

Plant tissue culture in the classroom

Tissue culture techniques are used extensively to grow many different plants for commercial and research purposes. New plants are grown from small pieces of plant tissue in a nutrient medium under sterile conditions. When conditions are suitable, plants can be induced to rapidly produce new shoots, which can be subdivided to produce more plants. The addition of suitable hormones can then induce root growth, and the plants can then be placed in soil and grown in the normal manner.

In the classroom, cauliflower florets give excellent results, with green shoots emerging within three weeks and roots developing within six weeks. The advantage of cauliflowers is that they will grow into a complete plant in the base medium, without the need for growth or root hormones.

In this activity you will produce clones (exact genetic copies) of a plant (cauliflower, rose, African violet or carnation) by tissue culture.

There are 6 major parts to this activity.

You will need:

Part 1

- 1 sachet of Murashige and Skoog Basal Salts with minimal organics medium (MSMO). If you wish to make up your own growing medium, use the recipe given at the end of this worksheet.
- 1 L distilled water
- 6 g of agar
- 1.5 L or 2 L container to prepare the growth medium in
- 100 × 35 mL polycarbonate tubes with screw lids

Part 2

- glass aquarium
- plastic sheet to cover the top of the aquarium
- adhesive tape
- bleach or strong disinfectant in a spray bottle

Part 3

- forceps or tweezers
- scalpel blade or razor blade
- 2 bottles of sterile water
- paper towel for cutting plants
- rubber gloves or surgical gloves
- pressure cooker

Part 4

- your chosen plant (cauliflower, rose, African violet or carnation)
- scalpel or razor blade
- beaker or jar to wash the plant material in
- detergent-water mixture
- sterilising solution such as a 1 in 4 dilution of White King bleach (4% available chlorine as sodium hypochlorite) in distilled water in a large beaker or jar

Part 5

- culture tubes from part 1
- sterile transfer chamber from part 2
- sterilised equipment from part 3
- sterilised plant material from part 4
- 2 or 3 beakers or jars of sterilised water

Part 6

- prepared culture tubes from part 5
- an area away from direct sunlight
- hormones such as BAP (benzylaminopurine) and NAA (naphthalene acetic acid) to stimulate growth and root development. (Commercial rooting hormone solutions and powders are available from hardware stores)

Biotechnology Online School Resource

What to do:**Part 1: Preparation of sterilised tubes of growing medium**

This will make 1 L of growth medium, which is enough to prepare about 100 growing tubes.

1. Dissolve the MSMO mixture in about 800 mL of distilled water while stirring the water continuously.
2. Weigh out 6 g agar and add it to the MSMO solution. Heat the solution gently while stirring until all the agar has dissolved. Add more distilled water to make the total volume up to 1 L.
3. Pour the still-warm medium into the polycarbonate tubes to a depth of about 15 mm.
4. Place the tubes (with lids sitting on the tubes but not tightened) in a pressure cooker and sterilise for 20 minutes. Cool the pressure cooker, then remove the tubes and tighten the lids.

Part 2: Preparation of a sterile transfer chamber

A suitable transfer chamber can be made from a glass aquarium turned on its side. Tape plastic sheet over the open side of the aquarium, and cut holes to allow hands to reach into the chamber. Plastic sleeves can be fitted to these holes to help prevent the entry of airborne spores into the chamber. The aquarium can be sterilised by spraying with White King bleach or similar.

Part 3: Preparation of sterile equipment

Sterilise the equipment by wrapping it in a paper bag and pressure cooking it for twenty minutes.

Alternatively the forceps and blades can be sterilised by dipping in bleach and then rinsing in sterile water, or dipping in alcohol and then placing in a flame.

Part 4: Preparation of the plant material

Your plant material must be sterilised to remove any bacteria or fungal spores. The ideal sterilisation process will kill all microorganisms, but not adversely affect plant material.

1. Cut cauliflower florets into small sections about 1 cm across. If using a rose or other cuttings, cut the shoots in 5–7 cm lengths.

2. Wash the prepared plant material in a detergent-water mixture for about 20 minutes. If using hairy plant material, scrub plant stems with a soft toothbrush to help remove fungi etc. The detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
3. Transfer the washed plant material to the sterilising solution. Shake the mixture for 1 minute and then leave to soak for 20 minutes

Part 5: Transfer of plant material to the medium

Use the sterilised rubber gloves and equipment from Part 3 for all of these steps.

1. Place the container of sterilising solution containing the plant material, the containers of sterile water, the sterilised forceps and blades, some sterile paper towel to use as a cutting surface and enough tubes containing sterile medium into the sterile aquarium.
2. Remove the sterilised plant material from the bleach solution, place on the paper towel and cut into suitable sized pieces (for cauliflower, about 2 to 3 mm across; for rose, 10 mm of stem with a bud attached).
3. Place the plant material in a container of sterilised water and wash thoroughly. Repeat the washing process in another container of sterile water (some methods suggest at least 3 washes with sterile water).
4. Take a prepared section of plant material in sterile forceps and place into the medium in the polycarbonate tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. Rose or other cuttings should be placed so that the shoots are level with the medium surface.
5. Replace the cap tightly on the tube.

Part 6: Growing the plants

6. Place the tubes containing plant sections in a light area of the classroom, away from direct sunlight, eg under a fluorescent light. New shoots should develop within 2 weeks, and should be well advanced in 3–4 weeks.
7. Roots can appear within 6 weeks on cauliflowers. Roses and other plants can be successfully grown using tissue culture techniques. However, once the shoots have developed they will need to be placed with the base of the shoot in a solution containing hormones to stimulate further growth and root development.

Murashige and Skoog Basal Salts with Minimal Organics Medium (MSMO)

Murashige and Skoog Basal Salts with Minimal Organics Medium (MSMO) is available from Sigma-Aldrich in Sydney. Phone (02) 9841 0555, fax (02) 9841 0500, or e-mail ausmail@sial.com and ask for Murashige and Skoog Basal Salts with Minimal Organics Medium (MSMO), catalogue number M6899.

It costs about \$30 for 10 litres. MSMO can also be ordered online:

<http://www.sigmaaldrich.com/catalog/search/ProductDetail/SIGMA/M6899>

Murashige Minimal Organics Medium recipe

| Inorganic salts | mg/L |
|---|-----------|
| NH ₄ NO ₃ | 1,650.00 |
| KNO ₃ | 1,900.00 |
| CaCl ₂ (anhyd) | 332.20 |
| MgSO ₄ (anhyd) | 180.70 |
| KH ₂ PO ₄ | 170.00 |
| Na ₂ EDTA | 37.25 |
| FeSO ₄ .7H ₂ O | 27.80 |
| H ₃ BO ₃ | 6.20 |
| MnSO ₄ .H ₂ O | 16.90 |
| ZnSO ₄ .H ₂ O | 5.37 |
| KI | 0.83 |
| Na ₂ MoO ₄ .2H ₂ O | 0.25 |
| CuSO ₄ (anhyd) | 0.016 |
| CoCl ₂ (anhyd) | 0.014 |
| Sucrose | 30,000.00 |
| i-Inositol | 100.00 |
| Thiamine.HCl | 0.40 |

The pH is adjusted to 5.7 using 0.1M HCl or NaOH.