

Enabling High-Precision Genetic Engineering in Poplar

Background

- *Populus* species are desirable crops for sustainable biofuel production. CRISPR/Cas9-based techniques have been actively applied to *Populus* for genetic and genomic improvements for traits such as increased growth rate and tailored lignin composition.
- CRISPR/Cas9 has been primarily used in the active Cas9 form to create knockouts in the transformable hybrid poplar clone ‘717-1B4’ (*Populus tremula* x *P. alba* clone INRA 717-1B4).
- Alternatively, CRISPR activation (CRISPRa) systems have not been evaluated in poplar species.

Approach

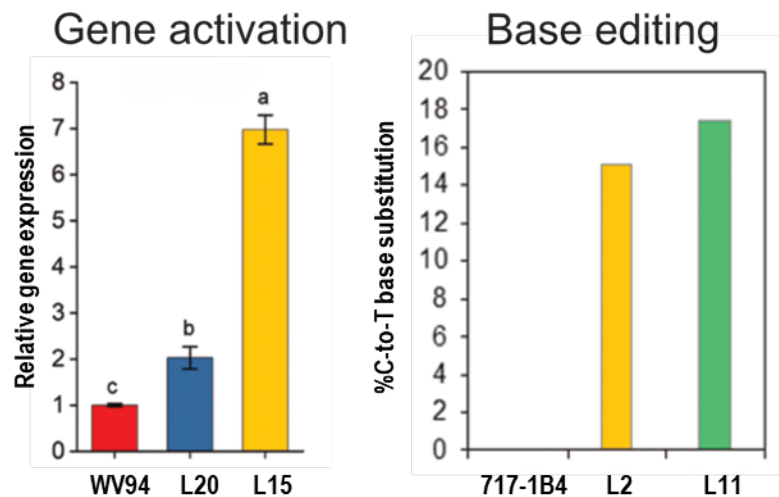
- Regulating gene activation: we employed a deactivated Cas9 (dCas9)-based CRISPRa technique to fine-tune the expression of two target genes (TPX2 and LecRLK-G), which play important roles in plant growth and defense response, respectively, in the 717-1B4 hybrid and *P. deltoides* ‘WV94’.
- Precise base-editing of single-nucleotide mutations: we applied Cas9 nickase (nCas9)-based cytosine base editor (CBE) to precisely introduce premature stop codons via C-to-T conversion, in the target gene PLATZ, which encodes a transcription factor involved in plant fungal pathogen response in poplar clone ‘717-1B4’.

Results

- We demonstrated the success of applying CRISPRa to up-regulate the expression (1.2-fold to 7.0-fold) of two endogenous genes, in two different poplar species with both transient protoplast and stable *Agrobacterium* transformation.
- We demonstrated precise base-editing with an (nCas9)-based CBE with an efficiency of 13%–14%.

Significance

- These results expand the application of CRISPR/Cas-based technologies in gene expression regulation and precise gene engineering in *Populus* sp., facilitating functional genomics research and genetic engineering in woody bioenergy crops.



CRISPR-mediated activation of the endogenous gene TPX2 in *Populus deltoides* ‘WV94’ and base editing (i.e., C-to-T substitution) of the endogenous gene PLATZ in hybrid poplar clone ‘717-1B4’ (*P. tremula* x *P. alba* clone INRA 717-1B4). L20 and L15 are transgenic events in the WV94 background. L2 and L11 are transgenic events in the hybrid poplar clone ‘717-1B4’ background.

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