

## **Title: Understanding the genetic basis of drought tolerance in bioenergy poplar**

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**Project Goals:** *Populus* serves as a promising biomass feedstock for a suite of industrial applications including biofuels conversion. Drought is one of the most important factors limiting cost-effective production of *Populus* biomass. It is imperative to overcome this obstacle to achieve sustainable production of *Populus* biomass. However, drought response in *Populus* is a complex trait requiring the regulation and coordinated interactions of many genes, and identification of genetic networks regulating drought response remains unaccomplished and is urgently needed to inform genetic improvement of *Populus* feedstocks for sustainable biomass production. The goal of this research is to determine the genetic basis of drought tolerance in bioenergy *Populus* enabling tree improvement and the wide-scale deployment of *Populus* for bioenergy in marginal and droughted environments. Fast growing feedstock crops are required for a future bioeconomy where plant-based biofuels, chemicals and biomass for Bioenergy with Carbon Capture and Storage (BECCS) will be utilized as part of a more sustainable, energy secure economy.

**Methods:** We will harness the natural genetic variation found in wild trees, adapted to different amounts of rainfall. Our main study population consists of 1,000 *P. trichocarpa* collected from a range of naturally droughted and wet environments, that already has a number of unique genomic and genetic resources for a tree species, that will be used here.

Drought tolerance, here defined as ‘*the maintenance of plant biomass production in the face of moderate and persistent drought stress*’ is a highly complex trait and we will use high throughput phenotyping, including UAV-mounted multi-spectral proximal analysis to unravel how drought tolerance varies across this wide population.

Using the latest computational tools and the extensive sequence, re-sequence and RNASeq resources available and new ones to be developed, we will identify multiple genetic loci linked to traits and resolve them through hierarchical network analysis to the level of cis- and trans-acting eQTL. We will validate our findings on these regulator cis-acting eQTN candidates for drought tolerance in fast transient assays using protoplasts and in longer-term whole tree experiments including accurate deployment of CRISPR-Cas9 gene edits. The innovation in this project comes from the rapid deployment of these approaches that will also benefit from findings in a second *Populus* natural collection from a range of wet and droughted sites across Europe for which early leads on target loci are already available for validation.

### **Objectives:**

(i). To deploy a large-scale experimental drought trial for up to 1000 unique genotypes of *Populus* equipping the sites with controlled irrigation and drought treatments that are fully automated and monitored.

- (ii) To test the hypothesis that a suite of traits identified for drought tolerance in *P. nigra* can be measured in drought and control treatments in the wide germplasm collection of *P. trichocarpa*.
- (iii) To use established and novel GWAS model approaches to identify gene loci linked to drought tolerance traits on interest in *P. trichocarpa*.
- (iv) To undertake comparative analysis of GWAS results for drought tolerance traits in *P. nigra* and *P. trichocarpa*.
- (v) Using RNAseq in *P. trichocarpa*, in droughted and control treatments to identify cis- and trans-regulated eQTN.
- (vi) Validate up to 50 cis-QTNs, from network hubs using transient protoplast assays.
- (vii) To establish Agrobacterium-based gene editing protocols in *Populus*.
- (viii) To utilize early leads from previous research to investigate at least 6 candidate genes for drought tolerance in *Populus*.
- (ix) To validate up to 20 candidate genes for drought tolerance in *P. trichocarpa* refined from the long-list tested in the transient assays for cis-acting hub gene targets.

**Impacts and benefits:** This research will deliver new genotypes of *Populus* that are better suited and able to tolerate droughted environments, that are more likely in the future. They will be ready to test at multiple sites at project end. At the same time, the genomic and genetic resources already available in *Populus* will be integrated into high throughput drought phenotyping and a complete atlas of gene expression related to drought stress for this natural population.

### **Publications**

**Taylor G., et al** (2019). Sustainable bioenergy for climate mitigation: developing drought tolerant trees and grasses. *Annals of Botany*, 124: 513-520.

**Muchero W. et al** (2018). Association mapping, transcriptomics, and transient expression and transient expression identify candidate genes mediating plant-pathogen interactions in a tree. *Proc Natl Acad Sci USA* 115: 11573-11578.

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