Characterization of the novel maize *carbohydrate partitioning defective* mutant *P135-21B*

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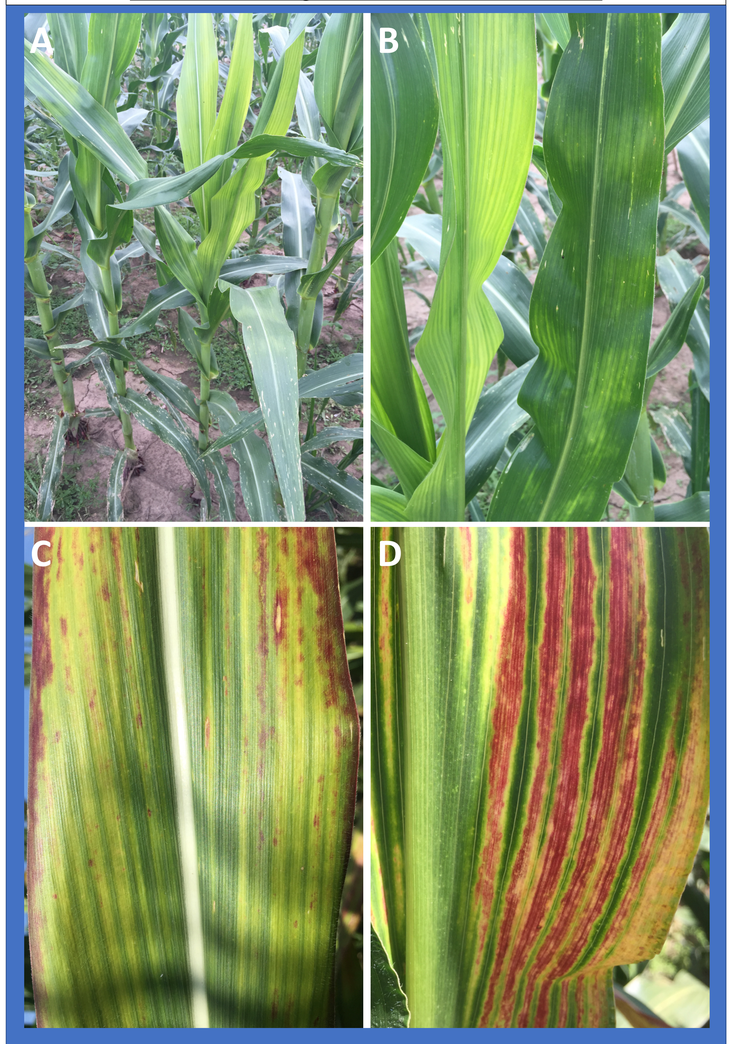
Introduction

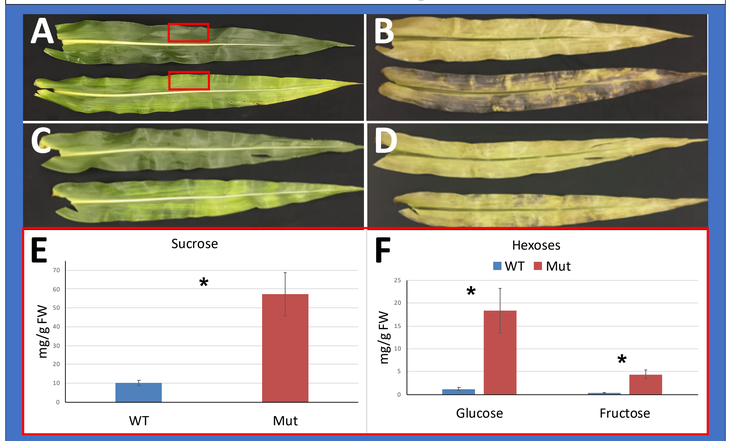
- *P135-21B*, a novel *carbohydrate partitioning defective* (*cpd*) mutant conditioned by a semi-dominant mutation, exhibits progressive basipetal leaf chlorosis and hyperaccumulates starch and soluble sugars.
- Aniline Blue staining of adult leaves suggests that the mutant phenotype may be caused by a partial blockage in the phloem due to hyperaccumulation of callose.
- *P135-21B*, which originated in the Va35 inbred, is conditioned by two independent loci in B73 and a single locus in Mo17. Rough mapping intervals were identified on Chromosome 1S and Chromosome 7S.

P135-21B exhibits progressive leaf chlorosis and anthocyanin accumulation



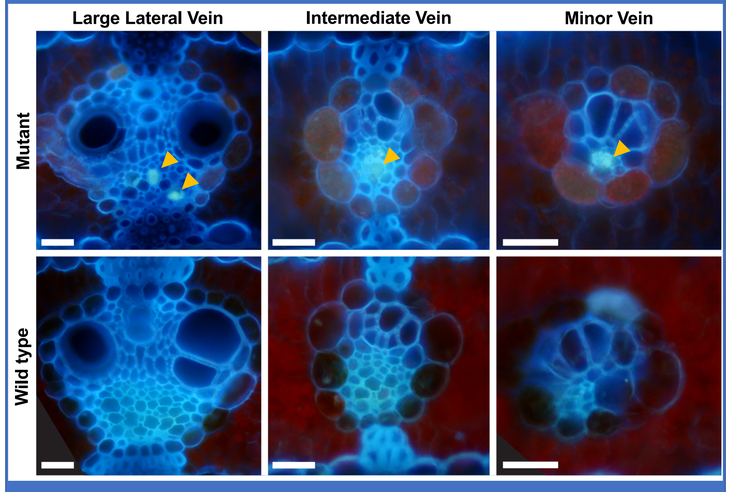
A. 8-week old *P135-21B* (B73) heterozygote exhibiting progressive chlorosis in upper canopy leaves and intermittent streaking and cross-banding in mid-canopy leaves.
B. Closer view of upper (left) and mid-canopy (right) leaves.
C. Leaf of 10-week old heterozygote showing strong intraveinal chlorosis and anthocyanin accumulation
D. Leaf of 10-week old heterozygote (Mo17) accumulating anthocyanin.

P135-21B hyperaccumulates starch and soluble sugars



A. Upper canopy leaves of *P135-21B* (B73) wild type (top) and chlorotic heterozygote (bottom). Boxes indicate positions sampled for soluble sugar analysis in Panels E and F.
B. Cleared and I_2KI stained upper canopy leaves. The heterozygote is hyperaccumulating starch in chlorotic regions (dark regions).
C. Mid-canopy leaves of *P135-21B* (B73) wild type (top) and chlorotic heterozygote (bottom).
D. Cleared and I_2KI stained mid-canopy leaves. Much less starch accumulation is apparent compared to the upper canopy.
E. Sucrose hyperaccumulates in chlorotic regions of heterozygotes relative to wild-type siblings. Error bars show standard error. *, t-test $p < 0.05$
F. Glucose and fructose hyperaccumulate in chlorotic regions of heterozygotes relative to wild-type siblings. Error bars show standard error. *, $p < 0.05$

P135-21B exhibits ectopic callose deposition in mature leaf phloem



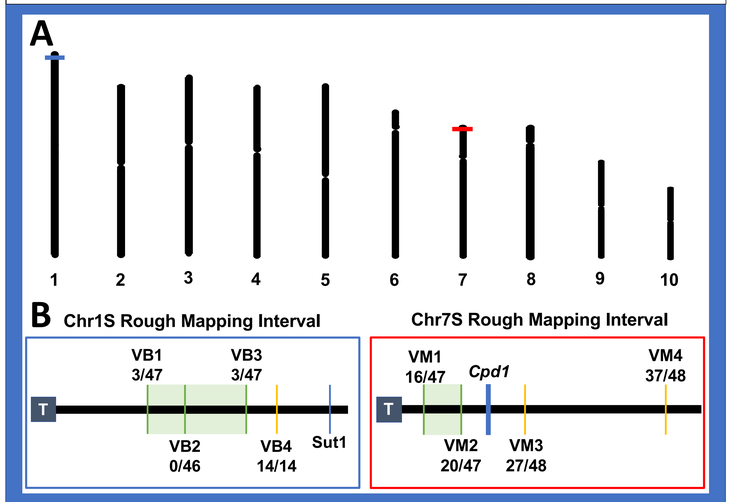
8-week old P135-21B (B73) heterozygote (Mutant) and isogenic wild-type sibling stained with Aniline Blue and visualized under UV light to visualize the cell wall polymer callose. The mutant is hyperaccumulating ectopic callose (arrowheads) within the phloem of all three vein classes.

P135-21B is conditioned by two independent loci in B73, and 1 locus in Mo17

Population	Total Mutants	Total WT	χ^2 1:1	χ^2 3:1
B73 x *P135/+*	113	349	$9.1e^{-7}$*	**0.86**
Mo17 x *P135/+*	275	322	**0.85**	ND

Individuals from multiple families of a segregating mapping population were scored for the mutant phenotype and analyzed for 1:1 or 3:1 segregation using a Chi-Squared Test. *, null hypothesis rejected.

Rough mapping intervals on Chr1S and Chr7S were identified



A. Overview of Bulked Segregant Analysis results for B73 x *P135-21B*/+ (Va35; Blue) and Mo17 x *P135-21B*/+ (Red) mapping populations.
B. Rough Mapping Intervals. Both intervals map near a known *cpd* mutant, but are novel. Mapping Legend: VB/VM, polymorphic DNA markers between VA35 (V) and B73 (B) or Mo17 (M); #/#, Recombinant Mutants/Total Mutants Screened; Boxed T, Telomere.

Experiments in Progress

- Develop new polymorphic PCR markers to fine map the intervals on Chr1S and Chr7S, and determine whether the second locus required to condition the *P135-21B* phenotype in the B73 mapping population is linked to Chr7S interval identified in the Mo17 mapping population.
- Use the tightly linked polymorphic markers to identify young mutant plants that are not yet showing the phenotype. Visualize the leaf veins with Aniline Blue to determine whether callose accumulation precedes starch and soluble sugar accumulation and may cause the phenotype.
- Generate higher order mutants by selfing to generate homozygotes and outcrossing to other semi-dominant mutations that display an ectopic callose phenotype, *Cpd4* and *Cpd1*. Measure starch, soluble sugar and callose accumulation in these families.

Acknowledgements

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