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APPLICATION REQUIREMENTS AND COSTS OF A TISSUE CULTURE FACILITY FOR THE NURSERYMAN

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INTRODUCTION

Plant cell and tissue culture technology has in recent years advanced so dramatically that today, not only does it serve as a research tool for plant scientists, but also has found a powerful niche in ornamental plant propagation. This technology had its origin in 1902 when Haberlandt postulated that if plant cells and tissues were excised and cultured on a nutrient medium under controlled environmental conditions, the phenomenon of cell totipotency should occur.

A number of major discoveries trace the development of plant tissue culture since then. It received a major stimulus when Morel (13) commercialized tissue culture of orchids. This encouraged scientists to explore its applicability for the propagation of diverse ornamental crops.

As a measure of its importance and significance in the nursery stock industry, the rate at which it is being adopted is indeed, remarkable. Reviewing the literature, one finds evidence of more than 50 plant genera presently being commercially propagated using the technique and at least another 20 awaiting commercialization. Additionally, some of these genera represent numerous species and cultivars, so that thousands of individual kinds of plants are available throughout the world. This compares with just four genera which showed a potential for tissue culture in 1979 (14).

ADVANTAGES OF TISSUE CULTURE

The advantages accruing to the nurseryman may be categorized as horticultural or business. The horticultural advantages include:

(i) its unmatched potential for the rapid asexual multiplication of uniform high quality plants;

(ii) the ability to produce crops independently of weather and seasonal influences;

(iii) the elimination of stock plant holding areas;

(iv) the ability to store genetic material.

The business advantages include:

(i) the potential to produce vast quantities of plants with short generation cycles in a limited space;

(ii) the enhanced image accredited to the nurseryman using sophisticated technology for plant propagation;

(iii) the strategic marketing advantage accruing through earlier distribution of new and elite genotypes;

(iv) access to international markets afforded by the legitimate movement across national frontiers.

APPLICATIONS OF TISSUE CULTURE

Rapid clonal propagation has emerged as the greatest application of plant tissue culture to the nursery industry. Genera hitherto deemed difficult to propagate are now readily available to nurserymen in virtually limitless quantities (10). In forestry clonal propagation of *Eucalyptus* is predicted to produce large economic gains (9). Other applications include the propagation of conventionally difficult to propagate plants (e.g. *Rhododendron* c.v. Brittannia), the production of elite plants with a high commercial value, and the reduction in time required to release new plant cultivars, a phenomenon particularly relevant to the hardy fruit nursery industry.

REQUIREMENTS FOR A TISSUE CULTURE FACILITY

The fundamental principles underlying successful tissue culture procedures involve the isolation of a plant organ or part (explant) from the mother plant, its culture under aseptic conditions in an appropriate environment, and the re-establishment of a high percentage of the microplants. Special laboratory and environmental facilities are required for this:

(a) A greenhouse structure for growing or forcing mother plants. For the nurseryman this is usually not an additional requirement.

(b) Suitably equipped laboratory areas for medium and plant

preparation, explant manipulation, and transfer and culture room/growth chamber facilities.

(c) A weaning facility complete with environmental control.

The following appliances are necessary:

(a) Laminar air-flow cabinet for aseptic manipulations.

(b) Autoclavê for culture vessel, media, dissecting instruments, and water sterilization.

(c) Balances: (i) electronic analytical to five decimal places, with 300 gram weighing capacity.

(ii) Top-loading electronic, analytical to two decimal places with 1500 gram weighing capacity; suitable for less accurate measurements.

(d) Stereoscopic binocular dissecting microscope for dissecting apical and axillary meristems, shoot tips and stem pieces, and for culture observation.

(e) Water purification system to provide quality water.

(f) Magnetic stirrer with hot plate facility to aid in dissolving and agitating chemicals.

(g) Refrigerator/freezer for storage of chemicals and stock solution.

(h) pH meter to measure the alkalinity or acidity of the unautoclaved nutrient medium.

(i) Spirit lamp/bunsen burner for flaming instruments.

(j) Trolley for transporting cultures.

(k) Miscellaneous instruments (scalpels, forceps, automatic pipette.).

(l) Supply of laboratory glassware, plasticware, culture vessels, and high quality analytical grade chemicals.

Laboratory. Three distinct laboratory areas must be created:

(i) preparation

(ii) transfer

(iii) culture.

The preparation laboratory is the general work area where chemicals are stored, culture media prepared and sterilized, and plant material made ready for disinfestation. Hence, it should be equipped with balances, water purifier, magnetic stirrer, pH meter, autoclave, and have adequate bench space and shelving for operation and storage. Other equipment should include a refrigerator/freezer. Any preparation laboratory must have an adequate supply of tap water and sufficient washing facilities. At least one large sink and draining board is necessary. Sufficient space should

be available to set up baths for acid and detergent treatment of glassware and for drying racks.

The transfer area is the nerve centre of the facility and ideally a totally sterile atmosphere is the optimum environment for this process. Unfortunately, this concept is uneconomic and for practical purposes laminar air-flow cabinets are used. Such cabinets should be sited within a self-contained room to minimize the risk of chance contamination. In a laminar air-flow cabinet, air is initially drawn through a pre-filter, followed by forcing through a highly efficient one and the air is subsequently directed either horizontally or vertically at a uniform rate over the work area. This maintains a sterile area, within which operatives may manipulate explants and microplants.

The growth chamber constitutes the third component of the plant tissue culture laboratory and they can range form simple environmentally-controlled commercial incubators to sophisticated computer-controlled phytotrons. The deployment of an inexpensive growth chamber of the structure described by Morgan and Clarke (12) is adequate, provided it is equipped with temperature, lighting, and daylength control. Although a temperature of 24°C ± 1°C is the most frequently adopted one for incubating tissue cultures, Gardiner *et al.* (5) used 20°C for *Pinus contorta*. Hence, the necessity to have a system with the facility for setting and maintaining any pre-determined value from 12°C to 30°C ± 1°C.

The usual method of providing lighting facilities is the incorporation of banks of fluorescent tubes suspended by flexible mountings from tiered benches. Such an arrangement will provide various illuminance values by raising or lowering the light banks or altering tube numbers. To capitalize on energy efficiency, the tube type selected should emit a high proportion of energy in the spectral range 550 to 600 nm.

Photoperiodic control is also necessary and is achieved using time-clocks. It should range from continuous darkness through multiple darkness/lighting combinations to continuous lighting.

Sterilization. The need to continually maintain sterility is fundamental to the success of the tissue culture process. It requires that the media, instruments, re-usable culture containers, (glass and plastic) transfer area, and the plant material be made and kept sterile. Excepting the latter two, sterilization is effected by autoclaving for 15 minutes at 121°C and a pressure of 103.4×10^3 Pa. However, Biondi and Thorpe (2) recommend a minimum of 15 minutes for 50 ml volumes rising to 40 minutes for 2,000 ml volumes.

It is important for the propagator to note that not all plasticware is autoclavable. Polycarbonate, polymethylpentene, and teflon are; polyvinylchloride, polystyrene, and acrylics are not. It is recommended that this factor be considered when obtaining cul-

ture containers.

Filtration. Filtration using bacteria-proof membranes is an alternative method for media sterilization. However, it is expensive and so is usually only used to sterilize substances destroyed by heat.

Disinfestation. Plants are invariably contaminated with microorganisms and therefore must be disinfested before inoculation onto the growth medium. The donor material is initially prepared by removing superfluous tissue followed by washing under running tap water, frequently for many hours. Surface disinfestation may be achieved using a variety of sterilants, most of which are used in low concentrations. Commercial household bleaches containing hypochlorite are often used at rates ranging between 0.5 and 5.0 per cent. The inclusion of a wetting agent is beneficial to ensure thorough wetting of the plant surface. Sterilants must be completely removed by washing in several changes of sterile distilled water.

Water. Tap water contains many contaminants (gaseous and particlate) and as such is unsuitable for tissue culture purposes. It is necessary for the nurseryman to obtain a supply of high quality water. Several methods for obtaining such water are available (2) including glass distillation and reverse osmosis. The former is cheaper to install and is the preferred source in the author's laboratory. However, it has high running costs and may produce sub-quality water if not carefully maintained.

Weaning. The re-establishment of autotropism ("self-sufficiency") of a high proportion of the tissue culture-derived microplants is central to the economics of the system. Seabrook (15) argued that the microplant ought to have a well developed root system prior to transfer to the *in vivo* state, while more recently the trend is toward the establishment of unrooted ones (19). Tissue-cultured plants are both physiologically and anatomically different from seedlings or softwood cuttings (16,17), differences which cause difficulties during the acclimation process and often lead to reduced survival and establishment rates. Several factors have been identified:

(i) poor vascular connections between shoots and roots thus reducing water conduction (7)

(ii) delayed functioning of the stomates and lack of structural epicuticular wax resulting in excessive water loss (6, 16)

(iii) poor development of the photosynthetic system (8)

(iv) culture medium composition and transfer from heterotrophic to photoautotrophic nutrition (3)

(v) disease susceptibility at this developmental stage (11).

The humidity levels in the *in vitro* environment frequently approach 100 per cent. Accordingly, Conner and Thomas (3) suggested that humidity should be gradually reduced in the weaning

chamber to minimize initial transpiration shock, and the risk of wilting and desiccation. High humidities may be maintained with the use of fog, intermittent mist, or an enclosed polyethylene case. Fogging has emerged as an ideal method for weaning microplants. Intermittent misting systems are less favoured since leaching of the leaves and root asphyxiation of the microplants tends to occur (1, 11). The use of polyethylene tents are also useful but are less favoured than fog.

As with humidity, temperature and light control are also important. Wong (18) suggested that an air and compost temperature related to that used *in vitro* should be maintained while low lighting levels during the initial photoautotrophic stage, followed by higher levels with plantlet development, has been suggested (4).

COSTS OF ESTABLISHING A TISSUE CULTURE FACILITY

The costs of establishing a tissue culture facility is highly variable and is influenced by many factors:

(i) Whether a building exists on the nursery that could easily be converted into a laboratory, or whether construction of a purpose-built one would be required. In the former instance, the conversion and refurbishment costs; in the latter, the construction costs.

(ii) Proximity to essential services such as electricity and water supplies.

(iii) The manufacturer, model, and specification of the equipment purchased—(portable versus fixed autoclave; small versus large laminar air-flow cabinet).

(iv) Presence or absence of a weaning facility. On nurseries, where propagation facilities exist, additional facilities are unlikely to be installed. However a weaning facility specifically for tissue-cultured plants is strongly recommended

The costs of establishing a minimum recommended tissue culture facility for the nurseryman are given in Table 1. Prices are given in pounds sterling and exclude VAT, but represent present (1988) prices in Ireland.

Table 1. Requirements and cost for establishing a tissue culture facility.

Laboratory*	£10,250.00
Culture room*	£ 2,550.00
Laminar air-flow cabinet (1.5 sq m)	£ 1,660.00
Balance (i) analytical (5 decimal places)	£ 1,440.00
Balance (ii) top loading (2 decimal places)	£ 940.00
Stereoscopic binocular microscope	£ 1,025.00
Autoclave (portable)	£ 350.00
Water still	£ 290.00
Glassware drier/oven	£ 340.00
Magnetic stirrer with hot plate	£ 215.00
Trolley	£ 215.00
Refrigerator/freezer	£ 200.00
pH meter	£ 100.00
Instruments (forceps/scalpels)	£ 85.00
Glassware (flasks/beakers/cylinders)	£ 430.00
Weaning facility (72 square meters)	£ 2,990.00
Initial supply of chemicals/plasticware and containers	£ 600.00
Total capital cost	£23,680.00
Annual charge @ 13% (× 0.1715)	£ 4,061.12

*Combined floor area 75 sq m

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SUCCESSES AND FAILURES WITH MICROPROPAGATED PLANTS: THE BLOOMS' EXPERIENCE

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Micropropagation is hardly new but is only now being accepted by the majority of the trade as a useful and standard method of propagation. Twenty years ago, it was barely talked about in the hardy nursery stock industry nor considered a viable alternative or replacement to more traditional methods. We looked upon it with a mixture of excitement and dread. On the one hand it had a potential benefit for producing hitherto difficult to propagate plants, apart from new or unusual forms—but on the other it seemed to open the way to very real dangers of overproduction.

Questions were asked like: Would it revolutionize propagation methods? Would it put the skilled propagator out of business? Would it make the rare plant common, bring down prices and flood the market?

At that time there was no way those questions could be answered. As a company we had to ask the question, "What was in it for Blooms?" Whether or not you like to face change and new technology, if you don't you will soon find progress passing you by. We had to avail ourselves of this science to help us produce items that were consistently in short supply and for rapidly building up stock of new plants. Plants in test tubes did seem rather far-fetched but it was exciting to consider getting new, rare, or unusual plants into