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Unveiling epigenetic regulation in the nitrogen-fixing symbiosis of *Medicago truncatula*

Nitrogen is an important macronutrient and of great significance since plants require it in large quantities to synthesize DNA, proteins, and other essential biomolecules. In legumes, Nitrogen-fixing bacteria, such as Rhizobium species, transform atmospheric nitrogen (N_2) into ammonia (NH_3) in specialized root nodules, which the plant utilizes for its growth. While significant transcriptomic studies have been conducted in legume plants, the role of epigenetics in regulating nitrogen fixation is still relatively underexplored. The goal of this study is to address this knowledge gap and understand the epigenetic mechanisms that regulate this process. The field of Epigenetics studies gene expression changes that are frequently mediated by processes such as DNA methylation and histone modification which do not alter the DNA sequence. Nevertheless, these alterations can significantly impact plant characteristics, including nitrogen fixation, by altering the expression of key genes involved in this process. The principal region responsible for active nitrogen fixation in the nodule is zone III, also known as the fixation zone.

This research focuses on discovering histone modifying genes that are actively regulated in the nitrogen fixation zone. To this objective, we have identified two genes that are highly expressed within the zone III. Using CRISPR technology, we are creating mutants and analyzing the physiological function of these genes to understand their role in symbiotic nitrogen fixation. In addition, we are cloning the 3 Kb upstream region of these promoters to analyze their symbiotic expression profiles. Through this research, we will understand how these genes contribute to symbiotic nitrogen-fixing and identify potential signaling pathways for enhancing nitrogen fixation efficiency in legume plants, resulting in minimizing reliance on synthetic fertilizers.