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Immunoprofiling of Cell Wall Carbohydrate Modifications During Aerenchyma Formation in Fabaceae Roots

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Understanding plant adaptation mechanisms to prolonged water immersion provides options for genetic modification of existing crops to create cultivars tolerant towards periodic flooding. An important advancement in understanding flooding adaptation would be to elucidate mechanisms such as aerenchyma air-space formation induced by hypoxic conditions consistent with prolonged water immersion. Aerenchyma formation occurs through programmed cell death (PCD) which may entail the chemical modification of pectic polysaccharides in root tissue cell walls via de-methyl esterification that enables cell wall degradation via enzymatic activity [1]. In this study, three Fabaceae species known to produce cavities in hypoxic conditions - *Pisum sativum* (green pea)*, Cicer arietinum* (chickpea)*, and Phaseolus coccineus* (scarlet runner bean) – were utilized to investigate the chemical modification of pectin in root vascular cells during aerenchyma formation.

Experimental design consisted of germination of 10 groups of 10-20 seeds, per species, in 2-liter beakers containing sterile vermiculite and deionized water for 5 days at 25ºC in constant darkness. Five seedling groups were flooded with deionized water on day 5 to stimulate aerenchyma formation, with another five groups in unflooded beakers serving as controls. Tissues 1.5 cm to 3 cm basally from the root tip were excised from experimental and control groups at five timepoints post-flooding (0, 12, 24, 36, and 48 hours), embedded in 3.5% agarose and sectioned at 100µm prior to immuno-labeling. Samples from control and experimental groups were stained with either LM19, JIM7 or JIM5 antibodies targeting pectin residues with differing degrees of de-methyl esterification. Secondary antibody labeling with Alexa Fluor® 647 fluorophore conjugates was viewed with an Olympus FV500 confocal light microscope to identify pectin localization patterns near root aerenchyma. Select control and flooded experimental samples from the 48-hour timepoint were pretreated for 2 hours at 50ºC with enzyme solution (4% Cellulase, 1% xylanase, 3% pectinase, 4% Viscoenzyme *L*), high pH buffer (0.1 M sodium carbonate, pH 11.4), or control buffer (0.05 M citrate buffer, pH 5.0) prior to LM19 primary antibody incubation, secondary antibody labeling with Alexa Fluor® 647 fluorophore and imaging on Olympus FV500 confocal light microscope.

The results of this study suggest that de-methyl esterification (DME) of pectin is a component of root stele aerenchyma formation in at least three legume species (Fig. 1A, D, G). Images of antibody binding suggest that areas of partially (JIM5) or fully methyl-esterified (JIM7) pectin are also present in the center of root steles but diminish in abundance or epitope availability during aerenchyma formation (Fig. 1B-C, E-F, H-I). This result supports the hypothesis that methyl-esterified pectin residues are less susceptible to the enzyme degradation needed for aerenchyma formation and must be DME prior to proper cavity formation. Additionally, the enhanced intensity of LM19 (DME pectin antibody) binding from enzyme pretreatment suggests that removal of cellulose and xylan (i.e. hemicellulose) may be required to promote DME – possibly through “unmasking” of the pectin residues in the cell walls (Fig. 2A, B, G, H, M, N). Future studies involving xylan removal may be particularly important due to similarity to the control treatment (sodium carbonate buffer) representing complete DME of pectin throughout the root cross-section (Fig. 2E, K, Q) and known association with pectin in other plants [2].

References:

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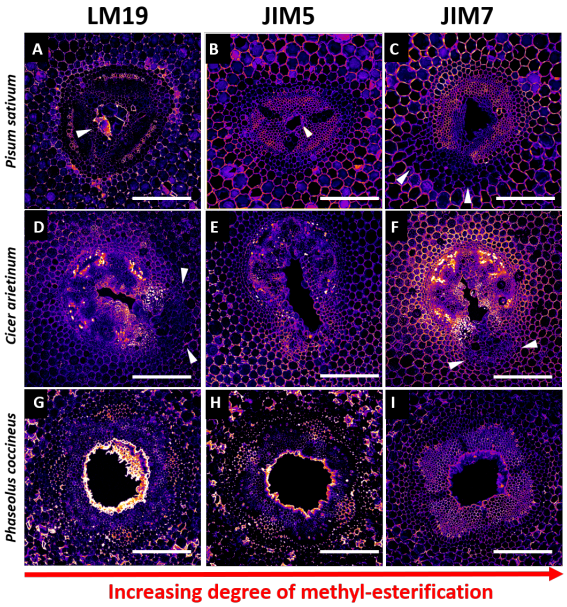

Figure 1. Pectin antibody immunolabeling patterns for three Fabaceae species. Scale bars = 50 µm.

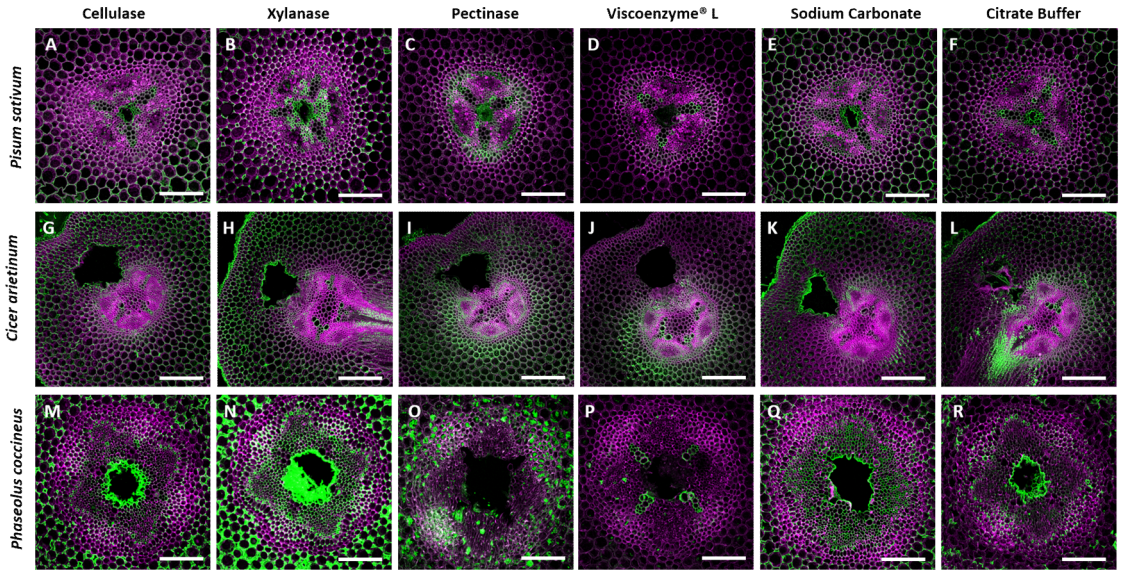

Figure 2. Effect of enzyme pretreatments on LM19 binding of 48-hour flooded roots. LM19 localization indicated in green, and aldehyde-induced autofluorescence indicated in magenta. Scale bars = 50 µm.

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