Who Turned Out the Lights?

Summary: This module guides students through the process of investigating reference and mutant strains of Arabidopsis to learn about how plants sense and respond to light. Through these activities students will learn about concepts such as growth and development, response to stimuli, mutations, phenotypes and genotypes.

Recommended Grade Level: Middle and high school

Duration: This module requires one day for preparation of materials, and eight days for completion of all student laboratory procedures and assignments.

Learning Objectives

Through this module students will:

- Plate and germinate four strains of Arabidopsis
- Make observations and compare phenotypes of mutant and reference strains grown in different conditions
- Collect and analyze data
- Determine the effect of different photoreceptor mutations on seedling growth and development
- Define concepts and terms associated with photomorphogenesis, the growing process, and plant anatomy.

Alignment with Next Generation Science Standards

NGSS	
Standards	-From Molecules to Organisms: Structure and Processes (MS-LS1-5, MS-LS1-8, HS-LS1-1 -Heredity: Inheritance and Variation of Traits (MS-LS3-1, HS-LS3-1)
Science & Engineering Practices	-Constructing explanations and designing solutions -Scientific investigations use a variety of methods -Asking questions and defining problems
Disciplinary Core Idea	-Structure and Function -Growth and Development of Organisms -Information Processing -Inheritance of Traits -Variation of Traits
Crosscutting Concepts	-Cause and effect -Structure and function

Supporting Resources

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

- Student handout: Laboratory procedures & assignments
- Grading rubric
- Growing Arabidopsis in the Classroom
- Greening the Classroom Terms & Conditions

Materials

4 strains of Arabidopsis seeds (See seed strain detail below)
Murashige and Skoog (MS) basal media (suggested: Item# M0404-1L, Sigma-Aldrich)

- NOTE: 1L of basal media is enough to prepare approximately 28 petri dishes

Agar (suggested: Item# A7921-100G, Sigma-Aldrich)
KOH (suggested: Item# P4494-50ML, Sigma-Aldrich)
pH meter (suggested: Item# HI98100, Hanna Instruments)
24 Petri dishes
Distilled water
Scale
1L glass beaker
Two 1L glass bottles
Wax paper
Toothpicks
Permanent marker (fine tip)
Aluminum foil
Plastic wrap
Parafilm – suggested:Item #1602181, Staples
Rulers
Magnifying glasses, optivisors or dissecting microscope
Gloves
Autoclave or microwave (required for Procedure 1)
Magnetic stir bar (required for Procedure 1.1 only)
Stir plate (required for Procedure 1.1 only)
Water bath (required for Procedure 1.2 only)
Autoclave, pressure cooker or oven (required for Procedure 2)
Growth space with fluorescent lights
Refrigerator

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, and serves as the reference strain for the three mutants used in this module.

- ***phyB-5*** (Catalog # CS6213) – This strain carries a recessive, homozygous ethylmethane sulfonate (EMS)-induced mutation in the phytochrome B gene. Phytochrome B is a photoreceptor involved in sensing red light. The phenotype of this mutant includes a long hypocotyl, elongated petioles, stems and root hairs, smaller leaves, fewer rosette leaves, longer main inflorescences with fewer siliques, and fewer lateral branches. This mutant flowers earlier than the reference strain.

- ***phyA-201*** (Catalog # CS6219) – This strain carries a recessive, homozygous EMS-induced mutation in the phytochrome A gene. Phytochrome A is a photoreceptor involved in sensing far-red light. The phenotype of this mutant includes an elongated hypocotyl and unexpanded cotyledons when grown in dark conditions, and a reduced greening response when dark-grown plants are placed in light conditions. Mutants grown in light conditions display a similar phenotype to reference strain plants.

- ***phyB-5, phyA-201*** (Catalog # CS6224) – This double mutant was generated by crossing *phyB-5* with *phyA-201*, resulting in recessive, homozygous EMS-induced mutations in both the phytochrome A and phytochrome B genes. These mutations affect the plant's ability to sense both red and far-red light. The phenotype of this double mutant includes slightly longer and longer hypocotyl length than the *phyB-5* mutant when grown in light and dark conditions respectively, poor development of cotyledons when grown in dark conditions, and reduced chlorophyll induction.

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 food 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.

This module explores how plants sense differences in their environment through the action of photoreceptors, and respond to differing conditions by modifying their growth and development. Light is essential for plant growth and survival. With the exception of parasitic species, plants do not obtain their energy from feeding on other organisms as animals do. Instead, plant utilize light energy to produce sugars through photosynthesis. Since light is a critical component of photosynthesis, it is important that plants can sense light and respond appropriately to maximize light capture.

Photoreceptors are light-sensitive proteins found throughout the plant and animal kingdoms. These proteins allow organisms to detect light, and pass a message that light has been detected to the network of molecules involved in producing a response. The response to light could involve activation or repression of genes, biochemical pathways, or hormones. In plants, these responses eventually result in differences in growth and development at the whole plant level.

Light is important during all phases of plant growth. When a seedling first germinates it relies on food reserves present in the seed for initial growth. However, as that food reserve diminishes, the seedling must initiate photosynthesis to generate its own food in order to survive. If a seed germinates in the absence of light, the resulting seedling will often respond by growing a long hypocotyl (stem), delaying the development of leaves, and delaying the production of chlorophyll. Chlorophyll is the green pigment responsible for light capture in photosynthesis. Thus, dark-grown seedlings tend to be tall and pale, with small or absent leaves. Conversely, seedlings germinated in light conditions tend to be short and green with larger leaves. In addition to affecting germination and plant architecture, the presence or absence of light also affects other important development stages including flowering, fruiting and senescence.

Scientists study plant responses to environmental stimuli, such as light, to learn about the factors controlling plant growth in general, and to study in detail the molecules involved in sensing the signal and producing the response. Insights gained from studying these types of molecules in plants can be translated to work on animals, including humans. Arabidopsis is well suited to this type of investigation. Many mutants of Arabidopsis are available allowing scientists to investigate the networks of genes and proteins involved in sensing and responding to light. There are three main categories of photoreceptors in plants that are responsible for sensing red/far-red, blue/UV-A, and UV-B light. This module investigates the effect of red/far-red photoreceptors, known as phytochromes, on the early stages of growth just after a seed has germinated. Phytochromes differ in the quality of light they sense and respond to. A range of phytochrome mutants of Arabidopsis are available from the Arabidopsis Biological Resource Center (ABRC). This module will investigate three phytochrome mutants in which specific phytochrome photoreceptors are absent, or non-functional.

Schedule of Procedures and Assignments

Day	Activity
Day 0	Procedure 1 - Prepare sterile media Procedure 2 – Sterilize toothpicks
Day 1	Procedure 3 – Plate seeds Begin cold treatment
Days 2-3	Continue cold treatment
Day 4	Procedure 4 – Transfer to growth environment Assignment 1 – Key terms and experimental design
Days 5-7	No activity
Day 8	Assignment 2 – Observations and measurements Assignment 3 – Display and interpret data Assignment 4 – Digging deeper

Laboratory Procedures & Assignments
Procedures 1 and 2 can be done immediately preceding or weeks in advance of Procedure 3. We recommend starting Procedure 3 on a Friday. This will allow the plates to be removed from cold treatment on Monday, and data collection to occur on the subsequent Friday.

NOTE: Gloves should be worn for all laboratory procedures.

PROCEDURE 1 – It is preferable that preparation of the sterile media is completed using an autoclave (Procedure 1.1). In the event that an autoclave is not available, the same result can be accomplished using a microwave oven (Procedure 1.2).

PROCEDURE 1.1 (Day 0) – Prepare sterile media using autoclave

1. Add 4.4 g of Murashige and Skoog Basal Medium to a beaker containing 0.8 L of distilled water and stir to dissolve. Add distilled water to a final volume of 1 L.
2. Check and adjust pH to 5.7 using 1M KOH.
3. Divide the media in half, placing 500 mL in two 1 L bottles. Add 5 g of agar per bottle. Keep the lid loose.
4. Place the bottles in an autoclave for 20 minutes at 121°C, 15 psi with a magnetic stir bar in the bottle.
5. Remove the bottles from the autoclave and place on a stir plate at low speed. Allow the agar medium to cool to 45-50°C (until the container can be held with bare hands). If available, perform steps 8 and 9 in sterile conditions within a laminar flow hood.
6. Divide the class into three groups. Each group will prepare a total of eight petri dishes (plates).
7. Using a fine point permanent marker, label the bottom of the plate with the group number, strain type and treatment (*e.g.* Group 1, Ler-0, dark). Note: Each group will prepare one light and one dark treatment for each of the four seed strains.
8. Pour enough media into the plates to cover approximately half of the depth of the dish.
9. Allow the plates to cool at room temperature for about an hour to allow the agar to solidify.
10. Once cool, wrap the plates in plastic wrap and store in the refrigerator until ready to use. Prepared plates can be stored for up to one month in the refrigerator prior to use.

PROCEDURE 1.2 (Day 0) – Prepare sterile media using microwave
NOTE: This procedure is written for 1000 or 1100 watt microwaves. Using a less powerful microwave will require slightly longer heating times.

CAUTION: The following steps involve bringing the media to a boil. Extreme caution should be used. This procedure should be completed by a teacher.

1. Add 4.4 g of Murashige and Skoog Basal Medium to a beaker containing 0.8 L of distilled water and stir to dissolve. Add distilled water to a final volume of 1 L.
2. Check and adjust pH to 5.7 using 1M KOH.
3. Divide the media in half, placing 500 mL in two 1 L bottles. Add 5 g of agar per bottle. Keep the lid loose.
4. Working with one bottle at a time (containing 500 mL media + 5 g agar), microwave the media for 4-5 minutes to bring the solution to a boil and dissolve all ingredients.
5. Allow the media to boil vigorously for approximately 5 seconds, however take care not to allow the solution to boil over. If the solution is close to boiling over, stop the microwave immediately.
6. When done, the solution should appear clear with no media granules visible. If the media is not clear, continue to microwave in short 10-15 timespans until all components are dissolved.
7. Once all of the ingredients have dissolved, microwave the solution for 15-20 seconds to bring it to a boil again. As before, the solution should boil vigorously but not boil over.
8. Bring the media to a boil two more times by repeating step 7 twice. Extreme caution should be used as the bottle will be very hot, and the media may superheat. If this occurs, the media may boil up when agitated even after the initial boiling has stopped.
9. Allow the bottle to stay in the microwave for many minutes before handling to reduce the risk associated with superheating. Use hot pad when removing the bottle from the microwave.

10. Place the bottle in a 50-56°C water bath and allow the media to cool (until the container can be held with bare hands). If available, perform steps 13 and 14 in sterile conditions within a laminar flow hood.
11. Divide the class into three groups. Each group will prepare a total of eight petri dishes (plates).
12. Using a fine point permanent marker, label the bottom of the plate with the group number, strain type and treatment (*e.g.* Group 1, Ler-0, dark). Note: Each group will prepare one light and one dark treatment for each of the four seed strains.
13. Gently swirl the bottle for 20-30 seconds, being careful not to introduce air bubbles into medium. Pour enough media into the plates to cover approximately half of the depth of the dish.
14. Allow the plates to cool at room temperature for about an hour to allow the agar to solidify.
15. Once cool, wrap the plates in plastic wrap and store in the refrigerator until ready to use. Prepared plates can be stored for up to one month in the refrigerator prior to use.

PROCEDURE 2 – It is preferable that the toothpicks be sterilized using an autoclave (Procedure 2.1). In the event that an autoclave is not available, the same result can be accomplished through the use of a conventional oven (Procedure 2.2) or pressure cooker (Procedure 2.3).

PROCEDURE 2.1 (Day 0) – Sterilize toothpicks in an autoclave

1. Wrap toothpicks in aluminum foil.
2. Place wrapped toothpicks in an autoclave on dry cycle.

PROCEDURE 2.2 (Day 0) – Sterilize toothpicks in an oven

1. Wrap toothpicks in aluminum foil.
2. Preheat oven to 320°F.
3. Place wrapped toothpicks in preheated oven for two hours.

PROCEDURE 2.3 (Day 0) – Sterilize toothpicks in a pressure cooker

1. Wrap toothpicks in aluminum foil.
2. Add one to two inches of water to the bottom of the pressure cooker.
3. Place wrapped toothpicks inside a beaker or other heat-proof container.
4. Place container in pressure cooker and set to 15 psi for 15 minutes.

PROCEDURE 3 (Day 1) – Plate seeds

NOTE: Work with one strain of seeds at a time to prevent cross-contamination. Use a new piece of wax paper and a new toothpick for each strain.

1. Sprinkle approximately 30 seeds of the selected strain onto a small piece of wax paper. Make sure you are working with the two petri dishes labeled for your selected seed strain.
2. Use a sterilized toothpick. Wet the tip of the toothpick by placing it gently on the surface of the agar. Touch the tip of the moistened toothpick to a seed to pick it up.
3. Touch the tip of the toothpick with the seed to the surface of the agar to place the seed on the media.
4. Repeat steps 2 and 3 until you have placed 15 seeds equally spaced on the surface of the agar medium.
5. Place the lid on the petri dish and wrap the edges with parafilm to prevent drying out.
6. Prepare a second petri dish with the same strain of seeds by repeating steps 2-5.
7. Repeat steps 1-6 for the remaining three strains of seeds. When complete, each group should have a light treatment and dark treatment petri dish for each of the four seed strains.
8. Each group should stack the four dark treatment dishes on top of each other and wrap the entire stack in aluminum foil. Be careful not to tip the dishes as this could displace the seeds. Label the stack with the group number and treatment (*e.g.* Group 1, dark).
9. Repeat step 8 for the light treatment dishes.
10. Place all prepared petri dishes in a refrigerator at approximately 4˚C for three days to promote uniform germination. This process is known as stratification.

PROCEDURE 4 (Day 4) – Transfer to growth environment

1. Remove all of the prepared dishes from the refrigerator.
2. Remove the foil (not the parafilm) from light treatment dishes and place them in a single row under fluorescent lights.
3. Do not unwrap the dark treatment dishes. Place the dark treatment stack in a drawer or closet in the same room as the light treatment dishes.
4. Allow plants to grow for four days.

ASSIGNMENT 1 (Day 4) – Key terms and experimental design

1. Define key terms associated with plant growth and development such as photomorphogenesis, photosynthesis, chlorophyll, photoreceptor, phytochrome, germination, senescence
2. List the control and variable for this experiment
3. Predict how the light and dark treatments will affect the reference and mutant plants

ASSIGNMENT 2 (Day 8) – Observations and measurement

1. Observe the seedlings in one of the light treatment and one of the dark treatment plates. Draw a seedling in your lab notebook and label the structures listed below:
    a. Hypocotyl, cotyledon, root, seed
2. Use a magnifying glass, optivisors or a dissecting microscope to observe the phenotype of the seedlings for the light and dark treatments for all four seed strains. Note the color of the hypocotyls and cotyledons, and the shape and size of the seedlings.
3. Note and illustrate any differences you notice between the reference strain and mutant seedlings for each treatment.
4. Use a ruler to measure the length of the hypocotyls of each seedling (in millimeters). Record your data in your lab notebook.

ASSIGNMENT 3 (Day 8) – Display and interpret data

1. Decide how to display the hypocotyl length data and create your visual.
2. Review the data and determine how the presence or absence of light affected each of the four strains of Arabidopsis.
3. Compare your interpretations to the predictions you made in Assignment 1.
4. Compare your results to those of the other groups in the class.
5. Use what you have learned to draw your conclusions about the importance of phytochrome A and B photoreceptors in plants.

ASSIGNMENT 4 (Day 8) – Digging deeper

1. Review scientific articles and/or credible online sources to learn more about how plants respond to the presence or absence of light. Write the citation for each of the sources reviewed in your lab notebook.
2. Answer the following questions:
    a. Why do plants grow tall and skinny when grown in the dark?
    b. Why are plants pale when grown in the dark?
    c. Why are there several types of photoreceptors in plants, and not just a single type?

References

Arabidopsis Biological Resource Center [ABRC]. (2016). Education and Outreach. Available online at https://abrcoutreach.osu.edu/

Briggs, W. R. & Olney, M. A. (2001). Photoreceptors in plant photomorphogenesis to date: Five photycrhomes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiology*, *125*, 85.

Mawphlang, O., & Kharshiing, E. V. (2017). Photoreceptor mediated plant growth responses: Implications for photoreceptor engineering toward improved performance in crops. *Frontiers in plant science*, *8*, 1181. doi:10.3389/fpls.2017.01181

Additional Reading

Nagatani, A., Reed, J. W. & Chroy, J. (1993). Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. *Plant Physiology*, 102(1), 269.

Reed, J. W., Nagatani, A., Elich, T. D., Fagan, M. & Chory, J. (1994). Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. *Plant Physiology*, 104(4), 1130.