

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

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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. Studies in model legumes such as *Medicago truncatula* and *Lotus japonicus* identified that (1) rhizobia colonize legume roots intracellularly following the recruitment of the arbuscular mycorrhizal (AM) signaling pathway known as the common symbiosis pathway (CSP), and (2) nodule formation (organogenesis) evolved from the lateral root developmental pathway, and is distinguished from it by the local cytokinin accumulation that requires the transcription factor Nodule Inception (NIN).

Comparative phylogenomics suggest that the root nodule symbiosis appeared once in the last common ancestor of the Fabales, Fagales, Cucurbitales, and Rosales, and lost multiple times within this “nitrogen-fixing clade”¹. *Populus* sp. are bioenergy crops and close relatives to the “nitrogen-fixing clade”. They retain several key 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 demonstrated that certain species of rhizobia can activate the AM signaling pathway in *Populus* sp. 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 *NIN* in *Populus* sp., generating overexpression and knock-down transgenic lines for the members of the *NIN* clade and symbiosis-related cytokinin receptors in *Populus* sp. Overexpression of some *NIN* genes altered root architecture in response to nitrate or cytokinin, and overexpression of cytokinin receptors occasionally produced nodule-like structures on *Populus* sp. roots treated with cytokinin. The initiation of lateral root organs by cytokinin is a critical feature distinguishing root nodules from lateral roots, since cytokinin inhibits the formation of the latter.

We are also characterizing the *cis*-regulatory elements of *NIN* using bioinformatic approaches such as Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) and identifying the targets of *NIN* and other symbiotic transcription factors using DNA affinity purification sequencing (DAP-seq). Furthermore, to understand if protein sequence changes to

NIN were required for nodulation, we are performing cross-species rescue experiments in the *M. truncatula nin-1* mutants.

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. Understanding the function and mechanism of action of *Populus* sp. *NIN* and other symbiotic genes will substantially aid in this process.

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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