Plant tissue culture

What is a sterile growing environment?
How is a plant clone produced?

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or multiple copies of cloned plants. This can be accomplished through the tissue culture of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of a genetic transformation of single plant cells, which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Tissue culture involves the use of small pieces of plant tissue (explants), which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for the explant type, plants can be induced to rapidly produce new shoots and new roots. These plantlets can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

Many types of plants are suitable for use in the classroom. Cauliflower florets particularly give excellent results since they can be grown into a complete plant in the basic tissue culture media, without the need for additional growth or root hormones. Green shoots are generally observable within three weeks, and roots develop within six weeks.

The most important part of this activity, however, is to maintain as sterile an environment as possible. Even one fungal spore or bacterial cell that comes into contact with the growth media will rapidly reproduce and soon completely overwhelm the small plant.

Students will maintain a sterile growing environment. Students will propagate many plants of the same genetic background.

Materials

- 1 Vial of Murashige Skoog (MS) media
- 1 L sterile distilled water
- 9 g of agar/L
- 30 g sucrose/L
- 1.5 L or 2 L container in which to prepare the growth medium
- Small amounts of 1M NaOH and 1M HCl to adjust the pH of the media
- pH paper (5.0–7.0 by tenths)
- Small petri dishes
- Aluminum foil
- Glass aquarium or box lined with plastic
- Plastic sheet to cover the top of the aquarium
- Adhesive tape
- 10% bleach solution in spray bottle (250 ml household bleach + 2250ml water)
- 70% alcohol in a spray bottle
- Forceps or tweezers
- Gloves
- Cutting equipment such as a scalpel blade or razor blade
- 2 gallons of sterile distilled water
- Hot plate
- Cauliflower florets
- Sterile paper towels or petri dishes for cutting
- Lidded containers in which to wash the plant material
- Detergent-water mixture: 1ml detergent per liter of water
- Sterilizing solution: 1–2% bleach Solution (5–10 ml household bleach + 500 ml water)
- 2 or 3 beakers or jars of sterile water
- A well-lit area away from direct sunlight or use full-spectrum gro-lights

Procedure

Preparation and sterilization of growing medium

These steps will make 500 ml of growth medium, which is enough to prepare about 65 growing tubes.
(We recommend that the teacher prepare the growth media).

1. Dissolve the MS mixture in about 400 ml of distilled water. Stir the water continuously while adding the salt mixture with a magnetic spin bar in the flask while on the hot plate. Add 15 g of sugar and continue to stir to dissolve. Adjust pH to 5.8 using drops of 1M NaOH or 1M HCl as necessary while gently stirring. Add distilled water to make the total volume up to 500 ml.
2. Weigh out 4.5 grams of agar and add it to the MS solution. Swirl the flask to mix and add a magnetic stir bar. Cover the flask with foil and heat with repeated stirring on the hot plate on high until the liquid is just boiling. Agar will boil over quickly.
3. Simmer the liquid for 30 minutes on low heat and let cool to touch.
4. Pour the still warm medium into the petri dishes until one-third full (be careful to keep the lid over the bottom dish). Cover immediately to cool. Stack culture dishes in sets of 6–8 dishes. Secure with tape.
5. Place the stacked culture dishes in an autoclave to sterilize.

Preparation: Sterile transfer chamber and equipment

1. A classroom transfer chamber can be made from a clean glass aquarium turned on its side. Scrub the aquarium thoroughly with a 10% bleach solution in a chemical hood if possible, making sure that you wear gloves and do not inhale the fumes. Rinse with sterile distilled water, turn upside down on a clean counter or paper towels and allow to dry.
2. Cut holes in a clean plastic sheet to allow arms to reach into the chamber and reinforce the cut edges with tape if necessary. Tape the clean plastic sheet over the open side of the aquarium making sure that the armholes are located at a convenient height. Plastic sleeves could also be fitted to these holes if you wish to make it easier to prevent the entry of airborne spores into the chamber. The finished aquarium chamber can be sterilized by spraying with 10% bleach solution just prior to each use and drying with sterile paper towel.
3. Wrap the forceps, scalpels, razor blades, paper towels and gloves (rubber or surgical) in aluminum foil, seal with tape and sterilize by placing in an oven at 350°F for 15 minutes. You can wrap each item separately or put together a "kit" so that each student will have their own sterile equipment to use.

   Alternatively, the forceps and blades can be sterilized by dipping in 10% bleach solution and then rinsing in sterile water. If you choose to dip in bleach and rinse in sterile water, it is best if fresh solutions are available for each student group since the water can easily be contaminated if care is not used. These liquid containers should only be opened only when they are inside of the sterile chamber.

Plant preparation

1. Sterilize your plant material to remove any bacteria or fungal spores that are present. The aim is to kill all microorganisms, but at the same time not cause any adverse damage to the plant material.
2. Cut cauliflower into small sections of florets about 1 cm across.
3. Wash the prepared plant material in a detergent-water mixture for about 20 minutes. This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
4. Transfer the washed plant material to the sterilizing chlorox solution. Shake the mixture for 1 minute and then leave to soak for 10–20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.

Note 1: At this point, the tissue is considered sterile. All subsequent rinses should be done with sterile water and all manipulations of the tissue performed with sterile instruments and supplies. Open one container at a time and never leave the lid off of any container longer than necessary.

Note 2: Many students will not fully appreciate the importance of carefully sterilizing explants and so there will be some cultures that become infected with bacterial or fungal growth. If you do not wish to emphasize this aspect of the laboratory students can be provided with plant materials that the instructor has already sterilized prior to use by the class.

Transfer of plant material to tissue culture medium

Use the sterile gloves and equipment for all of these steps.

1. Place the plant material still in the bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towel to use as a cutting surface and enough tubes containing sterile medium into the sterile aquarium. The outside surfaces of the containers, the capped tubes and the aluminum wrapped supplies should be briefly sprayed with 70% alcohol before moving them into the chamber.
2. Spray the gloves with a 70% alcohol solution and hands rubbed together to spread the alcohol just prior to placing hands into the chamber. Once students have gloves on and sprayed they must not touch anything that is outside of the sterile chamber.
3. Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2–3 minutes to remove the bleach. Pour off the water and repeat the washing process 3 more times.
4. Remove the sterilized plant material from the sterile water, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. Be sure to avoid any tissue that has been damaged by the bleach, which is apparent by its pale color.
5. Take a prepared section of plant material in sterile forceps and place into the medium in the culture dish. Cauliflower pieces should be partly submerged in the medium, flower bud facing up.
6. Replace the lid on the cuture dish.

Growing the plants

1. Place plant sections in a well-lit area of the classroom although not in direct sunlight. The shoots will grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 8–10″ overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not go over 82.4° F. New shoots should develop within 2 weeks and should be well advanced in 3 to 4 weeks. Check the tubes daily and discard any that show signs of infection (before discarding, add bleach into the tube). Roots can appear within 6 weeks on cauliflowers.
2. Working inside the sterile aquarium chamber, remove the lid from the culture dish. There will usually be several shoots that have arisen from each explant. *These shoots should be carefully separated by gently removing the whole explant from the media with sterile forceps and then separating the shoots by gently pulling them apart using two pairs of forceps.* Each shoot should then be placed into a tube of rooting media and the bottom of the shoot pushed into the media so that good contact is made. The cap is replaced and the shoots are then allowed to grow as in step 1 until roots are formed, usually within 2–3 weeks.

Potting the clones

1. Transfer plants with well-formed roots into soil.
2. Remove each plant from its tube of media and plant into a small pot containing a clean light potting mix. Gently wash off the agar medium prior to planting. The plants will still need to be protected at this stage since they are not accustomed to the drier air of the classroom when compared to the moist environment of the tube of media.
3. Place all of the pots onto a tray and cover lightly with a plastic dome or tent. Place the plants in an area with 12–16 hours of light (either natural or artificial) but not direct sunlight.
4. Remove cover after a week. Plants will acclimate to stronger light and drier atmospheric conditions.
5. Use these genetically identical plants to carry out other experimental tests knowing that one of the main variables in the experiment has been eliminated. Some of these tests could include looking at plant responses to low light levels, to drought or to saline soil conditions.

Assessment

1. Tissue culture uses a small piece of tissue from a mother plant to grow many new copies of the original plant. What is the term used to refer to this tissue sample?
2. Why is tissue culture used for propagation of some plants rather than just planting seeds?
3. What is a sterile environment?
4. Why is a sterile environment important in tissue culture?
5. How did you or your teacher sterilize the instruments that were used in this tissue culture activity?
6. Could we sterilize the plant tissue in the same manner? Why or why not?
7. What happens if you open your sterile plant container when it is not inside a sterile environment?
8. If some plants died, what steps caused the environment to become unsterile and what can be done to prevent this?
9. Tissue culture technology provides genetically identical plants. What value does this technology have for the agriculture industry? Can you think of specific applications for Kansas corn farmers?

Resources adapted with permission from the Kansas Corn Growers Association.