Unit-3

Pharmacognosy-I

B.Pharma 4 Semester Notes

UNIT-III

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.

Pharmacognosy - 1

Mr Chandresh Maurya (M.Pharm Pharmacognosy)

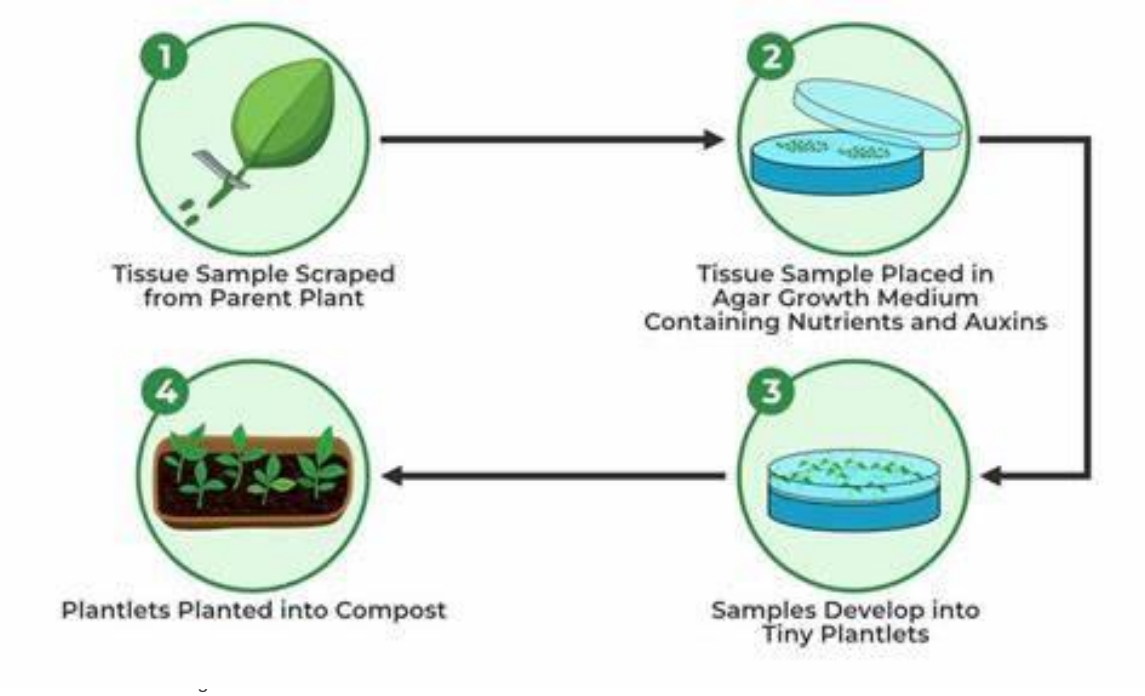
Plant tissue culture

Plant tissue culture is a technique used to grow plants under sterile conditions on a nutrient culture medium.

Plant tissue culture, also known as micropropagation.

It has a wide range of applications in research, agriculture, and pharmacognosy.

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs on a nutrient culture medium of known composition under sterile conditions



Historical Development

Year	Scientist	Development
1902	Haberlandt	1st proposed the concept of DTC (Plant tissue culture)
1904	Hanning	Establishment of embryo culture for 1st time.
1909	Kuster	1st observation of fusion cell
1922	Robins, Kotte	In-Vitro cultivation of root tips
1934	White	Permanent root culture for 1st time (tomato)
1934	Gavtheret	permanent callus culture using Vitamin B and auxins
1942	Gavtheret	Observation of secondary metabolites in PTC.
1953	Muir	Develop single cell culture

1955	Mothes & Kala	1st report of 2' metabolites production in liquid medium.
1965	Vasil and Hildebrandt	Regeneration of a plant from one single cell cultivation of tobacco cells
1977	Noguchi	Cultivation of tobacco cells
1908	Simon	Obtained success in regeneration of bulky callus buds and roots

Types of Cultures

There are several types of plant tissue cultures, including:

- Protoplast culture (Important)
- Callus culture (Important)
- Suspension culture
- Seed Culture
- Leaves Culture
- Root Culture
- Embryo Culture (Important)

1. Callus culture:

- It is culture of undifferentiated mass of parenchyma cell produced from an explant of a seedling or other plant part in agar medium under aseptic condition is known as Callus culture.
- Callus is densely aggregated, Uncontrolled, Under differentiated, Unorganized, aerated homogenous parenchymatous mass.

2. Embryo Culture:

Embryo is obtained or excised/isolated from ovule, seed or fruit.

↓

Transfer into nutrient medium (salts, sugar, etc) under aseptically

↓

Incubate at 25^0Cfor 16 Hours.

↓

Sub-culturing is done in fresh medium in the interval of 3-4 weeks

3. Protoplast culture:

- Protoplast are the naked cells without cell wall which are cultivated in liquid as well as on solid media.
- Protoplast can be isolated by mechanical or enzymatic method from almost all parts of the plant.
- Peeled leaft segments are treated with enzyme macrozyme and then treated with cellulose to isolate the protoplast.

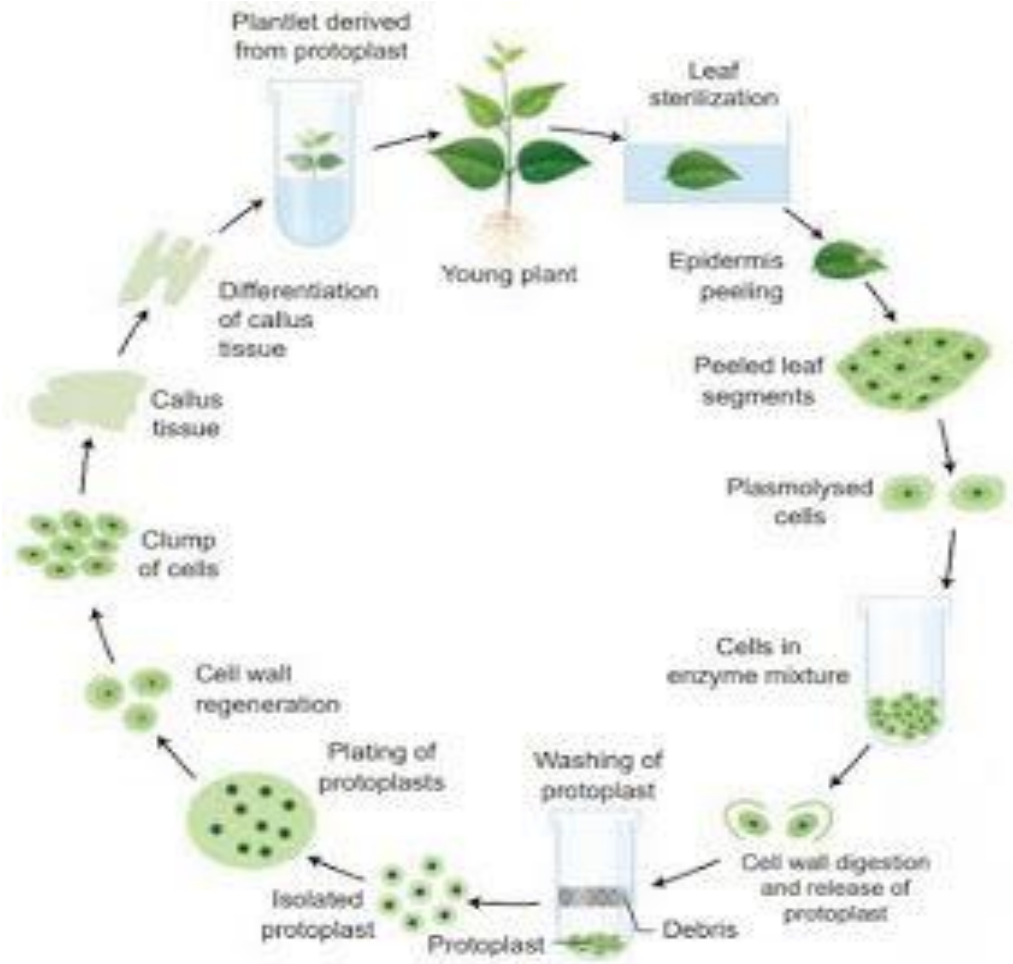


Figure 5.8: Protoplast Culture

Nutritional requirements of Plant tissue culture:

1. Inorganic nutrients:

- Macronutrient:
- Micronutrient:

2. pdf

3. Organic nutrients

- Nitrogen
- Vitamins
- Amino Acids

4. Antibiotic

5. Solidifying agent:

1. Inorganic nutrients:

Macronutrient: The macronutrients include six major elements as follows: Nitrogen (N), Potassium (K), Phosphorous (P), Calcium (Ca), Magnesium (Mg), Sulfur (S).

1. Nitrogen 2-20mmol/lit — Influences plant growth rate, essential in plant nucleic acids (DNA), proteins, chlorophyll, amino acids, and hormones.
2. Phosphorus 1-3 mmol/lit— Abundant in meristimatic and fast growing tissue, essential in photosynthesis, respiration.
3. Potassium 20 -30 mmol/lit— Necessary for cell division, meristematic tissue, helps in the pathways for carbohydrate, protein and chlorophyll synthesis.
4. Calcium 1-3 mmol/lit - Involved in formation of cell walls and root and leaf development. Participates in translocation of sugars, amino acids, and ties up oxalic acid (toxin)
5. Magnesium 1-3 mmol/lit - Involved in photosynthetic and respiration system. Active in uptake of phosphate and translocation of phosphate and starches.
6. Sulfur 1-3 mmol/lit - Involved in formation of nodules

Elements required in the life of a plant greater than 0.5 mmol/lit are referred as macronutrients

Micronutrient:

- Elements required in the life of a plant less than 0.5 mmol/lit are referred as micronutrients.
- Require in very small amounts for growth and development.
- Eg:- Iodine, Copper, Cobalt, Manganese, Iron, Zinc, etc.

2. pH:

- pH of the medium should be in a range of 5.6-6.0 before autoclaving the culture medium.
- Nutritional medium depends upon the type of plant tissue or cell which we used for culture.

3. Organic nutrients

Nitrogen:

- Most cultured plant cells are capable of synthesising essential vitamins but not in sufficient amount.

Vitamins:

- Cell in culture can synthesize in less amount so required externally in small quantity for better growth And Development.
- Eg: Thiamine (Vitamin B1), Nicotinic acid (niacin-Vitamin B3), Cynocobalamin (Vitamin B12), Riboflavin (Vitamin B1,), Folic acid (Vitamin M) 0.5 mg/lit, Biotin (Vitamin H), etc.

Amino Acids

- Some cultured plant-cells can synthesize all amino acids, none are considered essential. The most common sources of organic nitrogen used in culture media are amino acid mixtures, (e.g., casein hydrolysate), L-glutamine, L- asparagine, orginine, methionine and adenine. When amino acids are added alone, they can be inhibitory to cell growth.

4. Antibiotic

- It prevent the growth of microbes in plant tissue culture.
- Eg. Streptomycin, etc

5. Solidifying agent:

- They are used in the prepration of semi-solid or soled tissue culture medium.
- Eg: Agar, Gelatin, Pellets, Starch, Silica gel etc.

Applications of plant tissue culture:

- Micropropagation (Clonal Propagation)
- Synthetic seed
- Protoplast culture and somatic hybridization
- Hairy root culture
- Cryopreservation
- Tracing the biosynthetic pathways of secondary metabolites
- Generation novel compound from plant
- Respiration, organ function and metabolism in plant tissue culture can be studied.
- Plant improvement by studying diseases of plant and their elimination with the help of plant tissue culture.
- Mutant cell selection is done by addition of toxic substance to cells followed by isolation of resistant cells.
- Production of economical valuable chemicals by plant tissue culture which are not possible by other chemical methods.

Edible vaccines:

Vaccines:

- A disease antigen that stimulates the body to produce an antibody reaction but it is not strong to produce the diseases harmful effects. A vaccine is a biological preparation that establishes or improves immunity to a particular disease.

Edible Vaccine:

- **Transgenic plants are used as vaccine production systems.** The genes encoding antigens of bacterial and viral pathogens can be expressed in plants in a form in which they retain native immunogenic properties.
- It has also found application in prevention of autoimmune diseases, birth control , cancer therapy, etc. Edible vaccines are currently being developed for a number of human and animal diseases.

Plants used for edible Vaccine:

- Tobacco, Potato, Banana, Tomato, Rice, Lettuce, Soybean, Alfalfa, Muskmelon, Carrot, Peanuts, Wheat, Corn etc.

Advantages of Edible Vaccine:

- Needs no purification.
- Edible vaccine activates both mucosal and systemic immunity
- Heat-stable; do not require cold-chain maintenance.
- It is cheap and Administering oral vaccines would require little or no training at all.
- Easy to Administration.