doi:10.1017/S1431927620014348
Microsc. Microanal. 26 (Suppl 2), 2020

Investigation of Hemicellulose Alteration in Fabaceae Root Cell Walls During Flooding-Induced Aerenchyma Formation

Timothy Pegg, Daniel Gladish, Richard Edelmann and Robert Baker

Miami University, Oxford, Ohio, United States

Understanding plant adaptation mechanisms to prolonged water immersion provides options for improvement of existing legume crops to create cultivars tolerant towards periodic flooding. An important advancement in understanding flooding adaptation would be to elucidate the mechanism of aerenchyma air-space formation induced by hypoxic conditions consistent with prolonged water immersion. Aerenchyma formation occurs through programmed cell death (PCD) (Ni et al., 2019) that entails chemical modification of root cell wall components such as pectic polysaccharides (i.e. "pectin") and hemicellulose that enable cell wall degradation via enzymatic activity (Wu et al., 2018; Barnes & Anderson, 2018; Rost et al., 1991; Pegg et al., 2018). In this study, three Fabaceae species known to produce aerenchyma cavities in flooded, hypoxic conditions - *Phaseolus coccineus* (scarlet runner bean), *Pisum sativum* (green pea), and *Cicer arietinum* (chickpea) – were utilized to investigate the chemical modification of three types of hemicellulose cell wall components – xylan, fucosylated xyloglucan, and non-fucosylated xyloglucan – in root vascular stele cells during aerenchyma formation.

Experimental design consisted of germination of 5 groups of 10-20 seeds, per species, in 2-liter beakers containing sterile vermiculite and deionized water for 5 days at 25^{o}C 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 located 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, and 48 hours), embedded in 3.5% agarose and sectioned at 150µm prior to immuno-labeling. Samples from control and experimental groups were stained with either CCRC-M1, CCRC-M100 and LM10 antibodies targeting fucosylated xyloglucan, non-fucosylated xyloglucan, and xylan, respectively. Secondary antibody labeling with Alexa Fluor® 647 fluorophore conjugates was viewed with an Olympus FV500 confocal light microscope to identify hemicellulose localization patterns near root aerenchyma.

The results of this study demonstrate notable similarities and differences in hemicellulose labeling between each legume species tested. Antibody labeling of *P. coccineus* indicates that binding to fucosylated xyloglucan is limited within the stele during aerenchyma cavity formation (Fig. 1A), while nonfucosylated xyloglucan is bound more strongly in expanding edges of the aerenchyma (Fig. 1B) compared to control samples (Fig. 2A,B). *P. sativum* shows increased signal for non-fucosylated xyloglucan in cells immediately adjacent to aerenchyma (Fig. 1E) compared to non-flooded controls (Fig. 2E), but not for fucosylated xyloglucan (Fig. 1D, 2D). Neither *P. coccineus* or *P. sativum* demonstrated significant changes in xylan binding pattern (LM10) between flooded (Fig. 1C,F) and non-flooded (Fig. 2C,F) treatments. *C. arietinum* demonstrated a loss of signal at the expanding edges of the aerenchyma cavities for all three antibodies tested on the 24hr flooding treatment (Fig. 1G-I). Similar results were not observed in any of the antibodies applied to the non-flooded treatment group for *C. arietinum* (Fig. 2G-I).

The immunolabeling data suggests that removal of fucosyl groups from xyloglucan (CCR-M100 antibody) may be an important component of the modeling process in cells fated to be degraded by expanding root aerenchyma cavities. This process may share a similar function as the de-methyl-esterification mechanism for pectin that enables pectinase activity to cleave/degrade the pectin backbone structure after the removal of methyl ester groups (Gunawardena et al., 2001; Pérez-Pérez et al., 2019). Additionally, it appears that

C. arietinum aerenchyma formation may occur by an altered mechanism that requires more removal of hemicellulose components (Fig. 1G-I) compared to *P. coccineus* or *P. sativum* (Fig. 1A-F).

Future studies investigating enzyme pretreatment of root tissue sections with pectinase prior to hemicellulose antibody treatment may reveal possible "unmasking" events within the cell wall during aerenchyma formation. Such events could be required to remove polysaccharides binding to hemicellulose components that would otherwise prevent chemical alteration and/or enzyme activity needed to initiate cell wall degradation required for aerenchyma cavity enlargement. Notable, this could explain the differences observed in hemicellulose antibody labeling observed in this experiment between *C. arietinum* and *P. sativum* and *P. coccineus* by demonstrating that "unmasking" of hemicellulose occurs over a larger area of the root stele, and into the cortex, in *C. arietinum* compared to the other two legumes tested.

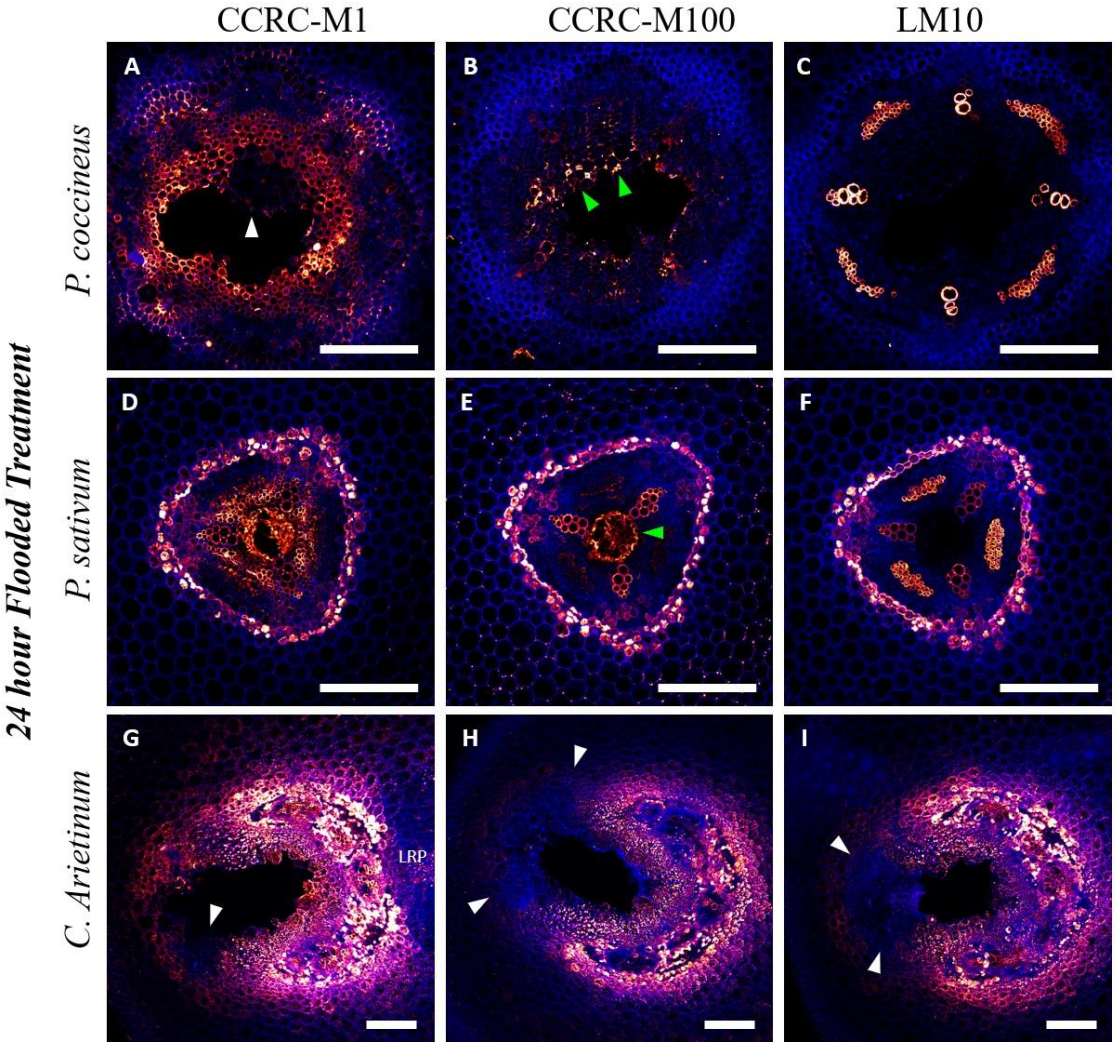

Figure 1. Hemicellulose immunolabeling patterns for three Fabaceae species scarlet runner bean (*Phaseolus coccineus*), pea (*Pisum sativum*) and chickpea (*Cicer arietinum*). (A-I) Legume root sections flooded for 24 hours. (A,D,G) Binding pattern for CCRC-M1 antibody against fucosylated xyloglucan. (B,E,H) Binding pattern for CCR-M100 antibody against non-fucosylated xyloglucan. (C,F,I) Binding pattern for LM10 antibody against xylan. Red/yellow = antibody binding pattern. Blue = fluorescence from aldehyde-based fixatives. White wedges show areas with a lack of antibody labeling. Green wedges show areas of increased antibody binding signal. LRP = lateral root primordia. Scale bars = 50 µm.

https://doi.org/10.1017/S1431927620014348 Published online by Cambridge University Press

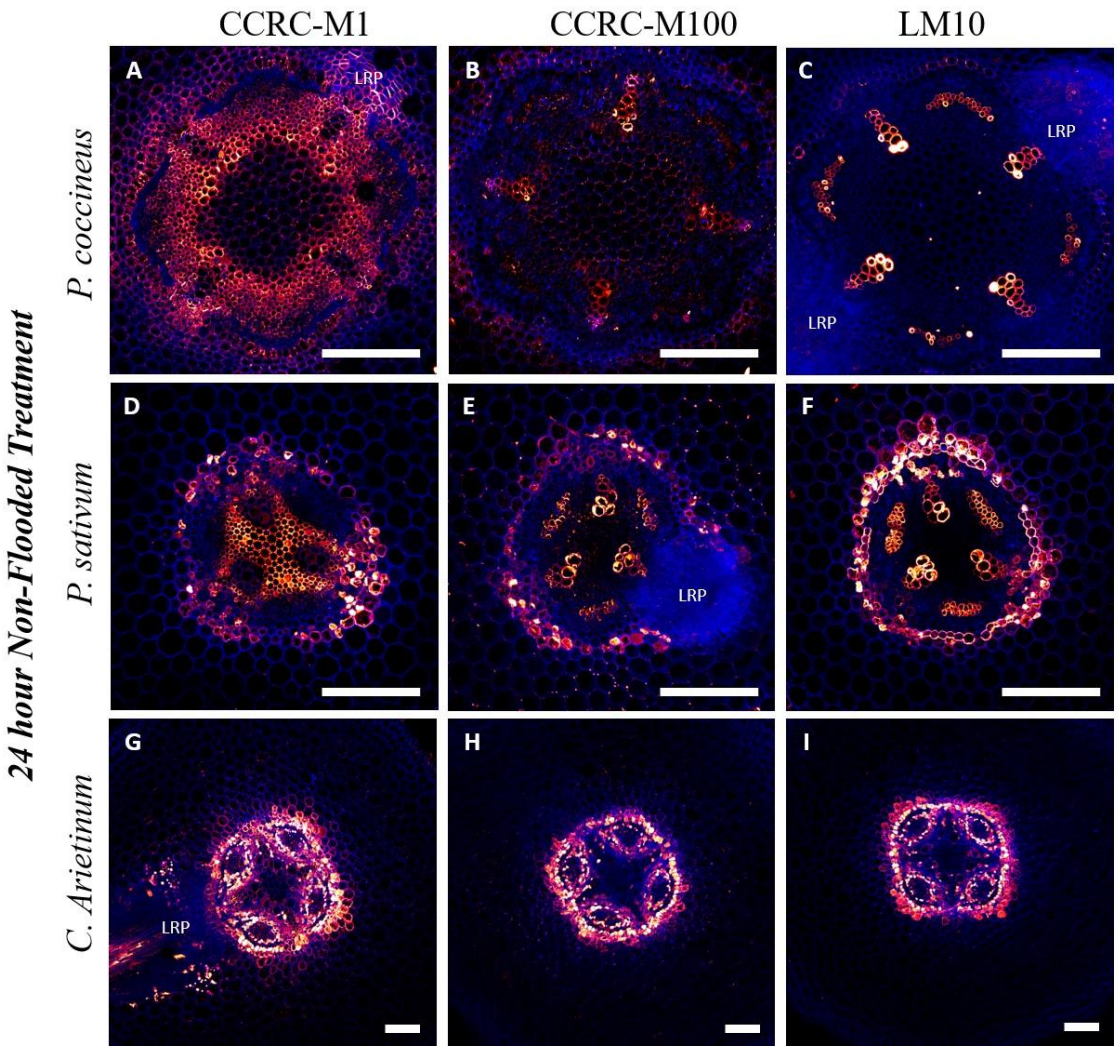

Figure 2. Hemicellulose immunolabeling patterns for three Fabaceae species scarlet runner bean (*Phaseolus coccineus*), pea (*Pisum sativum*) and chickpea (*Cicer arietinum*). (A-I) Unflooded legume root sections serving as experimental controls. (A,D,G) Binding pattern for CCRC-M1 antibody against fucosylated xyloglucan. (B,E,H) Binding pattern for CCR-M100 antibody against non-fucosylated xyloglucan. (C,F,I) Binding pattern for LM10 antibody against xylan. LRP = lateral root primordia. Scale bars = 50 µm.

References

Barnes, W. J. & Anderson, C. T. (2018). Release, Recycle, Rebuild: Cell-Wall Remodeling, Autodegradation, and Sugar Salvage for New Wall Biosynthesis during Plant Development. *Molecular Plant* **11**, 31–46. http://www.sciencedirect.com/science/article/pii/S1674205217302411.

Gunawardena, A. H. L. A. N., Pearce, D. M. E., Jackson, M. B., Hawes, C. R. & Evans, D. E. (2001). Rapid changes in cell wall pectic polysaccharides are closely associated with early stages of aerenchyma formation, a spatially localized form of programmed cell death in roots of maize (Zea mays L.) promoted by ethylene. *Plant, Cell & Environment* **24**, 1369–1375. https://doi.org/10.1046/j.1365-3040.2001.00774.x.

https://doi.org/10.1017/S1431927620014348 Published online by Cambridge University Press

Ni, X.-L., Gui, M.-Y., Tan, L.-L., Zhu, Q., Liu, W.-Z. & Li, C.-X. (2019). Programmed Cell Death and Aerenchyma Formation in Water-Logged Sunflower Stems and Its Promotion by Ethylene and ROS . *Frontiers in Plant Science* **9**, 1928. https://www.frontiersin.org/article/10.3389/fpls.2018.01928.

Pegg, T. J., Edelmann, R. E. & Gladish, D. K. (2018). *Progression of Cell Wall Matrix Alterations during Aerenchyma Formation in Pisum sativum Root Cortical Cells*.

Pérez-Pérez, Y., Carneros, E., Berenguer, E., Solís, M.-T., Bárány, I., Pintos, B., Gómez-Garay, A., Risueño, M. C. & Testillano, P. S. (2019). Pectin De-methylesterification and AGP Increase Promote Cell Wall Remodeling and Are Required During Somatic Embryogenesis of Quercus suber. *Frontiers in plant science* **9**, 1915. https://www.ncbi.nlm.nih.gov/pubmed/30671070.

Rost, T. L., Lu, P. & Gladish, D. (1991). The Occurrence of Vascular Cavities and Specialized Parenchyma Cells in the Roots of Cool-season Legumes. *Botanica Acta* **104**, 300–305. https://doi.org/10.1111/j.1438-8677.1991.tb00234.x.

Wu, H.-C., Bulgakov, V. P. & Jinn, T.-L. (2018). Pectin Methylesterases: Cell Wall Remodeling Proteins Are Required for Plant Response to Heat Stress . *Frontiers in Plant Science* **9**, 1612. https://www.frontiersin.org/article/10.3389/fpls.2018.01612.