PRODUCTION SHOOT-TIP CULTURE (MICRO-PROPAGATION)

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What does tissue culture really mean to the propagator? Tissue culture is a procedure in which an excised plant part is placed on an appropriate sterile culture medium, managed under controlled laboratory conditions, grown in a special disease-free environment, and ultimately develops into a new plant.

In starting a tissue culture laboratory, there should be sound objectives to justify the expense of setting up the costly facility and delegating trained personnel for its year 'round operation. Also, one must set up goals and reasons for operating the laboratory. Two important reasons for investing time and money into this type of project are:

I. Recovery of Disease-free plants. This is probably the most important reason for getting into tissue culture. By removing virus, bacterial and fungus pathogens which invade the plants, chances are vastly greater in restoring the plant or cultivar to its normal vigor and state of productivity. I must point out that by tissue culturing a plant, we are not assured of making it 100% disease-free. It is necessary to go through a complex indexing program before the plant is assured to be completely disease-free, but we find almost complete freedom from disease problems through the first culturing, and virtually none in successive generations of culture. We, therefore, try to culture basic stock once each year.

II. Rapid Clonal Multiplication. By using tissue culture techniques, one is able to rapidly increase plant production. This is very important to the grower who has plants that are slow and hard to propagate. Tissue culture also enables the grower to introduce new cultivars into the trade much sooner via this rapid means of multiplication. Another factor of tremendous economic significance is our ability to micro-propagate some plants, such as *Hosta* and *Gypsophila* every month of the year, as opposed to seasonal propagation with many of our herbaceous plants.

Our laboratory facility consists of a medium size room (16′ × 24′) divided into four smaller rooms: transfer room, chemical mixing room, growing room, and office record-keeping area.

Light, temperature, and nutrient media are the factors which control the proliferation of plant growth.

LIGHT: For shoot growth, low light intensity — around 100 ft-c (100 lux) — is used. High light intensity of 1000 ft-c (10,000 lux) is used for rooting. We use Gro-Lux lights, both wide spectrum and regular, controlled for a daylength of 16 hours. This daylength requirement can be varied if a given plant requires a different light period.

TEMPERATURE: The laboratory temperature is maintained at 70°F. The lights, however, create heat and, therefore, the laboratory requires air-conditioning for correct temperature control. The system is electrically heated, and all air is electronically filtered. So far we haven't varied the temperature but some plants might require other temperatures.

NUTRIENT MEDIA: The chemical nutrient medium consists of a modified Murashige-Skoog, using inorganic nutrients, vitamins, auxins, cytokinins, and agar. By modifying the auxin and cytokinin levels, shoots or roots are initiated. We prefer media which induce multiple shoots, thus giving us more rapid increase.

Stage I: The first step is the establishment of aseptic culture. Whatever plant part is used, certain procedures must be used in cleaning the plant. A Clorox solution (1-10 ratio) can be used for the washing cycle. The length of time for the washing can vary from 2 to 30 minutes depending upon the plant species. After washing comes rinsing, usually 2 times in sterile distilled water, then the shoot-tip or other plant part is placed in the test tube. In chrysanthemum and gypsophila, the shoot-tip is deep in the leaf-covered, growing point, so little contamination occurs. In fact, under our system, contamination has never been a problem.

Chrysanthemum × *morifolium* (garden mums), *Gypsophila paniculata* 'Bristol Fairy', *Ajuga* 'Burgundy Glow', *Phlox subulata* (creeping phlox) and *Dicentra spectabilis* (bleeding heart) are established by removing the shoot-tip from the growing point of the plant. In *Hosta plantaginea* 'Grandiflora' and *H. decorata* 'Thomas Hogg', the buds that develop below the soil surface are used in initially establishing the culture in stage I. The hostas are particularly hard to clean up for culture because the source of the original start comes from dormant buds which are under the soil surface. Persistent scrubbing in the Clorox solution is required prior to removal. We prefer starting with the dormant bud than with the juvenile bloom shoot, which some technicians use.

Stage II: After the plant has been established in the test tube and has grown for approximately one month, the next step involves separating the shoots, or dividing the plant, for further multiplication. At this same time, some shoots can also be

placed on the appropriate nutrient medium to promote roots.

Stage III: Two or three weeks after the plant is placed on the rooting medium, roots form. This is the step in which transition is made from the laboratory environment — the operation in which rooted plants are transferred from test tubes to the greenhouse. In order for the plant to adapt to its new environment (varying temperatures, moving air, and higher light intensities), the plant must go thru a re-adjustment period. This growing-on stage, in a sense, is similar to transplanting tender, fragile seedlings. Our procedure is to dibble them into small peat pots and place them under mist for 1 week or less; the survival rate is 100%. As plants quickly develop, they are shifted up to larger containers, or planted into stock beds for normal propagation procedures.

Micro-propagation (tissue culture) is a tool the modern plant propagator should not fail to use for producing plants, both for economic reason and for product-improvement. It is a must today. Not all plants respond to this system, but for those which do, we feel should be managed via tissue culture. As research continues there is promise many of the woody species will also be produced via tissue culture.

TISSUE CULTURE PROPAGATION OF DAYLILIES

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The Liliaceae is composed of many herbaceous perennial plants. It includes lilies, iris and other commercially important ornamentals. One flowering ornamental group, the *Hemerocallis*, known in the trade as daylilies, have been popular as herbaceous perennials for many years. The standard method of propagation is through division (1,2,4,6). This procedure, while it yields plants that are true-to-type, is a slow method of asexual propagation. The slow nature of propagation by division results in the better new cultivars never reaching the commercial market but remaining in collector and breeder gardens. We now describe a tissue culture method for rapid clonal multiplication of daylilies. When properly employed, the method yeilds uniform plants without genetic deviation.