WELCOME

TO

This is an Education Platform

We Provide PDF Notes for Pharmacy Students

Web Site http://www.fdspharmacy.in/

You tube https://www.youtube.com/c/FDSpharmacy

Telegram https://t.me/Fdspharmacy

App https://play.google.com/store/apps/details?id=com.FDSPharmacyMedia.FDSPharmacy

E-mail fdspharmacyinfo@gmail.com

PHARMACOGNOSY AND PHYTOCHEMISTRY – I

UNIT 3

TOPIC :

- **Plant tissue culture :**

  Historical development of plant tissue culture, types of cultures,
  Nutritional requirements,
  growth and their maintenance.
  Applications of plant tissue culture in pharmacognosy.
  Edible vaccines

Plant Tissue Culture

Plant Tissue Culture is a technique used to grow plant cells, tissues, or organs under sterile (aseptic) and controlled laboratory conditions on a nutrient culture medium.

- It is widely applied for micropropagation, genetic modification, conservation of rare species, and secondary metabolite production.

Key Features

- Carried out under aseptic (sterile) conditions.
- Requires a nutrient medium containing minerals, vitamins, plant hormones (auxins, cytokinins), and carbohydrates (usually sucrose).
- Uses explants – small pieces of plant tissue (leaf, stem, root, meristem, bud, etc.).
- Based on the principle of Totipotency – the ability of a single plant cell to regenerate into a complete plant.
- Produces clonal propagation (genetically identical plants).

Historical Development

- 1838–1839 (Schleiden & Schwann): Proposed *Cell Theory* – all plants are made of cells, each with potential to develop (concept of totipotency).
- 1902 (Gottlieb Haberlandt): Known as the *Father of Plant Tissue Culture*, first proposed growing plant cells in vitro (but unsuccessful in results).
- 1934 (Philip R. White): Successfully cultured *tomato root tips* in nutrient medium.
- 1941 (Gautheret, White & Nobécourt): Achieved *continuous growth* of undifferentiated plant cells (callus culture).
- 1950s (Skoog & Miller): Discovered role of auxins & cytokinins in organ formation (shoot & root differentiation).
- 1960s–1980s: Commercial development of micropropagation, protoplast culture, somatic embryogenesis.
- Present Day: Used in agriculture, horticulture, genetic engineering, and conservation biology.

Steps Involved in Plant Tissue Culture

Plant tissue culture involves several systematic steps carried out under aseptic and controlled laboratory conditions to ensure healthy plantlet development.

1. Selection & Preparation of Explant

- **Explant:** A small piece of plant tissue (leaf, stem, root, bud, meristem).
- **Selection:** Choose a healthy, disease-free, actively growing plant.
- **Preparation:** Wash explant with running water to remove surface dust and debris.

2. Sterilization

- Surface sterilization is essential to remove microbial contaminants.
- Common sterilizing agents:
  - 70% Ethanol (30 sec – 1 min).
  - Sodium hypochlorite (5–10 min).
  - Mercuric chloride (0.1–0.2% for 1–2 min, rarely used due to toxicity).
- **Rinsing:** Explants are rinsed 3–4 times with sterile distilled water to remove chemical residues.

3. Preparation of Culture Medium

- Standard medium: MS (Murashige and Skoog medium).
- Components:
  - **Macronutrients & Micronutrients** (N, P, K, Mg, Ca, Fe, Mn, Zn, Cu, B).
  - **Vitamins** (e.g., thiamine, nicotinic acid, pyridoxine).
  - **Carbon source** – usually sucrose (2–3%).
  - **Plant growth regulators**:
    - **Auxins** (e.g., IAA, NAA, 2,4-D).
    - **Cytokinins** (e.g., kinetin, BAP).
  - **Gelling agent** – Agar or gelrite.

- **pH adjustment:** Usually **5.6–5.8**.
- **Sterilization:** Done by autoclaving at 121°C (15 psi, 15–20 min).

4. Inoculation

- Sterilized explant is transferred onto culture medium.
- Done in a Laminar Air Flow (LAF) cabinet under aseptic conditions.
- Instruments (forceps, scalpel) are sterilized by flame or alcohol.

5. Incubation

- Cultures are incubated in a growth chamber with controlled conditions:
  - **Temperature:** ~25°C.
  - **Light:** 16-hour photoperiod, 8-hour dark cycle.
  - **Humidity:** 50–60%.

6. Callus Formation

- Explant undergoes cell division, producing an unorganized mass of undifferentiated cells (callus).
- Callus induction medium usually contains high auxin concentration.

7. Organogenesis / Somatic Embryogenesis

- By changing hormone ratios, callus differentiates into organs:
  - Cytokinin > Auxin → Shoot formation.
  - Auxin > Cytokinin → Root formation.
- Somatic embryogenesis: Development of embryos from somatic cells.

8. Sub-culturing

- Growing tissues are periodically transferred to fresh medium.
- Maintains nutrient supply and prevents accumulation of toxic metabolites.
- Promotes further growth, differentiation, and multiplication.

9. Hardening / Acclimatization

- Plantlets are gradually adapted to external environment:
  - First transferred to greenhouse/potting mix under controlled humidity & light.

- Later shifted to field conditions for normal growth.

Growth & Maintenance of Plant Tissue Culture

Growth of Plant Tissue Culture

- Growth in tissue culture refers to the increase in cell size, cell number, and development of organs/plantlets from an explant under sterile and nutrient-rich conditions.
- Growth is achieved through:
  1. **Cell Division (Mitosis):** Continuous multiplication of cells.
  2. **Callus Formation:** Development of an unorganized mass of undifferentiated cells.
  3. **Organ Formation:** Differentiation of callus into roots, shoots, or embryos depending on hormone balance.
     - **Cytokinin > Auxin → Shoots.**
     - **Auxin > Cytokinin → Roots.**
  4. **Plantlet Development:** Formation of a complete plant from callus or somatic embryos.

Growth is influenced by nutrient medium, growth hormones, and environmental conditions (light, temperature, pH, humidity).

Maintenance of Plant Tissue Culture

- Maintenance refers to keeping cultures healthy, viable, and actively growing for longer durations without contamination or nutrient depletion.
- Key requirements for proper maintenance:
    1. **Nutrient Supply:**
        - Periodic transfer of explants to fresh medium (subculturing).
        - Medium should contain essential macro & micronutrients, vitamins, carbon source (sucrose), and plant hormones.
    2. **Sterile Conditions:**
        - Strict aseptic techniques in laminar airflow chambers.
        - Sterilized glassware, media, and instruments to prevent microbial growth.
    3. **Controlled Environment:**
        - Temperature: ~25°C.
        - Light: 16 hours light / 8 hours dark cycle.
        - Humidity: 50–60%.
    4. **Regular Subculturing:**
        - Done every 4–6 weeks (varies by species).
        - Prevents nutrient depletion and accumulation of toxic metabolites.
    5. **Monitoring & Record Keeping:**
        - Observing for contamination, growth rate, callus/organ formation.
        - Recording passage numbers to maintain genetic stability.

Proper maintenance ensures continuous growth, genetic stability, and large-scale production of uniform plantlets.

Types of Plant Tissue Culture

Plant tissue culture is the technique of growing plant cells, tissues, or organs under sterile and nutrient-rich conditions. Depending on the explant used and the purpose, different types of cultures are practiced:

1. Callus Culture

- Callus culture is the growth of an unorganized, soft mass of dividing cells (callus) from any plant part (explant) on a solid nutrient medium.
- **Procedure**:
  - Explant (leaf/stem/root piece) is placed on nutrient medium containing hormones (auxin + cytokinin).
  - Cells divide continuously → form a lump of callus.
- **Applications**:
  - Micropropagation (cloning plants).
  - Mutation studies and genetic engineering.
  - Secondary metabolite production.
- **Examples**: Callus induction from carrot or tobacco leaf.

2. Suspension Culture

- In suspension culture, plant cells or small aggregates are grown while floating in a liquid medium.
- **Procedure**:
  - Callus is transferred to a liquid medium.
  - Culture is kept in a shaking incubator to keep cells suspended.
- **Applications**:
  - Production of useful chemicals (alkaloids, shikonin, taxol).
  - Cell research and protoplast preparation.
- **Example**: Shikonin production from plant cells.

3. Embryo Culture

- Embryo culture involves isolating and growing an immature or mature embryo in artificial medium to obtain a viable plant.
- **Procedure**:
  - Immature embryo is excised from seed before it aborts.
  - Placed on a nutrient medium for growth.
- **Applications**:
  - Breaking seed dormancy.
  - Rescue of hybrid embryos in plant breeding.
- **Examples**: Hybrid development in wheat × rye.

4. Anther / Pollen Culture

- Culture of anthers or pollens to produce haploid plants (having one set of chromosomes).
- **Procedure**:
  - Anthers/pollens are placed on nutrient medium.
  - They divide to form callus → haploid plants.
- **Applications**:
  - Production of haploid and doubled haploid plants for plant breeding.
  - Rapid development of pure lines.
- **Examples**: Haploid plants in rice, barley.

5. Protoplast Culture

- Protoplasts are plant cells without cell walls, obtained by enzymatic digestion.
- **Procedure**:
  - Plant cells treated with enzymes (cellulase, pectinase) to remove cell wall.
  - Naked cells (protoplasts) are cultured in nutrient medium.
  - They regenerate cell wall, form callus, and grow into plants.
- **Applications**:
  - Genetic engineering studies.
- **Examples**: Tomato + Potato = Pomato (somatic hybrid).

6. Meristem Culture

- Culture of the growing shoot tips or meristematic tissues to regenerate virus-free plants.
- **Procedure**:
  - Tiny meristem tip (0.1–0.5 mm) is isolated.
  - Cultured on suitable nutrient medium.
- **Applications**:
  - Production of disease-free plants.
  - Large-scale micropropagation of elite varieties.
- **Examples**: Banana, sugarcane, potato virus-free clones.

7. Organ Culture

- The culture of entire plant organs such as leaves, roots, or shoots in vitro.
- **Procedure**:
  - Organs are excised and placed on sterile nutrient medium.
- **Applications**:
  - To study organ development and morphogenesis.
  - To regenerate full plants from organs.
- **Examples**: Root culture to study hormone effects.

Applications of Plant Tissue Culture

- ✓ **Micropropagation** – Large-scale production of disease-free, uniform, high-yielding plants.
- ✓ **Germplasm Conservation** – Preservation of rare and endangered species (cryopreservation).
- ✓ **Genetic Engineering** – Used in transformation studies, protoplast fusion, production of transgenic plants.
- ✓ **Secondary Metabolite Production** – Cultivation of plant cells for alkaloids, glycosides, terpenoids, etc. used in pharmaceuticals.
- ✓ **Virus Elimination** – Meristem culture helps produce virus-free plants.
- ✓ **Hybridization Support** – Protoplast fusion creates somatic hybrids.
- ✓ **Crop Improvement** – Development of disease-resistant, stress-tolerant, and high-yield varieties.

Edible Vaccines

Edible vaccines are genetically engineered vaccines produced in edible plants (such as banana, potato, tomato, lettuce, maize, spinach) that express antigenic proteins from pathogens.
When consumed, these plant-derived antigens stimulate the immune system, providing protection against diseases.

Key Features

- **Oral delivery** – No injections required.
- **Cost-effective** – Cheaper than traditional vaccines (grown like crops).
- **Thermostable** – Stable at room temperature (no cold chain needed).
- **Needle-free** – Eliminates risks of needle use (pain, infection).
- **Mass production** – Can be scaled up using agricultural methods.

Working Mechanism

1. **Gene Insertion**
    - A pathogen-specific gene encoding an antigen is inserted into plant cells using:
        - *Agrobacterium-mediated transformation* or
        - *Biolistic (gene gun) method*.
2. **Plant Growth & Antigen Expression**
    - The modified plants grow and produce antigenic proteins in edible parts (fruits, tubers, leaves).
    - Example: *Potato producing Hepatitis B antigen*.
3. **Consumption & Immune Response**
    - When eaten, the plant releases the antigen in the gut.
    - Antigen stimulates:
        - **Mucosal immunity** → IgA antibodies in the intestine.
        - **Systemic immunity** → IgG antibodies in blood.

Advantages of Edible Vaccines

Advantage	Explanation
Needle-free	No pain, no risk of infection or cross-contamination.
Low cost	Grown like crops; no need for expensive purification.
Thermostable	Do not require refrigeration, unlike conventional vaccines.
Easy distribution	Can be produced locally in developing countries.
Dual benefit	Provide nutrition along with immunization.

Challenges & Limitations

Challenge	Explanation
Dosage control	Hard to standardize antigen concentration in plants.
Stability in digestion	Antigens may degrade in stomach acid and enzymes.
Public acceptance	Concerns about GMOs may reduce acceptance.
Regulatory hurdles	Long approval process for GM vaccines.
Allergenicity	Risk of allergic reactions due to plant proteins.

Examples of Edible Vaccines

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