Life in Bloom Advanced

Student Handout – Lab Procedures & Assignments

For the following procedures you will be working as a group. You will be assigned a group number and an experimental treatment. The materials listed for each procedure include the supplies your group will need to complete the activity.

PROCEDURE 1 – Prepare petri dishes

Materials

3 petri dishes	3 petri dish templates	12 pieces of filter paper
3 rulers	3 ball point pens	

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 three pieces of labeled filter paper per group using the provided templates, 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 from the date the plates are prepared)
    b. Treatment type (light/dark & water/GA)
    c. Replicate number
    d. Group number.

2. 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.

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.

4. Repeat steps 2-3 two more times to prepare all three plates.

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.

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. In this experiment your group will germinate five strains of Arabidopsis in a petri dish. You will explore the effect of mutations that affect different stages of GA synthesis (Figure 1).

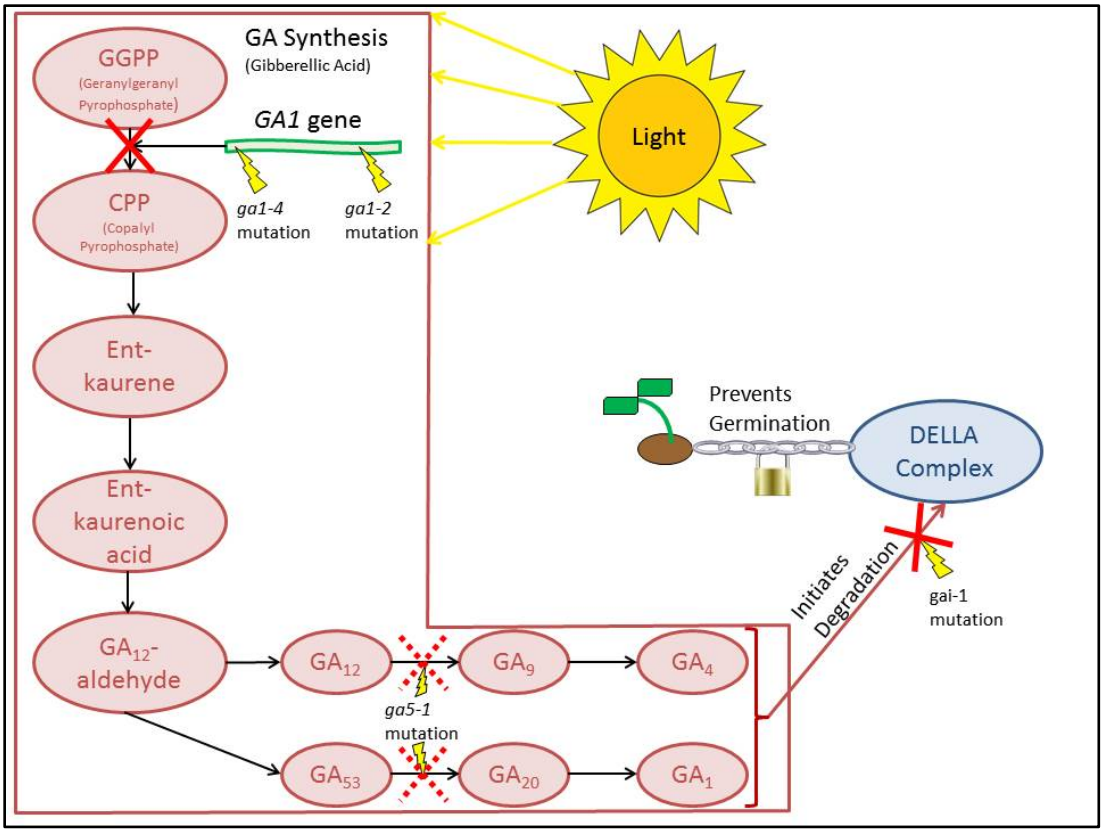

Figure 1. Gibberellic biosynthesis and signaling

Figure 1 shows a simplified version of the GA synthesis and signaling pathway. The parts of the pathway that are disrupted by the various mutants used in this experiment are each marked with a red (solid or dashed) X. The pink ovals in this figure represent the different compounds (called products) produced throughout the GA biosynthetic pathway. Several biologically active gibberellic acids are formed in the later stages of the pathway. GA4 is thought to be the main biologically active GA in many plants including Arabidopsis. The external GA supplied in these experiments is GA3.

Looking at Figure 1, you can see that the *ga1-4* and *ga1-2* mutations block the function of the *GA1* gene. The protein encoded by this gene acts early in the pathway and affects the production of all later products. The *ga5-1* mutation affects a protein that acts at a later stage of the pathway. This mutation affects the production of several GA products later in the pathway including GA9, GA4, GA20 and GA1 but does not affect production of earlier products including GA12 and GA53. The *gai-1* mutation does not affect the production of GA, but alters the sensing of and response to GA. When present, GA binds to a receptor that initiates the breakdown of DELLA proteins. Once these proteins have broken down, germination can occur. The gai-1 mutation prevents the breakdown of the DELLA complex.

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. Ler-0 is the parent plant for the four mutant strains used in this experiment.

ga1-2 (Catalog # CS3103) – This strain carries a mutation that affects the *GA1* gene, which encodes an enzyme involved in the early stages of GA synthesis.

ga1-4 (Catalog # CS3105) – This strain carries a mutation affects the same gene as in the *ga1-2* mutant *(GA1),* but the mutation is in in a different portion of the gene.

ga5-1 (Catalog # CS62) – This strain carries a mutation affecting the *GA5* gene which encodes an enzyme that acts late in the GA biosynthetic pathway, disrupting the production of some but not all GAs.

gai-1 (Catalog # CS63) – This strain carries a mutation affecting the GIBBERELLIC ACID INSENSITIVE (GAI) gene, which is involved in sensing and responding to GA.

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 genotype be affected by the group's treatment conditions?
    b. How will the germination rate of each genotype be affected by the treatment conditions being tested by other groups?

2. Define key terms related to genetics, growth, and development:

    Genotype, phenotype, reference strain, mutant/mutation, gene, germination, biosynthesis, synthesize, gibberellic acid, stratification, radicle, hypocotyl

PROCEDURE 2 – Plate seeds

Materials

3 prepared plates
5 wood toothpicks
1 permanent marker (fine tip)
30 mL GA3 solution (groups 2 & 4)
30 seeds of each genotype
3 parafilm strips
30 mL distilled water (groups 1 & 3)
1 pipette (groups 2 & 4)
5 sheets wax paper
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. Groups 1 and 3 - Use approximately 10 mL distilled water to thoroughly moisten the filter paper in each plate. Groups 2 and 4 – Use approximately 10 mL of GA3 solution to thoroughly moisten the filter paper in each plate.

2. Care should be taken to not over moisten the plates, 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.

3. Select three genotypes of seeds to start with. Assign a student to each of the selected genotypes. Use a permanent marker to note the genotype of each strain on a piece of wax paper. Place approximately 30 seeds of the selected genotype on the corresponding wax paper.

4. 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).

5. Repeat step 4 above until ten seeds have been plated in the appropriate space on the plate. Pass the plate to the next student to complete the same steps with their seed strain.

6. Repeat steps 4 and 5 until ten seeds have been plated in the appropriate space for all strains on all three plates. Once this is complete, the used wax paper and toothpick can be discarded.

7. Repeat steps 4 – 6 with the remaining two seed strains. When done, all five sections of the three plates should contain ten seeds each of the seed strains that correspond with the labels (Figure 3).

8. Care should be taken to not 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.

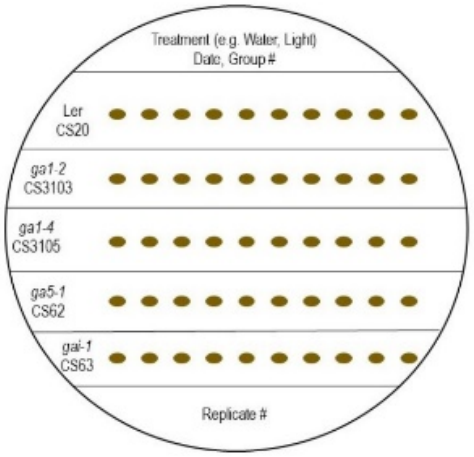

Figure 3. Petri dish layout

10. Stack the three plates on top of each other and wrap the stack in foil (Figure 4). Use a permanent marker to label the outside of the foil with your group number and treatment conditions.

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

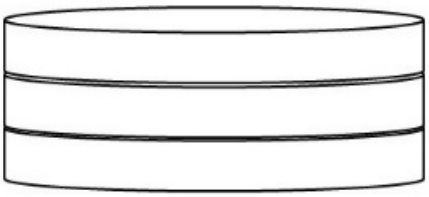
Figure 4. Stacked plates

PROCEDURE 3 – Transfer Plates

1. Take the plates out of cold treatment.

2. Groups 1 and 2 should remove the foil and place the plates, unstacked, under fluorescent light on a 24-hour light cycle.

3. Groups 3 and 4 should **not** remove the foil. These plates should be placed in a dark location (drawer or cabinet) in the same room as the light treatment plates.

ASSIGNMENT 2 – Collect Data

OBSERVING GERMINATION

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 2a), then the radicle breaking through the seed coat (Figure 2b). In later stages of germination, you will notice the development of root hairs, the hypocotyl (stem of a germinating seedling, Figure 2e), and the green cotyledons (seed leaves, Figure 2f) of the new seedling.

Figure 2. Stages of seed germination and seedling development in Arabidopsis (Mann *et al*., 2017).

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 areas.

2. Groups 1 and 2 should start collecting data on the day the plates are removed from cold treatment. Groups 3 and 4 will not collect data on their plates until the final day.

3. Groups 1 and 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 (Figure2b) 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.

4. Groups 3 and 4 – Confirm the data collected by Groups 1 & 2 for the first four days of data collection. Compare results, discuss, and resolve any differences in data.

5. Groups 3 and 4 – On the final day of data collection remove the dark treatment plates from the foil. Use a magnifying glass or dissecting microscope to observe the seeds in each plate. Count and record the number of germinated seeds per genotype.

ASSIGNMENT 3 – Data Analysis

Complete the following tasks in your lab notebook and datasheet.

1. Groups 1 and 2: Create X-Y scatter plots to compare the germination for each of the five genotypes on each of the five days of data collection.

2. Groups 3 and 4: Create bar graphs comparing the final germination of their treatment compared to the final germination of the corresponding light-grown treatment.

3. Review the data and figures for the group and draw conclusions about the effect of each mutation on germination. Write a summary of your findings and share with the class.

4. Determine if the data collected supports the hypotheses made in Assignment 1? Explain.

Review the background information included in Assignment 1, paying careful attention to Figure 6 and to where in the GA biosynthetic pathway each mutation occurs. Consider the results of your experiment. Use this information to answer the following questions.

5. What can you conclude about the GA1 gene based on the germination results for the *ga1-2* and *ga1-4* genotypes?

6. What can you conclude about the impact of the ga5-1 mutation on germination?

7. Compare the light and dark GA treatments. What differences do you notice? What can you conclude about the impact of the gai-1 mutation on germination?

8. Compare the class results with those of the example data. Identify and explain any differences in results between the two data sets.

Appendix I - Life in Bloom Advanced Student Data Sheet for Light Treatments

Group Number: _____ Treatment (water or GA solution):_____

Student Names: _____

Replicate #1

Genotype	Date:	Date:	Date:	Date:	Date:	Observations
CS20 *Ler*						
CS3103 *ga1-2*						
CS3105 *ga1-4*						
CS62 *ga5-1*						
CS63 *gai-1*						

Replicate #2

Genotype	Date:	Date:	Date:	Date:	Date:	Observations
CS20 *Ler*						
CS3103 *ga1-2*						
CS3105 *ga1-4*						
CS62 *ga5-1*						
CS63 *gai-1*						

Replicate #3

Genotype	Date:	Date:	Date:	Date:	Date:	Observations
CS20 *Ler*						
CS3103 *ga1-2*						
CS3105 *ga1-4*						
CS62 *ga5-1*						
CS63 *gai-1*						

Appendix II - Life in Bloom Advanced Student Data Sheet for Dark Treatments

Group Number: _____ Treatment (water or GA solution):_____

Student Names: _____

Replicate #1

Genotype	Date:	Observations
CS20 *Ler*		
CS3103 *ga1-2*		
CS3105 *ga1-4*		
CS62 *ga5-1*		
CS63 *gai-1*		

Replicate #2

Genotype	Date:	Observations
CS20 *Ler*		
CS3103 *ga1-2*		
CS3105 *ga1-4*		
CS62 *ga5-1*		
CS63 *gai-1*		

Replicate #3

Genotype	Date:	Observations
CS20 *Ler*		
CS3103 *ga1-2*		
CS3105 *ga1-4*		
CS62 *ga5-1*		
CS63 *gai-1*		