

- Tissue culture is *in vitro cultivation of plant cell or tissue* under aseptic and controlled environmental conditions, in liquid or on semisolid well-defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.
- This technique affords alternative solution to problems arising due to current rate of extinction and decimation of flora and ecosystem.

ADVANTAGES OF TISSUE CULTURE TECHNIQUE

- *Availability of raw material*
- *Fluctuation in supplies and quality*
- *Patent rights*
- *Political reasons*
- *Easy purification of the compound*
- *Modifications in chemical structure*
- *Disease-free and desired propagule*
- *Crop improvement*
- *Biosynthetic pathway*
- *Immobilization of cells*

GENERAL PROCEDURES INVOLVED IN PLANT TISSUE CULTURE

- *In vitro* culturing of plant tissue involves the following steps:
 - Sterilization of glassware tools/vessels
 - Preparation and sterilization of explant
 - Production of callus from explant
 - Proliferation of cultured callus
 - Sub-culturing of callus
 - Suspension culture



PLANT TISSUE CULTURE LABORATORY

REQUIRED FACILITIES FOR PLANT TISSUE CULTURE LAB:

In each laboratory where plant tissue culture techniques are used must have a number of facilities that include among others.

- Outdoor media preparation, sterilization and storage
- Washing room
- Media preparation room
- Water(distilled water)
- Transfer space (laminar air flow)
- Culture room
- Glass wares
- Microscope
- Shaker
- Autoclave chamber
- Tools dissection (spatula, scalpels,(tweezers)forceps, scissors)
- Shade or green houses
- Computer for record keeping


BASIC REQUIREMENTS OF A TISSUE CULTURE LABORATORY

- Equipment and apparatus
- Washing and storage facilities
- Media preparation room
- Sterilization room
- Aseptic chamber for culture
- Culture rooms or incubators fully equipped with temperature,
light and humidity control devices.
- Observation or recording area well equipped with computer for data processing.



EQUIPMENT AND APPARATUS

○ *Culture vessels and glassware*

- Many different kinds of vessels may be used for wing cultures.
 - **Callus culture** can be grown successfully in large test tubes (25 × 150 mm) or wide mouth conical flasks (Erlenmeyer flask).
 - In addition to the culture vessels, glassware such as **graduated pipettes, measuring cylinders, beakers, filters, funnel and petri dishes** are also required for making preparations.
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WASHING FACILITIES REQUIRED FOR A TISSUE CULTURE LABORATORY

- First and foremost requirement of the tissue culture laboratory is provision for **fresh water** supply and disposal of the **waste water**, and space for distillation unit for the supply of distilled and double distilled water and de-ionized water.
- Acid and alkali resistant sink or wash basin for apparatus / equipment washing and the working table should also be acid- and alkali-resistant.



WASHING ROOM:

- Washing room should have a sink.
- A desk made up of material resistant to acids and alkalis
- Drying racks and channels for demineralised or distilled water
- Space for the oven drying
- Equipments /washing machines and dryers
- Storage racks or equipment cabinets
- Disinfectants
- Oven

EQUIPMENT

- A **spirit burner** or **gas micro-burner** for flame sterilization of instruments
- An **autoclave** to sterilize the media.
- **Hot air oven** for the sterilization of glassware, etc.
- A **pH meter** for adjusting the pH of the medium.
- A **shaker** to maintain cell suspension culture.
- A **balance** to weigh various nutrients for the preparation of the medium.
- **Incubating chamber** or **laminar airflow** with UV light fitting for aseptic transfer of explants to the medium and for sub-culturing.
- A **BOD incubator** for maintaining constant temperature to facilitate the culture of callus and its subsequent maintenance.


MEDIA PREPARATION ROOM:

- In the media preparation room should be available space for storage of chemicals, glass culture and the lid
- Sturdy table or bench for storage of hot plate magnet stirrer
- Ph meter, scales and dispenser must be available
- Vacuum devices
- Distilling units
- Bunsen burner
- Refrigerator
- Freezer for storage of stocks, solutions and chemicals ,microwave ,gas stove
- Glass ware and other equipments
- All the chemicals utilized in the manufacturing of culture media
- Split AC

STERILIZATION ROOM

- For the sterilization of culture media, a good quality ISI mark autoclave is required and for small amount domestic pressure cookers, can also serve the purpose. For the sterilization of glassware and metallic equipments hot air oven with adjustable tray is required.

ASEPTIC CHAMBER/AREA FOR TRANSFER OF CULTURE

- For the transfer of culture into sterilized media, contaminant - free environment is mandatory.
 - modern laboratory have laminar airflow cabinet having vertical or horizontal airflow, arrange over the working surface to make it free from dust particles / micro-contaminants.
 - Inside the cabinet, there is arrangement for Bunsen burner and a UV tube fitted on the ceiling of the cabinet.
 - The advantage of working in the laminar airflow cabinet is that the flow of air does not hamper the use of Bunsen burner and moreover, the cabinet occupies relatively small space within the laboratory.
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INCUBATOR

- BOD incubators required to maintain the culture conditions should have the following characteristics:
 - Temperature range, 2–40°C
 - Temperature
 - Automatic digital temperature recorder
 - 24-h temperature and light programming
 - Adjustable fluorescent lighting up to 10,000 lux
 - Relative humidity range 20–98%
 - Relative humidity control ±3%



DATA COLLECTION AND RECORDING THE OBSERVATION

- The growth and maintenance of the tissue culture in the incubator should be observed and recorded at regular intervals.
- All the observations should be done in aseptic environment, i.e. in the laminar airflow. Whereas for microscopic examination, separate dust-free space should be marked for microscopic work. All the recorded data should be fed into the computer.