Life in Bloom Basic

Summary: This module guides students through the process of investigating the role of the plant hormone gibberellic acid (GA) in germination. Through these activities, students will attempt to germinate two known strains and one unknown strain of Arabidopsis with and without the addition of external GA, observe the results, and draw a conclusion as to the genotype of the unknown strain.

Recommended Grade Level: Middle and high school

Duration: This module includes three procedures and three assignments. It requires nine days for completion of all activities. For a more advanced experiment exploring the GA biosynthetic pathway and its role in germination, see Life in Bloom Advanced.

Learning Objectives

Through this module students will:

- Germinate two known strains and one unknown strain of Arabidopsis
- Make observations and compare the germination rate of different strains grown with and without external GA
- Collect, display, and interpret data
- Determine the effect of external GA on the two known strains of Arabidopsis
- Determine the genotype of the unknown strain of Arabidopsis
- Define concepts and terms associated with genetics, growth, and development

Alignment with Next Generation Science Standards

NGSS	
Standards	-From Molecules to Organisms: Structures and Processes (MS-LS1-5 & HS-LS1-3) -Heredity: Inheritance and Variation of Traits (MS-LS3-1)
Science & Engineering Practices	-Scientific investigations use a variety of methods
Disciplinary Core Idea	-Structure and function -Growth and development of organisms -Variation of traits
Crosscutting Concepts	-Structure and function -Stability and change

Supporting Resources

The following supporting resources are available for download from the ABRC website:

- Life in Bloom Advanced protocol video
    - Note: This video was developed for the Life in Bloom Advanced module. Though the details of the modules vary, this video will help you understand how to prepare the materials for this experiment.
- Student handout: Laboratory procedures & assignments
- Life in Bloom Basic petri dish template
- Grading rubric
- Data analysis spreadsheet
- Example Results
- Growing Arabidopsis in the classroom
- Greening the Classroom Terms & Concepts

Materials

Two known strains and two unknown strains of Arabidopsis seeds (see *Seed Strain* Details below)

Petri dishes (100x15mm) – One per student

Petri dish template – One per student

Round filter paper (9 cm, grade 8, coarse) – Four per student

Wood toothpicks – Three per student

Wax paper (approximately 10cm x 10cm) – Three sheets per pair of students

Parafilm strips – One per student

Aluminum foil – One sheet per pair of students

Magnifying glasses or a dissecting microscope

Permanent markers (fine tip) – One per pair of students

Rulers – One per student

Ball point pens – One per student

Lab notebook – One per student

200 µM Gibberellic acid solution (10 mL per group)

Pipette – One per pair of students, or one in a central location with the GA solution

Distilled water (10 mL per group)

Refrigerator

Growth space with fluorescent lights

Seed Strain Details

- **Landsberg erecta** (Ler-0, Catalog # CS20) - This laboratory strain contains an X-ray induced mutation in the *ERECTA* gene, which causes the plants to have a more upright growth habit. Ler-0 is widely used to generate mutants.

- **CS3103 -** This strain was generated by exposing Landsberg to fast neutrons. The resulting mutation prevents the plants from synthesizing their own gibberellic acid. Seeds of this strain will germinate with the addition of external gibberellic acid. The resulting dwarf plant has small, dark green leaves.

Background Information

Arabidopsis thaliana (Arabidopsis) was the first plant to have its genome completely sequenced. Although technically a weed, this plant has been transformed into an important model system for plant research, and a useful tool in teaching a variety of science concepts in K-12 and college level instruction. Arabidopsis is member of the Brassicaceae family and is related to a number of common crop plants including cabbage, radish and cauliflower. It is a small, relatively easy to grow plant with a fast life cycle, going from seed to mature plant in six to eight weeks.

Through this module, students will germinate one reference strain, one mutant strain and one unknown strain of Arabidopsis. Germination is the process by which a seed begins to sprout and grow into a seedling under favorable conditions. Variation in germination requirements leads to differences in seed dormancy. This module will explore the role of gibberellic acid in the process of germination. Gibberellic acid (GA) is a hormone that influences many 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 for germination and proper growth and development.

Because Arabidopsis seeds are so small, it can be difficult to detect the early stages of germination. A magnifying glass or microscope is needed to observe the tiny root tip (or radicle) as it emerges from the seed coat. Through the germination process, you will first observe an intact seed (Figure 1a), then the radicle breaking through the seed coat (Figure 1b). In later stages of germination, you will notice the development of root hairs, the hypocotyl (stem of a germinating seedling, Figure 1e), and the green cotyledons (seed leaves, Figure 1f) of the new seedling.

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

Schedule of Procedures and Assignments

Day	Activity
Week 1	
Day 1*	Procedure 1 – Prepare plates Assignment 1 – Form hypotheses
Day 2**	Procedure 2 – Plate seeds
Days 3 – 4	No activity, plates are in cold treatment
Week 2	
Day 5	Procedure 3 – Transfer plates Assignment 2 – Collect data
Days 6 – 8	Assignment 2 – Collect data
Day 9	Assignment 2 – Collect data Assignment 3 – Data analysis

NOTES:

*Procedure 1 (Day 1 above) can occur at any time before the start of the experiment.

**We recommend that Procedure 2 (Day 2 above) take place on a Friday. This schedule will allow the seeds to be placed in cold treatment over the weekend and moved to the growth area on Monday.

Laboratory Procedures & Assignments

PROCEDURE 1 – Prepare petri dishes

1. Divide students into groups of two and assign each a group number. Assign one student in each group the water treatment, and the other the GA treatment. Assign each group one of the two unknowns. Each student will prepare one petri dish (plate).

2. Prepare one piece of labeled filter paper per student using the provided templates, a ruler, and a ballpoint pen. The following details should be included on each labeled piece of filter paper: date the seeds will be plated, treatment type (water or GA), group number, unknown (A or B).

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

4. 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.

5. Each group will have two prepared plates, one for the water treatment and one for the GA treatment.

ASSIGNMENT 1 – Form Hypotheses

1. Review the seed strain and background information provided.

2. 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?

3. 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

Prepare 200 mL of a 200 µM GA solution before starting this procedure with the students:
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1. Add 0.0138 g (13.8 mg) GA to a 200 mL volumetric flask (or graduated cylinder).

2. Add enough 95% EtOH (ethanol) to dissolve the GA. Start by adding 1-2 mL 95% EtOH, swirl the container to dissolve. Keep adding 1-2 mL of 95% EtOH until the GA is fully dissolved.

3. Add enough distilled water to the container to bring the volume to 200 mL.

Prepared GA solution can be stored in a foil wrapped container for up to two weeks in the refrigerator (4°C). Exposure to light for extended periods of time can cause the GA to degrade.

For this procedure, each group will need 20 seeds each of CS20, CS3103 and their assigned unknown. Each student within the group should work with only one seed strain at a time. Students should use a new piece of wax paper and toothpick for each seed strain to prevent cross contamination.

1. Thoroughly moisten the filter paper. Students assigned to the water treatment should use approximately 10 mL distilled water only. Students assigned to the GA treatment should use approximately 10 mL of GA solution. 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. Drain excess liquid into a waste container or sink.

2. 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.

3. Moisten the tip of a wooden toothpick using the wet filter paper in the prepared plate. Students with the water treatment should only moisten their toothpick with water. Students with the GA treatment should moisten their 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 (see template for reference).

4. Repeat step 3 above until ten seeds have been plated in the appropriate space on the plate. Once both students in the group have plated 10 seeds of this genotype, the used wax paper and toothpicks can be discarded.

5. Repeat steps 2 – 4 with the remaining two seed strains. When done, all three sections of the plate should contain ten seeds each of the seed strain that corresponds with the label (Figure 2).

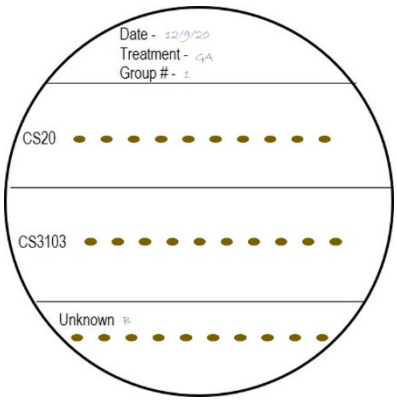

Figure 2. Sample of completed plate showing seed placement

6. 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.

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

8. Stack the 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.

9. 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

Provide students with a printed data sheet (Appendix I), have them prepare an excel data sheet, or use the pre-made excel sheet available for download with this module.

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.

ASSIGNMENT 3 – Data Analysis

Students can use the provided data sheet to generate graphs, create their own graphs using excel or plot the data on paper by hand. Remind students to label their axes, include a figure legend and title their graph.

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?

References

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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					