VARIATION WITHIN A CLONE DURING TISSUE CULTURE PROPAGATION

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Commercial plant propagators use vegetative propagation to increase the number of individuals of a superior clone. Traditionally, cuttings and grafts have been used to maintain and increase the numbers of individuals in a clone. More recently, tissue culture propagation has become economically feasible for some species. In either case, the integrity of the clone—its being ''true-to-type''—is a major objective for the propagator.

A clone is described by the International Code of Nomenclature for Cultivated Plants as a ''genetically uniform assemblage of individuals derived from a single individual.'' However, being ''genetically uniform'' does not necessarily mean that variation cannot occur within a clone. Tissue culture propagation represents a new challenge to the propagator to maintain the integrity of the individual clone. New challenges, but challenges from the same influences that induce clonal variation during standard vegetative propagation. Variation within a clone occurs by genetic, non-genetic, or epigenetic mechanisms (1). However, the frequency of clonal variation can be higher during tissue culture propagation compared to other types of vegetative propagation, requiring the propagator to be particularly aware of the appearance of ''off types''.

Understanding the mechanisms underlying clonal variation during tissue culture requires an understanding of the patterns of shoot formation found *in vitro*. Shoots form *in vitro* from axillary meristems or from newly-formed adventitious meristems. In general, the lowest frequency of variation is found in shoots formed from axillary meristems. Adventitious meristems can form directly from pre-existing cells in the stem, petiole, and leaf or indirectly from newly-formed callus cells. Indirect adventitious meristem formation from callus cells exhibits the highest frequency of variation.

Genetic variation in tissue culture (usually termed somaclonal variation) has been frequently documented and the subject has been recently reviewed (5). This type of variation is most commonly derived from meristems formed from callus cultures, but can occur during any adventitious shoot formation. Somaclonal variation is undesirable for clonal propagation, but it has recently been

exploited for the production of novel mutants. Generally, the frequency of mutation increases with an increasing number of subcultures. Variant individuals developed from callus cultures have been selected for increased disease resistance, tolerance to stress (salt, herbicides, etc.) and novel growth habit. Several new clones have been developed through somaclonal variation, particularly in vegetatively-propagated plants, including potato, sugar cane, and scented geranium (5). Besides the natural mutations that can be found during adventitious shoot formation, induced mutations using radiation or chemicals have also been used to introduce novel genetic variation into plants during tissue culture. More recently, foreign DNA has been introduced into somatic cells using *Agrobacterium* or a variety of physical means. As more desirable single gene traits are identified, DNA transfer will become an increasingly important source of genetic variation through tissue culture.

Non-genetic forms of variation are represented by chimeras, disease infected clones, and physiological variants. A chimera can be viewed as a genetic mosaic with the shoot meristem composed of a mixed population of genetically different cells. A shoot meristem has three distinct layers termed the L I, L II, and L III layers. A chimera occurs when one of these layers or a portion of a single layer contains cells with a different genetic makeup compared to the parent plant.

Chimeras, especially chimeras responsible for variegation, present a significant problem for propagators using tissue culture. Chimeral separation in tissue culture is very common and can be anticipated in any tissue culture system that utilizes adventitious meristem formation. The practical implications to the plant propagator are illustrated by the results from the tissue culture propagation of a variegated hosta (*Hosta sieboldiana* 'Francis Williams') (7). During tissue culture, chimeral separation occurred either due to the new meristem forming from a single histogen cell layer or from an unstable chimeral rearrangement of cells between histogen layers. The result was a chimeral rearrangement resulting in propagules that were all green, all gold, or plants with a green and gold chimeral makeup similar to the original clone. The best chance to maintain the integrity of a variegated clone during tissue culture is to use shoots derived from axillary meristems that maintain the chimeral histogen arrangement.

Disease infection usually creates a disease problem that is commonly detrimental and easily observable. However, pathogen infection can be masked, where the only visible symptoms are a reduction in growth rate or vigor. A viral infection can also induce a desirable variegation of leaves and flower petals very similar to

chimeras. Common examples are represented by tulip flower "breaks" and the variegated foliage form of flowering maple (*Abutilon pictum* 'Thompsonii'). Disease infection causes a change in the phenotype of the infected individual, but the genetic makeup of the plant has not been permanently altered and will revert to the original phenotype after the elimination of the pathogen. Disease infected clones propagate "true-to-type" from cuttings as long as the disease organism is present.

In variegated geraniums, Cassells and Minas (2) have shown that individuals behave differently in tissue culture depending whether the variegation is the result of a chimera or a virus infection. Chimeral geraniums responded as anticipated in tissue culture forming variegated plants from axillary meristems and undergoing chimeral separation from adventitious meristems forming both variegated and solid color individuals. With virus infected geraniums, the exact opposite was observed. Axillary meristems, which did not contain the virus, were all solid-colored. Shoots formed from adventitious meristems all contained the virus and were variegated. Obviously, the propagator must be aware of the source of variegation to determine an appropriate tissue culture protocol for the production scheme of variegated clones.

Physiological or phenotypic variation is a common form of non-genetic variation. There are numerous examples where plant growth and development (growth habit, flower size, flower color, etc.) are influenced by the environment. However, this type of variation is not a persistent change and the variant will revert to a "true-to-type" individual under a similar growing environment to other members of the clone.

In contrast to physiological variation, epigenetic variation represents a relatively stable change in the appearance of an individual in a clone regardless of the environment. Epigenetic variation is a stable change in the gene expression of an individual, not a change or mutation in existing genes. Epigenetic variants conform to the International Code of Nomenclature's definition of a clone by being "genetically uniform". However, these individuals can be extremely different in their appearance and this difference remains "true-to-type" during vegetative propagation. The most familiar form of epigenetic variation is represented by the juvenility phenomenon (3). Juvenile plants most commonly differ from the mature phase of the clone by growth habit, leaf shape, ease of root initiation from cuttings, and the ability to flower under normally inductive conditions.

Differences in the growth and development of tissue culture versus conventionally propagated individuals have been observed in the field (4). It is difficult to determine if these differences are

examples of physiological or epigenetic variation. The situation with blueberry tissue culture is a good illustration. Tissue-cultured blueberry plants showed a reduction in apical dominance and increased basal branching compared to conventionally propagated plants (8). It was suggested that there was a possible cytokinin carryover from the tissue culture environment. It is well-documented that applied cytokinin can increase basal branching in many woody plants, but these characteristics could also suggest a juvenile growth habit. Lyrene (6), using ease of root formation as an indicator of juvenility, observed that stem cuttings were easier to root from field-grown tissue culture propagated plants compared to conventionally propagated individuals of blueberry. He proposed that an epigenetic phase change was induced during tissue culture. It will remain difficult to ascribe these changes observed in growth and development in tissue culture to either a physiological or epigenetic variation until a more direct biochemical or molecular marker becomes available to describe epigenetic variants. Regardless, it remains for the plant propagator to determine if this change in growth and development significantly alters the clonal phenotype of the tissue culture plants making them an unmarketable product.

Tissue culture is another tool the plant propagator has to vegetatively propagate clonal plant material. Tissue culture is very useful for slow-to-multiply or difficult-to-propagate plants. However, it may not be appropriate in all situations. As with other methods of vegetative propagation, the burden of propagating "true-to-type" individuals remains with the propagator. Care should be taken to select a proper source plant and vigilant observations must be made both in the lab and in the field to prevent clonal variation of the propagated product.

LITERATURE CITED

1 Cassells, A. C. 1985. Genetic, epigenetic, and non-genetic variation in tissue culture derived plants, p 111-120 In: A. Schafer-Menuhr (ed). Advances in Agricultural Biotechnology In vitro techniques. Propagation and long term storage Martinus Nijhoff, Dordrecht

2. Cassells, A C. and G. Minas. 1983 Beneficially-infected and chimeral *Pelargonium.* implications for micropropagation by meristem and explant culture. *Acta Hort.* 131.187-297.

3 Hackett, W P 1985 Juvenility, maturation, and rejuvenation in woody plants. *Hort Rev* 7.109-155

4 Hartmann, H T , D. E Kester, and F. T Davies, Jr 1990. Plant Propagation Principles and Practices, 5th Ed Prentice Hall, New York.

5. Larkin, P J and W R Snowcroft. 1981 Somaclonal variation—a novel source of variability from cell cultures for plant improvement *Theor. Appl Genet* 60 197-214.

6 Lyrene, P M 1981 Juvenility and production of fast-rooting cuttings from blueberry shoot cultures *Jour Amer. Soc Hort Sci.* 106(3):396-398

7 Meyer, M M , Jr. 1980 In vitro propagation of *Hosta sieboldiana. HortScience* 15(6) 737-738

8 Read, P. E , D. K. Wildung, C A. Hartley, and J. M Sandahl 1989 Field performance of in vitro-propagated 'Northblue' blueberries *Acta Hort* 241.191-194.