Investigating Redundancy in the Elmo Family and Genetic Pinpointing of Adhesion Defects in *A. thaliana*

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For the summer of 2021, I worked to identify plants that contained two mutations within the Elmo family of proteins from a population of *A. thaliana.* The Elmo family consists of five small proteins that are predicted to be localized to the golgi membrane, and serve a function in cellular adhesion. The family was characterized after the discovery of Elmo1 by the Kohorn lab. This protein disrupts cellular adhesion when mutated, and was found using a red dye assay that stains plants with abnormal cellular adhesion. For this project, seed lines containing DNA inserts intended to disrupt each of the remaining four Elmo genes were obtained. The resulting plants were strategically crossed with each other in order to attempt to obtain plants containing two of the possible five mutations in the Elmo family. The double mutant candidates were the progeny of parents that were heterozygous for at least two separate mutations in genes coding for Elmo2 through Elmo5. Candidate plants were screened using a polymerase chain reaction intended to determine whether or not the gene was disrupted. Plants homozygous for the DNA inserts exhibit a similar trend in adhesion defects as the original mutant Elmo1 which was caused by an chemically induced mutation. This gives us confidence that the DNA inserts result in effective disabling of the target Elmo genes and proteins. An $Elmo1^{-/-}2^{-/-}$ double mutant had already been isolated and studied by the Kohorn lab. This double mutant experienced a disruption in cell adhesion that was much more drastic than either the single Elmo1 mutant or the Elmo2 mutant. This suggests that these two Elmo proteins have redundant functions in the regulation of cellular adhesion.

Only one of the five Elmo genes, Elmo4, has shown to exhibit an extreme adhesion defect such as an $Elmo1^{-/-}2^{-/-}$ double mutant when disrupted. By obtaining the other combination of double mutants in genes that show weak adhesion defects, the redundancy of the Elmo family of proteins can be studied. This gives valuable insight into the system of cellular adhesion that the Elmo family plays a role in. This past summer, every double mutant combination was isolated except for an $Elmo1^{-/-}5^{-/-}$. We intend to continue to cross pollinate $Elmo1^{-/-}$ and $Elmo5^{-/-}$ plants in order to obtain a double mutant in the F2 generation.

The project I contributed to also introduced a circle of DNA, known as a PKIR1.1 plasmid, into wild type *A. thaliana.* This plasmid was designed to splice the Elmo3 gene out of the *Arabidopsis* genome using the CRSPR/Cas9 system. The CRSPR/Cas9 system is a powerful tool that allows for the targeted removal of segments of DNA. The progeny of these plants will be screened using a red fluorescing protein that is present in the PKIR1.1 plasmids, as well as PCR designed to detect the gene deletion. These plants will be grown and analyzed similarly to the plants with DNA inserts in order to determine if a plant lacking any Elmo3 Proteins exhibits the same characteristics as a plant with a DNA insert in the Elmo3 gene. This allows for a rigorous understanding of the effect of a DNA insert within an Elmo gene, and ensures that the double mutations containing DNA inserts are an acceptable model for determining redundancy within the Elmo family.

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