A Gene-Editing Strategy for Overcoming Growth Defects in Plants with Engineered Cell Walls

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Background

- Lignification of secondary cell walls is a major factor conferring recalcitrance of lignocellulosic biomass to deconstruction for fuels and chemicals.
- Genetic modification can reduce lignin content and enhance saccharification efficiency, but usually at the cost of moderate to severe growth penalties associated with xylem development.

Approach

- We developed a method, using a single DNA construct, that uses CRISPR-Cas9 gene editing to knock-out expression of an endogenous gene of lignin monomer biosynthesis while at the same time expressing a modified version of the gene's open reading frame that escapes cutting by the Cas9 system and complements the introduced mutation in a tissue-specific manner.

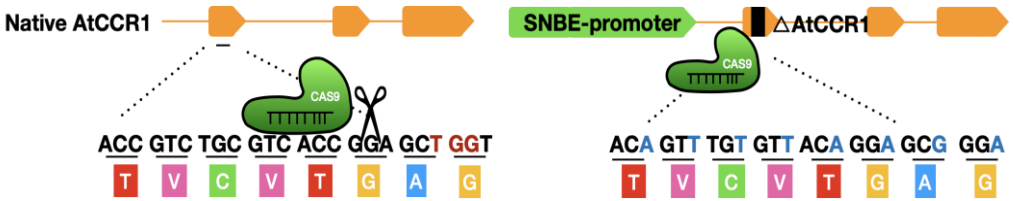
Outcome

- Applying these tools enabled the regeneration of Arabidopsis plants with reduced lignin, wild-type biomass yield, and up to 4-fold enhancement of cell wall sugar yield per plant.
- Plants were stable over at least 4 generations in both homozygous and bi-allelic heterozygous T1 lines.

Significance

- This method provides a general strategy for optimizing loss-of-function traits that are associated with growth penalties.
- This presents the opportunity to tune plant properties as optimal for conversion to biofuels and bioproducts while maintaining high plant yields.

Yu, H. et al. *Biotechnology for Biofuels* (2021) doi: 10.1186/s13068-021-02026-5



The CRISPR/Cas9 system with a sgRNA that targets Cas9 to the native *CCR1* gene along with vessel-specific expression of codon-modified CCR1 that encodes the same protein as native CCR1, driven by the xylem vessel specific promoter SNBE.

Tissue-specific complementation of the *ccr1-3* lignin pathway gene mutation maintains a 4-fold increase in saccharification without a decrease in growth

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