Background

According to the United States Department of Agriculture (USDA), corn was the largest crop grown in the U.S. in 2019 and is predicted to be the number one cereal in the world by 2020. Globally, corn has various end uses including various foods, animal feed, and ethanol for biofuel. Corn (or maize) is a very productive crop due to almost a century of intense improvement in the U.S., with yields steadily increasing since the 1930's. This improvement is due in part to advances in farming practices, but largely to genetics and breeding. The improved architecture, or shape, of maize makes it very amenable to large-scale production and harvest. One of the most effective strategies for boosting corn yield has been increased planting density, which improves with more narrow plants with upright leaf angles **(Figure 1)**.

Figure 1. Improvements to corn production since the 1930's include increasing planting density. *A comparison between a corn field from 1920 (left) and 2018 (right).*

Under dense-planting conditions, a more erect leaf angle enhances plant light interception in the lower canopy, and overall photosynthetic capacity, hence the ability to grow optimally (**Figure 2**).

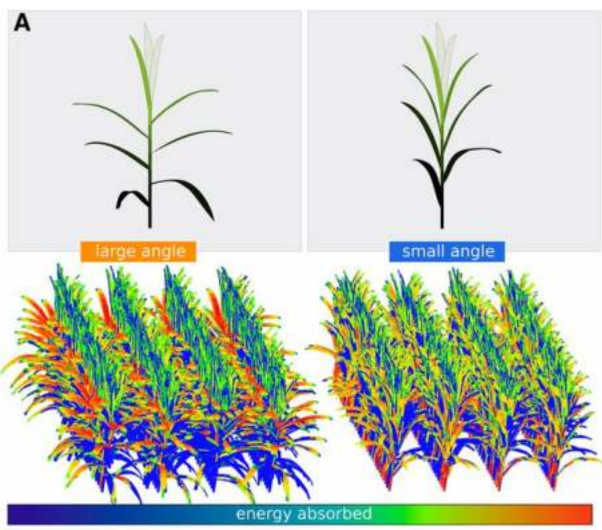

Figure 2. Leaf angle affects light distribution in crop canopies. *Schematic of corn plants and corn plots that vary in their leaf angles shows that light was distributed more uniformly and deeper into the canopies of plants with upright angles (from Truong, et al., 2015).*

Leaf angle in many cereal crops and other grasses is determined by a "boundary", which separates the leaf blade from the sheath, the part of the leaf that wraps around the stem. This boundary is referred to as the "ligule region", which includes a pair of wedge-shaped, lighter green auricles and the ligule, an epidermal fringe that wraps tightly against the inner leaves (**Figure 3**). Variation in the size and shape of auricles and ligule affect the ability of the leaf blade to bend back, and therefore leaf angle.

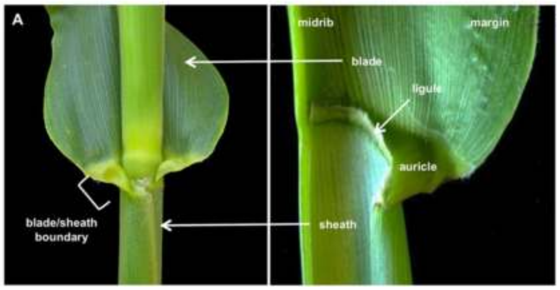

Figure 3. The structure of a maize leaf. *An intact maize leaf (left) and a leaf cut longitudinally along its midrib (right) show the distinct structures of a maize leaf including the sheath, the photosynthetic blade, and the ligule region that consists of a pair of auricles and the ligule (from Moon, et al., 2013).*

The leaf angle of a maize plant is an example of a "phenotype". Phenotype is largely controlled by an individual plant's "genotype", or genetic makeup, but can also be influenced by environmental factors. Over the years, scientists have identified genes that control development of the ligule region, and therefore leaf angle. Of interest to breeders are variations in the genetic code at these genes (or alleles), which make the plant have an optimal leaf angle for both dense planting and photosynthetic efficiency. Diversity panels of maize, which include a large variety of inbred lines with different genotypes, have been compiled and maintained by breeders and scientists. These panels retain rich genetic diversity that underlies extensive natural variation in phenotype, and provides a resource to mine for valuable alleles in breeding upright plant architecture.

Advances in high-resolution phenotyping technologies and analytics and in high-throughput sequencing are making it easier to link the diversity in phenotype to underlying sequence variants in a plant's genome. For example, it is anticipated that hand-measurements of individual leaf angles in a large field will be replaced by analyses of images captured by drone. This will rely on algorithms that not only capture phenotypic measurements of a single plant from an image (**Figure 4**), but of a whole field of plants and their influence on each other. The ability to link morphological phenotypes such as leaf angle and shape with physiological phenotypes (e.g., a plants thermal temperature in response to drought), crop improvement by breeding and/or engineering can be fast-tracked for food security in response to dynamic environments and rising world population.

Figure 4. Computer-assisted image analysis of maize seedlings. *Image analysis software can be used to extract features from an image (such as leaves) and collect phenotypic information such as leaf number and size (from Zhang et al., 2017).*

Genotype-to-Phenotype: Lab Component

This curriculum is intended to foster an understanding of the fundamental concepts linking a plant's genotype to its phenotype, and how that relates to crop improvement and food security. These same concepts and methods can be applied to studies of human disease, etc. The activities are focused around genotyping and phenotyping various lines of corn (maize) for seedling leaf angle. Leaf angle is an important agronomic trait that impacts planting density and light interception and absorbance in the lower canopy. Over the past 8 decades, corn production has exponentially increased in the US due to a number of factors, including farmers' selection of optimal phenotypes, such as planting density. A number of disciplines are integrated in current approaches to crop improvement; including molecular genetics and breeding, but also computer science, engineering, mathematics, and robotics. By joining these disciplines and advancing technologies, optimal phenotypes can be bred more quickly, helping to secure crop production in the face of uncertain climate fluctuations and world population increases.

This activity consists of:

- An overview lecture (or series of lectures) that inform on background topics related to genotype-to-phenotype.
- A lab component that phenotypes leaf angle in corn seedlings using both manual measurements and image analysis software.
- A lab component that amplifies a specific sequence of maize DNA using PCR, and determines the genotype of that individual based on a restriction endonuclease reaction.
- A series of checkpoint questions and summary points will help link the genotype with leaf angle phenotype.

The goal of this activity:

- Understanding how leaf angle of crops affect crop yields in agriculture.
- Learning both the advanced and traditional genotyping and phenotyping technologies.
- Knowing how to perform the basic molecular biology experiments, such as PCR, restriction endonuclease reaction, and running gel.

Key Terms and Definitions

genotype -- The genetic make-up of an organism's DNA (its genetic code).

phenotype -- A set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

quantitative trait -- A measurable phenotype that depends on the cumulative actions of many genes and the environment. These traits can vary among individuals over a range, to produce a continuous distribution of phenotypes. Examples include height, weight and blood pressure.

image analysis -- The extraction of meaningful information from images; mainly from digital images by means of digital image processing techniques.

Single Nucleotide Polymorphism (SNP) -- The most common form of DNA variation, alterations to a single base. If a SNP is within a gene, it can disrupt gene function. Most SNPs do not occur in genes, but can be associated with other types of DNA variation and so are used effectively as markers. The average frequency of SNPs in the human genome is approximately 1/1,000 base pairs (the human genome is over 3 billion base pairs!).

allele -- One of two or more alternative forms of a gene that arise by mutation, and are found at the same place on a chromosome.

Background references:

Truong SK, McCormick RF, Rooney WL, Mullet JE (2015) Harnessing Genetic Variation in Leaf Angle to Increase Productivity of Sorghum bicolor, Genetics, 201(3): 1229-1238

Moon J, Candela H, Hake S (2013) The Liguleless narrow mutation affects proximal-distal signaling and leaf growth, Development, 140: 405-412

Zhang X, Huang C, Wu D, Qiao F, Li W, Duan L, Wang K, Xiao Y, Chen G, Liu Q, Xiong L, Yang W, Yan J (2017) High-Throughput Phenotyping and QTL Mapping Reveals the Genetic Architecture of Maize Plant Growth, Plant Physiology, 173(3): 1554-1564