Ethylene modulation of reactive oxygen species signaling by flavonoid antioxidants in guard cells

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This project examined the role of ethylene in modulating signaling in guard cells, which regulate the aperture of stomatal pores on leaf surfaces. The hormone abscisic acid (ABA) triggers stomatal closure through a signaling pathway that generates hydrogen peroxide, a reactive oxygen species (ROS) second messenger. ROS are produced in transient bursts, and their accumulation must be minimized by antioxidants to keep concentrations from reaching damaging levels within the cell. Here we show that ethylene works antagonistically to ABA by inducing flavonol antioxidants in *Arabidopsis* and tomato guard cells. Flavonol accumulation in guard cells, but not surrounding pavement cells, was visualized with confocal imaging of a flavonol-specific dye. Consistent with flavonols in guard cells acting as antioxidants, increased levels of ROS were detected using the ROS sensor DCF in guard cells of *tt4*, an Arabidopsis mutant and *are*, a tomato mutant, which both have defects in flavonol synthesis. ROS levels are conversely decreased in guard cells of *aw,* a tomato mutant that synthesizes elevated levels of flavonols. Guard cells of the *are* and *tt4* mutants were more sensitive to ABA-induced closure than WT, while guard cells of the *aw* mutant are less sensitive. This suggests flavonols may dampen the ABA-dependent ROS burst that drives stomatal opening. Ethylene treatment of wild- type tomato and *Arabidopsis* plants increased flavonol accumulation in guard cells; however, no flavonol increases were observed in *Neverripe* and *ein2-5,* which have defects in ethylene receptors. Consistent with lower levels of ROS due to elevated flavonols, ethylene treatments delayed ABA- mediated stomatal closure. Together these results are consistent with ethylene modulating guard cell signaling by increasing flavonol accumulation and decreasing ROS levels.
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