

## 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.*

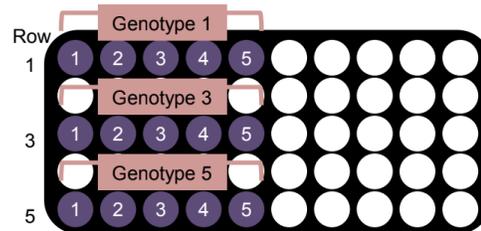
### MATERIALS

<b>Provide by the Danforth Center</b>	<b>Provide by the Student</b>
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 surface blended soil	Internet connection
Plant tags	Protection of plants from pets and wildlife
Protocol – How to grow corn and measure leaf angle manually	Scissors
Protocol – How to analyze plant images with ImageJ	Spray bottle, water can or hose
Protractor	Water
Sharpie	
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 surface 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 germinate. 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.

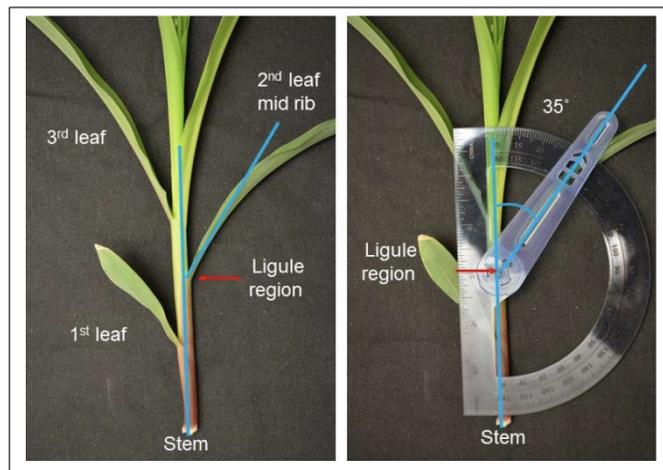
### **Part 2: Plant Care IMPORTANT! DO NOT overwater the planting trays.**

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 printed *phenotyping table* that is at the end of the protocol or downloaded from the ARE portal. 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. Use the notes section in the table to include any observations related to each seed/seedling (e.g., yellow spots, wilting, disease, etc.)
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 2<sup>nd</sup> and 3<sup>rd</sup> fully-expanded leaves from the bottom up. Check that the 1<sup>st</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> leaf angle of a corn seedling).
  - f. Record the leaf angle of the 2<sup>nd</sup> leaf in the phenotyping table.
  - g. Identify the 3<sup>rd</sup> 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 and the seedling replicate number (e.g. 138\_4.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. Go to Protocol 2 to continue with the image analysis of your photos to measure leaf angle using the software ImageJ.

## Part 5: Data entry and photos upload in the ARE portal

1. Access the student portal through the ARE website: <https://areportaldev.ddpsc.org/>
2. Use the username and password provided to you by your educator. Student login information can be found on the teacher portal.
3. To enter the team data, access the data entry form by clicking on the G2P Data Entry option on the left side menu of the portal.
  - a. Complete the information about your team and the environmental conditions of your experiment.
  - b. After you provide the information on the number of seeds planted, click on the Submit button. A second table opens.
  - c. Fill out this data table with the information you collected for each seed provided to your team including your notes.
  - d. Upload the associated photo of each plant on the same row of that plant's data.
  - e. Click on the submit button to save your data.
  - f. Check with your teacher to confirm that the data is available in the portal.

