Life in Bloom Advanced

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 one reference strain and four mutant strains of Arabidopsis in light and dark conditions both with and without the addition of external GA. Students will observe the results and draw conclusions about the effect of different mutations involved in the production of and response to GA.

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 less complex experiment exploring the role of GA in germination see Life in Bloom Basic.

Learning Objectives

Through this module students will:

- Germinate one reference strain and four mutant strains of Arabidopsis
- Make observations and compare the germination rate of different strains grown in light and dark conditions both with and without the addition of external GA
- Collect, display, and interpret data
- Determine the effect of mutations at different points along the GA biosynthetic pathway on germination
- Determine the effect of external GA on different mutants
- 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 from the ABRC website:

- Life in Bloom Advanced protocol video
- Student handout: Laboratory procedures & assignments
- Life in Bloom Advanced petri dish template
- Grading rubric
- Data analysis spreadsheet
- Example results
- Growing Arabidopsis in the classroom
- Greening the Classroom terms & concepts

Materials

Five strains of Arabidopsis seeds (see *Seed Strain Details* below)

Petri dishes (100x15mm) – Three per group

Petri dish template – Three per group

Round filter paper (9cm, grade 8, coarse) – 12 per group

Wood toothpicks – Five per group

Wax paper (approximately 10cm x 10cm) – Five sheets per group

Parafilm strips – Three per group

Aluminum Foil – One sheet per group

Magnifying glasses or a dissecting microscope

Permanent marker (fine tip) – One per group

Ruler – Three per group

Ball point pen – Three per group

Lab notebook – One per student

200 µM Gibberellic acid (GA3) solution – Approximately 30 mL per group (groups 2 & 4 only)

Distilled water – Approximately 30 mL per group (groups 1 & 3 only)

Pipette – One per group (groups 2 & 4 only) or one in a central location with the GA solution

Refrigerator

Dark growth space

Light growth space with fluorescent lights

Seed Strain Details

Landsberg erecta (Ler-0, Catalog # CS20) – This laboratory strain of Arabidopsis is widely used to generate mutants. It serves as the reference strain for this experiment.

ga1-2 (Catalog # CS3103) – This strain was generated by exposing Landsberg to fast neutrons. The resulting mutation affects the *GA1* gene, which encodes an enzyme involved in the early stages of GA synthesis, ent-copyl diphosphate synthetase, preventing the plants from synthesizing their own GA. Seeds of this strain will germinate with the addition of external GA.

ga1-4 (Catalog # CS3105) – This strain was generated by exposing Landsberg to fast neutrons. The resulting mutation affects the same gene as in the *ga1-2* mutant *(GA1),* but the mutation is in in a different portion of the gene. The plants cannot synthesize their own GA. Seeds of this strain will germinate with the addition of external GA.

ga5-1 (Catalog # CS62) – This strain carries an ethylmethane sulfonate-induced mutation affecting the GA5 gene which encodes the gibberellin 20 oxidase enzyme that acts late in the GA biosynthetic pathway, disrupting the production of some but not all GAs. The resulting mutant does not require the addition of external GA for germination, but germination is enhanced by GA.

gai-1 (Catalog # CS63) – This strain carries an X-ray induced semi-dominant mutation affecting the GIBBERELLIC ACID INSENSITIVE (GAI) gene, which encodes a transcription factor involved in sensing and responding to GA. This strain cannot respond to GA so the addition of external GA has no effect on this mutant.

Group Assignments

Group #	**Treatment**: Light/Dark, Water/GA solution
Group 1	Light, water
Group 2	Light, GA solution
Group 3	Dark, water
Group 4	Dark, GA solution

NOTES:

*Procedure 1 (Day 1 in the *Schedule of Procedures and Assignments*) can occur at any time before the start of the experiment.

**We recommend that Procedure 2 (Day 2 in the *Schedule of Procedures and Assignments*) 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.

Schedule of Procedures and Assignments

Day	**Activity**
Week 1	
Day 1*	Procedure 1 – Prepare plates Assignment 1 – Form hypothesis
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
Day 6 – 8	Assignment 2 – Collect data
Day 9	Assignment 2 – Collect data Assignment 3 – Data analysis

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 a 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 and four mutant strains of Arabidopsis in both light and dark conditions. Germination is the process by which a seed begins to sprout and grow into a seedling. Seeds require the right conditions to germinate. Temperature, light, and water are the external forces that influence germination. The plant hormone gibberellic acid (GA) is a critical component of the internal control of the development processes in plants. GA influences many aspects of plant growth and development including germination, flowering, and fruit development. Most strains of Arabidopsis can synthesize a range of biologically active GAs internally and do not require the addition of external GA for germination and proper growth and development. This experiment will explore the effects of several mutations affecting different stages of the GA biosynthetic pathway (Figure 1).

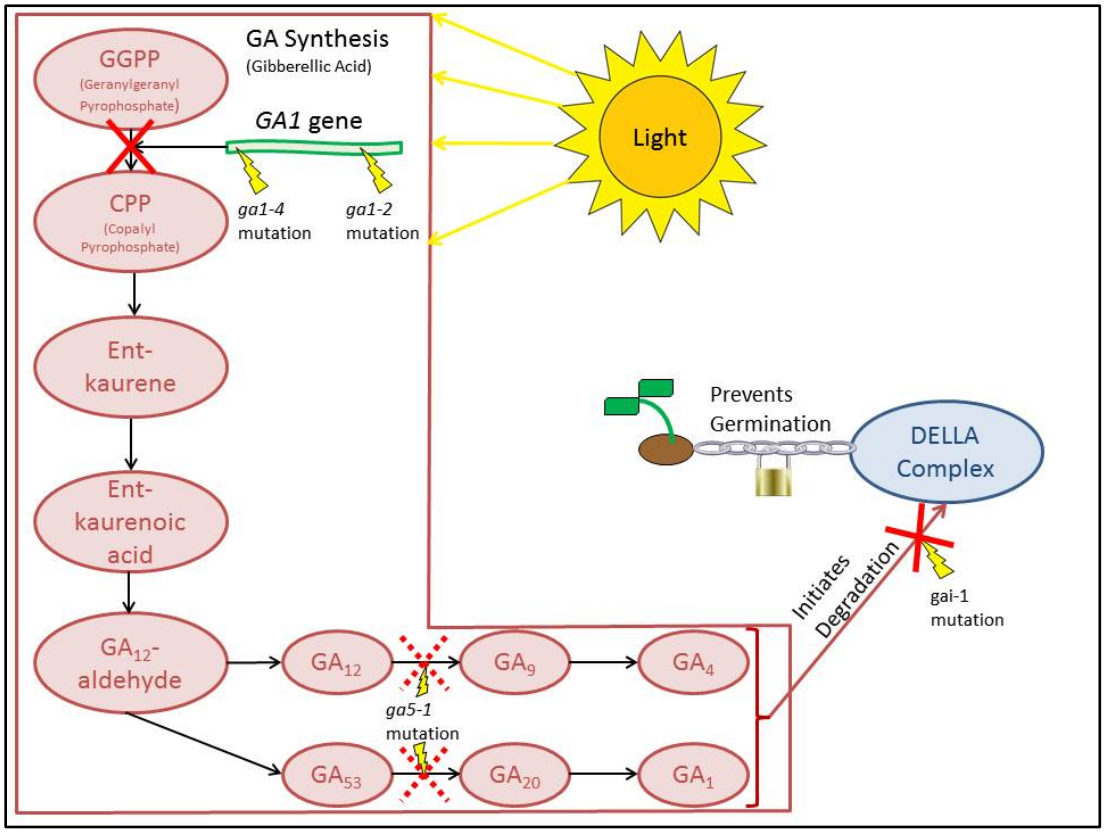

Figure 1. Gibberellic biosynthesis and signaling

Figure 1 shows a simplified version of the GA synthesis and signaling pathway indicating which part of the pathway is disrupted in the various mutants used in this experiment. The pink ovals in this figure represent the products in 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.

The *ga1-4* and *ga1-2* mutations block the function of the same gene early in the pathway affecting the production of all later products in the pathway. Results from this experiment will demonstrate that both regions of this gene are necessary for the gene to function, and that the GA precursor GGPP that is generated before the step affected by the mutation is not able to initiate germination. Neither of these strains will germinate in light or dark conditions without the addition of external GA.

The *ga5-1* mutation affects a later stage of GA synthesis. This mutation reduces 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 *ga5-1* strain will germinate more slowly than the reference strain when grown in light conditions indicating that there is still some GA activity in the mutant in light conditions, or that light is able to substitute for GA in promoting germination. Very little or no germination occurs in dark conditions. Addition of GA will promote germination in this strain in both light and dark.

The gai-1 mutation affects the sensing of and response to GA through the DELLA complex. When present, GA binds to a receptor that initiates the degradation of DELLA proteins. Once these proteins are degraded, germination can occur. When grown in light *gai-1* mutants germinate at lower levels than Ler or any of the GA biosynthetic mutants. This result indicates that light alone is not able to substitute for GA in promoting germination. Adding external GA to *gai-1* does not result in increased germination. This is because gai-1 mutants are able to produce GA, but the mechanism that allows the plant to sense and respond to GA is disrupted by the mutation.

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

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.

Laboratory Procedures & Assignments

PROCEDURE 1 – Prepare petri dishes

1. Divide the class into four groups. Assign each group a number that corresponds to an experimental treatment in the *Group Assignments* chart. Each group will prepare three petri dishes (plates) in order to run three replicates of their experimental treatment.

2. 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: date the seeds will be plated (may be different from the date the plates are prepared), treatment type (light/dark & water/GA), replicate number and group number.

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

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 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?

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

    Genotype, phenotype, reference strain, mutant/mutation, gene, germination, biosynthesis, synthesize, gibberellic acid, stratification, radicle, hypocotyl (See Greening the Classroom terms and conditions document for definitions)

PROCEDURE 2 – Plate seeds

Prepare 200 mL of a 200 µM GA3 solution before starting this procedure with the students:

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 30 seeds each of the five seed strains. Care should be taken to prevent cross contamination between seed samples. Individual students within the group should work with only one seed strain at a time. The group should use a new piece of wax paper and toothpick for each seed strain.

1. Thoroughly moisten the filter paper in each plate. Groups 1 and 3 should use approximately 10 mL distilled water only. Groups 2 and 4 should use approximately 10 mL of GA solution. 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.

2. 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.

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. Pass the plate to the next student to complete the same steps with their seed strain.

5. Repeat steps 3 and 4 until ten seeds have been plated in the appropriate space for all three strains on all three plates. Once this is complete, the used wax paper and toothpick can be discarded.

6. Repeat steps 2 – 5 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).

7. 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.

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

9. 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.

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

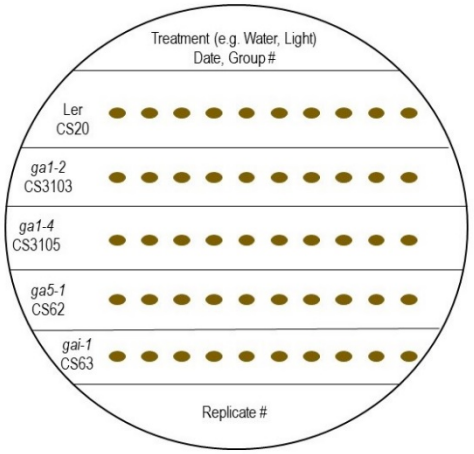

Figure 3. Petri dish layout

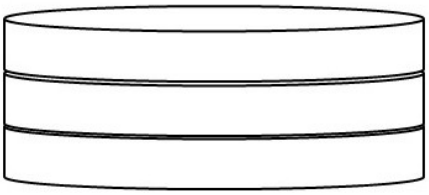
Figure 4. Stacked plates

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

Provide students with a printed data sheet (Appendices I & II), 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 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. Teacher Note: There should be no germinated seeds on the first day of data collection

    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.

    c. Since students in groups 3 and 4 will not be collecting data on their plates for the first four days of data collection, they should confirm the data gathered by groups 1 and 2. Have groups 3 and 4 also count the number of germinated seeds in groups 1 and 2's plates and compare results. Discuss and resolve any differences in data.

4. Groups 3 and 4 – On the final day of data collection (this would be Friday if the plates were removed from cold treatment on Monday) 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

Students can use the provided excel 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. 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.

   Expected results:

   - No germination in the water treatments for the *ga1-4* and *ga1-2* genotypes.
   - The *ga5-1* genotype germinates more slowly than wild-type on water.
   - Nothing germinates in the water/dark treatment plates for *ga1-2*, *ga1-5* and *gai* genotypes.
   - Adding GA to the dark treatment for the *gai-1* genotype will not rescue germination.
   - Adding GA to the dark treatments will rescue germination for all genotypes except *gai-1*.
   - Light is not an absolute requirement for all plants. The same can be said of GA. Seeds of certain genotypes grown in dark conditions may germinate at low levels in the presence of GA. Conversely, the presence of light can partially overcome the need for GA to initiate germination in some genotypes.

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

Review the background information, 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?

   The ga1-2 and ga1-4 mutations occur early in the GA biosynthetic pathway. Both mutations block the function of the GA1 gene. This demonstrates that both regions of the gene are necessary for the gene to function. It also demonstrates that the GGPP precursor to GA is not able to initiate germination.

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

   The ga5-1 mutation affects a later stage of GA synthesis. This mutation only causes a slight reduction in the germination of this genotype. This indicates that some of the GAs produced before this step in the pathway are biologically active in stimulating germination although not as effective as later products.

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?

   The gai-1 mutation reduces the seed's ability to sense and respond to GA. The gai-1 mutation completely blocks this genotype's ability to germinate in dark conditions. Both the water and GA treatments will germinate in light conditions, though at a lower rate than other genotypes.

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

References

Fleet, C. and Williams, M.E. (2011). Gibberellins. Teaching Tools in Plant Biology: Lecture Notes. *The Plant Cell (online)*.

Koornneef, M. and van der Veen, J.H. (1980). Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) heynh. *Theoretical and Applied Genetics*, *58(6)*, 257-263.

Mann, J.W., Larson, J., Pomeranz, M., Knee, E.M., Shin, D., Miller, J.A., Price, C.G., Crist, D.K., Grotewold, E., and Brkljacic, J. (2017). Linking Genotype to Phenotype: The effect of a mutation in gibberellic acid production on plant germination. *CourseSource*. https://doi.org/10.24918/cs.2017.18

Sun, Tp., Goodman, H.M. and Ausubel, F.M. (1992). Cloning the Arabidopsis GA1 locus by genomic subtraction. *The Plant Cell, 4(2),* 119-128.

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*		