High-throughput microscopy image analysis of plant stomata

Katie Murphy[1], Courtney Harmon[2], Doug Allen[2], and Malia Gehan[1]

[1]Affiliation not available
[2]Donald Danforth Plant Science Center

November 1, 2022

Abstract

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 dry weight in lipids in the form of triacylglycerol (TAG), 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.

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

Katherine M. Murphy[1]*, Courtney Harmon*[1]*, Doug Allen*[1]*, Malia Gehan*[1]

[1]*Donald Danforth Plant Science Center, St. Louis, MO*

ORCiD: 0000-0002-8891-4274

Keywords: stomata, abiotic stress, computer vision, biofuel, microscopy

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.