DESIGNING A PLANT MICROPROPAGATION LABORATORY

MARK P. BRIDGEN AND JOHN W. BARTOK, JR.[1]

Departments of Plant Science and Agricultural Engineering
University of Connecticut
Storrs, Connecticut 06268

Tissue culture is rapidly becoming a commercial method for propagating new cultivars, rare species, and difficult-to-propagate plants. From a few research laboratories several years ago, a whole new industry is emerging. Currently, the demand for micropropagated plants is greater than the supply with some plants. Some growers specialize in only the micropropagation of plantlets, leaving the growing-on to others; many growers are integrating a tissue culture laboratory into their overall operation.

In designing any laboratory, big or small, certain elements are essential for a successful operation. The correct design of a laboratory will not only help maintain asepsis, but it will also achieve a high standard of work.

FACILITIES

Careful planning is an important first step when considering the size and location of a laboratory. It is recommended that visits be made to several other facilities to view their arrangement and operation. A small lab should be set up first until the proper techniques and markets are developed.

A convenient location for a small lab is a room or part of the basement of a house, a garage, a remodeled office or a room in the headhouse. The minimum area required for media preparation, transfer and primary growth shelves is about 150 sq ft. Walls may have to be installed to separate different areas.

A good location includes the following:

1) Isolation from foot traffic.
2) No contamination from adjacent rooms.
3) Thermostatically controlled heat.
4) Water and drains for a sink.
5) Adequate electrical service.
6) Provisions for a fan and intake blower for ventilation.
7) Good lighting.

Larger labs are frequently built as free-standing buildings. Although more expensive to build, the added isolation from adjacent activities will keep the laboratory cleaner.

Prefabricated buildings make convenient low cost laboratories. They are readily available in many sizes in most parts of the coun-

[1] Assistant Professor and Extension Professor, respectively.

try. Built-in-place frame buildings can also be used. Consideration should be given to the following:

1) Check with local authorities about zoning and building permits.
2) Locate the building away from sources of contamination such as a gravel driveway or parking lot, soil mixing area, shipping dock, pesticide storage, or dust and chemicals from fields.
3) A clear span building allows for a flexible arrangement of walls.
4) The floor should be concrete or capable of carrying 50 pounds per square foot.
5) Walls and ceiling should be insulated to at least R-15 and be covered inside with a water-resistant material.
6) Windows, if desired, may be placed wherever convenient in the media preparation and glassware washing rooms.
7) The heating system should be capable of maintaining a room temperature at 70°F in the coldest part of winter.
8) A minimum ¾ in. water service is needed.
9) Connection to a septic system or sanitary sewer should be provided.
10) Air conditioning for summer cooling may be necessary.
11) Electric service capacity for equipment, lights and future expansion should be calculated. A minimum 100 amp service is recommended.

GENERAL LABORATORY DESIGN

Cleanliness is the major consideration when designing a plant tissue culture laboratory. Most companies are not aware of their losses from contamination, but estimates run from less than 1% up to 50%. When you consider the high value of the product, no losses from contamination are acceptable. Routine cleaning and aseptic procedures can decrease your losses to less than 1%. Laboratories should have easy to wash walls and floors. Acrylic or urethane epoxy wall paints can be used; cement floors can be painted with an epoxy or urethane floor enamel or have an inlaid linoleum installed. High efficiency particulate air (HEPA) filters or regular furnace filters can be installed over air intakes to the laboratory or on furnaces. If possible, an enclosed entrance should precede the laboratory; sticky mats can be laid there to help collect dirt from the outside, or shoes can be removed.

The traffic pattern and work flow in a laboratory must be considered in order to maximize cleanliness. The cleanest rooms or areas are the culture room, i.e. primary growth room, and the aseptic transfer area. It is best to design these rooms so they are not entered directly from the outside of a building. The media prepara-

tion area, glassware washing area, or storage area should be located outside these rooms. The primary growth room and aseptic transfer room should be enclosed with doors leading to each. Traffic through these areas can be minimized by installing pass-through windows. Ideally, the media preparation area would lead to the sterilization area, which would lead to the aseptic transfer room and eventually the primary growth room.

Unusual requirements for electricity and fire safety dictate that power installation be done by professional electricians. Most wiring will require 110 volts, but water treatment equipment and autoclaves may require 220 volts. Temperature and fire alarms are to be connected directly to telephone lines to give fast warnings of problems. An emergency generator should be available to operate essential equipment during power outages.

GLASSWARE WASHING AND STORAGE AREA

The glassware washing area should be located near the sterilization and media preparation areas. When culture vessels are removed from the growth area, they are often autoclaved to kill contaminants or to soften semi-solid media. The vessels can be easily moved to the washing area if the autoclave or pressure cooker is nearby. Locate the glassware storage area close to the wash area to expedite storage; these areas also need to be accessible to the media preparation area.

The glassware area should be equipped with at least one large sink; two sinks are preferable. Adequate work space is required on both sides of the sink; this space will be used for glassware soaking tubs and drainage trays. Plastic netting can be placed on surfaces near the sink to reduce glassware breakage and enhance water drainage. The pipes leading from the sink can be PVC to resist damage from acids and alkalis. Both hot and cold water should be available with water distillation and/or deionization devices nearby. Mobile drying racks can be stored nearby and lined with cheesecloth to prevent water dripping and loss of small objects. Locate ovens or hot air cabinets (75°C) close to the glassware washing and storage area. Dust-proof cabinets, low enough to allow easy access, can be used in the storage area.

MEDIA PREPARATION AND STERILIZATION AREA

The water source and glassware storage area should be convenient to the media preparation area. Benches, suitable for comfortable working while standing (34 to 36 in.) and deep enough (24 in.) to hold equipment listed below are essential. Their tops should be made with molded plastic laminate surfaces that can tolerate frequent cleanings.

There is a variety of equipment available for micropropaga-

tion laboratories; this equipment is generally located in the media preparation area. The equipment budget will determine the type and amount purchased. All laboratories need the following basics:

1) Refrigerator/freezer—This is needed to store chemicals and stock solutions. Small laboratories may find it adequate to use countertop refrigerators.

2) High quality water—Bottled water can be purchased inexpensively and placed in the media preparation area. Larger businesses may find it economical to obtain distillation or deionization devices; these would normally be located in the glassware washing area. Small, inexpensive, low production Pyrex distillation devices can be purchased by small businesses that want the convenience of a still, but not the cost.

3) Balances—High quality balances are essential for a micropropagation laboratory; this is one area where it is difficult to find an inexpensive substitute. A triple beam balance is useful for large amounts over 10 grams, but a balance that can measure down to 2 mg is essential. Most laboratories have both a microbalance and a less sensitive top loading balance; the latter can be used more quickly and efficiently for less sensitive quantities.

4) Hot plate/stirrer—At least one hot plate with an automatic stirrer is needed to make semi-solid media. This purchase can be eliminated by using a stove and hand stirring the media while it heats; however, the time saved by using a stirring hot plate is worth the money spent.

5) pH meter—This is needed to measure media pH. Some laboratories use pH indicator paper, however this method is considerably less accurate and could severely affect the results.

6) Aspirator or vacuum pump—Aspirators can be easily attached to a water source and used for filter sterilization of chemicals. They are also used to disinfest plant material. Vacuum pumps are faster and more efficient, but also more expensive.

7) Autoclave—An autoclave or pressure cooker is a vital part of a micropropagation laboratory. High pressure heat is needed to sterilize media, water, glassware, and utensils. Certain spores from fungi and bacteria will only be killed at a temperature of 121°F and 15 pounds per square inch (psi). Self generating steam autoclaves are more dependable and faster to operate.

8) Optional equipment—A variety of non-essential equipment is available for tissue culture laboratories; individual

needs and equipment cost will determine what can be purchased. Microwave ovens are convenient for defrosting frozen stocks and heating agar media. Dissecting microscopes are useful to have in the laboratory for meristeming, dissecting floral and shoot apices, and observing plant culture growth. Labwashers, or regular dishwashers, can be useful. Automatic media dispensers are helpful when pipetting large volumes of media.

PRIMARY GROWTH ROOM

Temperature, relative humidity, lighting units, and shelves need to be considered in the culture room. All of these environmental considerations will vary depending on the size of the growth room, its location, and the type of plants grown within it. For example, a small primary growth room located in a cool, North American climate, can be placed in an unheated or minimally heated basement. The ballasts from the fluorescent lights do not need to be separated; rather they can be used as a heating source. Excess heat can be blown out of the growth room and used to heat other parts of the basement or building. In this case, solid wood shelves with air spaces located between shelves are recommended to prevent the cultures on shelves above lights from becoming overheated. A larger growth room located in an above-ground location may need to have remote ballasts and/or a heat pump installed. Shelves in a larger growth room could then be glass or expanded metal.

Temperature is the primary concern in culture rooms; it affects decisions on lights, relative humidity, and shelving. Generally, temperatures are kept 76° ± 2°F. Heating can be accomplished by traditional heating systems supplemented with heat from light ballasts or space heaters. Cooling the room is usually a greater problem than heating; cooler temperatures can be obtained by installing heat pumps, air conditioners, or exhaust fans. Using outside windows to cool culture rooms invites contamination problems in the summer and humidity problems in the winter.

Some plant cultures can be kept in complete darkness; however, most culture rooms are lighted at 1 klux (approximately 100 ft-c) with some going up to 5 to 10 klux. The plant species being micropropagated will determine the intensity used. The developmental stage of the plants will also help determine if wide spectrum or cool white fluorescent lights are used. Rooting has been shown to increase with far-red light; therefore, wide spectrum lights should be used during stage III and cool-white lights can be used during Stages I and II. Automatic timers are needed to maintain desired photoperiods. Reflectors can be placed over bulbs to direct their light. Heat generated by the lights may cause condensation and

temperature problems. In addition to using procedures previously mentioned, small fans with or without polyethylene tubes attached, can be placed at the ends of shelves to increase air flow and decrease heat accumulation.

Relative humidity (RH) is difficult to control inside growing vessels, but fluctuations in the culture room may have a deleterious effect. Cultures can dry out if the room's RH is less than 50%; humidifiers can be used to correct this problem. If the RH becomes to high, a dehumidifier is recommended.

Shelving within primary growth rooms can vary depending upon the situation and the plants grown. Wood is recommended for inexpensive, easy-to-build shelves. The wood for shelves should be exterior particleboard or plywood and should be painted white to reflect the room's light. Expanded metal is more expensive than wood, but provides better air circulation; wire mesh of ¼ or ½ in. hardware cloth can be used but tends to sag under load. Tempered glass is sometimes used for shelves to increase light penetration, but it is more prone to breaking. Air spaces, 2 to 4 in., between the lights and shelves will decrease bottom heat on upper shelves and condensation in culture vessels. A room that is 8 ft high will accommodate 5 shelves, each 18 in. apart, when the bottom shelf is 4 in. off the floor. The top and bottom shelves may be difficult to work.

ASEPTIC TRANSFER AREA

In addition to the primary growth room, the aseptic transfer area needs to be as clean as possible. It is preferable to have a separate room for aseptic transfer; this decreases spore circulation and allows personnel to leave shoes outside the room. Special laboratory shoes and coats should be worn in this area. Laminar flow hoods or still-air boxes can be placed in this room and used for all aseptic work. Ultraviolet (UV) lights are sometimes installed in transfer areas to disinfect the room; these lights should only be used when people and plant material are not in the room. Safety switches can be installed to shut off the UV lights when regular room lights are turned on. Surfaces inside the aseptic transfer area should be smooth to minimize the amount of dust that settles. Several electric outlets are to be installed to accommodate balances, flow hoods, bacti-cinerators, and microscopes.