Sterile by Design: Genome Editing for Superior Hybrid Seeds

Some of the most widely cultivated crops worldwide are maize, rice, sorghum, and sunflower. More than half of the production of these crops comes from high-yielding hybrid varieties. Hybrid seeds are produced from crosses between two inbred parents, yielding hybrid progeny that perform better than either parent in traits such as yield, height, quality, and disease resistance.

Making the crosses required to produce hybrid seed commercially typically relies on Cytoplasmic Male Sterility, or CMS, defined as the maternally inherited inability to produce pollen. CMS is caused by mutant genes on the mitochondrial genome, which are inherited strictly through the maternal parent.

Using tobacco as a model system, we used genome editing tools to edit the mitochondrial genome in a manner that created a novel CMS trait. This was accomplished by repurposing the essential mitochondrial gene known as *atp1* (a gene necessary for producing the ATP energy required for cellular survival) to be a nuclear gene by placing it under the regulatory control of the 35S promoter of Cauliflower Mosaic Virus. This was followed by eliminating *atp1* from the mitochondria using custom-designed meganuclease constructs that targeted and cleaved *atp1*. Because the CaMV 35S promoter does not express well in immature anthers and developing seeds, two phenotypes were observed in otherwise normal-looking plants: (1) failure to produce pollen and (2) an abortive seed trait when the plants were fertilized using pollen from a typical plant.

In principle, the same strategy could be used to create CMS traits in virtually any crop species. To test this, we applied our system to tomatoes and showed that the CMS and abortive seed development traits were similarly manifest in this crop species. The current focus of our research is to reengineer the promoters or develop alternative strategies that separate the CMS trait from the abortive seed phenotype. Using tobacco as a model system, we have overcome the defective seed trait by combining mitochondrial genome editing with an RNAi-based strategy. As a result, we have created a complete three-line hybrid seed production system, including a CMS line, a Maintainer line, and a Restorer line. We are currently working on a CRISPR-Cas13 strategy to optimize our CMS system.