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Small molecules interfere with the tip-polarized growth of arabidopsis and tomato pollen tubes

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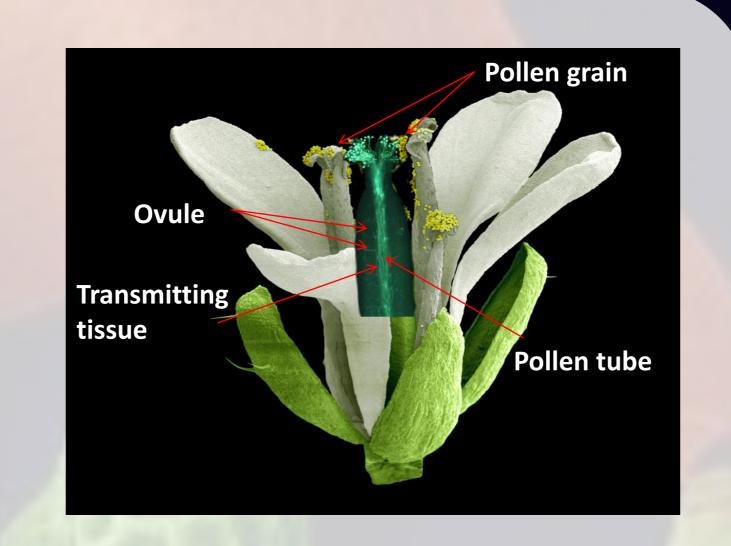
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I. Introduction

Sexual reproduction in land plants is of main economic importance because it allows the production of seeds. The process starts when the pollen grain lands on the stigma, rehydrates and produces a pollen tube, a tippolarized cell that grows through the transmitting tract tissue and delivers the two sperm cells to the ovule.



II. Problematic

To date, the mechanisms that occur during the **tip-polarized growth, guidance** and adhesion of pollen tubes through the female transmitting tract tissue are not well known.

III. Aim of this work

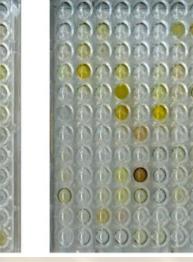
To investigate these different mechanisms in vitro.

using a chemical screen approach

IV. Chemical Screen:







Using a chemical screen of 300 small bioactive compounds originating from the CERMN (Centre d'étude et de Recherche du Médicament de Normandie) in 96-well plates

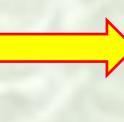


8 compounds were selected

- ✓ 3 for their abilities to perturb *Arabidopsis thaliana* pollen tube growth and shape.
- ✓ 5 for their inhibiting effects on the adhesion of pollen tubes *in vitro* on a matrix composed of an enriched-pectin fraction isolated from *Arabidopsis thaliana* pistils.

V. Materials and Methods

Flowers of *A. thaliana* and tomato (Solanum lycopersicum)



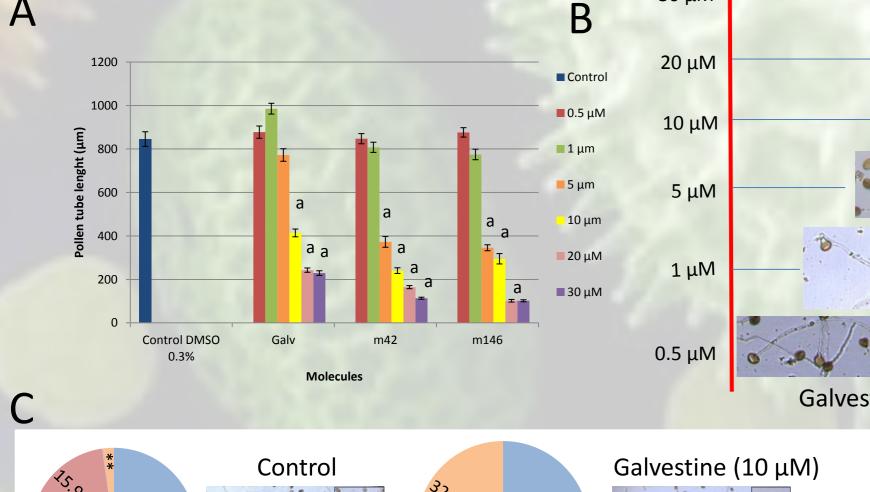
Extraction of pollen grains and culture for 6 h in a germination medium containing the selected compounds

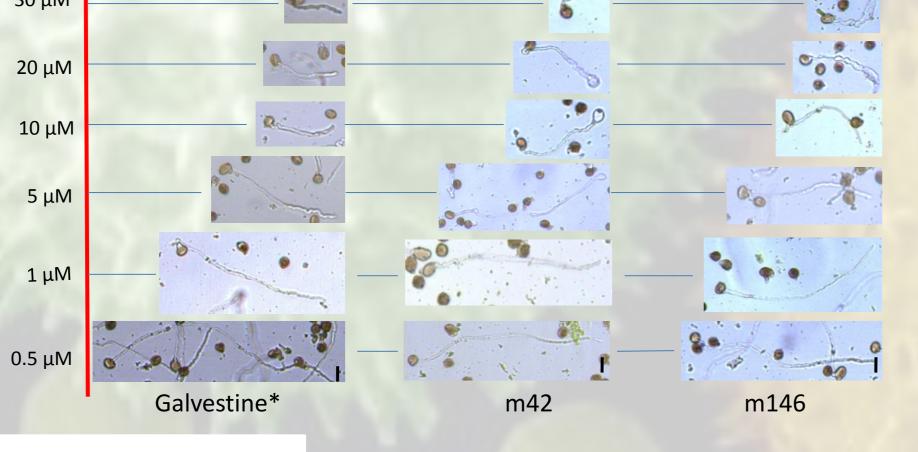


1/ Evaluation of the dose-dependent effect

2/ Select the concentration that induces a phenotype

VI. Effect on arabidopsis pollen tubes





Control Galvestine (10 μ M)

Mon-germinated GP

Normal PT

Early brust PT

Lately burst PT

PT with a phenotype

Figure 1: Dose effect of the compound on (A) the length and (B-C) the phenotype of arabidopsis pollen tubes.

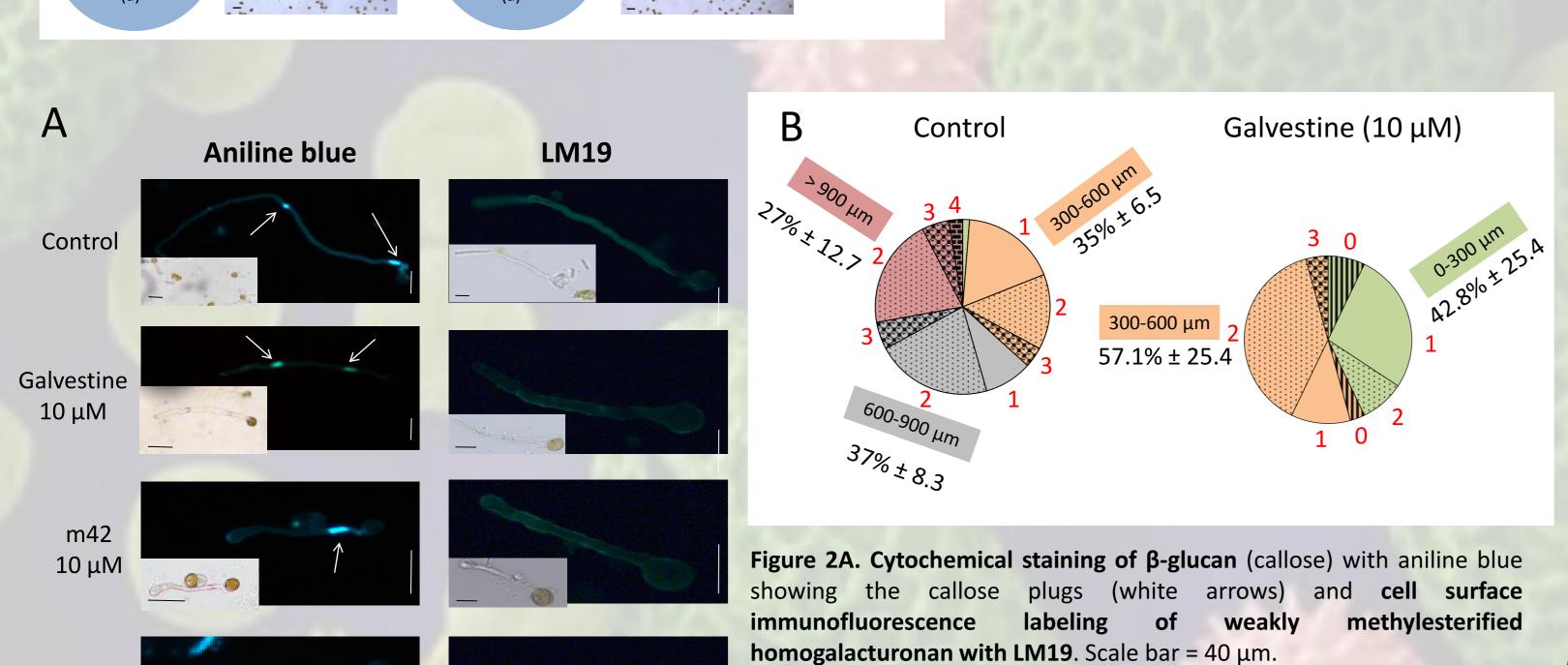
In B, scale bar = 20 μm and in C. scale bar = 40 μm. (expressed in %). PT : Pollen tube. PG: Pollen grain.

* Botté et al. (2011) Chemical inhibitors of monogalactosyldiacylglycerol synthases in Arabidopsis.

monogalactosyldiacylglycerol synthases in *Arabidopsis* thaliana. Nature Chemical Biology. 7: 834-842.

** Rate < 5%.

(a) Significant difference with the control.



VII. Effect on tomato pollen tubes

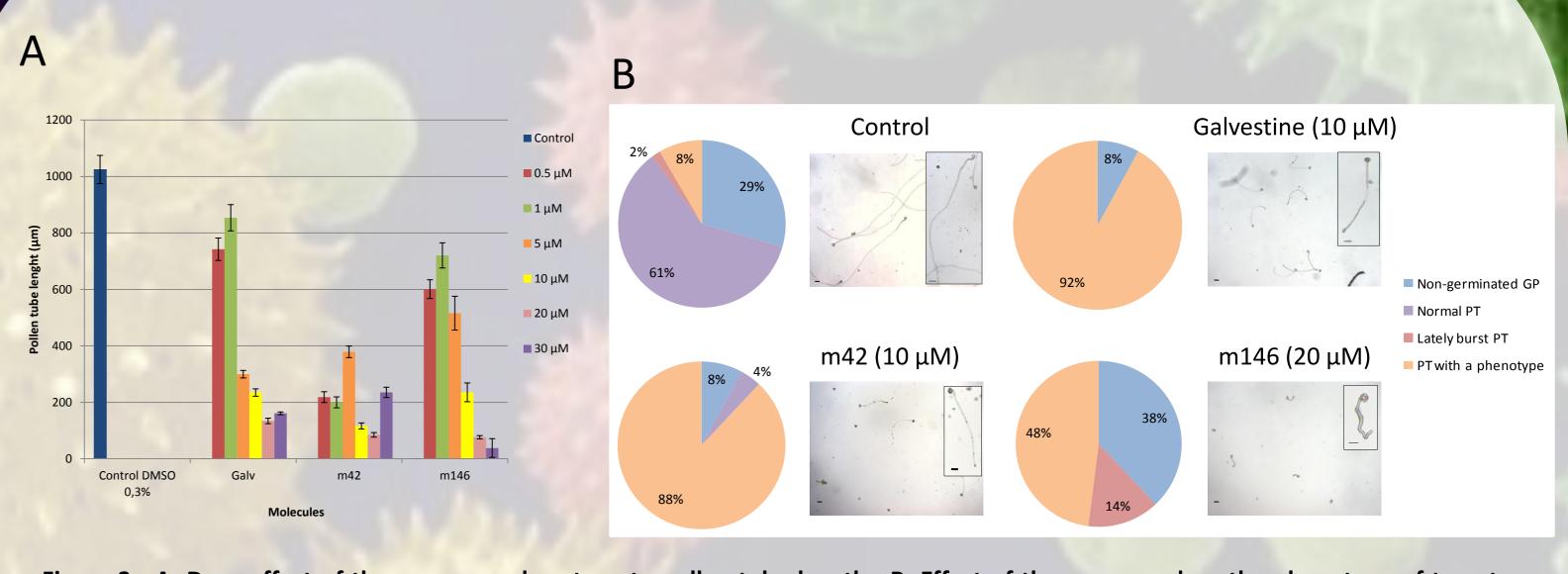


Figure 3: A. Dose effect of the coumpound on tomato pollen tube length. B. Effect of the compound on the phenotype of tomato pollen tubes (expressed in %). PT : Pollen tube. Scale bar = 40 μm.

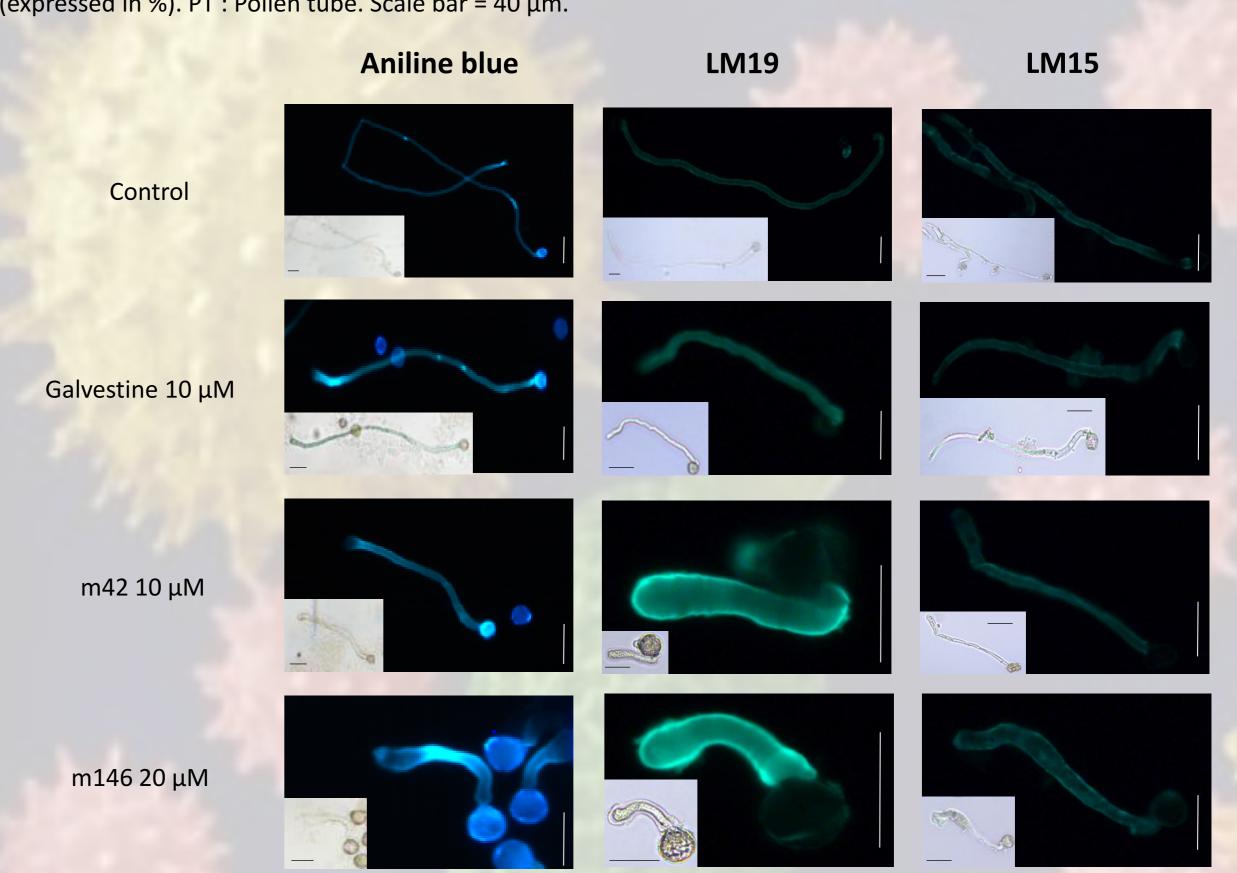


Figure 4: Cytochemical staining of β-glucan (callose) with aniline blue showing the callose plugs and cell surface immunofluorescence labeling of weakly methylesterified HG with LM19 and xyloglucan with LM15 (motif XXXG). Scale bar = 40 μ m.



 76.1 ± 6.3

VII. Conclusions and perspectives:

> The selected compounds have a dose-dependent effect on arabidopsis and tomato pollen tube growth.

Figure 2B. Effect of galvestine treatment on pollen tube length and the

number of callose plugs deposited (number in red).

➤In arabidopsis, the callose plug distribution is affected by galvestine treatment (a compound able to perturb galactolipid synthesis) and the pollen tube length is reduced. Similarly, the localisation of weakly methylesterified HG and xyloglucan was not affected in arabidopsis and tomato.

►In contrast, m42 and m146 have an effect on the tip-growth of arabidopsis and tomato pollen tubes with a noticeable accumulation of callose at the tip. M42 and m146 appeared to have an effect on the distribution of weakly methylesterified HG in tomato.

Effect of the compounds on the cytoskeleton organization of the pollen tube as well as the localization and characterization of the target of the compounds are in progress.



