**Non-Nodulating Mutants to Quantify Nitrogen-Fixation of Legume Crops**

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Legume plants recognise compatible rhizobia partners through the perception of signal molecules, called Nod factors. These molecules are recognised by cross membrane LysM receptor kinases called Nod factor receptors that are located on the host plants roots. The receptors form a complex comprised of NFR1 and NFR5. Perception of compatible Nod Factor signals initiates infection thread formation and nodule development, with knock-out mutations in either receptor resulting in a non-nodulation phenotype.
Non-nodulating legumes can be useful tools in crop breeding programs. They are only able to acquire nitrogen from the soil, compared with commercial varieties that source nitrogen from both soil and through nitrogen-fixation. These mutants can be used to measure the extent rhizobia nitrogen-fixation has on the nitrogen content of varieties that can form nodules.
We have identified NFR5 orthologs in mung bean (Vigna radiata) and cowpea (Vigna unguiculata). Using CRISPR/Cas9 and Agrobacterium tumefaciens mediated stable transformation, we are now generating knockout mutants in both species that can be used in future trials as a baseline for quantifying the extent nitrogen-fixation contributes to nodulating plants. Updates on current progress will be presented.

Nod factor receptors are a cross membrane LysM receptor kinase. They play a crucial role in recognizing rhizobial symbiotic signal molecules (nod factors) and initiate infection thread formation, leading to nodule development. The receptor complex comprises NFR5 and NFR51. Orthologs of NFR5 have been identified in various legumes, including L. japonicus, M. truncatula, P. sativum, G. max, A. hypogaea, and C. arietinum. Mutations in NFR5 cause significant disruption to nodulation.

The aim of this study is to identify the NFR5 ortholog in mung bean and generate CRISPR/Cas9 knockout plants, examining downstream impacts. We hypothesize that a CRISPR/Cas9-mediated knockout of NFR5 using an Agrobacterium tumefaciens mediated stable transformation system will produce a heritable nod- trait with no additional downstream effects.

Given the recalcitrance of many legumes to tissue culture techniques, optimizing current stable transformation methods is crucial. Agrobacterium-mediated transformation has emerged as the preferred option for legume stable transformation, although techniques vary among crops. In this study, we will explore and optimize Agrobacterium-mediated transformation for mung bean, focusing on factors such as explant type, co-cultivation conditions, and selection pressure. The identified NFR5 ortholog in mung bean will be targeted for knockout using CRISPR/Cas9, and the resulting mutants will be characterized phenotypically and genotypically to assess nodulation ability and any potential off-target effects. The resulting nod- mutant will provide a useful control to accurately quantify nitrogen fixation in similar crops.