VARIATION IN TISSUE CULTURE PROPAGATED PLANTS

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Variant or off-type plants resulting from tissue culture propagation are a concern of growers interested in using micropropagated plants (1, 2). This variation resulting from tissue culture has been reviewed comprehensively recently by several authors (4, 7). To determine how serious a problem such variant plants might be, it is useful to review the methods available for micropropagation and the sources of potential variation.

Propagation methods. Micropropagated plants can be produced from axillary or adventitious shoots or from somatic embryos. Production from axillary shoots is the most common method used and involves using plant growth regulators called cytokinins to prematurely stimulate the growth of buds in the axils of leaves on shoots growing in tissue-culture. Shoots produced from axillary buds are most likely to maintain genotypic and phenotypic fidelity. This method is used for a wide range of plants, such as *Dieffenbachia, Syngonium, Rhododendron, Acer*, and *Rubus*, for example.

Adventitious shoots can be produced either from pre-existing tissues (e.g. epidermis) or organs (e.g. leaves) or from callus (undifferentiated cells). Since adventitious shoots normally arise from single cells (7), chimeral forms of plants (e.g. leaf variegation, flower color) will not be phenotypically true-to-type. For this reason and for other concerns about genotypic and phenotypic fidelity, use of adventitious shoots for micropropagation is not common. However, such shoots are used for certain plants, for example African violet (*Saintpaulia*).

Somatic embryos develop from vegetative cells and thus should have a genotype identical to the plant from which they are derived. These embryos can be grown into plants just as are the zygotic embryos in normal seeds. Methods of using somatic embryos in artificial seeds are under development. Somatic embryos are not used yet for commercial micropropagation, but propagation systems based on somatic embryogenesis are being developed for a number of crops, e.g. daylily (*Hemerocallis*) (5). A major advantage of somatic embryos is the low unit cost so that they could be used both for crops that are normally seed-propagated because of cost considerations as well as for other crops. Thus, use of

somatic embyros is anticipated for vegetables, annual flowers, agronomic crops, and forest trees. Other crops will also have the potential for propagation from somatic embryos. For example, Preil (6) has worked extensively with poinsettias grown from somatic embryos. These plants grown are uniform, but differ somewhat from the parent cultivar in certain characteristics.

Types of variation. Variation resulting from tissue culture can be classified as genetic, epigenetic, or physiological. Genetic variation is that which arises as the result of a change in the genes of a plant, i.e. a mutation (3). Genetically different plants originating in tissue culture from somatic cells are called somaclonal variants. Such changes may be expressed as a difference in growth habit (dwarfing), variegation in leaves, difference in flower color, etc. The variation will persist through vegetative propagation and the variant characteristic will be transmitted to seedling progeny.

Epigenetic variation does not involve a change in the genetic makeup of the plant, but is the result of a change in expression of a gene or genes (3). Different morphological forms representing juvenile and mature states, e.g. in *Hedera helix*, are an example of epigenetic changes in plant development. Such changes are stable through vegetative propagation, although the stability will vary depending upon the species or cultivar, but are not transmitted through seed.

In micropropagated plants, an additional type of variation, physiological, can occur that tends to be transient in nature (3). Typically these changes, e.g. increased branching and vigor, disappear with time or following another cycle of conventional vegetative propagation. The basis for these changes is still unknown, but they may be epigenetic.

Control of variation. Several steps can be taken to reduce the amount of variation occurring in tissue culture. The first is to micropropagate only those cultivars that are genetically stable. Thus, many chimeral forms are not suitable for micropropagation, but the decision on whether a chimeral form is suitable must be made on a case by case basis. Some other genotypes will also be found through testing to be more likely to produce variants after micropropagation and should be cultured and evaluated with care. For example, highly polyploid types are usually unstable.

Since variation can increase with increasing time in culture, it is advisable to establish fresh material of a given cultivar in culture at regular intervals. These intervals probably should not be longer than three years and should be less for some crops.

The micropropagation system least likely to produce variants should be used. Thus, axillary shoot proliferation is preferable to adventitious shoot proliferation unless extensive trials have shown that plants growing from adventitious shoots are uniform, stable,

and phenotypically identical to the source cultivar. Use of plants produced by somatic embryogenesis will require extensive testing and is unlikely to be of importance for the nursery industry for some years to come.

Selection of the appropriate explants when subculturing clumps of shoots is significant, because callus tissue often develops at the base of a shoot clump. Adventitious shoots may arise from this callus so that it is safer to transfer only the upper portions of each shoot. This method reduces the rate of proliferation, however.

Composition of the medium used to grow the cultures *in vitro* must also be evaluated carefully, especially the type and concentration of growth regulators used. Cytokinins are of special concern because supra-optimal concentrations can stimulate adventitious bud formation and may contribute to some of the epigenetic variation seen in micropropagated plants.

An essential feature of any well-designed micropropagation program is the long-term evaluation of micropropagated plants. Naturally, with woody plants, this will involve considerable time, effort and expense, but ultimately it must be done to confirm that the micropropagated plants are phenotypically identical to the original clone. When this step is slighted or skipped, expensive problems can develop, as has been demonstrated already with several crops.

Furthermore, a testing program ensures that the identity of the micropropagated plants can be checked to ensure that no mislabeling occurred at any stage of the culture procedures.

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