

## Using CRISPR/Cas9 in Hairy Roots System to Explore the Gene Functions during Peanut Nodulation

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With the available allotetraploid peanut genomes, further functional genomics studies of the genes in peanut are very critical for peanut crop improvement. As an important legume crop, peanut plant forms root nodules to fix nitrogen through a symbiotic relationship with rhizobia. However, the nitrogen fixation efficiency in peanut is relatively low comparing with many other legume crops. In addition, rhizobia infect the peanut plants via an intercellular crack entry, which is a very different invasion path from the well-studied root hair invasion path as showing in model legume species and many other legume crops. Whether there is any connection between the crack entry or non-root hair invasion in peanut and low nitrogen fixation efficiency remains as a big question. Understanding the non-root hair rhizobial invasion process in peanut will not only reveal novel molecular mechanisms of an antecedent invasion process in legume symbiosis, but also will provide knowledge to enhance crop production while to reduce fertilizer input and promote sustainable agriculture.

CRISPR/Cas9 system is emerged as a robust tool for gene functional study and crop improvement, which haven't been extensively utilized in peanut yet. We have established a system to transfer the CRISPR-Cas9 constructs targeting genes involved in peanut symbiosis and nodulation to peanut hairy roots for gene function validation. As an example, we have applied the CRISPR/Cas9 in peanut hairy root transformation system to explore the function of Nod factor receptors (*NFR*) genes, which play an important role in initiating the rhizobia symbiosis in model legume species. Through gene sequence retrieval, qRT-PCR, making CRISPR construct, hairy root transformation, phenotypical evaluation of transformants, and target gene sequencing of transformants, we found that targeted genes were successfully edited by the CRISPR system though chimeric events existed. The mutants with editing in the two *AhNFR5* genes showed non-nodulating phenotype, thus validated the function of *AhNFR5* genes in nodule formation in peanut. The results demonstrated the efficacy of the genome editing tool established for targeted mutation in allotetraploid peanut, which can be used in peanut hairy root transformation system for peanut functional genomic studies, specifically on genes functioning in roots.