Micropropagation of Mother Stock Plants at Walberton Nursery©

Claire K. Hobbs
Walberton Nursery, Yapton Lane, Walberton, Arundel, W. Sussex, BN18 0AS U.K.

INTRODUCTION

Walberton Nursery is one of the founding members of the Farplants Group. The nursery was established in 1975 and is owned by David Tristram. Its main crops are herbaceous perennials including several that have been introduced or bred on the nursery including *Coreopsis grandiflora* 'Walcoreop', Flying Saucers™ blanket flower, *Crocosmia* 'Walcroy', Walberton Yellow™ montoretia, *Spiraea japonica* 'Walbuma', Magic Carpet™ Japanese spiraea, and *Erysimum* 'Fragrant Sunshine'.

The nursery has two small micropropagation laboratories on site. The first is used primarily for research and the production of virus-tested mother stock for in-house propagation and for other nurseries throughout the world. The second laboratory is currently used for the commercial production of two of our crops. In addition, there is a small isolation house where we are able to exclude the vast majority of pests and diseases and grow on plants from the laboratory ready for sale or use on the nursery.

BACKGROUND AND HISTORICAL PERSPECTIVE

Plant micropropagation is the technique of growing plant cells, tissues, or organs isolated from a mother plant and grown on artificial media (George, 1993). It is a useful tool allowing the rapid production of clonal plants using relatively small amounts of space and supplies. It may be used by anyone from enthusiastic amateurs multiplying plants in the kitchen at home to distinguished scientists working in elaborate laboratories (Kyte and Kleyn, 1996).

It was in the 1830s that the seeds of micropropagation as we know it today were sown with the development of cell theory and the notion that tissue had the potential to differentiate into any type of cells. In the 19th century the presence of plant hormones was deduced and callus formation was observed in wounded trees. By the early 20th century attempts were being made to micropropagate monocotyledons while immature embryos had been isolated and fertile plants recovered from brassicas.

Through the next 50 years, significant developments took place, including work on the role of plant hormones in the development of micropropagated tissue, the embryo culture of a wide range of subjects, and the culturing of root explants. By the 1970s the principles of micropropagation were established and its commercial use was becoming feasible for a wider range of plant subjects. The number of nurseries involved in plant propagation through tissue culture grew rapidly during the 1970s and 1980s as techniques developed and became more widely utilised (Kyte and Kleyn, 1996).

Micropropagation was first used on a large scale by the orchid industry, enabling growers to overcome issues such as unpredictable seed, taxa that were difficult to propagate, and virus-infected plants (Kyte and Keyn, 1996). Micropropagation is now widely used throughout the world for the commercial production of a wide range of subjects including *Heuchera*, *Hosta*, *Digitalis*, *Fragaria, Geranium*, *Lavandula*, *Nemesia*, and *Yucca*.

THE MICROPROPAGATION PROCESS

The basic procedure of micropropagation is relatively straightforward and consists of four main stages: explant establishment/initiation; multiplication; rooting; and acclimatisation/hardening off (Kyte and Kelyn, 1996).

A piece of tissue known as an explant is removed from the mother plant, carefully sterilised, and then placed in a test tube or similar sterile culturing environment. The explant may be a meristem, piece of stem, root, leaf, bud, seed, or flower part.

Once initiation has taken place, the explant is then able to grow and/or be divided to produce plantlets that can multiply almost indefinitely given proper care (Kyte and Kleyn, 1996). The plantlets are then rooted and acclimatised.

In its sterile environment the explant is given a balanced diet of nutrients and hormones often tailor-made to suit the particular species. The medium provides the major and minor nutrients and usually a carbohydrate (sucrose being the most common) to replace the carbon, which the plant normally fixes from the atmosphere by photosynthesis. Vitamins, amino acids, and plant growth regulators may be added to improve growth, and antibiotics may be added to control infections. A solidifying agent such as agar is used to thicken the medium and thus provide physical support to the explant (George, 1993). Although there are standard media mixes that may act as a starting point for micropropagation, the exact proportions of the ingredients can vary greatly between species.

Plant cells and tissues have the natural potential to put forth new growth and to multiply; micropropagation merely directs and assists this. New growth is usually initiated in meristematic tissue (undifferentiated cells that have not yet been programmed for their ultimate development). Such cells are located in the tips of stems and roots, leaf axils and margins, in stems as cambium, and in callus tissue. These cells are influenced by factors such as genetic make-up, light, temperature, nutrients, and hormones to differentiate into leaves, stems, roots, and other organs (Kyte and Kleyn, 1996).

USE OF MICROPROPAGATION IN PRODUCTION OF STOCK PLANTS

Advantages. Micropropagation has a number of advantages over conventional propagation in the production of mother stock plants. Advantages include:

- Once a plant is in culture it is relatively easy to maintain and it is possible to sustain healthy material for many years.
- It is possible to achieve greater uniformity than with cutting-raised plants.
- Laboratory stock requires less space than conventional stock plants and a very small stock can be maintained which can be quickly multiplied. However, if micropropagation is being used as the source for mother stock plants, space will still be required for these plants.
- Micropropagation allows rapid multiplication from one plant, which is particularly useful if only one plant is available, for example, where a plant is particularly rare or a single plant from a breeding programme. The multiplication rates of different species vary greatly. Houseplants may take only a few days to initiate roots while some rhododendrons may take 4 to 6 months. If a plant in micropropagation were able to double in numbers every month

then a single plant could create 1024 plantlets after 10 months and 2048 after 11 months (Kyte and Kleyn, 1996).

- It is possible to produce plants "out of season," and therefore year-round production of stock plants is possible. This can be particularly useful when exporting material to other parts of the world.
- Laboratory plantlets require less attention than stock plants on the nursery. Surveillance is necessary to check for signs of infection and progress and dividing and subculturing may be needed every 2 to 6 weeks, but the plantlets do not need watering, spraying, or weeding.
- Diseases, which can be transmitted through conventional propagation, may be eliminated through the procedures of micropropagation. External contaminants such as bacteria, fungi, and insects are removed when the explant is sterilised and using the apical meristem as the explant for the tissue culture may eliminate internal contaminants such as viruses. The apical meristem is a group of undifferentiated cells located at the microscopic tip of the dominant shoot. It is often virus free even in diseased plants because these meristematic cells haven't yet joined to the plant's vascular system and perhaps grow faster than a virus can spread. Thus if the few virus-free cells that make up the apical meristem are removed from the plant and placed in a culture they can grow and produce healthy, disease-free plants. This technique is known as meristem tip culture (Kyte and Kleyn, 1996).
- The ability to produce virus-free mother stock is a key advantage of micropropagation, particularly valuable in virus-prone plants such as *Nemesia*. Plant viruses may be observed by visible leaf symptoms; however, some latent viral symptoms may not be expressed in the plants when in culture and thus the virus testing of plants becomes essential. This may be done through the sap inoculation of an indicator plant with an extract from the infected plant tissue. Indicator plants such as *Chenopodium album* or *Nicotiana tabaccum* show symptoms for a wide range of viruses (Cassells, 1992). Sap inoculation is an easy and inexpensive method of determining the presence of viruses although results may take a few weeks. If a virus is detected, other, more detailed tests such as electron microscopy or enzyme-linked immunosorbent assay (ELISA) can be carried out to determine the exact virus present. Elisa tests are available for the detection of over 100 different plant viruses (Gugerli, 1992). If a virus is detected meristem tip culture may be employed to obtain virus-free material.

Disadvantages. Micropropagation also inevitably has its disadvantages:

- It is labour intensive and requires highly skilled staff, especially at the research stage, in refining nutrients, detecting, identifying, and overcoming contaminants. Unless there is an established procedure for tissue culturing a desired plant the labour required to establish the right medium formulations and growing conditions can be time consuming and therefore costly. Labour may account

for up to 80% of the costs of running a laboratory (Kyte and Kleyn, 1996). Most large-scale micropropagation is now done in developing countries because of their lower costs of skilled labour. Many U.K. laboratories that existed in the 1970s and 1980s have now closed, and those that remain tend to be very specialised.

- Genetic instability can be an issue with micropropagated plants: thousands of plants could be raised only to reveal a defect when mature, which may be due to a chemical imbalance or a mutation that has multiplied in culture (Kyte and Kleyn, 1996). However, rooting and growing on a number of individual "test" plants prior to large-scale production could detect this. Some species are more prone to mutation than others. Some mutations may occasionally be desirable and result in new cultivars.
- Some plants appear impossible to micropropagate; on some cases the right method, media formulation, and conditions have yet to be found.
- Although micropropagation can be used to clean up plants prone to diseases and viruses, plants within micropropagation are prone to biological contaminants such as bacteria found on or within explants or in the laboratory. Infections can spread rapidly on agar and destroy the plantlets. Such risks are minimised by using sound sterile techniques in the laboratory. However, some plants, for example, some hellebores, carry endogenous bacteria, which are very hard to eliminate.

SELECTION OF SUBJECTS SUITABLE FOR MICROPROPAGATION

A plant's growth characteristics in nature affect the ease with which it can be adapted to micropropagation, and as a rule, plants that show indeterminate growth are more successful in micropropagation than those that demonstrate determinate growth. Bulbs for example are difficult, especially as their growth underground means they are difficult to clean — meristems deep within bulbs make good explants but root explants are almost impossible (McCown and McCown, 1999). Unfortunately, assessing which plants will be suitable for micropropagation; how long they will take to establish a plant in culture and produce a rooted plant; and what the rate of multiplication will be can only be discovered with experimentation.

CONCLUSION

Micropropagation has potential not only as a source for mother stock plants but as a propagation method in general. It can be a little strange to see plants growing in test tubes in sterile laboratory conditions with no soil in sight, but they are still plants and it is fascinating to watch them develop in their microenvironment and produce roots. At Walberton Nursery we have certainly benefited over the years from being able to "clean up" plants prone to bacterial infection and viruses. Our successes include our micropropagated *Daphne tangutica*, which is healthier and more vigorous than those propagated conventionally; we have removed the bacterial problem from our *Photinia* crop; improved the quality of our *Scabious*; and produce high quality plants as the base for our mother stock plants.

LITERATURE CITED

Cassells, A.C. 1991. Problems in tissue culture contaminants, p. 31–44**.** In: P.C. Debergh and R.H. Zimmerman, Eds. Micropropagation: Technology and application. Kluwer Academic, Dordrecht, The Netherlands.

Cassells, A.C. 1992. Screening for pathogens and contaminating microorganisms in micropropagation, pp. 179–192. In: J.M. Duncan and L. Torrance, Eds. Techniques for the rapid detection of plant pathogens. Blackwell Science, Inc., Oxford, U.K.

George, E.F. 1993. Plant propagation by tissue culture (Part 1), 2nd Ed. Exegetics Ltd, Wiltshire, U.K.

Gugerli, P. 1992. Commercialisation of serological tests for plant viruses. In: J.M. Duncan and L. Torrance, Eds. Techniques for the rapid detection of plant pathogens. Blackwell Blackwell Science, Inc.

Kyte, L. and **J. Kleyn.** 1996. Plants from test tubes: An introduction to micropropagation. 3rd ed. Timber Press, Portland, Oregon

McCown, B.H., and **D.D. McCown.** 1999. A general approach for developing a commercial micropropagation system. In vitro Cell. Develop. Biol. 35:276–277.

Use of Cost Analysis to Improve Nursery Profitability©

Will George

Prenplants, Haven Road, The Haven, Billingshurst, West Sussex RH14 9BJ U.K.

INTRODUCTION

It is now possible to calculate the cost of every line of nursery stock on an individual nursery. The ease and accuracy of these costs will depend on the detail and quality of data collected by the nursery. In such a short paper it is not possible to fully cover all aspects of nursery stock costs. This paper will examine the most important factors that affect profitability and highlight the effects of time, space (crop density), yield, and waste on the profitability of nursery stock. The ways and means of allocating labour costs will also be reviewed.

Current Performance Within the Industry. Over the last few years the U.K. nursery stock industry has come under severe financial pressure. This has a number of causes. The market has slowed down due to adverse weather during key seasons and changes in consumer buying, resulting in over-production, both in the U.K. and in countries that export to the U.K. Many crops have been offered at low prices just to clear the backlog of plants.

At the same time, costs have been rising dramatically, and aggressive pricing policies from some of the larger multiple retailers are keeping prices of finished plants uneconomically low.

Tables 1 and 2 show the results from all nurseries within the Horticultural Trades Association's (HTA) Nursery Business Improvement Scheme (NBIS) for the 12 months to 31 March in each year. The NBIS is a scheme in which member nurseries can compare costs and other business data within local discussion groups. The data represent a comprehensive cross section of the industry and shows the trends within the nursery trade. Labour costs have increased dramatically over the last few years, as have transport, marketing, and sales costs, which are included in distribution. Despite a steady increase in productivity, the surplus available for extra income and investment has fallen.