

Aim 45

To Study the Plant Tissue Culture Laboratory

Requirements

Basic facilities and equipments :-

1. Autoclave
2. Laminar flow chamber
3. Balance
4. Magnetic stirrer with hot plate
5. Oven
6. pH meter
7. Water distillation tank
8. Refrigerator
9. Shaker
10. Microscope
11. Glassware
12. Chemical

Lab organization

1. Media room
2. Inoculation room
3. Washing room
4. Culture room

5. Green house

Nutrient culture media

1. Micro and macro nutrients
2. Vitamins
3. Sugar
4. Growth regulators
5. Agar
6. Distilled water

Lab requirement for plant tissue culture

For standard tissue culture laboratory following facilities are necessary.

1. Media Room:- Media room should have the facility for washing glassware and plastic wares. In the media room washed glass ware and plastic wares are stored and used for preparation of culture media.

A Media room should be equipped with washing benches, including a refrigerator, weighing balance, a hot plate, a magnetic stirrer, a ph meter, a vacuum pump, an autoclave, oven, water, distillation unit and shaker.

2. Washing: - The washing area should be in a corner of the media room, well isolated from inoculation and culture area. This should be provided with a working bench fitted with a large washing sink, running water tap and a geyser. This area should have plastic buckets to soak the lab ware and a clean cupboard to store them.

The glassware are washed by using detergents especially designed to remove all traces of acids to etc. finally they are rinsed in tap water and in distilled water. Sometimes the glass wares may need to be autoclaved before washing remove agar medium and to destroy microbial contamination.

3. Media Preparation and sterilization:-

This work is done in a media room and sterilization of equipment and media are done in oven and autoclave respectively.

4. Autoclave: - Most nutrient media are sterilized with the use of an autoclave. Autoclave is an apparatus for sterilization with steam. Provision exposure to sufficient, pressurized steam can. Destroy all microbes. An autoclave has a temperature range of 115-135°C. Good sterilizer results on time, pressure and volume of the of the object to be sterilized. Advantage of an autoclave are are speed. Simplicity. The additional destruction of virus and no absorption.

Disadvantage of change in pH

Components can separates out and chemical reaction can occur resulting in a loss of activity of media constituents.



Magnetic stirrer with hot plate



pH meter



Autoclave



Laminar air flow

Guidelines follow while using an autoclave:-

1. Test tube and flask containing between 20-30 ml. nutrient media is kept for 20 min at 121°C.
2. Flask containing 50-500 ml nutrient media for 25 min at 121°C.

3. Empty test tube flask and filter paper for 30 min at 121°C.

When autoclave, bottles, should not be tightly pack and their tops should be loosen. Aluminum cover above the equipment to avoid the water drops. The large volume must be sterilized for longer periods as the heat will take longer to penetrate that with smaller volume.

During and after autoclave following precaution should be taken into account:-

1. pH of the media is lowered by 0.3-0.5 units.
2. Autoclave at too high temp can caramelize sugar which may then be toxic.
3. Care should be taken when using the correction and duration of temperature.
4. It must be realized that volatile substance can be destroy by the use of autoclave and also the thermolabile hormones.
5. If nutrient media slopes are needed the test tubes must be placed on slopes to get set after autoclave.
6. It must be recognized to use deionized water in the autoclave as tap water contain too much calcium which become precipitate at the bottom of the autoclave.

Glassware and Teflon plastic ware may also be sterilized by dry heat in an oven at 160-180°C for 3 hour.

Inoculation room

Laminar air flow chamber:-It is necessary to carry out this preparation and cutting of cellulose etc. in a laminar air flow cabinet or inoculation cabinet. Research lab and commercial tissue culture lab always use laminar air flow chamber to limit the possibilities of infection.

A laminar air flow is one in which air is sucked from outside first being filtered through very fine filter before reaching the tables top of inoculation ion chamber. The air is first filtered through a coarse pro filter to remove large

particles. It is passed through a HEPA (high efficiency particulate air) filter which blocks the particles larger than 0.3 μm . The sterilized enough to keep the enclosed working area aseptic. The air flow can be regulate and florescent tube and UV emitting tube are also fixed in the roof of the cabinet. A gas supply is available on the table top for use in flaming this can be substituent by a spirits lamp.

In modern lab for laminar air flow cabinet in a special isolation room which is kept sterile and dust free by the use of filter. The room is pressurized so that non sterile air flow outside cannot enter to the room.

The filterers of the hood should be regularly vacuumed clean and replaced annually. The floor of the room containing the cabinet should be cleaned every day.

Culture room

This is an especially controlled environmental room to grow the culture where temp. Diurnal illumination, humidity are maintained. Room has:-

1. Air condition and heater to maintain the temperature at 25°C
2. Shelving made up of glass or wire with fluorecent tube (1000 lux).
3. A shaker for agitation of liquid culture.

It is devised to have a generator set for providing power to culture room in the event of electricity cut.