Plant tissue culture means the production of new plant from small pieces called explain, in an aseptic culture where the environment, as well as the nutrient and hormone levels for growth, is tightly controlled.

Gottlieb Haberlandt German botanist (1854-1945) first to culture isolated differentiated cells. Haberlandt established the first steps of plant tissue culture as it is known today. Haberlandt in the early 1900s proposed the concept of totipotency.

Plant tissue culture

PTC mainly depends upon the four principles

TOTIPOTENCY

Genetic potential of a plant cell to produce the entire plant

GROUND STATE

It refers to the normal state of the f cell. A cell may already be competent or incompetent

COMPETENCY

Cells retain the ability for differentiation and morphogenesis

DETERMINISM

The ability of a cell to respond to the stimulus initiates a developmental process leading to morphogenesis.

Advantages of plant tissue culture:

1. Single explant can be multiplied into several thousand plants in less than a year.
2. It can also be used to produce disease-free plants.

3. Micropropagation produces rooted plantlets ready for growth, saving time for the grower.
4. It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.

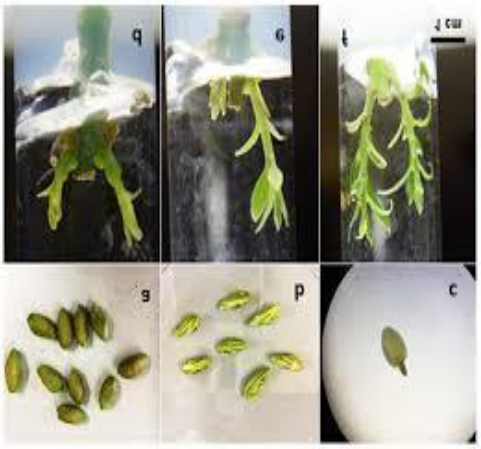
Disadvantages of plant tissue culture

1. It is very expensive.
2. It can have a labor cost of more than 70%.
3. An infected plant sample can produce infected progeny.
4. Not all plants can be successfully tissue-cultured often because the
5. A proper medium for growth is not known or the plants produce secondary metabolic chemicals that kill the explant.

Tissue culture has several critical requirements

1-Appropriate tissue (explant)

The explant is referred to as the cell or tissue (shoot tip, node) which is taken from a particular body and then placed in a culture medium for growth. In terms of plants, the explant is **the small pieces of plant parts and issues that are aseptically cut and then** they are kept in a nutrient medium.



2- Culture media

The culture media usually contain the following constituents:

A. Inorganic nutrients

B. Carbon and energy sources

C. Organic supplements

D. Solidifying agents

E. pH of medium

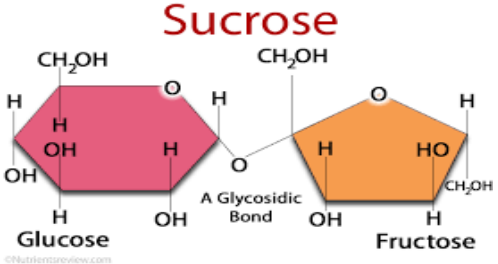
F. Plant growth regulators

A- Inorganic Nutrients:

- **Macronutrients:** any mineral required >0.5 mmol l^{-1}
  - **Nitrogen**: essential for amino acids, proteins, certain hormones, chlorophyll. Deficiency leads to the accumulation of anthocyanin.
  - **Phosphorus:** Essential for cell division, energy storage.
  - **Potassium:** Cell division, protein synthesis, nitrate reduction.
  - **Micronutrients:** any mineral required <0.5 mmol l^{-1}
    - Deficiency of micronutrients cause:
    - Reduced lignification (Cu, Fe)
    - Rosetting (Zn, Mn)

3-Carbon and Energy Sources:

Plant cells and tissues in the culture medium are heterotrophic and therefore, are dependent on the external carbon for energy. Among the energy sources, sucrose is the most preferred. During the course of sterilization (by autoclaving) of the medium, sucrose gets hydrolyzed to glucose and fructose.



4-Organic Supplements: The organic supplements include vitamins, amino acids, organic acids, organic extracts, activated charcoal, and antibiotics.

Vitamins:

Plant cells and tissues in culture (like the natural plants) are capable of synthesizing vitamins but in small quantities, inadequate to support growth. Therefore the medium should be supplemented with vitamins to achieve good growth of cells. The vitamins added to the media include thiamine, riboflavin, pyridoxine, folic acid, pantothenic acid, biotin.

Activated charcoal: Supplementation of the medium with activated charcoal stimulates the growth and differentiation of certain plant cells (carrot, tomato, orchids). Some toxic/inhibitory compounds (e.g. phenols) produced by cultured plants are removed by activated charcoal.

4 Solidifying agents

Agar is obtained from certain species of red algae and is commercially available in a powdered form. Gelrite is apolysaccharide derived from Pseudomonas bacteria that could be used as agar substitutes.

5 Media pH

Plant cells and tissue required optimum pH for growth and development in cultures. While preparing the media, thebe pH can adjusted to the requirement with 1.0 or 0.1 NHCl or NaOH.

6-Growth regulators

1 Auxins: induce cell division, cell elongation, and formation of callus in cultures. At a low concentration, auxins promote root formation while at a high concentration callus formation occurs.

2 Cytokinins: are involved in cell division, shoot differentiation, and somatic embryo formation.

3 Gibberellins: (GA_3) is the most commonly used for tissue culture. GA_3 promotes the growth of cultured cells, enhances callus growth

4 Abscisic Acid

In tissue cultures, exogenously applied ABA can affect (generally positively at low concentrations, while high concentrations inhibit) callus growth and organogenesis.

Kinds of nutrient media

1. Murashige and Skoog (MS) (herbaceous plants)
2. Linsmaier-Skoog media (herbaceous plans)
3. The woody plant medium (WPM) (woody plants)
4. Anderson (AND) medium (rhododendrons)
5. Gamborg (B5) medium (widely used for cell and tissue cultures).

3-Physical requirement

1- Temperature:

- Temperature of growth room is typically 25 ± 2 °C.
- For higher and lower temperatures special growth rooms should be prepared.

2- Light:

- Florescent: less than 1 K Lux ($0.014 \pm 2.58\ 10^{-3}$)
- LED light: More efficient than the previous type.
- Duration: 16 hrs light vs 8 hrs dark.