control acid injection. But the fundamental reading must still be the total alkalinity of the acidified water.

Sulphuric acid is the most practical acid to use. The (sulphate) sulphur provided would either be beneficial to your plants or at least not harmful. Nitric acid will often supply more nitrogen than is needed by your plants; this extra N will not allow you to hold back plants should you need to. The extra phosphorus provided if you use phosphoric acid will usually be too much for your plants and could lead to toxicities or interference with other nutrients. Hydrochloric acid must not be used as the extra chloride provided will increase the lushness of your plants, so making them more vulnerable to diseases, and/or it will burn leaf margins.

FURTHER READING

Handreck, K.A. and **N.D. Black.** 2002. Growing media for ornamental plants and turf. 3rd ed. University of New South Wales Press, Sydney, Australia.

Integrating a Tissue Culture Laboratory into a Nursery©

Ian Gordon
School of Agronomy and Horticulture, University of Queensland Gatton Campus QLD. 4343

INTRODUCTION

The successful introduction of many Australian native plants in tissue culture in commercial volumes has been full of pitfalls. Many papers have been published outlining research into the initiation and growth of native flowering plants but we are still to see large numbers of these plants in the commercial nursery and flower trade. Examples of plants such as *Ptilotus, Clianthus, Blandfordia,* and *Platycerium* are examples of native plants, which have been the subject of significant research, but we are yet to see any significant numbers of these plants available within the nursery and cut flower industries. Obviously, published research has not translated into commercial production of these plants.

There have also been some success stories with the introduction of some native plants into tissue culture production. *Anigozanthos*, *Asplenium*, and *Nephrolepis obliterata* 'Kimberley Queen'. However, there is clearly a major gulf between the small-scale success in a research project needed for publication and the development of reliable systems of commercial production in plant tissue culture.

This paper attempts to identify the operational issues in a plant tissue culture laboratory and how these can be integrated with commercial nursery production practices to ensure the successful commercial introduction of tissue-culture-produced lines into the commercial nursery and cut flower trade.

THE PLANT TISSUE CULTURE LABORATORY — FACTORS OF IMPORTANCE

The management of microbial contamination within the laboratory is the single most important factor in producing plants in the laboratory. A hygiene program must be implemented in all sections of the laboratory facilities to maintain freedom from contamination in cultures. Despite the title of this paper, I do not support the complete integration of a tissue culture laboratory within the physical confines of a nursery. The risk of microbial contamination increases dramatically if the laboratory is situated within the nursery.

In the case of the University of Queensland (UQ) Gatton campus, the Plant Nursery Unit and the Plant Tissue Culture laboratory are physically well separated and I see the issue of integration as one in which the practices carried out within the laboratory are integrated with the practices carried out after the plants arrive in the nursery.

THE INCUBATION ENVIRONMENT IN THE LABORATORY

A high light intensity and a high light quality are required to promote successful growth in the incubation laboratory. Many plants require a light intensity of 5000 lux or more to promote satisfactory growth. The light spectrum emitted by the lights is also significant in the quality of growth produced. If the light intensity is high enough and the light spectrum is emitting light in the photosynthetically active range (PAR), autotrophic growth can be induced in the growing cultures. If the cultures are photosynthesising during growth in the laboratory it is much easier for these plants to adapt to nursery conditions when the transfer is made.

Many laboratories use multi-tiered racks in the incubation rooms with fluorescent lights mounted on the underside of each tier of the rack. This means we are attempting to direct the light through opaque plastic lids on top of the culture containers. Obviously, not much of the light emitted by the tubes gets to the growth sites on the cultures. When the UQ Gatton Plant Tissue Culture was refurbished some years ago the fluorescent tubes were fastened onto the side walls of the incubation rooms so that the light is getting into the culture containers through the side walls. Light intensity can be adjusted based on the number of tubes, which are active. This means that the light levels can be matched to the individual species requirements.

In the UQ Gatton plant tissue culture laboratory the light intensity is in the range 7,500 to 10,000 lux. This is a high range figure by comparison with many other laboratories in Australia and I believe that the high light level we operate with is a major reason for the high success rate we achieve when cultures are deflasked into nursery media later on. The light intensity (as we approach winter) in the fog propagation house at Gatton is in the range of 15,000 to 20,000 lux so rooted plantlets can acclimatise to this light level very easily.

Daylength is another factor that must be adjusted and most laboratories seem to standardise on a 16-h light/8-h dark regime. Temperature should also be maintained at a standard 25 °C. Working with a standard and uniform environment throughout the year should mean that cultures will grow consistently at all seasons of the year. However, we regularly find that some plants show altered physiological status at some times of the year. In our laboratory cultures of *Grevillea* and *Caustis blakei* have much higher success rates during winter and lower success during the heat of summer.

A tissue culture laboratory will also benefit from the installation of a HEPA filtered-air system, which will provide a sterile filtered air supply through the facility.

HORMONAL ACTIONS AND INTERACTIONS

Most attendees will understand the hormonal effects of cytokinins and auxins during the different stages of culture. The anti-apical-dominance effect of cytokinins is utilised in producing short, multiple-branched plantlets which can assist in getting a high multiplication rate during the multiplication stage in the laboratory. Benzylaminopurine (BAP) is the most widely used cytokinin source for commercial tissue culture.

Auxins such as indolebutyric acid (IBA) which are the staple auxin formulations used in commercial stem cutting propagation are also the staple products used to induce roots to form on cultures prior to transfer to the nursery. The hormonal manipluation of cultures at the different stages of production are the keys to successful production of plants in the laboratory.

Using the correct compounds is important for success but using these compounds in the correct concentrations is equally important. There are many instances where too high a concentration of cytokinin can cause abnormal growth in cultures. Shoot vitrification often occurs in cultures of *Grevillea* when high cytokinin rates are used. There has also been a well-known instance in Australia of massive numbers of offtypes of bananas as a result of very high cytokinin levels.

TECHNICAL EXPERTISE IN COMMERCIAL TISSUE CULTURE

It is essential that a tissue culture laboratory have an experienced technical person who has a feel for culture production and manipulation in the laboratory. We members of I.P.P.S. look upon the professional plant propagator as a technically skilled professional who also has a degree of flair for the job of propagating plants. The tissue culture professional should be the equivalent of the experienced propagator with a strong science background but this person also needs the "flair" to understand the different ways to manipulate growth on cultures.

This technical expertise includes decisions on what plant material to use for initiation of cultures, the media combinations required by specific plants, the hormonal balance of culture media at the different stages, and developing easy methods of subculturing to produce commercial quantities of plants. Different stages of tissue culture production require different concentrations of minerals. For example, the multiplication stage in most plants requires high mineral levels but the mineral levels are often reduced by 50% at the root development stage. The technical manager of the laboratory has to be able to make decisions on these aspects of production so that commercial quantities can be consistently produced. Good records of production activities are essential so that future decisions can be based on successful historical information.

A science degree can provide the graduate with the scientific knowledge of tissue culture production but there will be many years of laboratory experience needed to develop that "flair" of the plant propagator.

ROOT FORMATION IN VITRO

It is my personal preference to obtain in vitro-rooted plants from the laboratory for deflasking in the nursery. Most soft-tissued plants will give almost 100% of cultures with a root system. Many woody plants do not respond so well and with plants such as *Grevillea* there may only be 60% to 70% of cultures with roots.

Some laboratory managers use activated charcoal in the in vitro rooting media. This is beneficial as it reduces the light intensity around the root zone and thus increases the rooting percentage.

WHAT DOES THE NURSERY MANAGER WANT FROM THE LABORATORY?

The success rate achieved by the nursery in deflasking rooted plantlets is strongly influenced by the quality of the plantlets delivered by the laboratory. Actively growing plantlets with some level of autotrophic growth will deflask more successfully than

weak and spindly plantlets. There are obviously many issues of nursery technique and facilities management that come into this equation as well. However, even the best nursery will not achieve success with poor quality plants from the laboratory.

There is considerable evidence from the UQ Gatton nursery that deflasking success rates in winter are often better and plantlet growth after deflasking is faster than in summer.

THE DEFLASKING PROGRAM IN THE NURSERY

The Nursery Transfer Room. An enclosed transfer room with a high standard of hygiene and good temperature control is desirable. It should be kept in a clean, hygienic condition. Good design will make it easy to manage hygiene.

The Container to Deflask Into. The end customer often drives this decision. Many of our customers want plants delivered in 50-mm round plastic tubes. This is not necessarily the best container for root development but it is popular and the customer is always right. Another customer we are about to commence supplying *Dianella* plants to has specified that the plants be delivered in a 100-cell tray. Cell trays make grading of plants more difficult and it is important for the staff doing the deflasking to practice size grading during the deflasking operation.

Deflasking Media. A high quality, pathogen-free medium is essential. It must also be reproducible from batch to batch so that product uniformity is maintained. We use a standard medium of 1 sphagnum peat : 1 perlite : 1 vermiculite (by volume). We use 1 $kg \cdot m^3$ of Mini Osmocote incorporated into the medium. This will be supplemented by regular liquid feeding later on.

The deflasking operation is relatively simple but it must be done quickly to avoid any drying or desiccation of plantlets during the deflasking operation. As soon as one tray is complete it should be moved into a high humidity propagation environment.

The Propagation Environment. Low light intensity is essential for newly deflasked plants. The UQ Gatton propagation house has a light transmission value in the range 10,000 to 20,000 lux. This is essentially 10% to 20% of outdoor ambient light. A warm temperature must be maintained. We use a bench-installed warm-water heating system set to maintain 25 °C. A high-pressure fogging system maintains the greenhouse humidity in the range of 80% to 90%. A misting system is also suitable as long as overwetting is avoided.

The propagation greenhouse must have a high standard of hygiene. Regular monitoring is important to ensure that problems are detected at an early stage. We use a routine spray program applied weekly in which we rotate four different fungicides and in some cases an insecticide is added to keep fungus gnats under control. These can be very troublesome during winter.

Residual Hormonal Effects. Many plants exhibit marked hormonal effects from the tissue culture media and these effects can continue after transfer to the nursery. Plants such as *Dieffenbachia* and *Dianella* show a multi-branched growth habit as a result of the residual cytokinin effect. This means that these plants look good in the market place.

Other plants frequently show a residual auxin effect that manifests itself as an apically dominant single-stemmed plant with no lateral branching. This habit of growth is not acceptable and the terminal growing point of these plants must be

removed to promote lateral branching. Given my Scottish heritage I asked myself the question "if I have to take these terminal shoots off the plants, can I use them as miniature cuttings to get a second batch of plants from the deflasked cultures"? The answer to this question is frequently "Yes, I can". We have achieved very good success with these ultra-soft cuttings on a number of different species. Miniature tip cuttings from *Actinotus* and *Metrosideros* 'Fiji' produce roots very quickly. Because the shoot tips have this residual auxin effect there is no need to apply auxin to the cuttings.

If the market place is looking for bushy, multiple-stemmed plants following tissue culture, there may be a need to pinch the shoot tips more than once to achieve this growth habit.

Supplementary Liquid Feeding. Two weeks after deflasking plants, we commence liquid feeding on a regular weekly cycle. A proprietary brand of liquid fertiliser with a complete range of major nutrients and trace elements is used.

Weaning Off the Fog. With most tissue-cultured plants we begin the process of weaning off the fog environment after 2 to 3 weeks in the fog house. Once it is clear that root growth is continuing to develop, the plants are moved to an adjacent greenhouse with a higher light intensity and a lower humidity to continue growth. Proper weaning from the effects of the fog house must be carried out before moving the plants. I have seen many examples of large-scale loss of plants through inadequate weaning from the fog or mist environment.

With many species of plants deflasked into our fog propagation environment we have a survival rate in excess of 90% and this high success rate is being achieved because we have successfully integrated the environmental and physiological factors of the tissue culture laboratory with the environmental, physiological, and management factors of the nursery unit.

Acknowledgement. I would like to acknowledge the assistance of Mr. Ross Bourne, Manager of the UQ Gatton Tissue Culture Laboratory, for his input into the development of this paper.