Characterizing new mutations of *ARF6* & *ARF8* in *A. thaliana*

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

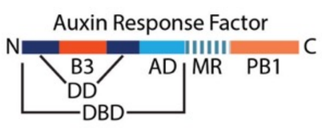
Rashmi Ramanujam, Jason W. Reed, Punita Nagpal
UNC-Chapel Hill, Department of Biology

Abstract

Auxin is a phytohormone that participates in various developmental and maturation processes in the *Arabidopsis thaliana* plant. The presence of the hormone induces ARF transcription factor activation through the turnover of Aux/IAA repressors in a conserved auxin signaling pathway. *ARF6* and *ARF8* genes are examined in this research. Single mutants, *arf6-2* and *arf8-8* resemble a WT Columbia phenotype. Double mutants, *arf6-2 arf8-8* have the most severe phenotype with growth arrest, closed flower buds, and indehiscent anthers. CRISPR-Cas9 was used to generate putative knock-out and gain-of-function mutations by targeting the N-terminus and C-terminus, respectively. Through flower dissections and statistical analysis, N-terminal mutations in *ARF6* and *ARF8* yielded an identifiable double mutant phenotype that resembles a "knock-out" of function of ARF proteins with arrested growth, closed buds, and indehiscent anthers. Putative gain-of-function (PB1 domain) mutants yield a weaker double mutant phenotype but have curved and elongated fruits. One PB1 domain mutant (ΔG) had a premature stop codon with a weaker phenotype than other PB1 mutants, potentially pointing to a "gain" of ARF function. With this research, the collective effect of ARF function and Aux/IAA repression on flower maturation and development can be understood.

Background

- *ARF6* and *ARF8* regulate stamen and gynoecium maturation and coordinate pollen release.[2]
- *arf6 arf8* double mutants have arrested buds, short stamen filaments and petals, and indehiscent anthers.[3]
- Single *arf6* or *arf8* mutants have weaker phenotypes than double mutants and have delayed petal and stamen growth.
- In a spring 2023 CURE class, CRISPR-Cas9 constructs were made to mutate *ARF6* or *ARF8 in arf8 and arf6* null mutant backgrounds.



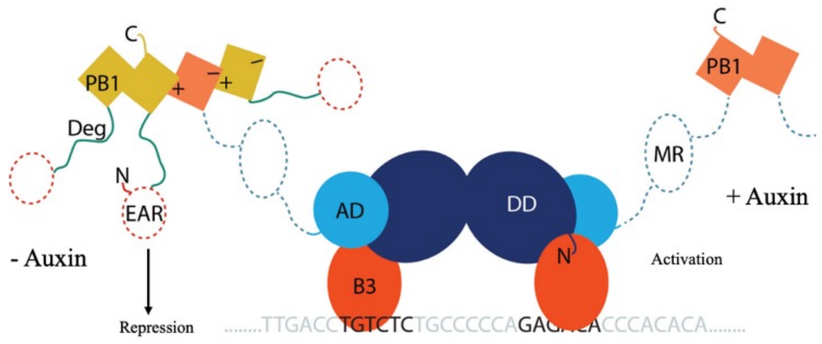


Figure 1. Auxin response mechanism.[1]

N-terminal *ARF6/8* mutations

- *arf6-x arf8-8* and *arf6-2 arf8-cko-x* mutant knock-out plant heights and organ lengths resemble those of the reference double null mutant *arf6-2 arf8-8*, with some organ length experimental variation.
  - Exception: Plant *arf6-cko-67C arf8-8* resembles a weak loss-of-function phenotype
  - Interest: Plant *arf6-2 arf8-cko-11B* has a 12 aa deletion and resembles the null.

Figure 2. *ARF6/8* knock-out mutant plant heights and organ lengths. A-C) *ARF6 KO* plants. D-F) *ARF8 KO* plants. Lowercase letters correspond with statistical analysis using the Tukey's HSD LS Means Differences test.

Acknowledgements:
- Dr. Jason Reed
- Dr. Punita Nagpal
- Undergraduates: Allison Musty, Anna Crist
- Spring 2023 CURE Class
- Jessica McAfee
- Dr. Eric Hastie

References:
1. Wright, R. C., & Nemhauser, J. L. (2015). New tangles in the auxin signaling web. F1000prime reports, 7, 19. https://doi.org/10.12703/P7-19
2. Nagpal, P., Ellis, C. M., Weber, H., Ploense, S. E., Barkawi, L. S., Guilfoyle, T. J., Hagen, G., Alonso, J. M., Cohen, J. D., Farmer, E. E., Ecker, J. R., & Reed, J. W. (2005). Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. Development, 132(18), 4107–4118. https://doi.org/10.1242/dev.01955
3. Reeves, P. H., Ellis, C. M., Ploense, S. E., Wu, M.-F., Yadav, V., Tholl, D., Chételat, A., Haupt, I., Kennerley, B. J., Hodgens, C., Farmer, E. E., Nagpal, P., & Reed, J. W. (2012). A Regulatory Network for Coordinated Flower Maturation. PLoS Genetics, 8(2), e1002506. https://doi.org/10.1371/journal.pgen.1002506

PB1 domain *ARF6/8* mutations

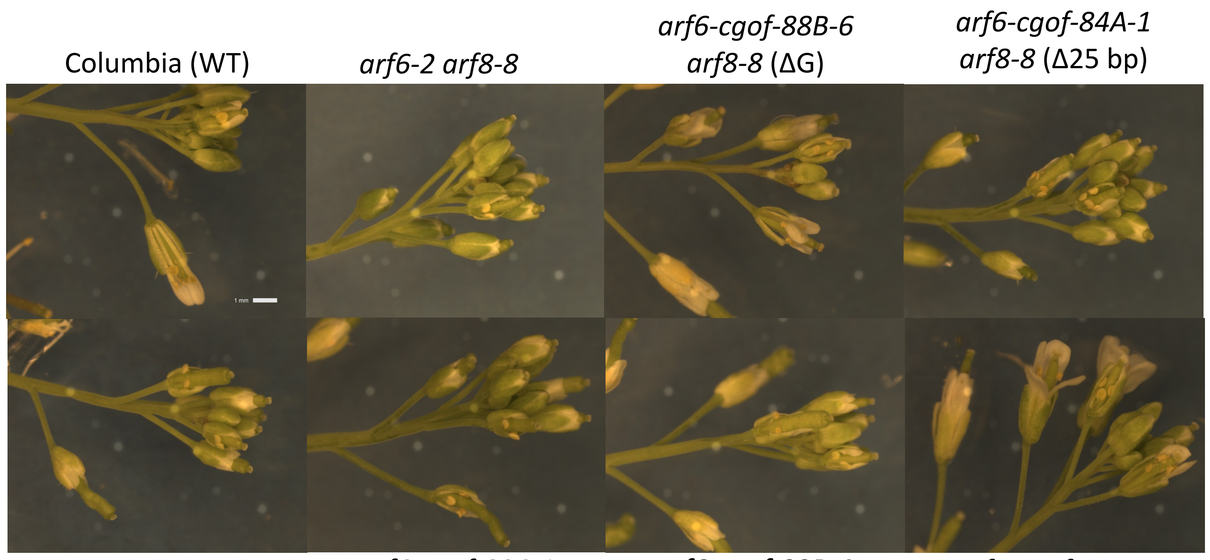


Figure 3. Inflorescence images of *ARF6* PB1 domain mutants. Scale bar in the "WT" panel corresponds to 1 mm.

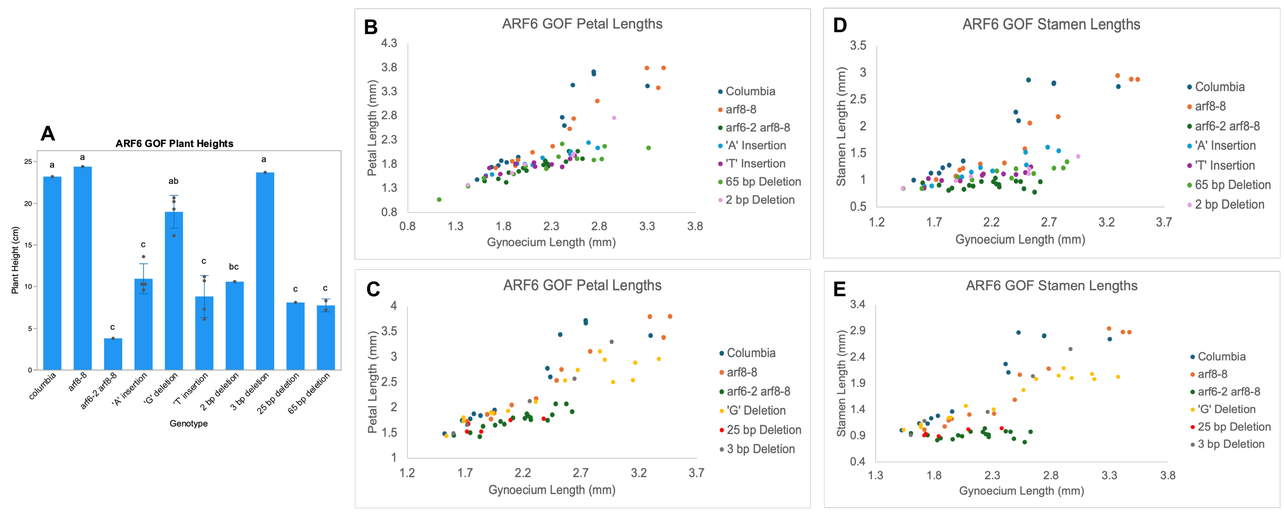


Figure 4. *ARF6* PB1 domain mutant plant heights and organ lengths. A) Plant heights. B-C) Petal lengths. D-E) Stamen lengths. Gynoecium length was used on the lateral axis due to flower buds being dissected in different growth stages. Lowercase letters correspond to statistical analysis using Tukey's HSD LS Means Differences test.

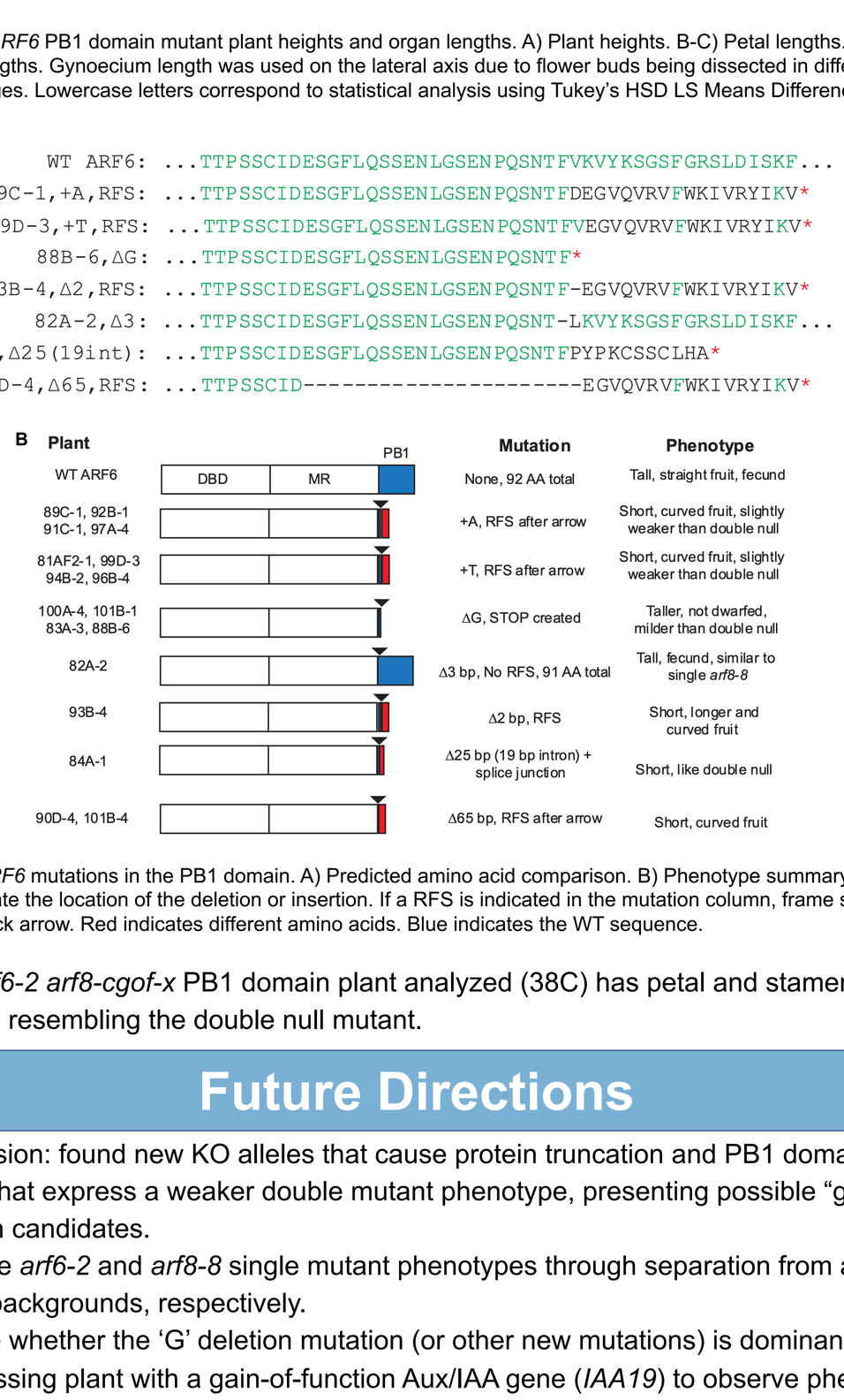


Figure 5. *ARF6* mutations in the PB1 domain. A) Predicted amino acid comparison. B) Phenotype summary. Black arrows indicate the location of the deletion or insertion. If a RFS is indicated in the mutation column, frame shifts occur after this black arrow. Red indicates different amino acids. Blue indicates the WT sequence.

- The *arf6-2 arf8-cgof-x* PB1 domain plant analyzed (38C) has petal and stamen lengths resembling the double null mutant.

Future Directions

- Conclusion: found new KO alleles that cause protein truncation and PB1 domain plants that express a weaker double mutant phenotype, presenting possible "gain" of function candidates.
- Observe *arf6-2* and *arf8-8* single mutant phenotypes through separation from arf8-8 or arf6-2 backgrounds, respectively.
- Explore whether the 'G' deletion mutation (or other new mutations) is dominant
  - Crossing plant with a gain-of-function Aux/IAA gene (*IAA19*) to observe phenotypes
- Assess growth phenotypes of PB1 domain mutants and WT lines under light variation
- Role of Aux/IAA repressors through CRISPR-Cas9 knock-out lines of *IAA* genes