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Addendum

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Abbreviations : AGP, arabinogalactan-protein; HG, Homogalacturonan; MAbs, monoclonal antibodies; PME, pectin methylesterase; RG-I, rhamnogalacturonan-I; TT, transmitting tract;

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Abstract

Plant sexual reproduction involves the growth of tip-polarized pollen tubes through the female tissues in order to deliver the sperm nuclei to the egg cells. Despite the importance of this crucial step, little is known about the molecular mechanisms involved in this spatial and temporal control of the tube growth. In order to study this process and to characterize the structural composition of the extracellular matrix of the male gametophyte, immunocytochemical and biochemical analyses of Arabidopsis pollen tube wall have been carried out. Results showed a well defined localization of cell wall epitopes with highly esterified homogalacturonan and arabinogalactan-protein mainly in the tip region, weakly methylesterified homogalacturonan back from the tip and xyloglucan and (1→5)-α-L-arabinan all along the tube. Here, we present complementary data regarding 1) the ultrastructure of the pollen tube cell wall and 2) the immunolocalization of homogalacturonan and arabinan epitopes in 16h-old pollen tubes and in the stigma and the transmitting tract of the female organ. Discussion regarding the pattern of the distribution of the cell wall epitopes and the possible mechanisms of cell adhesion between the pollen tubes and the female tissues is provided.

Introduction

Fertilization of flowering plants requires the delivery of the two sperm cells, carried by the fast growing tip-polarized pollen tube, to the egg cell. At every stage of the pollen tube development within the stigma, style and ovary, pollen tubes are guided to the ovules *via* multiple signals that need to pass through the cell wall of the pollen tube to reach their targets.[1-6]

The analysis of Arabidopsis pollen tube cell wall has recently been reported.[7] Results showed a well defined localization of cell wall epitopes with highly methylesterified homogalacturonan (HG) and arabinogalactan-protein (AGP) mainly in the tip region, weakly methylesterified HG back from the tip and xyloglucan and arabinan all along the tube. In addition, according to the one letter nomenclature of xyloglucan[8], the main motif of Arabidopsis pollen tube xyloglucan was XXFG harboring one *O*-acetyl group. In order to bring new information regarding the possible interaction between the pollen tubes and the female tissues, the ultrastructural organization of the pollen tube cell wall, the cytological staining and immunolocalization of the cell wall epitopes of the pistil and especially the transmitting tract (TT), a specialized tissue where pollen tubes grow, were carried out.

Scanning electron micrographs of pollen grain and pollen tube of Arabidopsis

Chemically fixed pollen tubes were observed with a SEM Zeiss EVO40 EP at 10 kV. The first two photographs show the change of shape of the pollen grain before and after imbibition in the liquid culture medium from an oblong to a spherical form (Fig. 1A, B). The pollen tube tip bulged from one of the three apertures (Fig. 1B) and a rapid polarized pollen tube cell elongation was observed (Fig. 1C). One hour after the imbibition, pollen tube length reached about 30 µm (Fig. 1C). Pollen tube growth is accompanied by the periodic deposition of callose plugs which maintain the tube cell in the apical region of the tube (Fig. 1D). The surface of the pollen tube did not appear smooth but instead showed cell wall material apparently oriented slightly parallel to the direction of the tube elongation (Fig. 1E). This contrasts with the view of the apical region of the pollen tube in which the cell wall showed a different organization (Fig. 1F).

Cell wall polysaccharides in pollen tube and pistil

Cytochemical staining and immunolocalization of cell wall epitopes in the pistil were carried out on 2 µm sections of flowers fixed with 4% formaldehyde and 1% glutaraldehyde, dehydrated in ethanol series and embedded in methacrylate resin. Samples were observed under Nomarski

differential interference contrast optics or fluorescence illumination on a Leica DLMB microscope equipped with FITC (absorption, 485–520 nm; emission, 520–560 nm wavelength). Pictures were acquired with a Leica DFC300FX camera. Pollen tubes were grown in liquid medium for 16-h and labeled with monoclonal antibodies (MAbs) as previously described.[7]

Weakly and highly methylesterified HGs were clearly detected along the whole tube with a weak (Fig. 2A) and a strong (Fig. 2B) labeling intensity at the tip, respectively. Cytochemical staining of carboxylated polysaccharides such as HG with toluidine blue (0.05% phosphate buffer pH 6.8) was performed on longitudinal sections of the pistil. Sections stained with toluidine blue showed a purple coloration of the tissues from the stigma, style, and ovary with a notably deeper staining within the TT and the ovules (Fig. 2C). Immunolocalization of weakly methylesterified HG with JIM5 exhibited a uniform labeling pattern over the different tissues (Fig. 2E). Immunolocalization of (1→5)-α-L-arabinan epitopes with LM6 showed a homogeneous fluorescence in all the tissues of the pistil except in the TT where the labeling appeared slightly denser (Fig. 2F) indicating the presence of branched rhamnogalacturonan-I (RG-I) and/or AGPs.

Discussion

Immuno-fluorescence of 16h-old pollen tubes with JIM5 and JIM7 showed similar labeling pattern seen with 6h-old pollen tubes [7] but with a tendency to fold during sample preparation because of the length of the pollen tubes. This labeling pattern is similar to the one observed with pollen tubes from other species such as potato, tobacco, petunia, jasmine and corn [8,9] with a dominant localization of the highly methylesterified HG at the tip and the weakly methylesterified HG epitopes behind the tip. These results are consistent with the theoretical model of action of the Pectin Methyl Esterases (PMEs) during pollen tube growth.[10,11] Indeed, variations in cell wall properties are likely to be related, at least in part, by the modulation of the level of methylesterification of HG. HGs are deposited in a highly methylesterified form in the tip region and demethylesterified by PMEs during the remodeling of the cell wall. The carboxyl groups of the HG, back from the tip, are able to complex calcium ions which could rigidify the cell wall.[12] In addition, (1→5)-α-L-arabinans, side chains of RG-I, are also abundant in Arabidopsis pollen tube cell wall, both at the tip and behind the tip.[7] Arabinan side chains of RG-I are thought to prevent HG polymers from forming tight association[13] and have been implicated in cell attachment.[14-16] These polymers may have similar action during the pollen tube growth at the cell walls

of both the pollen tube and the TT, also enriched in arabinans. Interestingly, arabinogalactan from the Trasmitting Tract Specific (TTS) AGP has been previously implicated in tobacco pollen tube growth and guidance.[17]

Taken together, these results suggest a strong regulation of the degree of methylesterification of the HG in the pollen tube and the female tissue cell walls probably acting as a regulator of the rigidification of the cell wall which may regulate polarized cell growth.[10,12] Similarly, changes in the degree of methylesterification of the HG in the TT cell wall may allow a local relaxation of the cell wall facilitating the intrusive growth of the pollen tubes in the extracellular matrix of this specialized tissue. Cell adhesion domains can have mechanosensory properties in eukaryotic cells.[18] Adhesion between Arabidopsis pollen tubes and the TT cells has been observed and may be controlled by arabinans from AGPs [19] and/or RG-I which might be an important cue for guiding the tube cells within the female tissue, as suggested with lily.[20] Investigations on mutant pollen defective in arabinosyltransferases and PMEs or plants with abnormal TT should provide new insights into the function of pectic HG and arabinan side chains of RG-I in pollen tube growth and adhesion.

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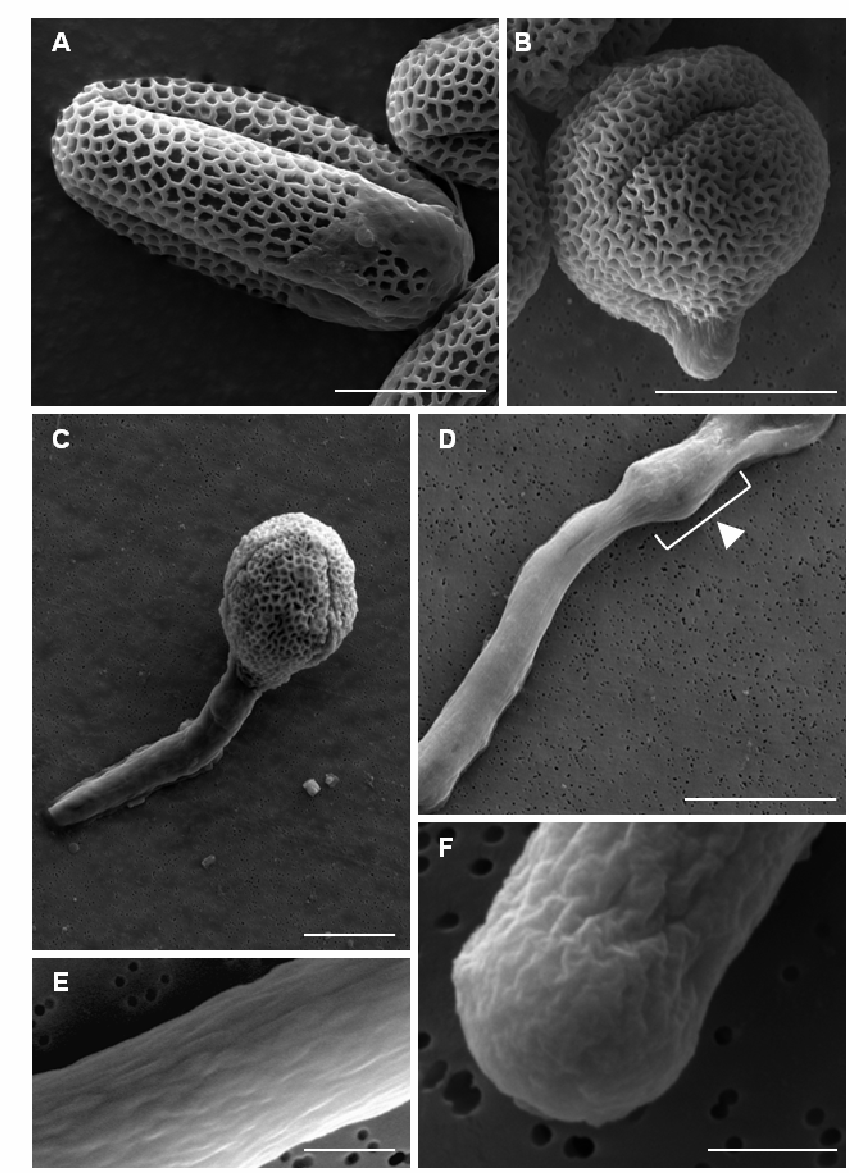

Figure 1. Scanning electron micrographs of Arabidopsis pollen grains and pollen tubes during growth. A, dehydrated oblong pollen grains showing the pollen coat at the surface of the exine. B, pollen tube tip emerging from hydrated pollen grain. C, one hour-old pollen tube. D, view of a callose plug (arrowhead). E, surface of the pollen tube cell wall back from the apical zone. F, view of the pollen tube cell wall in the tip region. Scale bars = 10 µm (A, B, C and D) and 2 µm (E and F).

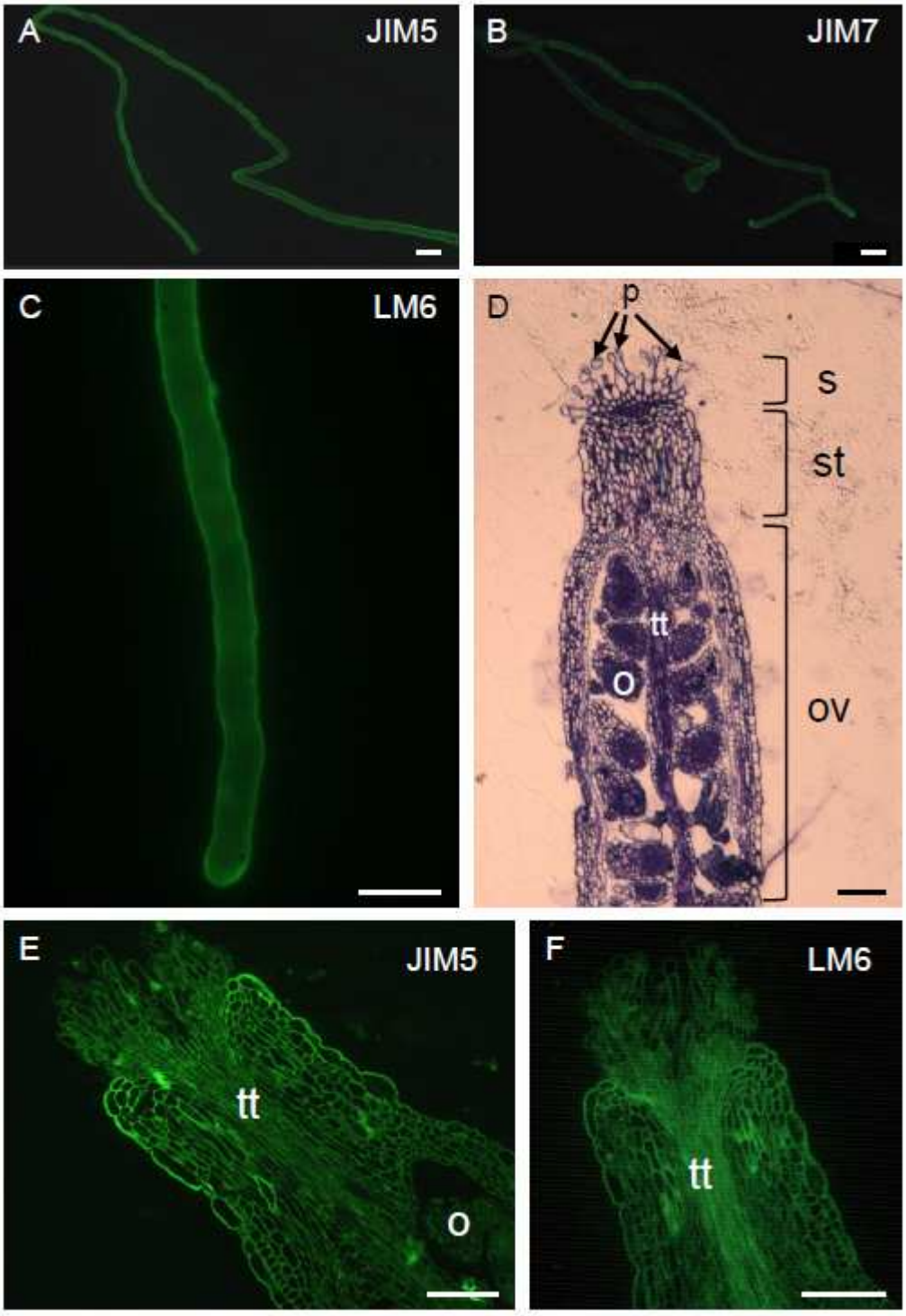

Figure 2. Cytochemical staining and immunolocalization of cell wall epitopes in the pollen tube and pistil. (A-B), Immunolocalization of weakly and highly methylesterified HG with JIM5 and JIM7, respectively. (C) Immunolocalization of (1→5)-α-L-arabinan epitopes with LM6. (D), Cytochemical staining of acid pectins with toluidine blue on longitudinal sections of the pistil. (E, F), Immuno-fluorescence labeling of the cell wall polymer epitopes in longitudinal sections of the pistil with the MAbs JIM5 (E) and LM6 (F). Pollen tubes were grown for 16h in liquid medium. Pistils were dissected from closed flowers (*i.e.* before pollination). Scale bars = 20 µm (A, B), 10 µm (C) and 50 µm (D-F). o, ovule; ov, ovary; p, papillae; s, stigma; st, style; tt, transmitting tract.