Characterization of the expression patterns of two receptor like kinasesin Medicago truncatula during root nodule symbiosis

Nitrogen fixation is a process that converts atmospheric nitrogen (N2) into a form that can be used by plants for growth and development. Through a symbiotic relationship with the bacteria rhizobia, Medicago truncatula, a model organism, offers an insightful look at the genetic components that allow this to happen. Medicago has many similarities to crop legumes such as Alfalfa and has a well documented genome making it a great organism to work with. In this study, efforts are being made to amplify the promoters of the two LRR-RLKs (Leucine Rich repeat-Receptor like kinases) genes: Medtr7g081410 and Medtr4g037720 using PCR amplification. A total of 3,000bp upstream sequence of the LRR-RLKs genes was targeted to amplify the promoter region. The amplified promoter sequence was then separated using agarose gel and electrophoresis. Golden Gate assembly protocol will be used to efficiently assemble multiple DNA fragments, using Type IIs restriction enzymes. The promoter sequences of both the genes will be cloned and introduced into M. truncatula plants, to study their expression in roots and nodules. Agrobacterium rhizogenes will be used for the transformation process, which induces hairy root formation. After the successful transformation, hairy roots will be cultivated in vitro, allowing for controlled monitoring of gene expression. B-glucuronidase (GUS) staining will be used as a reporter system to monitor and visualize gene expression and activity. The expression data of these two genes in the roots and nodules of M. truncatula plants will help to understand the role of these genes in symbiotic interactions, root development and nutrient uptake. Insight about the expression of these genes in nodules are of particular importance, as they harbor nitrogen-fixing bacteria which help to fix and utilize atmospheric nitrogen without synthetic fertilizer use.