CRISPR-Cas9- mediated gene knockout of defense response receptor in nitrogen-fixing legume model plant

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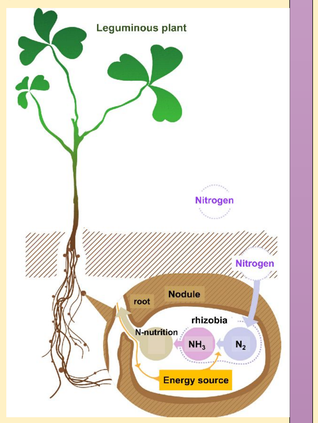
Background

- Medicago truncatula and rhizobia have a symbiotic relationship in which M. truncatula allows the rhizobia to invade its roots and form nodules to symbiotically perform nitrogen-fixing.
- LYM1 and LYM2 (defense receptor proteins) are thought to be cleaved by Dnf2 (Pi-PLC enzyme) during the initial rhizobia infection process.



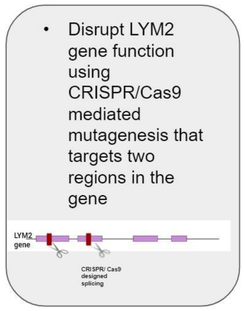
Question: What role does the LYM2 protein play in the infection process of legumes by rhizobia for nitrogen-fixing?

HYPOTHESIS: If the CRISPR/Cas9 machinery is successfully transformed into the plants, the PCR and sequencing will show a deletion in the LYM2 gene.

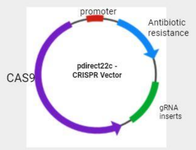
Experimental Design

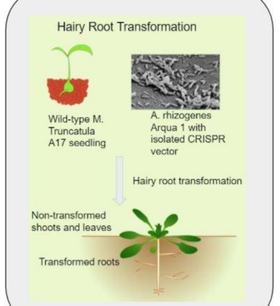
- CRISPR/Cas9 design via Golden Gate reaction
- Transgenic mutants via hairy root transformation

- Disrupt LYM2 gene function using CRISPR/Cas9 mediated mutagenesis that targets two regions in the gene



- gRNAs are assembled into the CRISPR construct via Golden gate reaction





- Confirm mutations via PCR and Sequencing

Results

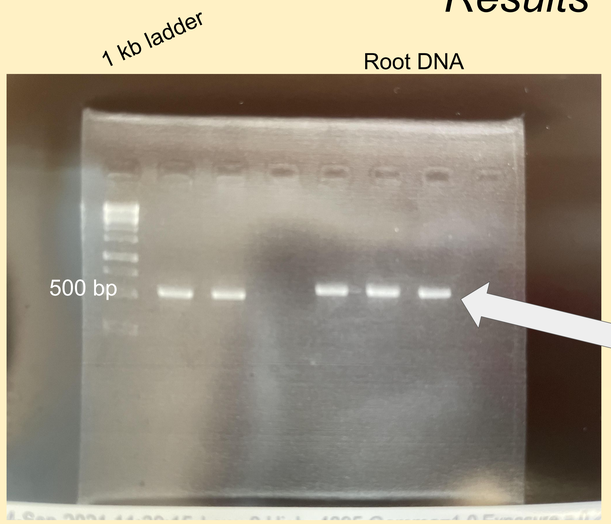


Figure 1. LYM2 CRISPR Vector 1 PCR and gel.

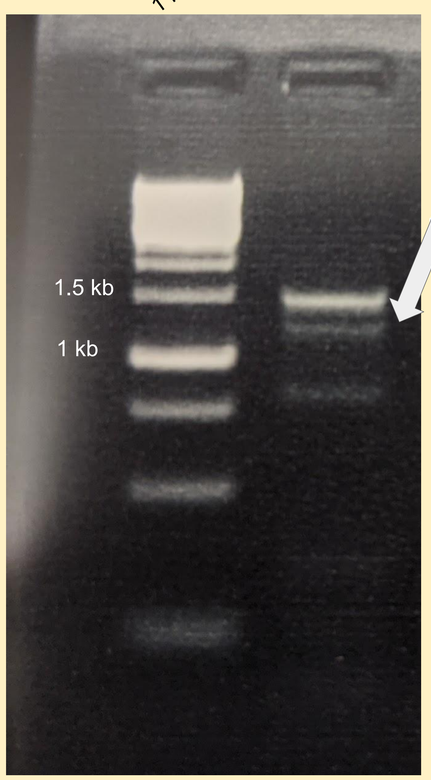


Figure 2. LYM2 CRISPR Vector 2 PCR and gel.

- Expected deletion DNA band 1200pb

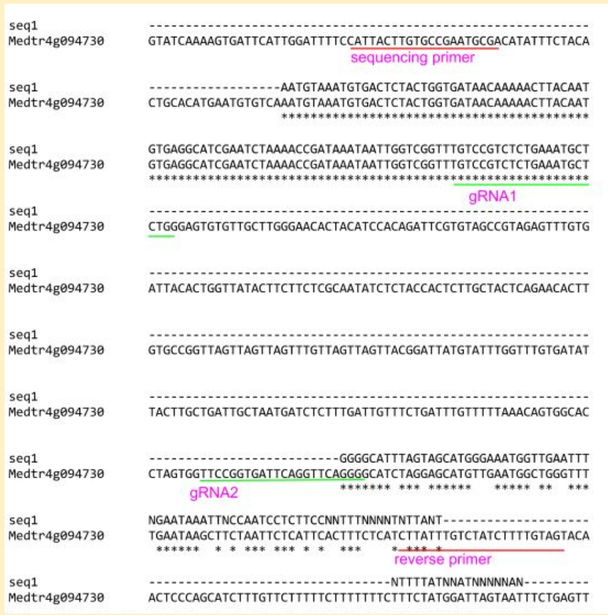


Figure 3. LYM2 CRISPR Vector 2 Sequencing.

Discussion

- PCR of transgenic root DNA from vector 1 does not show the expected deletion band size (Figure 1), implying that the CRISPR vector was not successful in transforming the roots.
- PCR of transgenic root DNA from vector 2 confirms expected band size (Figure 2). Further, the LYM2/ dnf2 root DNA has the expected deletion band in its sequencing results (Figure 3). This confirms that the CRISPR construct and the Cas9 machinery worked correctly and cut the gene into sizes that were expected.

Future directions

- Next, we will test the exact function of LYM2 proteins in its defense role in symbiotic nitrogen-fixing using the same methods.

Further, it has been suggested that LYM1 and LYM2 form a defense complex which can be further researched.

References and Acknowledgements

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