Ecotypic variation of stomatal size-density tradeoff in switchgrass (*P. virgatum*)

Brian J. Branstetter, Raiza Castillo-Argaez, Claudia Garnica-Diaz, Grace P. John.
Department of Biology, College of Liberal Arts and Sciences, University of Florida, Gainesville, FL

UF Biology
College of Liberal Arts and Sciences

Introduction

- Switchgrass (*Panicum virgatum*) is a C4 bunchgrass species with great potential to become a dominant biofuel crop.
- It can be grown in poor soil and does not need to be replanted annually.
- Populations naturally occur across a broad range in North America with wide variance in biomass yield across locally adapted ecotypes (Figure 1).
- Breeding efforts to increase yield focus intensively on recombining traits from select ecotypes to enhance growth rate and stress tolerance.
- Stomatal characteristics have been shown to contribute substantially to growth rate and stress tolerance across species and may provide an avenue for improving yield. However, trade-offs between stomatal size and density (SS-SD) have been observed in many systems and may constrain breed. Yet, this constraint has not been extensively researched within this species across ecotypes and the genetic drivers for shifts along the SS-SD axis have not been explained.

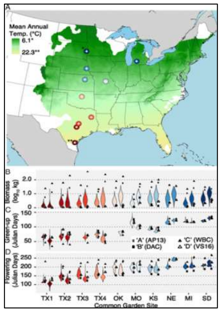

Figure 1, adapted from [2]. Planting sites and average temperature for the F0 parents used in this study. It shows data for their flowering time, green-up time, and biomass yield.

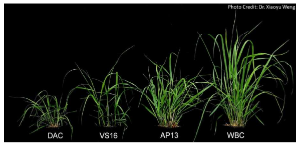

Figure 2, adapted from [1]. Fixed morphological variance across the switchgrass ecotypes used in this study. VS16 and DAC are upland variants characterized by a fast growth rate, short lifespan and small stature, while AP13 and WBC are longer lived, higher yield lowland variants.

Objectives

To measure anatomical traits of morphologically and functionally distinct switchgrass ecotypes and their hybrid offspring to quantify divergence related to local adaptation. This information can be used to inform the most beneficial switchgrass hybrids.

Methodology

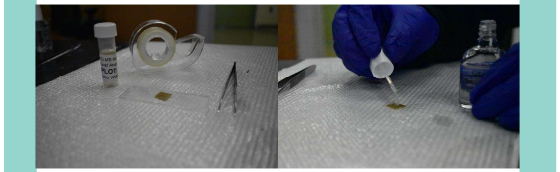

Figure 3. Epidermal peels were generated for over 100 leaf samples, representing the different ecotypes, grown in Colombia. Materials needed for a peel: microscope slide, leaf sample, forceps, and two-sided tape (left). First, nail polish is applied to both the abaxial and adaxial sides of a leaf sample (right). The nail polish adheres to the epidermal surfaces.

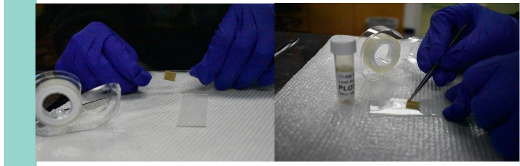

Figure 4. After the nail polish dries, two-sided tape is placed on both sides (left). Next, forceps are used to pull the leaf sample off the tape (right).

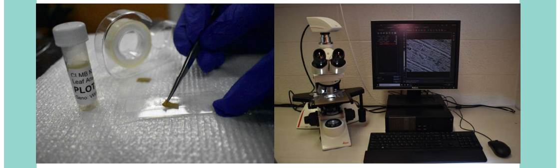

Figure 5. We used forceps to pull the leaf from the tape, which was placed on the microscope slide. Since the nail polish adheres to the epidermal surface, the epidermal layer should remain on the tape once the leaf is pulled off (left). We imaged epidermal peels with a light microscope, so that the anatomical traits could be measured.

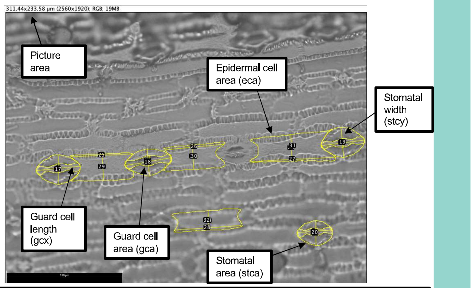

Figure 6. Sample scan of an epidermal peel annotated with the anatomical trait measured.

Results

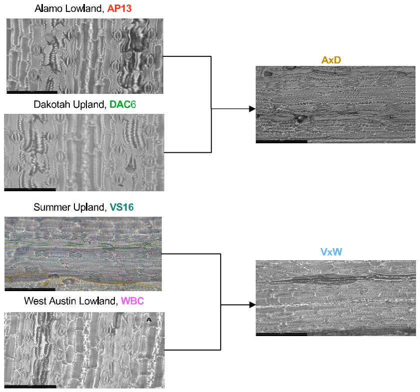

Figure 7. Light microscope scans showing the diversity in epidermal anatomy across upland (DAC and VS16) and lowland (AP13 and WBC) ecotypes and their hybrid offspring (AxD and VxW). Scale = 100 um.

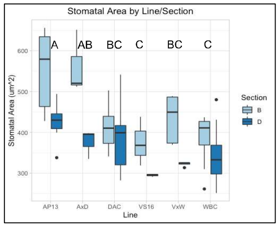

Figure 8. Paired boxplot of stomatal area on the abaxial (bottom) and adaxial (top) surfaces of leaves across ecotypes. The letters, derived from a post-hoc Tukey HSD test, denote significant differences in stomatal area between ecotypes. A one-way ANOVA found significant differences ($p < 0.001$) in stomatal size between at least two lines.

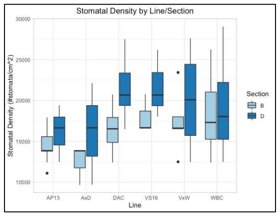

Figure 9. Paired boxplot of abaxial and adaxial data for stomatal density. A post-hoc Tukey HSD test found no significant differences between ecotypes. A one-way ANOVA found significant differences ($p < 0.05$) in stomatal density between at least two lines.

Results (continued)

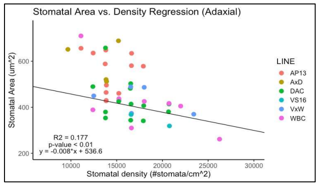

Figure 10. Linear regression of stomatal size vs. stomatal density for adaxial data across lines. Each data point represents the average stomatal size and density for one leaf's adaxial surface.

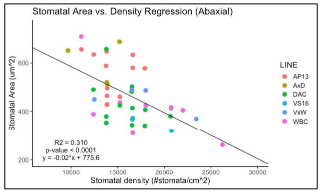

Figure 11. Linear regression of stomatal size vs. stomatal density for abaxial data across lines. Each data point represents the average stomatal size and density for one leaf's abaxial surface.

Conclusions

The regression analyses (Figures 10,11) show a consistent tradeoff between stomatal area and density independent of ecotypes. As the number of stomata on a leaf surface increases, the size of the stomates decrease. Likewise, if a leaf has fewer stomata, they are typically larger and farther apart. The tradeoff is noticeably stronger for abaxial data.

We found inconsistent variation in stomatal traits across ecotypes, such that AP13 possessed fewer, larger stomata than all other ecotypes (Figures 8,9). This relationship was expressed more strongly on the abaxial than adaxial surface. This suggests a dominant phenotype as the F_1 hybrid AxD, containing an AP13 allele, also shows few, large stomata on the abaxial side. We believe this has resulted from an adaptive shift towards improved water use efficiency (minimization of water loss while maximizing CO_2 conductance) to withstand stress over a longer growing season in lowland environments.

Given the suggested heritability of dominant stomatal traits, switchgrass may serve as an ideal model system to identify genes controlling stomatal anatomy and possible pleiotropic interactions using crosses in future genetic mapping studies.

References

1. The Juenger Laboratory. (2019). Retrieved March 8, 2021, from https://sites.cns.utexas.edu/juenger_lab
2. Lowry, D. B., Lovell, J. T., Zhang, L., Bonnette, J., Fay, P. A., Mitchell, R. B., Juenger, T. E. (2019). QTL × environment interactions underlie adaptive divergence in switchgrass across a large latitudinal gradient. *Proceedings of the National Academy of Sciences, 116*(26), 12933-12941. doi:10.1073/pnas.1821543116