Arabidopsis pollen tube growth and adhesion: Dissecting the pectin structure using an enzymatic approach

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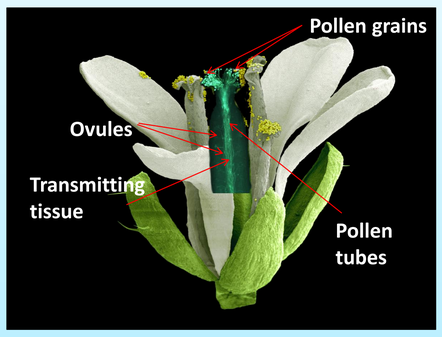
I. Introduction

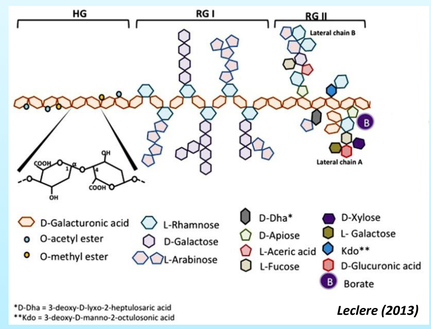
Guidance and adhesion are essential mechanisms for sexual reproduction in flowering plants. The process starts when the pollen grain lands on the stigma, rehydrates and forms a pollen tube that grows through the transmission tact tissue and delivers the two gamete cells to the ovule.
The female transmitting tract tissue plays a key role for the pollen tube as it serves as an adhesion matrix ensuring the physical support needed for its progress to the ovules.



To date, the involvement of the different components of the cell wall and their relative importance during adhesion is not known. In order to study the role of cell wall components in adhesion, we prepared different matrices, enriched in different classes of cell wall polymers and set up a robust and efficient *in vitro* adhesion test.

As the pectin-enriched matrix was the most efficient in promoting adhesion, we further investigated, using an enzymatic approach, what could be the minimal structure required for *in vitro* pollen tube adhesion.

Pectins are complex polymers composed of three major domains: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II). The main sugars are galacturonic acid (GalA)(HG and RG-I) that can be methyl and acetyl esterified and rhamnose (Rha), galactose (Gal) and arabinose (Ara) found also in RG-I.

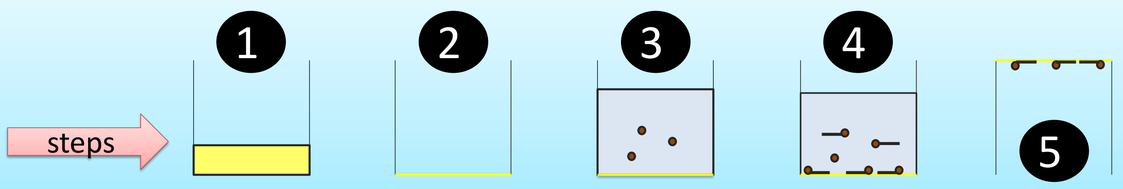


II. Aim of this work

To determine the minimal pectin structure required for *Arabidopsis thaliana* pollen tube adhesion *in vitro*.

III. Adhesion assay

Use of an *in vitro* adhesion assay in 96-well plates



1. Deposition of the matrix (70 µg).
2. Water evaporation.
3. Deposition of pollen grains in liquid germination medium.
4. Incubation at 22°C in the dark for 6h. Count the number of pollen tubes in contact with the matrix.
5. Washing step : Flip the plate, rinse 2 times with water, and count the number of remaining adhered pollen tubes.

IV. Results

1. The cell wall polymers that allow pollen tube adhesion are pectins

Table 1: Adhesion rate according to the origin of pectins and the extraction method.

Organs	Cell wall fraction	Extraction	Adhesion (%)
Flowers	Pectin-enriched	Imidazole	39.4
Leaves	**Pectin-enriched**	**Imidazole**	**31.1**
Leaves	Pectin-enriched	Ammonium oxalate	13.0
Leaves	Hemicellulose-enriched	Potassium hydroxide	2.3

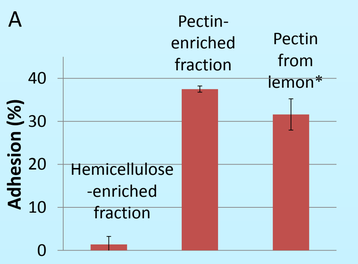
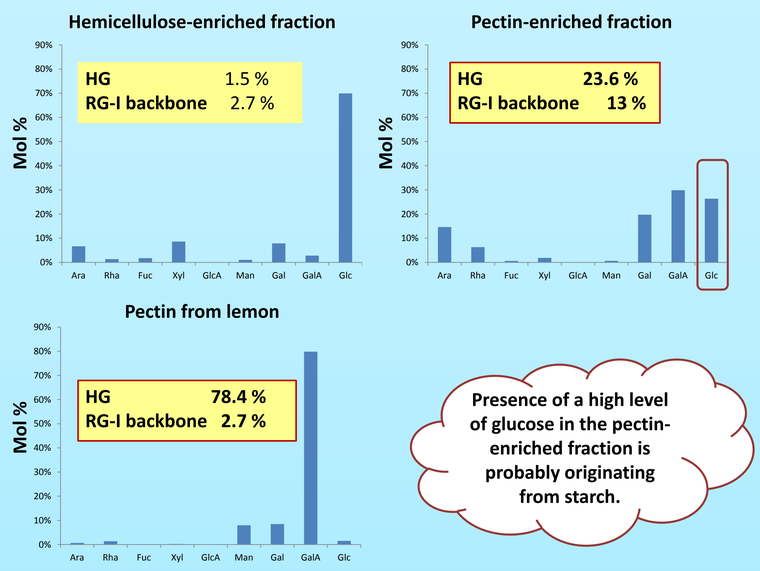


Figure 1: (A) Effect of pectin and hemicellulose-enriched fractions on pollen tube adhesion. (B) Monosaccharide composition by gas chromatography of the fractions. HG and RG-I backbone levels are indicated.
* Pectin from lemon, 85 % degree of methylesterification (DM) (Sigma-Aldrich®).



Presence of a high level of glucose in the pectin-enriched fraction is probably originating from starch.

2. Starch does not play a role in adhesion

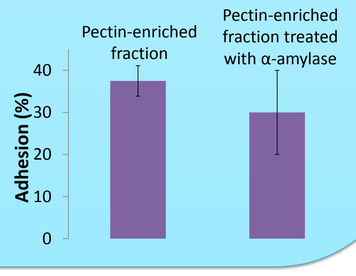
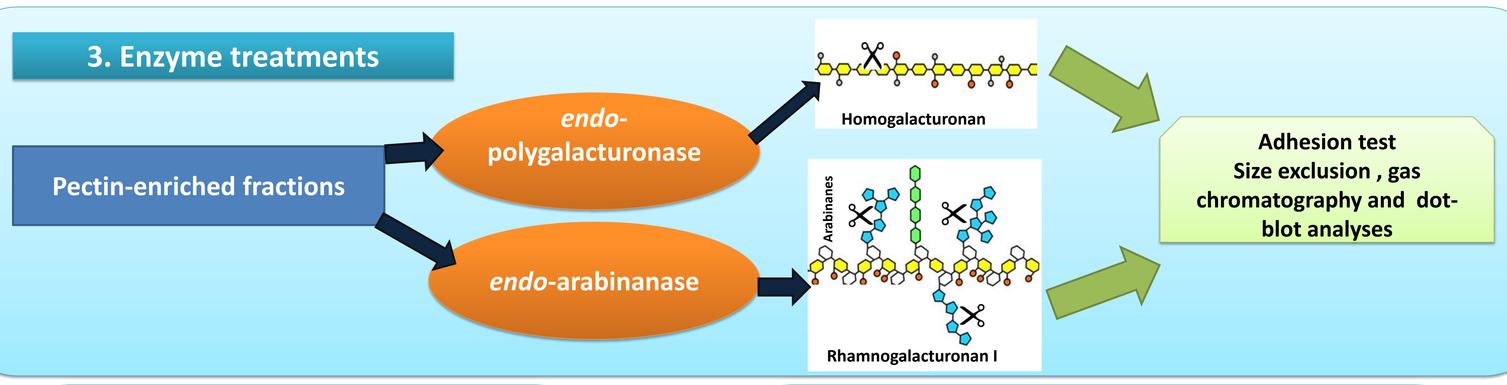


Figure 2: Effect of an α-amylase treatment of the pectin-enriched fraction on pollen tube adhesion.

3. Enzyme treatments



4. Cleavage of the HG backbone disrupts pollen tube adhesion except if the DM is high

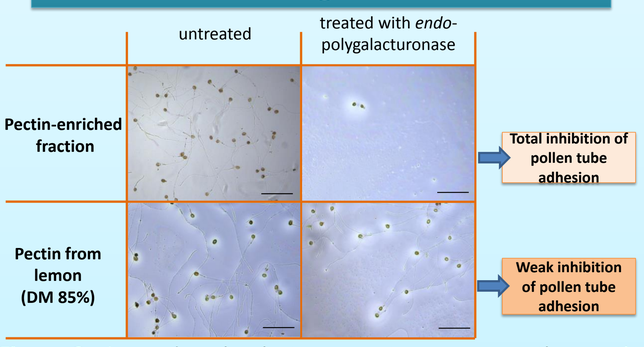


Figure 3: An *endo*-polygalacturonase treatment on the pectin-enriched fraction and on pectin from lemon (DM = 85%). Pictures show pollen tubes remaining on the matrix after the washing step. scale bar = 200 µm.

On HG with high DM, the *endo*-polygalacturonase is partially inefficient. The amount of degradation products and the loss of adhesion are low.

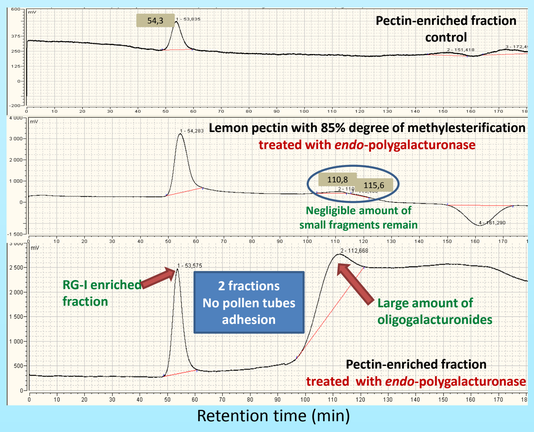


Figure 4: Size Exclusion Chromatography (SEC) performed on Sephadex-75G (fractionation range 3 x 10^3 – 8 x 10^4 Da) of untreated and treated pectin enriched-fraction and pectin from lemon with *endo*-polygalacturonase.

On HG with low DM (15,24%), the *endo*-polygalacturonase is efficient. The amount of degradation products and the loss of adhesion are important.

5. Role of rhamnogalacturonan-I backbone and arabinan side chains

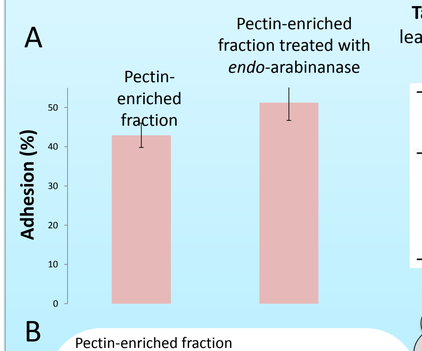


Table 2: Composition of pectin-enriched fractions from leaves and arabidopsis seed mucilage (extracted with cold water).

	Leaves	Mucilage
HG	23.5 %	not detected
RG-I backbone	13 %	97.7 %
RG-I side chains	34.4 %	2.3 %

Arabinan side chains and RG-I backbone appeared not to be implicated in pollen tube adhesion

No pollen tube adhesion

Figure 5: (A), Effect of an *endo*-arabinanase treatment of the pectin-enriched fraction on the adhesion. (B), Dot-blot with the LM6 monoclonal antibody which recognizes epitopes associated with arabinan side chains of RG-I.

6. Correlation between adhesion and HG levels in different pectin-enriched fractions

Positive correlation

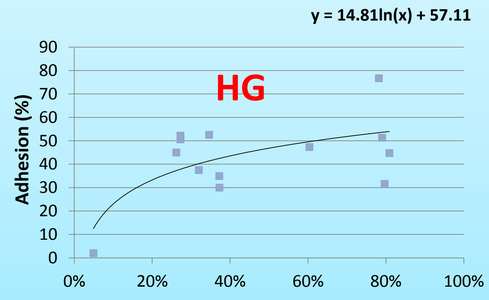


Figure 6: Effect of HG levels in pectin-enriched extracts on the adhesion rates.

VI. Conclusions and perspectives:

- ✓ Pectin-enriched fractions allow *Arabidopsis thaliana* pollen tube adhesion on an *in vitro* system.
- ✓ RG-I backbone and arabinan side chains do not promote pollen tube adhesion. Are galactan side chains of RG-I involved ?
- ✓ Long chains of HG are needed for adhesion. What is the minimum degree of polymerization of the HG to ensure adhesion?
- ✓ Pectin from citrus with high DM (85%) is able to promote adhesion. Is the degree of methylesterification of HG important?

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