B.Sc 3rd Year [VI Semester]: Paper 602: Unit V

Tissue Culture
Types, Techniques and Process

➢ What is Tissue Culture?

In biological research, tissue culture refers to a method in which fragments of a tissue (plant or animal tissue) are introduced into a new, artificial environment, where they continue to function or grow. While fragments of a tissue are often used, it is important to note that entire organs are also used for tissue culture purposes. Here, such growth media as broth and agar are used to facilitate the process.

* While the term tissue culture may be used for both plant and animal tissues, plant tissue culture is the more specific term used for the culture of plant tissues in tissue culture.

➢ Types of Tissue Culture

- Seed Culture

Seed culture is the type of tissue culture that is primarily used for plants such as orchids. For this method, explants (tissue from the plant) are obtained from an in-vitro derived plant and introduced in to an artificial environment, where they get to proliferate. In the event that a plant material is used directly for this process, then it has to be sterilized to prevent tissue damage and ensure optimum regeneration.

- Embryo Culture

Embryo culture is the type of tissue culture that involves the isolation of an embryo from a given organism for in vitro growth. *Note, the term embryo culture is used to refer to sexually produced zygotic embryo culture.

Embryo culture may involve the use of a mature of immature embryo. Whereas mature embryos for culture are essentially obtained from ripe seeds, immature embryo (embryo rescue) involves the use of immature embryos from unripe/hybrid seeds that failed to germinate. In doing so, the embryo is ultimately able to produce a viable plant.

For embryo culture, the ovule, seed or fruit from which the embryo is to be obtained is sterilized, and therefore the embryo does not have to be sterilized again. Salt sucrose may be used to provide the embryo with nutrients. The culture is enriched with organic or inorganic compounds, inorganic salts as well as growth regulators.

- **Callus Culture**

Callus - This is the term used to refer to unspecialized, unorganized and a dividing mass of cells. A callus is produced when explants (cells) are cultured in an appropriate medium - A good example of this is the tumor tissue that grows out of the wounds of differentiated tissues/organs.

In practice, callus culture involves the growth of a callus (composed of differentiated and non- differentiated cells), which is the followed by a procedure that induces organ differentiation.

For this type of tissue culture, the culture is often sustained on a gel medium, which is composed of agar and a mixture of given macro and micronutrients depending on the type of cells. Different types of basal salt mixtures such as murashige and skoog medium are also used in addition to vitamins to enhance growth.

- **Organ Culture**

Organ culture is a type of tissue culture that involves isolating an organ for in vitro growth. Here, any organ plant can be used as an explant for the culture process (Shoot, root, leaf, and flower).

With organ culture, or as is with their various tissue components, the method is used for preserve their structure or functions, which allows the organ to still resemble and retain the characteristics they would have in

vivo. Here, new growth (differentiated structures) continues given that the organ retains its physiological features. As such, an organ helps provide information on patterns of growth, differentiation as well as development.

There are number of methods that can be used for organ culture. These include;

- ***Plasma clot method*** - Here, the method involves the use of a clot that is composed of plasma and chick embryo extract (or any other extract) in a watch glass. This method is particularly used for the purposes of studying morphogenesis in embryonic organ rudiments and more recently for studying the actions of various hormones, vitamins and carcinogens of adult mammalian tissues.
- ***Raft method*** - For this method, the explant is placed on a raft of lens paper/rayon acetate and floated on a serum in a watch glass.
- ***Agar gel method*** - The medium used for this method is composed of a salt solution, serum as well as the embryo extract or a mixture of various amino acids and vitamin with 1 percent agar. The explant has to be subcultured every 5 to 7 days. The method is largely used for the study of developmental aspects of normal organs and tumors.

- ***Grid method*** - Grid method, as the name suggests involves the use of perforated stainless steel sheet, on which the tissue of interest is placed before being placed in a culture chamber containing fluid medium.

- **Protoplast Culture**

Protoplast -cells without cell walls. A protoplast is the term used to refer to cell (fungi, bacteria, plant cells etc) in which the cell wall has been removed, which is why they are also referred to as naked cells.

Protoplasts may be cultured in the following ways;

- Hanging-drop cultures
- Micro culture chambers

- Soft agars matrix

Once a protoplast has regenerated a cell wall, then it goes through the process of cell division to form a callus, which may then be subcultured for continued growth. Protoplast culture is an important method that provides numerous cells (single cells) that can be used for various studies. These include;

- Protoplast culture regenerated into a whole plant
- Development of hybrids
- Cell cloning
- Genetic transformations
- Membrane studies

In protoplast culture, a number of phases can be observed. These include;

- Development of a cell wall
- Cell division
- Continuous growth or regeneration to a whole plant

For plants, some of the special requirements include;

- Less amounts of iron and zinc and no ammonium
- Higher concentration of calcium
- High auxin/kinetic ratio for cell division and high kinetin/auxin ration for regeneration
- Glucose and vitamins

Some of the other types of tissue culture include;

- Single cell culture
- Suspension culture
- Anther culture
- Pollen culture

- Somatic Embryogenesis

Major Steps of Tissue Culture (Plants)

- Initiation Phase (Stage 1)

The initiation phase is the first phase of tissue culture. Here, the tissue of interest is obtained and introduced and sterilized in order to prevent any microorganism from negatively affecting the process. It is during this stage that the tissue is initiated in to culture.

- Multiplication Phase (Stage 2)

The multiplication phase is the second step of tissue culture where the in vitro plant material is re- divided and then introduced in to the medium. Here, the medium is composed of appropriate components for growth including regulators and nutrients. These are responsible for the proliferation of the tissue and the production of multiple shoots.*This step is often repeated several times in order to obtain the desired number of plants

- Root formation (Stage 3)

It is at this phase that roots are formed. Here, hormones are required in order to induce rooting, and consequently complete plantlets.

➢ Plant Tissue Culture

Tissue culture is applied in plant research for such purposes as the growing of new plants, which in some cases undergo genetic alterations. Here, the plant of interest is taken through the tissue culture process and grown in a controlled environment.

➢ The Process of Plant Tissue Culture

This process involves the use of small pieces of a given plant tissue (plant of interest). Once the tissue is obtained, it is then cultured in the appropriate medium under sterile conditions so as to prevent various types of microorganisms from affecting the process. The following is a general procedure for plant tissue culture

- **Medium preparation**

- The appropriate mixture (such as the MS mixture) is mixed with distilled water and stirred while adding the appropriate amount of sugar and sugar mixture. Here, sodium hydroxide or hydrochloric acid is used to adjust the pH - Contents used here will depend on the plant to be cultured and the number of tissues to be cultured.
- Agar is added to the mixture, heat and stirred to dissolve
- After cooling, the warm medium is poured into polycarbonate tubes (to a depth of about 4 cm)
- With lids sitting on the tubes, the tubes are placed in a pressure cooker and sterilized for 20 minutes

- **Plant preparation**

- Cut the plant part in to small pieces (e.g. cauliflower can be cut to florets of about 1cm across). On the other hand, such parts as the African violet leaves can be used as a whole.
- Using detergent and water, wash the plant part for about 20 minutes
- Transfer the plant part in to sterilizing Clorox solution, shake for a minute and leave to sock for 20 minutes
- Using a lid, gently discard the Clorox and retain the plant part in the container and then cap the container

- **Transferring the plant material to a tissue culture medium**

* 70 percent alcohol should be used for the sterilization of the equipment used and containers

- Open the container and pour sterile water to cover half the container
- Cover with a sterile lid again and shake the container for 2 to 3 minutes in order to wash the tissue and remove the bleach
- Pour the water and repeat this three times
- Using sterilized gloves, remove the plant part from the container and on to a sterile Petri dish

- Using a sterile blade cut the plant material to smaller pieces of about 2 to 3 mm across avoiding the parts that have been damaged by bleach
- Using sterile forceps, place a section of the plant in to the medium

Cauliflower - partly submerged in medium with flower bud facing up

Rose with shoots at level with medium surface

African violet leaf laid directly in surface of medium

*depending on the plant used, it is important to check and find out how it should be placed in the medium

Replace the lid/cap and close tightly

This procedure will result in the development of a callus, which then produces shoots after a few weeks. Once the shoots develop, then the plant section may be placed in the right environment (well lit, warmth etc) for further growth.

* Plant materials should be sterilized so as to remove any bacteria or spores that may be present.

For plants, the medium culture acts as a greenhouse that provides the explant with the idea environment for optimum growth. This includes being free of microorganisms, nutrients as well as the right balance of chemicals and hormones. Such media as BAP, TDZ are used while such hormones as IBA and IAA are used to induce growth. Some of the major reasons tissue culture is used for plants include;

- To produce large quantities of a given plant
- To accelerate the production of new varieties of a plant
- To maintain a virus free stock of the plant of interest

- **Technique for Plant In Vitro Culture**
  - ***Micropropagation*** - This technique is used for the purposes of developing high- quality clonal plants (a clone is a group of identical cells). This has the potential to provide rapid and large scale propagation of new genotypes.
  - **Somatic cell genetics** - Used for haploid production and somatic hybridization
  - ***Transgenic plants*** - Used for expression of mammalian genes or plant genes for various species it has proved beneficial for the engineering of species that are resistant against viruses and insects.

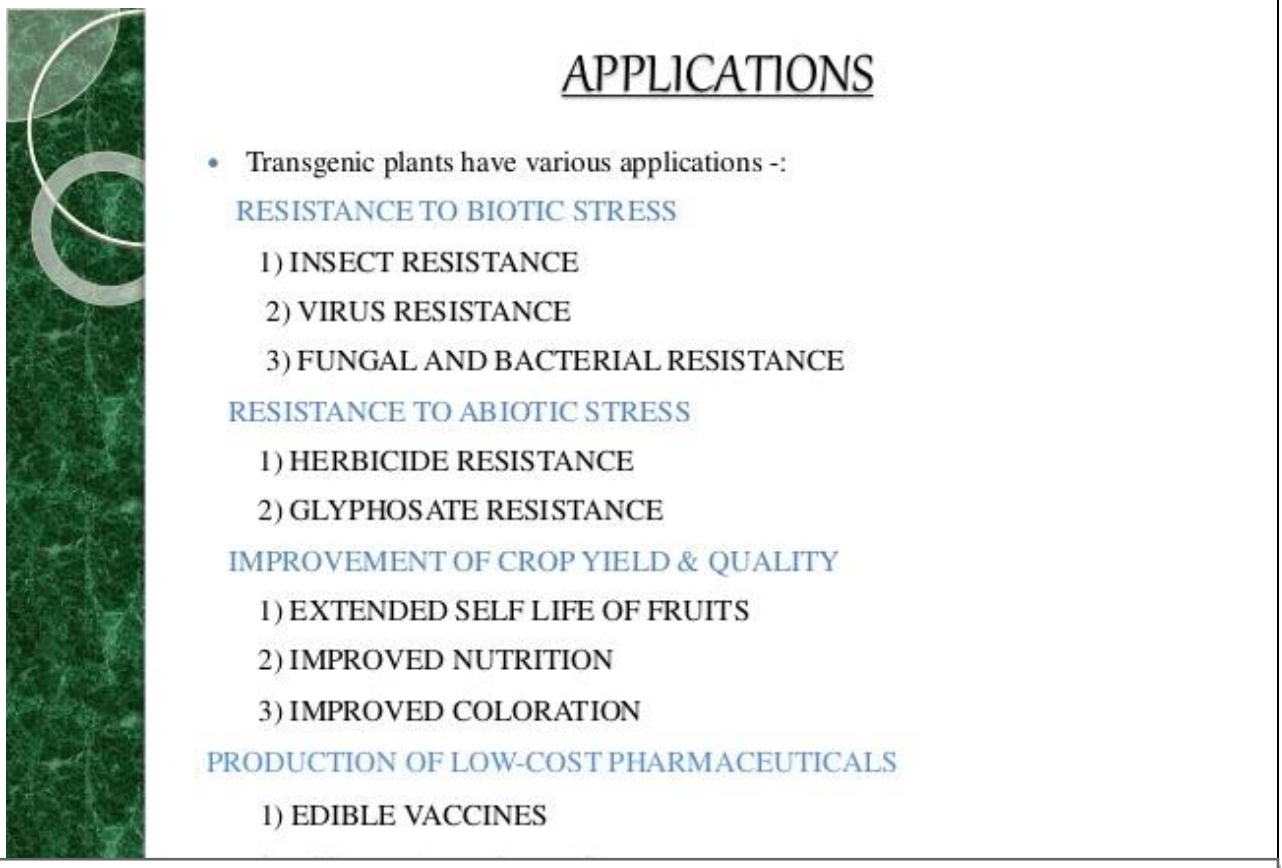
❖Conclusion

In reality, there are numerous methods used for tissue culture given that there are different types of tissues that require specific conditions for the culture process yield desired results. Both plant and animal tissue can be used for tissue culture purposes for a wide range of purposes. For instance, animal tissue culture may serve such purposes as preservation of an organ/tissue, studying the tutors or given tissues or for diagnosis purposes.

On the other hand, plant tissue culture may be used for cloning purposes, genetic modification of a given plant or simply to accelerate or increase yield of the plant of interest.

Tissue culture is therefore of great significance in biological studies due to its wide range of applications. The processes involved in tissue culture may be complex, requiring a lot of care to avoid such effects as contamination. Because of the complexities that may be involved in some of the steps, this may not be an experiment for everyone.

➢ Transgenic Plants:



Transgenic plants are **plants** that have been genetically engineered, a breeding approach that uses recombinant DNA techniques to create **plants** with new characteristics. They are identified as a class of **genetically modified** organism (**GMO**). The transgenic plants have various applications which have been outlined in the above chart. **Genetically modified crops** (**GM crops**) are plants used in agriculture, the DNA of which has been modified using genetic engineering methods. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species. Examples in food crops include resistance to certain pests, diseases, environmental conditions, reduction of spoilage, resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop.

➢ Haploids Plants:

- *What is a haploid plant?* Haploid plants originate from gametes (or gamete-like cells) that do not go through fertilization, but can still generate a viable individual. Therefore, haploids contain only the chromosome set found after meiosis in male (sperm cells) or female (egg cells) gametes. This chromosome set 'n' corresponds to only half of the chromosome set found in the fertilization product (zygote) and other somatic cells. Depending on whether the single set of chromosomes comes from the maternal or paternal side, the plant is referred to as maternal haploid and paternal haploid, respectively.
- *What is a doubled haploid (DH) plant?* In a DH plant, the chromosome set of a haploid plant has been doubled spontaneously or artificially. Chromosome doubling is necessary since haploid plants are generally frail, have reduced organ size and are not fertile. The most commonly used chemical agent to render haploid plantlets diploid is colchicine, which blocks cell division without blocking chromosome duplication. This treatment acts like a 'copy–paste' of the haploid genome into a diploid genome. Consequently, in DH plants all loci are homozygous. Chromosome doubling creates 'pure' homozygotes or fully inbred lines.
- *Why is doubled haploid technology impactful for agriculture?* Doubled haploid technology comprises both the production of haploid plants and the chromosome doubling process. It has become an important tool in plant breeding, since it shortens the time needed to create pure homozygous lines, which can either be released directly to farmers as cultivars or used as genitors (inbred lines) for the production of hybrid seeds. The primary advantage of DH plants is to possess a phenotypic stability due to the fact that all alleles are in a homozygous state. In short, DH technology increases the efficiency of plant breeding. The details technique in production of Haploid plants can be view in U tube link as given below:

https://www.youtube.com/watch?v=YlLp3MammNh8