One poplar 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase moonlight as transcriptional repressor to regulate lignin biosynthesis

Background

- The lignin in biomass restricts the access of cellulases and other cell wall degrading enzymes, which limits the conversion from biomass to biofuels and bioproducts. The understanding of the regulation of lignin biosynthesis in poplar is essential to overcome this barrier.
- EPSP synthase widely exist in plants and microorganisms. In the past decades, the only known function of EPSP synthase is to catalyze the production of aromatic amino acids. Here, we found that an additional helix-turn-helix (HTH) motif endows transcription activity to one poplar EPSP synthase (PtrEPSP-TF).

Approach

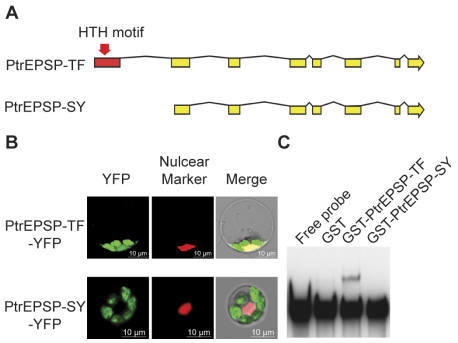
- We used genome-wide association mapping to define the linkage of PtrEPSP-TF and lignin biosynthesis. This linkage was validated by assessing *PtrEPSP-TF* overexpression poplars for lignin deposition, secondary metabolism, and gene expression levels. The molecular mechanism underlying this linkage was investigated by protoplast-based assays and biochemical studies.

Outcome

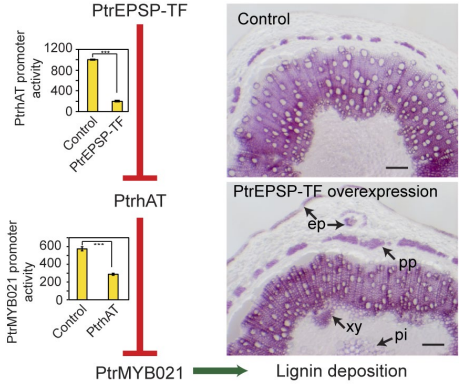
- Single Nucleotide Polymorphisms (SNPs) inside *PtrEPSP-TF* exhibited tight association with lignin content.
- *PtrEPSP-TF* overexpression poplars had increased deposition of lignin, accumulation of lignin-related metabolites, expression of lignin-related genes.
- The HTH motif was shown to be essential for the DNA binding and transcriptional repressor activity of PtrEPSP-TF.
- The expression of one master activator of lignin biosynthesis PtrMYB021 was found to be hierarchically regulated: PtrEPSP-TF directly represses the expression of *PtrhAT*. PtrhAT then directly represses the expression of *PtrMYB021*. Consequently, PtrEPSP-TF positively regulates *PtrMYB021* expression and lignin biosynthesis in poplar.

Significance

- For the first time, EPSP synthase is shown to co-function as transcriptional repressor.
- New strategy to manipulate lignin biosynthesis of poplar for better biofuel production.



The additional HTH motif endows new functions to PtrEPSP-TF. **A**) Protein structure of PtrEPSP-TF. **B**) Yellow fluorescent protein tagged PtrEPSP-TF exhibits nuclear accumulation. **C**) Electrophoretic mobility shift assay showing the DNA binding activity of PtrEPSP-TF.



PtrEPSP-TF activates *PtrMYB021* expression and lignin deposition. Left panel, the hierarchical regulation of PtrEPSP-TF, PtrhAT, and PtrMYB021. Right panel, ectopic lignin deposition (indicated by black arrows) in stems of poplars overexpressing PtrEPSP-TF. Lignin shows red color after staining.

Xie M *et al*. 2018. A 5-enolpyruvylshikimate 3-phosphate synthase functions as a transcriptional repressor in *Populus*. The Plant Cell DOI:10.1105/tpc.1800168

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