

NitFix: Phylogenomic Discovery and Engineering of Nitrogen Fixation into the Bioenergy Woody Crop Poplar

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Project Goals: The collaborative project "Phylogenomic discovery and engineering of nitrogen fixation into the bioenergy woody crop poplar" is focused on identifying the genomic novelties that enable the symbiotic relationship between nodulating plants and nitrogen-fixing bacteria to support genetically engineering this capability into bioenergy crops. Our specific aims are:

- (1) Phylogenomic discovery of the underlying mechanism of root nodule symbioses using a comparative phylogenetic framework to contrast related species that possess and lack the nodulation ability.**
- (2) Verification of molecular mechanisms of root nodule development in *Medicago truncatula* (nodulating) and poplar hairy root cultures (non-nodulating).**
- (3) Engineering nodulation capability into the bioenergy woody crop *Populus* spp. and testing the impact of these structures on nitrogen-fixation.**

Nitrogen (N) availability is critical for high biomass productivity of bioenergy crops, particularly in marginal lands. Despite the abundance of N₂ in the atmosphere and critical importance for growth and development, plants cannot access it. Instead, plants must absorb available N in the soil as nitrates or ammonium, provided through intensive fertilization in a process that is costly, environmentally damaging and potentially hazardous to human health. More efficient and cost-effective approaches are needed to enable bioenergy crops to acquire the N required to maximize growth while minimizing inputs and environmental impact. Some plants have the capability to obtain N through a mutualistic relationship with bacteria and archaea, which can convert N₂ in the atmosphere to NH₃. This capability is limited in the plant kingdom, and absent from most bioenergy crops. All flowering plant lineages known to undergo root nodule N-fixation with bacterial symbionts are within a single clade, where multiple origins and losses of symbioses have occurred.

Phylogenetic approaches and comparative genomics can be used to discover clade innovations and uncover genes that control N-fixing symbioses by comparing close relative species that have maintained or lost this ability. In order to achieve this goal, it is necessary to obtain a well-resolved and well-sampled N-fixing clade phylogeny. More specifically, we sampled 15,000 species from the N-fixing clade and are currently performing targeted-enrichment of DNA for 129 key functional genes involved in

nodulation, as well as over one hundred phylogenetic markers (further details can be found in the poster “NitFix: Global-Scale Phylogenomics of the Nitrogen-Fixing Clade”). In parallel we are pursuing a series of comparative genomic and transcriptomic approaches. These include the identification of conserved non-coding sequence (NCS) based on the alignment of 34 plant genomes, including nodulating species as well as an outgroup (details are described in the poster “NitFix: Identification of conserved non-coding sequences in nitrogen fixing plants”). We also characterized the root transcriptome and chromatin accessibility of the two species of key interest in this study, *Medicago truncatula* (nodulating) and *Populus* (non-nodulating), treated with lipochitooligosaccharides (Nod factors). These analyses identified a series of transcripts and regions of chromatin accessibility that respond to exposure to Nod factors within each species, as well as distinct differences in response between them. These may reflect differences in their ability to nodulate, and are being further evaluated in combination with genome comparisons, to detect target genes to be modified.

In parallel to the discovery of target genes, we are manipulating known genetic components involved in nodule development in a cell-specific manner in poplar (more details in the poster “NitFix: Engineering Root Nodule Symbiosis in *Populus* sp.”). The latter approach has involved developing root cortex and epidermis-specific promoters to modify the expression of genes involved in nodule development, such as Nodule INception (NIN) and cytokinin receptors. This targeted approach has resulted in a significant increase in the number of root lateral organs. We are currently characterizing these lateral organs in poplar and assessing their capability to support the establishment of a symbiotic relationship with N-fixing bacteria.

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