Title: Transgenic poplar analysis of native and synthetic inducible promoters for sensing abiotic stress and tissue specificity from poplar *cis*-regulatory elements.

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Project Goals: Sypro Poplar: Improving poplar biomass production under abiotic stress conditions: an integrated omics, bioinformatics, synthetic biology and genetic engineering approach.

The project goal is to attain robust biomass of trees under abiotic environmental stress conditions via genetic engineering. Abiotic stress-resistance genes, especially those for water deficit, salt, and temperature stress, should optimally be under inducible regulatory control. We are designing, constructing, and testing stress-inducible synthetic plant promoters to drive resistance genes. Omics data are used to discover *cis*-regulatory DNA motifs used to construct synthetic promoters. The synthetic promoters are then tested under appropriate stimuli in engineered plants to use toward the development of environmentally resilient poplar.

Abstract text: Abiotic stress can cause significant damage to plants, which is crucial to avoid if production of bioenergy tree crops is to be sustainable. Promoter engineering has been proposed as a promising solution to overcome the challenges caused by

various abiotic stressors: they can be useful to control the expression of stress resistance transgenes. Through previous studies, we synthesized 9 promoters (SD9-1, 9-2, 9-3, 13-1, 18-1, 18-3, SS16-1, 16-2, and 16-3) from poplar transcriptome data using a *de novo* DNA-motif-detecting algorithm. Their stress inducibility was identified in water-deficit and salt treatment assays using poplar leaf mesophyll protoplast transformation and agroinfiltration of *Nicotiana benthamiana* leaves. In this study, these 9 synthetic promoters were stably transformed into *Populus tremula* × *Populus alba* hybrid poplar, by which GFP inducibility was screened under osmotic stress conditions. Of 9 transgenic poplar lines each harboring a different synthetic promoter, SD18-1, SD9-2, SD9-3, and SS16-1, there was significantly induced GFP expression in both salt and PEG treatments.

Tissue-specific native and synthetic promoters were also designed from RNA-seq datasets from laser micro-dissected poplar leaf tissue after water-deficit or salt stress treatment. Three native promoters from water-deficit transcriptome data were separately fused with a GUS reporter gene and used to generate transgenic poplar. All three native promoters induced specific GUS expression in early developmental leaf stages of transgenic poplar. Specifically, the native promoter Potri.003G072800 was induced in leaf blades, but not in vascular tissue. Synthetic promoters were also designed for leaf-tissue-specific induction by constructing heptameric repeats of 10 bases from conserved sequence of native promoters. Additionally, the tissue-specific inducibility of synthetic promoters fused with a GFP reporter was screened in leaf mesophyll protoplasts, and two GFP inducible synthetic promoters were identified for leaf tissue expression.

The tested promoters will be utilized to develop stress-responsive and tissue-specifically-controlled bioenergy tree crops in the future.

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