Investigating the Molecular Function of Mutated *Arabidopsis* AS2 Gene

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Abstract

This study aims to more deeply understand the role of the *ASYMMETRIC LEAVES 2 (AS2)* gene in *Arabidopsis*. During leaf development, the AS2 protein keeps the *KNOX* genes turned off so that leaves can develop. We use CRISPR technology to create mutations in specific regions of AS2. These mutations are predicted to change the protein structure of AS2, which may result in a mutated leaf. The analysis of the mutation allows us to better understand how AS2 keeps the *KNOX* genes off.

Introduction

AS2 (ASYMMETRIC LEAVES 2) is a gene that encodes a protein regulating the expression of the *KNOX* genes in plants. *KNOX* genes are responsible for keeping cells in an unregulated state. Once leaves start to form the *KNOX* genes must be turned off and kept off for normal leave formation. AS2 functions to suppress *KNOX* gene expression during leaf development, although the exact mechanism remains largely unknown.

We created CRISPR tools to induce mutations in a specific region of the *Arabidopsis AS2* gene. CRISPR is a laboratory tool that causes mutations at specific regions of a gene. Once the tools are created, they are transformed into plants. The plants are then examined for mutant phenotypes. Plants with the mutant phenotype have their DNA sequenced to confirm a mutation in AS2.

Methods

Figure 1

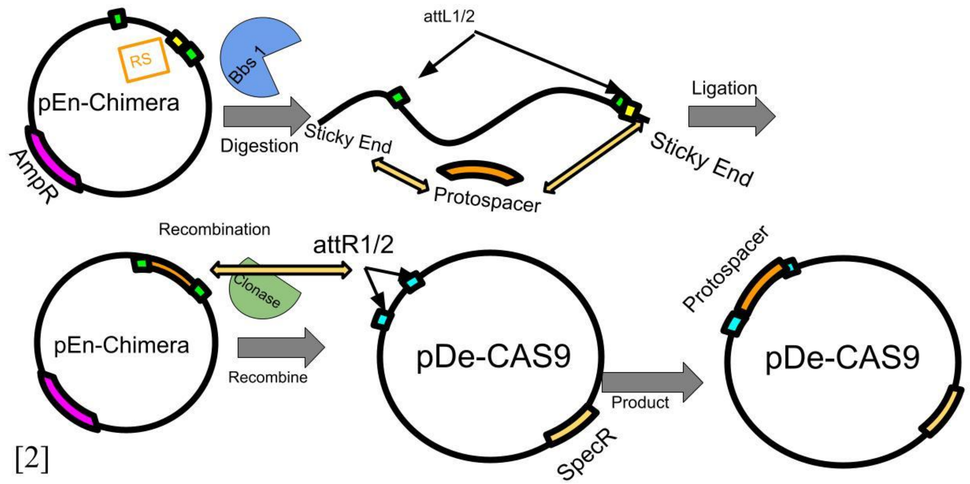

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Figure 1: Overview of CRISPR Tool Creation.

- Insert protospacers (0-2370) into Bbs 1 digested pEn-Chimera vector by ligation reaction
- Transform into E. coli bacteria plates
- Vector containing colonies are selected and E. coli colonies are cultured in liquid broth media culture
- DNA is isolated from these cultures
- Polymerase chain reaction (PCR) and gel electrophoresis employed to verify successful cloning of protospacers
- Resulting vector is replicated through PCR to amplify DNA segment for further experimentation

Results

Figure 2

(A) (B) (B) (C)

A. A computer model of the AS2 protein within the *Arabidopsis* plant.
B. A picture of an AS2 Mutant
C. A picture of a wild type plant.
D. PCR confirmation of the presence of the 0-2370 vector in E. Coli shown through gel electrophoresis

Conclusion

So far, the insertion of the 0-2370 segment into the pEn-Chimera has yet to be confirmed due to a failed transfer into the pDe-Cas9 vector. Once a successful transfer has taken place, the transformed vectors will be introduced into *Arabidopsis* plants to induce mutations within the *AS2* gene. This approach will shed light on AS2's role in proper leaf development.

References

[1] Machida, Y., Suzuki, T., Sasabe, M., Iwakawa, H., Kojima, S., & Machinda, C. (2021). Arabidopsis ASYMMETRIC LEAVES 2 (AS2): roles in plant morphogenesis, cell division, and pathogenesis. *Journal of Plant Research, 135*(1), 3-14. https:doi.org/10.1007/s10265-021-01349-6

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