

4. Kao, K.N., F. Constabel, M.R. Michayluk, and O. Gamborg. 1974. Plant protoplast fusion and growth of intergeneric hybrid cells. *Planta* 120:215-227.
5. Melchers, G. and G. Labib. 1975. Somatic hybridization of plants by fusion of protoplasts. I. Selection of light resistant hybrids of "haploid" light sensitive varieties of tobacco. *Molec. Gen. Genet.* 135:277-294.
6. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
7. Smith, H.H. 1972. Plant genetic tumors. *Progr. Exp. Tumor Res.* 15:138-164.
8. Smith, H.H. 1974. Model systems for somatic cell plant genetics. *Bioscience* 24:269-276.
9. Smith, H.H. 1976. Characterization of somatic hybrid plants and further exploitation of a selective system. "Cell Genetics in Higher Plants", Szeged, Hungary (Eds. D. Dudits, G.L. Farkas and P. Maliga) pp. 163-169.
10. Smith, H.H., K.N. Kao, and N.C. Combatti. 1976. Interspecific hybridization by protoplast fusion in *Nicotiana*: confirmation and extension. *Jour. Heredity* 67:123-128.
11. Street, H.E. *Methods and Applications in Tissue Culture*. Academic Press, New York. 1972.

## SOME POTENTIALS OF PLANT CELL AND TISSUE CULTURE

AUGUST E. KEHR

*National Program Staff-ARS  
U.S. Department of Agriculture  
Beltsville, Maryland 20705*

Plant tissue and cell culture techniques have advanced to the stage where their application to commercial plant propagation is imminent, and in quite a number of cases, is in actual practice. The purpose of this paper is to review some of these techniques and point out their potential practical value for plant propagators and others. These techniques include opportunities for: 1) rapid plant multiplication; 2) eradication of viruses and other tissue-borne pathogens from "old" cultivars; 3) production of homozygous lines; 4) long-time storage of germplasm; 5) transfer of genetic information with isolated DNA, and 6) more efficient plant breeding. Somatic hybridization of protoplasts to develop parasexual plant hybrids has been discussed by Dr. Smith, and the use of shoot-tip culture for plant propagation has been covered by Mark Cunningham.

### RAPID MULTIPLICATION OF PLANTS

Techniques are now being used to produce chrysanthemums by the billions, all free of any disease or pest. Four-year-old cultures still produce normal plantlets. In addition to

mums, tissue culture of shoot-tips is being used to multiply orchids and Boston ferns. It has been reported that one American nursery is producing over 50 to 100,000 plants of Boston ferns each month from tissue cultures. Bulb crops, such as hyacinths, lilies, and narcissus, are especially amenable to propagation in large numbers by these methods. Researchers in Florida are using this method for the rapid propagation of pathogen-free clones of aroid crops such as caladiums and taro. Though far from perfect, these methods are bases for the potential use of tissue culture in propagation of many plants in almost infinite numbers.

### ERADICATION OF PLANT PATHOGENS FROM "OLD" CULTIVARS

Plants which must be propagated from vegetative tissues often become infested with pathogens. Once infested, further propagation of the plant frequently propagates the pathogen, along with the plant, particularly in the cases of viruses. Fortunately, the growing tips of most plants are free of pathogens. Hence, if one cultures extremely small meristem tips, usually 1 mm in size, plant tissue cultures can be obtained which are free of the pathogens or which can be freed from pathogens. Once such pathogen-free tissue cultures are obtained, all succeeding plants derived from such cultures are likewise pathogen-free. By such means, viruses have been eradicated from plants, such as garlic, for which no virus-free material was previously known anywhere in the world. Because garlic never produces true seed, no method was known to develop virus-free stock (before the advent of tissue culture methods). Poplar trees freed from virus grow 10 to 30% faster than virus-infected poplars.

International plant introduction stations can control and eliminate viruses and pathogens more easily in tissue culture.

Likewise, the old potato cultivar 'Irish Cobbler' developed 150 years ago, was universally infested with virus X. Today, as a result of meristem tip cultures, virus-free 'Irish Cobbler' has been developed. Such virus-free clones most commonly yield 15 to 20% more than the usual clones.

Thus, today, using tissue culture methods, viruses and other disease organisms may be eradicated from horticultural cultivars which are universally infested. These techniques are now being used for developing disease-free cultivars of potatoes, dahlias, geraniums, gladiolas, and tree species.

### PRODUCTION OF PLANTS THAT BREED TRUE FROM SEED

Most woody plants and trees are extremely variable when grown from true seed. From the standpoint of most nurserymen,



this lack of uniformity is undesirable and is usually overcome by the more expensive method of vegetative propagation.

However, true breeding clones of plants may be developed by using the method of culture of pollen grains or unfertilized ovules. Tissues from pollen grains or ovules, when their chromosome complement is restored to the same number as the original parent plant, are homozygous or true breeding. Progeny grown from seed of such plants would be essentially comparable in uniformity to vegetatively propagated plants. This method appears especially desirable to produce true breeding plants such as trees that require many years between generations.

Although this method of producing true breeding plants has great potential, this technique cannot be applied today for most nursery crops. At present, unfortunately, the primary drawback is that tissue cultures of most woody plants will not easily regenerate into plantlets. Until this bottleneck is overcome, we cannot widely use this technique to develop true breeding woody shrubs — but that day is fast approaching.

#### LONG-TIME STORAGE OF PLANT TISSUES UNDER LIQUID NITROGEN

Of particular importance to horticulture is the potential for storage of vegetative material by tissue culture methods. For example, carrot tissue has been placed in tissue culture, frozen, stored in liquid nitrogen, thawed 2 years later, and used to regenerate normal carrot plants. This technique has also been used on morning glory, sycamore, and a few other plants.

This method could be effectively used to provide germplasm banks for collection of potatoes, fruit trees, and woody plants which now must be maintained by methods of vegetative propagation that are highly expensive and risky from the standpoint of diseases. Cryogenic techniques to store plant cells, including pollen, microspores, anthers, and ovaries have great potential. World genetic sources are disappearing rapidly. Unless they are protected, cultured, or stored, they may disappear forever.

#### TRANSFER OF GENETIC EFFECTS BY ISOLATED GENES OR FRAGMENTS OF DNA

The use of tissue culture methods for transfer of genetic potential among similar plant species and among widely divergent plant species is producing practical results. As one example, Dr. F.B. Holl of the National Research Council of Canada transferred the potential to fix atmospheric nitrogen from 'Trapper' pea (*Pisum sativum* L.) to 'Afghanistan', a pea cultivar that normally lacks nitrogen-fixing capabilities.

Gene transformation is accomplished by extracting DNA from a donor plant and "feeding" it to a receptor plant. Directed higher plant modification, in much the same manner that has been done for several years with bacteria, is an exciting potential. Many valuable plant characteristics that are selected for by nurserymen have a biochemical-genetic basis that conceivably could be transmitted from donor plants to recipient plants, thereby circumventing the conventional methods of plant breeding.

## PLANT BREEDING

Plant breeding by today's conventional means is relatively slow and expensive. I am convinced that plant tissue culture methods now being developed will enable plant breeders of the future to accomplish as much in the space of a small room as now requires huge field acreage. The desired results will be accomplished in a small fraction of the time now required. A flask developed by shaker culture of plant tissue contains many millions of cells, each cell having the potential of a full grown plant. By subjecting such flasks of cells to appropriate selection pressures, plants could be selected for the desired character from a random population composed of millions of potential plants. The highly successful techniques hitherto available only to bacterial genetics are now potentially applicable to all higher plants.

Today, somatic mutations in plants are rare. They are rare because only the mutations which occur in one cell, the apical cell, can be expressed. Mutations occurring in the billions of other cells making up the plant parts are not expressed in normal plant growth. With tissue culture methods, this "locked up" variability in the somatic cells is freed and may be readily expressed. The potentials of such unlocked variability in plant improvement go beyond our imagination. These potentials will be achieved in the near future. They await only the day when we can achieve the goal of growing a full-grown plant from any cell in tissue culture, a goal which today has been achieved with relatively few plants.

In summary, these exciting developments and remarkable breakthroughs in plant cell and tissue culture offer new opportunities and new horizons for those who possess the foresight to capitalize on them. We must aggressively seek out and purposefully use these new methods, new ideas, and new products of research.