Thursday Evening, December 13, 1979

The Thursday evening educational program on tissue culture was convened at 7:30 p.m. with Dr. Elton M. Smith serving as moderator.

GETTING STARTED IN TISSUE CULTURE — EQUIPMENT AND COSTS

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Abstract. A pilot tissue culture laboratory can be set up for less than $1000 if alternatives of materials and equipment are investigated. Some construction skills are needed to accomplish this, although no charge has been added for such labor costs to this proposal.

Tissue culture as a method of propagation is presently used on a rather limited scale compared with its potential use. Among the reasons for this are 1) a specialized knowledge of plant anatomy and morphology is required, 2) a strict regimen must be followed to maintain asepsis, 3) all plants cannot as yet be propagated by this technique, and 4) expensive, sophisticated equipment and costly, specially designed growth rooms are needed to grow the tissues and plants during the reproduction and replication phases. The latter reason will be considered in this paper, since costs are frequently given as the reason many people with adequate knowledge do not attempt tissue culture. A 1976 article (1) stated that "for a good commercial lab, equipment alone would cost $30,000." Another article (2) at about this same time indicated that a fully equipped lab could cost up to $250,000 and, if inflation is applied to these figures, the price might be nearly doubled today. But with a little ingenuity and a look at how things were done before we had sophisticated machines, perhaps a basically equipped lab can be set up for less than $30,000. Let's examine the laboratory equipment needed and consider what alternatives are available to reduce costs and perhaps permit a pilot operation to be set up for under $1000.

Autoclave. This unit is needed to sterilize nutrient media, glassware and occasionally tools. In a medium to large culture lab it would be used 2 or more times a week depending upon

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the size of the operation. A good autoclave with a chamber, 16 × 16 × 24 inches, will cost between $12,000 and $26,000 new, depending upon the degree of automation ordered. Smaller table models are available but these still cost a minimum of $2200 to $2400 and the working chambers are quite small, usually 9 to 12 inches in diameter, which makes their use somewhat inconvenient and their cost still prohibitive. Since an autoclave is basically a sophisticated pressure cooker, why not use a pressure cooker? You won't have all the convenience of an autoclave but it will do an admirable job, and the cost for a canning size cooker varies from $32 for one you have to heat on a stove to about $200 for one with an electric heater built in. Cost of "autoclave": $40-$200.

Hood or Isolation Chambers. These units are used to provide a clean work area in which new tissues or plant parts can be dissected out and placed in culture, and for making routine transfers and divisions of cultures already started. The more sophisticated types costing up to $4000 have large, open work areas which provide a constant flow of filtered air to reduce the possibility of contamination by spores and other organisms in the air. Smaller table top models cost from $450 to $1200. My original isolation cabinet was homemade in 1964 at a cost of $12 for materials. In 1976, two of my students built another isolation cabinet for their use in a special problem course, and their total cost of materials was under $20. A box 24 inches wide, 16 inches deep and 20 inches on the back side was constructed of ½ inch plywood and painted with epoxy paint. A sheet of window glass set a 60° angle served as the face of the cabinet and this was sealed to the plywood with silicon windshield sealer. A sheet of window glass large enough for the case to sit on serves well as the floor of the cabinet and is much easier to keep aseptically clean during use than would be a painted wood surface. A single bulb fluroescent fixture and a UV germicidal lamp could be installed, and this will increase the cost to about $45 for materials. The fluorescent lamp gives good lighting for working in the cabinet, and the UV lamp is turned on 30 to 45 minutes before starting work in the cabinet (but turned off while working in the cabinet) to help in maintaining sterility. Cost - $45.

Binocular Dissecting Microscope. Prices vary from $225 to $1500. I would advise against buying the cheaper models; for in an advanced lab a good "binoc" will cost $500 to $800. It is used for examining buds and tissues and dissecting out apical buds or small organs in an early stage of development. For much of the work done in a tissue culture lab high magnification is not essential. A headband binocular magnifier with 3X to 5X magnification and a focal distance of at least 3 inches is often adequate for those instances where magnification is needed. Cost - $17.

Growth Room. This can be as elaborate as a whole new building or clean room completely outfitted for growing tissues. For the advanced lab, the room would be completely sealed except for an exhaust and entry for its air supply filtered through HEPA filters to eliminate air borne contaminates. All walls and shelves should be painted white to reflect light and to make maximum use of the light supplied. The room should have its own heating and air conditioning systems. The ballasts for the fluorescent lights should be located so that they do not pose a fire hazard. However, the heat given off by them should be utilized in the winter to heat the growth room or adjacent work areas, and during the summer the heat should be dumped to the outside atmosphere to reduce air conditioning costs. A beginning lab can utilize any room where temperatures can be maintained between 20° and 27°C (68° and 81°F). This could be an unused portion of an office or storeroom or an extra room in a home. Racks or shelves with fluorescent lights suspended 14 to 18 inches above them will need to be installed. Two shelves 2 × 8 ft with two 4 ft shoplight fluorescent fixtures each, with 2 cool-white lamps suspended above them would provide adequate space to get started. The 32 sq ft of area would accommodate any one of the following: 2400 18 cm or 1500 25 cm tubes in racks; 700 jelly or baby food jars or 336 olive jars. Since many room situations would serve to get started, only the cost of 1 sheet of plywood ($12), 4 shoplights ($16 each) and a time clock ($20) to regulate light duration at the usual 16 hr light is considered. Total Cost - $96.

Autopipettor. Used to apportion tissue culture medium into tubes and bottles. These are nice to have, and a large lab should have at least one, but at $650 to $750 each they are unnecessary for the starting lab. A 2-liter aspirator bottle with the spout at the very bottom (cost $14.50 each) with soft tubing which can be easily pinched to stop liquid flow and of a diameter to fit the aspirator bottle spout (3/8 inch I.D. gum rubber tubing, cost $4.50 for a 12 ft package) will serve very well. A large funnel with about a 6 inch diameter (glass or plastic) to pour prepared medium into the aspirator bottle and a pinch clamp ($4.00/dz) to control liquid flow are also needed. A piece of plastic pipe with holes bored in it can be fitted to the end of the rubber tubing which will allow 2 to 4 tubes to be filled at once. Total cost of the alternative - $23.

Culture Vessels. Many glassware alternatives are available which can be used for growing tissues. During the past 15 years I have tried and discarded many types. Plastic disposable vessels are also available in many configurations but I would not recommend them for any but the largest commercial labs and then only in certain instances. Although the manufacturers claim they are relatively inexpensive, I believe them to be rather costly unless large quantities are purchased and used. For a beginning lab I'd suggest the following:

Unit	Size	Amount	Cost
Culture tubes	25 × 100 mm	2 Gr.	$ 76.00
Kaputs	25 mm	Pkg/500	26.00
16 oz. straightsided flint jars	89-400	2 Gr.	56.00
Petri dish (bottom only)	100 × 10 mm	2 Gr.	152.00
			$310.00

A beginning lab could get by with even less expense by using baby food jars and their lids or ordering metal lids for the 89-400 flint jars rather than using the Petri dish bottoms as closures. Metal lids with paper liners should have the liners removed before use. The advantage of the Petri dish as a closure is that more light is available to the plant tissue in the jar.

Tools. Small instruments are needed for isolating buds and tissues as well as for routine subdivision and maintenance of cultures. Three sizes of forceps will handle almost any tissue culture manipulation; 300 and 200 mm straight tipped and 115 mm curved tipped forceps. A beginning lab would not need the 300 mm forceps; these are needed when large, deep bottles such as Mason jars are used in the lab. Cost of 2 pair each of 115 mm and 200 mm forceps - $13.50.

Dissecting needles can be purchased with wood handles for $2.15/dz but a metal needle holder with replaceable tips is preferable; the holders are $2.15 (2 recommended), and 36 replaceable needles cost $2.60. Cost - $6.90.

Knives are needed for cutting and dividing the tissue. For the starting lab, one No. 3 ($3.90) and a No. 7 ($4.75) dissecting knife handles are suggested with one package of 100 No. 11 knife blades ($20.50) which will fit both handles. Cost $29.15.

An alcohol lamp is used for flaming tools and the necks of tubes and jars. A small glass one costs $1.90 and if carefully handled should last many years. A brass alcohol burner which will not break costs $13.00. Cost - $1.90; Total Tool Cost - $51.45.

Chemicals for Preparing Media. The specific medium to be used will vary somewhat with the plant or plants to be propagated. The lab has three options; 1) to purchase prepared media formulations[1] designed specifically for plant tissue culture, 2) to

[1] Flow Laboratories Inc., 1710 Chapman Ave., Rockville, MD 20852; Grand Island Biological Co., P.O. Box 200, Chagrin Falls, OH 44022; KC Biological Inc., P.O. Box 5441, Lenexa, KS 66215.

prepare all media from individual chemicals or 3) to purchase prepared salt mix for media but add agar, sugar, growth regulators and vitamins. With prepared media the lab has few optins open to it for making media constituent changes, but its preparation may be as simple as pouring the contents of a package into water and heating it to dissolve the materials. This option would require as little as $50 to $100 outlay. If the lab elects to prepare its own media, a balance in addition to chemicals must be purchased. This option also requires a knowledge of chemistry and of media used for plant tissue culture. An adequate balance for this type of lab will cost $1000 to $3000 and an array of needed chemicals, $300 to $500. The third option will give the beginning lab considerable latitude in medium formulation and is also an excellent system for a small research lab. A supply of agar, auxins (IBA and NAA), cytokinins (kinetin and benzyl adenine), vitamins (thiamine, nicotinic acid, pyroxodine) and glycine can be purchased from chemical supply houses or companies selling prepared media formulations. Sugar purchased from the grocery is far less costly than that from a chemical supply house and unless critical research is being done it is quite satisfactory. A balance, though desirable, is not necessary for this option but it would be necessary to hire a chemically knowledgeable person to prepare stock solutions of the auxins, cytokinins, vitamins and glycine. The needed quantities of each could be measured volumetrically with a pipette. The agar and sugar can be measured dry by making several weighings of the amount to be used (e.g. 7 g/l agar) and marking the volumes on a clear plastic pill vial. Rather than weighing, one volume of each of these components is used per liter of medium being prepared. The cost of this option would probably be $100 to $150.

Water. Contaminants in some tap water supplies could cause failures of tissues in culture. Double glass distilled water is preferred for media preparation; cost of a glass still is between $600-$1800. For the small beginning lab, the purchase of 5 gal carboys of distilled water to be used only for preparing media is the least costly route, but there is a continuing cost and occasionally water of questionable quality may be obtained. An alternative between these two extremes is to purchase a disposable mixed bed ion exchanger (Ultra-pure mixed bed, cost $33), an organic removal cartridge (cost $26), two wall brackets for mounting them (cost $18) and 1 or 2, 5 gal carboys for storing treated water. Depending on the minerals in your water supply, these cartridges will normally process in excess of 1000 gal of water before needing replacement. Total Cost - $77.

pH Control of Media. The price of pH meters to measure the acidity of culture media begin at $210; the better instruments cost \$600 to \$900. For the small or beginning lab pH narrow range indicator paper which covers the pH range from 2.9 to 4.2 and 4.9 to 6.5 will work satisfactorily. Since most media are adjusted to pH 5.6 to 5.8 the lab could get by with just the latter pH range paper; a 2 roll dispenser of these papers costs \$3. Culture media are usually around pH 4 as prepared and are most commonly adjusted to pH 5.6 to 5.8 for use. Sodium hydroxide (1N) is added drop wise to increase the pH value and HCl (1N) can be used to adjust the pH downward if the desired pH value is overshot. These two chemicals are available as 1N *solutions for \$6/liter. Total cost for pH control* - \$18.

Material for Achieving and Maintaining Asepsis. This is the basic requirement for all tissue culture work. A laundry bleach such as Clorox is the most common material available for reducing or eliminating contaminants on the surfaces of tissues, tools, isolation cabinets, etc. (Usually it is diluted 1:9 v/v, Clorox:water, for immersing tissues to be cultured, but dilutions from 1:4 to 1:19 v/v may occasionally be used.)

Methyl alcohol can be used for sterilizing tools by dipping and flaming and for running the alcohol lamp used to flame the tools. (Ethyl alcohol is preferable because it is less toxic but application to the Alcoholic Beverage Commission must be obtained for purchasing it for manufacturing use, and its controls are rather stringent.)

A "scrub soap" such as used by hospitals or dentists is a good investment. The hands and forearms are washed to the elbows with soap prior to beginning work in the transfer hood. A common soap can be used but the "scrub soaps" have various biocides added to better reduce organisms on the skin. A small supply of all three (bleach, alcohol and soap) should cost \$3 to \$5.

Miscellaneous Materials and Equipment. Equipment is needed in which to heat the culture medium during preparation; laboratory beakers of various sizes are preferred though ceramic cookware can also be used. Aluminum pans and utensils are not suitable, although stainless steel could be used. Larger vessels for heating should have a 2-liter capacity. An assortment of pyrex kitchen measuring cups will serve for the smaller glassware for measuring liquids but should not be used for heating the medium. Estimated cost - \$75.

Racks for tubes can be made from 1″ or 2″ Styrofoam sheet insulation; a cutter can be made from conduit or thin wall tubing for cutting holes in the Styrofoam which will accommodate the size tubes being used. The sheet of Styrofoam is cut into appropriate sizes to hold a predetermined number of tubes. I cut the sheet into 4 × 12 inch pieces which will hold 18 25 cm tubes or 30 18 cm tubes. Thin aluminum sheet or other material is sheared to a size about ¼ to ½ inch smaller dimensions than the Styrofoam and two holes are drilled at each end for passing a wire through to attach it to the Styrofoam tube holder with wire. Material cost per 2″ thick rack is 45¢; 50 racks = $22.50.

CONCLUSIONS

A producing pilot tissue culture laboratory can be set up for less than $1000 (Table 1). This proposal does make the assumption that the person setting it up has some minimal construction abilities and has some knowledge of tissue culture techniques. Before setting up a tissue culture lab the company executive who will oversee the operation and the individual who will be in charge of it should take a course in plant tissue culture methods such as that offered by the Alton Jones Center at Lake Placid, New York. Prior to this, however, the company should have made some critical analysis of its production system to determine that a tissue culture laboratory is a needed addition to their production system (3).

Table 1. Estimated total costs for starting a tissue culture laboratory.

Pressure cooker	$ 40	Water treatment	$ 77
Isolation hood	45	pH control	18
Magnifier	17	Asepsis materials	5
Lights & shelving	96	Misc. cooking & measuring glassware	75
Apportioning equip.	23	Tube racks	23
Culture vessels	310		
Tools	52		$931
Medium chemicals	150		

LITERATURE CITED

1. Anon. 1976. Plant propagation by tissue culture moves from academic theory into commercial production. *Florist*. June, 72-81.
2. Anon. 1977. Tissue culture comes to Texas. *So. Florist & Nursmn*. May 6, p. 24-26.
3. McGuire, J.J. 1979. Tissue culture — is it for every wholesale nurseryman? *R.I. Nurseryman's Newsletter*, No. 63, Fall.

CAMERON SMITH: We have had back luck with hydride paper. You can build a pH meter with two integrated circuits for $25 plus about $29 for the glass electrode. I would consider building one because the hydride paper ages and goes out of calibration.

GUS MEHLQUIST: Where can you buy these materials.

LEN STOLTZ: From any of the supply houses such as Fisher Scientific. Use your letterhead.

BILL CUNNINGHAM: I might point out that a Bunsen burner or alcohol lamp is not a satisfactory way to sterilize tools. An incinerator is better.

LEN STOLTZ: You are right; however, it will burn up your tools which is costly. We use 95% ethyl alcohol because we had contamination problems with 70%.

DICK ZIMMERMAN: Alton Jones offers two courses on tissue culture. One course is 3 days long and the other is 2 weeks.

PROGRESS ON *IN VITRO* PROPAGATION OF RED MAPLE[1]

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Abstract. Tissue culture experiments were conducted using shoot-tips (5 to 10 mm) or single node sections to devise an *in vitro* propagation scheme for red maple (*Acer rubrum*). Inconsistent proliferation of axillary buds on shoot-tip explants occurred when they were aseptically cultured on modified Linsmaier and Skoog (LS) medium containing kinetin (K), 6-benzylaminopurine (6·BAP) or 6(ν-ν-dimethylallylamino)-purine (2ip) at 0.1, 1.0, 5 or 10 mg/l.

Actively growing shoots were a prerequisite for a high percent rooting of proliferated shoots. Eighty-five percent of shoots cultured on ½ strenght LS + 0.5 mg/l indolebutyric acid (IBA) developed roots within 10 days. Phenolic secretion that inhibited growth was controlled by preconditioning explants on potato dextrose agar or LS basal medium by a series of 3-day subcultures.

In the past few years, research efforts on *in vitro* propagation of woody plant species have experienced increased activity. Research reports have been issued on apple (1,5,6,7,12), almond (11), blackberry (3), bougainvillea (4), pear (5), and plum (5). In addition, Winton (13) in a recent review stated that at least 37 angiosperm and 19 gymnosperm trees have been regenerated as individual rooted shoots. This listing may, upon initial examination, appear impressive but for many speices regeneration was low in frequency, from embryonic tissues, or from callus. Winton's list (13) includes *Betula*, *Citrus* spp., *Hamamelis*, *Populus* and *Ulmus americana* that are of interest to nurserymen. An earlier review by Abbott (2) presents a summary of present *in vitro* successes with evergreen and conifer species.

Commercial cultivars of red maple (*Acer rubrum*) are generally propagated by budding or grafting onto red maple seedlings. In many horticultural species, this propagation technique

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