Lab: 5

Practical Biotechnology

Plant Tissue culture Technique

is a collection of techniques used to maintain or grow plant cells, tissues, or organs under sterile conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method. known as micropropagation

Micropropagation: multiplication of plants from vegetative parts by using tissue culture nutrient medium.

The plant tissue culture was the basis of many applications:

1-plant breeding and conservation of germplasm

2-Production of free diseases plants

3- Industrial production of natural plant products

4-Rapid clonal propagation

5-Genetic engineering

Plant tissue culture procedure

1. Selection of Explants.

Explant A small tissue excised from any part of the plant is called an explant which is the starting point. It can be initiated from any part of **the** plant- root, stem, petiole, leaf, or flower.

2. Surface Sterilization of Explants:

This Means the elimination of all microorganisms or contaminated factors that affecting on the growth of explant in the culture media, because of its rapid growth and its competition the explant on the nutrients and secrete toxic substances into the media and thus absorbed by the plant then death.

The procedure of surface sterilization:

Collected explants were thoroughly washed with 2-4 drops of surfactant for 10min. Under running tap water. After that explants were subjected to chemical sterilization (ethanol alcohol and sodium hypochlorite) in the laminar airflow.it is then washed 3-4 times with sterilized double water.

Also, we can obtain sterilized explants from the culture of aseptic seeds and then used its aseptic seedling. This method was important for easy seed sterilize without affecting its viability.

The use of sterile chemicals depends on :

* The sensitivity of the cultured explant

* The optimal time used

* Easily it's removed with distilled water washing process.

3. **Inoculation of explant:**

transfer of sterile explant on the nutrient medium under **an aseptic condition** (free from contamination).

4. Incubation:

Growing the culture in the growth chamber. After the defined period of incubation, an unorganized and undifferentiated (no root and shoot) mass of cells called **callus** is obtained from each explant.

5. Transfer of callus and Regeneration.

6. Hardening:

Gradual exposure of plantlets to environmental conditions. Tissue culture plants are highly sensitive to tolerate natural environmental conditions. They have to be slowly adapted to a normal atmosphere. So first they are to be grown in greenhouses.

7. Transfer of plantlets to the field.

Callus culture

The callus is an unorganized undifferentiated mass of cells produced from an explant of a seedling. Callus can be **subcultured** indefinitely by transferring the callus to the same fresh medium, Subculturing needs to be done every 3-5 weeks .

Initiation of callus :

There are three stages to initiate callus

1-Induction of growth

2- Division phase

3- Differentiation

The callus is sub-cultured because:

(a) The nutrient may be decreased.

(b) Medium drying.

(c) Cell metabolites may accumulate and cause toxicity. Active growth can be maintained even after several subcultures .

** The condition and requirements for plant tissue culture technique

It is found in specific laboratories for this technique and contains three regions:

1) *Washing and media preparation region*: In this region :

1-Preparation all types of media that is used in this technique.

2-excised the explant from the mother plant for culture

3-the primary sterilization of the explant done by washing it in water and soap.

The area should contain large sinks, a region resist to acids and alkalis, racks, distilled water, and double-distilled water. Space for drying, ovens, pipette washers, and storage cabinets.

2) Transfer region:

In this region, all procedures in plant tissue culture happen (seed surface sterilization, seeds culture, subculture, etc) in the **laminar airflow chamber.**

"laminar airflow chamber" The chamber is provided with two UV sterilizing lamps (one small, one big) and a fluorescent lamp**,** Laminar air flow has a number of small blower motors to blow air which passes through a number of HEPA (high- efficiency particulate air) filters. Such filters remove particles larger than 0.3 μm. The ultraclean air is free from fungal and bacterial contaminants, Before starting work, laminar air flow is put on for 10-15 minutes.

3) Culture ROOM (Incubator) :

In this room all types of cultures were under controlled conditions of temperature, humidity and light quality and, duration.