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Cell-Type Specific Manipulation of the Monocot Stomatal Complex: Employing amiRNA Mediated RNAi to Study Guard and Subsidiary Cells

The stomatal complex in monocot grasses features a unique structure compared to dicots, consisting of a pair of guard cells flanked by two subsidiary cells. Stomata are microscopic pores on the leaf surface responsible for gas exchange and water management. The configuration of monocot stomata enables faster stomatal responses compared to dicots. While previous research has focused on dicot stomata, recent studies of monocot grass stomata have shown the need for cell-type-specific genetic tools. This project explores the potential of RNA interference (RNAi) to target reduced gene expression to stomatal complex cells. The goal is to reduce expression of cell wall biosynthesis genes specifically in guard cells or subsidiary cells using RNAi; therefore, one must determine whether the artificial microRNAs (amiRNA) synthesized in these cell types can migrate into adjacent epidermal cells, causing undesired side effects in the plants such as reduced epidermal cell expansion. To test for migration of amiRNA, the following approach will be taken. First, a construct with a ubiquitous gene promoter driving eGFP expression will be created and transformed into Brachypodium distachyon. GFP expression in all cell types will be verified by fluorescence microscopy. Next, eGFP downregulation constructs driven by cell-specific promoters, BdMUTE for guard cells and BdPOLAR for subsidiary cells, will be created. These promoters will express artificial microRNAs (amiRNAs) that target GFP mRNA for degradation. The plants expressing GFP in all cell types will be used as the genetic background to insert the amiRNA constructs. A loss of GFP fluorescence in the guard cells or subsidiary cells due to degradation of the GFP mRNA is predicted. Fluorescence microscopy of epidermal cells surrounding the guard cells and subsidiary cells will be performed to determine if a quantified decrease in fluorescence exists; if so, it suggests migration of amiRNAs to the surrounding epidermal cells is the cause. The application of this research to benefit society includes the development of drought-resistant monocot crops—including wheat, rice, and corn, to address global food shortages.