Generating CRISPR Mutations in the *ASYMMETRIC LEAVES1* Gene of Arabidopsis

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Introduction

This project targets the *AS1* (Asymmetric Leaves1) gene in *Arabidopsis thaliana*, which we believe works in conjunction with the *AS2* gene to keep the *KNOX* genes turned off. Plants have both *AS1* and *ASF1* genes, while humans and most animals only have *ASF1*. Previous studies have found that *HIRA* interacts with *ASF1* in animals, but no evidence exists for the interaction in plants, leading to the theory that this is what allows plant cells to un-differentiate. It is thought that together *AS1* and *HIRA* promote leaf development by repressing *KNOX* genes, which control the undifferentiated cell state[1].

We believe that differences in differentiation occur because plant *HIRA* binds to *AS1*, not *ASF1*. We hope that by disrupting *AS1* and *HIRA* binding through CRISPR/CAS mutations, we can gain more insight into how the binding influences differentiation[2].

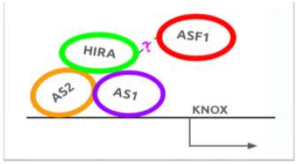

Image 1: theoretical binding of AS1 and 2 to HIRA and ASF1

It is thought that the non-myb domain C-terminal region of *AS1* is what binds *HIRA*, but we don't know exactly where in the region this occurs, or what amino acids are involved. Our mutations are intended to disrupt this binding, and help us narrow down what binds where[2].

Methods

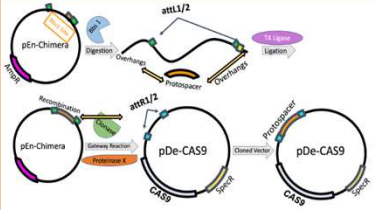

Image 2: Diagram of the cloning process[5]

Cloning methods: pEn-Chimera was digested with the restriction enzyme Bbs I. Protospacers *AS1 29-0* or *22-9* were combined with the digested pEN-Chimera. The insertion of the protospacers into pEN-Chimera was confirmed through PCR and gel electrophoresis tests[3].

Transformed cells are selected using antibiotics, cultured, and then the Arabidopsis flowers are dipped into the solution. The plants are then grown until there are seeds that can be harvested and planted[4].

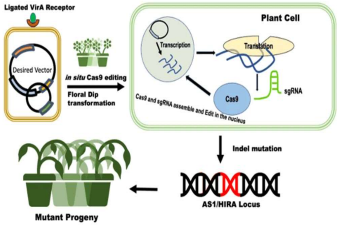

Image 3: Transfer of vector into plants[5]

CRISPR Explained

CRISPR stands for clustered regularly interspaced short palindromic repeats, and are short repeated DNA sequences in the E. Coli genome. It's part of the bacteria's viral defense system, allowing the bacteria to cut up invading viruses and store some of the DNA. If re-exposed to the same virus, the bacteria will recognize it through an enzyme called Cas9 that uses the stored DNA to recognize viruses. CRISPR has since been modified to be able to target and cut selected gene sequences in a variety of organisms, allowing researchers to remove genes from a genome, leaving a gap where they can also insert a different gene if needed[6].

Results

We have successfully transferred our vectors into Arabidopsis seeds, and now have fully-grown mutant plants. We are currently working on analyzing the genotypes and phenotypes of our CRISPER mutants

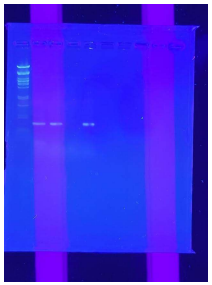

Image 4: Example of gel electrophoresis, used to confirm the presence of protospacer and primers

Image 5: Spectinomycin plates displaying growing bacterial colonies, this is a sign our PDE-cas9 transformation has worked

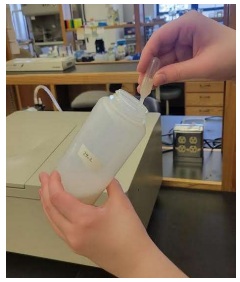

Image 6: Infiltration medium containing the transformed agrobacterium and carrier liquid

Image 7: Dipping the Arabidopsis plants into the suspended agrobacterium

Image 8: Plated seeds from dipped plants, seeds containing our protospacer will be immune to the herbicide in the plate and grow, seeds without mutations will not grow

Image 9: the same plate after about a week, the green plants are showing resistance to the herbicide plate, indicating the presence of our protospacer. They should contain AS1 mutations

Image 10: My first transgenic plant after being transplanted, this plant should display *AS1* mutations

Image 11: Example of the transplanting process with wild type plants, plants are repotted and given more space to promote growth

Future Directions

Moving forward, we hope to continue working on modeling *AS1* and other proteins, doing more analysis of our plants, and potentially crossing my *AS1* mutants with *HIRA* mutants created by other research groups within our lab.

Image 12: A model of the *AS1* protein generated from an amino acid sequence using the I-TASSER-MTD protein prediction program. Region 1 (red) contains the MYB domain, and region 2 (blue) is the very end of the protein

Images 13 and 14: wild-type and *AS1* mutant plants

References

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