Plant tissue culture
Lab 2

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- **Plant tissue culture** means the production of new plant from small pieces called explant, in aseptic culture where 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 cell. Haberlan nt tissue culture as it known today. Haberlandt ept of totipotency.

Principle of Plant tissue culture

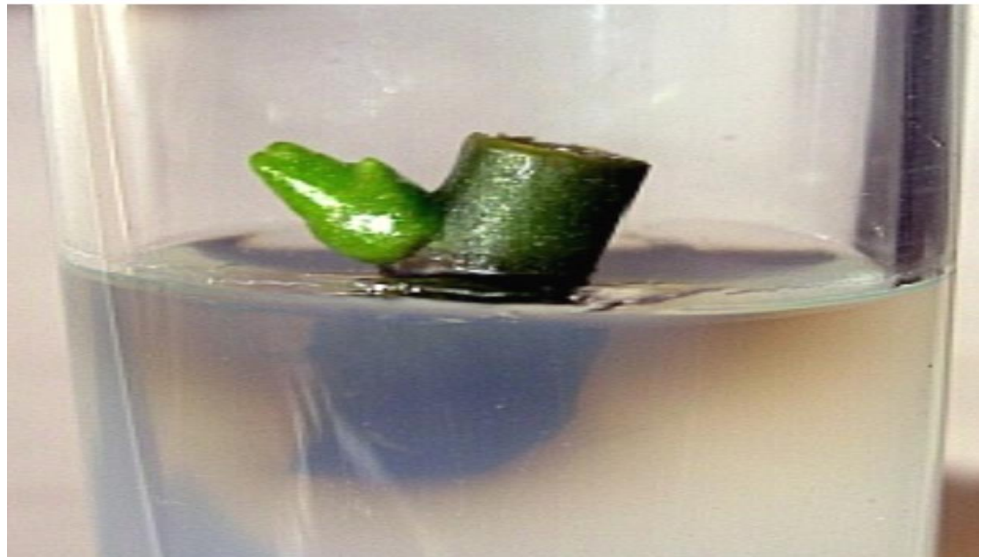
PTC mainly depends upon the four principles

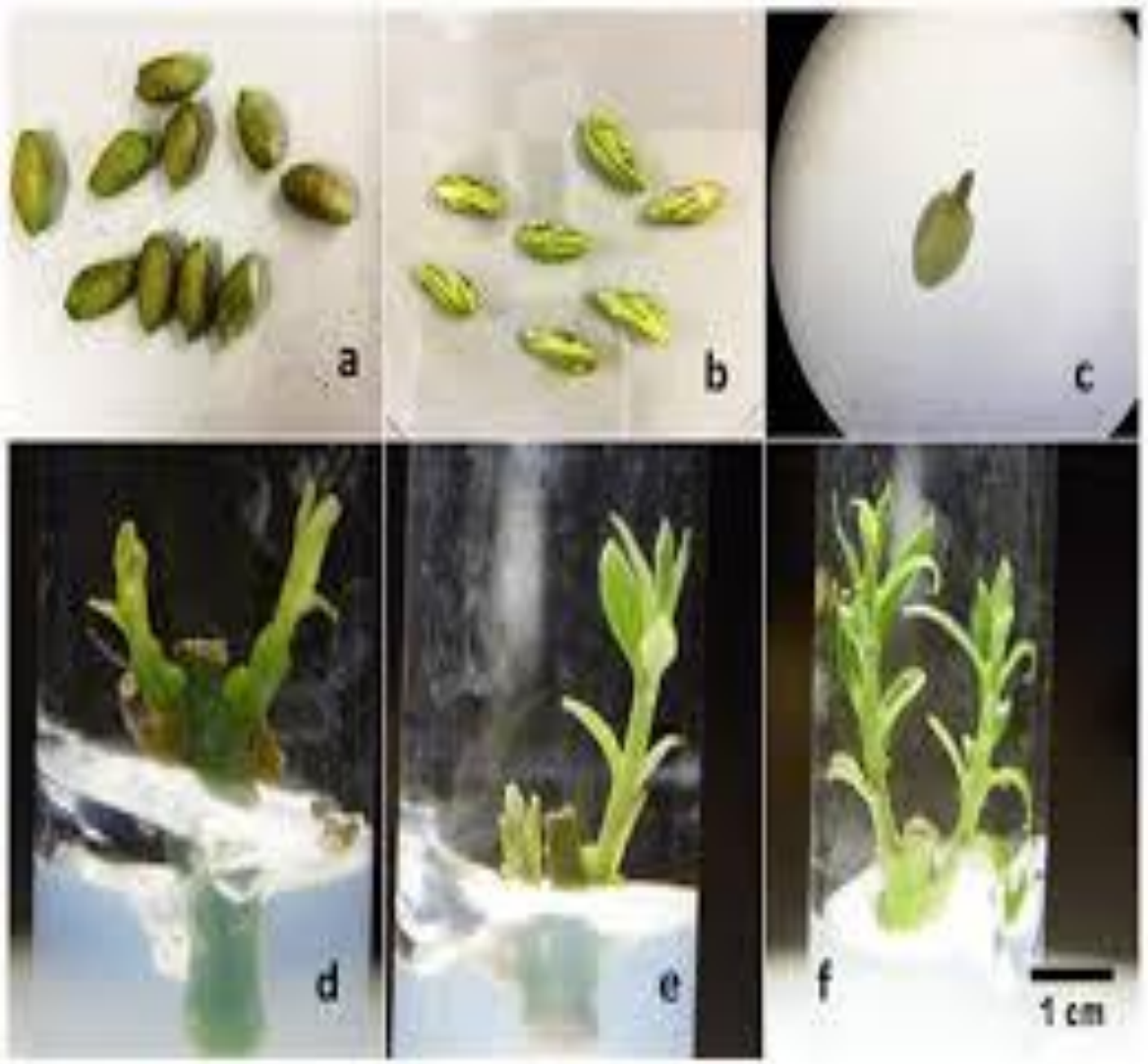
- TOTIPOTENCY
  - Genetic potential of plant cell to produce the entire plant
- GROUND STATE
  - It refers the normal state of cell. Cell may already be competent or incompetent
- COMPETENCY
  - Cells retain ability for differentiation and morphogenesis
- Determinism
  - The ability of a cell to respond to stimuli that initiates a developmental process leading to morphogenesis.

PTC Requirements

1-Appropriate tissue (explant)

Explant is referred to as the cell or tissue(shoot tip, node, bud root, leaf, etc.) 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 part and issues that are aseptically cut and then** they are kept in a nutrient medium.




a
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PTC Requirements

2- Chemical requirements (Culture media)

The culture media usually contain the following constituents:

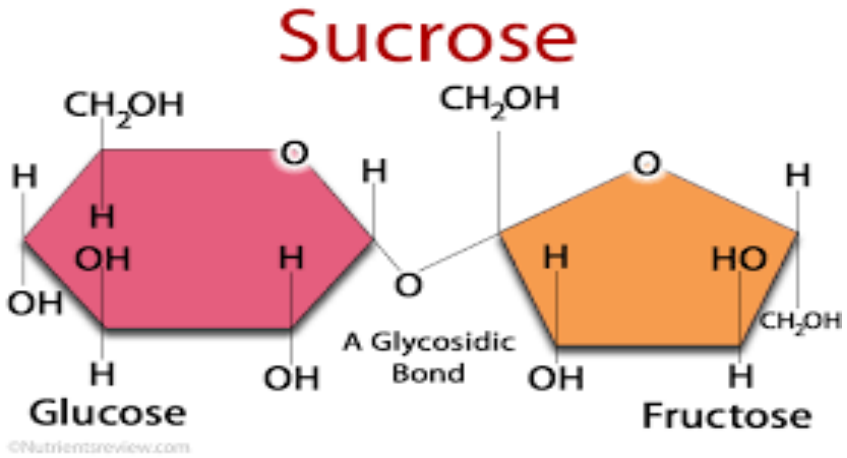
1. Inorganic nutrients
2. Carbon and energy sources
3. Organic supplements
4. Solidifying agents
5. pH of medium
6. Plant growth regulators

1 Inorganic Nutrients:

- Macronutrients: any mineral required >0.5 mmol l^{-1}
  - **Nitrogen**: essential for amino acids, proteins, certain hormones, chlorophyll. Deficiency leads to 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)

2-Carbon and Energy Sources:

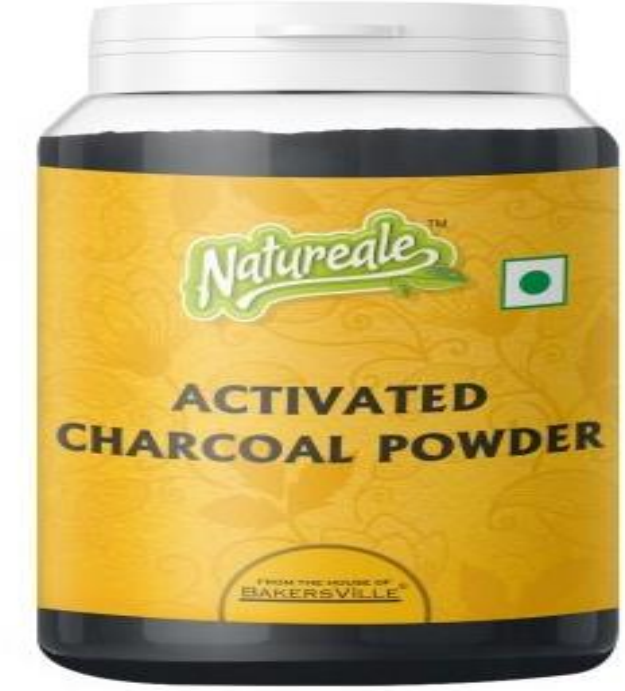
- Plant cells and tissues in the culture medium are heterotrophic and therefore, are dependent on the external carbon for energy. Among the energy sources, sucrose is the most preferred. During the course of sterilization (by autoclaving) of the medium, sucrose gets hydrolyzed to glucose and fructose.



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

- **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.
- Myo-inositol (MI): is used as second messenger in auxin pathway through storing IAA as IAA-myo-inositol. In PTC MI may act as a precursor in the formation of pectin and hemicellulose in cell wall synthesis.

- **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 commercially available in a powdered form. Gelrite is apolysaccharide derived from Pseudomonas bacteria could be used as agar substitutes.

5- Media pH

Plant cells and tissue required optimum pH (5.7) for growth and development in cultures. While preparing the media, the pH can adjusted to the requirement with 1.0 or 0.1N HCl 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 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 1965) (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).


A
ms
b
g


B


Murashige & Skoog Medium
Murashige & Skoog Medium

- **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.

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 labour 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 proper medium for growth is not known or the plants produce secondary metabolic chemicals that kill the explant.