How does the structure of the phloem cell wall contribute to whole-plant carbohydrate partitioning?

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Carbohydrate partitioning is the process by which sugars move from source tissues, e.g., mature leaves, to sink tissues, such as the roots. These sugars are transported in the form of sucrose and later turned into glucose polymers and cellular building blocks such as cellulose. Cellulose is the largest form of carbon storage and is deposited in plant cell walls. The COBRA gene has been identified to affect the deposition of cellulose in *Arabidopsis thaliana*. COBRA proteins help assemble the cellulose microfiber structure of the cell walls during plant development, and *cobra* mutants have been characterized to have root defects.

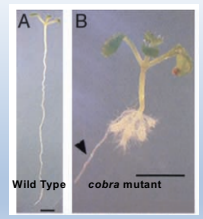
A gene that functions in carbohydrate partitioning in maize encodes a COBRA gene: *Brittle stalk2-Like3 (Bk2L3)*. In maize, a mutation in this gene was identified as *carbohydrate partitioning defective 28 (cpd28)*. Here we investigated the abundance of specific cell wall epitopes using monoclonal antibodies against known cell wall structural components. The data presented should help illuminate how BK2L3 contributes to phloem cell wall architecture and ultimately contributes to whole-plant carbohydrate partitioning.

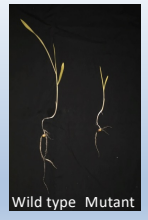
cpd28 mutant plants are dwarves with chlorotic leaves

Wildtype Mutant

The *cpd28* is dwarfed compared to the wild type and hyperaccumulates sugars in the leaves (unpublished).

cpd28 and *cobra* mutants demonstrate decreased growth

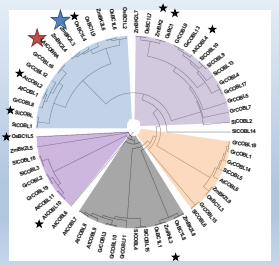




Schindelman, et al. 2001

The *cobra* mutants in Arabidopsis showed stunted growth as does *cpd28* (right).

Arabidopsis COBRA and BK2L3 (CPD28) are closely related



BK2L3 is a member of the cobra family, which has been described in Arabidopsis as well as other plants such as tomato and cotton. The mutants of this family typically have reduced growth as seen in maize and Arabidopsis. The BK2L3 gene is indicated with the blue star and the Arabidopsis gene with a red star.

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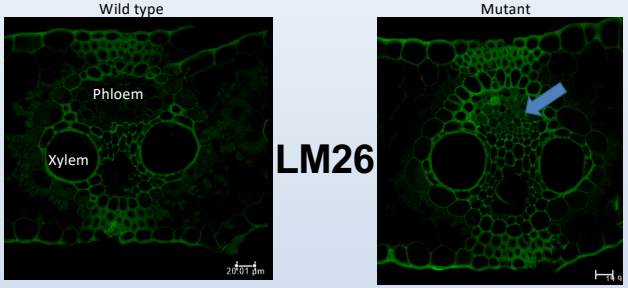
Imaging cell wall differences

Immunolocalization

To examine if there are cell wall changes between the wild type and mutants in the maize *Bk2L3* gene (first identified as the *carbohydrate partitioning defective28* mutant), I am conducting immunolocalization experiments using antibodies raised against known cell wall epitopes. The immunostaining results are visualized using fluorescence microscopy to compare mutants to the wild type.

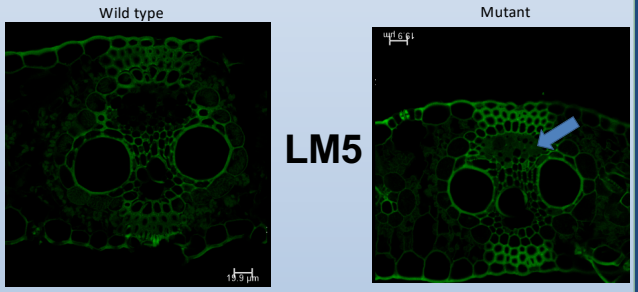
Rhamnogalacturonan-I
Galactan: LM26 branched (1,6-Gal) (1→4)-β-D-galactan

Wild type Mutant



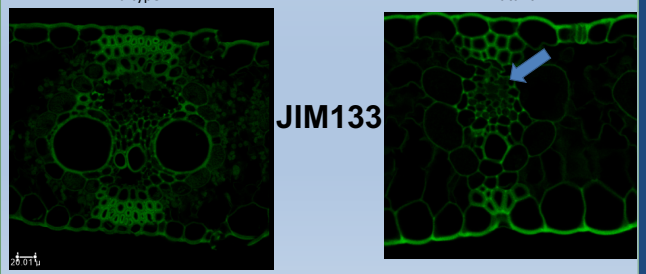
Rhamnogalacturonan-I
Galactan: LM5 (Nonreducing End) (1→4)-β-D-galactan

Wild type Mutant



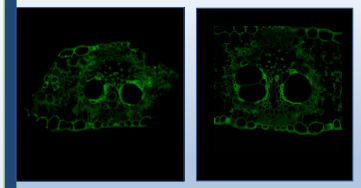
Rhamnogalacturonan-I
Galactan: JIM133

Wild type Mutant



These results demonstrate alterations in the cell wall structure of the phloem tissue in the mutants (blue arrows). This is seen using all three antibodies which all bind to the galactan portion (pectin) of the cell wall. This could be due to a structural compensation for the decreased cellulose content in the mutants (not shown).

No changes with non-galactan antibodies in mutants

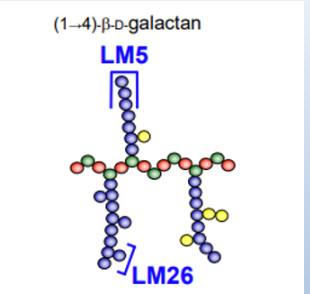


Wild type Mutant

LM6 – Arabinan: (1→5)-α-L-arabinan

We imaged many other antibodies, including LM10, LM2, and JIM14 and observed no differences between mutant and wild type siblings.

Monoclonal antibodies against pectin



It is now known that **LM5** binds to the non-reducing end of pectic galactan. The recently isolated MAb **LM26** binds to a branched epitope of pectic galactan with a 1,6-galactosyl residue on the 1,4-galactan backbone.

"JIM133 selectively binds all tested β-1,3-linked galactooligosaccharides, with tolerance for various β-1,6-linked arabinose and Gal substitutions. Thus, JIM133 can be used to detect the nonreducing ends of the β-1,3-linked galactan backbone in AG structures of arabinogalactan proteins (AGPs)."

Ruprecht et al. 2017

Future directions

- We are performing a third biological replicate with LM5, LM26, and JIM133 to further investigate these results.
- Another project that we are conducting includes a root growth study to test the function of the *Bk2L3* gene. We know that Arabidopsis *cobra* mutants have decreased growth. Transgenic complementation testing using the maize *Bk2L3* gene in a *cobra* mutant background will be analyzed for their root growth phenotype.
- Further experiments in progress are to study the Arabidopsis *Cobra* gene and its mutant *cob4* null allele. Experiments to be done include sugar quantifications using HPAE-PAD protocols to test if Arabidopsis *cob* mutants also hyperaccumulate sugar like the maize *cpd28*.
- Additional immunolocalization experiments using other antibodies directed against different cell wall polymers will be performed.

Summary

- Early promising results found significant alterations in the cell wall structure of the phloem tissue in the mutants, which may contribute to the carbohydrate partitioning defective phenotype observed.