Protocol: Growing and Phenotyping Corn Seedlings

OVERVIEW

In this exercise, maize seeds from various genetic backgrounds (genotypes) will be grown to seedlings, phenotyped for their leaf angle and photographed. Plant phenotyping involves the measurement of physical traits in plants. There is a wide range of variation in leaf angle across genotypes, from very obvious to not visible. Students will be given a set of maize genotypes with known and unknown leaf angle phenotypes. The data collected by the students will be evaluated together. Measurements recorded by students will be integrated into a data analysis pipeline that performs genome association studies (GWAS) to study genetic variants in different individuals to see if any variant is associated with a phenotypic trait, and to perform genomic selection. The results from these analyses will be used to help make predictions on leaf angle in mature corn plants. The students become an integral part of the project through this activity.

MATERIALS

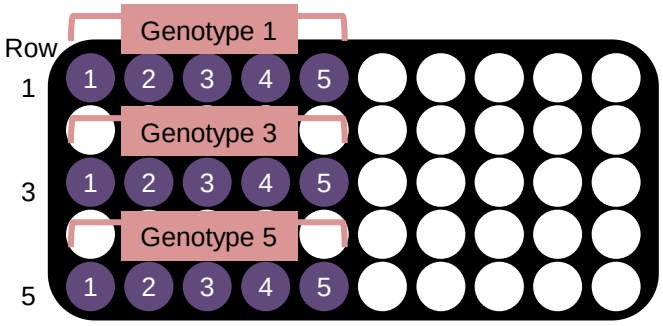
Provide by the Danforth Center	**Provide by the Student**
5x10 cone tray with lid	Area to grow plants with plentiful sunlight (outdoors or indoors)
Black paper	Cell phone camera or other digital camera
Corn seeds (15 seeds, 5 from each of 3 genotypes)	Computer
Metro Mix 360 with turface blended soil	Internet connection
Plant tags	Plastic container
Protocol – How to grow corn and measure leaf angle manually	Protection of plants from pets and wildlife
Protocol – How to analyze plant images with PlantCV	Scissors
Protractor	Spray bottle, water can or hose
Sharpie	Water
Thermometer	

Part 1: Planting Corn Seeds in Soil Trays

IMPORTANT! Schedule your planting date for phenotyping at 16-18 Days after sowing.

1. Fill half of the rows 1, 3, and 5 of a clean 5x10 cone tray with potting soil (Metro Mix 360 with turface blended soil). This step is pre-prepared at the Donald Danforth Plant Science Center.
2. Moisten the soil by misting with water from a spray bottle or using the “mist” setting on a hose. Hold the spray nozzle about 1 foot above the tray so soil does not fly out of the pots.

3. Each half row fits five seeds from a genotype (See diagram below for the arrangement in a tray). Label each cone in the tray using a plant tag. The tag should include the seedling ID (genotype number and seedling number) and the date of planting (See diagram below to label your tag for each seedling). The seedling number represents each of the seedling replicates within a genotype and helps to track individual seedlings throughout the experiment.



4. Use a sharpie marker to punch a 1 cm deep hole in the soil and plant a seed in each cone as labeled. Using a plastic container, sprinkle a light layer of soil (about 0.5 cm) over the seeds. **Note: do not plant the seeds too deep.**

5. Mist the pots with water again. Be careful to hold the spray nozzle one foot above the tray so that you do not displace soil or the seeds.

6. Once you finish planting, **place your tray in direct sunlight** outdoors or indoors by a window. Avoid shaded environments. **Note: keep your tray protected from pets and or wildlife.**
7. Cover the trays with the clear plastic lids until germinating seedlings emerge from the soil. The lid helps keep the humidity high to encourage germination. It takes about 4 to 5 days to see the seedlings geminate. After the plants have emerged, remove the lid to expose the seedlings to your outdoor/indoor environment.
8. Use the thermometer to measure the temperature at which your plants will grow. If you are growing the plants indoor, take the temperature once at the planting date. This assumes that the indoor temperature will not change much during the growing period. If you are growing the plants outdoor, it is expected a more variable temperature. Take measurements at low and high temperatures a couple of times during the growing period and determine what have been the minimum and maximum temperatures that the plants were exposed to. Record the growth temperature in the data table at the end of this protocol.

Part 2: Plant Care

IMPORTANT! DO NOT overwater the planting trays.

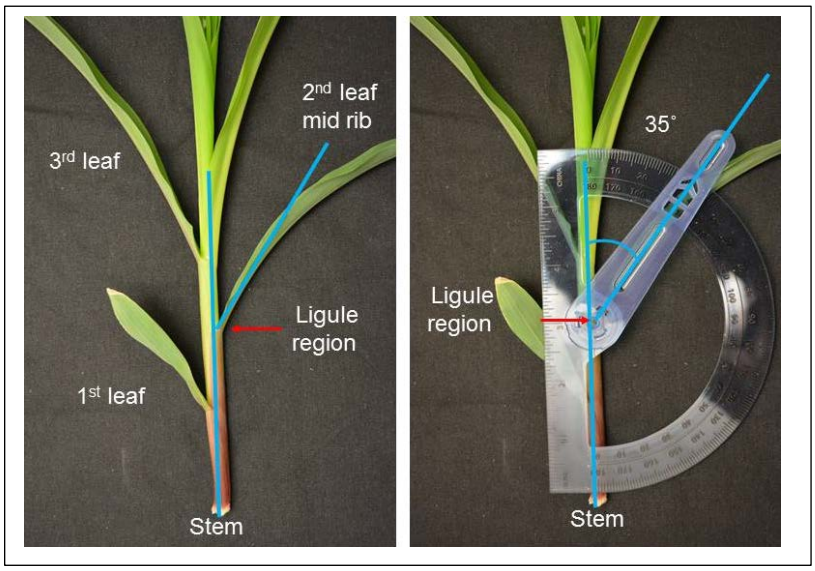
1. Add 1.5 L of water to the bottom of the tray. Typically, before germination you only need to water the tray when you see the soil obviously dry out. Once the seeds start germinating, you might need to water the tray every other day. Seedlings need more water when they become bigger and you might need to water them every day after 14 days after sowing.
2. After watering the tray, wait about one hour and then pour off any excess water into the sink. **Corn does not like to sit in standing water.** The soil should feel moist - not dry or too wet / muddy. If the soil in one cone ever feels dry, you can slowly and gently water that cone from the top as well.

Part 3: Data Collection of Leaf Angle

1. After planting, check on your plants daily and record the date of germination using the *phenotyping table* that is at the end of the protocol. Corn seedlings typically emerge around 4-5 days after sowing. If you observe an obvious delay in seed germination for a genotype comparing to the other genotypes planted, adjust the phenotyping date for the delayed genotype accordingly. Make sure the plants have the first three leaves expanded.
2. For the first week after germination, try to observe the seedlings every other day. After that, observe your plants weekly.
3. Phenotype the leaf angle of the second and third leaves in the corn seedlings at 16-18 days after sowing. **Note: Plants that have the first three leaves fully expanded are ready for phenotyping. Wait if not.**
    a. Cut a seedling at its root-shoot junction (at the soil line).
    b. Lay the seedling on a flat surface on top of the black paper provided. Make sure the leaves naturally fall on either side of the stem. **Note: keeping the natural curve of the leaf is very important for the accuracy of data collection.**
    c. Identify the 2nd and 3rd fully-expanded leaves from the bottom up. Check that the 1st leaf has not detached from the seedling when cut. If it does, just save this leaf for the activity on the second protocol about plant imaging. Keep track of which leaf is which.

d. Place the midpoint of the protractor at the region where a leaf meets the stem. We refer to this as the *ligule region* (see diagram below).
e. Line up the stem with line zero on the protractor, and the mid rib of the 2nd leaf with the mid-line of the swing arm on the protractor. Read the degree at where the swing arm crosses the number scale (See the example below showing the measurement of the 2nd leaf angle of a corn seedling).
f. Record the leaf angle of the 2nd leaf in the phenotyping table.
g. Identify the 3rd leaf and repeat steps **d** to **f**.

4. After recording the leaf angle measurements of a seedling, take a photo of it (go to Part 4 below for instructions).
5. Continue measuring leaf angle (steps a. to g.) and photographing all seedlings (Part 4).



Part 4: Photograph the Seedlings

1. Before photographing a seedling:
   a. Check that leaves have the natural curve. **This is very important for the accuracy of data.**
   b. Make sure that the first leaf is present in the photo. If it detached when the seedling was cut, place it close to its original position, opposite to the side of the second leaf.
   c. Place the camera lens parallel to the flat surface.
   d. Make sure the photo frame includes the whole seedling.
   e. Place the plant tag in the photo view as far away from the seedling as possible.
   f. Check that the image is focused.
2. Take the photo.
3. Rename each image using the genotype number, the seedling number and the two capital letters from the first letter of your first and last names (e.g. 138_4_SA.jpg). **Make sure that the numbers in the plant tag and the numbers in the image name are the same.**
4. Repeat steps 1 and 2 to photograph and label each seedling.
5. Upload the photos in your Google classroom space. You should have received an email with a Classroom invite with a link to your space and instructions.
6. Go to Protocol 2 to continue with the image analysis of your photos to measure leaf angle using the software PlantCV.

Phenotyping Table

Student name: ____________________ **School name: ______________________**

Growth environment (outdoor or indoor): _______________

Growth temperature (°C or °F): _________________
(Include maximum and minimum temperatures for outdoor environment)

	Tag label			**Germination Date**	**Phenotyping Date**	**2nd Leaf Angle (°)**	**3rd Leaf Angle (°)**
	Genotype no.	**Seedling no.**	**Planting Date**				
Example:	138	4	6_31_2020	7_5_2020	7_20_2020	35	40

Keep in mind the format for the dates:

month_date_year