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HOW FAR DO WE GO? FUTURE DEVELOPMENTS AND OPPORTUNITIES IN MICROPROPAGATION

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INTRODUCTION

There is no doubt that amazing advances have been made since plant tissues were first cultured *in vitro* in the 1930's. Orchid propagation by both seed and meristem culture (mericlone) was an early use of these techniques. Florist crops and pot plants probably still account for the largest number of plants propagated in culture. Increasing use of micropropagation techniques is being made in hardy ornamental nursery stock and plantation crops with considerable effect being expended in investigations in micropropagation of forest species.

Currently at least 205 laboratories are in operation worldwide (3), but it is difficult to distinguish between production and research laboratories, making any realistic output estimate impossible. There are a number of units in operation or planned with a production capacity of 5 to 20 million plantlets. The theoretical capacity of a facility and what is actually produced are often widely different and the logistics of the very large units present enormous problems.

The rapid development of micropropagation and interest in its possibilities, resulted in a crisis of confidence in the 1970s. Micropropagation had begun to be perceived as a panacea for all problems but a credibility gap grew between the theory and what was actually delivered. Nurserymen became disillusioned as contracts were not always met and insufficient account was taken of limitations of the technique. These problems have been, in many cases, overcome, but there are five outstanding problems to be faced before the technique can be fully used.

The first is synchronous development *in vitro*. We need to be able to understand and control the physiology of the plant more exactly.

Secondly, the least spoken of, yet most immediate problem, in commercial production is chronic contamination *in vitro*. Both bacterial and fungal problems occur with, in some cases, insects such as mites acting as vectors.

Thirdly, it is essential to aim for consistent high yield of quality plantlets. In the past insufficient attention has been paid to grading. Work within the Ministry of Agriculture's Agricultural Development and Advisory Service has demonstrated the importance of grading and we are understanding more of the effects *in vitro* conditions can have upon grading and growing on.

With some 50 per cent of production costs attributable to labour, efficient labour utilisation and management is essential. Savings in this area can be highly significant but should not be at the expense of quality.

Finally, it is essential for realistic business strategies to be adopted; over-capitalisation and too rapid a growth rate can create problems. Similarly a realistic marketing plan is required.

FUTURE DEVELOPMENTS

1. Automation. Aspects of automation will become increasingly important with simple aids and ergonomics such as media dispensing and container handling systems from the food industry; other aids and work study can be exceptionally cost effective. Developments in containers, especially the use of various types of plastic, may result in significant improvements in systems without high capital costs.

A number of possibilities present themselves using computer technology and robotic arms. A key component of this approach is an accurate vision system to guide the robot arm. It is possible to site the system in a sterile cabinet to ensure aseptic manipulations (5) and a number of groups are investigating these possibilities around the world.

Solutions will be technically possible but with high costs, especially of programming, the economics may be questionable in many cases.

2. Liquid Culture Systems. These offer a number of possibilities as they are potentially very flexible. Thin stationary liquid cultures have been used for some foliage plants and laboratory shakers have been crudely scaled up to demonstrate the potentially large increases in shoot production, such as 7-fold in fuchsia and amelanchier (7). Aeration of liquid systems can increase growth and yield in orchids (Pennell, unpublished data) with a 3-fold increase in protocorms. The natural progression of these studies would be to utilize bioreactors (fermenters) as is the case in work on alfalfa embryos (11).

Another approach would be a system using liquid media

together with an inert support such as agar, fibres, or granules. Maene and Debergh (8) adopted this approach, of a secondary liquid phase, with a range of species including cordyline, philodendron, magnolia, and spathiphyllum, initially to improve elongation and rooting. The concept has been used by others (2) where shoots of *Pinus radiata* have been maintained in the same culture vessel on agar, with liquid replenishments, for 18 months. An automated plant culture system based on replenishment of liquid media has been developed and can be monitored and controlled by computer (14). Such a system is almost *in vitro* hydroponics, in which manual handling of cultures is reduced to the absolute minimum and is only needed for the initiation of cultures and singulation (if required) of shoots prior to weaning.

The use and investigation of these potential options require a detailed knowledge of the various physiological systems which are used in micropropagation systems and especially their limitations.

3. Somatic embryogenesis. Literally, *in-vitro* embryo formation without pollination and with embryos often arising from a single cell. The main studies in this area have concentrated on carrot and celery although progress has been made with some plantation crops, notably the palms, using this approach. Palm embryoids develop into plantlets *in vitro* prior to transfer to compost (12). The key problems are that not all species will respond in this way. The development and synchronization of embryo production is difficult to control and developing delivery systems with high germination rates can be difficult.

Theoretically the long term aim would be to create an embryo which can be fluid-drilled (6) into compost or alternatively be encapsulated (9). Encapsulation consists of forming an artificial seed coat around an embryo consisting of a gel matrix and could enable seeding machines to be used.

4. Adventitious shoot systems. Many ornamental herbaceous plants have the ability to develop secondary meristems and adventitious shoots from plant organs, *Saintpaulia* leaves for example. Where species respond in this way, it may be possible to develop systems where mechanical cutting and transfer of tissues may be possible. Extreme examples of this approach are in the propagation of *Davallia* and *Platycerium* ferns (4).

Stock cultures are added to a sterile blender and homogenised for a few seconds. Fragments of tissues are then dispensed into culture containers. The potential yields from this system are enormous but are limited by:

- (i) very few species survive this type of treatment;
- (ii) cellular debris produced may have toxic effects;
- (iii) limited number of plants can produce adventitious shoots;
- (iv) uniformity of plants produced can be variable *in vitro* and

in the field with dangers of phenotypic and genetic variation;

(v) a long regeneration period may be required;

(vi) at some stage plantlets will probably still require singulation;

(vii) sterility is essential but may be difficult to maintain.

Nevertheless, this could be a valid approach in some circumstances.

5. Axillary shoot systems. Stimulating axillary branching or culturing nodal sections is the most stable and widely used system in micropropagation. There is scope for manipulating plant development by changing culture conditions. Most significant advances in systems using this type of plant development will centre on reducing labour at transfer by the use of a secondary liquid phase or by the development of robotics.

6. Specialization. Micropropagation companies will specialize in young plants and move away from pure contract work. There will be a trend to buy—in research and development to make best use of resources. A number of relatively small nursery units are likely to be set up for the micropropagation of a limited range of plants and be treated much like a mist unit, frame yard, or any other propagation facility.

7. Spin-off from micropropagation. There will be better utilisation of microplants on nurseries to make best use of growth rates and changes in plant form. Also, exploiting the observed juvenility and enhanced rootability of cuttings from microplants could have significant effect upon propagation generally.

The application of micropropagation techniques to conventional systems such as inducing juvenility in stock plants or the use of hydroponic culture in propagation are worth investigation.

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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).