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Literature Review

Nitrogen-fixing symbiosis is a mutually beneficial relationship between bacteria and plants. Bacteria called rhizobia perform nitrogen fixation, providing legumes with a source of usable nitrogen, while the plants provide protection, and for the bacteria's energy and nutrient needs. All organisms need nitrogen, as it is one of the main elements in protein and nucleic acids. However, while nitrogen is abundant in Earth's atmosphere, it exists in a form that is inaccessible to plants and animals—nitrogen gas. Only prokaryotes are able to modify the nitrogen into compound forms like ammonium that plants and animals can incorporate. Thus, this process of nitrogen fixation is important both in natural ecosystems and agriculture.

While it is uncertain why eukaryotes seem unable to fix nitrogen, the major enzyme used in this process, nitrogenase, is inhibited by oxygen. Thus, prokaryotes apply strategies such as living in places with little oxygen, burning oxygen extremely quickly, or, in the case of rhizobia, forming a symbiotic relationship with plants that can shield them from free oxygen. *Medicago truncatula*, the barrelclover is a well-established model organism for legume research. It spreads root hairs into the soil that curl to trap rhizobia they encounter. It then grows a channel that allows the bacteria to form an infection thread into the plant. Once inside the plant, rhizobia is engulfed by plant membranes through endocytosis, forming symbiosomes that essentially become ammonium-producing organelles. *M. truncatula* also forms nodules to which it sends leghemoglobin to trap free oxygen and prevent it from disrupting the bacteria's nitrogen fixation.

Various types of genes are necessary for the transition to symbiosis. The relationship is initiated by an exchange of flavonoids from the plant and lipochitooligosaccharides called Nod factors (NF) from rhizobia (Oldroyd, 2013). The plant detects Nod factors with receptors that have LysM domains which facilitates nodule organogenesis Other downstream effects in *M. truncatula* include a spike in Ca-Calmodulin-dependent protein kinase (CCamK) which results in calcium oscillation, and phosphorylation of CYCLOPs. This signal cascade is essential for nodule organogenesis (Wang, 2022).

In addition to Nod factors, the legume's receptors recognize bacterial surface exopolysaccharides (EPS). These signals are required for infection progression and proper infection thread formation after Nod factors have been detected (Kawaharada et al., 2015). Furthermore, maturation of symbiosomes after rhizobia have penetrated deep into plant tissues require workable copies of the plant gene, DNF1. It codes for a subunit of the signal peptidase complex (SPC), a 4-subunit protein complex that cleaves signal peptides off newly translated proteins entering the endoplasmic reticulum. This protein modification is essential for protein secretion, which controls maturation of the symbiosome (Wang, 2022).

Many steps in these pathways are still poorly understood, as are the necessary molecular components and mechanisms necessary for nitrogen-fixing symbiosis. Thus, candidate genes were tested for a potential role in this process. The hypothesis is that the genes Nod26.1 and Nod26.2 are required due to being related to previously-established essential nodulation genes. The genes will be tested through genetic knockout in *M. truncatula* using the CRISPR/Cas9 system to determine if the deletion impacts symbiosis with rhizobia.

If the CRISPR construct is successfully cloned against the gene and the gene is deleted from the plant as expected, then an inability of the plant to enable rhizobia symbiosis would indicate the importance of that gene's function in this process. The plant's failure to form a successful relationship with the bacteria could be manifest in several ways, including a failure to capture bacteria with root hairs, a failure to extend the infection thread, inhibited nodule organogenesis, or disrupted leghemoglobin production/localization. Were such a case observed, it could be inferred that the gene in question plays an important role in that function. If no such effect is observed to any degree, then perhaps the gene has little or no effect, or the plant has alternative methods to achieve successful symbiosis.

Works Cited

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