

sense. That is to say, unless you have dedicated personnel who do not mind tedious, repetitious, daily work, most laboratories will have turn-over problems. I have found that if the owner knows micropropagation in theory and in actual practices, he will not necessarily find himself in a position of having to hire overqualified personnel. He is able to hire less qualified persons who can be trained, and thus he may be able to lessen turn-over problems. Also, once a laboratory is firmly established and can pay well, including provision of worker incentives, then everything that the laboratory does and seeks to do, including research can challenge the employees to good performance. Detail work, in the perspective of learning and achieving, can become an adventure.

In closing, I would like to say that with all of the trials and tribulations I have experienced in starting and operating a commercial tissue culture laboratory, I can testify to pleasures that are denied to those who are not willing to venture into a new enterprise. The pleasure of learning is, to me, undeniable — a very great personal value.

HOW CAN WE GET MICROCUTTINGS OUT OF THE LAB?

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The current use of in vitro techniques for rapid clonal propagation is the most advanced area of plant tissue culture. According to Murashige, by 1978 there were at least 100 facilities engaged in commercially propagating a variety of plants through tissue culture. Nearly all of them arose within the previous 5 years, and several new ones continue to emerge each year (6). It is difficult to obtain accurate production numbers from these commercial micropropagation laboratories. Conservative annual United States estimates range from 14 million units (flower crops, ornamental foliage and ferns) to 55 million (all agronomic crops).

Commercial propagators using plant tissue culture techniques produce plants through adventitious shoots and/or enhanced axillary branching pathways. The in vitro propagation steps are as follows:

- Step I. Establishment of an aseptic tissue culture of a plant.
- Step II. Rapid numerical increase of organs or other structures.

- Step III. Preparation of propagule for successful transfer to soil, involving rooting of the shoot cuttings, hardening of plants, and initiating the change from the heterotrophic to the autotrophic state.
- Step IV. Establishment in soil of a tissue culture derived plants, either after undergoing a Stage III pre-transplant treatment, or in certain species, after direct transfer of plants from Stage II into soil (5).

The tissue culture process is expensive and requires the right combination of facilities, materials, personnel, and plants. Labor has been shown to be the major cost factor in the production of tissue culture grown plants. Anderson and Meagher (1) in 1978 reported that labor, excluding supervisory salaries, was 30.8% of the cost of producing a crop of lilies and that Stage III labor use was 75% of the total labor cost. In 1977, Anderson, Meagher and Nelson (2) reported that wages, supervisory and administrative salaries amount to 65.9% of the total cost of producing a crop of *Brassica oleracea* L. Therefore changing the tissue culture steps to reduce labor is a direct way to reduce the cost of production.

Donnan (3) in 1978 suggested the elimination of a laboratory rooting phase to reduce production costs. He determined that it is possible to take Stage II-3 cultures of *Begonia* × *rex-cultorum* 'Merry Christmas' to the greenhouse, separate out at least 10 plants per culture and plant them. By eliminating Stage III a reduction of 56% in labor cost is realized. Similarly shoots of *Ficus* may be cut from cultures in the laboratory and stuck directly into the rooting medium in the greenhouse. Plants without roots that do not have to be removed from a rooting container and medium can be handled at a faster rate when compared to the conventional method. Additional savings would come from the elimination of Stage III media cost, media preparation labor cost, and the overhead cost per plant when grown in the culture room. Although the elimination of a laboratory rooting Stage III makes costs more reasonable, there remains a great need for determining the specific environmental requirements for successful re-establishment of propagules in the greenhouse.

It has been clearly established that tissue culture derived plants differ considerably from conventionally produced seedlings. Grout and Aston (4) reported that tissue culture produced cauliflower plants have a modified leaf anatomy, where the palisade mesophyll tissue is absent or minimal. In addition, poor vascular connections occur between the root and shoot in vitro and little epicuticular wax is present. Just as in Stage III, the successful re-establishment of unrooted shoots in

the greenhouse requires rooting shoots which have developed in Stage II, hardening the plants to moisture stress, and converting the plants to a more autotrophic form of growth. Detailed studies on the environmental conditions favorable to re-establishment of unrooted shoots in the greenhouse are few.

Donnan (3) reported on rooting uniform unrooted shoots of *Saintpaulia ionantha* 'Marshmallow', *Begonia* × *rex-cultorum* 'Merry Christmas', and *Nephrolepis exaltata* 'Bostoniensis' from Stage II tissue cultures. These shoots were planted into four greenhouse transplant media in seed trays. Some trays were covered with a clear plastic top and placed on a greenhouse bench while others were placed uncovered in a polyethylene covered tent. He indicated that conventional mist systems raise the relative humidity of the atmosphere surrounding a plant. In such situations the moisture content of the growing medium becomes excessive and the CO₂ and O₂ levels become abnormal with subsequent inhibition of root development. In contrast a tent-like structure can maintain high relative humidity without excessive overhead misting required. His results also showed the great variation in growth of unrooted tissue culture grown plants in the greenhouse environment due to rooting medium. A commercially prepared soilless mix, Redi-Earth, from W.R. Grace Co. was better than sand, peat plus styrofoam bead mix, and Plant-Gar plus peat plus styrofoam bead mix for *Saintpaulia* and *Begonia*, whereas *Nephrolepis* shoots did not adapt well to any of the treatments. Plants in tents did better than individually covered trays.

In 1981 a review of synthetic foam growing media from The Smithers Company was initiated by the author to determine whether existing foam products could satisfy both in vitro and in vivo rooting needs. Foam slabs could replace agar as the physical support system. Foam could be saturated with root inducing nutrient formulations, then flushed to remove the sugar containing medium before transferring rooted plants in foam slabs to the greenhouse. A systems approach to getting plants out of the laboratory could be envisioned that would allow for the efficient handling of plant materials as groups of plants instead of individuals. Rooting shoots from Stage II cultures directly in the greenhouse might be feasible for more difficult-to-root plants if a foam rooting medium had the proper balance of open and closed cells which governs saturation and drainage. This gives a proper moisture level for improved rooting.

Foam samples, ¾" thick were cut from slabs of test materials listed in Table 1 to fit 1 qt Mason jars or Magenta AG7 containers. Distilled water was added to moisten the foam. Vessels containing the foam samples were autoclaved at 121°C,

15 p.s.i., for 15 minutes to determine physical changes. Results are reported in Table 1.

Table 1. Oasis® Grower Product Review.

| Product name | Withstands autoclaving | Leaching required | Roots penetrate foam medium, emerge from foam |
|-----------------------------------|------------------------|-------------------|---|
| Rootcubes® Growing Medium | Yes | Yes | Yes |
| Horticubes® Growing Medium | Yes | No | Yes |
| Horti III | | No | No |
| Mod. Rootcubes® Growing Medium | Yes | No | No |

Products were evaluated for practical use in vitro. Oasis® Rootcubes® Growing Medium used for the rooting of poinsettia cuttings and other vegetatively propagated flowering plants is sterile, economical, and easy to handle in a greenhouse situation. However, the material must be leached by flushing with water to remove residual neutralizing salts from manufacturing. These can cause stem damage to sodium sensitive plants. Foam units could not be economically dried after the leaching step, which makes in vitro use impossible. Known nutrient medium composition could not be added to a wet foam medium. A no-leach phenolic foam was developed by The Smithers Company for actual rooting tests.

Stage II stock cultures of *Philodendron × wend-imbe*, an easy-to-root species, were obtained from Phyto-tech Lab. in Torrance, California. Preliminary screening of this no-leach foam against traditional Stage III agar rooting medium was done by placing ¾ in thick slabs of foam in 1 qt Mason jars with 100 ml of liquid rooting solution. Jars were covered with aluminum foil and sterilized by autoclaving at 121°C for 15 minutes. After cooling uniform ¾ in shoots were aseptically taken from Stage II stock cultures and inserted in the foam or agar medium, 24 shoots per jar. Planted jars were placed in the culture room environment of 27°C, 16 hour photoperiod and 3000 lux. Although shoots began to root into the foam slabs within 7 days, no roots emerged from the no-leach foam. Therefore transplanting to greenhouse was not possible.

Additional tests were run using 4 in × 4 in Flow Lab. PlantCon containers, but not under controlled aseptic conditions. Slabs of foam were cut to fit the vessel; distilled water or 50 ppm nitrogen solution (Peters 20-20-20) was added before inserting the *Philodendron* shoots. Shoots were removed from Stage II stock cultures and rinsed in distilled water to remove any agar medium. Each PlantCon contained 36 shoots. Four ⅛ in holes were made in the PlantCon lid to allow for air circu-

lation and to reduce the condensate which collected in the container while in the culture room environment. Again, while shoots began to root into the foam slabs, no roots emerged from the no-leach foam units.

Similarly, shoots placed in Horti III foam slabs in PlantCon containers with distilled water began to root into the foam but no roots emerged. Cubes were cut open to expose the roots which were brown and clubby. Foliage color became increasingly yellow in the distilled water treatments. Tests done on the water drained from the test foam indicated a drop in pH from 6.4 to 2.68. Dilute sodium hydroxide was added to the distilled water until the starting solution level was 10.12 but after a 15 hour soaking period the water drained from the foam was 3.2. No further tests were made.

The last foam growing medium to be reviewed was Oasis® Horticubes® Growing Medium. This product is used for vegetable seed germination, N.F.T. and/other hydroponic growing systems as well as rooting of foliage cuttings in commercial propagation greenhouses. Rooting tests were conducted with Stage II *Philodendron* shoots in PlantCon containers as described above. Shoots began to root into the foam within 7 days and were ready for transplanting after 14 days. Plants with fertilizer were greener and more vigorous at time of transplanting than those without nutrients. Additional rooting tests were performed in the laboratory with *Syngonium podophyllum* 'White Butterfly' and *Gerbera jamesonii* 'Apple Blossom' Stage II shoots with positive results. Consequently rooting tests were transferred from the laboratory and controlled culture room environment to actual greenhouse conditions for larger scale trials.

Horticubes® Growing Medium was cut into 10 in × 20 in sheets, ¾ in thick. Sheets were grooved to make 630 minicubes, ½ in square. These could be used in shallow trays at a spacing of 453 "cubes" per sq ft or separated into individual "cubes" and placed in commercially available plug trays, 288 wells per 11 in × 22 in tray, 171 units per sq ft. To date shoots from Stage II cultures of *Gerbera*, *Syngonium*, *Dieffenbachia*, *Spathiphyllum*, *Ficus*, *Philodendron*, and *Eucalyptus* have been successfully rooted in Horticubes® in the propagation greenhouse environment, thus eliminating the costly labor step of Stage III in vitro.

Additional testing through pilot programs with micropropagators is underway to further determine specific plant and greenhouse requirements for the foam system. Culture media formulation to enhance direct rooting of microcuttings is needed as well as determination of cultural practices (light intensi-

ty and duration, temperature, moisture, relative humidity) during acclimitization and rooting procedures.

The polyurethane foam medium can be autoclaved to meet in vitro needs as well as a combined Stage III+IV in vivo rooting process. The material is sterile through manufacturing procedures and has a 50% drainage factor. This high drainage reduces the likelihood of excessive moisture content, creating a more favorable rooting environment. The material will readily absorb nutrient solutions and can be flushed for quick changes in nutrient composition. Shoots rooted in "cubes" are easy to handle and lend themselves to handling through automation equipment already in use by bedding plant nurserymen today. The use of synthetic rigid foam systems may be the more cost efficient, labor saving way to get millions of microcuttings out of the laboratory.

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CUTTING PROPAGATION OF *METASEQUOIA* *GLYPTOSTROBOIDES*

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In a world where it seems that every time we turn around we hear of another life form that is near extinction or has become extinct it is nice to read or talk about a life form that