Immunolocalization of pectin and arabinogalactan protein epitopes in unpollinated ovules of *Beta vulgaris* L. genotypes

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INTRODUCTION AND AIM OF THE STUDY

The effective production of haploids and doubled haploids by the use of unpollinated ovules embryogenesis depends on many factors, from which the donor plant genotype seems to be the most important. But the mechanisms which determine the gynogenesis induction in particular sugar beet (*Beta vulgaris* L.) genotypes are still unknown. The presence and changes in plant cell wall composition have previously been described in relation to morphogenetic potential. Especially pectins and arabinogalactan proteins (AGPs) are the major cell wall components implicated to the development and differentiation of plant cells and tissues. Above mentioned compounds are widely distributed throughout the plant kingdom and occur either in intercellular spaces, plasma membranes and certain cytoplasmic vesicles.

The aim of this study was to compare the occurrence and localization of pectin and arabinogalactan protein epitopes in unfertilized ovules isolated from *Beta vulgaris* L. genotypes of different embryogenetic potential.

MATERIALS AND METHODS

Vernalized roots of *Beta vulgaris* L. breeding lines were obtained from the Sugar Beet Breeding Company (KHBC), Breeding Station in Straszków, Poland. The plants were grown in a field during the normal season (Fig. 1A-D). Four genotypes with different embryogenetic potential: low (Fig. 2A-H) and high (Fig. 2I-P) were selected for immunocytochemical analyses. The unfertilized ovules were received from unopened floral buds of maternal genotypes at the culture initiation stage.

The unfertilized ovaries where fixed in the mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M Pipes, pH 7.2. After extensive washing with the buffer, the material was dehydrated in an ethanol series, and embedded in LR Gold resin as described previously by Wiśniewska and Majewska (2007). The 0.5 -µm sections were cut with a RM 2155 microtome, collected on slides, and dried on a warm plate. For the *in situ* immunocytochemistry the sections were labelled with monoclonal antibodies: LM2 (binging ß-GlcA- the component of AGP) and JIM7 (that binds to highly methyl-esterified linkage glucans. Binding sites of particular antibodies were visualized by FITC-conjugated antibodies. For morphological observations the sections were stained with the mixture of 0,5% toluidine blue, 0,5% methylene blue, 0,5% Azur B in and propidium iodide (Fig. 3A-H).

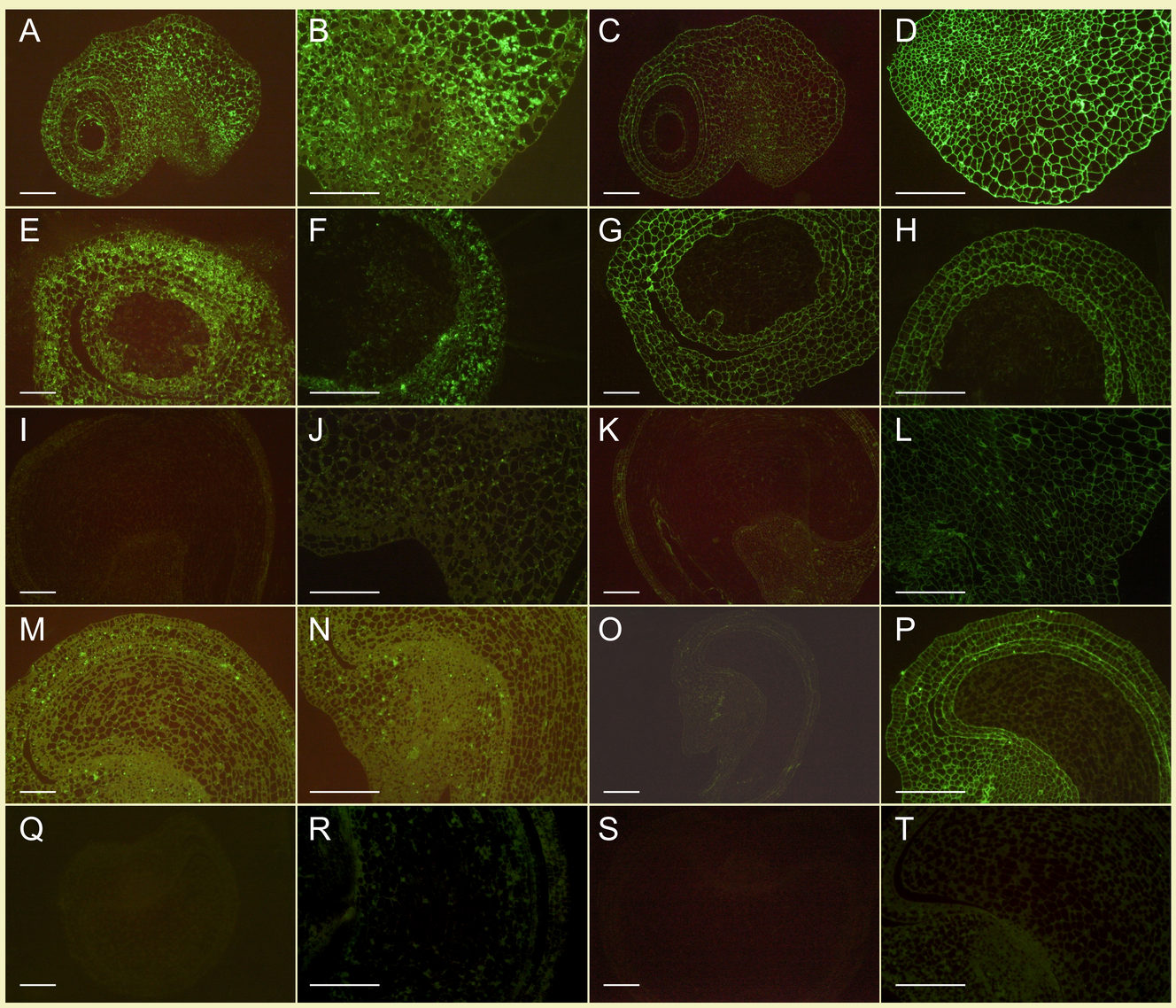
RESULTS

The presence and relative aboudance of carbohydrate epitopes typifying AGPs and pectins have been determined for unpollinated ovaries of *B. vulgaris* (Fig. 2A-T). Comparison of breeding lines with the two embryogenetic potential abilities revealed that both similarities and differences existed between them in terms of the occurrence and localization of pectin and arabinogalactan protein epitopes. Observed differences between ovaries involved epitopes that react with LM2 antibodies, which could be detected in high levels in ovary cells with low embryogenetic potential (Fig. 2A, B, E, F) and scarcely represented in ovary cells with high embryogenetic potential (Fig. 2I, J, M, N). The similarities involved the widespread of epitopes recognized by pectic motifs recognized by JIM7 in unfertilized ovary cells (Fig. 2C, D, G, H, K, L, O, P). Control reactions performed by omitting the primary antibodies resulted in a lack fluorescent signal (Fig. 2Q-T). The differences in starch grains localization among analysed plant material could be observed. Higher amounts of starch grains were detected in ovary cells with low embryogenetic potential (Fig. 3A-D) and lower amounts of starch grains were presented in ovary cells with high embryogenetic potential (Fig. 3E-H).

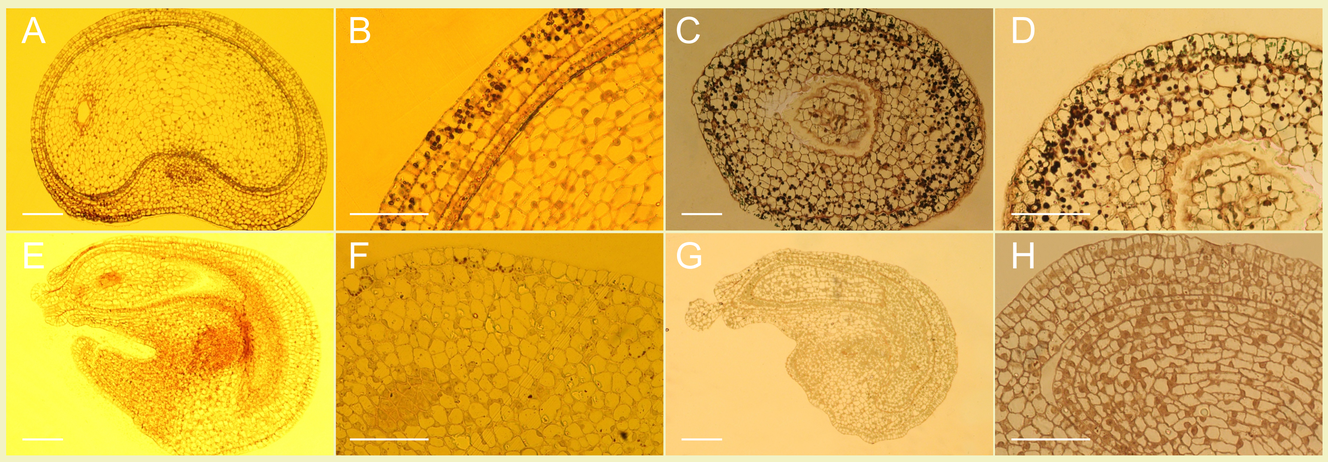
CONCLUSIONS

The study results indicated that the occurrence and localization of arabinogalactan protein epitopes differed in unfertilized ovules displaying different embryogenetic potential. Arabinogalactan proteins and pectins certainly have important functions in differentiation capacity during cell development. Whether the high and low presence of epitopes recognized by LM2 reflect only different starch localization or other cell properties remains to be determine.

Figs. 1A-B. Donor plants of *Beta vulgaris* L. during field cultivation (**A**) and before ovaries isolation (**B**).
Figs. 1C-D. Unpollinated ovaries of *Beta vulgaris* L: *in vivo* (**C**) and *in vitro* (**D**) conditions.



Figs. 2A-T. Immunolabeling of *Beta vulgaris* L. unpollinated ovules isolated from selected genotypes with low (**A-H**) and high (**I-P**) embryogenetic potential with LM2 (**A**,**B**,**E**,**F**,**I**,**J**,**M**,**N**) and JIM7 (**C**,**D**,**G**,**H**,**K**,**L**,**O**,**P**) antibodies. Cells of genotypes with high embryogenetic potential showed more intense green fluorescence then cells of genotypes with low embryogenetic potentail in reactions with LM2 antibody. Strong labeling of cells within genotypes of both embryogenetic potential was observed for reactions with JIM7 antibody. Control reactions (**Q-T**) were performed by omitting primary antibody. Bars = 100



Figs. 3A-H. Morphology of *Beta vulgaris* L. unpollinated ovules isolated from selected genotypes with low (**A**,**B**,**C**,**D**) and high (**E**,**F**,**G**,**H**) embyogenetic potential. Bars = 100 µm.