

Rapid optical profilometry and computer vision of leaf epidermal structure applied to genetic and environmental control of stomatal patterning in model C₄ species

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Genetically tractable model systems closely related to bioenergy grasses need to be developed to drive the crop improvement required for large scale, ecologically sustainable bioenergy production. *Setaria viridis* is an ideal candidate C₄ panacoid grass. The objectives of this project are to utilize genomic, computational and engineering tools to begin the genetic dissection of drought and density response in *S. viridis*. This will be achieved through: 1) Quantitative trait and association genetics; 2) novel controlled environment and field phenotyping combined with molecular and chemical profiling; 3) development of metabolic and gene networks; 4) development of transformation technologies; 5) reverse genetic testing of candidate genes.

Leaf epidermal structures, including stomata and hairs, play key roles in leaf function. Stomatal and hair patterning are highly regulated developmental processes in response to both environmental and genetic signals. Modern quantitative genetics approaches have not been fully applied to understanding epidermal structures due the laborious nature of phenotyping methods. C₄ grasses are agriculturally and ecologically important, in large part due to their high water use efficiency. Yet, little is known about the mechanisms controlling stomatal and hair patterning in this key plant functional type. We have developed and applied a rapid method of assessing stomatal and hair patterning in two model C₄ species – maize and setaria. The leaf surface is scanned in less than two minutes with a modified confocal microscope, generating a quantitative measurement of a patch of the leaf surface. We have developed an algorithm for automatically detecting stomata in epidermal surfaces through training of a pattern-recognition neural network. We have validated this rapid phenotyping technique in: (1) diverse *Zea mays* inbreds grown at ambient and elevated ozone using free-air concentration enrichment (FACE) technology; and (2) in a recombinant inbred line (RIL) population resulting from the cross of *Setaria italica* x *Setaria viridis*. Variation in stomatal patterning among founder lines of the NAM population of *Z. mays* was reproducible between field and greenhouse conditions at Illinois and Purdue. QTL for stomatal patterning and epidermal surface roughness were identified in *Setaria*.

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