Life in Bloom Basic

Student Handout – Lab Procedures & Assignments

For the following procedures you will be working with a lab partner. You will be assigned a group number and an unknown (A or B). One person in your group will be responsible for the water treatment and the other for the gibberellic acid (GA) treatment. The materials listed for each procedure include the supplies your group will need to complete the activity.

PROCEDURE 1 – Prepare petri dishes

Materials

2 petri dishes with lids	2 petri dish templates	8 sheets of filter paper
2 ball point pens	2 rulers	

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 dish is referred to as plating.

1. Prepare one piece of labeled filter paper per student using the provided template, a ruler, and a ballpoint pen. The following details should be included on each labeled piece of filter paper:
    a. Date the seeds will be plated (may be different than the date the plates are prepared)
    b. Treatment type (water or GA)
    c. Group number
    d. Unknown (A or B).

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

3. Place the 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.

ASSIGNMENT 1 – Form Hypotheses

Review the following background information and seed strain details.

BACKGROUND INFORMATION

The seeds you will be working with in this experiment are from a plant known as *Arabidopsis thaliana*. Arabidopsis is a weed that can be found growing in many different habitats around the world. It belongs to the same plant family as many familiar vegetables including cabbage, radish, and cauliflower. Arabidopsis plants are small and relatively easy to grow. The plants have a fast life cycle. Once planted, a seed will grow into a mature plant in approximately six to eight weeks.

Even though it is a weed, Arabidopsis is very important to scientists. It was the first plant to have its genome completely sequenced, which means that scientists know a lot about the genes that make up its DNA. Arabidopsis is a model system for plant science research, which means that scientists can take what they learn from studying Arabidopsis and apply that knowledge to other more complex and/or important plant species.

In this experiment you will work with your lab partner to germinate three strains of Arabidopsis in a petri dish. Germination is the process by which a seed begins to sprout and grow. If or when a seed will germinate is controlled by a variety of environmental and genetic factors. This experiment will investigate the role of gibberellic acid (GA) on germination. GA is a hormone that affects many different aspects of plant growth and development including germination, flowering, and fruit development. Most strains of Arabidopsis can synthesize GA internally and do not require the addition of external GA to germinate, grow, and develop.

Arabidopsis seeds are very small. It can be hard to tell if a seed has germinated at first. Use a magnifying glass or microscope to view your seeds. The thing you will see when your seeds germinate is a tiny root tip (radicle) breaking through the seed coat (Figure 1b). As germination continues you will notice the development of root hairs, the stem of the seedling (hypocotyl), and the green seed leaves (cotyledons).

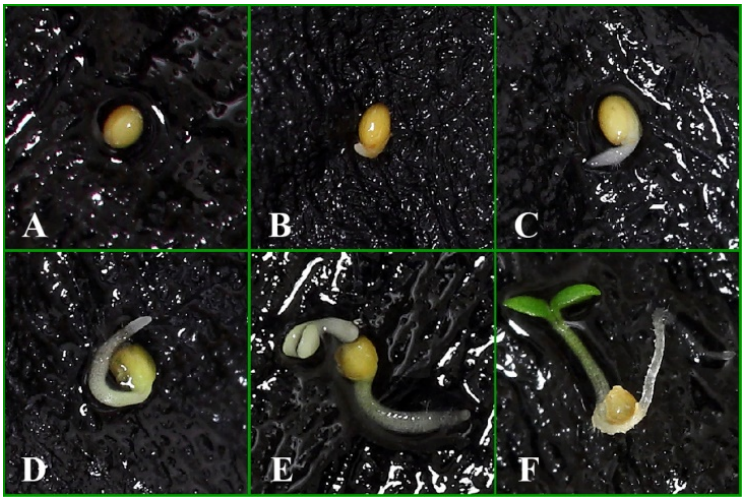

Figure 1. Stages of seed germination and seedling development in Arabidopsis (Mann *et al*., 2017).

SEED STRAIN DETAILS

Landsberg erecta (Ler-0, Catalog # CS20) – This strain of Arabidopsis is known as Landsberg or Ler-0. It was created in a laboratory using X-rays to cause a mutation that makes the plant grow very upright. Researchers often use Ler-0 to create new mutants.

CS3103 – Ler-0 is the parent plant for this strain of Arabidopsis. CS3103 has a mutation that prevents the plant from making its own gibberellic acid. The seeds will germinate if external gibberellic acid is added.

Complete the following tasks in your lab notebook.

1. Form a hypothesis for each prompt below. Be sure to explain your reasoning to support each hypothesis.
    a. How will the germination rate of each known genotype be affected by the water treatment?
    b. How will the germination rate of each known genotype be affected by the GA treatment?

2. Define key terms related to genetics, growth, and development:
   Genotype, reference strain, mutant/mutation, germination, dormancy, synthesize, gibberellic acid, stratification, radicle, hypocotyl

PROCEDURE 2 – Plate seeds

Materials

2 prepared plates
6 wood toothpicks
1 pipette
1 permanent marker (fine tip)
20 seeds each of CS20, CS3103, unknown
10 mL GA solution
2 parafilm strips
3 sheets wax paper
10 mL distilled water
1 sheet aluminum foil

NOTE: To prevent cross contamination be sure to use a new piece of wax paper and toothpick for each seed strain.

1. Water treatment – Use approximately 10 mL distilled water to thoroughly moisten the filter paper.

2. GA treatment – Use a pipette to remove approximately 10 mL of GA solution from the provided container. Thoroughly moisten the filter paper.

3. Drain excess liquid into a waste container or sink. Care should be taken to not over moisten the plate, as excess moisture could cause the seeds to shift from the designated location and/or promote the development of mold in the plate.

4. Select one genotype of seed to start. Note the genotype with a permanent marker on the corner of a piece of wax paper. Place approximately 20 seeds of the selected genotype on the wax paper.

5. Moisten the tip of a wooden toothpick using the wet filter paper in the prepared plate. If you are responsible for the water treatment, you should only moisten your toothpick with water. If you are responsible for the GA treatment, you should moisten your toothpicks with GA solution. Use the wet tip of the toothpick to pick up a single seed from the wax paper and place it in the corresponding space on the plate.

6. Repeat step 5 above until ten seeds have been plated in the appropriate space on each plate. Once both students in the group have plated 10 seeds of this genotype, the used wax paper and toothpicks can be discarded.

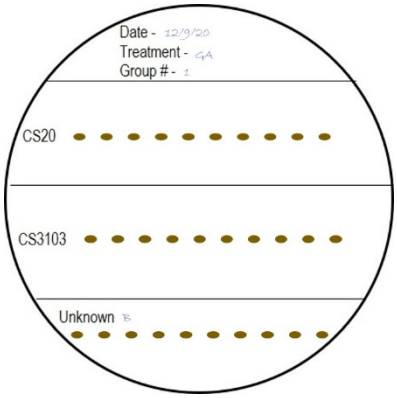

Figure 2. Sample of completed plate showing seed placement

7. Repeat steps 4-5 with the remaining two seed strains. When done, all three sections of both plates should contain ten seeds each of the seed strain that corresponds with each labeled section (Figure 2).

8. Care should be taken not to tip the plates sideways once the seeds have been plated. Prepared plates should be transported and stored horizontally to avoid displacing the seeds.

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

10. Stack your group's two plates on top of each other and wrap the stack in foil (Figure 3). Label the outside of the foil with your group number and/or names.


Figure 3. Example of how to stack prepared plates.

11. Place the wrapped plates in a refrigerator for 2-5 days. This process is known as cold treatment or stratification.

PROCEDURE 3 – Transfer Plates

1. Take the plates out of cold treatment and remove the foil. Unstack the plates and place them under fluorescent light on a 24-hour light cycle.

ASSIGNMENT 2 – Collect Data

Complete the following tasks and record the requested data on your data sheet. Your teacher will either provide you with a data sheet or ask you to create your own.

1. Record the date the plates are removed from cold treatment and placed in the growth area.

2. Starting on the day the plates are removed from cold treatment, use a magnifying glass or dissecting microscope to observe the seeds in each plate. Count and record the number of germinated seeds per genotype daily.

    a. If you see the root radicle (Figure1b) count that seed as germinated. For the purposes of this experiment, it is important to be conservative in your counting. If you are unsure if a seed has germinated, do not count it.

    b. Condensation may form on the lid of the petri dish, making it difficult to see the seeds. If this happens, it is okay to remove the lid for data collection. Be sure to replace the lid and rewrap the edges with parafilm so that the filter paper does not dry out.

Figure 1. Stages of seed germination and seedling development in Arabidopsis (Mann *et al*., 2017).

ASSIGNMENT 3 – Data Analysis

Complete the following tasks in your lab notebook and data sheet.

1. As a class, aggregate the data collected for each plate in each treatment. Determine the daily class average for each genotype.

2. Plot the daily class average for each genotype in the water treatment on a X-Y scatter plot. Plot the results for the GA treatment in a different X-Y scatter plot.

3. Review your results. Does your data support the hypotheses you made in Assignment 1? Explain.

4. Based on what you observe, determine if your unknown is CS20 or CS3103. How did you come to this conclusion?

Appendix I - Life in Bloom Basic Student Data Sheet

Group Number: _____ Unknown: A or B (circle one)

Water treatment – Student Name: _____

GA treatment – Student name: _____

Water Treatment					
	Date:	Date	Date:	Date:	Date:
CS20					
CS3103					
Unknown					
Class average – CS20					
Class average – CS3103					

GA Treatment					
	Date:	Date	Date:	Date:	Date:
CS20					
CS3103					
Unknown					
Class average – CS20					
Class average – CS3103					