MICROPROPAGATION EXERCISES IN TEACHING PLANT PROPAGATION

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Most students in university-level plant propagation courses are aware of the rapidly developing field of tissue culture as it relates to the propagation of plants. Many are eager to have some first-hand experience with the techniques involved.

In our plant propagation course at the University of California, Davis, which is given in the spring quarter, we are presently accommodating about 150 students with five 3-hour lab sections per week. In the lab we cover the usual aspects of propagation by seeds, cuttings, grafting and budding, layering, etc. Into this we have interjected two exercises using aseptic culture techniques. The first is handled during our two laboratory sessions on seed propagation in which the students sterilize and plant cymbidium orchid seeds, on nutrient agar as is done commercially. After seeding, the flasks are placed in growth chambers for germination and development of the seedlings. In the second exercise the students cut apart cymbidium orchid shoot-tip protocorms, transferring a portion to rotating flasks for further proliferation. The remaining protocorms are planted in nutrient agar in flasks for formation of roots and shoots and development of the plants. The orchid protocorm exercise is included during one of our two laboratories on cutting propagation. The ability of cymbidium orchids to regenerate new plants has been known, of course, for many years starting with the work of Morel in developing virus-free plants (4,5,6).

By using both seeds and protocorms, we are able to relate the aseptic culture techniques to both sexual and asexual propagation.

FACILITIES

Since no room space was available for setting up transfer chambers, a six station unit was constructed from a war surplus metal field telephone exchange. Figure 1 shows outside and inside views of this reconstructed chamber. Students work in the transfer chamber by placing their arms through holes over which plastic is draped (Figure 1).

Dust particles in the chambers are reduced by maintaining filtered air (5 micron filters) under positive air pressure and by the use of ultraviolet lamps operating when the chambers are not in use. A thermostatically controlled electric heater, to-

gether with an air conditioning unit, maintain the temperature in the chamber at approximately 70°F.

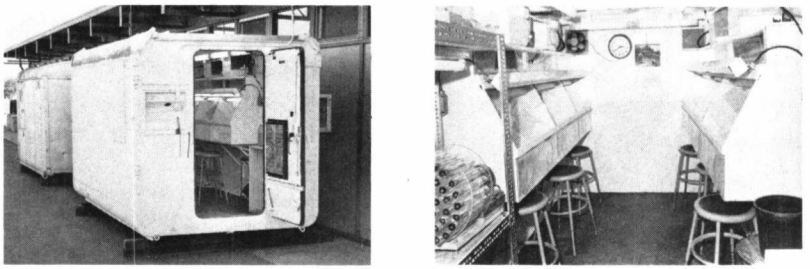

Figure 1. *Above.* Outside view of 6-station aseptic transfer chamber developed from war surplus telephone exchange unit.
Below. Inside of chamber showing transfer stations where work is done under filtered pressurized air.

PLANTING ORCHID SEEDS

The students are furnished the material and equipment shown in Figure 2 for planting the orchid seeds. Each student is supplied with a vial which contains a small amount of orchid seed. He is also furnished a 125 ml flask containing sterilized nutrient agar. The composition of this is basically the Knudson C medium as developed for germinating orchid seed (3).

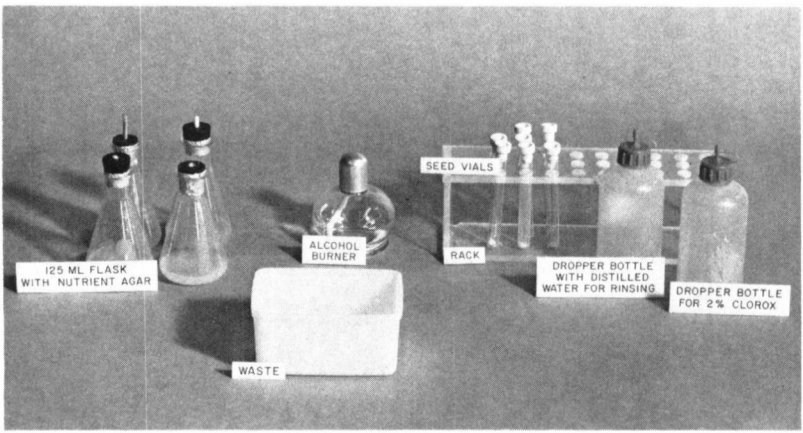

Figure 2. Equipment and material provided each student for sterilizing and planting orchid seeds.

$Ca(NO_2)_2 \cdot 4H_20$	1000 mg/l	$MnSO_4 \cdot 4H_20$	7.5 mg/l
$(NH_4)_2SO_4$	500 mg/l	$FeSO_4 \cdot 7H_20$	25 mg/l
KH_2PO_4	250 mg/l	sucrose	2%
$MgSO_4 \cdot 7H_20$	250 mg/l	agar	1.5%

This medium has been modified (2) by replacing the KH_2PO_4 with 18 ml of potassium phosphate buffer prepared by combining 97.5 ml of 0.1 M KH_2PO_4 solution (13.6 gm in 1 liter water) and 2.5 ml of 0.1 M K_2HPO_4 solution (17.4 gm in 1 liter water). This maintains the pH at 5.3 without further adjustment. To this, 1 ml of a micro-nutrient solution is added as given below.

$MnSO_4 \cdot 4H_2O$	1.81 g	$CuSO_4 \cdot 5H_2O$	0.08 g
H_3BO_3	2.86 g	$(NH_4)_2MoO_4$	0.09 g
$ZnSO_4 \cdot 7H_2O$	0.22 g	Distilled water	995 ml

Iron can be supplied in several ways: iron tartrate (1 ml of a 1% stock solution); inorganic iron ($FeCl_3 \cdot 6H_2O$, 1 mg/l) or $FeSO_4$, 2.5 mg/l; or chelated iron. Chelated iron can be supplied as NaFeEDTA, 25 mg/l, or by mixing Na_2EDTA and $FeSO_4 \cdot 7H_2O$ in equimolar concentrations to give 0.1 mM Fe.

The students then sterilize the seed, using aseptic techniques, with 2% Clorox according to the following directions (1).

1. Fill tube containing seeds 1/2 full with sodium hypochlorite (2% Clorox) solution, add stopper, and shake for 5 minutes. This will sterilize the seeds. Use mild wetting agent (as 7x) to aid in wetting seeds.

2. Invert test tube, loosen stopper slightly, and slowly drain out disinfectant solution, rotating tube so that seeds adhere to sides.

3. After draining, add sterile water to fill tube less than half full and shake several times. Slowly drain out rinse solution, rotating tube so that seeds adhere to sides. Then add 20 drops sterile water.

4. Remove stopper from sterilized flask, flame opening, then pour water and seeds onto nutrient agar. Replace stopper in flask and give it a few gentle swirls to distribute seeds.

5. Write name on flask with wax pencil.

The flasks containing the seeds are then placed in a growth chamber held at about 70°F with about 200 ft-c light intensity. This chamber is shown in Figure 3. The appearance of the germinated seeds after 8 weeks is shown in Figure 4. Growth of the orchid seedlings is very slow, but by the end of the quarter the students can determine fairly well the results of their work.

Infection in the nutrient medium was a problem until we rigorously enforced the directions that before they began work the students must wash their hands and arms thoroughly above the elbow, rinsing them well with an alcohol-iodine solution. To prepare 1 liter of this solution mix 737 ml of alcohol and 263 ml of distilled water. About 6 crystals of iodine are dis-

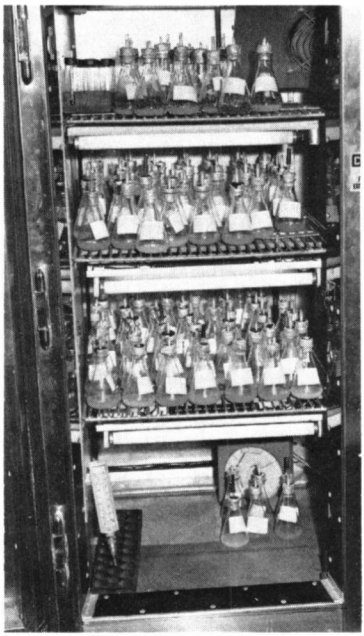

Figure 3. Growth chamber containing flasks of orchid seeds planted by students on nutrient agar.

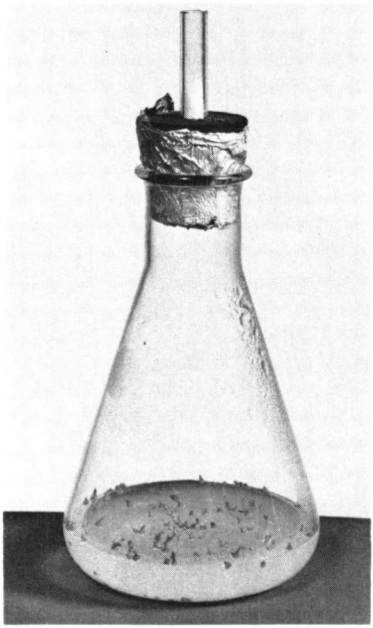

Figure 4. Appearance of germinated orchid seeds after about 10 weeks.

solved in alcohol before water is added. Arms and hands should be washed immediately before working in transfer chambers. Some flasks still become infected, but this is rare.

A teaching assistant stays with each group of students in the transfer chamber from the beginning to the end of the exercise explaining the procedures to be followed.

DIVIDING AND PLANTING CYMBIDIUM ORCHID PROTOCORMS

Several hundred protocorms are prepared in advance of this exercise from orchid shoot tips so that each student will have about six well-developed protocorms for his use. Using the same transfer chambers as described above, the students are provided with the equipment illustrated in Figure 5; i.e. flasks of protocorms to be divided; Petri dishes; tweezers and scalpel for cutting up the protocorms; small nutrient agar bottles for placing the developing protocorms for further growth; and a liquid medium for placing the divided protocorms for further proliferation. The same aseptic techniques as used in planting the orchid seeds are required for the protocorms.

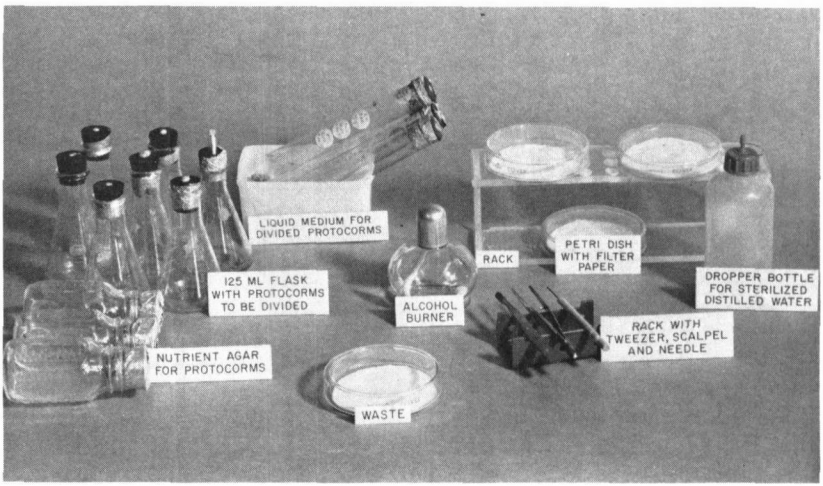

Figure 5. Equipment and material provided each student for dividing and planting orchid protocorms under aseptic conditions.

The protocorms planted in the nutrient agar bottles for growing on are placed in a growth chamber at 70°F and 200 ft-c as shown in Figure 6. The appearance of these plants after about 7 weeks is shown in Figure 7. The protocorms make much more rapid growth than do the orchid seedlings.

Figure 6. Growth chamber containing flasks of nutrient agar on which the orchid protocorms have been planted by the students.

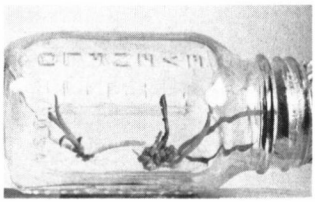

Figure 7. Growth of protocorms on nutrient agar after about 8 weeks.

Two rotating devices are used for maintaining the cut-up protocorms for further proliferation, as shown in Figure 8. One holds 125 ml flasks; the other holds 2.5 × 20 cm test tubes.

The solid medium used for growing the established protocorms is basically that of Wimber (6), as follows:

	Grams		Grams
KNO_3	0.525	ferric tartrate	0.03
$CaHPO_4$	0.20	tryptone	2.00
KH_2PO_4	0.25	sucrose	20.00
$(NH_4)_2SO_4$	0.50	agar	12.0
$MgSO_4$	0.25	water	1000 ml

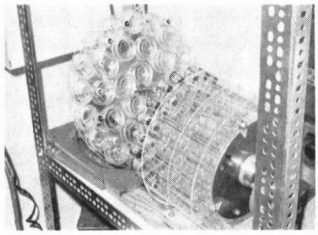

Figure 8. Two rotating devices for holding flasks (*left*) and test tubes (*right*) in which divided protocorms are placed for further proliferation.

The liquid medium used in the rotating vessels for further protocorm proliferation is the same as given for the solid medium except the agar is omitted.

These two exercises in orchid propagation, using aseptic culture techniques, one involving sexual propagation and one asexual propagation, provide an excellent means for developing many concepts during the classroom discussions preceding and following the actual laboratory work. The results have been highly successful and give the students considerable satisfaction in using aseptic culture methods.

It would be desirable, of course, to have the students gain more experience in media preparation, sterilization techniques, and other procedures such as callus cultures and shoot tip excision but time is not available to do this when these exercises are inserted into an already busy laboratory schedule. The exercises described would be a good introduction to an advanced course in tissue culture techniques in plant propagation.

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