Characterizing new mutations of ARF6 & ARF8 in A. thaliana

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 Cterminus, 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 ( $\Delta G$ ) 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.