

# REDUCED WALL ACETYLATION C (RWA-C) – A Target Gene for Overcoming Biomass Recalcitrance in Populus

## Background

- Plant lignocellulosic biomass is a vital alternative feedstock source for bioenergy. However, the acetylation of xylan in the secondary cell walls impedes the deconstruction and conversion of biomass to biofuels. Previous studies have shown that *REDUCED WALL ACETYLATION* (RWA) genes are directly involved in the acetylation of xylan but the regulatory mechanism of RWA is not fully understood.

## Approach

- We screened the *Populus* activation-tagged population to identify transgenic lines with altered cell wall chemistry phenotypes and subsequently determined the gene that was responsible for the observed phenotypes. We further employed a combination of molecular, genetic and biochemical approaches to investigate and validate the underlying regulatory mechanism.

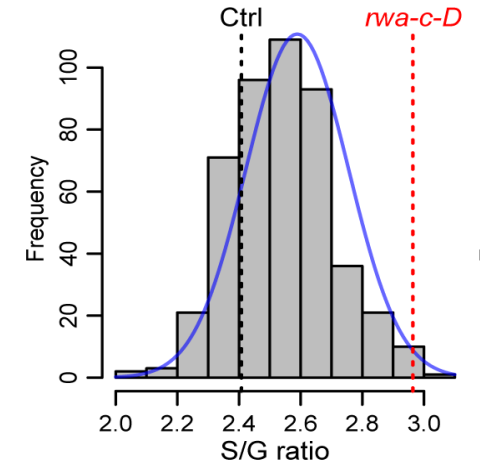
## Results

- A member of the *RWA* gene family, designated as *RWA-C*, was determined via molecular cloning and transgenic study to be responsible for the increased syringyl/guaiacyl (S/G) lignin ratio phenotype observed in the original activation-tagged line.
- Overexpression of *PtrRWA-C* increases the level of xylan acetylation, increases lignin content and increases S/G ratio in the *Populus* transgenic plants, resulting in reduced saccharification efficiency.
- Gene co-expression network and expression quantitative trait loci analysis indicated that the expression of *RWA-C* is not only regulated by the secondary cell wall hierarchical regulatory network but also by an AP2 family transcription factor HRD.

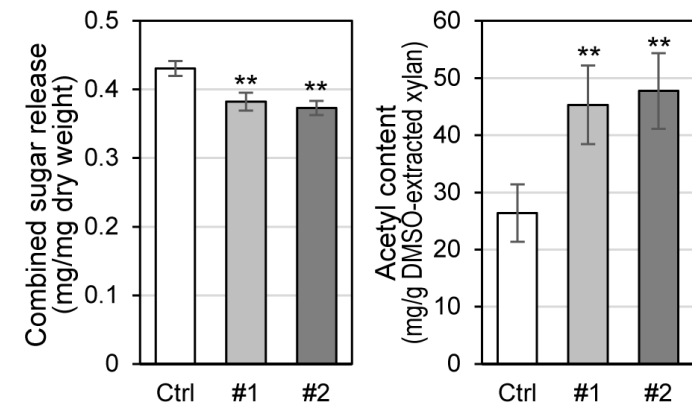
## Significance

- These findings provide novel insights into the functional role of *RWA-C* in xylan acetylation, lignin biosynthesis and saccharification.
- Genetic engineering or synthetic biology approaches manipulating *RWA-C* offers a strategy for altering cell wall properties for enhanced conversion of biomass into biofuels in *Populus* and other plants.

Zhang J et al. (2023). *Plant Physiology*, 10.1093/plphys/kiad377.



The distribution of syringyl/guaiacyl (S/G) lignin ratio in the *Populus* activation-tagged population. The black and red dash lines indicate the values of wild type control (Ctrl) and the selected activation-tagged line (*rwa-c-D*), respectively.



*Populus* transgenic lines overexpressing *RWA-C* display reduced sugar release and increased xylan acetylation. Asterisks indicate significant differences between transgenic lines (#1 and #2) and control (Ctrl) plants.