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CRISPR-Cas9 Mediated Targeted Mutagenesis to Assess the Function of *MtBAK1* and Expression in the Roots and Nodules of *Medicago Truncatula.*

Small signaling peptides are powerful regulators of diverse biological processes. In plants this includes nitrogen acquisition through the modulation of root development and interactions with beneficial bacteria, such as rhizobia. Peptides are short fragments of longer polypeptide chains that bind to cell surface receptors, initiating downstream signaling cascades. Plant peptides predominantly bind to receptors in the LRR-RLK (Leucine-Rich Repeat Receptor-Like Kinases) family, triggering biochemical activities. However, not only the receptor but also functional co-receptors are required for signal transduction. These co-receptors can be shared by multiple signaling pathways. In the non nodulating model plant *Arabidopsis thaliana,* AtBAK1 (Brassinosteroid insensitive1-associated receptor kinase) is a co-receptor necessary for plant associations with pathogenic bacteria and the brassinosteroid hormone signaling pathway. In the legume *Medicago truncatula*, there are six orthologs of the Arabidopsis BAK1, making it challenging to determine its function in plant symbiosis signaling pathways. BAK1, a membrane-bound co-receptor, activates its kinase domain through transphosphorylation when bound to ligand-stimulated transmembrane receptors. Using the CRISPR-Cas9 reverse genetic approach, this study targets all five orthologs of the MtBAK1 gene in *Medicago truncatula*, aiming to characterize BAK1's function in root nodule symbiosis. The study will clone three guide RNAs into the pDIRECT23C vector backbone using the golden gate cloning system. Correct assembly of all three guide RNAs will be verified through sanger sequencing. In addition, efforts will be made to amplify the promoters of the following genes: Medtr2g008360.1, Medtr2g008370.1, Medtr2g008380.1, Medtr2g008390.1, and Medtr2g008400.1. The Golden Gate cloning method will be employed to efficiently and precisely assemble multiple DNA fragments, utilizing the unique properties of Type IIs restriction enzymes. This method will facilitate the introduction of these amplified promoter sequences into *Medicago truncatula* plants, which are model legumes, to study their expression in roots and nodules. The transformation process will involve the use of *Agrobacterium rhizogenes*, which induces the formation of hairy roots that are genetically modified to express the target genes. Once the transformation is successful, these hairy roots can be cultivated in vitro, allowing for controlled monitoring of gene expression. GUS (β-glucuronidase) staining will serve as a reporter system to visualize gene expression and activity, enabling an assessment of the effectiveness of the gene constructs introduced through Golden Gate cloning. By analyzing the expression levels of the targeted genes in both the roots and nodules, insights can be gained into their roles in plant development, symbiotic interactions, and nutrient uptake. The nodules are particularly important, as they house nitrogen-fixing bacteria that enhance the plant’s ability to utilize atmospheric nitrogen. Techniques such as quantitative PCR or gene expression assays may be employed to quantify expression levels, providing valuable data on how these genes contribute to the overall growth and function of *Medicago truncatula*.