PLANT PROPAGATION USING IN VITRO CULTURE TECHNIQUES

MARGARET E. MARSTON

Department of Agriculture and Horticulture
University of Nottingham School of Agriculture
Sutton Bonington, Loughborough, England

INTRODUCTION

The emphasis of this paper is on the production of plants under aseptic conditions. As the principles of *in vitro* culture have been reviewed by Murashige (7) in an earlier volume of the IPPS Proceedings and as technological aspects have recently been discussed by Marston (4, 5), this introduction merely serves as a reminder of some of the more important points.

Propagation in flasks or test-tubes entails the growing of a small portion of a plant, the explant, on or in an artificial nutrient medium, generally composed of mineral salts, vitamins, growth substances and sugars which have beeen dissolved in distilled water. Unless, however, precautions are taken to ensure that everything is sterilised, bacteria and fungi flourish and multiply so fast under these conditions that they swamp and kill the explants. To avoid this, glass containers, with the appropriate volume of medium measured into them, are sterilized in an autoclave or, on a small scale, in a pressure cooker and plant material is surface sterilized using a suitable disinfectant. Explants are then removed and placed in the sterilized flasks or test-tubes which are opened in clean air in a special transfer room, where only filtered air enters, or under a transfer hood, or in a cabinet with an open front where filtered air flows over the bench towards the operator. The glass vessels must then be quickly closed using a sterilized stopper or cover to prevent the entry of unwanted organisms.

Actively growing plant material needs air — there are various ways of supplying this. The explant may be placed on the medium which has been made semisolid by adding agar gel, or just over it by standing it on a bridge made of folded filter paper whose legs stand in the liquid medium. In these instances the apparatus is, of course, stationary. Alternatively, the explant may be put in the liquid medium and aeration provided by attaching the containers to revolving wheels or by standing them on a platform of a specially designed shaker. According to requirements, light or darkness is given and generally a temperature of about 25° C is maintained.

Seeds and excised embryos. About fifty years ago, Knudson (3) developed a method for the germination of seeds and growth of young seedlings of orchids on a nutrient medium. Before then, the

production of orchids from seed often resulted in failure as their embryos are not fully developed and there are virtually no food reserves. Today, excised embryos of other species may be cultured by plant breeders in cases where seeds may not develop to maturity as, for example, when crossing certain species of *Lilium* or making generic hybrids in orchids.

Small excised portions of plant material. Plant breeders may also find it helpful to use an *in vitro* culture technique as one step in a series of operations. For instance, when breeding cauliflowers one particular selected plant would only yield a limited amount of seed, but if it could be bulked up by vegetative means the amount of seed would be increased. Pow (8) of the National Vegetable Research Station, Wellesbourne has described how small pieces of curd, 3 to 7 mm in diameter, may be cultured to produce plants which when transferred to pots and later to open ground grow normally to produce curds and then seed. Similarly, the small buds which are exposed when leaves of a Brussels sprout are removed, one by one, may be excised and grown *in vitro* to give plantlets.

Small slices cut from stems of asparagus spears may also result in plants, although asparagus is much more difficult and tedious to culture than cauliflower or Brussels sprouts. Nevertheless, Takatori *et al.* (12) have developed a technique which may be used to bulk up an outstanding plant and is, in fact, being used at The John Innes Institution.

At the 18th International Horticultural Congress in Israel in 1970, M. Ziv and A.H. Halevy presented a paper on the production of corms from thin slices of flowering stems of *Gladiolus* and, later, showed flasks full of leaves and corms to those participants who visited the Faculty of Agriculture of the Hebrew University at Rehovot.

Meristem culture. Mention must be made of the large number of plants that have been cleaned up from virus diseases by removing, and culturing, the apical tip, which is usually less than 0.5 mm in height. In this way virus-tested chrysanthemums, carnations, strawberries, etc. have been obtained. It is due to Morel who was working with a *Cymbidium* cultivar infected with a mosaic virus disease that we have the present so-called method of "meristem culture of orchids". Morel (6) noticed that excised shoot tips of Cymbidium cultured *in vitro* did not continue to elongate and behave rather like a miniature cutting, but developed into a green spherule on which green protuberances then appeared. The exciting part of this discovery was that he was able to obtain from these "protocorms" either more and more new protocorms or plantlets, according to the conditions he imposed.

Since then, propagators have speculated on the future of plant propagation and have wondered what other plants might respond. Hackett (1) and Hackett and Anderson (2) have been successful, in a

somewhat similar way, with carnations and Walkey and Woolfitt (13) have developed the technique for *Nicotiana rustica* L.

Walkey and Woolfitt (14) have recently reported a somewhat different technique for the mass production of cauliflowers. Small pieces of curd are placed in a nutrient medium and are then broken up into barely visible specks by vigorously shaking. When shaking ceases plantlets develop.

Cell suspensions. Steward's work at Cornell with aseptic cultures is well-known. For example, with carrots small pieces of tissue are bored from the edible root and cultured in specially designed flasks attached to wheels which rotate about a horizontal axis. Cells slough off to give a suspension of free cells, consisting of individual cells and clumps of cells. Under certain defined conditions these become organized again, but not to form the same tissue as before. Instead, "embryoids" form $\frac{1}{M}$ these behave rather like seeds, each producing a shoot and root and developing into a plant. Steward (9) has recently discussed the implications of this technique and the refinements necessary for extended use in crop production.

Wilmar and Hellendoorn (15) have described some fundamental work with asparagus (*Asparagus officinalis* L.) and now great interest is being shown in possibly extending this technique to other species.

Pollen cultures. During the last few months, there have been reports in the press headed "pollen grain plants" or "one parent plants". Indeed, a recent issue of the New Scientist carried an article (11) which discussed the significance of haploid plants. John Innes Institution also had an exhibit, showing how progeny may be obtained from pollen, at Chelsea Flower Show in May, 1970. So far, success has been reported with tobacco, a species of *Datura*, and with rice. Sunderland (10, 11) suggests that traditional plant breeding might, one day, be transformed and lead to improved varieties of crop and horticultural plants. The technique involves the removal of immature anthers at a precise stage of development and culturing these on a defined medium. Eventually, the anthers burst open to release not pollen but many multicellular plants. They continue to grow as if they were seedlings but, having developed from pollen, carry only half the genetical information compared with the parent plants, that is they have only half the number of chromosomes. Plants so raised are sterile and propagation must be by vegetative means.

If a chromosome-doubling agent, such as the drug colchicine, is applied to a germinating seed the chromosome number may be doubled. Current work (10, 11) is establishing that if colchicine is added to the nutrient medium and the anthers left on this for a short time the plantlets ultimately produced may be diploid. Since in this case the diploid is the result of doubling a haploid, it must necessarily be homozygous, even if the original plant were heterozygous. The

important implication here is that such plants will be true breeding and may be multiplied by seed.

This method is likely to be of outstanding importance if it is successfully developed for plants which are difficult to propagate vegetatively, such as the oil, coconut and date palms. If the technique became successful for a wide range of plants the present position regarding vegetative propagation might radically change.

The future. Before we can look too far into the future the question of variation must be determined. Research workers with tissue cultures are well aware that the number of chromosomes of cells in culture may vary but if an explant develops directly into one plant, this should be a replica of the original. As far as is known, "meristem culture" of orchids reproduce the individual, but we must proceed with caution when many plants are produced following the production of callus and cell suspensions. Where there is stability, propagation using *in vitro* culture techniques offers the possibility of easy shipment from one country to another and storage of cultures in the laboratory while bulking up to produce large numbers of the most desirable cultivars.

LITERATURE CITED

1. Hackett, W.P. 1966. Application of tissue culture to plant propagation. *Proc. Inter. Plant Prop. Soc.,* 16: 88-92
2. Hackett, W.P. and Anderson, J.M. 1967. Aseptic multiplication and maintenance of differentiated carnation shoot tissue derived from shoot apices. *Proc. Amer. Soc. Hort. Sci.,* 90: 365-369
3. Knudson, L. 1922. Nonsymbiotic germination of orchid seeds. *Bot. Gaz.,* 73 (1): 1-25
4. Marston, M.E. 1969. *In vitro* culture — a technique for propagators. *Gdnrs' Chron.,* 166 (8): 15-17
5. Marston, M.E. 1969. Technology of *in vitro* culture. *Gdnrs' Chron.,* 166 (9): 14-15
6. Morel, G.M. 1964. Tissue culture — a new means of clonal propagation of orchids. *Am. Orchid Soc. Bull.,* 473-478, June, 1964
7. Murashige, T. 1966. Principles of *in vitro* culture. *Proc. Inter. Plant Prop. Soc.,* 16: 80-88
8. Pow, J.J. 1969. Clonal propagation *in vitro* from cauliflower curd. *Hort. Res.,* 9 (2): 151-152
9. Steward, F.C. 1970. Cloning cells and controlling the composition of crops. *Progress,* 54 (2): 44-51
10. Sunderland, N. and Wicks, F.M. 1969. Cultivation of haploid plants from tobacco pollen. *Nature,* Lond. 224: 1227-1229
11. Sunderland, N. 1970. Pollen plants and their significance. *New Scientist,* 47: 142-144

12. Takatori, F.H., Murashige, T. and Stillman, J.I. 1968. Vegetative propagation of *Asparagus* through tissue culture. *HortSci.*, 3 (1): 20-22
13. Walkey, D.G.A. and Woolfitt, J.M.G. 1968. Clonal multiplication of *Nicotiana rustica* L. from shoot meristems in culture. *Nature*, Lond., 220: 13461347
14. Walkey, D.G.A. and Woolfitt, J.M.G. 1970. Rapid clonal multiplication of cauliflower by shake culture. *J. Hort. Sci.*, 45: 205-206
15. Wilmar, C. and Hellendoorn, M. 1968. Growth and morphogenesis of *Asparagus* cells cultured *in vitro*. *Nature*, Lond., 217: 369-370