TISSUE CULTURE AND PLANT PROPAGATION: COMING DOWN TO EARTH

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The use of tissue culture. The technique of plant tissue culture has captured the imagination of the nursery industry, the media, and the general public. To the layman, the use of aseptic methods brings an aura of science and science fiction to the seemingly mundane business of plant propagation. To the nurseryman, the very high multiplication rates which can be achieved *in vitro* are most attractive from the commercial viewpoint.

Progress in the horticultural applications of plant tissue culture has been spectacular in the last 15 years (9,11). Aseptic methods of vegetative propagation have become standard procedures for production of pathogen-free materials and for the routine multiplication of many high-value ornamentals. A large number of species can now be regenerated *in vitro* by the induction of somatic embryos or adventitious organs and the technique of micropropagation is assuming special importance in horticulture (6).

The commercial value of aseptic methods for propagation of ornamentals such as orchids and ferns is well established, and a number of other specialized applications of plant tissue culture have proved themselves in the market place However, in the present atmosphere of enthusiasm for tissue culture it is well to remember that it is just a *technique*. As with any technique in plant propagation, tissue culture is appropriate in some instances and inappropriate in others, and its use is founded upon the requirements of the crop or plant concerned.

Genetic variation in vitro: The case of fruit crops. Most deciduous and evergreen fruits are highly hererozygous outcrossers and they do not breed true from seed. The object of plant propagation in fruit crops is to perpetuate specific genotypes and high fidelity reproduction is the basic requirement of all methods of propagation. There is risk in the use of tissue culture for clonal propagation of fruit crops because genetic changes, both gross and subtle, can and do occur *in vitro*.

Many fruit cultivars arose as bud sports (somatic mutations) and are chimeric in structure (5). Rearrangements in chimeric structure during organogenesis or embryogenesis would change the nature of the cultivar. A warning is contained in the observation of Barlass and Skene (1,2) that shoots

produced *in vitro* by fragmented apices of the grapevine are of both axillary and adventitious origin. It follows that the use of this method for propagation of cultivars which are periclinal chimeras is likely to produce solid mutants and solid normal plants, as well as the original chimera

It has been assumed hitherto that micropropagation, a technique which avoids the genetically-unstable callus stage, is a high-fidelity method of plant multiplication. This assumption has yet to be proven in orchard trials. A degree of variation in the ornamentals which are produced by aseptic methods may be quite acceptable but variation in named cultivars of apple, grapes, or citrus, especially in economically-significant characters, could have serious consequences for growers and for the nurserymen who supply the trees.

Genetic variation which arises in cultivars as a result of the procedures of tissue culture, although highly undesirable for plant propagation, is of considerable interest in plant improvement. Recent research on regeneration of crop plants from cells and protoplasts has revealed the presence of much useful covert genetic variation which is not expressed in conventionally-propagated plants (10,12,13). Cabernet Sauvignon vines which were raised in this laboratory from somatic embryos (8) are exhibiting considerable variation in the field (7). These results, although preliminary, raise the possibility of greatly expanding the scope of clonal selection in fruit crops by exploiting the somatic heterogeneity of long-established asexually propagated cultivars through the use of tissue cultures.

Two contributions to this conference are concerned with the application of plant tissue cultures to the genetic improvement of fruit crops. Mr. K. Rajasekaran (University of Sydney) will review the regeneration *in vitro* of grapevine species, hybrids, and cultivars by embryogenesis and organogenesis. Included will be a description of plantlet production from isloated ovules and anthers. Mrs. Sridevy Sriskandarajah (University of Sydney) will describe methods for inducing prolific adventitious rooting *in vitro* in the apple cultivars, Jonathan, Granny Smith, and Delicious. These cultivars are very difficult to root by conventional methods. Aseptic methods of propagation of own-rooted apples (14) were developed primarily for use in a mutation-breeding project, but if the trees produced *in vitro* remain true-to-type, these methods could be of interest to fruit tree nurserymen.

Developments in conventional methods of propagation. The decision to use aseptic methods for plant propagation should be made only where it is clear that conventional meth-

ods are inadequate or unsuitable. Another contribution from the University of Sydney, by Dr. Peter Goodwin, will illustrate (i) the importance of defining the objectives in a plant propagation programme, and (ii) the selection of a mode of propagation which is appropriate to the achievement of these objectives. With the potatoes, it is possible to produce very large numbers of very small plants by use of tissue culture, but Dr. Goodwin will show that propagation by single node cuttings is a much superior method for rapid multiplication of pathogen-free material, particularly for potato improvement programmes in underdeveloped countries.

Developments in conventional methods of plant propagation, especially in the rooting of cuttings, have been overshadowed in recent years by interest in tissue culture. Clonal selection for ease of rooting, conditioning of mother plants to enhance the regenerative capacity of cuttings, and rooting *in situ* of shoots on hedged trees are all topics of considerable significance for propagators of hardy nursery stock. Up to now most research on these subjects has been with fruit trees, notably by Dr Brian Howard (4) and his colleagues at East Malling Research Station, England. The time is ripe to extend these approaches to woody ornamentals, including native Australian species A start has been made at the University of Sydney. Dr J. Clemens and Mr G.P. Lamont will present some of their results on propagation of, respectively, *Grevillea* spp. and *Boronia serrulata*, later in this Conference.

CONCLUSIONS

Tissue culture is an important new tool for the plant propagator. Rapid multiplication of scarce material, including newly-bred cultivars, and propagation of bulb plants, orchids, and ferns are areas in which aseptic techniques have much to offer. The experienced plant propagator is a craftsman and the mark of the craftsman is that he chooses the right tool for the job. Sledgehammers are not needed to crack nuts and tissue culture is not needed for most propagation tasks in general nursery work.

Tissue culture is an indispensable tool for research in genetics and morphogenesis, and for production of pathogen-free stock. It is also a vehicle for the production and exploitation of genetic variation in crop and ornamental species, and it is in this sphere that tissue culture holds greatest promise for the advancement of horticulture.

LITERATURE CITED

1 Barlass, M and K G M Skene 1980 Studies on the fragmented shoot apex of the grapevine I The regenerative capacity of leaf primordial fragments *in vitro J Exp Bot* 31 483-488

2 Barlass, M and K G M Skene 1980 Studies on the fragmented shoot apex of the grapevine II Factors affecting growth and differentiation in *vitro J Exp Bot* 31 489-495

3 Heinz, D J , M Krishnamurthi, L G Nickell and A Maretzki 1977 Cell, tissue and organ culture in sugarcane improvement In Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture Eds Reinert, J and Bajaj, Y P S Springer-Verlag, Berlin, Heidelberg, New York pp 3-17

4 Howard, B H 1979 Plant propagation In Rept East Malling Res Stn for 1978 (1979) pp 71-82

5 Janick, J and J N. Moore 1975 (Eds) Advances in Fruit Breeding. Purdue Univ Press, W Lafayette, pp 623

6 Jones, O P 1979 Propagation *in vitro* of apple trees and other woody fruit plants Methods and applications *Scientific Hort* 30 44-48

7 Mullins, M G and P R Hedberg Field performance of Cabernet Sauvignon grapevines produced from somatic embryos in *vitro Ann Rept Dept Agron Hort Sci* , Univ Sydney, 1980 8 31

8 Mullins, M G and C Srinivasan 1976 Somatic embryos and plantlets from an ancient clone of the grapevine (cv Cabernet Sauvignon) by apomixis *in vitro J Exp Bot* 27 1022-1030

9 Murashige, T 1974 Plant propagation through tissue culture *Ann Rev Plant Physiol* 25·135-166

10 Orton, T J 1980 Chromosomal variability in tissue cultures and regenerated plants of *Hordeum Theor Appl Genet* 56.101-112

11 Pierik, R L M 1979 *In vitro* culture of higher plants Kniphorst Scientific Bookshop Wageningen pp 149

12 Shepard, J F , D Bidney and E Shakir 1980 Potato protoplasts in crop improvement *Science* 28 17-24

13 Skirvin, R M 1978 Natural and induced variation in tissue culture. *Euphytica* 27 241-266

14 Sriskandarajah, S and M G Mullins 1980 Micropropagation of the Granny Smith apple Factors affecting root formation in *vitro J Hort Sci* 56 71-76

NURSERY RECORD KEEPING IN PROPAGATION

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The aim in plant propagation is to achieve a 100% result. All of us, as we have developed our techniques over the years have made many mistakes and learned many lessons. Having learned from these experiences, we have developed skills