What More Can Tissue Culture Do for Us?

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For nearly 30 years plant propagators and growers around the world have utilized the benefits of plant micropropagation. Indeed, some of these nursery people even have developed their own tissue-culture laboratories to mass produce choice selections for their own nursery, while others have decided to work with independent micropropagation labs. Many areas of horticulture have benefited from this technology.

This paper is not intended to be a review of plant micropropagation. Rather, it is an examination of the relatively unexploited benefits plant tissue culture may provide our plant growing industry. In the following text we will examine two areas:

1) Producing and maintaining high health plants.
2) Producing, screening, and preserving beneficial variation.

PRODUCING AND MAINTAINING HIGH HEALTH PLANTS

A number of pathogenic disease organisms may be found systemically in plants. They may be slightly or severely debilitating to the plant. These pathogens may include: bacteria, phytoplasma, and viruses.

There are systemic bacteria that can be quite toxic to plants. Reuther (1988) used meristem culture to successfully free *Pelargonium* free of systemic *Xanthomonas* bacteria. A scheme for screening and maintaining *Pelargonium* free of this bacteria was also developed.

Citrus with a phytoplasma (mycoplasma) infection were freed using meristem apex grafting as reported by Murashige (1972). This technology might be useful in freeing many selections of *Syringa* from the ash yellows phytoplasma.

The list of plant pathogenic viruses is quite long. Several herbaceous and woody ornamental plants have been shown to be affected by this group of pathogens. Morel was one of the first researchers to use plant tissue culture techniques to rid certain orchids of virus. This landmark work was then used to develop micropropagation protocols for *Cymbidium* orchids. Although meristems may not always be free of virus, the isolation and culture of meristems has proven to be a time-tested method to help rid plants of select viruses. By combining meristem culture with heat therapy or possibly chemotherapy, a greater degree of success may be achieved for freeing the select plant of virus. Using heat therapy, meristematic tissue or shoot cultures may be grown in an environment of elevated temperatures to free shoot tissue of virus. Plant cultures may be grown in an environment that is gradually exposed to elevated temperatures, up to a maximum of nearly 42°C. A wide variety of ornamental and small fruit plants have been freed of virus using meristem culture including: *Daphne*, *Fragaria*, orchids, *Rubus*, *Vaccinium*, *Dendranthema* (syn.*Chrysanthemum*), *Dianthus*, and *Petunia*.

PRODUCING, SCREENING, AND PRESERVING BENEFICIAL VARIATION

There are several fascinating and useful tissue-culture methods to induce or capture beneficial variation. They include:

1) Somaclonal variation;

2) Gene insertion technologies;
3) Use of plant mutagens;
4) Embryo culture;
5) Production of polyploid plants.

Somaclonal Variation. Somaclonal variation is clonal variation produced using any tissue-culture method (i.e., callus, shoot, embryo, or protoplast culture). There are several spectacular ornamental plants that are somaclones. Many choice *Hosta* selections that are now commercially available are somaclonal variants. *Daphne* 'Briggs Moonlight' is a highly variegated woody plant somaclone that I selected several years ago. This showy plant is a somaclonal variant that first appeared in a solitary shoot culture of *Daphne ×burkwoodii* 'Somerset'. A number of factors affect the occurrence and degree of somaclonal variation. They include:

1) Type of tissue used to initiate cultures;
2) In vitro method of propagation;
3) Type of growth regulator used and its concentration;
4) Number of subcultures or length of time in culture;
5) Genetic stability of the stock plant.

1) *Type of Tissue Used to Initiate Cultures.* Meristematic tissue is generally much more genetically stable and homogeneous than nonmeristematic tissue. Nonmeristematic explants that have been used by researchers to initiate cultures include: callus, protoplasts, floral parts, leaves, and stems without vegetative buds. Shoots produced adventitiously from these initially nonmeristematic explant tissues are likely to be variants from the original source genotype.

2) *In Vitro Method of Propagation.* As mentioned previously, adventitious shoot production may induce a greater frequency of somaclonal variation. Using an in vitro propagation method that encourages the production of adventitious shoots will ultimately cause a greater percentage of somaclones. This is especially true with shoots that arise from callus tissue or basal stem callus in shoot cultures. Shoot cultures and nodal stem culture are the most reliable micropropagation methods of preserving the genetic integrity of a select genotype.

3) *Type of Growth Regulator Used and its Concentration.* The use of plant growth regulators may lead to a greater percentage of somaclonal variants. Auxins, such as 2,4-dichorophenoxyacetic acid (2,4-D), are often added to cultures to maintain and enhance growth of callus or suspension cultures. As a rule, the use of powerful auxins such as: 2,4-D, 4-amino-3,5,6-trichloropicolinic acid (picloram), and 4-chlorophenoxyacetic acid (4-CPA) will cause greater somaclonal variation than using auxins such as: 3-indole-acetic acid (IAA) and 3-indolebutanoic (indolebutyric) acid (IBA).

Cytokinins enhance the frequency of cell division in vitro and as a consequence may encourage somaclonal variation. The use of potent cytokinins at high concentrations can lead to accelerated rates of adventitious bud formation.

4) *Number of Subcultures or Length of Time in Culture.* The longer cultures are kept actively growing and subcultured inside the tissue-culture lab, the greater the possibility variation will occur within those cultures. This is especially true when culturing nonmeristematic tissues, such as callus or suspension cultures. Frequent reinitiation from select stock plants will often minimize variation.

5) *Genetic Stability of the Stock Plant.* The genetic stability of the stock plant has a profound influence on the potential for somaclonal variants to be formed. Some mother plants may be inherently less genetically stable than others and as a consequence much more likely to produce variants. In addition, if the mother stock is chimeral in nature, the likelihood of off-types formed due to rearrangement of the chimera is very probable.

Gene Insertion Technologies. It is not the intent of this paper to examine this subject in depth. However, the use of various delivery systems to insert genes has made it possible to increase the genetic variation of select plants using foreign genes. We have seen the development of plants with enhanced color, longevity, herbicide tolerance, and disease and pest resistance. Many challenges still exist in this area, not the least will be the political and social acceptance of genetically modified organisms (GMO) developed from these technologies. This is a very controversial issue outside the United States especially with regards to genetically engineered food, fiber, and forestry crops. Many countries will not allow imports or growing of GMO within their borders. It will be interesting to see if this will be less of an issue with genetically modified ornamental plants produced from these technologies.

Use of Plant Mutagens. The very controlled and stable nature of the in vitro environment allows for easy exposure of plant tissue to mutagens. Generally, these cultures have rapidly growing tissue that is very receptive to a variety of mutagens. The large population that can be exposed in a small space increases the likelihood that the treatment will be a success. Mutagens can be either physical or chemical in nature. Handro (1981) mentions that physical mutagens can be either electromagnetic or particle radiation in nature. Chemical mutagens are very capable of inducing variation when used with in vitro cultures. Due to their nature, chemical mutagens are very potent materials, with many being carcinogenic, and must be handled appropriately.

Embryo Culture. The culturing of embryos using the embryo rescue technique has not been used with many woody or herbaceous ornamental crops. Embryo rescue is a technique developed to save genetic variation developed from crossing of relatively unrelated plants. In wide crosses, such as between two genera, the embryos often do not develop due to post zygotic incompatibility. In this case, the zygote is produced, but not accepted by the endosperm. As a consequence, the embryo not receiving adequate nutrients, aborts or withers. Using embryo rescue the percentage of success is usually quite low. But it certainly can be a method to preserve desirable and valuable variation produced by the plant breeder.

Production of Polyploid Plants. A simple definition of a polyploid is a cell that carries more than two sets of chromosomes in its nucleus. Most plants carry two sets of chromosomes in their nucleus. For example, the China rose (*Rosa chinensis*) has two sets of seven chromosomes, or a total of 14 in every somatic cell. Like *R. chinensis*, many plants contain two sets of chromosomes and are considered to be diploid (for two sets). The number of sets of chromosomes can be increased using an appropriate agent and increases to three sets (triploid), four sets (tetraploid), six sets (hexaploid), and eight sets (octoploid) are not uncommon.

In general, compared to their diploid counterparts, polyploid plants have:

- Foliage that is thicker, wider and generally deeper in color.
- Stems that are thicker and more stout.
- Flowers that are larger, but not twice as large. Petals increase in width greater than in length. Blossoms have greater substance due to thicker petals. Color changes in the petals can be slight or dramatic.
- Fruit size may or may not be significantly larger in size.
- Polyploidy can have a profound effect on plant sterility. Treatment of sterile diploids often yield extremely fertile tetraploids. And conversely, treatment of fertile diploids often result in sterile tetraploids.

Polyploidy can be induced in vitro using a variety of antimitotic agents, including: colchicine (Blakeslee and Avery, 1937) and oryzalin or 3,5-dinitro-N4,N4-dipropylsulfanilamide. Goldy and Lyrene (1984) developed a very effective protocol to produce polyploids of blueberries (*Vaccinium* sp.) using colchicine. Polyploids were induced by exposing in vitro microshoots to low levels of colchicine. Polyploids with their thicker shoots were screened visually in the culture vessels. Polyploids were later confirmed by chromosome counts using a microscope. In addition to microscopic verification, polyploidy can be confirmed using DNA flow cytometry. This technique measures the amount of DNA present in the unknown plant relative to a standard. It can be an extremely accurate measure for cells with high ploidy levels.

Tissue culture is a valuable tool for providing more new, unusual and healthy plants to our gardens and landscapes. Although variation from micropropagation is definitely not desirable, when used correctly it may be a useful source of exciting plants for the future.

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