Title: Mechanism of ROS Regulation in ABA-Induced Stomatal Closure

Abscisic acid (ABA) induces stomatal closure in response to drought stress by binding to its receptor and initiating a signaling cascade that reduces turgor pressure within guard cells that surround stomata. This signaling pathway includes synthesis of reactive oxygen species (ROS) through activation of respiratory burst oxidase homolog (RBOH) enzymes. Genetic evidence in *Arabidopsis* has shown that ROS produced by RBOHD and RBOHF are necessary for guard cell signaling and stomatal closure. To ensure productive signaling while preventing guard cells from reaching oxidative stress, ROS levels must be tightly regulated. Plants employ many antioxidant mechanisms to maintain ROS homeostasis, which include plant specialized metabolites called flavonols. In mutants with decreased flavonols ROS levels are increased, as judged using two fluorescent ROS sensors, and the rate of ABA-dependent guard cell closure was enhanced, while the rate of light dependent guard cell opening was impaired. In mutants with elevated flavonols, ROS levels and the rate of ABA dependent guard cell closure were reduced. ABA treatment also activates two MAP kinases (MPK9 and MPK12) that positively regulate ABA-induced guard cell closure. ABA treatment of *mpk9-1/mpk12-1* mutant guard cells increased ROS levels in the nucleus and cytoplasm similar to that of wild-type guard cells under the same treatment, suggesting that these MAP kinases function downstream of the RBOHD/F ROS burst. Rapid increase in RBOH-produced ROS is not only a response to ABA, but to many other environmental signals. Therefore, we are using genetic and chemical approaches to understand the mechanisms and spatial controls of ROS production, ROS homeostasis, and how these ROS signals regulate guard cell signaling.