Plant tissue culture

Plant tissue culture is a technique of culturing plant cells, tissues and organs on synthetic media under aseptic environment and controlled conditions of light, temperature, and humidity. The development of plant tissue culture as a fundamental science was closely linked with the discovery and characterization of plant hormones, and has facilitated our understanding of plant growth and development. Furthermore, the ability to grow plant cells and tissues in culture and to control their development forms the basis of many practical applications in agriculture, horticulture industrial chemistry and is a prerequisite for plant genetic engineering.

Historical development

1. German botanist Gottlieb Haberlandt was the first person to culture isolated, fully differentiated cells in 1898.
2. Hanning(1904) Embryo culture of selected crucifers.
3. Robbins (1922), Kotte In Vitro culture of root tips
4. Laibach (1925) Use of embryo culture techniquein inter-specific crosses of Linum
5. White (1934-37) Successful culture of tomato roots importance of vitamins in growth media
6. Snow, Gautheret (1934-1939) Importance of auxin as growth regulator
7. Gautheret, Nobecourt, White (1939) Successful establishment of continuously growing cultures.
8. Van Overbeek (1941) Use of coconut milk containing a cell division factor first time in Datura
9. Skoog and Tsui (1951), Miller et al (1955), In vitro cell differentiation, discovery of Kinetin
10. Morel and Martin (1952) Application of micro grafting to obtain virus free plants
11. Muir (1953) Isolation and culture of single cells from plants
12. Skoog and Miller (1957) Discovery of principle of hormonal control of the organ formation in tissue culture
13. Reinert and Steward (1958-59) First report on somatic embryogenesis
14. Jones et al (1960) Hanging drop method of cell culture ϖ Bergmann (1960) Bergmann cell culture technique
15. E C Cocking (1960) Isolation of protoplasts by enzymatic degradation method
16. Murashige and Skoog (1962) Development of MS media
17. Guha and Maheshwari (1964) Production of First haploid plant by anther culture
18. Power et al (1970) Protoplast fusion
19. Takabe et al (1971)Rgeneration of first plant from protoplast
20. Carlson et al (1972) First report on inter-specific hybridization through protoplast fusion

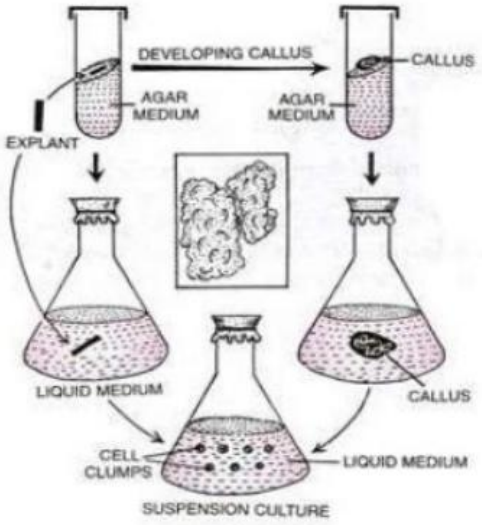
Types of plant tissue cultures

1. **Callus Culture**

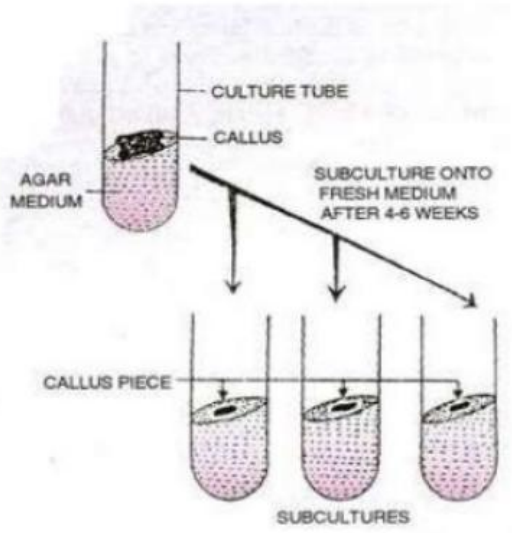
In Callus culture, cell division in explant forms a callus. Callus is irregular unorganized and undifferentiated mass of actively dividing cells. Darkness & solid medium gelled by agar

stimulates callus formation. The medium contains the auxins and BAP (Benzyl amino purines). Both are growth regulators (Hormones). This stimulates cell division in explant. Callus is obtained within 2-3 weeks.

Callus culture and Sub culture



Initiation of callus and suspension cultures.



Schematic representation of subculturing.

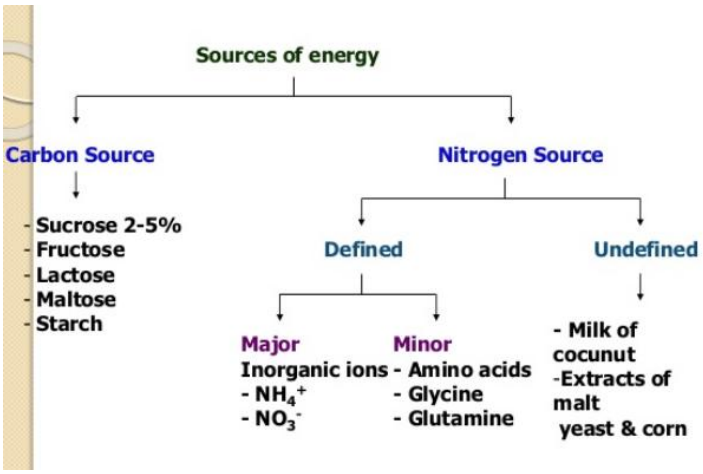
After sufficient time of callus growth on the same medium following changes will occur;
Depletion of nutrient in the medium
Gradual loss of water
Accumulation of metabolic toxins
Hence for maintenance of growth in callus it is necessary to subculture the callus.
Subculture should be repeated after 4-5 weeks.

2. **Single Cell Culture**
   As stated earlier, cells derived from a single cell through mitosis constitute a clone and the process of obtaining clones is called cloning (asexual progeny of a single individual make up a clone).
3. **Root tip culture**
   Tips of the lateral roots are sterilized, excised and transferred to fresh medium.
   The lateral roots continue to grow and provide several roots.
   After 7 days that are used to initiate stock or experiment.
   Thus the root material derived from a single radical.
   Such genetically uniform root cultures are referred to as a clone of isolated roots.
4. **Leaves culture**
   Leaves (800µm) may be detached from shoots, surface sterilized and place in healthy condition for long period.
   Growth rate in the culture depends on their stages of maturity at excision.
   Young leaves have more growth potential then the nearly mature ones.
5. **Shoot tip culture**

The excised shoot tips (100-1000µm long) of many plant species can be cultured on relatively simple nutrient media.
This media must contains growth hormones and will often form roots and develop into whole plants.

6. **Complete flower culture**
   Flowers (2days after pollination) are excised, sterilized by immersion in 5% calcium hypochloride, washed with sterilized water.
   Transfer this to culture tubes containing an agar medium.
   Fruits, which develop are smaller than their natural counterpart, size can be increases by supplementing the medium with appropriate combination of growth hormones.
7. **Anther Culture**
   Young flower buds are removed from the plant & surface sterilized.
   The anthers are then excised and transferred to an appropriate nutrient medium.
   The plantlet are formed after 4-5 weeks of inoculation.
   Many plantlets are produced from the single anther.
8. **Pollens culture**
   Pollen grains are removed from the anther.
   Anthers are placed in a 5ml liquid medium in petri dish.
   Petri dishes containing the pollen grains in the culture media are sealed with parafilm & incubated at 28ºC in dark for 14 days.
   3-4 weeks may be required to obtain haploid plantlets.

Nutritional requirements, growth and their maintenance.



Composition of Culture media

Cultre Media is composed of

Inorganic nutrients which includes macronutrients like nitrogen, phosphorous, potassium, calcium etc. and micronutrients like boron, copper, iron, manganese, zinc etc.

Organic nutrients includes Vitamins like Vitamin B1, B6, B3, B5 etc. Amino acids like L-arginine, L-asparagine, L-cysteine HCL, L-glutamine etc, Carbon source like glucose or maltose, Growth hormones/regulators like auxin, cytokinins and gibberellins, ethylene, abscisic acid.

Others media substances like protein hydrolysates, yeast extaracts, fruit (e.g. banana) extracts, coconut milk, solidifying agents like agar, alginate, gelatin etc., Iron source e.g.EDTA, Antibiotics.

pH of the medium should be in a range of 5.6-6.0 before autoclaving the culture medium

Inorganic nutrients:

Mineral elements play very important role in the growth of plant Function of nutrients in plant growth. Essentially about 15 elements found important for whole plant growth have also been proved necessary for the growth of tissue(s) in culture.

Macronutrient:

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

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

Micronutrient:

Elements required in the life of a plant less than 0.5 mmol/lit are referred as micronutrients.

Overall the plant thrives on seventeen elements out of which four like carbon, hydrogen, oxygen and nitrogen are derived from the atmosphere and the rest thirteen microelements like Boron, copper, iron, manganese, zinc, cobalt, molybdenum, nickel, aluminium, iodine, ferrous, sodium, chlorine.

A media lacking in these micronutrient does not support healthy and wholesome growth and the plant exhibits deficiency symptoms such as pigmentation, absence of vessels, presence of narrow cambial zone, cellular hypertrophy and symptoms of chlorosis due to absence of ferrous and sulphur.

Iron (Fe)-1 mM/l - Involved in Cell division, respiration, chlorophyll synthesis and photosynthesis. Eg. FeNaEDTA = sodium salt of EDTA.

Manganese (Mn) 20-90 mM/l - Involved in Cell elongation, regulation of enzymes and growth hormones. Assists in photosynthesis and respiration.

Boron (B) 2-5100 mM/l responsible for cell division and cell elongation

Copper (Cu) 0.1 mM/l

Molybdenum (Mo) 5mM/l

Cobalt (Co) 0.1 mM/l

Zinc (Zn) 1.5-30 mM/l

Iodine (I) **Nickel** (Ni), **Aluminum** (Al),
Ferrous, chlorine (Cl) and sodium (Na) are also required

Organic nutrients

It includes Nitrogen substances, Vitamins, Amino acids, Carbon source, Growth hormones/regulators

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

Vitamins-

Thiamine (Vitamin B1) - essential as a coenzyme in the citric acid cycle. It is required mostly in tissue culture and is considered to be essential. Thiamine hydrocholoride in 0.1- 1mg/lit concentration is required.

Nicotinic acid (niacin-Vitamin B3) 0.5 mg/lit, Pyridoxine (Vitamin B6) 0.5 mg/lit, Calcium pentothenate (Vitamin B5) 0.1 mg/lit, are known to improve growth of the tissue culture material.

Myo-inositol - part of the B complex, in phosphate form is part of cell membranes, organelles and is not essential to growth but beneficial and have important role in many biosynthetic pathways.

Cynocobalamin (Vitamin B12), Riboflavin (Vitamin B1,), Folic acid (Vitamin M) 0.5 mg/lit, Biotin (Vitamin H), p-amino benzoic acid (PABA), Ascorbic acid (Vitamin C), α- tocopherol (vitamin E) are added in special cases but their exact role is not yet well established.

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.

Tyrosine has been used to stimulate morphogenesis in cell cultures but should only be used in an agar medium. L-tyrosine - stimulates shoot formation.

Supplementation of the culture medium with adenine sulfate can stimulate cell growth and greatly enhance shoot formation.

Carbon source

Carbohydrates are used in tissue culture media as an energy source of carbon. Most plant tissue culture are nonautotropic and are therefore entirely dependent on an external source of carbon.

The most commonly used carbon source is Sucrose (2-5% or 20-30 g/lit)

Glucose and Fructose are used for good growth.

Maltose and raffinose are used in some cases.

In general excised dicotyledonous roots grow better with sucrose where as monocots do best with dextrose (glucose).

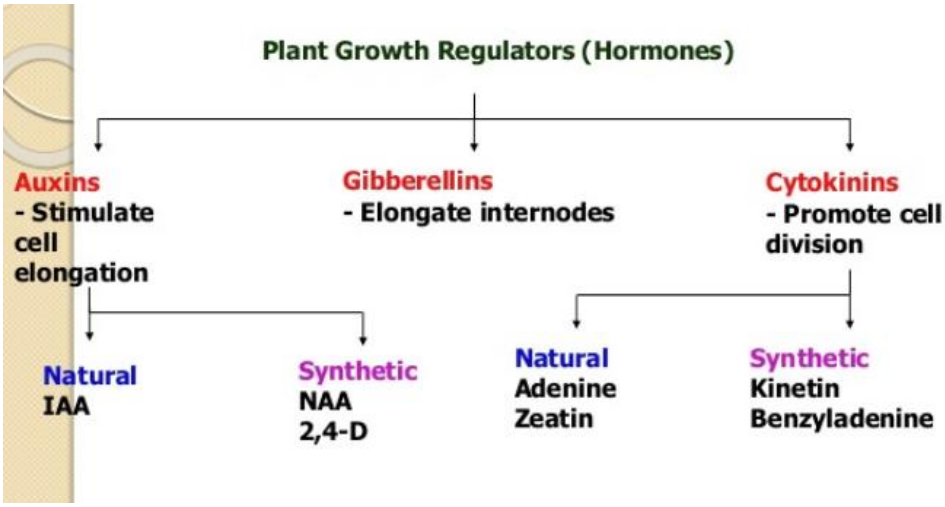
Other carbohydrates like mannose, sorbitol, pentoses, sugar alcohol, glycols, hexoses, uronic acid, lactose, galactose, potato starch, grain starch and even glycosides can be used depending on the experimental conditions.

Growth hormones/regulators/ Modulators

The success of plant tissue, cell and organ culture will depends on the amount of plant hormones and growth substance added into nutrient medium. ϖ Auxins, ethylene, abscisic acid, cytokinins and gibberellins are commonly recognized as the five main classes of naturally occurring plant hormones.

The requirement of these hormones varies considerable with their endogenous levels.

Other plant hormones like polyamines, jasmonates, salicylates are also used depending on the experimental conditions and plants to be cultured.



Applications of plant tissue culture in Pharmacognosy

1. Production of phytopharmaceuticals and secondary metabolites.
    a. Biotransformation (Biochemical Conversion)
    b. Plant cell immobilization
    c. Genetic transformation (Transgenic plant)
    d. Elicitors
2. Micropropagation (Clonal Propagation)
3. Synthetic seed
4. Protoplast culture and somatic hybridization
5. Hairy root culture
6. Cryopreservation
7. Tracing the biosynthetic pathways of secondary metabolites
8. Generation novel compound from plant
9. Respiration, organ function and metabolism in plant tissue culture can be studied.
10. Plant improvement by studying diseases of plant and their elimination with the help of plant tissue culture.
11. Mutant cell selection is done by addition of toxic substance to cells followed by isolation of resistant cells.
12. Production of economical valuable chemicals by plant tissue culture which are not possible by other chemical methods.

Production of phytopharmaceuticals and secondary metabolites Secondary plant metabolites like alkaloids, terpenoids, flavonoids, lipids, oils, tannins, anthraquinones, flavones, napthaquinones, vitamins, proteins, anticancer agents, antiviral agents etc. are isolated from plant tissue culutre.

Edible vaccines

Vaccine: Definition .
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.
Vaccines can be

PROPHYLACTIC (e.g. to prevent the effects of a future infection by any natural or "wild" pathogen)
THERAPEUTIC (e.g. vaccines against cancer)

Ideal vaccine
It should not be toxic or pathogenic.
Low levels of side effect.
It should not contaminate the environment.
It should not cause problems in individual.
Technique of vaccination should be simple.
It should be cheap.

Edible Vaccine

In the 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.
Initially thought to be useful only for preventing infectious diseases, 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.
As Hippocrates said , Let “thy food be thy medicine”

Advantages of Edible Vaccine

1. As oral vaccines provide “mucosal immunity” at various sites by secreting antibodies. In edible vaccines Dont need to worry about re-use, misuse and lack of sterilization. Thus, low risk of infection.
2. It is cheap and Administering oral vaccines would require little or no training at all.
3. Most importantly, they trigger the immunity at the mucosal surfaces such as mouth which is the body’s first line of defense.
4. Needs no purification.
5. Edible vaccine activates both mucosal and systemic immunity
6. Heat-stable; do not require cold-chain maintenance.
7. If the local/native crop of a particular area is engineered to produce the vaccine, then the need for transportation and distribution can be eliminated.
8. Edible vaccine holds a great potential . It reduces the cost of transportation and refrigeration. It neglect the needle and complicated way of vaccine administration. For many disease the research in going on in many countries funded by their government or industry. Significant challenges are still to be overcome before vaccine

crop can become a reality. There are some safety concerns which need to be overcome in near future…

Plants used for edible Vaccine

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

Examples

Transgenic potato expressing norwalk virus antigen showed seroconversion.

Transgenic potato with CT-B gene of Vibrio cholerae was shown to be effective in mice.

Mice fed with tobacco expressing MV-H could attain antibody titers five times the level considered protective for humans. MV-H edible vaccine does not cause atypical measles, which may be occasionally seen with the current vaccine.

Transgenic rice, lettuce and baby food against measles are also being developed.

For hepatitis B, parenteral VLPs could invoke specific antibodies in mice. First human trials of a potato based vaccine against hepatitis B have reported encouraging results. The amount of HBsAg needed for one dose could be achieved in a single potato.

Mice immunized intramuscularly with doses of purified H5, VLPs were protected against influenza virus.