PLANT TISSUE CULTURE—WHERE IS IT GOING?

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INTRODUCTION

The last ten years have established micropropagation as a commercial production technique for horticultural crops. Zimmerman estimates that approximately half the production has been in foliage crops, a quarter in woody ornamental plants and shade trees, and the remaining quarter shared by fruits and non-woody flowering plants (7). The first part of the 1980s recorded a huge increase in both capacity and production of micropropagules. In the second half of the decade overall U.S. production has probably increased only slightly, with the largest gains in the micropropagation of woody ornamentals, shade trees, and fruit crops. The micropropagation industry appears to be in a maturation phase, experiencing consolidation and market development. Market development has focused on maintaining or improving quality, dependability, and position.

Micropropagation is usually considered a part of the biotechnology industry. Like micropropagation, the other components of the biotechnology industry such as companies specializing in genetic engineering are also in a maturation and consolidation phase. Now is an appropriate time to consider what will be necessary for stimulating future growth in micropropagation. In this paper, we will discuss possible scenarios.

PATHWAYS FOR EXPANSION

Reducing the Cost per Unit. Tissue culture is a labor intensive industry and thus has had to rely on a high $/unit return to make a profit. The recent expansion of the woody ornamental segment of the market continues this trend as the value of a shade tree micropropagule is much higher than a foliage plant micropropagule. If techniques existed to reduce the $/unit costs, then other markets could be accessed, e.g. annuals, vegetables, and forest crops. Some

attempts have been made to automate medium preparation and handling of culture vessels. This is only a small step in the right direction. The primary requirement is a fusion of biology with engineering to utilize robotics and bioreactors. Unfortunately, the current standard biological system used in commercial micropropagation is not readily adaptable to engineering solutions for labor cost reduction. The cutting of shoots in the cultures and transferring explants to fresh medium is an important phase of quality control. The growth variation in culture across the many crops currently micropropagated makes the development of an inexpensive and universal robotic system difficult. Additionally, most bioreactors are not adapted to plants; rather they have been designed for microorganisms like yeast and bacteria. Thus, large-scale commercial automation without creation of new biological technologies seems remote.

Creation of New Biological Technologies. At least four systems are being investigated; if they can be successfully applied, huge new markets could be accessed. For example, conifers, especially temperate pine, spruce, and fir, have not proven adaptable to shoot culture methods. Both the ornamental and forestry markets would welcome reliable clonal propagation techniques for these genera. A second feature is that these new biological techniques appear much more amenable to automation than standard shoot culture.

Four techniques being explored by research laboratories are:

1) *Somatic Embryogenesis*—this is the adventitious development, in sterile culture, of true (seed-like) embryos (Figure 1). Unlike zygotic embryos from seed, these would be clonal and thus exact duplicates of each other (2). A somatic embryogenic system was developed for carrots in the 1950's but the first report of an embryogenic system for conifers (pine) occurred in 1985, a recent breakthrough (6). Examples of current research are spruce, pine, monocots (corn and other grains), and vegetables (celery).

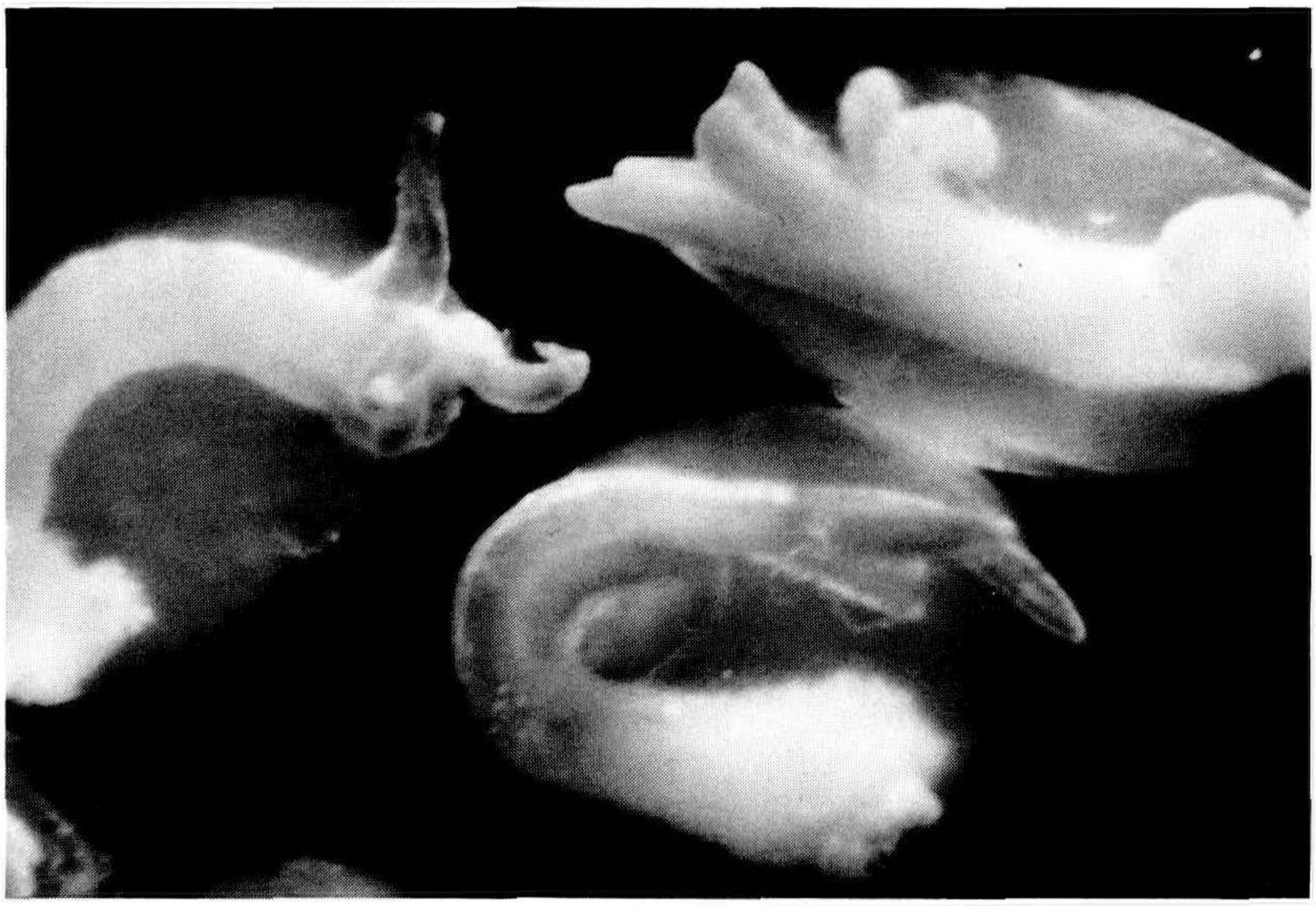

Figure 1. Spruce somatic embryos encapsulated in a gel-like substance (alginate) to prevent desiccation until plantlets are established. The hope is these could be handled much like true seed.

2) *Nodules*—this is an organogenic system where adventitious buds are developed from self-replicating, cell formations exhibiting a high degree of cellular organization (vascularization) (4). Examples of current work on nodules (Figure 2) include poplar, spruce, and some perennials.

Figure 2. A culture of nodules showing all the phases of shoot differentiation. These nodules were differentiated in a bioreactor. The most developed shoot has also formed an adventitious root. Reprinted from Plenum Publishing Corp., Lit. Cite 4.

3) *Meristemoids*—these are dense meristematic masses that can be divided into individual meristems (1). Radiata pine is an example of current research.

4) *Specialized Structures*—these take a variety of forms, potato microtubers would be an example (Figure 3). Other work is being done on bulbs and corms.

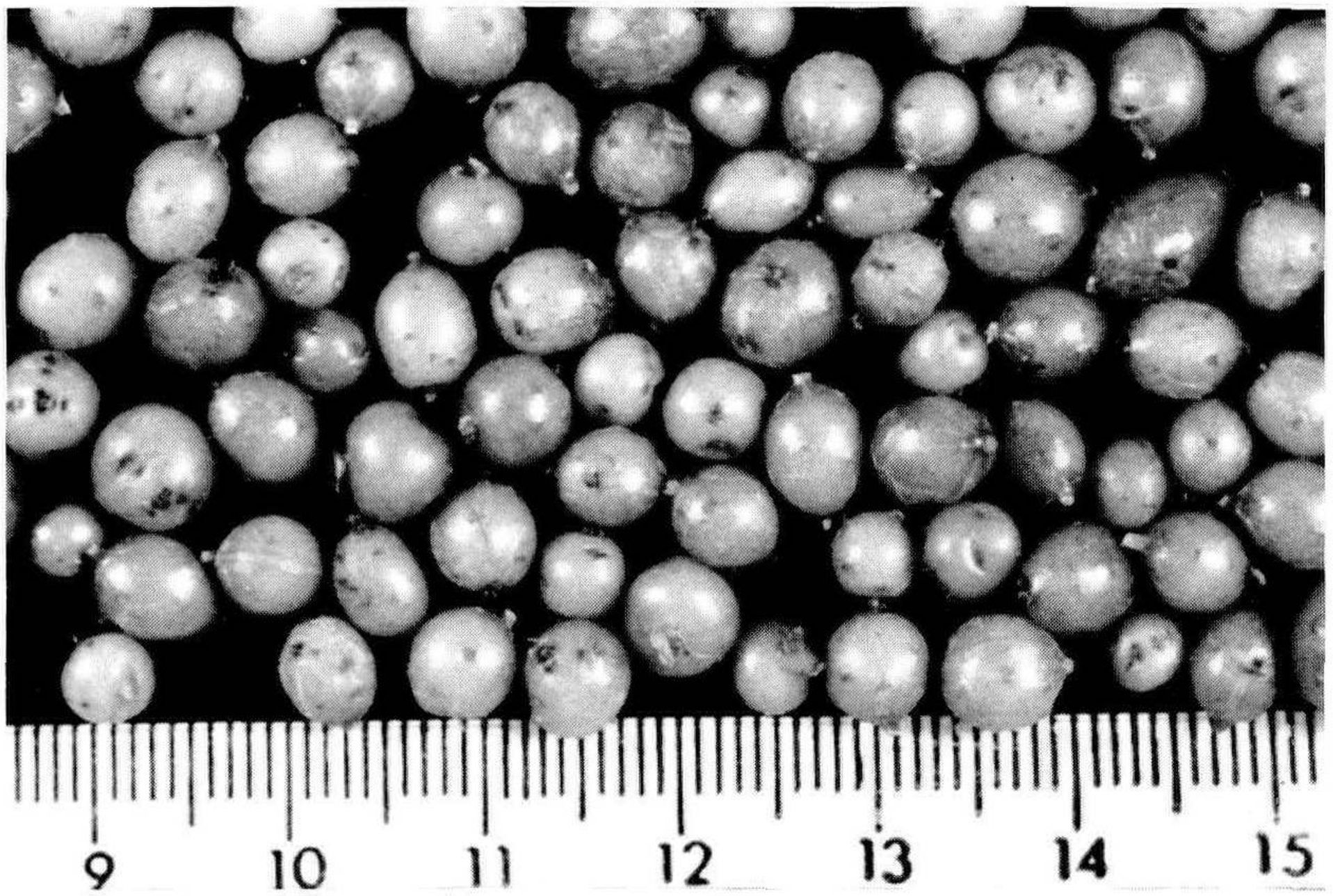

Figure 3. Potato microtubers produced in a bioreactor culture system.

All four of these biological technologies have parallel technical problems that must be understood before they become commercially viable.

1) *Difficulty in Establishing the Starting Cultures.* With embryogenesis, starting explants are often limited to embryonic material. This can be complicated by the narrow period of time in which a developing zygotic embryo is able to be induced to form somatic embryos. In spruce, for example, the time window for optimum embryogenic induction may be less than 2 weeks. Unfortunately, with woody perennials it is important to be able to work with mature material where desirable characteristics can be identified. The other three systems have been similarly recalcitrant to establish self-generating and sustaining cultures using a variety of crops.

2) *Uniform Development.* For commercial purposes, on the order of 90% of a culture must be at a uniform stage of maturity. Such uniformity has not been easily obtained.

3) *Conversion to Plants*. Regenerating uniform and vigorous plantlets has been difficult and rates low or variable. In part, the difficulty is due to a poor understanding of cellular differentiation and controlling mechanisms (4, 6).

New Products. The third and perhaps most widely publicized method for micropropagation to enter a major expansion mode is the creation of new products. This is already an important mechanism in the ornamental plant micropropagation industry where laboratories work closely with plant breeders and can assist in the rapid introduction of new clones. Genetic engineering of new plants could lead to the domination of existing markets as well as creation of new markets. Woody plants, especially trees, make ideal candidates for genetic engineering when you consider the immense obstacles facing traditional breeders; long juvenile periods, large size, complex genetics, lack of basic inheritance information, and species diversity. Two questions have to be answered: 1) Do the techniques exist to biologically engineer new woody plants, and 2) Have genes been identified for important characteristics?

Plant tissue culture is the supporting sister of this new thrust in biotechnology since most gene transfers have relied on sterile cultures as the starting material. Further, once the genetic transformation has occurred, plant regeneration, multiplication, and ex vitro establishment are required by techniques familiar to individuals involved in micropropagation (3). The feature that makes the genetic transformation approach so attractive with trees is the ability to add individual and unique characteristics to our best specimens without altering the desirable traits for which that specimen was originally selected. This would provide a tremendous advantage in plant improvement programs.

The techniques to genetically modify trees involve an assortment of laboratory procedures, mostly transferred from work with herbaceous plants. The most elegant technique is genetic transformation involving the insertion and expression of isolated genes. This has been done using biological vectors like *Agrobacterium tumefaciens* (crown gall disease) or mechanically.

The most sophisticated and successful technique currently available involves bombardment with gold or tungsten pellets. The pellets are coated with the desired genetic information and "shot" into the plant cells, where the genetic information is incorporated into the plant genome.

The second question, the identification and isolation of specific genes, is a greater obstacle. The positive aspect is that genes can be borrowed from organisms throughout the biological kingdom—bacteria, algae, animals, other plants. The negative aspect is that

characteristics determined by many genes (e.g. hardiness) are not yet readily manipulated.

It is now apparent that genetic engineering of woody plants can benefit several areas in crop improvement. We would like to briefly describe a few of the general areas we envision as beneficial:

1) *Resistance to Chemical Agents*. A number of traits are known to be controlled by specific genes, some of which have already been isolated from plant and non-plant sources. Resistance to herbicides (e.g. glyphosate) is one of these and while this may not be of special significance to ornamental growers, it may be important in the forestry and vegetable industries. Other genes, for example—tolerance to salt or heavy metals, have yet to be identified but are not outside the realm of possibility.

2) *Resistance to Biological Pests*. This is of enormous interest to the nursery community and insect resistance genes have and are continuing to be identified. In particular, the endotoxin gene in *Bacillis thuringiensis* (Bt) that conveys resistance to lepidopteran pests is available. Resistance to disease appears to be much more difficult. The use of genes encoding viral coat protein RNA was shown to limit damage caused by viruses in transformed cucumber, tomato, alfalfa, and potato. This approach could be of value with trees that are subject to viral epidemics (6). In all cases, the target pest must be chosen very carefully so that the chances of promoting the development of resistance is minimized. This is a crucial consideration with long-lived perennial plants.

3) *Change in Plant Form*. Traits like dwarfness, branch scaffold structure, and flower color all seem potential candidates for genetic engineering via genes controlling hormone or enzyme production. Compact forms of some ornamental shrubs (*Rhododendron*) have already been isolated using somaclonal techniques. Genetic manipulation of hormonal status without altering critical growth patterns will be a necessary goal.

4) *Sexual Characteristics*. At present genes to control plant fertility or sterility are not well understood but identification and isolation of these genes is a possibility. Sterile plants would eliminate the drain of the plant reserves into reproduction and in others would eliminate aesthetic problems (messy fruits) or hazards (acorn on sidewalks). Indeed, sterility may be a regulatory requirement of many genetically engineered woody plants.

5) *Environmental Stress Resistance*. Field testing of transformed strawberry plants with increased frost tolerance has already occurred. Very recent work on cotton and drought stress has pinpointed some controlling factors such as fibrous root structure.

Other possibilities for genetic modification of trees include a variety of techniques such as haploid culture, somatic hybridization

through protoplast fusion, and stimulation of mutations through callus (somaclones). These techniques will be less predictable and will require more evaluation than genetic transformants, yet can be useful adjuncts to conventional woody plant improvement programs. For example, disease resistance appears to be much more approachable using somaclonal variation.

SUMMARY

While the micropropagation industry may have given the impression of stagnation for the last few years, we believe it has, in fact, been a period of maturation. The maturation has been expressed as an improvement of quality, dependability, and market service. With the introduction of automation tooled for biological systems, new plant culture techniques, and new bio-engineered plants, the micropropagation industry is expected to be invigorated. Individuals working in the field of micropropagation are excited about the potential advances we have discussed and are waiting impatiently for these techniques to be applied commercially, especially to the field of ornamental horticulture.

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