



Denise Rogers<sup>1</sup>, Thomas Lübberstedt<sup>2</sup>, Memiş Bilgici<sup>2</sup>

<sup>1</sup>Huguenot High School, Richmond City Public Schools

<sup>2</sup>Department of Agronomy, Iowa State University, Ames, IA, 50011-1051, USA



### Abstract

We measured stomatal area-size, density, length, and width on both the first and ear leaves of maize. There is significant difference between first leaf and ear leaf. However, first leaf measurements reliably mirror ear leaf traits. Close correlations were found for stomatal area and density across leaf stages. Stomatal density ( $r = 0.72$ ) and Length ( $r = 0.70$ ) are closely correlated between first and ear leaves. Stomatal area is negatively correlated ( $r = -0.56$ ) revealing a relationship. Low-yield hybrids have higher stomatal density, but lower stomatal area-size. High-yield hybrids have smaller stomata density, but higher stomata area-size in Kentucky Blue Grass-PGC.

### Background

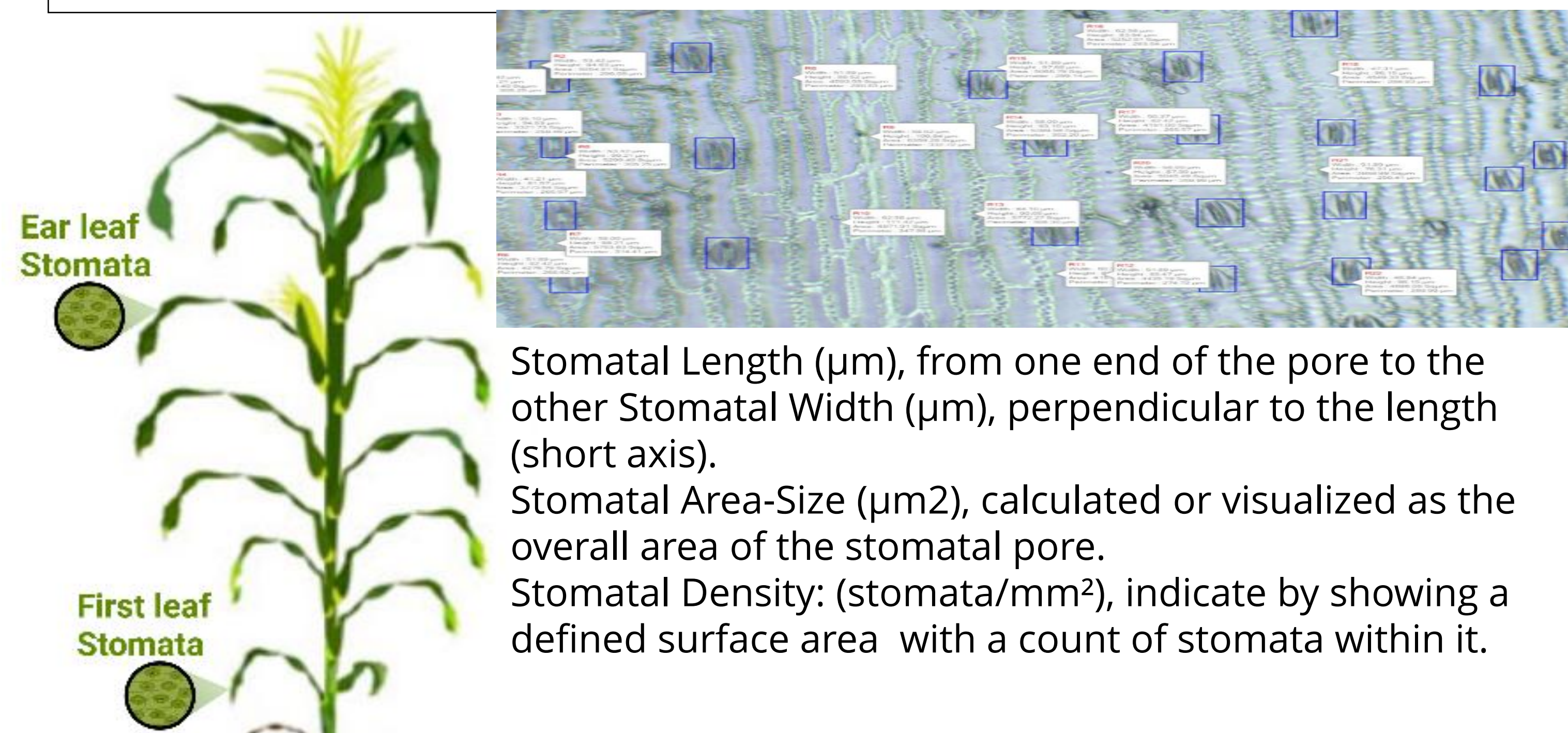
Stomata control gas exchange and water loss in plants, directly influencing growth and yield. In maize, little is known about how stomatal traits on early leaves relate to those on mature leaves like the ear leaf, which is critical for grain filling in the presence of perennial cover crops. Insights from this research can help inform breeding programs aimed at enhancing yield and resource efficiency.

### Objectives

Determine whether stomatal traits measured early in development (first leaf) can predict traits in the ear leaf and overall grain yield. Compare stomatal density, size, length and width in first and ear leaf of maize plants under PGC field conditions. Assess variability in stomatal traits across two leaves, developmental stages, and maize genotypes in PGC.

### Methods

Collected 10 hybrid leaf samples for stomatal measurements and 2 replicates per genotypes were used. Nail polish imprint method with transparent tape was performed. 3 stomata samples were collected from each hybrid.



The diagram shows a maize plant, highlighting two specific leaves where stomatal traits are measured: Ear leaf Stomata: A leaf located near the developing ear, typically a mature and physiologically active leaf. First leaf Stomata: The basal leaf that emerges early in development. Each leaf has an inset showing a microscopic view of stomata, emphasizing that measurements such as density, length, width, and area were taken from both locations.

### Figures

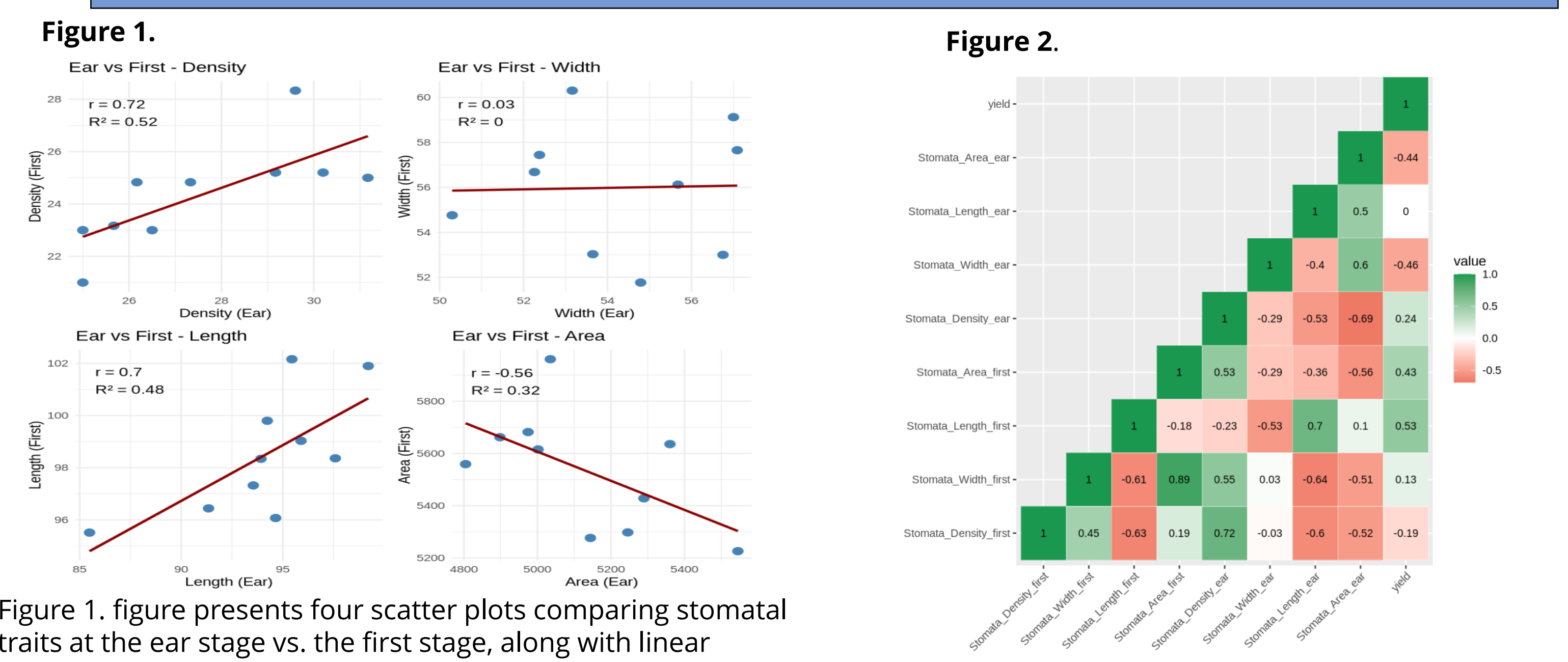


Figure 1. figure presents four scatter plots comparing stomatal traits at the ear stage vs. the first stage, along with linear regression lines, Pearson correlation coefficients ( $r$ ), and coefficients of determination ( $R^2$ ). Each subplot evaluates the consistency or relationship of a particular stomatal trait between the two stages.

Figure 2. shows a correlation heatmap visualizing the relationships among several stomatal traits and yield.

### Classroom Applications

*Virginia Science Standards of Learning Curriculum Framework* BIO.5 The student will investigate and understand that there are common mechanisms for inheritance.

Key ideas

- c) the variety of traits in an organism are the result of the expression of various combinations of alleles;
- d) meiosis has a role in genetic variation between generations;
- e) synthetic biology has biological and ethical implications.

Essential knowledge and practices:

predict possible genotypes and phenotypes of non-Mendelian traits (BIO.5 c) identify sources of genetic diversity and explain how it can be an advantage for populations (BIO.5 c)

Activities: Punnett squares-exploring variations in corn leaves, meiosis microscopy in maize tassels and electrophoresis

### Future Collaborations

Establish the relationship between stomatal traits, leaf position, developmental stages, environmental conditions, and uniform maize breeding background.

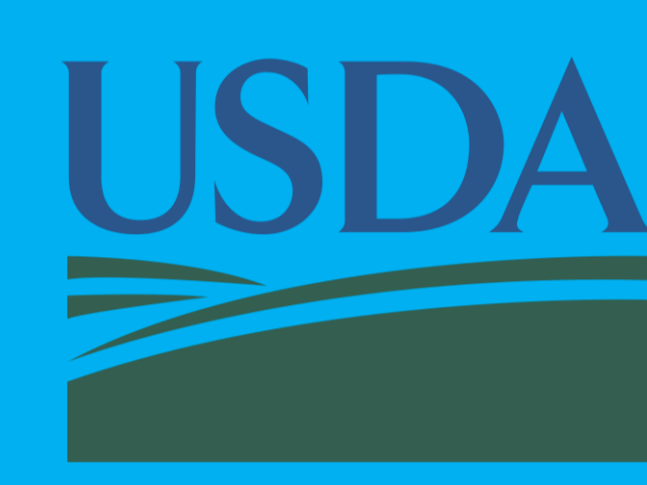
Early-stage screening can be implemented for rapid phenotyping. This will save time, energy and resources in large-scale maize breeding programs.

### Acknowledgements

I would like to thank the REGENPGC Project, my P.I., Professor Dr. Thomas Lübberstedt, and mentor, Memiş Bilgici, for helping me to understand the significance of the PGC research. In addition, I also appreciate all members of the Lübberstedt lab for their assistance throughout the summer. Special thank you to Sarah Pfeffer, Research Scientist II, for providing me with all of the much needed lab training. Lastly, I thank Maureen Griffin and Eric Hall for selecting me to be a part of this wonderful research experience.

### Results & Conclusion

Leaf position: there is a significant difference between the first leaf and ear leaf, but it is highly correlated and predictable. First leaf measurements reliably mirror ear leaf traits. Moreover, low-yield hybrids have higher stomata area-size but lower stomata density. High-yield hybrids have smaller stomata area-size but higher stomata density. Breeding implications for maize: early first-leaf screening accelerates genotype evolution and saving time and resources while targeting optimal stomatal phenotypes for improved yield and stress resilience in PGC. Ear-leaf and first-leaf stomatal area tend to depress yield (negative coefficients and correlations). There is a negative correlation between stomata area-size and density. Targeted selection for optimally smaller stomata area-size and more sparsely distributed stomata density could improve both yield and resilience. First-leaf stomata are easy, fast, and cheap to measure—perfect for early culling.



RegenPGC is supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-68012-35923 from the USDA National Institute of Food and Agriculture. Any opinions, findings, conclusions, or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

