PLANT TISSUE CULTURE — POSSIBLE APPLICATIONS IN THE NEW ZEALAND NURSERY INDUSTRY

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Over the past 10 years or so there has been considerable interest in the prospect of using tissue culture methods in modern plant propagation practice (1, 2, 3, 4, 5, 6, 7, 8, 9, 10). Progress in the tissue culture field has indeed been exciting and it is now possible, with some cultivars of some species, to grow complete plants from single cells and even from protoplasts (i.e. cells from which the cell wall has been removed). Such areas are still only research tools which allow us to investigate in more detail the processes of cell growth and differentiation.

There are, however, two applications of tissue culture which have already found a place in plant propagation. Firstly, in the production of virus-free (disease-free or high-health) propagating material and, secondly, in the rapid clonal multiplication of selected plants. It is about these applications that I will speak today. I will describe some of the principles and problems involved, the work we are doing at Plant Physiology Division, D.S.I.R. and, finally, some prospects for the future.

Virus-free Propagating Material. Selected desirable plant types are usually propagated vegetatively using cuttings, grafting, budding or simple plant division. Using any of these methods there is a very real risk of propagating virus-infected material since symptoms are often masked or show only seasonal expression. It has been found that many horticultural cultivars are almost, if not entirely, infected with one or more viruses; typical examples are daphne, lily, and some cultivars of chrysanthemum, carnation and rose. In order to obtain virus-free material we must, first of all, have adequate facilities for virus indexing using an electron microscope, inoculation, or grafting to indicator plants and other specialised techniques. These methods will sometimes indicate specimens apparently free of known virus diseases and these specimens can be used for subsequent propagation.

High temperatures (usually around 37-40°C) will inactivate many viruses and new shoot material on heat-treated plants will often be free of virus. The preceding paper presented by Mr. Ken Davey illustrated the use of thermotherapy in berry fruit propagation. The general principle involved appears to be to raise the temperature as high as the plant can tolerate and yet still continue growth and to maintain this temperature until virus inactivation

has occurred. Any treatment which can enhance plant survival at high temperatures is likely to increase the effectiveness of thermotherapy. Work in Australia has shown that elevated carbon dioxide concentrations enhance plant survival at high temperatures and there are good indications that reduced root temperature may also be beneficial. To determine optimum conditions for thermotherapy controlled environment facilities such as the Climate Laboratory of the Plant Physiology Division D.S.I.R. should prove invaluable.

An alternative approach to obtain virus-free plants has been shoot tip culture. In the vicinity of the apical or axillary bud meristems, virus is either absent or much reduced in concentration. If the shoot tip consisting of the meristematic dome and one or two leaf primordia is carefully dissected under a microscope and cultured under suitable conditions the resulting plant has often been found to be free of virus.

A combination of thermotherapy and meristem tip culture have been shown to be more effective than either method alone. This combination is now applied routinely in many centres in Europe and the U.S.A., for flower crops such as carnations and chrysanthemums and also for strawberries, potatoes and hops. The potential for these techniques is very promising and progress is limited principally by the time and facilities required to investigate the specific requirements for each crop. It is essential to emphasise that the virus-free status of plants produced by these methods must still be thoroughly checked by virus indexing. As mentioned above virus indexing involves the use of expensive and sophisticated instrumentation and techniques. To be certain that a virus is eliminated may take months or even years in cases where the virus expression is seasonal or where the virus has been attenuated (i.e. rendered less virulent). Often only one plant is grown from each shoot tip. Subsequent propagation may be either by traditional means or by tissue culture methods as discussed later.

To date these techniques have been used mainly in research stations and universities. Overseas some large commercial firms are establishing their own laboratories and specialist tissue culture services are also being established. In Great Britain the Nuclear Stock Association (Ornamentals) Ltd. works in close association with the Glasshouse Crops Research Institute at Littlehampton. In Australia the Victorian Plant Research Institute has a section working on ornamental plants which produces virus-indexed stock for sale to both commercial and amateur growers.

Providing that both interest and support from the industry is forthcoming we hope that arrangements can be made whereby the Plant Physiology Division, in conjunction with the Nursery Re-

search Centre at Massey University, will be able to perform a similar function. Virus-free stocks of commercially important plants could be produced and maintained for distribution to the industry.

Rapid Clonal Propagation. The term micropropagation has been proposed to cover tissue culture methods of plant propagation. When a shoot tip is cultured, changes in the hormonal balance can produce either a single rooted plantlet, a proliferation of shoots or development of undifferentiated callus. The precise conditions required for each species (and sometimes for each cultivar) must be determined by trial and error. Generally the class of hormones known as *auxins* promote root initiation at low concentrations and enhanced callus formation at higher concentrations. A second class of hormones, the *cytokinins*, tend to inhibit root formation except at very low concentrations and often induce multiple shoot formation at higher concentrations.

Alteration of development can also result from agitation of cultures. If a culture is placed in a liquid medium and rotated so that the tissue is gently tumbled root and shoot development is retarded. In orchid cultures protocorms proliferate and in chrysanthemum cultures "leafy" callus develops. If rotation is stopped or if cultures are transferred to a medium solidified with agar, shoot development proceeds rapidly.

So far these methods have only been applied to a limited number of species but in these cases the multiplication rates are impressive. For instance Earle and Langhans (4) compared conventional propagation of chrysanthemums by cuttings which can produce 30,000 plants from a single plant in one year with two methods of micropropagation. Multiple shoot formation from shoot tips could produce at least 9 million plants and a "leafy" callus system over 90 billion plants in a single year. Murashige, *et al.* (9) compared the 50 to 100-fold increase per year by division of a gerbera plant with the 1 million-fold increase attainable by tissue culture.

None of the published calculations of multiplication rate have so far included an analysis of labour and costs. Application of micropropagation methods in the nursery will depend on the value placed on the plants produced. In the orchid industry, tissue culture was readily adopted because of the extremely slow rate of traditional propagation methods and the high value of selected clones. But when plants can be readily grown from cuttings, as for example with chrysanthemums, can tissue culture grown plants compete in price with traditional cuttings? I don't know the answer, but obviously the total number of plants required would greatly influence the costings. The growing space for stock plants is saved in micropropagation but the plantlets produced are initially smaller and more fragile than traditional cuttings.

Where stocks of new cultivars are being increased for release, the time for stock buildup may be reduced from 2 years to less than a year for chrysanthemums (4). In the case of gerbera or bulb crops, such as gladioli or lilies, natural rates of increase are very slow and the rapid rates of increase that can be achieved with tissue culture (2, 9, 10) then become more attractive.

It is possible to carry out small scale tissue culture work with relatively simple facilities and equipment. Numerous orchid hobbyists have successfully mericloned their favorite orchids with only minimal capital outlay but, of course, they have no indication of the virus status of the plants they are propagating. Progressing from a backyard propagator to a full-time commercial undertaking requires considerable financial commitment, and economies of scale quickly become evident. If you look at any orchid magazine you will find advertisements for mericloning services with minimum fees and charges which become less as numbers increase. The difficult steps are the initial culture establishment and reliable virus indexing, not the subsequent subculturing steps required for large scale production.

Research at Plant Physiology Division. I am one of a group of workers concerned with aspects of plant tissue culture and genetic engineering. I began my micropropagation work by looking at methods of clonal propagation of asparagus.. Here was a process crop with considerable export potential, which showed great variability among plants grown from seed and was badly damaged by root rot infections. When I commenced this work I envisaged large scale production of elite plants. Over the past few years, however, higher density planting and direct seeding has raised plant populations from about 20,000 to more than 100,000 plants per hectare. I now see the use of tissue culture in the propagation of parent plants, selected on the basis of desirable progeny, which would be used to establish a bed for seed production.

Shoot tips approximately 1 mm long from primary or secondary laterals of asparagus spears can be rooted readily but survival of these rooted plantlets on transfer to soil is variable. Survival appears to depend on the development of crown buds and thick storage roots and the factors controlling their formation on the tissue culture plantlets are not understood at present.

In New Zealand several lily breeders have produced many fine hybrids of *Lilium auratum* x *L. speciosum* and there appears to be a good export market for these hybrids if they can be produced cheaply enough free from virus. However it appears that at least one virus known as Lily Symptomless Virus (LSV) is widespread in these hybrids. As the name implies this virus causes no visible symptoms in most cultivars, but in conjunction with either Tulip Breaking Virus or Cucumber Mosaic Virus, serious and damaging effects can be clearly seen. Work from the U.S.A., in

particular, has indicated lily buds developed on lily scale pieces in tissue culture may be free of virus. Although cultivars differed in the percentage of virus-free bulbs obtained, the results from the mid-century hybrid, 'Enchantment' were promising and this cultivar showed a rapid rate of multiplication in culture (2). With Dr. Ken Milne of Massey University, I have been checking whether bulbs grown on scale pieces are free of virus and also investigating the use of tissue culture for rapid propagation.

When bulb scales are cut up and placed in a modified Murashige and Skoog medium, many small bulblets form and later develop leaves. Based upon electron microscope examination many of these plantlets appeared to be free of rod viruses but following transfer to potting medium in the glasshouse, virus levels appear to build up rapidly in some plants. Much work remains to be done to survey the lilies in order to determine which viruses are present in New Zealand and the economic importance of these viruses. When virus-free bulbs have been produced, methods to maintain these bulbs in a virus-free condition have yet to be tested.

In the Climate Laboratory at the Plant Physiology Division we have 24 growth rooms in which temperature, humidity, light level, photoperiod, nutrient application, and carbon dioxide level can be controlled automatically. Dr. Roger Slack, Dr. Ken Milne, and myself are investigating factors which will enhance plant growth during thermotherapy. We are testing the effects of root cooling and carbon dioxide enrichment to 900 ppm, the normal level being 300 ppm. The present experiment is being carried out with chrysanthemums but subsequent experiments will include other plant types such as roses and daphne.The results of these experiments will be assessed on the basis of plant growth at temperatures near 40^{0}C, on our ability to grow shoot tips from heat-treated plants, and the presence or absence of virus after treatment.

We have already checked out micropropagation methods for chrysanthemums and carnations and are presently attempting to culture shoot tips of roses and daphne.

The Future. In this paper I have dealt with developments in two areas of the tissue culture field which I feel should have an impact on the nursery industry in the near future. There are other, perhaps more exciting, areas involving genetic engineering which may eventually lead to new plant types but to predict results from these developments requires a crystal ball.

Of the two areas I have discussed, I believe that the production of virus-free stock to be the one of most immediate concern. The plants I mentioned were mainly herbaceous because these are the plants that have been studied most widely in tissue culture.

Obviously there is need for work to be done on more woody species such as roses and daphne and, no doubt, you can think of a dozen or more plants that you would like to obtain in a virus-free condition. However, the amount of work involved for each plant is large and best prospects can perhaps be expected by exchange of both information and plant material with other workers overseas.

In New Zealand most nurseries handle a wide range of plant material and are relatively small by international standards. With the exception of orchid specialists, there are probably very few growers who could justify establishing facilities for tissue culture on economic grounds at this time. Possibly orchid specialists who are mericloning for other growers might expand their services to handle other types of plants. Other growers who are contemplating specialisation of production might well consider the potential of tissue culture for rapid clonal propagation. At the Plant Physiology Division we hope to be able to advise on techniques and problems associated with individual crops. If required, specific problems could be investigated and workshops on tissue culture methods could be organised.

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