Germination

Student Handout – Lab Procedures & Assignments

NOTE: For the following procedures, the class will be divided into six groups. Each group will be assigned a number and treatment type by the instructor. The materials listed for each procedure include the supplies each group will need to complete the activity.

Group Assignments

Group	Cold Treatment	Light Treatment
Group 1	No stratification	Light
Group 2	No stratification	Dark
Group 3	3 day stratification	Light
Group 4	3 day stratification	Dark
Group 5	7 day stratification	Light
Group 6	7 day stratification	Dark

PROCEDURE 1 – Prepare petri dishes

Materials

3 petri dishes with lids
12 pieces of filter paper
Ball point pen
Template
Ruler

NOTE: Throughout the experiment, petri dishes will often be referred to as plates. The process of placing seeds on the filter paper inside of the petri dishes is referred to as plating.

1. Prepare three pieces of labeled filter paper for your treatment using the provided template, a ruler and a pen. The following details should be included on each labeled piece of filter paper: the date the seeds will be plated, treatment type, group number and replicate number.

2. Stack three blank pieces of filter paper on top of each other. Press this stack of papers into the bottom of an empty plate. Use your finger or the end of a capped pen to press the edges tightly into the corners of the plate.

3. Place one labeled piece of filter paper into the plate on top of the blank papers. Press around the edges for a smooth fit. A properly prepared plate will have a total of four pieces of filter paper (three blank, one labeled) firmly secured in the bottom of the plate.

PROCEDURE 2 – Plate seeds

Materials

5 strains of Arabidopsis seeds (30 seeds of each)
5 toothpicks
3 strips of Parafilm
Aluminum foil (except Group 1)
Growth space
3 prepared petri dishes
5 pieces of wax paper
Permanent marker
Refrigerator

NOTE 1: Each student in the group should work with only one genotype of seed at a time. Use a new piece of wax paper and toothpick for each genotype to prevent cross contamination.

1. Add enough distilled water (approximately 8-10 ml) to the filter paper within the plates to thoroughly moisten all four layers of paper.

2. Be careful not to add too much water to the plate. Excess water could cause the seeds to shift from their designated location and/or promote the mold growth in the plate. Drain excess water into a waste container or sink.

3. Select one genotype of seed. Note the genotype with a permanent marker on the corner of a piece of wax paper. Place approximately 30 seeds of the selected genotype on the wax paper.

4. Thoroughly moisten the tip of a wood toothpick with water. Use the moistened tip of the toothpick to carefully pick up a single seed from the wax paper and place it in the corresponding space on the plate (see template for reference).

5. Repeat step 4 above until ten seeds have been plated in the appropriate space on all three plates. Dispose of the wax paper and toothpick.

6. Repeat steps 3 – 5 with the remaining four genotypes until all five genotypes have been plated on all three plates.

7. Do not to tip the plates sideways once the seeds have been plated. Prepared plates should be transported and stored horizontally to avoid displacing seeds from their designated areas.

8. Place the lid on each plate and seal edges with Parafilm to prevent the filter paper from drying out.

9. Handle your completed plates based on your assigned treatment as outlined in the table below. Plates grown in light conditions should be kept in 24 hour continuous light for the duration of the growth period.

Group #	**Group Protocols**
Group 1	1. Place plates in the light growth area
Group 2	1. Stack plates and wrap with aluminum foil. 2. Label the outside of the foil with your group number. 3. Place in the dark growth area (e.g. drawer or cabinet within the same room as the light growth area)
Group 3 Group 4 Group 5 Group 6	1. Stack plates and wrap with aluminum foil. 2. Label the outside of the foil with your group number. 3. Place the plates in a refrigerator at approximately 40°F.

ASSIGNMENT 1 – Form Hypotheses

Review the information below about each seed strain and the collection location. If time allows, do additional research about each location.

- **Columbia** (Col-0, Catalog # CS70000) – The exact history of this strain of Arabidopsis is unclear. However, the Col-0 genome was completely sequenced and is used as a reference for comparison with the genome sequences of other strains of Arabidopsis. This strain has been maintained in the laboratory for many generations, grows well in laboratory conditions, and has relatively low levels of seed dormancy. The observed flowering time for this strain is 22 days after planting. Col-0 serves as the reference strain for this experiment.

- **Fei-0** (Catalog # CS76412) – This natural variant was collected along a roadside in the village of Santa Maria da Feira, Portugal at X:-8.54, Y: 40.92, Z: 140. Observed flowering time for this strain is 21 days after planting.

    - **Santa Maria Da Feira, Portugal** is a coastal city with a mild, temperate climate. The average temperature is 49°F in the winter and 66°F in the summer. Average annual precipitation is approximately 39 inches with 77% humidity.

- **Kly-4** (Catalog # CS76384) – This natural variant was collected in a steppe in Kolyvan, Russia at X: 82.55, Y: 51.32, Z: 505. Observed flowering time for this strain is 31 days after planting.

    - **Kolyvan, Russia** is a rural village in the Kuryinsky district of Russia. Specific weather data for this small village is hard to find, so the following statistics are for larger cities in the same region of the country. The average temperature is 18°F in the winter and 76°F in the summer. In the winter there are fewer than four hours of sunshine per day. Average annual precipitation is 18 inches with 68% humidity.

- **Lag2-2** (Catalog #CS76390) – This natural variant was collected in a grazed pasture near a rock pile in Lagodechi, Georgia at X: 46.28, Y: 41.83, Z: 508. Observed flowering time for this strain exceeds 55 days after planting.

    - **Lagodechi, Georgia** is a small town located at the foot of the Greater Caucasus Mountains. The average temperature is 45°F in winter and 85°F in summer. Average annual precipitation is approximately 19.5 inches with 64% humidity.

- **Xan-1** (Catalog #CS76387) – This natural variant was collected along a partially shaded roadside and open pasture in Xanbulan, Azerbaijar at X: 48.80, Y: 38.65, Z: 37. Observed flowering time for this strain exceeds 55 days after planting.

    - **Xanbulan, Azerbaijan** is a small village within the Lankaran Rayon. The climate is sub-tropical, with average temperatures of 48°F in the winter and 85°F in the summer. Average annual precipitation is approximately 8 inches with 70% humidity.

Complete the following tasks in your lab notebook.

1. Define key terms related to genetics, growth and development:
   Genotype, phenotype, natural variant, germination, dormancy, radicle, cotyledon, hypocotyl

2. Form a hypothesis for each prompt below. Be sure to explain your reasoning to support each hypotheses.

    a. How will the germination rate of each genotype be affected by each of the six treatments?
    b. Which genotype will have the highest and lowest germination rate for each treatment?
    c. Which treatment will result in the highest germination rate for the largest number of genotypes?
    d. Which treatment will result in the earliest germination for each of the six treatments?

PROCEDURE 3 – Groups 3 & 5 - Transfer Plates

1. Remove the wrapped plates from the refrigerator after the appropriate number of days have passed.
    a. Group 3 – Plates should remain in the refrigerator for three days
    b. Group 5 – Plates should remain in the refrigerator for seven days
2. Unwrap the plates and place them under a fluorescent light on a 24 hour light cycle.

PROCEDURE 3 – Groups 4 & 6 – Transfer Plates

1. Remove the wrapped plates from the refrigerator after the appropriate number of days have passed.
    a. Group 4 – Plates should remain in the refrigerator for three days
    b. Group 6 – Plates should remain in the refrigerator for seven days
3. Do not unwrap the plates.
4. Place the plates in a dark location (e.g. drawer or cabinet) in the same room as the light growth area.

ASSIGNMENT 2 – Groups 1, 3, 5 - Collect Data

Record the following information on your group data sheet or individual lab notebook.

1. Record the date you moved your plates into the light growth area.

2. Use a magnifying glass or microscope to observe the seedlings on a daily basis for seven days after placing them in the light growth area.

    a. Count and record the number of germinated seeds per genotype daily.

        i. Count a seed as germinated when you observe a broken seed coat and the radicle has emerged from the seed (Figure 1b, below).

        ii. If condensation on the lid of the plate makes data collection difficult, the lid can be removed. Be sure to replace and reseal the lid after each data collection session.

3. Observe and note the phenotypes of the seedlings for each genotype in each treatment. Pay attention to the shape and size of the seedling, as well as the color of the hypocotyl and cotyledon.

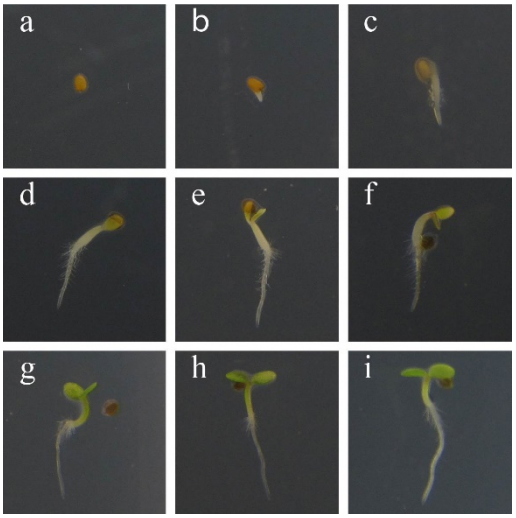


Figure 1. Stages of seed germination and seedling development in Arabidopsis. This image is reproduced as a courtesy and with permission from Dr. Annkatrin Rose, Department of Biology, Appalachian State University.

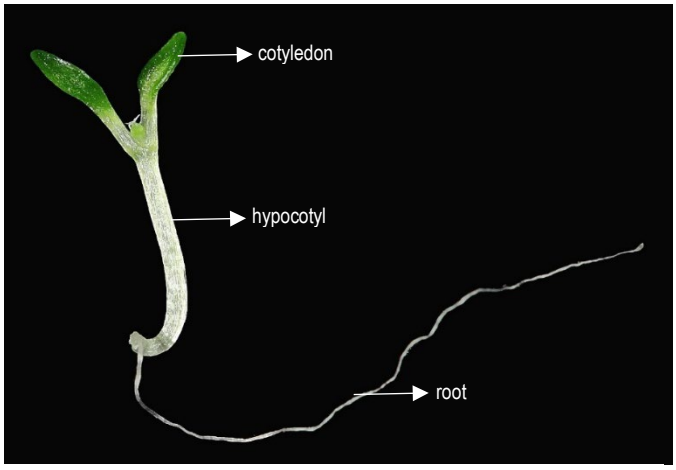


Figure 2. Anatomy of an Arabidopsis seedling.

ASSIGNMENT 2 – Groups 2, 4, 6 - Collect Data

Record the following information on your group data sheet or individual lab notebook.

1. Record the date you moved your plates into the dark growth area.

2. Collect no data for the first six days after placing the plates in the dark growth area.

3. On day seven, remove the foil from the plates and observe the seedlings.

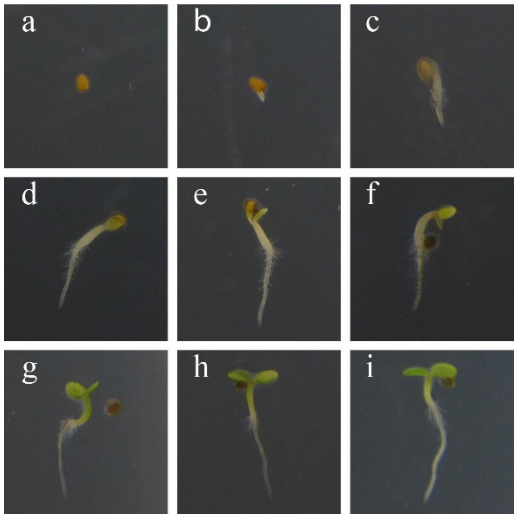
4. Use a magnifying glass or microscope to observe the seedlings.

    a. Count and record the number of germinated seeds per genotype.

        i. Count a seed as germinated when you observe a broken seed coat and the radicle has emerged from the seed (Figure 1b).

        ii. If condensation on the lid of the plate makes data collection difficult, the lid can be removed.

5. Observe and note the phenotypes of the seedlings for each genotype in each treatment. Pay attention to the shape and size of the seedling, as well as the color of the hypocotyl and cotyledon.



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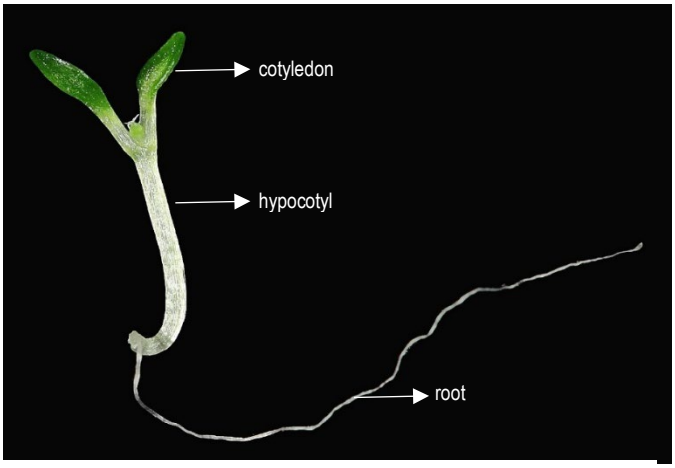

Figure 2. Anatomy of an Arabidopsis seedling.

ASSIGNMENT 3 – Analyze Data & Formulate Conclusions
Complete the following activities using your spreadsheet and lab notebook.

1. Calculate the daily germination rate of each genotype for each replicate.

2. Calculate the mean and standard deviation of the three replicates.

3. Use the means and standard deviations to generate a scatter plot to compare the germination rate among the five genotypes within each treatment. In this plot, the Y axis will represent the germination percentage and the X axis will represent the days of evaluation after transfer to the growth area.

4. As a class, create a new scatterplot comparing the effect of the six treatments on the germination rate of each genotype. In this plot, the Y axis will represent the germination percentage and the X axis will represent the days of evaluation.

5. Compare the final germination rates of different genotypes under different conditions.

6. Review the data about the shape, size and color of the seedlings.

7. Answer the following questions:

    a. Does the qualitative data you collected about the appearance of the seedlings give you any insights into the effect each treatment had on the overall health of the developing plants?

    b. Do different genotypes have different requirements for light and stratification in order to germinate?

    c. How would you summarize the light and stratification requirements of each of the five genotypes?

    d. Do certain treatments increase the speed of germination?

    e. Are some genotypes affected more than others by the extended cold treatment?

    f. Which genotypes have more dormancy?

    g. How many days of stratification are sufficient to break dormancy and attain near 100% germination in each genotype?

    h. Were there dark-germinating genotypes?

    i. Discuss the effects of the possible interactions between genotype, stratification and light on seed germination.

    j. Based on what you observed, predict which genotypes will be more likely to survive under winter conditions.

    k. How could seed dormancy give plants an adaptive advantage over other plants in different harsh environmental conditions?

    l. Why is seed dormancy an important economic trait for agricultural production?

8. Revisit your original hypotheses. Does the data you collected support or disprove your hypotheses?