The Impact of Mutations in the *Arabidopsis* Apetela (AP3) Gene

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Abstract

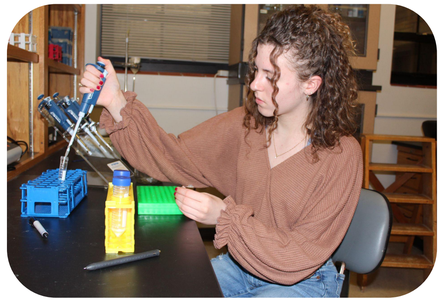
The purpose of this research is to understand the molecular functioning of the *Arabidopsis thaliana* Apetela (Ap3) gene. We created mutations in two sites of the gene, AP3-3 and AP3-5. These are predicted to change Ap3 protein structure, which may result in a mutated flower. Analyzing the effects of new mutations allows an understanding of protein formation both in plants and humans.

Introduction

AP3 is the gene that encodes a protein involved in the proper development of floral anatomy in *Arabidopsis*. The function of AP3 is to specify petal and stamen identities[2]. The stamen is the plant's male reproductive organ, responsible for producing pollen. The petals attract pollinators and protect the floral reproductive organs. In the diagram below[1], the darker colors show the amount of AP3 gene expression in a mature *Arabidopsis* flower. CRISPR technology will be used to mutate two regions of AP3. The CRISPR-Cas9 systems with the genetic guide sequences are then transformed into the *Arabidopsis* plants. The CRISPR system then makes mutations in the AP3 gene, at sites AP3-3 and AP3-5.

AP3 gene expression in a mature Arabidopsis flower

Methods

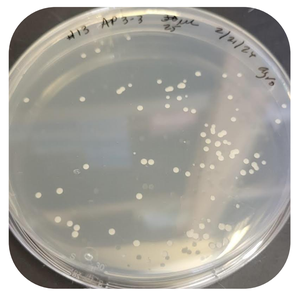


The methodology used in this experiment includes cloning the AP3-3 and AP3-5 protospacers into a pEn-Chimera vector. This vector was transformed into the common bacteria *Escherichia coli*. DNA was isolated from the cultures and then the cloning of the protospacers were confirmed through PCR and gel electrophoresis. The protospacers were recombined into a pDe-CAS9 vector, which was confirmed using gel electrophoresis, showing bands of DNA at 5.9, 5, and 3.8 kilobases. 25 µL of agrobacterium cells were electroporated with 1 µL of the pDe-CAS9 and the agrobacterium was plated.

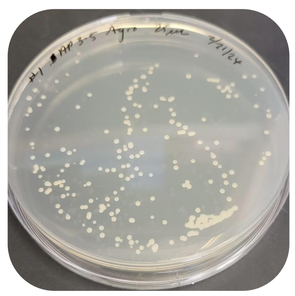
Results & Future Work

The agrobacterium showed growth on plates indicating positive results from the electroporation. This leads into the next steps of the experiment, including dipping the *Arabidopsis* plants into the agrobacterium, which is commonly used to transfer genes into plants using horizontal gene transfer[3]. The plant's flowers will be dipped into the agrobacterium, which will transfer the pDe-CAS9 vector to cut and replace part of the AP3 gene (AP3-3 or AP3-5, depending upon which vector it is sequenced with). While waiting for the plants, we will generate 3D models of the normal protein and examine the structure using modeling software. Pictured to the right are the petri plates from February 21, 2024, with AP3-3 (A) and AP3-5 (B) transformed agrobacterium colonies.

(A)



(B)



Conclusion

In an exploration of the *Arabidopsis* floral genes in particular, the procedures will be repeated for the AG1 and AP1 gene fragments. The findings of this experiment are expected to be shared to support the widespread biological research of the *Arabidopsis* plant. Due to *Arabidopsis* being related to cabbage, broccoli, and other economically important crops[1], it is beneficial to understand the protein formation among similar organisms.

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Dr. Tara Phelps-Durr and mentee Hazel Frans

References

[1] Arabidopsis: The model plant. bio0202 - Members of the Multinational Arabidopsis Steering Committee. (2002). https://www.nsf.gov/pubs/2002/bio0202/model.htm

[2] Locus: AT3G54340. AT3G54340(AP3). (n.d.). https://www.arabidopsis.org/servlets/TairObject?id=39428&type=locus

[3] Matveeva, T. V., & Otten, L. (2019). Widespread occurrence of natural genetic transformation of plants by Agrobacterium. Plant molecular biology, 101(4-5), 415–437. https://doi.org/10.1007/s11103-019-00913-y