Who Turned Out the Lights?

Student Handout – Lab Procedures & Assignments

PROCEDURE 1.1 – Prepare sterile media using autoclave

Materials

Autoclave
One 1L glass beaker
Scale
Plastic wrap
Murashige and Skoog (MS) basal media
Stir plate
Two 1L glass bottles
pH meter
Distilled water
Agar
Magnetic stir bar
8 petri dishes (per group)
Fine tip permanent marker
1M KOH
Gloves

1. Put on gloves.
2. Add 4.4 g of Murashige and Skoog Basal Medium to a beaker containing 0.8 L of distilled water and stir to dissolve.
3. Add distilled water to a final volume of 1 L.
4. Check and adjust pH to 5.7 using 1M KOH.
5. Divide the media in half, placing 500 mL in two 1 L bottles.
6. Add 5 g of agar per bottle. Keep the lid loose.
7. Place the bottles in an autoclave for 20 minutes at 121°C, 15 psi with a magnetic stir bar in the bottle.
8. 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 10 and 11 in sterile conditions within a laminar flow hood.
9. Using a fine point permanent marker, label the bottom of eight petri dishes with your group number, strain type and treatment (*e.g.* Group 1, Ler-0, dark). Each group will prepare one light and one dark treatment for each of the four seed strains (reference strain Ler-0, mutant *phyB-5*, *phyA-201*, and a double mutant strain *phyB-5, phyA-201*).
10. Pour enough media into each petri dish to cover approximately half of the depth of the dish. Place the lid on the petri dish.
11. Allow the petri dishes to cool at room temperature for about an hour to allow the agar to solidify.
12. Once cool, wrap the petri dishes in plastic wrap and store in the refrigerator until ready to use.

PROCEDURE 1.2 – Prepare sterile media using microwave

Materials

One 1L glass beaker
Scale
Plastic wrap
Microwave
Two 1L glass bottles
pH meter
Distilled water
Hot pads
8 petri dishes (per group)
Fine tip permanent marker
1M KOH
Water bath

NOTE: This procedure is written for 1000 or 1100 watt microwaves. Using a less powerful microwave will require slightly longer heating times.

CAUTION: This procedure involves bringing the media to a boil and extreme caution should be used. Much of this procedure will be performed by the teacher. The steps below are for the student portion of this procedure.

1. Using a fine point permanent marker, label the bottom of eight petri dishes with your group number, strain type and treatment (*e.g.* Group 1, Ler-0, dark). Each group will prepare one light and one dark treatment for each of the four seed strains (reference strain Ler-0, mutant *phyB-5*, *phyA-201*, and a double mutant strain *phyB-5, phyA-201*).

2. You can begin working with the sterile media once it has cooled to the point where the bottle can be handled with bare hands. If available, perform step 3 and 4 in sterile conditions within a laminar flow hood.

3. Gently swirl the bottle for 20-30 seconds, being careful not to introduce air bubbles into medium. Pour enough media into the petri dishes to cover approximately half of the depth of the dish. Place the lid on the petri dish.

4. Allow the petri dishes to cool at room temperature for about an hour to allow the agar to solidify.

5. Once cool, wrap the petri dishes in plastic wrap and store in the refrigerator until ready to use.

PROCEDURE 2 – Sterilize toothpicks

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).

Materials

One of the following: Autoclave, oven or pressure cooker
Toothpicks (prepare at least four per group)
Aluminum foil
Glass beaker (Procedure 2.3 only)

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 – Plate seeds

Materials (per group)

4 strains of Arabidopsis seeds (30 seeds of each)
4 sterilized toothpicks
8 strips of parafilm
Permanent marker
8 prepared petri dishes
4 pieces of wax paper
2 large pieces of aluminum foil
Refrigerator

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. Choose one seed strain to start with. Locate the two petri dishes labeled for your selected seed strain.

2. Sprinkle approximately 30 seeds of the selected strain onto a small piece of wax paper.

3. Wet the tip of one sterilized 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.

4. Touch the tip of the toothpick with the seed to the surface of the agar to place the seed on the media. Be careful not to push the seed into the media.

5. Repeat steps 3 and 4 until you have placed 15 seeds equally spaced on the surface of the agar medium.

6. Place the lid on the petri dish and wrap the edges with parafilm to prevent drying out.

7. Prepare the second petri dish with the same strain of seeds by repeating steps 3-6.

8. Discard the used wax paper and toothpick.

9. Repeat steps 1-8 for the remaining three strains of seeds. When complete, your group should have a light treatment and dark treatment petri dish for each of the four seed strains.

10. 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 your group number and treatment (*e.g.* Group 1, dark).

11. Repeat step 10 for the light treatment dishes.

12. 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 – 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 – Key terms and experimental design
Complete the following tasks in your lab notebook:

1. Define key terms associated with plant growth and development:

   Photomorphogenesis, photosynthesis, chlorophyll, photoreceptor, phytochrome, germination, senescence

2. List the control and variable for this experiment.

3. Make a prediction about how the light and dark treatments will affect each of the different strains of Arabidopsis.

ASSIGNMENT 2 – Observations and measurement
Complete the following tasks in your lab notebook:

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 seedlings for the light and dark treatments for all four seed strains. Make notes about the color of the hypocotyls and cotyledons, and the shape and size of the seedlings for both treatments of all four seed strains.

3. Illustrate and make notes about any differences you notice between the reference strain (Ler-0) and the 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 – Display and interpret data
Complete the following tasks in your lab notebook:

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 your class.

5. Use what you have learned to draw your conclusions about the importance of phytochrome A and B photoreceptors in plants.

ASSIGNMENT 4 – Digging deeper
Complete the following tasks in your lab notebook:

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 you review 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?