Discovery of Novel *Cis*-Regulatory Elements Responsive to Salt-Stress in Hybrid Poplar for Designing Inducible Synthetic Promoters

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Project Goals: In SyPro Poplar we intend to (i) study the functions of selected stress-responsive genes; (ii) discover novel motifs and construct stress-responsive synthetic promoters; and (iii) use these promoters to drive the expression of genes shown to confer abiotic stress tolerance in a variety of crops and develop abiotic stress-tolerant poplar seedlings in a coordinated fashion. We will use a combinatorial gene stacking approach with key transgenes driven by stress-responsive synthetic promoters to confer stress resistance. Our plan is to develop a series of abiotic stress-responsive synthetic promoters comprised of the stretch of DNA containing multiple copies of abiotic responsive *cis*-motifs upstream of a core-promoter in which abiotic stress specific transcription factors (TFs) bind to their cognate sequences to drive transcription under multiple abiotic stresses. The aim is the development of transgenic trees with sustained photosynthetic activity and increased biomass production under individual and the simultaneous occurrence of water deficit, increased soil salinity and elevated temperatures.

Advanced -omics data combined with plant synthetic biology technologies are powerful tools to discover novel motifs associated with abiotic stress responses and to construct synthetic promoters to regulate stress-coping genes expression specifically to the stress conditions. In the present study, we designed synthetic promoters responsive to salt-stress using novel *cis*-regulatory elements in hybrid poplar (*Populus tremula* x *Populus alba*). Poplar-transcriptome data[1,2] were used as input for computational-based motif discovery that are being developed as a KBase module. The computational goal is to identify conserved minimal motifs in native plant gene promoters are responsible for upregulated gene expression under salt-stress. Through this process we identified a highly conserved 20-base-long motif, which we refer to as motif 16. Three native promoter variants, which included motif 16, were fused to a green fluorescent protein (GFP) reporter gene. Gene constructs were transfected into poplar mesophyll protoplasts and subjected to salt treatment, which resulted in GFP signal induction after 24 hr. The endogenous gene expression of the three genes in poplar leaves was confirmed by qRT-PCR using greenhouse-grown poplar plants under salt-stress. A synthetic promoter, comprising a 7-repeat (heptamer repeat) of the motif 16 that we placed upstream of the cauliflower mosaic virus (CaMV) -46 35S (minimal) promoter, which was used to drive a GFP reporter. We also tested another synthetic inducible promoter candidate containing heptameric repeats of the ABRE motif (ACGTG, a canonical motif responsive to salt-stress), in the same construct architecture. Each

synthetic promoter strongly induced GFP expression after 24 hr under salt-treatment in poplar mesophyll protoplasts. We are currently in the process of producing transgenic poplar with constitutively-expressed GFP and GFP under the control of three native and two synthetic salt-inducible promoters. Furthermore, we are producing a permutational library using motif 16 to produce 5-base-long fragments for the purpose of discovering the minimal core-motifs responsible for salt-stress induction. The top-performing synthetic promoters will be subsequently deployed to control key abiotic stress resistance genes in poplar with the goal of producing robust and sustainable feedstock.

References

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