Expression profiling and localization of wall polysaccharides in the olive pollen during *in vitro* germination

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Polysaccharides are structural components of the pollen tube wall [1]. These compounds are essential in pollen function since they participate in the germination mechanism [2] and the pollen tube adhesion to the pistil tissues [3]. At the same time, they largely control pollen tube wall permeability, to allow passage of sporophytic proteins that determine compatibility in many species [4].The aim of this study was to analyze the expression pattern and localization of several wall polysaccharidic components, including pectins, arabinogalactan proteins (AGPs) and callose, in the olive pollen during *in vitro* germination. For this purpose, we use biochemical methods in combination with confocal laser scanning microscopy (CLSM). After Western blot analysis, LM5 antibody allowed us to identify up to seven galactose-rich pectins within a range of molecular mass of 58-92 kDa, whereas LM6 antibody bound to a pectin of about 253 kDa that contains $[(1\rightarrow5)$-α-L-arabinose$]_5$ motifs. During *in vitro* germination, levels of galactan-type pectins significantly decreased, whereas the opposite tendency was observed for the arabinose-rich pectin. CLSM experiments with LM5 antibody showed a preferential fluorescence in the outer region of pollen grain apertures, forming a ring-shaped structure around the pollen tube, although a slight fluorescence was also observed at the wall of the apical region of the pollen tube. The LM6 antibody showed an intense fluorescence signal in the cell wall all along the pollen tube, as well as in the apertural regions of the pollen grain. High (15-80%) and low esterified pectins were detected using JIM7 and JIM5 antibodies, respectively. The antibody JIM7 recognized an esterified pectin of 290 kDa that increased its synthesis during *in vitro* germination. This pectin was located in the cytoplasm and the apertural region of the hydrated pollen, and in the pollen tube wall at the apex. The JIM5 antibody bound to a pectin with a molecular mass of about 264 kDa. It was located in the intine and the material adhered to the exine of the pollen grain, and along the outer layer of the pollen tube wall, from the basal region to the subapical zone. On the other hand, the expression pattern and distribution of AGPs was studied using JIM13 and JIM14 antibodies. JIM 13 specifically bound to β-D-glcpA-(1→3)-α-D-galpA-(1→2)-L-Rha residues of polysaccharides attached to the polypeptide of an AGP of 318 kDa and a cluster of AGPs in the range of 70-120 kDa. The antibody JIM14 was able to recognize the L-arabinose residues in the carbohydrate chains covalently attached to the polypeptide of an AGP of 270 kDa, whose expression levels decreased during *in vitro* germination. We also found a differential cell localization depending of the antibody used. The JIM13 antibody produced an intense green fluorescent signal in the outer layer of the pollen tube wall and the plasma membrane along the pollen tube. JIM14 showed fluorescent labelling in the apex region of the pollen tube only. Finally, callose was located using sirofluor staining. The callose was distributed along the pollen tube, between the plasma membrane and the pectic wall. The dynamic of pollen tube wall synthesis in the olive is discussed on the basis of our findings here.

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