NAPPN Annual Conference Abstract: High-throughput microscopy image analysis of plant stomata

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BodyText:

High-oil tobacco varieties have been recently engineered to produce increased leaf oil content for future food and fuel needs. An engineered variety of *Nicotiana tabacum* produces ~30 percent of leaf weight in lipids in the form of triacylglycerol, a significant increase relative to the less than 1 percent storage oil normally found in wild-type leaves. This high-oil tobacco also accumulates oil bodies in stomatal guard cells. In order to understand the impact of oil on guard cell shape, aperture, and dynamics, we have co-opted computer vision tools in PlantCV to create an accurate, flexible, and high-throughput method for microscopy image analysis of stomata. To this end, leaf impressions are made with silicone putty; clear nail polish peels of the putty impressions are imaged using light microscopy. Binary thresholding followed by point-and-click regions of interest and morphology calculations provide stomatal counts, aperture, and other shape characteristics. Applying this method to high-oil tobacco demonstrated reduced stomatal aperture but the same number of stomata per unit leaf area, providing a mechanistic explanation of high-oil tobacco responses to high temperature and water deficit stresses.