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Tissue Culture Propagation: Where We Are, Where We're Going©

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A brief history of micropropagation will be presented, followed by the current state of the art, with suggestions for further applications that may aid the modern plant propagator. The concept of totipotency, espoused by Haberlandt and named by Steward, is the foundation upon which the fundamental practices of micropropagation have been built. The basic requirements of auxin and cytokinins delineated by Thimann and Went, and Skoog's lab, respectively, provided the impetus for research leading to the creation of micropropagation laboratories throughout the world. Commercial labs are often affiliated with nurseries or other production operations, but many, such as those producing orchids and plants for indoor use, focus entirely on micropropagation. Genetically engineered plants not only depend upon modern molecular genetics, but also require the practical application of micropropagation and plant tissue culture. Future developments will aid the determination of genetic fidelity of both micropropagated and conventionally propagated plants. Efficiencies will be achieved through use of bioreactors, flow-through photoautotrophic systems, and yet-to-be-discovered methodologies. Examples of potential applications for the practical propagator suggest that the use of micropropagation will continue to be one of many tools available to the plant propagator of the future.

INTRODUCTION

In the last half of the 20th Century, propagation of a wide range of plants by tissue culture (micropropagation) became accepted commercial practice. Following Haberlandt's (1902) description of the basic principles of plant tissue culture, great progress has been made. These principles revolved around the concept of totipotency, a term proposed by Steward (1968). The rapid strides achieved in the field of micropropagation of many plant species have been made possible by the research accomplishments of numerous dedicated scientists. Although it is impossible to list all of these accomplishments in this brief overview, some of the more significant contributions are important, both from a historical perspective and because in many cases they have become standard practices in plant propagation. Haberlandt's exploration of plant cell culture, although cells failed to divide in his experiments, stimulated efforts by scientists throughout the world to attempt to define conditions required for successful production of viable plant cell, organ, and tissue cultures, ultimately leading to the use of micropropagation for practical multiplication of economically important plants.

The primary reasons that Haberlandt's experiments did not result in multiplication of intact plants was the fact that the roles of auxin and cytokinin had yet to be discovered. The identification of indoleacetic acid (IAA) and its analogs by Thimann and Went (1934, 1935) led to culture of tomato roots by Gautheret and Nobecourt [referred to by White (1963)] in France and White in the U.S.A. (White, 1934; 1963). Practical plant micropropagation, however, was not yet achieved until

the discovery (and naming) of cytokinins, along with description of the importance of auxin: cytokinin balance for successful micropropagation, by Skoog's group at the University of Wisconsin (Skoog and Tsui, 1948; Skoog and Miller, 1957). Numerous listings of studies describing rates and choices of auxins and/or cytokinins have been published, including those of Murashige (1974), deFossard (1981), and George and Sherrington (1984).

WHERE ARE WE NOW?

As noted earlier, commercial laboratories were developed for the purpose of rapidly propagating plants via tissue culture, or micropropagation. Many commercial nurseries that started tissue culture labs later abandoned them, having discovered that they were too costly to operate, or that it was more efficient to buy micropropagated plants ("starts," liners, transplants) from a business that specialized in micropropagation. Although some large commercial labs have been established in other countries, many plants have not been imported into the U.S.A. from such labs, because of shipping costs and plant quarantine regulations, which in turn provide advantages to domestic labs in this competitive business. Table 1 provides data on the state of the micropropagation industry in 1996, as compiled by Richard Zimmerman (Zimmerman, 1996). Much change has occurred since those data were compiled, and as a result, no up-date has been published to date. A few pertinent changes in this dynamic industry can be noted as follows:

- A significant number of the commercial tissue culture labs reported by Zimmerman are no longer in business; this is especially true for labs that were listed in the "small" category.
- Several of the labs in the "large" or "extra large" category in 1996 have become even larger.
- Some of the larger labs have narrowed their focus to include fewer species or cultivars, probably because those genotypes proved to be more efficiently propagated and more profitable than those dropped from production.
- Species and cultivar focus has been an ever-changing dynamic, as plants became more or less fashionable to use in landscapes, as houseplants, or in production horticulture.
- A number of labs have been exceptionally opportunistic, obtaining rights to propagate newly bred or selected cultivars, often ones that are newly patented or protected by other legal means.
- A few examples exist where two or more nurseries pooled resources to support a micropropagation lab dedicated to propagating plants specifically for those enterprises.
- Some labs were purchased by other labs to obtain rights for propagation of specific plants or to take advantage of well-developed markets.
- In spite of shipping costs and plant quarantine barriers, sizeable off-shore production has evolved, especially in countries that have low input costs, such as labor.

Riordan (1996) published a similar compilation for European plant tissue culture laboratories, a project funded by the European Union's "COST 822" program. At that time, there were over 500 laboratories recorded, but he indicated that this

Table 1. Summary of production of micropropagated plants by commercial laboratories in the United States. Data collected March and April, 1996. Numbers are thousands of plants, based on estimates provided by laboratory managers/owners (Zimmerman, 1996).

Region	Fruit and nuts	Vegetables	Ericaceous Plants	Trees	Shrubs	Herbaceous perennial	Foliage	Orchids	Flower/ GH	Misc	Total
Eastern U.S.	1,431	3,692	373	211	850	4,388	1,200	272	6	25	12,448
Florida	10	0	0	450	2,030	1,030	47,975	278	4,900	975	57,648
W. Central U.S.	50	4,849	0	0	1,400	1,320	0	495	0	1,715	9,829
Pacific Northwest	1,970	1,130	3,620	2,240	1,990	2,080	0	0	0	1,270	14,300
Calif. & Hawaii	260	3,191	0	1,120	1,010	630	14,520	2,765	2,581	560	26,637
Total	3,721	12,862	3,993	4,021	7,280	9,448	63,695	3,810	7,487	4,545	120,862

number reflected a drop from previous years (already being influenced by growth of the industry in developing countries?). Production by these laboratories was over 179 million plants, with *Prunus* representing the most frequently reported group of plants propagated. In 2003, an up-dating of these data was done as part of the "COST" project (O'Riordan, 2003).

WHERE ARE WE GOING?

It is clear that off-shore micropropagation will continue to grow and have an increasing impact on the North American propagation industry. Micropropagation labs in developing countries are focused on exploiting an export market, in addition to serving domestic needs. The export market drives a significant part of their enterprise which generates much-needed foreign exchange. Examples of new and expanded labs abound in India, Philippines, Malaysia, Indonesia, and Thailand. Labs exist that project future production to be in excess of several million units destined for export, with production of ornamental plants that will directly compete with laboratories in the U.S.A., Canada, and Europe. Orchid production from countries such as Thailand and Malaysia has been an important part of world-wide production for several decades.

What are some likely scenarios to evolve in the future that may be of interest to plant propagators?

- Micropropagation labs will continue to produce plants that have regeneration problems (banana, potato, bamboo, and others), and newly bred or selected clonally propagated plants, at least until sufficient numbers can be built up for conventional propagation methods to take over production.
- Heat treatment of intact stock plants prior to in vitro culture, a now-standard treatment to produce plants free of specific pathogens following the pioneering work of Morel (1948, 1960), will remain important. A thorough review of this approach has been published by TenHouten, et al. (1968). In work with dahlia, use of in vitro heat treatment to obtain potentially virus-free meristems has also been investigated (Read, 1990). The meristem thus obtained is very small, but can be successfully cultured. Such in vitro heat treatment may even be used following conventional heat treatments of the stock plant, thus gaining even greater leverage in creating disease-free plants.
- **Stock Plants**. The significance of the plant from which explants are taken will become more important. Many failed attempts to reproduce the results reported from one laboratory to another can be attributed to the inability to duplicate the source material. Practical nursery propagators have long known that the stock plant affects the relative rate of adventitious root production by cuttings. A better understanding of the factors influencing stock plant growth and physiology can therefore enhance micropropagation success (Read, 1990; Read et al., 1979; Murashige, 1974).
- **Forcing Solution Technology.** The use of forcing solutions has been demonstrated to be an effective method of delivering plant-growth-regulating chemicals into potential explants of woody

plants (Read et al., 1984, 1986). This technology involves immersing the bases of cut woody stems into solutions containing 2% sucrose and 8-hydroxyquinoline citrate at the rate of 200 mg·L^{-1} (Fig. 1). Chemicals intended to affect budbreak and in vitro responses may be added to such solutions, for example, GA_3 to influence budbreak and cytokinins and/or auxins to cause organogenic response (Figs. 1 and 2; Yang and Zhang, 1987; Read and Yang, 1989). Inclusion of silver-containing compounds (silver nitrate, silver thiosulfate) has been shown to reduce time to budbreak and enhance the frequency of shoot regeneration. (Hamooh and Read, 2000).

Figure 1. Illustration of the forcing solution technique for producing softwood growth for use as explants. Solutions contain 2% sucrose and 8-hydroxyquinoline citrate at 200 mg·L^{-1} of solution. Stem bases are freshly cut and solutions replaced every 3 days.

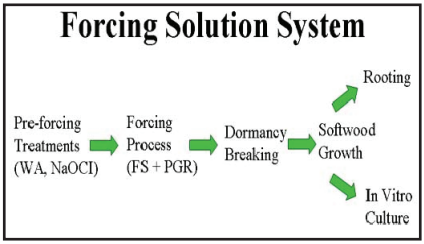

Figure 2. Schematic diagram illustrating the application of the forcing solution technique for producing explants and softwood cuttings from cut woody stems.

- **Forcing Large Stem Segments.** Use of large branches excised from juvenile portions of trees and shrubs has also been shown to be an acceptable method for obtaining explant material (Henry and Preece, 1997; Preece and Read, 2003). Softwood shoots suitable for explants to culture in vitro are obtained by laying the large stems horizontally in flats or beds of perlite under intermittent mist (Van Sambeek et al., 1997; Van Sambeek and Preece, 1999). These forcing technologies appear to offer valuable tools for the practical propagator of woody plants.
- **Improved Acclimatization.** Failure of micropropagated plantlets to adapt to "real-world" conditions of higher light and lower humidity than that experienced under in vitro conditions has long been a major challenge to the practical application of micropropagation (Read and Fellman, 1985; Brainerd and Fuchigami, 1981). Many approaches to solving this problem, such as the creation of a "controlled environment rooting facility" employed for ex vitro rooting (Read and Fellman, 1985; Nas and Read, 2003) and the photoautotrophic systems proposed by Kozai (1988, 1991).
- **Medium Development Research.** Attempts to mimic the manner by which embryos or intact plants are nourished in nature

have often been the basis for medium development research. Nas and Read (2004) reported on successful approaches to development of a suitable medium for hazelnut (*Corylus*) tissue culture based on the constituents of the hazelnut seed. In another approach, Jorge et al. (2007) have demonstrated effective use of leaf extracts of *Macuna pruriens* for its culture in vitro. It is proposed that exploration of seed constituents and plant extracts will lead to development of new and more precise media for efficient micropropagation of many important plants.

- **Applications of Biotechnology.** Enhancement of micropropagation efficiency and improved verification of genetic fidelity of clonally propagated plants are likely accomplishments as technologies advance and greater sophistication is brought to bear on problems facing scientists and practical propagators. An overview of early research involving the use of biotechnology for practical regulation of growth and development was presented by Preece (1991) and approaches to transformation of floriculture crops was reported by Robinson and Firoozabady (1993). They pointed out the potential for insertion of genes for plant hormone biosynthesis which could result in modifications in plant morphology useful in many plant systems, including applications to modern landscape designs. Use of biotechnology to control somaclonal variation may also be of great value, both to avoid undesirable off-types and to take advantage of novel characteristics and traits (Preece 1991). Random amplified polymorphic DNA (RAPD) analysis has been employed to assess clonal stability in hazelnut (Nas et al., 2004). Use of RAPD and other biotechnological approaches of a similar nature offer encouraging prospects for study of somaclonal variation and even greater control when conducting research on micropropagated plants.
- **Transformations with *Agrobacterium rhizogenes*.** Transformations with *Agrobacterium rhizogenes* offers promise to plant propagators, since it can confer the ability for cuttings to root more readily (Rugini, 1990; White and Sinkar, 1987). Cheng (2007) has provided excellent further insights into genetically engineering plants to enhance adventitious rooting of cuttings of woody plants.

In the future, it is likely that other useful plant modifications can be achieved through genetic engineering and biotechnological approaches. Reducing leaf senescence by use of a mutated ethylene gene has been accomplished by transformation of chrysanthemum (Satoh et al., 2007) and increased vase life in carnation has been commercialized by genetic modifications (Chandler, 2007). Another example reported by Ahn et al. (2007) used biolistic transformation to successfully modify an oriental lily with a CMV anti-viral gene.

The application of bioreactors for practical micropropagation, as noted by Ziv (Ziv, 1992; Ziv and Naor, 2006) will no doubt continue to be expanded and refined for plant propagation. Shohael et al. (2007) have presented an excellent review on the potential application of large-scale bioreactors. Other potentially valuable contributions of micropropagation in the future include establishment of practical produc-

tion laboratories in developing countries (Read and Chishimba, 1997), multiplication and eventual removal of endangered species from endangered status (Zachary, 1981; Szendrak et al., 1994) and possible commercialization of in vitro production of flowers (and possibly other horticultural commodities) (Ziv and Naor, 2006; Tran Thanh Van, 1973).

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