Final Result: Characterization of the role of the NPF1B gene in plant root nutritional symbioses

Project: Characterization of the role of the NPF1B gene in plant root nutritional symbioses
University of Vermont
APPROVED as of 06/13/2025

Final Result

Project Director

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Primary Critical Issue

Foundational and Exploratory Research in Agriculture

Fiscal Year

2024

Project Start & End Date

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Organization

University of Vermont

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VT-H02607

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1021202

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Research Capacity Fund (Hatch)

In 2-3 sentences, briefly describe the issue or problem that your project addresses.

In nature, plants obtain much of their mineral nutrients from symbioses with soil microbes, forming **mycorrhizal roots** with specialized fungi and **nitrogen-fixing root nodules** with rhizobium bacteria; a goal of sustainable agriculture is to cultivate these ancient associations to reduce reliance on commercial fertilizer. We have identified a gene, *NPF1B*, associated with both mycorrhization and nodulation, expressed specifically in **infected cells**, the **sites of nutrient transfer** between symbiotic partners. To learn how the same gene can function in two different symbioses, we will examine alternative splicing of the *NPF1B* gene in both mycorrhization and nodulation.

Briefly describe in non-technical terms how your major activities helped you

achieve, or make significant progress toward, the goals and objectives described in your non-technical summary.

To learn the biological function of NPF1B, we determined its pattern of expression, to see when and where the NPF1B gene functions. We found that its expression pattern is strictly limited to infected cells within the symbiotic nodule, suggesting an important role in the cells involved in nutrient transfer. We also found that the function of NPF1B and its close relative, MtLATD/NIP, has diverged, and that neither the NPF1B promoter nor its coding sequence can substitute for LATD/NIP, suggesting that it may perform a different biochemical function, possibly transporting a different substrate or interacting with different molecular partners.

Using a bioinformatic analysis of 71 species, we found that patterns of retention and loss of *NPF1B* were correlated with retention or loss of the ability to form the mycorrhizal symbiosis, an ancient symbiosis important for phosphate nutrition of plants. Statistical analysis showed that mycorrhization is dependent on the presence of *NPF1B* and that retention of *NPF1B* in the genome is dependent on the ability to form mycorrhizae, indicating a fundamental role in this very important and ancient nutritional symbiosis.

We showed that the expression of NPF1B and the closely related LATD/NIP gene are both confined to nodules and to root tips. Both are also expressed in the earliest stages of lateral root and nodule development. We found that the LATD/NIP gene is expressed strictly in the meristem (region of cell division) of both roots and nodules. Curiously, the NPF1B gene is expressed in the adjacent region to that of LATD/NIP in both nodules and lateral roots. In nodules, it is expressed in the infection zone, where the rhizobium bacteria first enter nodule cells, just behind the meristem. In lateral roots, it appears to be expressed in the first part of the elongation zone, just adjacent to the region of cell division where LATD/NIP is expressed.

To answer the question about alternative splicing, we performed long-read direct RNA sequencing experiments on both nodule tissue and root tips. We found that NPF1B does in fact have an additional exon at the 5' end of the gene that was previously unknown. This region does not have an obvious parallel in the closely related LATD/NIP gene. Our long-read RNA seq data will provide an important dataset for the field.

As part of our gene expression studies, we found that the length of LATD/NIP expression in the root tip correlated with growth rate: slow growing roots had shorter zones of *LATD/NIP* expression and fast-growing roots had longer zones, consistent with the observation that slow-growing roots have a shorter and less active meristems than fast-growing roots. Together, these paint the picture of LATD/NIP as a gene that functions within dividing cells to provide a nuanced control of root growth. Turning up or down LATD/NIP function provides the plant with a mechanism to differentially regulate root growth across the root system, slowing growth in some branches, while stimulating it in others.

To learn more about the function of the NPF1B and MtLATD/NIP gene in different environments, we took advantage of a biodiversity panel of M. truncatula collected across their native range. We carried out a Genome Environment Analysis to identify variants of the MtNPF1B and MtLATD/NIP gene associated with differences in root and nodule growth under conditions of different water availability and other soil characteristics. Using this approach, we found 5 sequence changes in *LATD/NIP* SNP's correlated with variables related to water drainage or retention and 2 in MtNPF1B correlated with Precipitation Seasonality and Annual Mean Temperature.

These findings are consistent with LATD/NIP's known role in root elongation and root hair growth, key traits for obtaining water from the soil, and suggest that LATD/NIP could be an important target for plant breeding to improve water use efficiency. The expression of NPF1B in the infection zone of the nodule suggests that it may play an important role in the establishment of this nutritional symbiosis.

Briefly describe how your target audience benefited from your project's activities.

The target audience includes scientists interested in the mechanism of nutrient acquisition in plant nutritional symbioses as well as those interested in sustainable agriculture and soil health. We presented our work which will help them to understand how genetic mechanisms control plant nutritional symbioses.

Briefly describe how the broader public benefited from your project's activities.

We have designed laboratory exercises based on this symbiotic interaction for undergraduate courses geared towards students with an interest in plants and agriculture and identified a role for the MtNPF1B gene in the important mycorrhizal symbiosis and in infection in symbiotic nitrogen-fixation, both important components of sustainable agriculture. Several undergraduate students have learned about plant biology research and beneficial plant-microbe interactions by shadowing graduate and undergraduate students working on this research project.

Comments (optional)

Opportunities for Training and Professional Development:

This has provided an opportunity for graduate student Julie Raiguel to develop expertise in molecular biology and genetics, as well as in establishment of symbiotic plants in the lab. As Covid restrictions on lab occupancy eased, an undergraduate, Annie Gilland, started working with Julie on this project, learning molecular skills and testing the hypothesis that introns regulate the expression of this gene for her Honors thesis. Brailey Buntin, a first-generation college student, has continued this project, again learning basic molecular biology skills and experimental design. David Green, a Masters student, learned bioinformatics using data from this project and Emma Danza, an Accelerated Masters student in microbiology, did her undergraduate research on this project, developing valuable molecular biology and experimental design experience.