PHARMACOGNOSY-I

UNIT 3 NOTES

PLANT TISSUE CULTURE

- HISTORICAL DEVELOPMENT
- TYPES OF CULTURE
- NUTRITIONAL REQUIREMENTS
- GROWTH AND DEVELOPMENT
- APPLICATION OF PLANTS TISSUE CULTURE
- EDIBLE VACCINES

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Plant Tissue Culture

- Plant tissue culture is a technique used to grow plant cells, tissues or organs in sterile and controlled environment on a nutrient culture medium.
- This method is commonly used for the propagation of plants, genetic modification and conservation of rare or endangered species.

Key Features

- Aseptic (sterile) conditions
- Nutrient medium containing minerals, vitamins, hormones and carbohydrates.
- Use of explants (small pieces of plant tissue, such as leaf, stem, root or meristem)
- Can lead to clonal propagation (producing genetically identical plants)
- It is based on the principle of totipotency – the ability of a single plant cell to develop into a whole plant.

Historical Development Of Plant Tissue Culture

① **1838 - 1839 - Schlieden and Schwan**

Proposed the cell theory, stating that all plants are made of cells and each cell has potential to keep develop into a whole plant (totipotency concept).

② **1902 Gottlieb Haberland**

known as father of Plant cell culture, he first proposed the idea of growing plant cells in vitro, though he was unsuccessful in practical results.

③ **1934 Phillip R. White**

Successfully cultured root tips of tomato plants in a nutrient medium.

④ **1941 - Gautheret, White and Nobécourt**

Achieved continuous growth of plant cells (mass of undiffenetiated cells) on synthetic media.

⑤ **1950s - Skoog and Miller**

Discovered the role of plant hormones (auxins and cytokines) in organ formation - roots vs shoots.

⑥ 1960s - 1980s Commercialization

Techniques like micropropagation, protoplast culture, and somatic embryogenesis developed and applied on a commercial scale.

⑦ Present day

Widely used in agriculture, horticulture, genetic engineering, and conservation microbiology.

STEPS INVOLVED IN PLANT TISSUE CULTURE

① Selection of explant & Preparation of explant

- Explant : A small piece of plant tissue (e.g., leaf, stem root, bud)
- Selection : Choose a healthy, disease - free plant.
- Preparation: Wash explants with running water to remove surface dust.

② Sterilization

- Surface sterilization : Treat explants with disinfectants like:
  70 % ethanol (30 sec to 1 min)
  0.1 % - 0.2 % mercuric chloride or 1% - 2% sodium hypochlorite (5 - 10 minutes)
- Rinsing : Rinse 3-4 times with sterile distilled water to remove traces of chemicals.

③ Preparation of culture medium

- Use standard nutrient medium (e.g., M.S. medium - Murashige and Skoog).
- Medium containts :
  - Micronutrients and Macronutrients
  - Vitamins
  - Carbon source (usually sucrose)
  - Plant growth regulators (Auxins & Cytokinins)
  - Gelling agents (Agar)

Adjust pH (usually 5.6-5.8) and sterilize by autoclaving.

④ Inoculation

- Transfer sterilized explant to sterile culture medium.
- Performed in a laminar airflow cabinet under aseptic conditions.

⑤ Incubation

- Culture tubes or jars are incubated in growth chamber.
- Condition maintained :
  - Temperature ~ 25°C
  - Light 16 hrs light/ 8-hours dark cycle
  - Humidity 50-60%.

⑥ Callus Formation

Depending on medium and explant :

- Callus Formation - Unorganized mass of cells
- Organogenesis - Development of roots, shoots or embryos.

⑦ Subculturing

Transfer growing tissues to fresh medium periodically to:

- Promote further growth
- Induce differentiation (shoot/root development)

⑧ Hardening (Acclimatization)

Gradual adaptation of plantlets to external conditions:

- Transfer to soil or potting mix in a greenhouse.
- Provide controlled humidity and light.
- Later transfer to field conditions.

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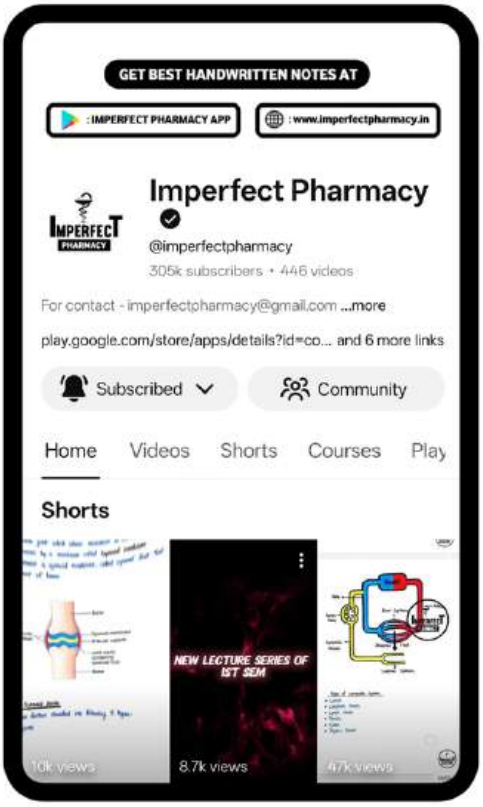

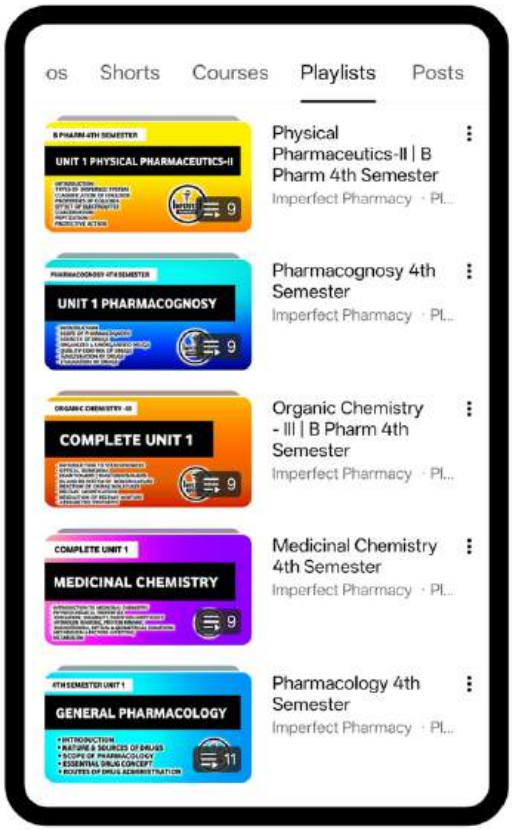

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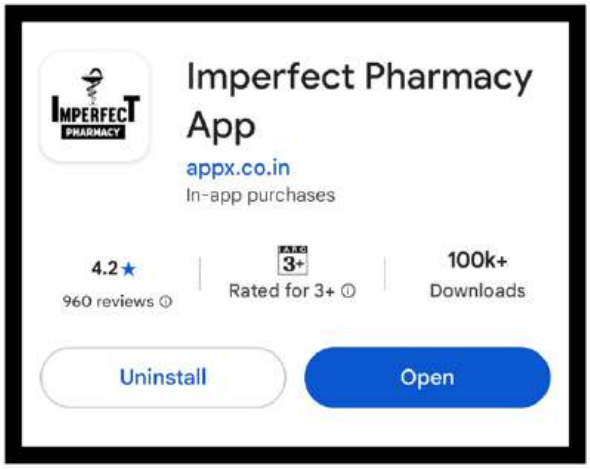

GROWTH & MAINTENANCE

① Growth of Plant tissue Culture

- Growth in tissue culture refers to the increase in size, number of cells, and development of plant parts (like roots, shoots, or embryos) from the explant under sterile and nutrient-rich conditions.
- It includes:
  - Cell devision (mitosis)
  - Callus formation
  - Organ formation (roots / shoots)
  - Development of complete plantlets.

② Maintenance of Plant tissue Culture

- Maintenance means keeping the cultures healthy and active for long periods of providing:
  - Proper nutrients
  - Sterile conditions
  - Ideal environmental conditions (light, temperature, humidity)
  - Regular subculturing (transferring to fresh media)

Types Of Plant Culture

① Callus Culture

Callus culture means growing a soft, white or yellow mass of unorganized cells (called callus) in a laboratory from any part of the plant.

Procedure :
- We take a small piece of plant (like leaf or stem) ⟶ called explant.
- We keep it on a solid nutrient medium (with hormones like auxin and cytokinin).
- Cells divide and form a lump of cells = callus.

Applications :
- To grow new plants (micropropagation)
  For gene editing.
- To study mutations or variations.

Example : Callus from carrot or tobbacco leaf.

② Suspension Culture

Suspension culture is when single cells or small cell groups float in a liquid medium and grow.

Procedure :
- We take callus and transfer it into liquid nutrient solution.
- We keep it in a shaking machine so that cells stay suspended.
- Cells grow and multiply in liquid.

APPLICATION :
- To produce useful chemicals (like alkaloids)
- For cell research
- To prepare protoplasts

EXAMPLE : Production of shikonin (a naturally occuring napthoquinone) from plant cells.

③ EMBRYO CULTURE

Embryo culture means growing the baby plant (embryo) from a seed before it dies or fails to grow naturally.

PROCEDURE :
- Immature embryo is taken out from seed.
- It is grown on special nutrient medium.

APPLICATION :
- To grow plants quickly.
- To breaks seed dormancy.

EXAMPLE : Hybrid of wheat and rye.

④ Anther Or Pollen Culture

Here, male parts of the flower like anthers or Pollens are used to grow haploid plants (having only one set of chromosomes).

Procedure:
- Anthers or Pollens from young flowers are placed on culture medium.
- They devided and form callus → later develop into plant.

Applications:
- To make haploid and doubled haploid plants quickly.
- Used in breeding programs to save time.

Examples: Haploid plants in rice or barley.

⑤ Protoplast Culture

Protoplast in a plant cell without its cell wall.
Protoplast culture means growing plants from such cells.

Procedure:
- Plant cells are treated with enzymes to remove cell wall.
- The soft, naked cells (protoplasts) are kept in medium.
- They grow → devide → make callus → then plant.

APPLICATIONS :
- To make hybrid of different species (called somatic hybrids).
- To genetic engineering.
- For studying how cells behave

EXAMPLE : Tomato + Potato = Potato (somatic hybrid).

⑥ MERISTEM CULTURE

- Meristem are the growing tips of a plant (like shoots tips).
- This technique grows whole plants from just a small meristem.

PROCEDURE :
- A tiny shoot tip (0.1 – 0.5 mm) is taken out.
- It is cultured on a nutrient medium.

APPLICATIONS :
- To make disease-free plants.
- For cloning high-yielding varities.

EXAMPLE : Banana, sugarcane, potato ⟶ virus-free production.

⑦ ORGAN CULTURE

This is the culture of entire plant organs like leaf, roots, shoots etc.

PROCEDURE: Organs are cut and grown on medium under sterile compounds.

APPLICATIONS:
- To study how organ develop
- To regenerate full plant from a root/shoot.

EXAMPLE: Root culture to study hormone response.

Application Of Plant Culture Tissue

① **Micropropagation**

Large-scale production of plants through tissue culture, useful for multiplying rare or endangered species.

② **Plant Breeding**

Tissue culture can be used to produce double haploids, which can speed up the breeding process.

③ **Genetic Engineering**

Plant tissue culture is used to introduce new genes into plants, allowing for the development of transgenic crops.

④ **Production Of Secondary Metabolites**

Plant tissue culture can be used to produce valuable compounds like alkaloids, glycosides, and phenolics.

⑤ **Conservation Of Plant Genetic Resources**

Tissue culture can be used to conserve rare or endangered plant species.

⑥ **PATHOGEN - FREE PLANTS**

Tissue culture can be used to produce pathogen-free plants, reducing the risk of disease transmission.

⑦ **IMPROVED CROP YIELDS**

Plant tissue culture can be used to produce high-quality planting material, leading to improved crop yeilds.

EDIBLE VACCINES

Edible vaccines are genetically engineered vaccines produced in edible plants (such as bananas, potatoes, tomatoes and lettuce) that contain antigens to stimulates an immune response when consumed.

KEY FEATURES

- Oral delivery - No need for injections
- Cost-effective - Cheaper than traditional vaccines
- Stable at room temperature - No cold storage required
- Needle-free - Eliminates needle associated risks.

WORKING MECHANISM

① GENE INSERTION

- A gene encoding a pathogen antigen (e.g. from bacteria or viruses) is inserted into a plant using genetic engineering (e.g. Agrobacterium-mediated transformation or biolistics).

② PLANT GROWTH & ANTIGEN PRODUCTION

- The plant express the antigen in its edible parts (fruits, leaves, tubers).
- Example- Hepatitis B antigen produced in potatoes, rabies antigen in spinach.

③ Consumption & Immune Response

- When eaten, the antigen is released in the gut.
- Mucosal immunity (IgA) and systemic immunity (IGA) are triggered.

Advantages Of Edible Vaccines

Advantage	Explanation
Needle-free administration	No pain, no risk of needle contamination
Low production cost	Grown like crops, no expensive purification needed.
Thermostable	No refrigeration required (unlike traditional vaccines).
Eeasier distribution	Can be grown locally in developing countries.
Dual Benefit	Provides nutrition along with immunization.

Challenges & Limitations

Challenge	Explanation
Dosage control	Difficult to standardize antigen levels in plants.
Stability in digestion	Antigens may degrade in the stomach (acid & enzymes).
Public acceptance	GMO concerns may reduce consumer willingness.
Regulatory hurdles	Strict approval processes for genetically modified plants.
Allerginicity risk	Some plants proteins may trigger allergies.

Examples Of Edible Vaccine

Vaccine Target	Plant Used
Hepatitis B	Potato, Banana
Rabies	Spinach, tomato
Norovirus	Tobacco, Potato
E. coli (diarrhea)	Maize
Measles	Lettuce, Carrot

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