Abscisic Acid Drives Stomatal Closure through Increases in Hydrogen Peroxide in Mitochondria

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Abscisic acid (ABA) drives stomatal closure to minimize water loss due to transpiration in response to drought. ABA rapidly increases the accumulation of guard cell reactive oxygen species (ROS), though the subcellular locations and identity of these ROS has received limited study. We utilized chemical probes and genetically-encoded biosensors to monitor different subcellular compartments where ABA increases hydrogen peroxide (H_2O_2) in guard cells. ABA increased ROS levels in several subcellular locations including chloroplasts, cytosol, and nuclei by way of the generic redox probe, dichlorofluorescein (DCF). Intriguingly, ABA also increased fluorescence of both DCF and an H_2O_2-selective probe, Peroxy Orange 1, in mitochondria that colocalized with Mitotracker dyes. Mitochondrial ROS increases were lost in guard cells of an ABA receptor mutant, consistent with mitochondrial ROS increases being dependent on the canonical ABA signaling pathway. ABA treatment of guard cells transformed with a genetically-encoded H_2O_2 reporter (roGFP2-Orp1) targeted to the cytoplasm or the mitochondria (mt-roGFP2-Orp1), revealed significant ABA-induced H_2O_2 increases in these locations. Consistent with ROS changes in mitochondria functioning in stomatal closure, the *abo6* mutant, with a defect in mitochondrial electron transport, has elevated ABA-induced ROS in mitochondria and enhanced stomatal closure. These effects can also be phenocopied with rotenone, which increases mitochondrial ROS, while the mitochondrially targeted antioxidant, MitoQ, is sufficient to dampen the effect of ABA on mitochondrial ROS accumulation and stomatal closure. ABA-induced ROS accumulation in guard cell mitochondria was also lost in mutants with defects in genes encoding ROS producing Respiratory Burst Oxidase Homolog (RBOH) enzymes and reduced by treatment with the RBOH inhibitor VAS2870, suggesting a role for RBOHs in ABA-increased ROS in guard cell mitochondria. These results demonstrate that ABA signaling elevates H_2O_2 accumulation in guard cell mitochondria which are required to drive stomatal closure.