# Characterization and Analysis of Mutants Defective in Nodulation and Symbiotic Nitrogen Fixation in the Model Legume Plant *Medicago truncatula*

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## Introduction

The current state of the agriculture is perilous because the widely practiced farming techniques are depleting the nitrogen supplies from the soil. Most plants rely on the nitrogen in the soil for nitrogen fixation, despite the fact that the largest supply of nitrogen exists in the atmosphere, taking up 78% of the atmosphere's makeup. Legume plants are unique because of their ability to form a symbiotic relationship with the soil bacteria rhizobia. Rhizobia infect legume plants and form structures called “nodules” on the roots. Symbiotic nitrogen fixation (SNF) is the natural process that occurs inside the nodules, where rhizobia capture and convert atmospheric nitrogen into the usable form, ammonia. Understanding this process of SNF by finding all the essential genes will help us to transfer SNF process to non-legume plants, which could potentially lead to the creation of farming techniques that are exceedingly more eco-friendly. We are using a forward genetics method in the model legume plant *Medicago truncatula*. Using tobacco *Tnt1* retrotransposon, thousands of *M. truncatula* mutants were created by the Noble Research Institute. By screening ~4000 mutants, Dr. Veerappan isolated more than 200 mutants that are defective in SNF. Here I present data on the phenotypic characterization of the wild type and mutants defective in SNF. Wild type plant phenotypes are green shoots, large, ovoid-shaped and reddish pink nodules whereas the mutants show strong nitrogen deficiency (reddish purple shoots) and also small, round, white nodules (Nod+;Fix-). Each mutant studied contains approximately 20-100 mutations. I will analyze *Tnt1* mutant database and design PCR primers to identify the causative mutation responsible for the defective of SNF phenotypes present in mutants.

## Methodology

The seeds were scarified, sterilized, and vernalized for 5 days, then germinated for 2 days in the dark. They were then grown on the aeroponic system in the presence of nitrogen for 5 days. The plants were then grown in the presence of non-nitrogen containing media for 7 days. At 14 days post germination, the media was inoculated with rhizobia. Images were taken for phenotypic characterization of mutants compared to wild-type, at 14 days post inoculation using a stereomicroscope equipped with a digital camera. At 21 days post inoculation, the rest of the plants were removed from the aeroponic system and root lengths and nodule numbers were measured. Nodules from each plant type were stained with 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-GAL) stained in preparation of sectioning in order to determine the presence of rhizobia bacteria in the nodule.

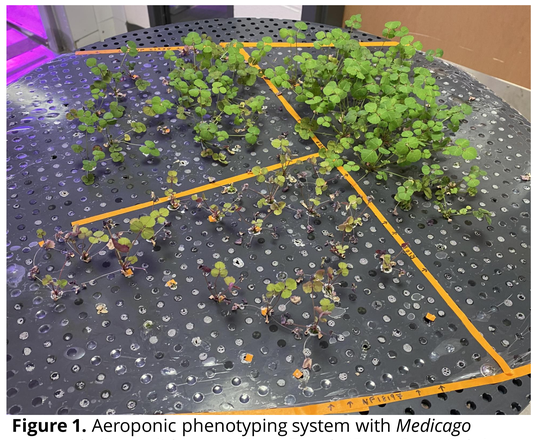

**Figure 1.** Aeroponic phenotyping system with *Medicago truncatula* lines wild-type, NFxxxx7 and NFxxxx0 at 16 dpi.

## Results

### Nodule pictures

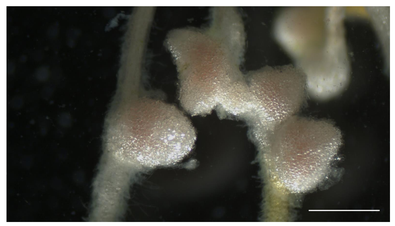

**Figure 2.** Wild-type nodule picture at 14 dpi. Scale bar set at 1 mm. Images captured by

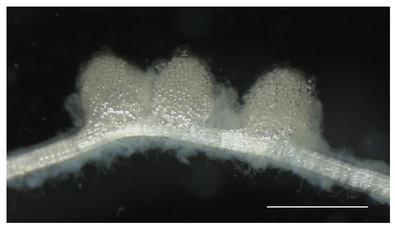

**Figure 3.** NFxxxx7 nodule picture at 14 dpi. Scale bar set at 1 mm.

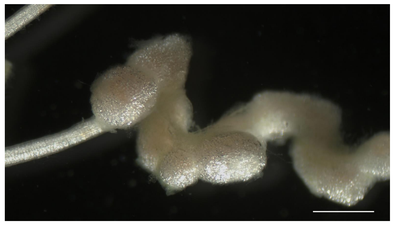

**Figure 4.** NFxxxx0 nodule picture at 14 dpi. Scale bar set at 1 mm.

### X-GAL staining

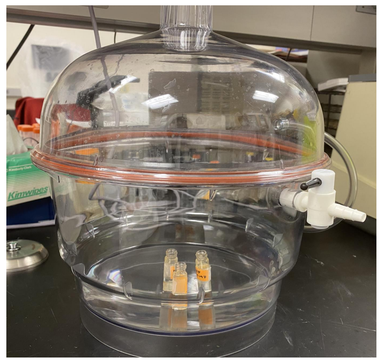

**Figure 5.** X-GAL staining of the wild-type and the mutants. Nodules were fixed using glutaraldehyde and stained with X-GAL staining to visualize rhizobia inside the nodules.

### Plant pictures

**Figure 6.** Phenotypes of wild-type (left) and mutant line NFxxxx7 (right) 23 dpi. Scale bar set at 1 cm.

**Figure 7.** Phenotypes of wild-type (left) and mutant line NFxxxx0 (right) 23 dpi. Scale bar set at 1 cm.

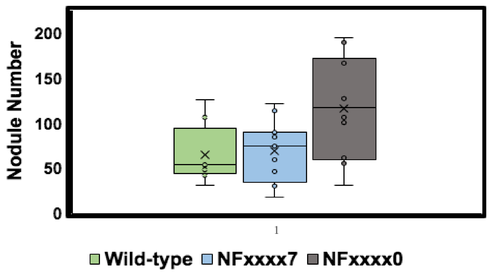


**Figure 8.** Box plot of nodule numbers of wild-type, NFxxxx7, and NFxxxx0. Data recorded from 8 wild-type plants, 11 NFxxxx7 plants, and 10 NFxxxx0 plants. A t-test (2-tailed, 1-type (paired) provided p-values of 0.793 for NFxxxx7 and a p-value of 0.030 for NFxxxx0 when compared to wild-type.

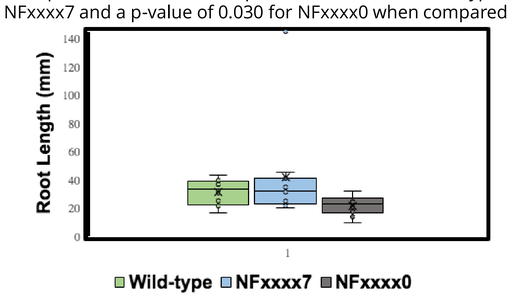


**Figure 9.** Box plot of root length of wild-type, NFxxxx7, and NFxxxx0. Data recorded from 8 wild-type plants, 11 NFxxxx7 plants, and 10 NFxxxx0 plants. A t-test (2-tailed, 1-type (paired) provided p-values of 0.370 for NFxxxx7 and a p-value of 0.029 for NFxxxx0 when compared to wild-type.

## Summary & Future Work

- Symbiotic mutants were isolated in the primary mutant screen Noble Research Foundation, LLC.
- Mutant line NFxxxx7 displayed defective SNF phenotypes including spherical white nodules (Nod+/Fix-) nodule and reddish-purple vegetative parts.
- Mutant line NFxxxx0 did not display defective SNF phenotypes. This mutant produced pinkish white (Nod+/Fix+/-) nodules and the absence of purple vegetation but produced increased nodule numbers.
- Nodule numbers and root lengths of each line were recorded.
- X-GAL staining was successful and vibratome sectioning of nodules to visualize rhizobial occupancy will be performed in the future.
- Future steps includes identification and PCR validation of Tnt1 insertion mutations in mutants.

## References

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