Workshop Activity 1
Subculture of an Orchid Seedling

BACKGROUND

The term "plant tissue culture" is generally used in a broad sense to include axenic (microbe-free) culture of plant cells, tissues, organs or entire plants, usually on synthetic nutrient media. A sound understanding of plant physiology is necessary to design appropriate tissue culture techniques and careful aseptic technique is absolutely essential for success in plant tissue culture because microbial contaminants may rapidly outgrow and destroy the plant cells on rich nutrient media.

Plant tissue culture is used in relatively complicated recent biotechnologies such as plant genetic engineering and also in simpler, well-established commercial techniques for rapid propagation of valuable plants. For example, many species of orchids are valuable for the ornamental plant and cut flower trades. Seed production is profuse, but very few seedlings develop under field and glasshouse conditions. However, if mature seeds are aseptically transferred onto an appropriate tissue culture medium, virtually every seed may produce a seedling which can be subcultured and grown until it is sufficiently robust to transfer into a pot for normal growth and flower production.

In this exercise, you will have the opportunity to work in a laminar air flow cabinet to aseptically subculture an orchid seedling from a batch culture vessel into an individual growth tube; this is a rapid routine operation in commercial tissue culture laboratories. You may keep the subcultured seedling, and if your technique has been good, you should subsequently be able to transfer it to potting mix and nurture your own flowering orchid plant from tissue culture.

PROCEDURE

1. Label your culture tube with the orchid species provided, the date and your name, and proceed to the laminar flow area with your workshop leader.

2. As demonstrated by your workshop leader, carefully transfer a selected seedling into your culture tube. Use stringent aseptic technique!

3. If your culture tube subsequently develops microbial contamination, discard it immediately. If your aseptic technique was good (no contamination) and you would like to produce a flowering orchid plant from your seedling, follow the after-care instructions after the workshop.
AFTER-CARE

1. Incubate the culture tube under aquarium lights or on a window ledge which does not receive direct sunlight. Your seedling should show substantial growth over several months.

2. Early next September, carefully remove the seedling and agar from the culture tube, and gently remove all the agar from the roots in a tray of lukewarm water or fungicide solution.

3. Transfer the plant to a 3-5cm plastic pot containing a well-drained orchid potting mix (commercial mixes frequently contain perlite, styrofoam, charcoal and pine bark pieces). A thin surface layer of peat or sphagnum moss may be helpful to hold moisture until the plant becomes established.

4. Water the potted plant immediately with fresh fungicide solution. Some growers also include “Formula 20” to stimulate root development.

5. Cover the pot with a plastic bag, previously shaken with water, to maintain a high humidity.

6. Keep the covered pot under similar light and temperature conditions to the culture tube (as above), and gradually expose the potted plant to lower ambient humidity (by punching holes in the bag, or gradually raising the bag, or uncovering the plant for an increasing period each day) over a period of a week.

7. Mist the uncovered plant and pot daily with water from a hand-spray for several weeks (or indefinitely if humidity is low) and water every 2-3 days, alternating water with a dilute liquid fertiliser. Always allow the pot to drain freely after watering or your plant will rot and die.

8. Repot each year, using an appropriate size pot as your orchid grows. Under suitable growth conditions (around 50% sunlight) your plant will be ready to flower after several years.

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**QUESTION:** Why is it necessary to maintain a high humidity when plants from tissue culture are first transferred to pots?

**QUESTION:** What are some of the likely sources of contamination when you are handling the culture inside the laminar flow cabinet?