Evolution of root nodule symbiosis & engineering of symbiotic nitrogen fixation in *Populus* sp.

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https://nitfix.org/

Project Goal: Transfer the root nodule symbiosis from legumes to the bioenergy crops *Populus* sp.

Legumes (Fabales) and close relatives of the Fagales, Cucurbitales, and Rosales can associate efficiently with nitrogen-fixing bacteria, in symbioses that lead to the development of root nodules. Legumes, in particular, host bacteria called rhizobia in their root nodules. Genetic studies in model legumes such as *Medicago truncatula* and *Lotus japonicus* identified that (1) rhizobia colonize legume roots intracellularly through the recruitment of the arbuscular mycorrhizal (AM) signaling pathway and (2) root nodules evolved from the recruitment of the lateral root developmental pathway. Essential mechanisms connecting these two processes are cytokinin signaling and the NIN (Nodule INception) protein.

Recent comparative phylogenomic studies suggest that these root nodule symbioses appeared once in the last common ancestor of the Fabales, Fagales, Cucurbitales, and Rosales, and has been lost multiple times within this monophyletic group often called the "nitrogen-fixing clade"[1]. *Populus* sp. are bioenergy crops and close relatives to the "nitrogen-fixing clade". In particular, they seem to possess all the genes known to be required for nodule symbiosis, including *NIN*[2]. *Populus* sp. is easily transformable and represents an excellent model for synthetic biology approaches.

We identified rhizobia and their diffusible signals that can activate the AM signaling pathway in *Populus* sp., as they do in legumes. We studied *Populus* sp. responses to these signals using cell biology (calcium spiking) and transcriptomic approaches (RNA-seq).

We are also working on characterizing the role of cytokinin signaling and the *NIN* genes in *Populus* sp. We overexpressed and knocked-down the *NIN* genes and cytokinin receptors in *Populus* sp. Overexpression of some *NIN* genes and cytokinin receptors led to an increase in lateral root development and, in some cases, the development of nodule-like structures on *Populus* sp. roots. Interestingly, the development of these root lateral structures is activated by cytokinins, which is a critical feature distinguishing root nodules from lateral roots. We are also characterizing the *cis*-elements targets of NIN and other symbiotic transcription factors using ATAC-seq and DAP-seq. We are actively working on trying to get these nodule-like structures colonized by rhizobia.

In the long term, engineering a nitrogen-fixing root nodule symbiosis in *Populus* sp. would greatly enhance biomass productivity on marginal soils and the sustainability of bioenergy production.

References

1. A Resurrected Scenario: Single Gain and Massive Loss of Nitrogen-Fixing Nodulation; van Velzen *et al*., Trends in Plant Science, 2019
2. Phylogenomics reveals multiple losses of nitrogen-fixing root nodule symbiosis; Griesmann *et al*., Science, 2018

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