PlantingScience Foundations of Genetics: Traits, Variation, and Environment in Rapid Cycling *Brassica rapa*

Teacher's Guide

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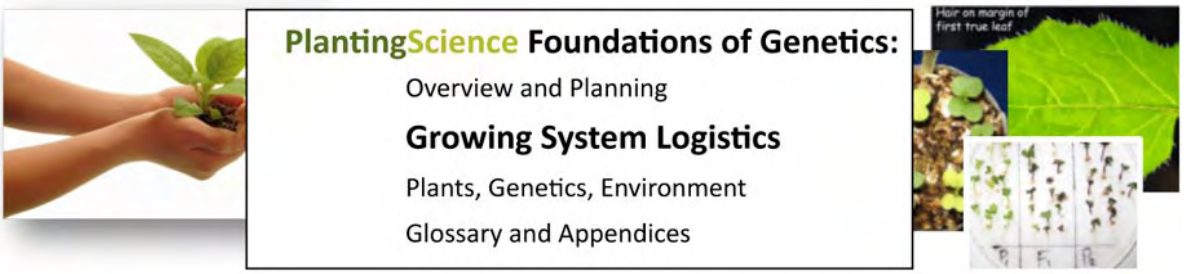
Each section is available for download in pdf format from www.PlantingScience.org.



The Environment

Here we focus on setting up your growing system, including light, and soil / nutrition, and water system. This is also a perfect opportunity to explore with your students the environmental needs of plants. Plants need light, air, water, appropriate temperature, and nutrition to photosynthesize and grow.

Light

Continuous fluorescent lighting is required (lights on 24 hours a day). Instructions are contained here for constructing light boxes from plastic crates, aluminum foil, and a screw-in fluorescent light bulb. It is also acceptable to have banks of long fluorescent lighting.
See http://www.fastplants.org/grow.lighting.php for more information.

One light box should be adequate to house 12 bottles with 6 plants each for a total of 72 plants of the F2 generation seed stock. If you choose to grow the parent and F1 lines of plants as well, you will need additional lighting.

Air

Plants need air in order to photosynthesize. This requires little attention, since air surrounds us.

Water

Plants should be watered with distilled water (see instructions contained here) so they have a constant water supply in their reservoir.

Temperature

Consistent room temperature is desired, with optimum temperature 65 - 78°F (18 - 26°C). Keep a thermometer near the plants and check the temperature regularly.

Nutrition

In this experiment, we vary the nutrition environment by having 2 treatments (high and low nutrition). Seeds will be planted in a mixture of seedling starter soil (with as little fertilizer added as possible) and vermiculite (or perlite). Then, a controlled amount of fertilizer is added in the form of Osmocote pellets. The nutrition levels vary by a factor of 4:1 (the high level has 4 times the fertilizer as the low level). See instructions contained here.

Growing System

The seeds are planted in soil, fertilizer, and water that are contained in a growing system of your choice. Instructions here describe bottle growing systems constructed of plastic bottles. For more information on other growing systems, see http://www.fastplants.org/grow.lighting.php.

Light Box Construction for Growing Plants

Overview

Two plastic filing crates (AKA "milk crates") will be stacked on top of each other, long side horizontal. The open sides will face each other, and one side of each will be cut out to create an opening for our access. A screw-in fluorescent light will be fixed at the top of the two crates, and they will be lined on the sides with aluminum foil.

Materials for each light box (need one light box per class):

From office or big-box store:

- 2 plastic file crates approx. 17" X 14" X 11½"
- Scissors
- Double-sided transparent tape ½" wide
- Single-sided transparent tape ½" wide
- 10 small binder clips
- Aluminum foil
- Exacto knife
- Ruler
- Felt marker
- "Utility" saw with hacksaw blade
- Pocket knife (or other small, stiff, sharp blade)
- Fluorescent screw-in bulb, 150 W equivalent (40-42 W)
- Socket plug
- 6-12' extension cord

Brands of crates

Some of the supplies (Velcro not used)

Light bulb and materials

Light Box Construction Procedure

1. Using a 'utility' saw with a hacksaw blade in it, cut out a front panel of each of two stacking crates.

2. On the outside bottom of <u>one</u> of the crates, trace an approximately one inch diameter circle on the plastic center panel of the 'Office Depot' crate. Then with a small stiff sharp blade, carefully puncture repeatedly the plastic until the circle is cut out. This is the trickiest part of the whole construction. The hole that is made is to fit and attach the compact fluorescent light with the socket plug to the upper section of the plant light box. If the base of the light bulb doesn't fit in the hole, you can enlarge the hole by shaving small amounts of plastic off the hole circumference with the knife blade until the base of the lamp fits through the hole. <u>The hole should not be so large that the socket plug goes through it.</u>

In the case of a 'Staples' brand stacking crate, the bottom of the crate is slotted such that a small sheet of thin rigid plastic, such as that cut with scissors from a plastic milk jug or from the bottom of a plastic 'picnic' plate can be cut to fit the center slot of the top crate. The hole for the light is then cut in the small plastic sheet.

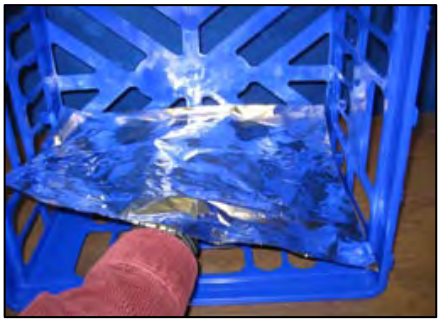
3. Prepare for lining the modified crates with foil sheets. Place short (~1 inch) pieces of ½ inch wide double sided clear tape at nine locations, corners, middle sides and middle, of each of the four interior walls of each of the two crates.

4. Stack the two crates aligned as in the photo, with openings facing each other, and the cut out sides facing you. Align the smooth rims, and apply two small clamps to each corner as illustrated.

5. Observing the crate orientation will inform you on the dimensions of the wall foil pieces to be cut.

6. Cut appropriate sized foil pieces. A metal straight edge ruler enables easy cutting the foil by tearing it against the edge. Each of the two crates will require two pieces of foil 12 X 10 inches and two pieces 15 X 12 inches.

7. Lower the precut foil pieces to fit on each wall. Smooth out and press foil to stick on tape.

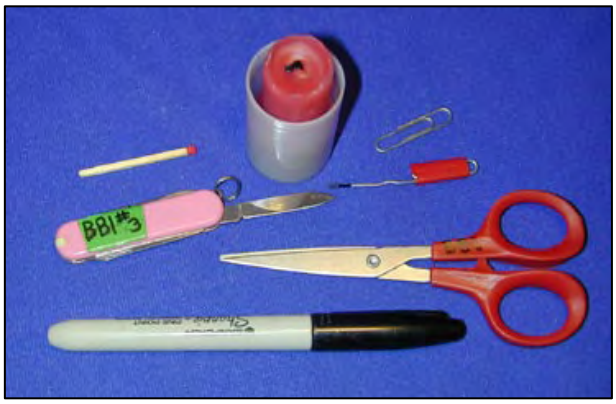


8. Affix the light by inserting the light bulb through the hole in the top of the light box so that the bulb is facing inside. Affix it by screwing the socket plug, which is outside the light box, to the base of the light bulb. This way, the light bulb is inside, and the socket plug is outside the light box. The socket plug can later be plugged into the extension cord to reach an electrical outlet.

9. Create a foil curtain to be clipped to the front opening of the light box. Curtain dimensions are 12 X 20 inches. The curtain can be made significantly more durable by taping the edges and centers with strips of ¾ inch single sided clear tape, as illustrated. The curtain is secured to the top of the PLB with small binder clips.

Constructing the Bottle Growing System

Materials (for each class)

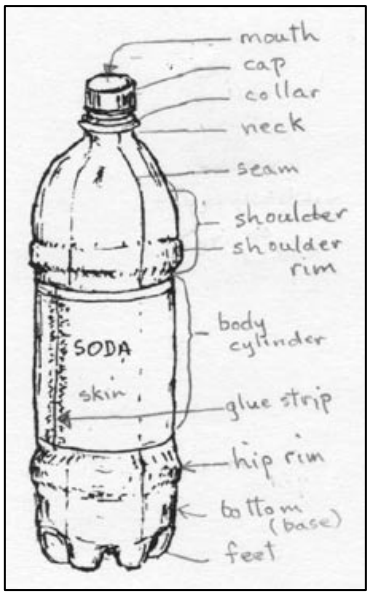


- 12 *clear* plastic bottles approx. 20 oz each with plastic lids (standard Pepsi and Coke bottles and many water bottles work well).
- Scissors
- Paper clips
- Pocket knife
- Felt marker
- Hair dryer (optional)
- Drill with 3/16" drill bit
- 3.5 yards of 4 mm acrylic or nylon 'macrame' cording found in craft stores
- Matches (or lighter) and candle

Procedure

1. Remove label on 12 bottles by using a hair dryer to melt the glue (optional).

2. Using a felt marker, draw a line around each of the 36 bottles approximately 1 cm below the "shoulder rim" as shown. Save both parts of the bottles along with cap.



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3. Cut each bottle on the marked cutting ring. Before inserting the scissors, puncture the bottle with a knife or cutting blade and make a 1-2 inch cut on the line of the cutting mark. Then insert scissors in the way illustrated, with the top scissor blade inserted into the cut in the bottle. The cut is made by cutting downwards on the bottle rather than cutting upwards. Cutting downwards will give a smoother cut than by cutting upwards.

4. Drill holes in the bottle caps using a drill press (or hand drill), or melting them with a hot 'nail poke' (a small beheaded nail inserted in the end of a short piece of twig) heated in a flame (gas stove, burner or candle)..

5. Cut one wick for each bottle from the cording, 4-5" long. Seal the frayed ends of the wick in a flame. Thread the wicks through each bottle cap.

6. Assemble the Bottle Growing System as shown, with top inverted like a funnel. The bottle cap with water wick is seated in the bottle reservoir that will hold the water and nutrients.

Preparing Soil and Fertilizer, and Planting Seeds

Materials (for each class)

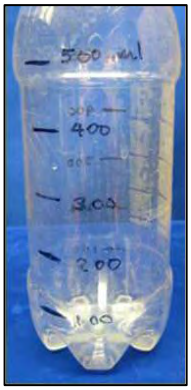
- 12 Bottle Growing Systems (see instructions above)
- Plastic bottles for watering bottles (can be 20 ounce bottles as above, or 2 liter bottles) – quantity is your preference to have in the class
- Beaker or other graduated vessel for measuring water volume
- Small bag of seedling starter planting mix with as little fertilizer as possible (Brands preferred include 'RediEarth', and 'Jiffy Mix' containing >50% finely milled peat moss, fine Vermiculite, lime and wetting agent, and are recommended because they do not have added starter fertilizer in their formulation)
- Tub to mix soil in
- 2 tablespoons to distribute soil and Vermiculite into bottles
- Bag of coarse grade horticultural Vermiculite (preferred) or Perlite
- Bottle of Osmocote pellets
- Brassica seeds
- Labels to place in the soil of each bottle, such as a plastic or wooden applicator stick
- Black plastic or aluminum foil (to wrap the bottles in)
- Thin flat plastic (e.g. from milk jug) for making stencils
- Single hole-punch
- Scissors
- ¾" clear tape for dispensing seeds and pellets
- Note paper
- Colored labeling tape for bottles
- Sharpies
- Light box (made in Activity 1A)

About the fertilizer

The environmental condition we are varying is nutrition level, and we do that with fertilizer. It is important the fertilizer is in equal proportions of Nitrogen, Phosphate, and Potassium (N-P-K). Osmocote® is a readily available fertilizer that offers slow-release pellets. (See Appendix A, p. 55, for an alternative fertilizer.) The two treatments we use (high and low nutrient levels) are in a 4:1 proportion. The high level will receive 4 pellets per plant; the low level will receive 1 pellet per plant. (See Experimental Design, p. 11)

Prepare:

1. **Prepare water bottles.** Remove labels from plastic bottles with a hair dryer, and drill a hole in the plastic lid. Mark levels of water as shown, using a beaker or other graduated vessel to measure volume of water. Put lid on for use as shown.



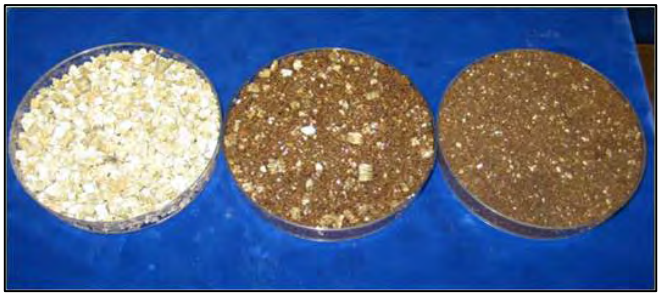
2. **Prepare seed dispensing tapes** for each group of students. Seed dispensing tape ensures that seed will not be spilled, mixed or lost. Pour the required number of seeds from the seed envelope onto prepared dispensing tapes made from ¾ inch clear tape. Note the loop of tape that enables tape to be peeled back so fingers can easily roll seed off tape.

3. **Prepare Osmocote® pellet dispensing tapes** for each Bottle Growing System. There will be either 6 or 24 pellets per bottle, depending on whether it has low or high nutrition, respectively.

4. **Prepare plastic stencils for seed placement**. Using the plastic from a milk jug, create a seed spacing die as shown for each team of students. Cut a circle with scissors; then use a paper hole-punch to cut 6 evenly spaced holes in it. A loop of tape on top of the disc enables it to be easily placed and removed from the root-medium.

5. **Prepare soil mixture.** In a tub, make a 50:50 mix of: a) coarse Vermiculite (or Perlite), and b) commercial 'seedling starter' planting mix. Moisten this mixture slightly. Use a tablespoon to distribute.

6. **Set out Vermiculite for distribution.** Use a tablespoon to distribute.



Vermiculite 50:50 Mixture Seedling Starter

A word about the root medium:

Our goal is to have as little fertilizer as possible in the soil. Why? Because we want to carefully control the nutrient level through our Osmocote pellets. So we add Vermiculite to the soil mix to dilute whatever fertilizer may be in there. We also layer the Vermiculite above and below the soil in the Bottle Growing System. The aeration provided by the Vermiculite also facilitates root growth.

Sowing the Seeds

1. Assemble the Bottle Growing System as shown, with top inverted like a funnel. The bottle cap with water wick is seated in the bottle reservoir that will hold the water and nutrients. Identify which bottles will have high and low nutrient levels, and label top of plastic with Sharpie.

2. Fill the bottle funnel with root media. First, add 5 heaping tablespoons (~100 cc) of Vermiculite (preferred) or Perlite.

3. Add Osmocote pellets: 6 pellets in the low nutrient treatment (1 pellet per plant), and 24 pellets in the high nutrient treatment (4 pellets per plant). Spread evenly across surface.

4. Next, add 4 heaping tablespoons (~80 cc) of slightly moistened 50:50 mixture of Vermiculite 'seedling starter' mix.

Level of Osmocote pellets

5. Place the plastic spacing die in each bottle, on top of the soil as shown.

6. Place 1 seed through each hole.

7. Cover the seed with two tablespoonfuls of Vermiculite, and smooth it over the surface to the rim of the bottle funnel.

8. Use a plastic label stick to label whether the bottle contains a high or low nutrient treatment.

9. Label the Team the bottle belongs to.

10. Soak the entire root medium ("soil") well with water until it begins to drip from the base of the wick. A little sediment will drip from the funnel at this first soaking. Pour it off and fill the reservoir with sufficient water to reach the bottle cap on the growing funnel. See photo.

11. To exclude algal growth in the reservoir solution, wrap the reservoir in black plastic (cut from a trash bag) or aluminum foil.

12. **Important!** Use caution when picking up and handling the bottle growing system. Be sure to grasp at the base area and not at the top of the reservoir near the growing funnel. If the reservoir is grasped near the top, it will collapse in your hand, driving the funnel with the plants out and into the air!

13. Place the bottle growing systems in your plant light box on a foil with light on 24 hours per day.

Caring for the Plants

Thin Plants

Plants will begin to emerge in ~48 hours of sowing if the temperature in the box is 22-28° C. If there are more than 6 plants in the bottle, thin by snipping the extra plants at soil level. There should be 6 plants in each bottle. Below left are plants 3 days after sowing; below right are plants 6 days after sowing.

Labeling

Once plants have been thinned to six in each bottle, they should be numbered sequentially on a tape wrapped around the rim of the growing funnel. Each plant in a particular environmental (nutritional) subpopulation should have a unique number from 1 to 36. In our example, the two different environments are also labeled using different colored tape.

Tending *Brassica* Plants through Their Life Cycle

Check reservoirs every day or two and add the appropriate water to keep reservoirs filled to the inverted cap level. Remember that plants will use more water as they grow. Be particularly sure to fill all reservoirs before the weekends. Keep a record of how much water is used.

If available, put a thermometer in your light box and monitor the temperature. An indoor/outdoor type thermometer (Radio Shack) works well. If the temperature in the box is above the optimum range (22 - 28° C), open one or more of the panels in your upper crate by cutting and folding back the foil near the top of the sides and back of the light box.

You can also place bamboo stakes in the pots and gently tie plants to it to keep them from falling over.

The photo at right shows typical *Brassica* F2 progeny at ~16 days after sowing. The one at left (yellow label) has been in the low nutrient environment. The one at right (green label) has been in the high nutrient environment.