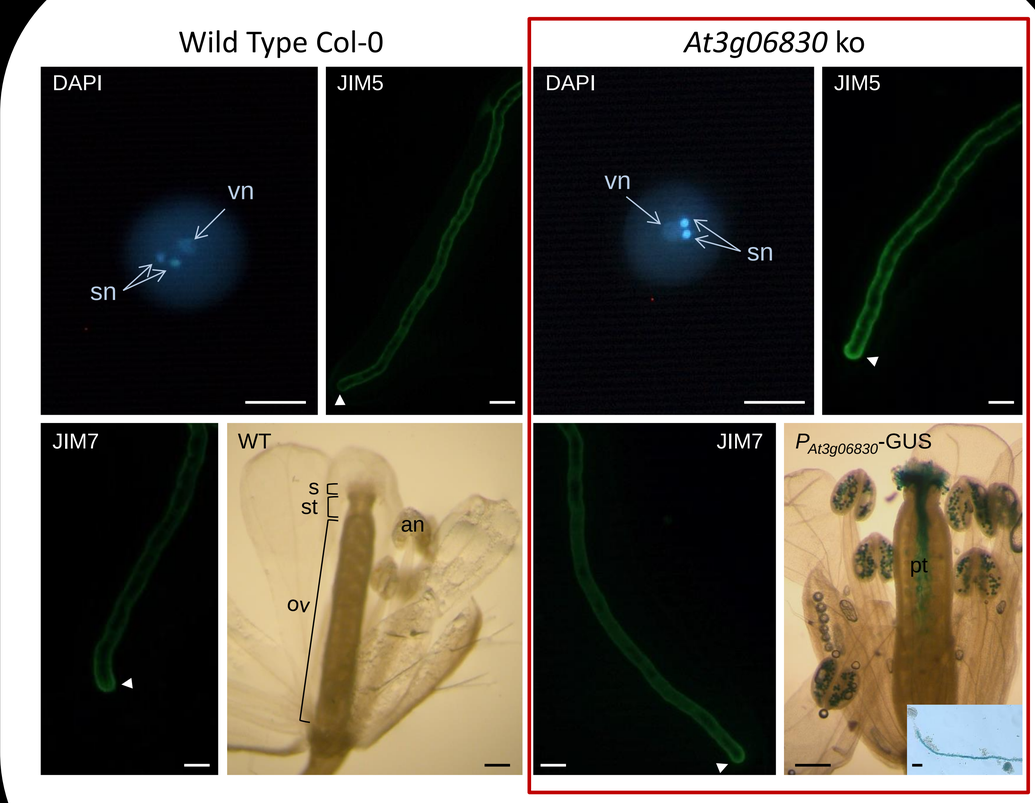

Figure 3. DAPI staining, immunolocalisation of weakly and highly methylesterified HG and i*n vivo* and *in vitro* GUS staining. sn, sperm nucleus, vn, vegetative nucleus; s, stigma; st, style; ov, ovary. Scale bars : 10 µm for DAPI, JIM5 , JIM 7 and bottom right insert ; 150 µm for WT and $P_{At3g06830}$ –GUS flowers.

References :

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, et al. 2008 Genevestigator V3: a reference expression database for the meta-analysis of transcriptomes. Advances in Bioinformatics 420747

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, et al. 2007 An "Electronic Fluorescent Pictograph" Browser for Exploring and Analyzing Large-Scale Biological Data Sets. PLoS ONE 2(8): e78