## *Practical 9*

# VISIT TO A TISSUE CULTURE LABORATORY

## EXERCISE

9.1 : Visit to a tissue culture laboratory to learn about its compartments, and equipments required for establishment of such lab

## OBJECTIVES

- To get acquainted with basic requirements of a tissue culture lab
- To know different parts/components of a tissue culture laboratory
- To know important instruments required in a tissue culture laboratory
- To conduct different steps of micropropagation in tissue culture laboratory

**Delivery schedule:** 04 periods

**Student expectations/learning objectives**

- Getting acquainted with various components and basic requirements of a tissue culture laboratory
- To learn media preparation, and inoculation procedures for micropropagation

**Pre-learning required:** Brief knowledge about micropropagation of horticultural crops.

**Handouts/material required/equipment & tools:** A laboratory notebook and pen, various types of laboratory equipment and accessories.

**Procedure/methodology**

Students will be taken to a tissue culture laboratory, where different components, equipments and accessories used in tissue culture laboratory will be shown to them. Students will note down all the details in their notebooks.

## INTRODUCTION

Tissue culture is a old term applied to micropropagation. Propagation of plants under aseptic and controlled conditions of environment and nutrition under lab conditions is called as 'micropropagation' or tissue culture. Although, vegetative propagation of plants has been practiced for centuries and many improvements in conventional methods have been made over the years. However, now, the tissue culture technique i.e ., micropropagation has expanded its scope and potential on a commercial scale. Micropropagation is suitable for the rapid and large-scale clonal multiplication of elite material. The technique has been referred as micropropagation because the size of the tissue in culture is very minute as compared to conventional vegetative cutting or any other plant part. The meristem explant used for micropropagation is about 0.1-0.5 mm size having only one or two leaf primordia. With the advancement in science and technology, micropropagation technique has also been standardized for many plants, and it is now widely used for multiplication of many horticultural plants. The plant part to be cultured is called as explant, for which artificial culture medium is required. Since all these actions are performed under controlled conditions, hence, a well-equipped laboratory is required. In general, the key organization and facilities of most tissue culture laboratories comprise of;

- Working space to carry out the routine laboratory work.
- Washing sinks and drain racks.
- Hot-water arrangements.
- Ovens to dry the washed glasswares.
- Cabinets or shelves for the safe storage of clean glassware, chemicals, etc.
- Inoculation room for carrying out aseptic transfers of explants or for subculturing.
- Sterilization facility: autoclaves, steamers and ovens for sterilizing media, solutions, distilled water, glassware and instruments.
- Incubation room, to incubate the tissues and explants in a controlled environment (where light, temperature and humidity are controlled)
- Continuous supply of distilled and double distilled water (laboratory grade purified water).
- Various equipments and accessories.

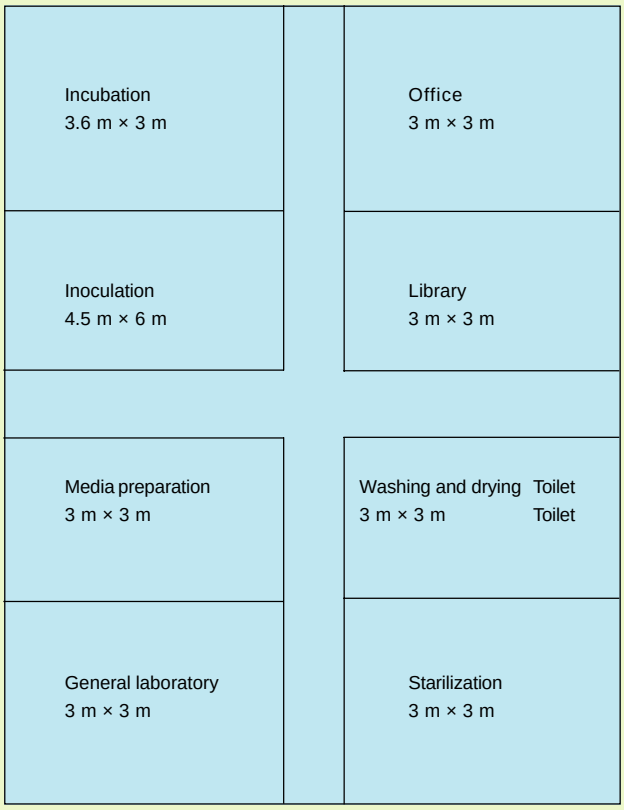
A typical tissue culture laboratory can be divided into separate rooms according to the operations and functions, which are to be carried out during the course of work such as office, incubation room, media preparation room, inoculation room etc.

## Washing-and-drying room

The washing area should contain large sinks, some lead-lined to resist acids and alkalis, draining boards, and racks, and have access to distilled water. Space for drying ovens or racks and storage cabinets should also be available in the washing area.

## Sterilization room

The room should preferably have 2 autoclaves, a horizontal and a vertical autoclave. This room should also be provided with a sink and all essential service lines. This room should be well ventilated by fitting exhaust-fans. The oven may also be accommodated in this room.



Layout of a typical tissue culture laboratory

## General laboratory

Tables of both standing and sitting height are necessary in this room for plugging tubes and wrapping the glassware before sterilization. This room should be provided with a refrigerator and deep-freeze for storing the perishable compounds, stock solutions, etc. The working tables can be placed adjacent to the walls of the room to accommodate the appropriate equipments.

## Media preparation room

This room should get a sufficient and continuous supply of distilled water through water purification system, a water storage carboy for storage, pH meter and balances. In addition, 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 should be available. Other necessary equipments may include vacuum filtration unit and Bunsen burners with a gas source.

## Inoculation room

In this room, the inoculation cabinet or laminar air-flow cabinet may be kept. Air is forced into the unit through a dust filter then passed through a HEPA filter. A 0.3-?m HEPA filter of 99.97-99.99% efficiency works well. The air is then either directed downward (vertical flow unit) or outward (horizontal flow unit) over the working surface. The constant flow of bacteria-free filtered air prevents

non-filtered air and particulate matter from settling on the working surface. The floor of this room should be covered with linoleum or tiles to facilitate proper cleaning. It is essential that the atmosphere in this room should be free from contaminants. This room should preferably be air-conditioned because it ensures good air-circulation and helps in maintaining constant temperature. A generator of an emergency power supply is essential wherever cultures are incubated.

## Incubation room

After the aseptic transfers, all types of tissue cultures should be incubated under conditions of well-controlled temperature, humidity, air circulation, and light quality and duration. The explants and tissues have to be incubated at a specified temperature, the most desirable being 25±2°C, which can be maintained with air-conditioners. The temperature should be constant throughout the entire culture room (i.e., no hot or cold spots). During winters, controlled heating system should be provided additionally.


Inner view of a tissue culture lab

Cultures are placed on wooden or metal slotted angle racks, preferably painted white for brighter illumination. Cool daylight fluorescent tubelights (40 watts) can be fitted on the racks with a timing device to adjust the photoperiod required for the growth of cultures. These illuminated racks can be arranged in rows and along the room. Both light and temperature should be programmable for a 24-h period. Sometimes, cultures have to be incubated in continuous darkness, for which there should be adequate provision. This is often done by using black curtains to cut-off the light. Some of the racks can also be used for hardening the clones or differentiated plants after transfer to small pots from the aseptic to normal environment. The culture room should have fairly uniform forced-air ventilation, and a humidity range of 20-98% controllable to ±3 per cent. A rotary shaker of variable speed ranging from 80-220 rpm to take 100 ml or 250 ml Erlenmeyer flasks should also be housed in one corner of the room.

## Laboratory equipment/ items

The following items can be seen in a common tissue culture laboratory:

| Equipment/ items | Function |
|---|---|
| Water purification system | Purification of water for media preparation |
| Electronic balance | Measuring out biochemicals and media |
| pH meter | Measurement and adjustment of media pH |

| | |
|---|---|
| Hot plate/stirrer | Mixing & heating media and stock |
| Refrigerator/freezer | Storage of stock solutions, media, hormones |
| Laminar Air Flow | Provide a sterile atmosphere to transfer cultures |
| Aluminum foil | Used to wrap instruments prior to sterilization, cover vessels |
| Beakers | Mixing solutions |
| Wash bottles | Rinsing instruments, beakers, transplants from tissue culture |
| Culture tubes/vessels | For culturing and maintaining of explants/ plantlets |
| Culture tube racks | Holding culture tubes |
| Closure | Sealing culture tubes/ culture vessels |
| Filtration system, vacuum; disposable syringe filter | Sterilization of heat liable stock solutions |
| Forceps | Transferring tissue |
| Micropipettes | Measuring out stock solutions |
| Scalpel handle | Cutting explants |
| Scalpel blades | Cutting explants |
| Autoclave | Sterilizing media and instrument |
| Sterilizer, dry heat with glass beads | Sterilizes instruments in laminar air flow between transfers |
| Hot air oven | For drying of glasswares |
| Shakers | For continuous shaking of liquid culture |
| Non-absorbent cotton | Used as closure of test tubes or conical flasks |
| Culture trolley | For accommodating culture racks or culture vessels |

Water purification system

Autoclave

Electronic balance

Freezer

Hot air oven


Laminar air flow

pH meter

Hot plate cum magnetic stirrer

Shaker

Some common equipments required in a tissue culture laboratory

Culture trolley

Vacum filter

Bead sterilizer

Syringe filter

Culture vessels

Chemicals and reagents

Micropipette

Aluminum foil

Non-absorbent cotton

Scalpel and forceps

Some commonly used items/accessories in a tissue culture laboratory

## STUDENT'S ACTIVITIES /EXERCISES

- Visit a tissue culture laboratory and make a list of its components and their role.
- Visit a tissue culture laboratory and make a list of equipments and important items along with their functions/roles
- Practice the following in tissue culture laboratory: explant preparation, media preparation and culture (inoculation) establishment of some horticultural crops.

## RESOURCE MATERIAL


- Paranjpe, S.V. (1993). Tissue Culture Laboratory. In: Handbook of Plant Tissue Culture (Ed. Mascarenhas, A.F.). Publication & Information Division, ICAR, New Delhi.
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