during their holiday periods in the Laboratory gives them understanding of the practical aspects of this exciting area.

We are now approaching the end of a second year since we established the Laboratory, and it is with some excitement that we look to the future as we foresee the development of many new crops along with the increase in the numbers of many basic crops currently in production.

Plans are already underway for the development of a new Laboratory in association with a new nursery. This will include 5 work stations and 3 culture rooms. It would also include a basic laboratory area as well as facilities for media preparation, as we must be aware of the possibility, due to changes of ownership, for failure of other companies to meet our needs. Should media preparation be necessary within our own Company for commercial use, we must safeguard ourselves. This would not mean the expense of all the equipment, but at least the basic space.

Tissue culture, or micropropagation, is presently the most exciting, challenging, and demanding aspect of horticulture and, putting aside all of this, it is one of the most demanding on the dollar. Anyone who is involved in it will surely agree that the money spent in what is a small square footage area, along with the cost of running such an operation, is sizeable.

Nevertheless, it is possible to make a profit, but it is necessary that crop planning and projections be carefully estimated. Records as to actual performances must be kept so that a case history of crops can be developed and, therefore, a plan for future production can be applied. Tissue culture can be fun, fascinating, and frustrating but, what's more, it can be financially disastrous without careful planning.

AN OVERVIEW OF A COMMERCIAL PLANT TISSUE CULTURE LABORATORY IN HAWAII

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We are a specialized laboratory with over 90% of our work dealing with the mericloning of orchids. The other 10% entails the mericloning of other ornamentals, such as *Anthurium andraeanum*, *Cordyline*, *Dieffenbachia*, Spathiphylum, and bromeliads.

In sharing my experiences with you I will cover:

(1) Planning and building the laboratory

(2) Culture and management

(3) Production and marketing

(4) Personnel

As I have just returned from a visit to various tissue culture laboratories in the Philippines, Thailand, and Singapore, I will also touch upon the facilities and problems I saw in the Southeast Asian laboratories.

First of all, about planning and building the laboratory. When we started in 1977, we had very little information on hand to guide us. If we had had ample funds, we could have hired a professional consultant, engaged a private contractor, and thus perhaps lessened our problems. But we accepted the challenge of starting something new and did most of the work ourselves. To begin with, we visited a number of commercial laboratories in the U.S. and also some University research facilities. We wanted to get some first-hand observation and knowledge of as many facets of these operations as possible. One thing we were particularly interested in was the type of equipment being used by the various laboratories.

In planning, we had to clarify in our minds what the functions of our laboratory would be, keeping in mind expansion possibilities. Our basic function remained, of course, to produce large quantities of economically feasible, clonable ornamental plants. After exploring dozens of plant genera, we finally settled on orchids, *Anthurium*, *Cordyline*, *Dieffenbachia*, and *Spathiphyllum* as the main plants we would focus on. Of these, we are now cloning mostly orchids. The other four plant types were cloned for mother block purposes only.

Once we pinned down the function of our laboratory, we set to work building the facility. This involved planning for size and location of various work areas, preparing for positive aseptic conditions, allocating space for culture shelves, (allowing for shelf height and size) lighting, air conditioning, shakers, laminar flow hoods, traffic flow patterns, etc. . . . All of these and more had to be worked out in detail.

Today our facility occupies 1,800 square feet of working laboratory space. Our transflasking room is approximately 300 square feet, with five laminar flow hoods, two 18,000-BTU air conditioners, and UV lights on the ceilings. We also made provision for installing a suction fan with absolute HEPA filters to bring in outside air, and an exhaust fan to interchange the inside air in the laboratory in order to clear up any build-up of alcohol in the room.

Within the inner laboratory, our explanting room of 72 square feet contains our dissecting microscope. There is also a sink for the sterilization of explants. In addition, we have an interior office and library with two four-tiered lighted shelves. Here we place some of our PLB's mother flasks for evaluation, and here we do our research. This room is 12 × 12 feet.

Our largest room is the culture room. This is 20 × 36 feet. It houses the following:

- Three units of a four-wheeled roll-a-drum rotating shaker, with a capacity of revolving 3,000 test tubes when filled;
- Two units of gyratory shakers, one holding 50-ml flasks and the other designed for 250-ml flasks; and
- Units of single roll-a-drums for research purposes.

The remaining space in our culture rooms holds six sets of 54-in × 8-ft shelves with six tiers of shelving. These are all lighted with four sets of Cool White or Gro-Lux lamps per shelf. The lights are timed to be on for 12 hours only. Our total lighted shelf space has a capacity of holding 13,000 500-ml flasks — more if we interspersed these flasks with our 250-ml mother flasks. (In contrast, the Bangkok Orchid Centre in Thailand, which has a laboratory three times larger than ours in square footage, has a culture room holding 20,000 bottles at a time. The tissue culture laboratory of Multicos Orchids in Singapore was acceptalbe but the one at the University of Philippines at Los Banos was unacceptable, in my opinion.)

Our glassware room of 120 square feet has enough shelving to hold 5,000 flasks, together with our chemicals and other laboratory supplies. We use around 25,000 or more flasks in our operation at a cost of $2.00 per flask. (In contrast, the Bangkok laboratory accommodates around 50,000 bottles at a cost of only 10¢ each. That is a real plus in savings in glassware alone.)

For preparation and sterilization of glassware, for media preparation, and for storage cabinets, we have allocated a room of 400 square feet. This room also houses our autoclave, distiller and de-ionizer, pH meter, electronic balance, microwave oven, blender, propane stove, refrigerator, chemicals, and additional glassware.

Our energy bill averages about $1,200 a month. On the whole, I feel we have a fairly efficient laboratory complex, with our procedures well optimized and our operations properly maximized.

Let me now turn to some problems in culture and management . . .

One of the interesting things about learning is discovering what questions to ask. We've identified some questions of importance to us regarding procedures and techniques of in-vitro propagation. For instance:

- What part of the plant should we try to excise in order to get the proper explant tissue to start with? Or . . .
- How do we get the tissues to produce shoots or other structures that will ultimately grow into a viable plant?
- Among the multitude of factors involved in micropropagation, what are important to measure and balance? . . .

These are factors such as:

— Cytokinins and auxins
— Strength of various chemicals, whether liquid or solid
— pH of media
— Speed of rotation of shakers
— Light intensities
— Temperatures . . . etc. . . .

The key factor in all of this is when to begin and when to stop the various stages of culture. All of these questions require some research on the part of the laboratory, in order to arrive at satisfactory procedures. For instance . . . with our Vacin and Went media, we have 11 different variations being used, just for orchid culture.

The many problems associated with commercial tissue culture operations often can be alleviated by careful planning. Standard operating procedures have to be developed; specific goals have to be set regarding the final form of the product that is to be marketed.

In our case, because we have 8 acres of land for growing and a retail outlet in the heart of Waikiki, we are hopeful that we can sell all of our products — the orchids, the anthuriums, and the bromeliads — in an integrated way. We are at present planning the grow-out and retail operations. We plan to surround our retail outlet with a ¼-acre exotic garden consisting of orchids, bromeliads, and other landscape plants. Our products will be in test tubes, baby food jars; they will be in flasks, community pots; they will also be individual 2-inch cell paks and 4-inch nearly blooming plants, to full flowering plants, ending up with the cut flower stages. What I am saying is that we hope to be one of the few commercial laboratories in our field that will be dealing with the full circle of plant propagation — cloning the plants and growing them to different stages and finally retailing the various products in Waikiki — where more than 3 million tourists visit annually. Our packaging concept is such that we will have products for both

the domestic and the foreign tourist. The U.S. tourist will be able to buy certified, nursery-grown plants; the foreign tourist will be able to purchase test-tube plants that will clear their plant quarantine restrictions.

In operating any tissue culture laboratory, a careful study of the potential market for any of the lab's products must be made before any significant production is initiated. In short, markets determine the best possible program for the laboratory; markets dictate the proper operating procedures to use. In addition, a monitoring program must be installed to insure that the planned procedures are being followed for a consistently high-quality product.

If planning is done well, redundant as well as extra work can be eliminated. This will result in increased efficiency — that is, more actual work results for the same amount or even less effort. This leads to more plants being produced, a larger share of the market, and a resultant higher profit margin. Because of the "newness" of the plant cloning technology as applied to commercial enterprises, studies are needed to determine "fitting" the lab's products into existing practices. In other words, a concerted effort must be made to study the potential market for this new technology. Due to the variations found in each potential crop, specific questions must be answered before the production of any crop is initiated. Questions include the following:

(1) Where is the market for the plant? In our case, as mentioned earlier, because we are not only a commercial micro-propagator but also a grower with good acreage, and a retailer with an ideal location, we can afford to produce plants which are uneconomical for wholesaling, but which can be very profitable for retailing. For example, our *Anthurium andraeanum* plants in baby food jars prepared for retailing to the foreign tourists.

(2) What parts of the market can be integrated with the products of the laboratory? Our operation integrates the culture and the marketing of a full gamut of products — from test-tube plantlets to blooming plants and cut flowers.

(The Bangkok Orchid Growers Cooperative, which is owned by 500 orchid growers farming 6,000 acres, grosses well over 30 million dollars annually. Their tissue culture facilities are the largest that I have seen. They are using eight laminar flow hoods, have seven standing types of autoclaves, they employ 25 workers and have a culture room capacity of 20,000 bottles on triangle-arranged, three-tiered racks. The main function of these facilities is to mericlone and to propagate by seed their various orchid genera for the growers. A few years back, they were selling only the larger cut flower sprays. Today, over 60% of their export cut flower sales are geared to supermarkets in Denmark and Germany. By mixing five sprays of *Dendrobium* and *Arachnis* or *Arandas*, and then adding sword fern stems,

they have created an arrangement packaged for this European market. This is a small example of integration with fabulous economic results.)

(3) Is the plant to be cloned conducive to this kind of technology? And when processing is completed, is the plant still a commercially viable product? Our answer to this question is the key reason we are cloning either award orchids or cut flower cultivars.

(4) What advantages does propagating a certain plant in-vitro have over the standard methods? Will cloning help remove viruses or other plant pathogens? We concentrate on specific orchids in large part because of our high rate of success in explanting and the short period needed for PLB's proliferation. In dendrobium mericloning, we get close to an 80% "take," which is just as good as the world's largest orchid laboratory in Bangkok. Also, we have been successful in culturing out the *cymbidium* mosaic and *odontoglossum* ringspot viruses.

(5) What is the complete cost of production to deliver the finished prouct? In dendrobium orchids, or in the rapid proliferation plants, such as the reed epi's and miniature oncidiums, we estimate our production costs to be approximately 10¢ per mericlone in 5,000 lots, and we are wholesaling them at 25¢ each. For cattleyas, the production cost is double and more, because our "catch" rate is only about 50%. This rate is better than for Bangkok orchids, as they are successful in only 30% of their cattleya explants. I believe their low rate is a matter of techniques or procedures.

(6) What is the future market potential of any cloned plant? And what are the parameters that can affect its future market? Say, in orchids, what will the consumer preferences be in style or in color five years from now? Will the demand continue to be for large flowers for corsages and the smaller flowers for blooming plants, as it is presently?

(7) Is there another crop that is more profitable to clone in the coming years? A crop with better potential than, say, orchids? A crop that can bring a higher unit of return for a longer period of time? We are already looking into the intergenerics, such as the warm climate odontocidiums, aspoglossums, vuylstekearas, maclellanaras, and miltocidiums, to name a few, and the miniature cattleyas, including their intergenerics. The main reason we are doing this is that the newness of these plants and the lack of competition in these genera makes exploration attractive. Also, the present trend in home ownership is in high-rise cubbyholes rather than single-family dwellings with lots of yard space, thus necessitating the growing of smaller plants.

All of these questions impinge upon planning the present and future operations of a commercial tissue culture operation. In addition, for our laboratory, the limited space found in the present facilities must be a consideration, as well as the limited capital available for research and/or improvements. Until such a time when expansion in both space and capital can occur, these limitations will assert considerable influence on our analysis of potential crops and potential growth.

To me, the greatest error any laboratory can make is to produce a crop uneconomically just for the sake of prestige. In the long run, prestige probably won't make money. On the other hand, research is a must. However, it cannot be achieved until there is sufficient capital or cash flow from the products generated by one's laboratory.

I firmly believe that at this developmental stage of commercial ornamental production via tissue culture, the bulk of research should be at the University level, with support from private industry. Research work such as anther culture, chromosome doubling, protoplast fusion, etc., would be of extreme importance to our field.

Earlier I talked about culture and management. That means that a commercial laboratory such as ours must be able to increase the propugule at least four- or five-fold within a year's span, and then have the work scheduled for output accordingly. We must also be successful in growing the plants out from an in-vitro condition into in-vivo nursery conditions, without any appreciable loss from plant mortality. I firmly believe that if the quality of production in the laboratory is good (this means good roots and shoots), then the acclimatization process after the plant leaves the laboratory will be easier and quicker under an increased humidity mist system. Our experience has been that dendrobium explants can be cultured into flowering orchids in less than 30 months.

All of the problems I have discussed and the questions I have cited, must be resolved and commercial procedures optimized before we can say definitely that the cloning of plants, whether orchids or any other genera, is economically viable. My wish is that more persons in the plant cloning field will publish their data oftener and share their knowledge more willingly. It is only through research and shared knowledge that this young industry can survive and thus contribute to a dramatic growth in commercial micropropagation of plants desirable to mankind.

Lastly, a brief word about personnel. It has struck me over and over again that micropropagation is — and will probably always be — a labor-intensive type of operation in a special

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.