CSIR NET Life Science Unit 6

Plant Tissue Culture and Micropropagation

In animals, once the cells become differentiated into specialized cells, they cannot be reversed to form the same cells which they were before differentiation (differentiation is irreversible in animals). However, **Gottlieb Haberlandt** said that all the plant cells are **totipotent** (i.e., each differentiated cell has the ability to convert back into its undifferentiated state (this is called **De-differentiation**), which can then again undergo differentiation to form a new plant out of it (this is called **Re-differentiation**).

In-plant tissue culture the small fragment of plant or tissues (explant) from any part of the plant is taken and grown in the *in-vitro* system in an **artificial nutrient medium** to generate a complete plant.

The artificial nutrient medium can be a solid gel-like or a liquid medium and consists of the following -

1. a) Inorganic nutrients- Macronutrients (N, P, K, Mg, Ca, S) and Micronutrients (Fe, Mn, Zn, B, Cu, Mo).
2. b) Carbon source- Sucrose
3. c) Solidifying agent for solid medium- Agar (benefit of using agar- It does not react with media components, not digested by plant enzymes).
4. d) Antibiotics to inhibit the growth of microbes- For example- Kanamycin
5. e) Growth regulators- Auxins, Gibberellins, Abscisic acid, Ethylene, Polyamines, and Jasmonic acid.

Types of plant tissue cultures

a) Callus culture- When tissues or cells are grown on an agar medium, they undergo unorganized divisions in them to form an unorganized and undifferentiated mass of cells which is called a callus. Later, the addition of hormones and other required chemicals convert this callus into a complete plant, and this process is termed a callus culture.

b) Embryo culture- First demonstrated by Hanning. Isolation of an embryo from a young seed and then culturing it in an *in-Vitro system* to form a complete plant is referred to as embryo culture.

Uses of embryo culture -

1. Shortens the time period for the development of a plant. A normal cycle of a plant- Plant à Flowering à Young seed à Mature à Seed germination to form a new plant. Now, as we can see, when we isolate embryos from a young seed, we save time.

2. Overcomes seed dormancy issues.

c) Haploid culture- It is used to develop haploid plants from diploid plants.

1) Anther/ Pollen culture- Firstly demonstrated by Guha and Maheshwari in the development of the *Datura innoxia* plant through this culture. Anthers (haploid) are collected from a plant and surface sterilized, and then grown on a nutrient medium. Pollen grains inside the anther divide and a callus are formed. The callus is later differentiated to form roots and shoots. The plant formed in this way will also be haploid.

Uses - Production of haploid plants and homozygous diploid plants by using colchicine, which reduces the breeding cycle.

2) Ovary/ Ovule culture- Similarly, when an unfertilized ovule (haploid) is used to produce a complete haploid plant, it is called ovule culture.

Uses - Production of haploid plants and used for *in-vitro* fertilization events.

Difference between plant tissue culture and micropropagation?

Mostly the two terms are used synonymously, but there is a difference between the two. Micropropagation is defined as the propagation (increase in number) of plants from a small amount of plant material, whereas tissue culture is the first step in this process.

Tissue Culture

G. Morel in 1960 first used the tissue culture technique for the micropropagation of orchid plants.

5 stages in micropropagation-

1. Explant preparation

2. Callus formation

3. Development of shoot

4. Development of root

5. Transfer of plants to the greenhouse

Use of tissue culture

1. Quick large-scale production of similar kinds (clones) of superior quality plantlets by micropropagation.

2. Producing virus-free plants- Let's suppose we have a superior quality plant, and now it has disease in it. So, we can use the apical meristem of this plant to grow multiple superior quality plants like it was.

(Note - Meristems remain free from viruses).

1. Conservation of germplasm- Storage of tissue or part of a plant by cryopreservation (storing in liquid nitrogen at -196° C), which can later be grown into a complete plant by tissue culture. This is helpful because conventional methods include storing seeds at ultra-low temperatures for germplasm conservation, which is not beneficial because many plants do not produce seeds or produce short-lived recalcitrant seeds. Moreover, storage of roots and tubers requires large space, and they lose viability in a short time period.

1. Large-scale production of secondary metabolites- Secondary metabolites produced by plants are of great use for medicinal purposes. But, they are produced by plants in small amounts. Using tissue cultures these plants can be manipulated in labs to produce large amounts of secondary metabolites of our interest. Secondary metabolites are produced using tissue culture at a large scale- Digoxin (Cardiac medicine), Diosgenin (Antifertility drug), and Taxol (Anti-cancerous drug).