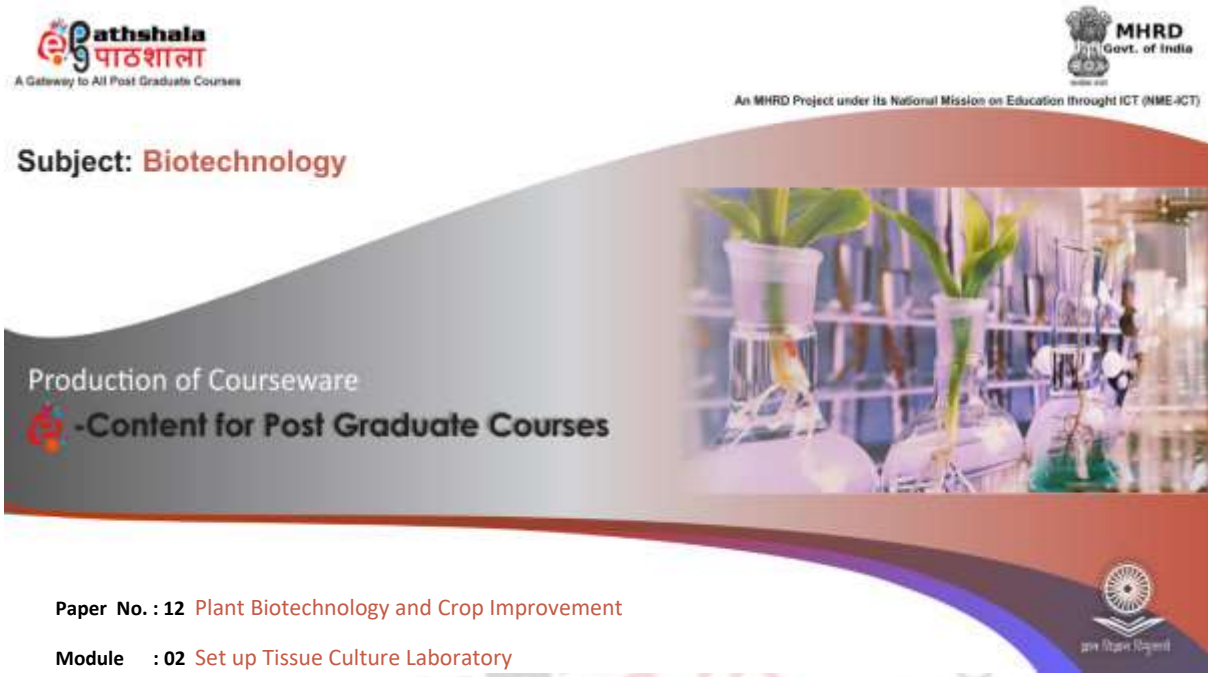

Paper No. : 12 Plant Biotechnology and Crop Improvement

Module : 02 Set up Tissue Culture Laboratory

Principal Investigator:	Dr Vibha Dhawan, Distinguished Fellow and Sr. Director The Energy and Resources Institute (TERI), New Delhi
Co-Principal Investigator:	Prof S K Jain, Professor, of Medical Biochemistry Jamia Hamdard University, New Delhi
Paper Coordinator:	Dr Vibha Dhawan, Distinguished Fellow and Sr. Director The Energy and Resources Institute (TERI), New Delhi
Content Writer:	Dr Vibha Dhawan, Distinguished Fellow and Sr. Director The Energy and Resources Institute (TERI), New Delhi
Content Reviewer:	Dr Nidhi P Chanana, Fellow The Energy and Resources Institute (TERI), New Delhi

Description of Module	
Subject Name	Biotechnology
Paper Name	Plant biotechnology and crop improvement
Module Name/Title	Set up Tissue Culture Laboratory
Module Id	02
Pre-requisites	
Objectives	
Keywords	

Setting up a Tissue Culture Laboratory

Table of contents

Learning objective

For any tissue culture work a well equipped laboratory is required, depending on the size and nature of operations, the requirement may vary both in terms of size and investments.

In this module, you will learn about the basic infrastructure that is required for research or a commercial laboratory. The laboratory consists of an area for storing glassware, plasticware and chemicals; an area where washing of used glassware / plasticware can be done; area for drawing of washed vials; media preparation area where equipment and chemicals used for media preparation are kept; autoclave for sterilization; Inoculation room and laminar air flow cabinet where aspectic manupulations are carried out; growth rooms for maintaining cultures and an area for acclamatization of plantlets are required.

Tissue culture laboratory, whether for research or for commercial purpose, must have provision for certain basic facilities. The laboratory should consist of:

1) Store/storage facilities for keeping glassware & chemicals
2) Washing area
3) Area for media preparation
4) Autoclave for sterilization
5) Media Store
6) Laminar air flow cabinets for aseptic manipulations
7) Growth rooms for maintaining cultures under controlled conditions of light and temperature
8) Area for observations of cultures
9) Transfer area
10) Acclimatization

1. Store/storage facilities for keeping glassware & chemicals

Plant tissue culture requires large numbers of chemicals; variety of glassware and plasticware and inventory of the same needs to be maintained. Some of the chemicals are not available locally and are thus imported calling for maintaining adequate stock. Most laboratories work out a reorder level to remain well equipped and not suffer from any delays. Like any other store, shelves / almirahs are integral part. Also, some of the chemicals are to be stored at low temperature and thus provision for double door refrigerator/ deep freezer is made.

2. Washing area

The washing area should have adequate supply of good quality running tap water. Depending upon the size of the laboratory, washing area can either have separate tanks for soaking or used glass ware/ plasticware or may manage with plastic buckets / tubs. Many laboratories opt for glassware washing machine but most prefer to go for manual as the volumes that can be washed in automatic machines are too small and energy costs are very high. Even countries in the west, where manpower cost are comparatively high, preference is given for disposable vessles than cleaning them with automatic machines. In many laboratories where volumes are reasonable, soaking followed by washing with brushes is followed. While laboratories having large volumes, brushes on the motor are employed. The first step in washing involves dipping in mild acid solutions (not always essential) followed by dipping in soap solution; manual / brushing on the motor washing; washing with tap water and final rinse with RO / good quality water.

Depending on the location,ground water / potable water can be used for washing but final rinse is to be given with RO / deionized water. This is essential as any residue sticking to the glassware of the water such as detergent, inorganic or organic salts can have determinatal impact on growing tissue. For protoplast / molecular biology experiments, final rinse is to be given with mili-Q water. However, this being expensive is restricted to only the glassware which is to be used for sophisticated high precision research experiments.

In many laboratories hot air ovens are kept to dry glassware but commercial laboratories avoid this step and washed glassware is kept upside down atleast overnight.

It is desirable to have washing area separated from the media preparation area. The two areas, however, should be linked.

3. Area for media preparation

Media room can be considered as the kitchen of the tissue culture facility. Usually, it consist of a working table in the centre and benches along the walls. The tops of the working table is usually covered with wood / laminated board. Use of granite / stone is avoided as it leads to brackage of glassware with minimal of mishandling. The height of the tables and benches should be suitable for working while standing as well as sitting on stools. The benches are required to keep small equipments such as balances (top pan macro balance and analytical micro) for weighing chemicals; pH meter; magnetic stirrer; hot plate; media dispenser; gas stove; etc. Above the benches, shelves are kept to store chemicals which are used for media preparation. Inside the media preparation room, provision is made for keeping either a double door refrigerator or a refrigerator and a deep freeze. Depending on the size of the laboratory either an autoclave is kept inside the media room or provision for separate media sterilization room is made.

For accurate measurement of various constituents of culture media, balances are required. Top pan balance is used for measuring larger quantities of chemicals (in gms), including inorganic salts; sugar; agar, etc. Analytical balance is used for measuring smaller quantities (in mgs) including growth regulators. pH meter is used for measuring and adjusting the hydrogen ion concentration of the culture media. Adjusting pH medium is essential not only for solidification of agar, but also for ensuring availability of various salts. To prepare media, agar and sugar are weighed and put in borosilicate flask with RO/ Distilled / double distilled water. The flask is put either in an autoclave and temperature build to 80 – 100°C or agar melting is done on a gas stove. The agar – sugar – water is heated in stainless steel pans with constant stirring. In the melting agar – sugar – water solution, macro / micro / iron / vitamin stock solution are added and final volume is made. pH of the media is adjusted with IN NaOH or IN HCl solution. Depending on the objective, range of glass vials are used – ranging from test-tubes disposable jars / glass bottles, etc. The pouring can either be done manually or through a media dispenser. The amount of medium to be poured in each vial depends on the size of the vial; number of explants and duration for which culture is kept. In test – tubes, 20 ml media is poured while in 400 ml jar, 50 ml of

medium is poured.

4. Autoclave for sterilization

Autoclave is either kept inside the media preparation area or is kept in a separate room. While the media preparation room can be air-conditioned, autoclave, it is kept in a well ventilated room as it generates lot of heat. In commercial tissue culture labs, double door autoclaves are used in which the prepared media is loaded from one side and after sterilization, unloaded in the media store which is part of the clean area. The size and shape of the autoclave again is dictated by the scale of the plant tissue culture being practised. A smaller size autoclave is required to engage with molecular biology / protoplast culture. On the other hand in laboratories practing micropropagation, large autoclaves are required. Autoclave is an equipment for generating steam under pressure. At pressure of 15 Ibs; and temperature of 121° C for 15 – 25 min. (depending on the quantity of media), the media gets sterilized. For sterilizing contaminated cultures, 30 – 40 min. duration is chosen. The dry cycle is run for sterilizing instruments, lab coats; plates for dissection, etc. The duration of dry cycle is usually one hour. To avoid spreading of contaninants, it is advisable to autoclave contaminated jars for an hour and immediaterly discard agar.

5. Media Store

In large tissue culture facililites a provision for separate room is made which is fitted with UV lights and adequate shelves. Sterilized media is stored for at least 3 days prior to inoculation. The double door autoclave, opens directly in media store and media which it is still in molten stage is shifted to shelves. If slants are to be made (specially in case of test tubes) to increase the surface area, it is done in the media store. This area is a part of clean area and is maintained under class 100,000 level.

6. Inoculation area

While smaller laboratories have transfer hoods fitted with UV lights, laminar air flow cabinets are common. They can be kept either in a quite corner of the general laboratory or in larger laboratories separate transfer room is created. Transfer room has high level of cleanliness and is comparable to operation theatre in a hospital as all aseptic manupulations are to be carried out here. This area has restricted entry and all those working here follow hygine.

7. Laminar air flow cabinets for aseptic manipulations

All the aseptic manipulations are carried out inside the Laminar Airflow cabinets. There is a unidirectional flow of sterile air through HEPA filters that flows in the cabinet where cultures are prepared and transferred to the media. They are also fitted with UV lights to prevent growth of microbes in the cabinet while it is not in use. The culture media / mother cultures are provided to operators / researchers on a trolley which also serve as a size bench for them. It is advisable to have fire extingushers in this area as there is use of heat / fire and rectified spirit. Till few years back, spirit lamps were used to sterilize instruments that were dipped in rectified spirit and flamed. However, these have been replaced with hot bead sterilizer where temperature of 200 °C. is maintained and after every operation, instruments are kept in hot beat steralizers for a couple of minutes to sterilize them. The laminar air flow cabinets are monitored continuously for the air pressure and strict schedule is followed for cleaning fo pre-filters. While working, operators surface sterilize their hands and arms with rectified spirit; wear cap and face mask to avoid any infection.

8. Growth Rooms

Growth rooms are rooms in which culture are maintained under controlled conditions of light and temperature. The growth room typically has one entry and has no windows. To maintain high level of cleanliness this room is maintained under class 100,000 by passing air through filters; has plastic paints on the walls and have air ducks opening at individual shelf level to maintain uniform temperature. The temperature is constantly maintained and fluctuation of as low as ± 2 ° C is premisible. Usually light is provided by cool white florecent lamps which is gradually getting replaced by CFL / LED bulbs. Precautions are taken that there is no heating of cultures that are kept above the rack. In many laboratories use of glass / transparent fiber glass is favoured so as to maximise light. Since cost of maintaining this room is fairly high, racks on rail are practiced in many commercial tissue culture units. Depending on the nature of the experiments, shaking machines (horizontal / rotary) are prevalent. BOD incubators with or without shaking provisions are also used in research laboratories. You will learn about specific requirements for suspension culture / single cell culture/ protoplast culture in the subsequent chapters.

9. Area for observations of cultures

While in research laboratories no separate provision is made in commercial laboratories, a separate room is made for taking observations and observing cultures under the microcopes. Compound microscope enables detection of bacteria and fungi in culture; a provision for stereo microscope can also be made for dissecting small size meristematic dome for getting clean explants from virus infected plants. However, these operations are carried out in laminar flow cabinet.

10. Transfer area

A provision for separate room is made where cultures are washed of agar and transplanted to potting mix. Many a times, potting mix is sterilized & thus provision for boiler is made. The plantlets are usualy transferred in portrays.

11. Acclimatization

The plantlets produced under *in vitro* conditions, although green in colour, does not photosynthesize effectively and lack mechanisms to control water loss. These plantlets thus upon transplantation, must be gradually and carefully acclimatized to the natural conditions.

In tissue-culture propagation, greenhouse is required: to raise and maintain mother plants; and to harden the plantlets gradually to the natural environment. In the greenhouse, the humidity near the pad is close to 95 – 100% and at the other end (far side) is ~ 80%. The temperature of the greenhouse is usually ± 10 ° C of the ambient temperature. During certain periods (peak summer and winter), providing conducive temperature is a challenge as most species thrive between 20 – 30 ° C. In many regions, where temperatures are high, green houses are covered with shade net and have vents which are opened for part of the day. In cold regions, use of heaters is prevalent. Further,once the plants are hardened but outside conditions are very harsh, small plantlets are transferred to polyhouses for secondary hardening. Polyhouses are low cost structures where condusive temperatures for plant growth are provided with some control on humidity levels.

Conclusion

In this module, you have learnt about the basic requirements to set up a tissue culture laboratory. There are some basic structures which are required by all who are practising tissue culture. In most Universities, part of the infrastructure is build on the centralized basis (for example, facilities for autoclaving; growth rooms; green house, etc.), while others are customized to the research requirement. The entire infrastructure demands high level of cleanliness. Also, the instruments must be calibrated from time to time to get consistent results. Proper record keeping and good observation skills are essential to succeed with plant tissue culture.