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BIOTOOLS FOR SURVIVAL

INTRODUCTION-

BIOTECHNOLOGY, in recent years, has created unprecedented opportunities, not only for the manipulation of biological systems for the benefit of mankind, but also for understanding studies to understand the fundamental life processes. Concequently, it has become the world's fastest growing and the most rapidly changing technology.

Origin and definition -

The origin of biotechnology can be traced back to prehistoric times, when microorganisms were already used for the processes like fermentation. Although a molecular biologist may consider CLONING OF DNA to be the most important event in the history of biotechnology, the latter has actually been rediscovered in 1970 s third time during the present century. In 1920 s CLOSTRIDIUM ACETOBUTYLICUM was used by Chaim weizmann for converting starch into butanol and acetone; the latter was essential component of explosives during World war. This raised hope for commercial production of useful chemicals through biological processes and may be considered as the first rediscovery of biotechnology in the present century. The production of "PENCILLIN" antibiotic discovered by ALEXANDER FLEMING in 1929 on a large scale from cultures of pencillium notatum marked the second rediscovery of biotechnology. This was the beginning of an era of antibiotic research. The third rediscovery of biotechnology is its recent reincarnation in the form of recombinant –DNA TECHNOLOGY, which led to the development of a variety of gene technologies and is thus considered to be the greatest scientific revolution of this century.

BIOTECHNOLOGY, as word indicates is the product of interaction between the science of biology and technology. It is believed that biotechnology in future may become a major force for human existence. Already the products of biotechnology are playing a very important role in employment, productivity trade, economics, and the quality of human life through out the world. Fermentation, by some

microorganisms, formation of yoghurt and cheese from milk and vinegar from molasses, production of antibiotics like penicillin from certain fungi and the processes of baking and brewing are include in old biotechnology. The techniques of RECOMBIANT DNA and POLYMERASE CHAIN REACTION cell culture and fusion and bio processing which became possible only through the researchers in molecular biology have been described as new biotechnology.

BIOTECHNOLOGY IS "the controlled use of biological agents, such as microorganisms or cellular components, for beneficial use"

or

"the integrated use of biochemistry ,microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of microorganisms, cultured tissue cells and parts thereof"

Some of the biotechnological programs undertaken are:

1.Automated bioscreening.
2.Genetical improvement of pharmaceutical microorganisms.
3.Engineering of a series of organisms for specific industrial use.
4.Developing immobilized cell and enzyme systems for chemical process industries.
5.Improved production of vitamin B12.
6.Manufacturing fructose from inexpensive forms of glucose.
7.Bio processing alkenes to valuable oxides and glycols.
8.Production of plants resistant to herbicides viruses, insects and other pests.
9.Production of photo synthetically efficient plants.
10.Production of biopesticides and biofertilisers.
11.Developing a vaccine to prevent colibacillosis, a wide spread disease of newborn calves and piglets.
12.Human gene therapy.

Tissue culture techniques in biotechnology

An important aspect of all biotechnology processes is the culture of either the microorganisms or plant and animal cells or tissues and organs inartificial media.

Anther culture is used for haploid breeding, gametic and somatic cell tissue cultures are used for tapping gametoclonal and somaclonal variation or for production of artificial seeds. Transformation of protoplasts in culture leads to the production of useful transgenic plants. Embryo culture technique has also helped in extending the range of distant hybridization for plant breeding purposes. Similarly, animal cells are used for multiplication of superior livestock suing an variety of techniques like cloning of superior embryonic cells, transformation of cultured cells leading to the production of transgenic animals and invitro fertilization and transfer of embryos to surrogate mothers.

Gene technology as a tool:

More recently extensive use of newly discovered *POLYMERASE CHAIN REACTION* (PCR) has been made for gene technology This technology has become a major thrust area of present day researchers and some developed countries are encouraging researchers in this field as a matter of national priority.

Hybridoma and monoclonal antibodies in biotechnology-

Enzyme conjugated antibodies are being used for detection of viruses both in plants and animals including humans using Enzyme linked immunosorbent assay tests. Immunotoxins are being produced from gene fusions so that the toxic drugs meant for killing tumour cells may be carried to the target sites with the help of specific antibodies.

Biotechnology in medicine:

A large number of vaccines for immunization against dreadly diseases DNA PROBES AND MONOCLONAL ANTIBODIES for diagnosis of various diseases, DNA probes and monoclonal antibodies for diagnosis of various diseases and human growth hormone and other pharmaceutical drugs for treatment of diseases are being released. DNA fingerprinting and auto antibody fingerprinting techniques are also proving a great boon in forensic medicine for identification of criminals like murderers and rapists through the study of DNA or antibodies from blood and semen stains urine tears, saliva perspiration or hair roots. Etc.

Biotechnology and protein or enzyme engineering:

Another very important area of biotechnology is protein engineering that will lead to the production of enzymes and storage of proteins. In this area the production engineer first prepares a computer aided protein model for a specific function and then prepares a synthetic gene that will produce this desired protein in a predictable manner. Biotechnology has also provided us with a remarkable technique in the form of immobilized enzyme systems, which allowed the production of a variety of substances e.g. production of high fructose corn syrup using an immobilized enzyme glucose isomerase.

Biotechnology and metabolic engineering:

One of the major objectives of biotechnology research is the use of living systems for the production of metabolites at the industrial scale. The opportunity to introduce the heterologus genes and regulatory elements made metabolic engineering a very fascinating area of research.

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Biotechnology in agriculture-

Biotechnology has also revolutionized research activities in the area of agriculture which includes, plant cell tissue and organ culture, genetic engineering lead to transformation followed by regeneration of plants to give transgenic plants carrying desirable traits like disease resistance, insect resistance and herbicide resistance eventually this may also be used for increasing photosynthetic efficiency, nitrogen fixing ability, improved storage proteins, hybrid crops, crops for food processing etc. Somatic hybrids between sexually incompatible species permitting transfer of desirable traits from wild or unrelated crop species to crop plants; Transgenic animals produced in mice, pigs, cows. Etc
It is suggested that some of these eventually by used in bioreactors to produce drugs through their milk, blood, or urine; this area is sometimes described as molecular farming.

Biotechnology and environment:

Biotechnological methods have been devised for some environmental problems
Like pollution control, depletion of natural resources for nonrenewable energy, restoration of degraded lands and biodiversity conservation. Microbes are being developed to be used as biopesticides, biofertilisers, biosensors, and recovery of metals cleaning of spilled oils and for a variety of other purposes. Biomass is being produced and used as renewable source of energy, by capturing solar energy. Tissue culture and genetic engineering mycorrhizae root nodulation are also being used for reclamation of degraded lands.

Biotechnology in India-

On the recommendations of the SCIENCE ADVISORY COMMITTEE to parliament, Government of India constituted a National biotechnology Board under the department of science and Technology to coordinate and encourage research in this direction.

National Biotechnology Board (NBTB), decided to compile a list of biotechnologists in India and abroad working in the following fields.

1. Genetic engineering
2. Photosynthesis
3. Tissue culture
4. Enzyme engineering
5. Alcohol fermentation and
6. Immunotechnology.

An international Centre for Genetic Engineering and Biotechnology for developing countries under the auspicies of United Nations has been established. An international Institute of Biotechnology having parallel to those of ICGEB was established in U.K. It provides educational training through different methods including a course for the degree of Master of Biotechnology. Biotechnology centers in India have also been established at Indian agricultural Research Institute (IARI), New Delhi, National Dairy research institute of biotechnology (NDRI), karnal and Indian veterinary research institute (IVRI), Izatnagar.

With the inception of National Biotechnology Board and Department of Biotechnology enormous effort for growth and development of biotechnology in India has made to create scientific infrastructure in terms of both physical facilities and trained manpower.

Areas of research-

During the last more than 5 years, products, processes, and services in the field of biotechnology in India has become a reality. The successful use of embryo transfer and split embryos technology has become possible to give birth to improved buffalo calves. DNA fingerprinting using bkm probe has been developed. The diagnostic kit for filarial has been released and others for more than a dozen diseases are in development. An animal birth control product, which can be injected in males, has been developed at NII NewDelhi. A number of other male and female birth control vaccines are in different stages of evaluation.

A number of vaccines against diseases are being developed. The most important developments in these connections are the

establishments of Bharat immunological and biological corporations and Indian Vaccine Corporation. Micro propagation of elite trees has been made possible at commercial scale for bamboos at university of DELHI for teak eucalyptus and sandal wood through two pilot plans, one at TATA Energy research institute, NewDelhi and other in National CHEMICAL laboratory in Pune through the efforts of several laboratories and private companies, commercial prodution of elite plants through tissue culture has become possible for following other crops ornamentals, pomogranaite, vegetable crops, sugarcane Etc.tissueculture propagation of cardamom and oil palm has also been standardized for commercial application.
Biopesticides and biofertilizersare being developed on priority to meet the nitrogen demands and also to protect the soil structure. Aquaculture technology developed for farming prawn and major carps; economically viable technology giving higher yields are being developed for farmers. A yeast strain capable of producing ethanol has been isolated by IMTEC Chandigarh which will be shortly used in breweries. Seri culture involving improvement in production of quality of Indian silk has become possible.

The above is the brief account of facilities developed and achievements made in India in the field of biotechnology.

Plant tissue culture (PTC) techniques:

Plant tissue culture (PTC) techniques are used for growing plants in a sterile controlled environment for the purpose of mass-production, germplasm preservation, plant breeding, physiological studies, and genetic engineering. By using plant hormones and other growth regulators, small plant parts can be induced to produce hundreds of small “plantlets”, which can later be grown in a greenhouse, in the field, or as house plants.
In the process of learning PTC techniques, we learn about plant growth, morphology, nutrition, hormones, interaction of microorganisms, aseptic methods, laboratory safety, and organizational skills. Use of plant tissue culture has been limited in the past by the need for expensive equipment (laminar flowhood, analytical balance, and autoclave). However, by using biocides such as PC (Preservative Chemical) or NaDCC (sodium dichloro-s-triazinetrione is a spa and swimming pool disinfectant), expensive equipments are no longer essential. PTC experiments can be conducted in the room or home with little problem of

contamination using inexpensive household supplies (microwave oven, baby food jars, bleach, plastic storage box, etc.).

Supplies in Your room, Home Kitchen, or Local Discount Store?

Normal supplies

- pint and quart jars
- food coloring (optional)
- forceps (6 “ or longer)
- African violet leaves
- microwave-proof plate
- plastic or cardboard box
- hydroponic fertilizer
- baby food jars
- isopropyl or ethyl alcohol
- measuring spoons
- florist’s tape
- kitchen knife (about 6” long)
- baking soda
- pyrex pie pan (about 8”)
- goggles and gloves
- dusk mask, apron, and shoes
- dish detergent
- table sugar
- bleach and vinegar
- salad plate

Inexpensive pint jars filled with 70% ethanol, bleach, and sterile water are used to disinfect plant material. Baby food jars are used as media containers to culture plant material.

Supplies Not in Your Typical Store?

- Murashige and Skoog (MS) medium
- Benzlaminopurine (BAP), a plant hormone that induces shoot formation
- Naphthaleneacetic acid (NAA), a plant growth regulator that induces root formation
- Preservative Chemical (PC), a biocide that reduces contamination
- Agar, for solidifying the medium
- Polypropylene baby food jar caps (if you are using a microwave oven)
- Plastic (regular) pint jar caps (if you are using a microwave oven)
- pH papers

Where can you find these items?

You can get them from departmental, Medical and chemical supply stores in every town.

Safety Recommendations:

You need to teach yourself some basic laboratory skills and discuss lab safety including: the safe handling and disposal of alcohol and bleach solutions, disinfecting forceps and knives with alcohol (flame sterilization is not recommended), preparation of media, and the use of protective clothing such as latex gloves, goggles, latex aprons, dusk masks, and leather or tennis shoes.

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Material Safety Data Sheets (MSDS) provide information on the safe handling of chemicals. These are required for any chemical used in a room, and are obtained from the internet, manufacturers, and chemical supply stores.

BACKGROUND:

Plants have been vegetatively propagated for a very long time. Separating rootstocks, grafting,rooting branches and leaves are all ways to vegetatively propagate plants, by-passing the seedstage .
Tissue culture is a newer method that enables more control of environmental factors and has provided the evidence that entire, fertile, seed-producing plants can be cloned from single somatic cells. Depending on the plant, tissue cultures might be produced from any of these parts: Apical meristem, Flowers, Ovary, Pollen , Stem, Leaf, Root, and even Seed. Generally, dicots have been more successfully tissue cultured, but recently monocots like rice have been put into culture. Just this year (1999-2000) rice researchers reported that they could transform rice to have the capability of producing a precursor for vitamin A. Golden colored rice has the potential to reduce blindness in developing nations.
Tissue culture is the method that begins the process for making genetically engineered plants through recombinant DNA technology. However, for decades before recombinant DNA technology started being applied to plants, plant cultures were genetically modified using mutagenic chemicals like colchicine, which often generated larger plants with multiple sets of chromosomes (polyploidy) or by treatment with X-rays to induce mutations via physical breaks in the chromosomes, translocations or changes in the nucleotide sequence (though the mechanisms of mutation at the molecular level were not known at the time these treatments were being used). Plant tissue cultures can be grown in agar medium or liquid medium. On agar, a solid substrate, the plants can more easily develop roots and shoots. In suspension culture, the plant material is generally shaken continuously though gently. Bits and pieces break off starting new clumps. These clumps can be pipetted onto an agar surface or the clumps can be coated with a variety of materials. Seed potatoes are actually clumps of suspension cells.
Using plant parts (explants) scientists now study the nutritional and regulatory requirements for plant development and cell differentiation and for determining how plants respond to their environment at the molecular level, including how plant cells defend themselves from pathogens(fungi, bacteria, viruses). Many common food crops and household plants are vegetatively propagated through grafting or tissue culture. Grapes, seedless fruit and roses are generally grafts. Potatoes, African violets, asparagus fern are routinely propagated from tissue cultures. In fact, 95% of the potatoes we eat are generated from “seed” embryos of apical meristem suspension cultures.
LAB DESIGN

Any laboratory in which tissue culture techniques are performed, regardless of the specific purpose, must contain a number of basic facilities. These usually include the following:

• A general washing area
• A media preparation, sterilization, and storage area
• An aseptic transfer area
• Environmentally controlled incubators or culture rooms
• An observation/data collection area.

Washing Area: The washing area should contain large sinks, draining boards, and racks, and have access to deionized /distilled water. Space for drying ovens or racks, automated dishwashers, acid baths, pipet washers and driers, and storage cabinets may be necessary in the washing area, depending on the work being performed.

Media Preparation Area: The media preparation area should have ample storage space for the chemicals, culture vessels and closures, and glassware required for media preparation and dispensing. Bench space for hot plates/stirrers, pH meters, balances, water baths, and media-dispensing equipment should be available. Other necessary equipment may include air and vacuum sources, Bunsen burners with a gas source, refrigerators and freezers for storing stock solutions and chemicals, a microwave or convection oven, and an autoclave or domestic pressure cooker for sterilizing media, glassware, and instruments.

In preparing culture media, analytical grade chemicals should be used and good weighing habits practiced. To insure accuracy, an exact, step-by-step routine should be developed for media preparation. This routine should be contained in a complete media preparation checklist required to be completed by all media preparers, even for the simplest media.The water used in preparing media should be highly purified though deionization and/or distillation. Tap water is not recommended because it may contain undesirable salts and dissolved gases, microorganisms (algae, fungi, bacteria), and particulate matter (silt, oils, organic matter, etc.). Water used for plant tissue culture should meet, at a minimum, the standards for type II reagent grade water, i.e., be free of pyrogens, gases, and organic matter and have an electrical conductivity less than 1.0 µmho/cm.

The most common and preferred method of purifying water to type II standards is a deionization treatment followed by one or two glass distillations. The deionization treatment removes most ionic impurities, and the distillation process removes large organic molecules, microorganisms, and pyrogens. Three other methods that will produce type II purity water are absorption filtration, which uses activated carbon to remove organic contaminants and free chlorine; membrane filtration, which removes particulate matter and most bacterial contamination; and reverse osmosis, which removes approximately 90% of the bacterial, organic, and particulate matter as well as about 90% of the ionized impurities.

Transfer Area: Under very clean and dry conditions, tissue culture techniques can be successfully performed on an open laboratory bench. However, it is advisable that a laminar flow hood or sterile transfer room be utilized for making transfers. Within the transfer area there should be a source of electricity, gas, compressed air, and vacuum.

The most desirable arrangement is a small dust-free room equipped with an overhead ultraviolet light and a positive-pressure ventilation unit. The ventilation should be equipped with a high-efficiency particulate air (HEPA)

filter. A 0.3-µm HEPA filter of 99.97-99.99% efficiency works well. All surfaces in the room should be designed and constructed in such a manner that dust and microorganisms do not accumulate and the surfaces can be thoroughly cleaned and disinfected. A room of such design is particularly useful if large numbers of cultures are being manipulated or large pieces of equipment are being utilized. Another type of transfer area is a laminar flow hood. Air is forced into the unit through a dust filter then passed through a **HEPA filter.** The air is then either directed downward (vertical flow unit) or outward (horizontal flow unit) over the working surface. The constant flow of microbe-free filtered air prevents non-filtered air and particulate matter in the room from settling on the working surface. The simplest type of transfer area suitable for tissue culture work is an enclosed plastic box commonly called a glove box. This type of culture hood is sterilized by an ultraviolet light and wiped down periodically with 70% alcohol when in use. This type of unit is used when relatively few transfers are performed.

Culture Room: All types of tissue cultures should be incubated under conditions of well-controlled temperature, humidity, air circulation, and light quality and duration. These environmental factors may influence the growth and differentiation process directly during culture or indirectly by affecting their response in subsequent generations. Protoplast cultures, low-density cell suspension cultures, and anther cultures are particularly sensitive to environmental condition. Typically, the culture room for growth of plant tissue cultures should have a temperature between 15° and 30° C, with a temperature fluctuation of less than ±0.5° C; however, a wider range in temperature may be required for specific experiments. It is also recommended that the room have an alarm system to indicate when the temperature has reached preset high or low temperature limits, as well as a continuous temperature recorder to monitor temperature fluctuations. The temperature should be constant throughout the entire culture room (i.e., no hot or cold spots). The culture room should have enough fluorescent lighting to reach the 10,000 lux; the lighting should be adjustable in terms of quantity and photoperiod duration. Both light and temperature should be programmable for a 24-hr period. The culture room should have fairly uniform forced-air ventilation, and a humidity range of 20-98% controllable to ±3%. Many incubators, large growth chambers, and walk-in environmental chambers meet these specifications.

Low Cost Plant Tissue Culture Preparation Methods

Given certain basics there are many options for procedure, equipment and supplies for plant tissue culture. Some decisions will be based upon the amount of time and money available, others are merely a matter of personal preference. Catalogs, such as Sigma, Carolina Biological, or Edmund Scientific for are essential for reference or purchasing are very important resources.

OPTIONS The following discussion lists some of the various options you have as you begin the tissue culture of plants. Options used in in the sample protocol are identified with an asterisk (*).

Which plant?

Perhaps the easiest decision you need to make is which plant you would like to place in culture. To begin with I would suggest one of the following:

- Boston fern (runner tip)
- Rex Begonia {petiole segment}
- Kalenchoe (stem tip)
- Strawberry (runner tip)
- African violet , leaf part
- Arrowhead plant , stem tip
- Orchid plant , meristem tip
- Banana suckers-meristem

LOW COST ENERGY OPTIONS

Use of natural light Artificial lighting of cultures in the growth rooms is one of the most expensive and inefficient methods in tissue culture technology. The lights, chokes, fixtures, timer controls, equipment to handle high electrical load, and their operation and maintenance add to high costs. Moreover, artificial lighting generates heat that has to be dissipated by cooling and air-conditioning further adding to the electrical load. Although special fluorescent tubes are used to compensate for the red and far-red part of natural daylight, artificial light quality does not match that of natural light under which the plants are ultimately grown. Also, the cool fluorescent lights used for illumination provide minimal energy required for photosynthesis. As a result, in vitro plants adapt to low-light intensity, and have a reduced growth rate. Plants can adapt to a wide range of conditions by changing their metabolism and structures. They develop structural and anatomical features in leaves and stems, e.g. cuticle and wax on leaves, thickness of the leaf, fewer and closed stomata, and thickening of epidermal cells of the stems, that allow survival in the harsh environment. However, once they adapt to a set of conditions, re-adaptation to new conditions is rather slow or difficult. Plant tissues formed and adapted to low light conditions are usually fragile and may becomevitrified, leading to poor survival under field conditions. This can be a major disadvantage in the plant hardening process, and later establishment in the field. Under artificial light of low intensity, plants have low reserves, and a poor root system. On transfer to soil, the in vitro formed roots have to adjust to soil solutes of varying pH. The usual response of the in vitro formed roots is that they stop functioning in soil and new roots are formed, which take over the function of the original roots. If new roots do not emerge, the plant dies. One method to circumvent these negative effects is to culture the in vitro plants under natural light, during their last phase in liquid medium, based on half- or quarter-strength MS salts without sugar and vitamins, under either aseptic or non-aseptic conditions. If roots or root initials are not formed, the medium can be either supplemented with auxins (IAA, IBA), or shoots dipped in a solution of rooting hormones. This procedure provides much stronger and healthier plants with a high survival rate..

Changing the method of illumination from artificial to natural light is a decisive low cost option in tissue culture.This not only reduces electricity and capital costs, but also improves the plant quality. Expensive artificial lights can be

replaced in several ways. One option is to grow the in vitro cultures in diffused natural light under plastic or glass. This works very well in temperate climates, but under tropical conditions, heat build-up has to be reduced by installing thermostat-controlled exhaust fans.

Low cost temperature regulation :

Maintaining in vitro cultures at a regulated temperature with air conditioners adds to the cost but does not contribute to specific plant quality. In fact, as in the case of artificial lighting, plants grown under a narrow temperature range are at a disadvantage during hardening and later under the field conditions. Elimination of this factor significantly contributes to reduction in electrical costs. Contrary to the common belief that the day- and night-temperature in the growth room must be strictly controlled at an even level, many in vitro growing plants can tolerate wide fluctuations in temperature. High daytime temperatures of up to 28-30oC and as low as 10-12oC at night do not damage plant growth. On the contrary, fluctuations in temperature promote better growth. In vitro growing plants of banana and potato, kept at 16-41oC at 750 µmol m-2 s-1 under natural light, showed as good or better growth than in the controlled growth room

Reducing energy costs for water and autoclaving

Normally, distilled water is produced from water stills operated by electricity. Some water stills and autoclaving require a three-phase connection. For small facilities, it is prudent to operate the units on a single-phase electrical connection. In small units, tap water may be used after autoclaving rather than distillation. Pressure cookers heated with gas can also be used where capital is a constraint. In case of large-sized facilities, autoclaves and water distillation equipment operated on electricity is still the most economic.

Reducing energy in the glasshouse and hardening area

Electrical usage can be reduced significantly from hardening plants in open shade if glasshouses are not available. In vitro grown plants can be hardened by covering them with net-shades after transfer to soil or by placing containers in shade under thatched or plastic- covered open huts. The plants hardened under natural light are sturdy, and withstand transplantation better in the field. In the case of culture in plastic containers, the lids are removed, and containers are covered with a thin plastic sheet, and left for 3-4 days in shade. The medium is then removed by washing with tap water, and the plants are retained for another 3-5 days in the shade before transfer to soil.

REDUCING LABOUR COSTS

Production of plants based on tissue culture technology and their subsequent growing is a labour intensive system. In developed countries, labour is a major cost factor in micropropagation. On the other hand, in developing countries, labour is relatively less costly, which is a major advantage. Yet, in developing countries, increasing the efficiency of production is relevant to reduce the cost of tissue-cultured plants. In developing countries, the access to capital is not easy and borrowing is costly with high interest rates, which adds to cost of production. Hence, it is important to maintain high labour efficiency, especially when the operators in the culture transfer room are not highly trained. The typical profile of a tissue culture plant production system shows that 40% costs are for labour, 10% for materials, 20% for overheads, and 30% for sales, general and administrative activities. The manual transfer

of explants in culture vessels has the upper ceiling of production per operator at about 5000 operations or transfers per day. However, this peak rate is rarely achieved. In general, 2500 transfers per operator per day are considered to be good productivity, which is strongly influenced by the type of culture vessel used. For example, the transfers per person using test tubes are much lower than that in Petri dishes and wide mouth culture vessels. Once the efficiency of labour in transferring number of propagules per hour has been achieved to the maximum possible, there is little scope to improve efficiency unless automated or semi-automated systems are introduced. These are based on the use of liquid media and bioreactors, and mechanization to handle propagules. Such systems have been developed and can be integrated into the production under certain conditions (Osmoteck, 2000). Accordingly, such a system can reduce cost of production by 50% in a 20 million-unit production facility. The labour cost comes down from conventional to semi-automated model by 70% and in fully automated model by 75%. The system based on the use of plastic bags increased throughput per person and reduced labour cost per propagule by 60% . Also, the improvement in contamination control increased multiplication rate and improved survival during hardening and produced more saleable plants. In some cases, the multiplication rate increased from 1.5 to 5, reducing labour cost of tissue culture by 96% per plant before hardening. To reduce labour costs, such systems can be combined so that the multiplication phase is in the bioreactor and the last stages in plastic bags.

To maintain high efficiency of the operators under laminar flow hoods, they should work not more than 4 hours per day and preferably on a single bench; afterwards they should undertake other operations, such as washing, drying of containers or work in glasshouse. The provision of music in culture transfer rooms also increases the efficiency. Usually, female operators are more efficient than males in the transfer rooms and for potting of plants. This contributes to the gender based rural employment.

Reducing overhead costs

Overhead costs for commercial scale tissue culture-based plant production include salaries of managers - directors, scientists, marketing managers, market promotional expenses, payment of commissions, and perks to the management. Much of these cannot be avoided. The large-sized companies can afford high overhead costs that match large volume of business. However, smaller units have to rely on a few permanent managers, but may hire the expertise of the specialists as and when required.

EXPLANT SOURCE

Plant tissue cultures are initiated from tiny pieces, called explants, taken from any part of a plant. Practically all parts of a plant have been used successfully as a source of explants. In practice, the “explant” is removed surgically, surface sterilized and placed on a nutrient medium to initiate the mother culture, that is multiplied repeatedly by subculture. The following plant parts are extensively used in commercial micropropagation.

Shoot-tip and meristem-tip culture:

Shoots develop from a small group of cells known as shoot apical meristem. The apical meristem maintains itself, gives rise to new tissues and organs, and communicates signals to the rest of the plant. Shoot-tips and meristem-

tips are perhaps the most popular source of explants to initiate tissue cultures. The shoot apex explant measures between 100 to 500µm and includes the apical meristem with 1 to 3 leaf primordia. The apical meristem of a shoot is the portion lying distal to the youngest leaf primordium, and is ca.100µm in diameter and 250µm in length with 800-1200 cells. In practice, shoot-tip explants between 100 to 1000µm are cultured to free plants from viruses. Even explants larger than 1000µm have been frequentlyused. The term "meristem-tip culture" has been suggested to distinguish the large explants from those used in conventional propagation.

Nodal or axillary bud culture:

This consists of a piece of stem with axillary bud culture with or without a portion of shoot. When only the axillary bud is taken, it is designated as "axillary bud" culture. Floral meristem and bud culture: Such explants are not commonly used in commercial propagation, but floral meristems and buds can generate complete plants. Other sources of explants: In some plants, leaf discs, intercalary meristems from nodes, small pieces of stems, immature zygotic embryos and nucellus have also been used as explants to initiate cultures.

Cell suspension and callus cultures:

Plant parts such as leaf discs, intercalary meristems, - stem-pieces, immature embryos, anthers, pollen, microspores and ovules have been cultured to initiate callus. A callus is a mass of unorganized cells, which in many cases, upon transfer to suitable medium, is capable of giving rise to shoot-buds and somatic embryos, which then form complete plants. Such calli on culture in liquid media on shakers are used for initiating cell suspensions. Liquid suspension cultures maintained on mechanical shakers achieve fast and excellent multiplication rates. However, in commercial micropropagation, calli are cultured mostly in bottles and flasks kept on semi-solid or liquid media. To a limited extent, bioreactors have become popular for somatic embryogenic cultures. It is considered that some day robotics could be adapted to bioreactor- based micropropagation.

PROCESS OF MICROPROPAGATION

The process of plant micro-propagation aims to produce clones (true copies of a plant in large numbers). The process is usually divided into the following stages:

Stage 0- pre-propagation step or selection and pre-treatment of suitable plants.

Stage I - initiation of explants - surface sterilization, establishment of mother explants.

Stage II - subculture for multiplication/proliferation of explants.

Stage III – shooting and rooting of the explants.

Stage IV - weaning/hardening.

These stages are universally applicable in large-scale multiplication of plants. The individual plant species, varieties and clones require specific modification of the growth media, weaning and hardening conditions. A rule of the thumb is to propagate plants under conditions as natural or similar to those in which the plants will be ultimately grown ex-vitro.For example, if a chrysanthemum variety is to be grown under long day-length for flower production, it is better

to multiply the material under long-day length at stages III and IV. There is a wide option to undertake production of plant material up to a limited number of stages. For example, many commercial tissue culture companies undertake production up to Stage III, and leave the remaining stages to others.

Pre-propagation stage

The pre-propagation stage (also called stage 0) requires proper maintenance of the mother plants in the greenhouse under disease- and insect-free conditions with minimal dust. Clean enclosed areas, glasshouses, plastic tunnels, and net-covered tunnels, provide high quality explant source plants with minimal infection. Collection of plant material for clonal propagation should be done after appropriate pretreatment of the mother plants with fungicides and pesticides to minimize contamination in the in vitro cultures. This improves growth and multiplication rates of in vitro cultures. The control of contamination begins with the pretreatment of the donor plants. They may be prescreened for diseases, isolated and treated to reduce contamination. The explants are then brought to the production facility, surface sterilized and introduced into culture. They may at this stage be treated with antibiotics and fungicides as well as anti-microbial formulations, such as PPM. The explants are then culture indexed for contamination by standard microbiological techniques, which are occasionally supplemented with tests based on molecular biology or other techniques.

Stage I

This stage refers to the inoculation of the explants on sterile medium to initiate aseptic culture. Initiation of explants is the very first step in micropropagation. A good clean explant, once established in an aseptic condition, can be multiplied several times; hence, explant initiation in an aseptic condition should be regarded as a critical step in micropropagation. More than often, explants fail to establish and grow, not due to the lack of a suitable medium but because of contamination. The explants are transferred to in vitro environment, free from microbial contaminants. The process requires excision of tiny plant pieces and their surface sterilization with chemicals such as sodium hypochlorite, ethyl alcohol and repeated washing with sterile distilled water before and after treatment with chemicals. After a short period of culture, usually 3 to 5 days, the contaminated explants are discarded. The surviving explants showing growth are maintained and used for further subculture. In herbaceous plants e.g. potato, chrysanthemum, carnation, streptocarpus, strawberry, and African violet; the explant sources are meristems, apical- and axillary buds, young seedlings, developing young leaves and petioles, and unopened floral buds. The following low cost options can be adapted to initiate explants:

Sterile instrument technique

This method assumes that most of the deep-seated meristems and those covered by leaves or other integuments (e.g. floral bracts) are sterile. In this procedure, the explant is washed with sterile water, rinsed in ethanol, and instruments are sterilized every time they touch the surface of the explant, and the explant is moved to a new location on the dissection stage. Surface sterilization technique This is by far the most commonly used method. The explants are washed in sterile water, rinsed in ethanol, and surface sterilization is achieved by using chemicals with chlorine base. Calcium or

sodium hypochlorite based solutions, 1-3% (v/v) are usually used for soft herbaceous materials. A cheap and ready-made sterilant is 5-7% solution of 'Domestos'- a toilet disinfectant which contains 10.5% v/v sodium hypochlorite, 0.3% sodium carbonate, 10.0% sodium chloride and 0.5% (w/v) sodium hydroxide and a patented thickener). The explants are washed in sterile distilled water before and after sterilization. Other surface sterilants used include mercuric chloride (avoid its use as far as possible, since it is highly toxic), hydrogen peroxide, and potassium permanganate.

For soft tissues

1. Wash explants from perennial plants for 1-2 hr in tap water. Eliminate this step for material from glasshouse grown plants.
2. Wash in sterile distilled water three to four times for 5 to 10 minutes each.
3. Dip in 95% ethanol for 3 to 5 seconds.
4. Wash once again with sterile distilled water for 5 minutes.
5. Surface-sterilize in 5% 'Domestos' (v/v) for 20-25 minutes.
6. Wash with sterile distilled water three times for 10 minutes each.
7. Drain water droplets by placing on pre-sterilized blotting paper.
8. Transfer explants singly to the medium. N.B.: Sterilize forceps each time to transfer explants to avoid cross-contamination.

For woody stems (e.g. roses, hardy shrubs, and trees):

1. Collect stems, shoots, buds and store at 5 0C till needed.
2. Rinse in ethanol for 3 to 5 seconds.
3. Rinse in 1-% sodium hypochlorite (20% bleach) for 10 minutes.
4. Place lower parts of stems in flasks in 2% sucrose and 200 PPM 8-hydroxyquinoline citrate at 23+2 0C. For items collected in September/October, add 50-PPM GA3. After that l0 PPM GA will help break the dormancy.
5. Re-cut the bottom of stem and replace the solution after 2 days.
6. Excise the softwood from the developed shoots and use material for explants or for rooting.
7. Surface-sterilize as in the above protocol.

Do not forget to sterilize forceps and scalpel every time for the transfer of explants to fresh solutions. Use sterile containers in the protocol of surface sterilization. If explants become brown or pale at the end of the protocol, reduce the strength of 'Domestos' to 2.5%. Alternatively, dip explants in 10% 'Domestos' for 2 minutes and then proceed to surface sterilize with 3-5% 'Domestos' for 20 minutes. If basal contamination is observed after 2-3 days of culture, explants can sometimes be rescued by removing the basal end by making a single cut with a sharp scalpel and re-culturing on fresh medium.

Stage II

Stage II is the propagation phase in which the explants are cultured on the appropriate media for multiplication of shoots. The primary goal is to achieve propagation without losing the genetic stability. Repeated culture of axillary and adventitious shoots, cutting with nodes, somatic embryos and other organs from Stage I leads to multiplication of propagules in large numbers. The propagules produced at this stage can be further used for multiplication by their repeated culture. Sometimes it is necessary to subculture the in vitro derived shoots onto different media for elongation.

Stage III Thein vitro shoots obtained at Stage II are rooted to produce complete plants. If the proliferated material consists of bud-like structures (e.g. orchids) or clumps of shoots (banana, pineapple), they should be separated after rooting and not before. Many plants (e.g. banana, pineapple, roses, potato, chrysanthemum, strawberry, mint, several grasses and many more) can be rooted on half-strength-MS (Murashige and Skoog, 1962) medium without any growth-regulators. Good sturdy well-rooted plants are essential for high survival during weaning and later transfer to soil. This stage is labour intensive and expensive. The process of in vitro rooting has been estimated to account for approximately 35–75% of the total cost of production. Efforts should be made to combine rooting and acclimatization stages.

Stage IV

At this stage, the in vitro micropropagated plants are weaned and hardened. This is the final stage of the tissue culture operation after which the micropropagated plantlets are ready for transfer to the greenhouse. Steps are taken to grow individual plantlets capable of carrying out photosynthesis. The hardening of the tissue-cultured plantlets is done gradually from high to low humidity and from low light intensity to high intensity conditions. If grown on solid medium, most of the agar can be removed gently by rinsing with water. . Plants can be left in shade for 3 to 6 days where diffused natural light conditions them to the new environment. The plants are then transferred to an appropriate substrate (sand, peat, compost, etc.), and gradually hardened. Low-cost options include the use of plastic domes or tunnels, which reduces the natural light intensity and maintains high relative humidity during the hardening process. If the plants are still joined together after rooting, these should be planted as bunches in the soil and separated after 6 to 8 weeks of growth.

DELIVERY TO THE GROWERS

The delivery of the rooted and hardened small micro-propagated plants to growers and market requires extra care. In some cases, plant losses can occur during shipment and handling by growers. This is particularly true when the plants are not fully hardened and rooted or not grown for sufficient duration after transfer to soil. Growers should be given clear instructions how to handle the material provided. Apart from the economic loss, poor survival of planted material erodes the confidence of growers in the technology. The transfer of individual plants to soil in black plastic or polythene bags is widely used as a low-cost option to provide fully-grown banana plants directly to farmers in many developing countries.

PHYSICAL COMPONENTS OF TISSUE CULTURE TECHNOLOGY

The physical components of a typical plant tissue culture facility include equipment and buildings with preparation room, transfer room, culture or growth room, hardening and weaning area, soil-growing area (greenhouses, plastic tunnels), packaging and shipping area, and related facilities –office and store for chemicals, containers and supplies. Careful planning is the first important step when considering the size and location of a facility. The size of the physical components of a tissue culture facility will vary according to its functional needs – the

volume of production. It is recommended that an existing facility should be visited to view the layout and operational needs before starting a new facility. A number of low-cost alternatives can be used to simplify various operations and reduce the costs in a tissue culture facility.

INTRODUCTION

In designing any laboratory, big or small, certain elements in its design and layout are absolutely essential for its successful operation. Correct design of a laboratory will not only reduce contamination, but also achieve a high efficiency in work performance (Bridgen and Bertok, 1997). Properly planned and designed laboratories can reduce both the operational and energy costs. A tissue culture laboratory must be designed to accommodate the equipment and its use in the various stages of micropropagation in the most efficient manner. This chapter describes planning the functional needs of an average-sized micropropagation facility. The information given below would allow better planning and stream-lined functioning of the facility, and thereby lead to cost reduction.

General consideration for location A convenient location for a small laboratory can be a room or part of the basement of a house, a garage, a remodeled office or a room in the header house. The minimum area required for media preparation, transfer and primary growth shelves is about 14 m2. Walls should be installed to partition different areas. Before setting up a commercial micropropagation unit, it is essential to check out the area keeping in mind the climate, and access to water, electricity, transportation, and infrastructure for supplies. A temperate climate is usually better suited to tissue culture ventures. This greatly reduces the cost of cooling required to maintain the temperature for optimum growth of the cultures. The availability of electricity and water is of utmost importance, and should be taken into consideration while choosing the location of the facility. For example in India, of the 76 commercial tissue culture units, nearly 52 are located in and around the cities of Bangalore and Pune, where the climate is moderate. Hence, cooling is required only during certain periods of the year. However, in cities like Delhi, which have extremes of climate, tissue culture facilities require both heating and cooling. In a facility, which produces five million plants, the electricity cost per thousand plants is around US $0.30 in Bangalore and Pune; the same is about US $0.80 in Delhi. Disruption of power and water supply causes major breakdown in the smooth running of tissue culture units. Poor quality water adds to the cost of media.Location consideration should also include a check with local authorities about zoning and building permits before construction begins. The buildings should be located away from sources of contamination such as a gravel driveway, parking lot, soil mixing area, shipping dock, pesticide storage, and dust and chemicals from fields. Sanitation of the area is important in selecting location. The units should be located in areas where insect populations are minimal and air has low dust and pollen counts. In the case of export-oriented units, these should be near an international airport, which will reduce the time lag between packaging and shipment. This is critical to assure timely delivery of quality tissue culture products. For new ventures, the size of the facility should be kept small until the market acceptance is ensured.

Design and lay out of thelaboratories

Plant tissue culture laboratories have specific design requirements. Careful initial planning is, therefore, a prerequisite for successful running of a facility. The location and design of the laboratories should take into account isolation from foot traffic, control of contamination from adjacent rooms, thermostatically controlled heating and cooling, water supply and drains for a sink, adequate electrical service, provisions for a fan and intake blower for ventilation, and good lighting. Large sized facilities are frequently built free standing. Although more expensive to build, the added isolation from adjacent activities keeps the laboratory clean. Prefabricated buildings make convenient low-cost laboratories. They are readily available in various sizes in many countries. Prefabricated buildings assembled on site can also be used. A single span building allows for a flexible arrangement of walls for dividing into convenient sized rooms. The floor should be of concrete or capable of carrying 170.5 kg/m2 (50 pounds per sq. foot). Walls and ceiling should be insulated to at least R-15, and covered inside with water-resistant material. Windows, if desired, may be placed wherever convenient in the media preparation and glassware washing rooms. The heating system should be capable of maintaining room temperature at 200C during the coldest part of winter. A minimum of 2cm pipes should be used for water supply. Connection to a septic system or sanitary sewer should be provided. Air conditioning requirements should carefully estimated. Electrical service capacity for equipment, lights and future expansion should be calculated. For safety reasons, the electrical installation should be carried out professionally. Most electrical wiring will require 220 Volts, and autoclaves 230/250 Volts.

The areas such as the media preparation room, inoculation room and growth chambers should be isolated as 'clean zones'. The office, storage area, staff centre and packaging rooms can be maintained under ordinary conditions. The working areas must be demarcated according to the activities involved in the facility. Cleanliness is the major consideration when designing a plant tissue culture laboratory to minimise contamination. A positive pressure module should be installed to circumvent air intake from outside. Routine cleaning and aseptic procedures can decrease contamination losses to less than 1%. An enclosed entrance should precede the laboratories, and sticky mats should be placed to collect dirt from shoes.

The traffic pattern and workflow in the laboratory must be considered to maximise cleanliness. The cleanest rooms or areas are the culture room (aseptic transfer area) and the growth room. There should be no direct access to these rooms from outside. The media preparation area, glassware washing and storage areas should be located away from these rooms. The growth room and aseptic transfer rooms should be fitted with see-through doors and should be adjacent to each other. Traffic through these areas should be minimal and restricted to the personnel working under laminar flow cabinets. Ideally, the media preparation area leads into the sterilisation area, which leads into the aseptic transfer room, and eventually to the growth room. Temperature and fire alarms must be connected directly to telephone lines to give fast warnings. An emergency generator should be available to operate essential equipment during power breakdown.

ESSENTIAL EQUIPMENT

The basic equipment in most tissue culture facilities includes the following:

Autoclave An autoclave is basically a large-sized but sophisticated pressure cooker, and is used for the sterilisation of the medium, glassware and instruments. Autoclaves of different sizes are available commercially. High-pressure heat is needed to sterilise media, water, and glassware. Certain spores from fungi and bacteria are killed only at 1210Cand 1.05kg/sq.cm (15 pounds per sq. inch) pressure. Self-generating steam autoclaves are more dependable and faster to operate.

Laminar airflow chamber The laminar flow chambers provide clean filtered air that allows cultures to be handled under contamination-free environment. Several types of laminar flow chambers are sold on the market and are available in different sizes. The laminar-flow cabinets are located in the culture transfer area. Some large-sized laboratories have sterile rooms in addition to laminar flow cabinets.

Other equipment

The sterilisation of instruments, such as the forceps, scalpel holders and blades is achieved with either gas flamed burners or with glass-bead sterilizers. The medium preparation room usually has the following equipment. A refrigerator-freezer to store chemicals and stock solutions, weighing scales for large amounts of over 10 g, and an analytical balance with1 mg accuracy, a magnetic stirrer for the agitation, and a pH meter. Small laboratories may locate the refrigerator under the workbench to save space. High quality balances are essential in a tissue culture laboratory. Most laboratories have top loading balances, which allow quick and efficient weighing. A hot plate with an automatic stirrer is needed to for preparing the media before autoclaving. The pH meter is needed to determine pH of the media. Some laboratories use pH indicator paper, however this method is considerably less accurate, and can severely affect the results. An aspirator can be attached to a water tap for filter sterilisation of chemicals and for surface sterilisation of the plant material. However, vacuum pumps are faster and more efficient, but also more expensive.

A drying oven is required to keep glassware such as beakers, flasks and cylinders, and is also useful for dry sterilisation of scalpels and glassware, such as Petri dishes, pipettes andothers. The media containing carbon sources (e.g. sugars) and growth regulators are sterilised in the autoclave, but sometimes, aseptic filtration is better to avoid breakdown of heat-labile chemicals. The water still is also located in the medium preparation area. To prepare media, distilled or de-ionised water is generally used, although tap water can be used in some cases.

OPTIONAL EQUIPMENT

A variety of non-essential equipment is used in tissue culture laboratories. The specific requirements determine what need to be purchased. Microwave ovens are convenient for defrosting stock solutions and pre-heating agar media. Most laboratories have a dissecting microscope to excise small explants. Laboratory glassware washers or regular dishwashers can be used for replacing manual labour. Automatic media dispensers are helpful to pipette pre-set volume of media. A gyratory shaker or a reciprocal shaker is necessary if micropropagation is based on liquid media or suspension

cultures. Computers, photocopiers and fax machines are helpful for easy data management and maintenance of records. Some of the equipment may be costly, but goes a long way in saving time and labour and is essential for rapid communication in the competitive world.

ACTIVITY SPECIFIC REQUIREMENTS:

Based on the different activities of a tissue culture, a facility can be divided into semi-clean, clean and ultra-clean areas. The semi-clean areas comprise of the washing room, office and staff restrooms, where there is no need for maintaining sterile conditions. The clean areas encompass the media preparation and sterilisation rooms, which have to be sufficiently clean. High sterility has to be maintained in the culture transfer rooms and the growth rooms, which constitute the ultra-clean areas.

Glassware washing and storage area

The glassware washing area should be located near the sterilisation and medium preparation rooms. This area should have at least one large sink but two sinks are preferable. Adequate workspace is required on each sides of the sink; this space is used for glassware soaking and drainage. Plastic netting can be placed on surfaces near the sink to reduce glassware breakage and enhance water drainage. The outlet pipe from the sink should be of PVC to resist damage from acids and alkalis. Both hot and cold water should be available and the water still and de-ionisation unit should be located nearby. The choice of electrical washers should be based on the projected use, durability, reliability and cost, and service availability. In India and some other developing countries, where labour is relatively cheap, washing is done manually. The washing room should be swapped periodically. Mobile drying racks can be used and lined with cheesecloth to prevent water dripping and loss of small objects. Ovens or hot air-cabinets should be located close to the glassware washing and storage area. Dust-proof cabinets and storage containers should be installed to allow for easy access to glassware.When culture vessels are removed from the growth area, they are often autoclaved to kill contaminants and to soften semi-solid media. It should be possible to move the vessels easily to the washing area. The glassware storage area should be close to the wash area to expedite storage and access for media preparation.

Media preparation and sterilisation area

The media preparation room should have smooth walls and floors, which enable easy cleaning to maintain a high degree of cleanliness. Minimum number of doors and windows should be provided in this room but within the local fire safety regulations. This reduces cost and contamination. The media preparation and sterilisation can be carried out in the same area but preferably in different rooms, which need not be separated with doors. Media preparation area should be equipped with both tap and purified water. An appropriate system for water purification must be selected and fitted after careful consideration of the cost and quality. A number of electrical appliances are required for media preparation; hence, it is essential to have safety devices like fire extinguisher, fire blanket and a first aid kit in the media preparation room. A variety of glassware, plastic ware and stainless steel apparatus is required for measuring, mixing, and media storage. These should be stored in the cabinets built under the worktables and taken out for

use as and when required. This would save the cost and space for building storage shelves. The use of glassware should be kept at a minimum, as it will help in reducing losses due to breakage. As far as possible, plastic ware and stainless steel vessels should be used, as they are much cheaper and more durable than glassware. The water source and glassware storage area should be in or near the medium preparation area. Work bench tops, suitable for comfortable working while standing should be 85 to 90 cm high and 60 cm deep. The workbench tops should be made with plastic laminate surfaces that can tolerate frequent cleaning.

Sterilisation room

The sterilising room should be in continuation with the media preparation room. The layout must be planned in such a way that it ensures the smooth movement of the containers from the washing to the media preparation and sterilisation room. The sterilisation room must have walls and floors that can withstand moisture, heat and steam. An exhaust should be fitted to remove the warm and moist air. The exhaust fan should have an outer cover to prevent entry of outside air. The fan cover should open only when the fan is in operation. In small tissue culture facilities, costly autoclaves can be replaced by simple pressure cookers. However, for large volume media making, horizontal or vertical autoclaves should be installed. Double door autoclaves, which open directly into the media storage room, may be costly but reduce contamination. A cheaper alternative is to transfer the sterilised media to the adjoining room through a hatch window.

Transfer room The most important work area is the culture transfer room where the core activity takes place. The transfer area needs to be as clean as possible and be a separate room with minimal air disturbance. Walls and floors of the transfer room must be smooth to ensure frequent cleaning. The doors and windows should be minimal to prevent contamination, but within local safety code. There is no special lighting requirement in the transfer room. The illumination of the laminar airflow chamber is sufficient for work. Sterilisation of the instruments can be done with glass-bead sterilizers or flaming after dipping in alcohol, usually ethanol. The culture containers should be stacked on mobile carts (trolleys) to facilitate easy movement from the medium storage room to the transfer room, and finally to the growth room. The chair seats of the transfer operators should be comfortable, as they have to work for long periods in the same place. Fire extinguishers and first aid kits should be provided in the transfer room as a safety measure. The personnel should leave shoes outside the room. Special laboratory shoes and coats should be worn in this area. Ultraviolet (UV) lights are sometimes installed in transfer areas to disinfect the room; these lights should be used only when people and plant material are not in the room. Safety switches can be installed to turn off the UV lights when regular room lights are turned on.

Growth room

Growth room is an equally important area where plant cultures are maintained under controlled environmental conditions to achieve optimal growth. It is advisable to have more than one growth room to provide varied culture conditions since different plant species may have different requirements of light and temperature during in vitro culture. Also, in the event of the failure of cooling or lighting in one room, the plant cultures can be moved to another

room to prevent loss of cultures. In the growth room, the number of doors should be minimal to prevent contamination. There is no need for windows in the growth room, except when natural light is used. When artificial lighting is used, the external light can interfere with the photoperiod and temperature of the growth room. Depending on the amount of available space and cost, the culture containers can be placed on either fixed or mobile shelves. Mobile shelves have the advantage of providing access to cultures from both sides of the shelves. The height of the shelves should not exceed 2m. High shelving requires step-up stools to place and remove cultures, being dangerous and time consuming. The primary source of illumination in the growth room is normally from the lights mounted on the shelves. Overhead light sources can be minimised, as they would be in use only while working during the dark cycle. Plant cultures may not receive uniform light from the conventional downward illumination. Lights directly fitted to the racks create uneven heat distribution. This leads to high humidity within the culture containers, which in turn can cause hyperhydricity. Sideways illumination is an alternative, which requires less number of lights, and provides more uniform lighting. But care has to be taken not to break the lights while moving the cultures across the shelves.

CONTROL OF GROWING CONDITIONS

Controlled temperature, lighting and relative humidity, and shelving need to be considered in planning the growth room. These vary depending on the size of the growth room, its location, and the type of plants cultured. For example, a small growth room located in the cool North American climate can be placed in an unheated or minimally heated basement. The chokes (ballasts) of the fluorescent lights need not to be separated; rather they can serve as a source of heat. Excess heat can be dissipated from the growth room, and used for heating other areas in the basement. In such a situation, solid wooden shelves with space between shelves can be used and prevent culture vessels on shelf above the lights from becoming over-heated. However, a large growth room located above ground needs to have the light chokes installed outside the room. Shelves in large growth room can be of glass or metal wire mesh.

Temperature control

Temperature is a primary concern in growth rooms; it affects decisions on installation of lights, control of relative humidity, and type of shelving. Temperature in the growth room is usually controlled with air conditioners. Generally, temperatures are kept around 220C. Heating is provided from conventional heating systems and can be supplemented with heat from light chokes. In most developing countries, cooling the growth room is usually a bigger problem than heating. Cooling can be provided with heat pumps, air conditioners and exhaust fans. Using open windows to cool culture rooms leads to contamination during summer and humidity problems in winter.

Lighting control

Some plant cultures can be kept in complete darkness; however, most culture rooms need to be illuminated at 1 Klux [134.5 µmole/m2/s (microeinsteins per second per sq. centimetre or approximately 1076 foot candle) with some up to 5 to 10 Klux (672-1345 µmole/m2/s). The plant species and/or propagation scheduling determines the light intensity. The developmental stage of the

plants also determines if wide spectrum or cool white-fluorescent lights are to be used. Rooting is strongly influenced positively with far-red light; therefore, wide spectrum lights should be used during Stage III and cool-white lights during Stage I and II. Automatic timers are needed to maintain the desired photoperiod. Reflectors can be placed over bulbs to direct the light downwards and evenly. Heat generated by lights may cause condensation and temperature problems. Small fans placed at the end of the shelves increase airflow and decrease heat build-up. Reflective glossy paint on the walls provides an even light distribution as well as reduces the number of lights required. Relative humidity (RH) is difficult to control inside the culture vessels, but wide fluctuations in the growth room 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 too high, a dehumidifier is recommended.

Shelving

Shelves within the growth rooms vary depending upon the situation and the plants grown. Frames for the shelves can be made from 1.25cm (half-inch) thick angle iron. Shelves built from rigid wire mesh to allow maximum air movement and minimise shading should be used. Wood is inexpensive to build shelves. The wood for shelving should have smooth exterior, and should be painted white to reflect light. Expanded metal is more expensive than wood, but provides better air circulation. Tempered glass is sometimes used for shelves to increase light penetration, but it is more prone to breaking. Air spaces of 5 to 10cm between the lights and shelves decrease heat on upper shelves and reduce condensation in culture vessels. A room that is 2.4m high will accommodate 5 shelves, each 45cm. apart, when the bottom shelf is 10 cm above the floor.

GREENHOUSE FACILITY

A critical stage in plant tissue culture is the interim phase between the laboratory and field conditions. In vitro derived plants need to be gradually hardened to field conditions. Plant hardening is usually carried out under greenhouse that ensures high survival of the tissue-cultured plants in the field. There are three types of greenhouses: Ground to ground, Gable, and Quonset type. The most commonly used greenhouse is the Quonset type. It contains movable or fixed benches with hardening tunnels on them. The size of the greenhouse must be based on the scale of production. Greenhouse glazing can be of glass orfibreglass. Polyethylene films or sheets of polycarbonate or acrylic can also be used. Air inflated double polyethylene covering is the most economic). Appropriate light, shading and blackout systems can be achieved with supplementary lighting. Drip irrigation systems, misting and fogging can be installed as needed. Greenhouses erected in warm climates should have fan-assisted drip pad cooling especially during summer. Greenhouses in colder climates need to be heated. Floor and bench systems can be used for heating and cooling the air. Low cost plastic pipes can be used to circulate warm air, which are adequate and cost effective.

PACKAGING AND SHIPPING

A separate area should be designated for packaging in a commercial tissue culture unit. Packaging materials such as cardboard cartons and labels should be stored in this area. The type of packaging of a particular plant depends

greatly on the temperature zones through which the consignment has to pass from the point of shipment to its destination. For example, if the consignment of tissue-cultured plants is passing anywhere within temperate zones, it is only necessary to take care of the frost conditions and pack accordingly. But if the consignment is passing from a tropical country to a temperate country, it becomes necessary to take care of the temperature zones in different places through which the consignment passes. Thus, the type of packaging of tissue-cultured products varies with plant and destination. For example, in a package of 22 kg, about 40,000 lily plantlets can be packed. However, in the same parcel, only 20,000 Spathiphyllum plantlets or 10,000 Syngoniumplantlets can be packed because of the higher respiration rates in these species. The heat build-up is much more in Spathiphyllum and Syngonium plants than the dormant lilies. Similarly, only 8000 Gerbera or 7000 Cordyline plants can be packed in the same package. Cooling material such as ice, dry ice and other material take up much of the packaging space.

Before loading and shipping, the packed items should be properly counted and rechecked. The cartons containing the cultures for shipping to the customer should be properly labelled with the names and addresses of the consignor and the consignee, and the details of the commodity (storage temperature, handling, etc.). Adequate care must be taken while packaging large consignments, so that there is no disturbance or damage during transit. To prevent any sort of delay, ensure that the consignment is accompanied by documents such as invoice, packing list, import permit, phytosanitary certificate and Generalized System of Preferences (GSP).

OFFICE AND STAFF AREA

In any commercial tissue culture unit, an office is a prerequisite to manage the facility. The office should be large enough to accommodate a filing cabinet, computer, a photocopier and a fax machine for keeping records of cultures, shipments and to handle correspondence. Other activities like maintaining inventory, ordering fresh supplies and accounting can also be done in the office area. In a moderately sized production unit, office area can also double as reception area. The employee must be provided with changing room and rest room (washroom). The latter are absolutely essential in the event of spills. Tables and chairs must be provided for tea and lunch breaks outside the laboratory area. The staff area must also have lockers, fire extinguishers and first-aid supplies. Food and drink vending machines can also be provided.

COST OF FACILITY

The building of a tissue culture facility includes the cost of land, construction, electrical installation and plumbing. If the available funds are limited, well-designed laboratories can be established by modifying existing structures. For example, a two or three room house or a trailer with appropriate modifications can be converted into a medium sized micropropagation facility. Depending on the production and storage capacity, a facility can be small- less 100,000 plants, medium- 100,000 to 500,000 plants, or large-scale- 500,000 to 2,000,000 plants per annum.

The initial investment required for setting up a low cost, medium scale tissue culture laboratory (ca. 195 m2) in India is given in Table. Land is sold at a premium, especially near large cities. In India, the land and building costs work out to about US $62500. A house, ca. 195 m2, can be converted into a

tissue culture facility with a capacity of 200,000 plants. The rental charges for such a structure would be around US $5000 (Rs 2,25,000) per year. The initial investment would thus include only plumbing and electrical work, reducing the capital cost to US $2750 (Rs 123 750). Thus, in large cities, it would be more economic to rent than build the premises (Table). If the hardened tissue-cultured plants were the end products, an additional investment of US $20,000 (Rs9.00,000) would be required to erect a greenhouse. If the production is limited to-in-agar and ex-agar products (rooted or non-rooted micro-cuttings), greenhouse would not be required.

Table. Cost of setting up a tissue culture facility in India :

Requirement Cost	(US $)	(Rs.lacs)	(US $)	(Rs.lacs)
	Rented building		Purchased building	
Infrastructure	2,750	123,750	62,437	28,09,665
Equipment	13,885	624,825	13,885	624,825
Accessories	4,193	188,685	4,193	188,685
Furniture	7,333	329,985	7,333	329,985
Miscellaneous	521	23,445	521	23,445
Total	**28,682**	**1289,790**	**88,369**	**39,76,605**

The basic equipment will cost US $14000. The consumables, such as glassware, chemicals and disposable items would require another US $4200. Besides the fixed capital, a commercial tissue culture unit has the recurring expenses of rent, building maintenance, electricity and overheads. The estimated cost of furniture that includes racks with illumination source for growth room, cupboards, benching and chairs would be US $7400. Other miscellaneous items such as labelling and sealing machines would cost US $50.
For small scale facility of operation this could be brought to almost half the cost of the medium scale investment meaning with a total capital outlay not exceeding USD –17,700 or Rs. 800,000 exclusive of the green house.

Scale of operation

The physical components required for the functioning of a commercial tissue culture laboratory can be either scaled up or down according to the interests of the propagator. The correct design of a laboratory, big or small, will help maintain asepsis, thereby increasing the efficiency of the unit and achieving a high standard of work. A micropropagation company in India converted a three-room apartment into a medium-sized tissue culture laboratory.
Plant such as Spathiphyllum,Syngonium,Ficus, hosta, calla lily, gerbera, and cordyline, were produced on a commercial scale. Delivery of the tissue-cultured plants to the tune of 2.5 million per year has been

made from this unit by adopting various low cost alternatives. Similar tissue culture facilities, aptly called 'bio-factories', in villages of Cuba produce up to one million banana plants and 2.5 million sugarcane plants annually.
Significant cost reduction for large-scale production can be achieved with automation and mechanisation. Although, automation of certain steps of micropropagation has been investigated for the past 20 years, its commercial use has not been adopted. The capital costs of such automated systems have prevented their application.
Thanks.

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