TISSUE CULTURE IN PLANT RESEARCH AND THE ORNAMENTALS INDUSTRY

JONATHAN SUTTON[1]

Plant Research Institute
Department of Agriculture, Victoria

In recent years plant tissue culture has experienced a boom period both in research and commerce. Research workers around the world continue to find new applications of the technique with the result that some areas of research have progressed rapidly. Industry in turn has been quick to adopt tissue culture techniques where improved production can be realised.

In this paper some of the more recent advances will be discussed. Also, because enquiries have been received from growers interested in using tissue culture in their own operation, some basic requirements for starting such work will be outlined.

When we talk of plant tissue culture we are usually referring to any kind of plant tissue growing on a sterile nutrient solution under aseptic conditions.

Depending on the aim of the exercise, the growing tissue may subsequently differentiate into recognisable plant organs such as roots, shoots, petioles and leaves, or it may continue to produce a mass of undifferentiated callus tissue.

Usually the type of growth can be determined by changing the balance of hormones in the nutrient solution as shown by Earle and Langhans (2) in their work on the propagation of chrysanthemums *in vitro*.

A wide range of plant material has now been grown in culture. Ornamental plants include carnations, chrysanthemums, gerberas, geraniums, freesias, irises and lilies. However, each plant species has particular nutrient requirements and much time is often spent trying different nutrient combinations to achieve good growth of the cultures.

One aspect of this work which may have important implications for the future concerns the use of tissue cultures as a food source (3). Given the right conditions the growth in culture of plant cells of food crops may be extremely rapid and weight gains have surpassed those of field or container-grown plants.

With such a system operating, nutrients could be fed in at one end, and vitamin rich plant material harvested at the other.

Such studies are in their infancy. However, already good growth has been achieved with tissue cultures of bean, cucumber, endive, parsley, lettuce, spinach and potato tuber.

---

[1]Senior Plant Pathologist

Several important medical applications of plant tissue culture were recently outlined by Kehr (4) of the US Department of Agriculture. He referred to the production of insulin which has proven difficult to manufacture artificially. Up to the present it has been obtained from animal sources. However, recent work indicates that it can be conveniently obtained from tissue cultives of *Momortica charantia* and as a result this source may be of considerable future importance. Other substances which can be produced in this way include cardiac glycosides, the reported anticancer drug "camptothecin" and a wide range of alkaloids. Medical industries are quick to adopt and develop such methods of producing drugs.

It is interesting to note that tissue cultures to date have been mostly of herbaceous plants. By contrast few successful tissue cultures from woody plants have been made. Their requirements seem to be more complex and it has proven more difficult for healthy callus growth to be initiated and sustained. Widely divergent parts of many plants can be grown in culture. Healthy callus growth has been obtained from leaves, stems, shoots and roots as well as various storage organs such as carrot and potato tubers (1).

This phenomenon may be an extension of wound tissue formation. In response to localised damage, some differentiated cells may recommence dividing to form wound tissue.

Furthermore, each new cell thus formed seems to have genetic coding and physiological flexibility necessary to recreate a plant similar to the parent. This has been shown by Vasil and Hildebrandt (9) who produced tobacco plants from a single tobacco cell in microculture in fresh nutrient solution. In studies such as these single living cells are suspended in a drop of nutrient solution in a special glass slide.

In this way they can be examined with the aid of a good microscope and changes which occur in the cell can be noted.

TISSUE CULTURE IN PLANT DISEASE STUDIES

Techniques of this kind have proven particularly valuable in studying the interaction of some disease organisms with plant cells. Where the invading organism exists within living plant cells and a delicate balance exists between the two; this interaction is difficult to study using other methods. Typical diseases which may conveniently be studied this way are those caused by viruses, mycoplasmas and obligate fungal pathogens such as *Plasmodiophora brassicae*. This organism causes the "club root" disease in a range of cruciferous plants (10). These diseases represent the situation where the invaded plant cells sustain the growth of the pathogen as well as carrying out their normal function.

Often, particularly where cells are infected with viruses, crystal-like inclusion bodies can be seen within the cells. Using single cell techniques these can be closely observed (7).

In studies using sugar cane, plants were grown from single cells. From one cultivar, plants were produced with characteristics differing from those of the parent. This could indicate the presence of different cell clones within the parent and hence the possibility of selecting superior strains. However, an alternative possibility which must not be discounted is that genetic aberrations may have occurred early in the growth of some cultures, giving rise to the non-uniform types.

TISSUE CULTURE FOR THE RAPID MULTIPLICATION OF PLANTS

The use of tissue culture for the rapid propagation of some ornamental plants is already being used widely by some sections of the horticultural industry. In recent years much progress has been made, particularly with some plants notoriously slow to multiply vegetatively.

It should be emphasised that the tissue culture process is relatively complex and specialised facilities are required, some of which will be referred to later. Therefore, the technique is generally considered only when simpler methods of propagation have proven unsatisfactory.

The propagation of orchids is a noteworthy example where alternatives were not available. Using the conventional back bulb division it could take 10 years to get a dozen or so good sized divisions. Morel (5) in France found that orchids could be multiplied indefinitely by growing corm pieces in nutrient tubes and continually sectioning them. Each new piece became what he called a "protocorm" which could be sectioned further.

When sufficient plantlets were obtained they were allowed to develop without further sectioning until they could be transferred to a suitable orchid potting medium where growth continued to maturity.

In 1968 a similar technique was described for the rapid vegetative propagation of asparagus (8). Asparagus, which is generally propagated through seeds, shows extreme variability in the yielding ability of individual plants.

To avoid this variability, attempts were made to propagate by segmenting established and selected crowns. However the practice is very slow and commercially not feasible. It was found that actively growing spears could-be-cut into sections, surface sterilised, and grown on a nutrient medium. In the dark, such sections produced a mass of callus tissue at the base. The callus tissue was then divided and placed into separate flasks containing a modified nut-

rient medium. These cultures were grown under fluorescent lights. After about one month shoots and roots had developed to the stage where planting out was possible.

The process, therefore, consisted of three main stages:

1. The induction of callus from freshly excised spear sections
2. The initiation of plantlets from the callus
3. The further development of plantlets into plants of transplantable size.

For the three steps, the basic nutrient was the same. However, the growth factor supplement and light intensity was varied. Before transplanting it was found very important to "harden" plants off with an acclimatisation process, otherwise serious losses occurred. Hardening was achieved by opening the culture in the greenhouse. It was also necessary to flush with a dilute Hoagland's solution to avoid contamination. Using this technique, numerous plants similar to the parent in vigour and productivity, were obtained. In later work with asparagus up to 62% of cultures developed roots and shoots within three months.

Tissue culture has also been used for the rapid propagation of the gerbera. This is a flower of increasing economic importance. However, as with asparagus, propagation by seed results in seedlings which are not uniform due to the heterozygous nature of the plant. Vegetative propagation by division is too slow to be commercially practical.

In some recent work (6), a method appropriate for commercial application was described for the rapid propagation of gerberas. It was found that, on suitable nutrients, gerbera shoot tips produced numerous miniature divisions which could be separated and recultured. Gerbera shoot tips seem difficult to decontaminate. However, about 20% of the cultures were not contaminated and this was sufficient to start the propagation process.

The examples just given of rapid propagation of orchids, asparagus and gerberas, serve to illustrate typical situations where tissue culture has had or will have a major impact. It should be noted that, as well as avoiding problems of seedling variability and slow propagation rates, the technique has incidental advantages in that fungal and bacterial pathogens are generally absent. For these reasons, use of plant tissue culture for plant propagation in the coming years is likely to be extensive.

MERISTEM (SHOOT-TIP) CULTURE FOR THE PRODUCTION OF PATHOGEN-TESTED PLANTS

To obtain plants free of detectable diseases, several methods are available. Usually meristem culture is only used when simple methods do not work well.

Plants propagated by seed are usually virus-free, even though the parent plant may be infected with several viruses. However, the method is often unsatisfactory because of seedling variability previously mentioned.

A second alternative exists where crops are not wholly infected. Local tulip crops represent such a case where plants free of tulip-breaking virus can be found in many crops.

By careful selection and testing, healthy plants can be isolated and used as a source of clean propagation material. It is important that selected plants are maintained in an environment where deterioration will not occur. Since many plant viruses are spread by aphids, insect proof cages are an important aspect of this approach. Unfortunately plants free of diseases cannot always be obtained using methods such as these. For this reason, the discovery, some years ago, that meristem culture could be used to produce plants apparently free of diseases, was received with great interest.

Since that time much progress has been made with the result that crops such as carnations, chrysanthemums, potatoes and several others, can now be obtained free of diseases.

Meristem culture is a particular type of tissue culture. It involves dissecting out the growing tip or meristem from a bud. Because meristems are small (about 0.2-0.4 mm) this work must be done with the aid of a good dissecting microscope. After removal from the bud the growing tip is transferred to a tube containing sterilised nutrient solution. Provided the correct nutrient solution is used and that light and other factors are in proper balance, the meristem continues to grow and differentiate until a small plant is formed. The work is done under aseptic conditions to prevent contamination from bacteria and fungi.

The reasons are not well understood why, using this technique, plants can be produced free of diseases. It seems that virus multiplication is inhibited where plant cells divide rapidly such as the meristematic regions of buds. Furthermore, the cut which removes the meristem causes wounding at its base, thus stimulating more cell division to occur.

It is important to realise that only a proportion of plants produced in this way are free of virus diseases. Therefore, testing meristemmed plants for residual diseases is a vital part of the technique.

Our work with carnations indicated that viruses are eliminated more efficiently if, before meristems are cut, the plants are heat treated. Heat treatment at 37.5°C (100°F) for periods in excess of 4 weeks seemed to result in more vigorous growth of meristems, less contamination and a higher proportion of virus free plants.

REQUIREMENTS FOR TISSUE CULTURE WORK

A number of commercial organisations, in particular those concerned with the propagation of orchids, have been quick to utilise tissue culture to increase production.

Some of the facilities and equipment needed for such an operation are included in the following:

1. A clean, draught-free room or laboratory for contamination-free work.
2. Chemicals and an accurate balance for the preparation of nutrient solutions.
3. Facilities for sterilizing nutrient solutions. Steam-operated autoclaves are suitable for this purpose. A pressure cooker can be used for small amounts.
4. Glassware for use in preparing and storing nutrient solutions and for holding tissue cultures. Suitable caps are necessary to preevent contamination and drying out of the cultures.
5. A variety of dissection instruments.
6. Both light and dark areas with temperature control, where cultures can be held during growth.

Using facilities and equipment such as those referred to, and with an appreciation of aseptic handling of cultures, most commercial propagators could use tissue culture for the rapid propagation of some plants.

However, to extend the technique to the commercial production of virus-free plants by meristem culture is not so easy. Firstly, a good binocular microscope is essential so that meristems can be excised from buds with minimal damage. Secondly, a hot box may be necessary to heat-treat parent plants before taking meristems. Finally, because testing of plants following meristem cultures, is essential, methods to identify infected plants must be available.

Virus tests can often be done using "indicator plants." In these tests, sap from plants to be tested, is wiped onto the leaves of sensitive indicator plants in the presence of an abrasive.

If viruses are present in the sap, the indicator plants may react with characteristic leaf symptoms after 6-10 days.

Access to an electron microscope may be a great advantage, enabling tests for some viruses to be completed in minutes. Unfortunately, most propagators do not have access to equipment such as this. For these reasons, it seems likely that the production of virus-tested stock will continue to be done at suitably equipped research institutes such as the Victorian Plant Research Institute, at least for the foreseeable future.

CONCLUSIONS

In conclusion I foresee that interest in plant tissue culture will continue to grow. As a tool, aiding research, its role is already well established. In the future, application of the techniques will widen, enabling progress to be made in many areas of research previously neglected.

As a production method for elite planting stock, it will mean improved yields and more reliable and consistent production for some crops.

As a means of obtaining disease-free plants, the technique has already had major successes and for many crops seriously affected by virus diseases the technique represents a light at the end of the tunnel.

The story of plant tissue culture even at this early stage is one where great progress has been made largely through the cooperative efforts of industry and research organisations around the world.

LITERATURE CITED

1. Barker, W. G. 1968. Behaviour *in vitro* of plant cells from various sources within the same organism. *Can. Jour. Bot.* 47(8): 1334-1336.
2. Earle, E. D. and R. N. Langhans 1973. Propagation of chrysanthemum *in vitro*. 1. Multiple plantlets from shoot tips and the establishment of tissue cultures. *Jour. Amer. Soc. Hort. Sci.* 99(2):128-132.
3. Hildebrandt, A. C. 1967. Plant cell culture as a potential food source. Activities Report. Fall issue 18(2) 110-118. Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.
4. Kehr, August 1975. New developments in plant cell and tissue culture. *HortScience* 10(1):4-5.
5. Morel, G. 1965. Clonal propagation of orchids by meristem culture. *Cymbidium Society News* 20(7).
6. Murashige, Toshio, Maria Serpaa and Jeanne B. Jones 1974. Clonal multiplication of gerbera through tissue culture. HortScience 9(3):175-180.
7. Singh, M. and A.C. Hildebrandt, 1967. Inclusion bodies produced by three strains of tobacco mosaic virus in tobacco callus cultures. *Acta Phytopathologica, Academiae Scientarium Hungaricae* 2(1):3-9.
8. Takatori, Frank H., Toshio Murashige and James I. Stillman, 1968. Vegetative propagation of asparagus through tissue culture. HortScience 3(1):20-23.
9. Vasil, V. and A. C. Hildebrandt, 1965. Differentiation of tobacco plants from single, isolated cells in microculture. *Science*. 150:889.
10. Williams, P. H., M. N. Reddy and J. O. Strandberg, 1969. Growth of noninfected and *Plasmodiophora brassicae* infected cabbage callus in culture. *Can. Jour. Bot.* 47(8):1217-1221.

QUESTIONS

In reply to questions it was stated that they believe they now have two virus-free clones of daphne. These are from selection, not tissue culture. They have been tested for 10 known daphne viruses and are now being multiplied. Seedling

daphne have not been tested for virus but they are probably free of it. It was emphasised that freedom from virus does not guarantee that they will not become infected. The aim is to inform growers of the ways that virus is spread so that they can then reduce this spread.

PROPAGATION AND CULTURE OF AFRICAN VIOLETS

HENRY C. JACKSON

East Malvern "Idaho" Nurseries
East Malvern, Victoria

African violets are fast becoming a favourite house plant in Australia. I have been growing them at "Idaho" Nurseries since 1957. The only cultivars that were available at that time were a single blue named "Blue Boy," a single pink (unnamed) and a double pink called "Bud's Pink Waltz." I grew these cultivars until 1961. Then the late Dr. Sydney Crawcour went to America and contacted several growers. On his return I started to import named cultivars and over the next few years I imported some 300 cultivars.

Propagation. African violets can be propagated by three methods, i.e. seed, division, and leaf cuttings. The quickest and most successful way to reproduce named cultivars is by leaf cutting. If you are growing named cultivars much time can be saved when making up orders by arranging them in alphabetical order, starting with propagation and following on into potting. By this method several dozen plants can be picked out in a short time.

Leaf cuttings are taken all year round from stock plants that are healthy and flowering true-to-type. I strip the plant of all mature leaves. All petioles are cut at a 45^0 angle about 1" to 1¼" from the leaf. The end of the petiole is split for about ½"; this gives a greater rooting and shoot initiation area. All cuttings are then dipped in Mancozeb for protection against decay of the leaves; cuttings are planted in a mixture of 50% peat moss and 50% Styrene foam (or perlite); enough lime is added to bring the pH to 7. Trays are placed on the top bench of the glasshouse using natural light and a temperature of 65F^0 to 70^{0}F. As soon as the cuttings are rooted, feeding is started with ¼ strength liquid fertiliser every watering.

Depending on the time of year, plantlets usually appear by 1 to 2 months and are usually ready to pot off singly into 2" pots in about 3 months. Never be in too much of a hurry to break up the clumps; let them grow to about 2" high before splitting into single plants. You should get between 4 and 8 plantlets from each leaf. I pot up all plantlets into 2" pots, irrespective of whether they have roots or not. It makes no difference for all young plantlets grow evenly.

Let the plantlets in the 2" pots grow to a good size, about 3" to 4" leaf span before potting on into 3¼" pots. This will save a lot of