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Cell Adhesion and Growth of *Arabidopsis thaliana* Pollen Tubes

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Introduction

Against a backdrop of climate changes and population growth, new issues are emerging such as how to feed the world's population. It is therefore vital to understand the mechanisms of sexual reproduction in plants. These mechanisms can then be worked on to improve the production/quality of seeds, which form the basis of human nutrition [1].

During sexual reproduction, two cell adhesion mechanisms are involved. When the pollen grain lands on the flower's pistil, it adheres to the stigma and becomes imbibed so that it can germinate (Figure 1). From this pollen grain emerges a pollen tube. As the pollen tube travels, it adheres to the female transmitting tissue of the style, which constitutes the second adhesion mechanism (Figure 1).

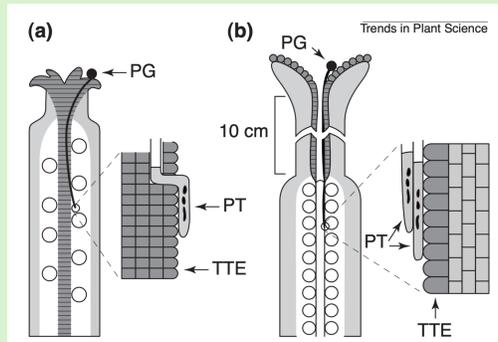


Figure 1: Path of the pollen tube in (a) solid and (b) hollow styles [2]. PT: Pollen Tube; PG: Pollen Grain; TTE: Transmitting Tract Epidermis

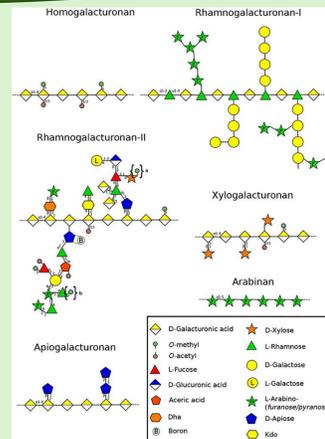


Figure 2: Structure of the different pectin domains in the plant cell wall [3].

Several molecules playing a role in this adhesion event have been identified such as the SCA (stigma/stylar cysteine-rich adhesin) protein [4]. In addition, numerous AGPs (Arabinogalactan Proteins) have been characterized in the transmission tissue, which may indicate a possible role in the adhesion of the pollen tube to this tissue [5].

Finally, pectins (Figure 2) have also been shown to play a major role in cell-cell attachment, particularly homogalacturonans.

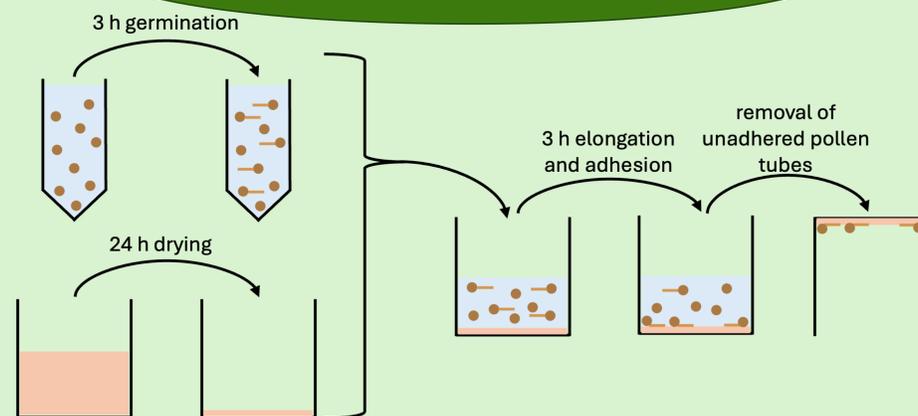
Given the quantity of pectins in the cell wall of the pollen tube and the transmitting tissue, it is very likely that pectins play a role in pollen tube/tissue of transmission adhesion.

For this reason, the aim of this project is to identify the pectin motifs involved in the adhesion of the pollen tube to the style.

Materials and methods

The adhesion matrix was composed of pectins extracted from *Arabidopsis thaliana* col-0 leaves in two different ways: with imidazole-HCl or with ammonium oxalate. Prior to these extractions, the cell wall extracts were detached with successive DMSO and α -amylase treatments.

For the pollen tube adhesion protocol, pectins in water were deposited at the bottom of a well in a 96-well plate.



The pollen grains were germinated during 3 h in a 1.5 mL Eppendorf and then transferred to the wells with the matrix.

After 3 h in the well, photos of the pollen tubes were taken under an inverted optical microscope, then the plate was inverted and rinsed with the germination medium.

The adhered pollen tubes were again observed under an inverted optical microscope.

Results

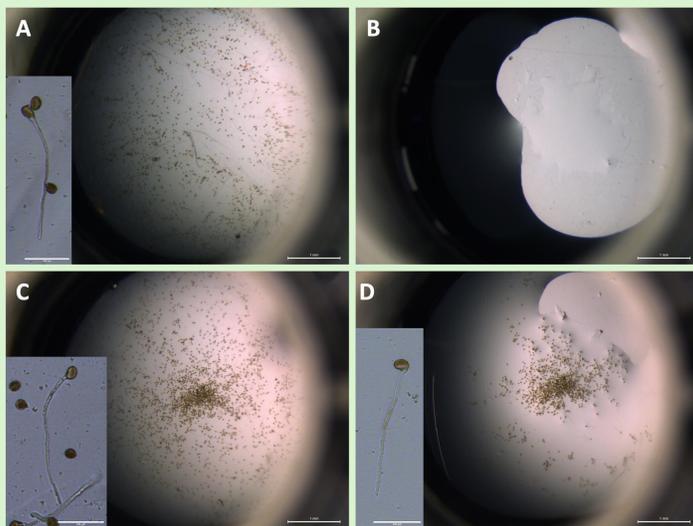


Figure 3: Adhesion tests of *Arabidopsis thaliana* pollen tubes. A: Pollen tubes after 6 h of germination on a hemicellulose-enriched matrix. B: The well in A after rinsing with the germination medium. C: Pollen tubes after 6 h of germination on a pectin-enriched matrix extracted from *A. thaliana* leaves using ammonium oxalate. D: The well in C after rinsing with the germination medium. Scale bar: 1 mm for wide shots and 100 μ m for zoom shots

Figure 3A shows that the pollen grains have germinated and the pollen tubes have elongated on the hemicellulose-enriched matrix. However, Figure 3B shows that there are no more pollen tubes after rinsing.

The hemicellulose-enriched matrix did not allow pollen tube adhesion.

On the other hand, in Figure 3C, as with the hemicellulose matrix, germinated pollen grains and pollen tubes can be seen, and these pollen grains with their pollen tubes can be seen again in Figure 3D.

The pectins have enabled the pollen tubes to adhere.

On the other hand, in Figures 4A and 4B, there are no obvious abnormal phenotypes of pollen tubes.

The pectin-enriched matrices were also analysed by gas chromatography (GC) to determine their monosaccharide compositions (Table 1), and it was found that they are largely composed of homogalacturonans (55 and 48%, respectively).

Homogalacturonans can be expected to play a role in adhesion.

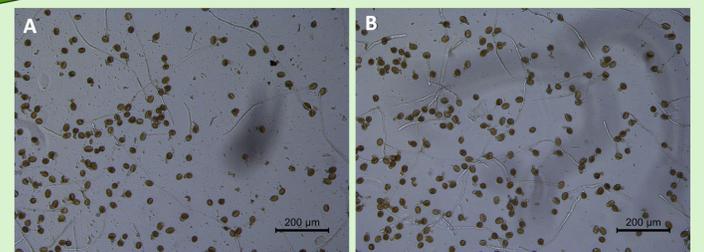


Figure 4: Pollen tubes after 6 h of germination A: Pollen tubes on a hemicellulose-enriched matrix; B: Pollen tubes on a pectin-enriched matrix. Scale bar : 200 μ m

Table 1: Monosaccharide composition (mol%) of pectins extracted with ammonium oxalate or imidazole-HCl

Name of monosaccharides	Pectins extracted with ammonium oxalate	Pectins extracted with imidazole-HCl
Arabinose	9	12
Rhamnose	7	10
Fucose	1	1
Xylose	4	5
Mannose	2	Not Detected
Galactose	13	12
Galacturonic Acid	62	58
Glucose	2	Not Detected
Glucuronic Acid	Not Detected	1
HG % = GalA-Rha	55	48
RG-I % = 2Rha+Gal+Ara	36	44

Conclusions and Perspectives

Conclusions

- Cellulose and hemicellulose extracted from *Arabidopsis thaliana* Col-0 leaves do not promote pollen tube adhesion,
- Pectins extracted from *Arabidopsis thaliana* Col-0 leaves enable pollen tubes to adhere to the matrix.
- Pectins are mainly composed of homogalacturonans. We can therefore hypothesize that homogalacturonans play a role in cell adhesion, but it is also possible that other components of pectins such as rhamnogalacturonan-I (36 and 44% in ammonium oxalate and imidazole-HCl, respectively) (Table 1) play this role.

Perspectives

- Enzyme digestion of the different pectin domains to determine the fractions that allow pollen tube adhesion
- Prepare adhesion matrices from pectins extracted from cell wall biosynthesis deficient mutants and test them for pollen tube adhesion
- Study the intracellular mechanisms of pollen tubes when they adhere to determine those involved in cell adhesion

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