Characterization of *Medicago truncatula* Plant Mutants Defective in Symbiotic Nitrogen Fixation

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**Introduction**

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. Inside the nodules, *rhizobia* capture and convert atmospheric nitrogen into usable form ammonia by a natural process called symbiotic nitrogen fixation (SNF). Understanding this process of SNF by finding all the essential genes will help us to transfer SNF process to non-legume plants, which would decrease cost and increase environmental safety of crop production. 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. We will present data on the phenotypic characterization of mutants NFxxx34, NFxxx97, NFxxx46, NFxxx50, and NFxxx52, defective in SNF in comparison to the wild-type R13B. 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 small, round, white nodules (Nod+Fix−). Each mutant studied contains approximately 20-100 mutations, and in order to determine which mutation causes the defects of SNF, we will analyze Tnt1 mutant database and design PCR primers to further the identification of the causative mutation.

**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 length, and nodule numbers were measured. Nodules from each plant 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.

**Results**

**Nodule pictures**

- Figure 2. Wild-type nodule picture at 14 dpi. Scale bar set at 1 mm. Images captured using stereomicroscope.
- Figure 3. NFxxx97 nodule picture at 14 dpi. Scale bar set at 1 mm.
- Figure 4. NFxxx50 nodule picture at 14 dpi. Scale bar set at 1 mm.
- Figure 5. NFxxx34 Wild-type like nodule picture at 26 dpi. Scale bar set at 1 mm.
- Figure 6. NFxxx34 Mutant nodule picture at 26 dpi. Scale bar set at 1 mm.
- Figure 7. NFxxx52 nodule picture at 26 dpi. Scale bar set at 1 mm.
- Figure 8. NFxxx46 nodule picture at 26 dpi. Scale bar set at 1 mm.
- Figure 9. 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.

**X-GAL staining**

- Figure 10. Phenotypes of wild-type (left) and mutant line NFxxx97 (right) 23 dpi. Scale bar set at 1 cm.
- Figure 11. Phenotypes of wild-type (left) and mutant line NFxxx50 (right) 23 dpi. Scale bar set at 1 cm.

**Plant pictures**

- Figure 12. Box plot of root length of wild-type, NFxxx97, NFxxx50, and mutant and wild-type of NFxxx34. Data recorded from 8 wild-type plants, 11 NFxxx97 plants, 10 NFxxx50 plants, 6 wild-type-like NFxxx34 plants, and 8 mutant NFxxx34 plants. A t-test (2-tailed), 1 degree of freedom, p-values: 0.793 for NFxxx97, 0.0352 for NFxxx50, 0.00376 for NFxxx34 wild-type like, and 0.237 for NFxxx34 mutant, when compared to wild-type.
- Figure 13. Box plot of root length of wild-type, NFxxx97, NFxxx50, and mutant and wild-type of NFxxx34. Data recorded from 8 wild-type plants, 11 NFxxx97 plants, 10 NFxxx50 plants, 6 wild-type-like NFxxx34 plants, and 8 mutant NFxxx34 plants. A t-test (2-tailed), 1 degree of freedom, p-values: 0.370 for NFxxx97, 0.287 for NFxxx50, 0.188 for NFxxx34 wild-type, and 0.984 for NFxxx34 mutant, when compared to wild-type.

**Summary & Future Work**

- Symbiotic mutants were isolated in the primary mutant screen in Noble Research Foundation, LLC.
- Mutant line NFxxx97 displayed defective SNF phenotypes including spherical white nodules (Nod+Fix−) nodule and reddish-purple vegetative parts.
- Mutant line NFxxx50 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.
- Mutant lines NFxxx34, NFxxx52, and NFxxx46 displayed defective SNF phenotypes, as previously described.
- 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 analyzing Tnt1 database to identify Tnt1 insertion mutations in mutants.
- Purify genomic DNA and validate the Tnt1 insertions by PCR amplification

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**References**


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