[PDF] Plant Tissue Culture: Definition, Method, examples, advantages

Introduction

Plant tissue culture was a recent addition to the plant breeding techniques that emerged in the 1950s. It is very important to crop improvement programmes and plant biotechnology in general. It is becoming more popular as a successful way of propagation that utilizes tissue scraps from plant media and cell technologies to grow new plants in artificial environments. It is increasingly being used as a substitute for vegetative plant propagation. Bacterial and fungal diseases are typically absent in plants that are grown in vitro.

The in vitro aseptic culture of cells, tissues, organs, or complete plants under strict dietary and environmental controls is known as tissue culture, and it is frequently used to create plant clones. The resulting clones are exact replicas of the chosen genotype.

A properly set up culture facility and nutritional media are necessary for the entire operation. The creation of nutritive medium including inorganic and organic salts, supplemented with vitamins, plant growth hormone, and amino acids as needed are some of the phases in this procedure, as well as incubation and inoculation, as well as the sanitation of explants, glassware, and other accessories.

History of tissue culture:

In 1902, German botanist Gottlieb Haberlandt made the initial argument for the significance of isolated plant tissue and cell culture. As the founder of plant tissue culture, he is revered.

He utilised Lamium Puroureum and Eichhornia crassipes tissue, Ornithooalium epidemis, and Pulmonaria Mollissima epidermal hairs. He cultivated them in a specific salt solution containing sucrose and saw the cells develop. The cells survived for as long as a month. They expanded in size and structure, their cell walls thickened, and the chloroplasts—which had previously lacked starch—began to contain starch. None of the cells split, though.

The reason for his failure was that he was working with highly differentiated cells and lacked access to the modern growth hormones required to trigger cell division in mature cells. Hanning (1940) opened a fresh line of inquiry, which eventually grew into a significant applied field of in-vitro methods.

General plant tissue culture processes include:

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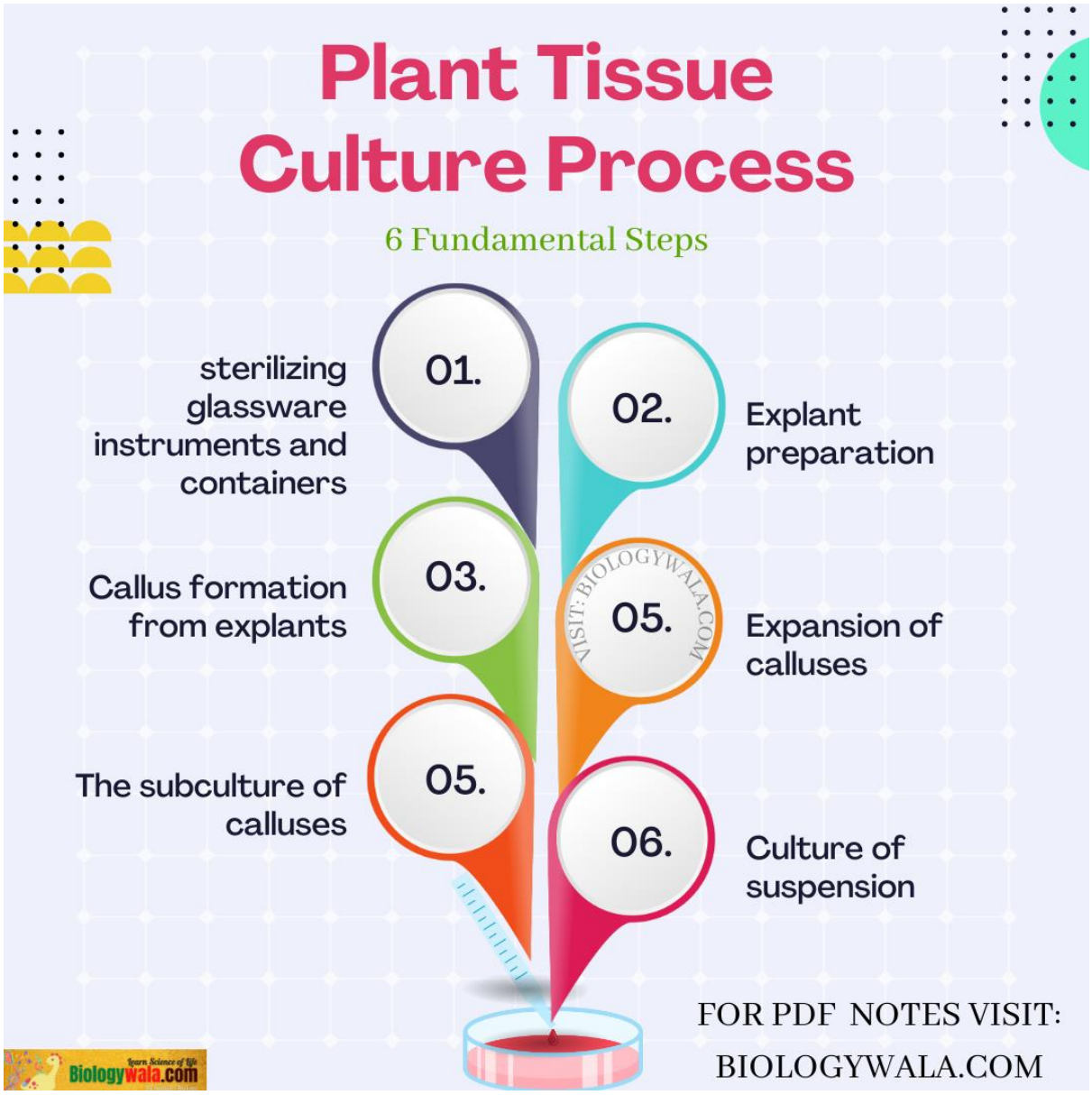
1. sterilizing glassware instruments and containers;
2. preparing and sterilizing explants;
3. producing calluses from explants;
4. promoting the growth of cultured calluses;
5. subculturing calluses; and
6. suspension culture.



1. Sterilizing glassware instruments and containers

Tools and vessels used for glassware sterilization should all be Pyrex or Corning. The glassware should be immersed in sodium dichromate sulfuric acid solution for the entire night. The following morning, glassware should be rinsed with distilled water followed by fresh running tap water, and then placed inverted in plastic buckets or trays to drain any extra water. The glassware is dried in a hot air oven at a high temperature of 1200 c for a half-hour to an hour. After sterilization, empty containers are wrapped in aluminium foil to avoid reinfection. Aluminium foil is also used to wrap stainless steel and metal tools (such as forceps, scalpels, and blades) and pads of cotton wool are inserted into the pipettes' openings before they are either wrapped in aluminium foil or placed in an aluminium or stainless-steel box. Typically, sterilizing takes between one and four hours.

2. Explant preparation:

An explant is a piece of the plant's body that has been removed to create a culture. Plants that are grown under regulated environmental conditions can produce explant. These plants are homozygous and typically pathogen-free. Any part of the plant, including the root, stem, leaf, or meristematic tissue like the cambium, as well as floral components like the anthers and stamens, may be used as an explant. The explant's age plays a significant role in callus development. Younger tissues work better than older tissues. With the use of a sharp knife, a sufficient section of the plant is taken, and the dried and mature portion is separated from the immature tissue.

When grains and seeds are utilized to prepare explants, they are immediately sterilized and placed in a nutritional medium. The obtained seedlings must be used for explant preparation after germination. Explant surface sterilization Chromic acid, mercuric chloride (0.11%), calcium hypochlorite (1-2%), and alcohol (70%) are employed to sterilize the explant's surface. Typically, the tissue is rinsed with distilled water after being submerged in the sterilizing agent solution for 10 to 15 minutes. The tissue is then rinsed with sterile water to remove the sodium hypochlorite before undergoing a second 20-minute sodium hypochlorite treatment. For inoculation, this tissue is utilized. By exposing the explants to aqueous sterilized solutions of various concentrations, they are sterilized.

3. Callus formation from explants

Onto a predetermined media that is housed in flasks, the sterile explant is transferred aseptically. The flasks are moved to the BOD incubator for culture maintenance. A temperature adjustment of 25–20 c is made. Callus (undifferentiated amorphous cell mass) formation requires some level of light. Normally, 3 to 8 days after incubation, enough callus is formed.

4. Expansion of calluses

If the callus is fully established, it should be divided into small pieces and moved to a new medium with a different hormone content that promotes growth. Proliferation medium is the term used to describe the medium that is utilized to produce additional calluses.

5. The subculture of calluses

To sustain the vitality of the cells, the callus should be routinely moved to a new medium after it has grown sufficiently. The sub-culturing will be carried out every 4-6 weeks.

6. Culture of suspension

In suspension culture, distinct cells are uniformly suspended in liquid media. Callus is put in a liquid medium for suspension culture preparation, where it is continuously stirred to keep the cells apart. A rotating shaker device placed inside the incubator can be used to stir things up at a pace of 50 to 150 rpm. Subculturing is possible if a sufficient number of cells have been produced.

The Essentials for Plant Tissue Culture

A tissue culture laboratory should contain the following standard basic equipment for tissue culture technique:

1. Instruments and machinery

2. laundry and storage areas

3. space for media preparation

4. the sterile chamber

5. An aseptic culture chamber

6. Cultivation chambers or incubators that are completely furnished with equipment to regulate temperature, light, and humidity

7. A well-equipped computer in the observation or recording space for data processing

1. Instruments and machinery

Cultured glassware and vessels for wing cultures, a variety of vessels can be utilized. Wide-mouth conical flasks or big test tubes (25150mm) can be used to develop callus cultures satisfactorily. Glassware used for preparations includes graduated pipettes, measuring cylinders, beakers, filters, funnels, and Petri dishes in addition to the culture vessels. The glasses should all be made of corning or Pyrex.

Equipment: forceps, scalpels, and scissors for cutting explants from plant sections that have been removed and transferring them.

2. laundry and storage areas

For aseptically transferring explants to the medium and subculturing, use an incubator or laminar air flow with a UV light fitting. An autoclave sterilizes the media. A hot air oven to sterilize glassware. A pH meter to adjust the pH of the medium. A shaker to

maintain cell suspension culture. a scale to weigh different nutrients for the medium's preparation. A spirit burner or gas micro burner for flame sterilization of instruments.

The availability of freshwater supply, wastewater disposal, and space for a distillation unit for the delivery of distilled, double-distilled, and de-ionized water are the most important requirements for a tissue culture laboratory. The working table should also be acid and alkali resistant, as should the sink or wash basin used for washing apparatus and equipment.

With the hot air oven, washing machine, pipette washers, and plastic bucket or steel tray for soaking or drainage of the detergent bath or additional water, there needs to be enough room. Dried glassware should be stored in a separate dust-proof cabinet or cupboard. In the washing, drying, and storing areas, cleanliness is required.

3. Preparation area for media

The space needed for a media preparation room should be large enough to house the chemicals, lab equipment, culture vessels, and weighing and mixing equipment, as well as a hot plate, pH meter, water baths, Bunsen burners with the gas supply, microwave oven, autoclave, or domestic pressure cooker, as well as a refrigerator and freezer for storing prepared media and stock solutions.

4. Sterile environment

A decent-grade autoclave with an ISI mark is needed to sterilize culture media, while small domestic pressure cookers can also be used. Glassware and metallic equipment must be sterilized in a hot air oven with an adjustable tray.

5. Aseptic culture chamber

It is essential to work in a contaminant-free environment when transferring culture onto sterile media. The most basic transfer area design calls for a regular tiny wooden hood with a glass or plastic door that is either sliding or hinged and fitted with an ultraviolet tube. It is simple to position this aseptic in a serene area of the lab.

To keep the work surface free of dust particles and other contaminants, modern laboratories use laminar airflow cabinets with either vertical or horizontal airflow.

6. Cultivation chambers or incubators

The development and differentiation of cultured tissues are influenced by environmental variables. A typical incubation chamber or area should be equipped with

24-hour temperature and light control systems. To keep the temperature at 25–20 c, air conditioning or room heaters are needed. In terms of the length of the photo session, the light needs to be adjusted. The ideal humidity range is between 20% and 90%.

Shelves must be made such that culture vessels can be placed on them or in trays without interfering with the regulation of light, temperature, or humidity. To keep experiment data and assure identity, a label needs to adhere to each tray and rack

Plant tissue culture applications| Plant tissue culture examples:

1. Haploid production
2. Germplasm preservation
3. In vitro hybridization-protoplast fusion
4. Transgenic plants

1.Haploid production

The sporophyte of haploid plants has a single pair of chromosomes, or the "gametophytic number of chromosomes," or n. As opposed to diploids, which have two sets (2n) of chromosomes, this. For the creation of homozygous lines (homozygous plants) and the advancement of plants in plant breeding programmes, haploid plants are extremely important.

Two broad categories can be used to categorize haploids:

a. Monoploids (mono haploids): These are haploids from diploid species, such as maize and barley, that have half as many chromosomes.
b. Polyhaploids: From a polyploid species, haploids with half as many chromosomes are considered polyhaploids, such as wheat and potatoes.

2. Germplasm Conservation

Germplasm, in its broadest sense, refers to the genetic material that is passed on to progeny by germ cells. Breeders use germplasm as the starting point to create a variety of crops.

Hence, germplasm preservation becomes important in all breeding programmes. Primitive man developed the habit of storing certain seeds or vegetative propagules from one season to the next when he learned about the value of plants for food and shelter.

The in vitro preservation of germplasm mostly uses three methods:

a. **Cryopreservation (freeze-preservation)**
b. **Cold storage**
c. **Low-pressure and low-oxygen storage**

Greek: krayos-frost; translated as "preservation in the frozen state," cryopreservation is the preservation of things in their frozen state. By lowering the temperature in the presence of cryoprotectants, the principle of cryopreservation is to bring plant cell and tissue cultures to a zero metabolism or non-dividing state.

Mechanism of Cryopreservation:

The freeze-preserving method relies on the transformation of water from a liquid to a solid state within the cells.

Due to the inclusion of salts and organic molecules, the freezing point of cell water is substantially lower (even as low as -68°C) than that of pure water, which is approximately 0°C.

The metabolic functions and biological deteriorations in the cells and tissues nearly halt when stored at low temperatures.

Limits and Safety Measures for Effective Cryopreservation

It is crucial to have a solid technical and theoretical understanding of living plant cells as well as the cryopreservation process.

Cold Storage:

The main goal of cold storage is to preserve germplasm at low, non-freezing temperatures (1–9°C).

In contrast to full cessation during cryopreservation, the growth of the plant material is slowed down when it is stored in freezing temperatures.

Cold storage is therefore seen as a slow-growth germplasm conservation technique. This method's main benefit is that no cryogenic harm is done to the plant material (cells/tissues).

Low-Pressure and Low-Oxygen Storage:

- Low-pressure storage (LPS) and low-oxygen storage (LOS) have been developed for germplasm conservation as substitutes for cryopreservation and cold storage.

Uses of Germplasm Storage: Plant breeders and biotechnologists benefit greatly from germplasm preservation.

1. Upkeep of stock cultures A variety of plant species' plant materials (cell/tissue cultures) can be cryopreserved, maintained for a long time, and used as and when required. In contrast, in vitro cell line maintenance requires regular sub-cultures and transfers to maintain vitality. Hence, germplasm storage is the best way to prevent sub-culturing and keep cells and tissues alive for a long time.

2. Cryopreservation is the best method for maintaining cell cultures that create secondary metabolites over a lengthy period (e.g. medicines).

3. Plant materials devoid of disease (pathogens) can be frozen and multiplied as needed.

Refractory seeds can be kept alive for a very long time.

4. Conservation

Applications of Germplasm Storage:

For biotechnologists and plant breeders, the germplasm repository has become quite useful.

1. Upkeep of stock cultures: Plant materials (cell/tissue cultures) from various species can be cryopreserved and kept for several years before being used as needed. In contrast, in vitro cell line maintenance requires regular sub-cultures and transfers to maintain vitality. Hence, germplasm storage is the best way to prevent sub-culturing and keep cells and tissues alive for a long time.

2. Cryopreservation is the best method for maintaining cell cultures that create secondary metabolites over a lengthy period (e.g. medicines).

3. Plant materials devoid of disease (pathogens) can be frozen and multiplied as needed.

Refractory seeds can be kept alive for a very long time.

4. Cultural somaclonal and gametoclonal variant conservation.

5. It is possible to preserve plant materials from threatened species.

6. Pollen preservation for increased longevity.

7. It is possible to store rare germplasms created through somatic hybridization and other genetic alterations.

- **Protoplast isolation**

Protoplasts are plant cells without a cell but with a plasma membrane. The fusing of similar or dissimilar species is possible in protoplasts, and the resulting organism can grow into the entire plant.

Almost every plant element, including leaves, tubes, tubers, root nodules, endosperms, pollen mother cells, pollen, pollen tetras, and embryogenic or non-embryogenic suspension culture, can provide protoplast.

Male and female gametes have lately been found to contain viable protoplast.

Hanstein first used the term "PROTOPLAST" for the live material encased in the cell membrane in 1880.

The outer plasma membrane of the isolated protoplast is completely exposed, making it extremely fragile. The only barrier separating the interior of the living plant cell from the outside world is the plasma membrane.

It is possible to isolate protoplast.

Protoplast isolation methods:

- Mechanical (non-enzymatic)
- Direct enzymatic method
- Sequential enzymatic (two-step)

- **Transgenic plants production:**

plants that have undergone genetic modification and have had foreign or source genes inserted or introduced into desired or targeted plants

Transformation refers to the process of creating transgenic plants (i.e., uptake of foreign DNA by plant cells.) thus this method is referred to as the transformation method.

It is sometimes referred to as genetic alteration or genetic engineering.

- Herbicide resistance
- Insect resistance
- Virus resistance
- Altered oil content
- delayed ripening of fruits
- tolerance to drought, cold, and salinity
- Pollen control
- Enhanced shelf life
- Pharmaceutical & edible vaccines
- Biotic & Abiotic stress tolerance
- Nutritional quality

Advantages of tissue culture| Importance of tissue culture:

1. The accessibility of raw materials
2. Variations in quality and supply
3. Patent rights
4. Political considerations
5. Simple compound purification
6. Alteration of chemical composition
7. Desired propagule that is free of disease
8. Crop development
9. The biosynthetic process
10. Cell immobilization

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