

**“SyPro Poplar”**  
**Improving Poplar Biomass Production under Abiotic Stress Conditions:**  
**An Integrated Omics, Bioinformatics, Synthetic Biology and Genetic Engineering Approach**

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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.**

Abiotic stresses such as drought, high temperature and salinity are major causes for decreased crop yield, including decreased biomass from bioenergy feedstocks. Producing crops with robust phenotypes under variable growth conditions has proven to be challenging, with abiotic stress tolerance proving to be among the most challenging traits. Given the low input requirements needed for competitive bioenergy feedstock cultivation, irrigation and other mitigation is not economically feasible. Therefore resistance to stresses will likely be required to be genetically encoded within crops. The development of multi-stress tolerant plants is critical for both food and energy security with predictable and environmentally-independent biomass production being the most important trait<sup>1</sup>. In this regard, advanced -omics and plant synthetic biology methods are promising ways to accumulate favorable alleles associated with stress tolerance in a plant genome. Although various genes relevant to plant responses to different types of abiotic stresses have been characterized and used to improve plant productivity under stress, only limited success has been achieved in producing stress-tolerant bioenergy crops<sup>2</sup>. To date, constitutive overexpression (using CaMV 35S or ubiquitin promoters) of single genes has been the most common strategy for improving abiotic stress tolerance in plants. However, this strategy has had a relatively limited success due to involvement of multiple pathways in plant adaptation to stress and possible negative pleiotropic effects on plant growth<sup>3</sup>. For that reason, tissue-specific and stress responsive synthetic promoters can be designed to respond specifically to different stress conditions, effectively inducing transgene expression in plants while maintaining a low activity under normal growth conditions. Compared to natural promoters, synthetic promoters provide higher (or lower) expression level with high specificity, lower basal expression level, shorter length, and less sequence homology to any host genomic sequences<sup>4</sup>. However, the use of integrated strategies, including -omics, bioinformatics and biotechnological tools, for the development and testing of synthetic promoters has been restricted. Thus, construction of synthetic promoters with appropriate tissue specificity and inducibility is a nascent science. Moreover, to date, synthetic promoters have been mainly used to drive expression of reporter genes under controlled conditions. In SyPro Poplar, we aim to construct novel monopartite and tripartite synthetic tissue-specific promoters (by multimerization of novel abiotic responsive cis-elements) capable of driving transcription of novel stress-responsive genes under multiple abiotic stresses and under field conditions.

The proposed research program will focus on poplar leveraging its vast suite of genomic resources, including several high quality reference genomes for strategically-important species, and re-sequenced genomes, transcriptomes, proteomes, and metabolomes for large population samples. Moreover, it is a prevalent woody feedstock for improved next-generation biofuels, representing the most productive native trees in the northern hemisphere. Poplar has distinct advantages over other biofuels crops because the timing of harvest is very flexible, necessitating minimal long-term storage at processing facilities and thereby solving some major logistical challenges for biofuel production<sup>5</sup>.

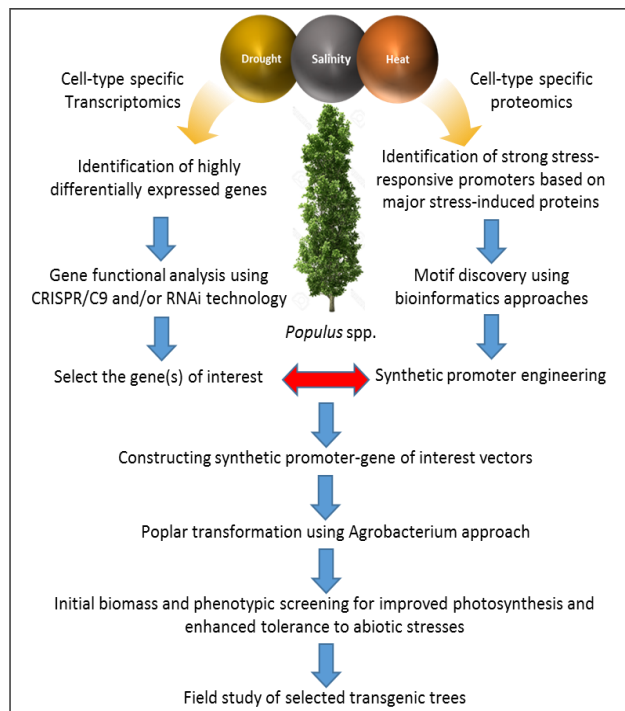
In SyPro Poplar we intend to integrate omics, bioinformatics, synthetic biology and genetic engineering approaches to develop transgenic poplar trees with sustained photosynthetic activity and increased biomass production under individual and the simultaneous occurrence of water deficit, increased soil salinity and elevated temperatures.

*Populus tremula x alba* clone INRA 717 1-B4, as a model genotype with extremely high transformation efficiency and favorable silvicultural characteristics, has been propagated and used by SyPro Poplar team for initial test experiments and method optimizations including laser capture microdissection (LCM) and tissue specific proteomics based on newly developed PNNL capabilities, nanowell-based preparation in one-pot for trace samples (nanoPOTS), small-volume sample preparation, and ultrasensitive liquid chromatography-mass spectrometry (LC-MS). Preliminary genomics analysis and motif discovery of available poplar data is in progress using bioinformatics tools such as BioMart and MEME. To build a prototype in the first year of the project, vector construction and transformation of clone INRA 717 1-B4 with some selected stress-responsive genes under the control of both constitutive and stress-responsive inducible promoters is also in progress.

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**Figure 1.** SyPro Poplar workflow.