SYLLABUS

B.Sc VI SEM

Plant and Environmental Biotechnology

Unit I	**Topic** **Introduction to and history of plant tissue culture** **MS Media for plan tissue culture** **Use of Growth regulation** **Cell division an cell cycle** **Selection and maintenance of callus** **Single cell culture**
Unit II	**Cytodifferentiation** **Micropropogation** **Organogenesis** **Somatic Embryogenesis** **Synthetic seed and its application** **Anther and Ovary culture** **Production of haploids and their uses** **In vitro Pollination and Fertilization**
Unit III	**Protoplast isolation** **Testing of viability** **Regeneration of protoplas and protoplast fusion** **Markers for selection of hybrid cell**

	Practical application of somatic hybridization **Introduction to Cybrids and Transgenic plants** **Use of A. tumifaciens and A. rhizogenes** **Transfection methods** **Advantages of Transgenic Plant**
Unit IV	**Conventional fuels- firewood plant, coal gas** **Animal oils and their impacts** **Methenogenic bacteria and biogas** **Microbial hydrogen production, solar energy** **Gasohol experiment** **Plant based petroleum industry** **Cellulose degradation and their EVS impact** **Microbial leaching of Copper and Uranium** **Biorecovery of petroleum- MEOR** **Bioremediation and Biodeterioration- Petroleum** **Lether, textile and paper**
Unit V	**Biopesticides- Bacterial and fungal** **GM crops containing insecticidal genes** **Biofertilizers- Nitrogen fixers, PSB** **Mycorrhiza and VAM** **Biosensors and Biopolymers**

	Biofilm and Bioplastics **Biochips and Bioindicators** **Biological weapons and Bioterrorism**

UNIT-1

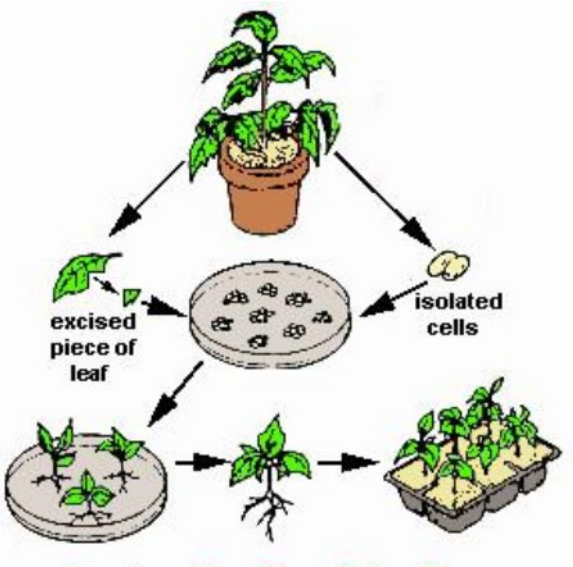
Q.1 Introduction and history of Plant tissue culture:

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as microprapogation.

1. Introduction:

* Most methods of plant transformation applied to GM crops require that a whole plant is regenerated from isolated plant cells or tissues which have been genetically transformed.
* This regeneration is conducted in vitro so that the environment and growth medium can be manipulated to ensure a high frequency of regeneration.
* In addition to a high frequency of regeneration, the regenerable cells must be accessible to gene transfer by whatever technique is chosen.
* The primary aim is therefore to produce, as easily and as quickly as possible, a large number of regenerable cells that are accessible to gene transfer.
* The subsequent regeneration step is often the most difficult step in plant transformation studies.
* However, it is important to remember that a high frequency of regeneration does not necessarily correlate with high transformation efficiency.
* This chapter will consider some basic issues concerned with plant tissue culture in vitro, particularly as applied to plant transformation.
* It will also look at the basic culture types used for plant transformation and cover some of the techniques that can be used to regenerate whole transformed plants from transformed cells or tissue.
* Plant tissue culture, the growth of plant cells outside an intact plant, is a technique essential in many areas of the plant sciences.
* Cultures of individual or groups of plant cells, and whole organs, contribute to understanding both fundamental and applied science.
* It relies on maintaining plant cells in aseptic conditions on a suitable nutrient medium.
* The culture can be sustained as a mass of undifferentiated cells for an extended period of time, or regenerated into whole plants.
* The starting point for all tissue cultures is plant tissue, called an explant. It can be initiated

from any part of a plant - root, stem, petiole, leaf or flower - although the success of any one of these varies between species.
* It is essential that the surface of the explant is sterilised to remove all microbial contamination.
* Plant cell division is slow compared to the growth of bacteria and fungi, and even minor contaminants will easily over-grow the plant tissue culture.
* The explant is then incubated on a sterile nutrient medium to initiate the tissue culture.
* The composition of the growth medium is designed to both sustain the plant cells, encourage cell division, and control development of either an undifferentiated cell mass, or particular plant organs.
*The concentration of the growth regulators in the medium, namely auxin and cytokinin, seems to be the critical factor for determining whether a tissue culture is initiated, and how it subsequently develops.
* The explant should initially form a callus, from which it is possible to generate multiple embryos and then shoots, forming the basis for plant regeneration and thus the technology of micropropagation.
* The first stage of tissue culture initiation is vital for information on what combination of media components will give a friable, fast-growing callus, or a green chlorophyllous callus, or embryo, root or shoot formation.
* There is at present no way to predict the exact growth medium, and growth protocol, to generate a particular type of callus.
* These characteristics have to be determined through a carefully designed and observed experiment for each new plant species, and frequently also for each new variety of the species which is taken into tissue culture.
* The basis of the experiment will be media and protocols that give the desired effect in other plant species, and experience.



Overview of the Tissue Culture Process

2. History of Plant Tissue Culture:

-History of Tissue Culture Techniques - The in vitro techniques were developed initially to demonstrate the totipotency of plant cells predicted by Haberlandt in 1902. Totipotency is the ability of a plant cell to perform all the functions of development, which are characteristic of zygote, i.e., ability to develop into a complete plant.

- In 1902, Haberlandt reported culture of isolated single palisade cells from leaves in Knop's salt solution enriched with sucrose.
-The cells remained alive for up to 1 month, increased in size, accumulated starch but failed to divide.

- Efforts to demonstrate totipotency led to the development of techniques for cultivation of plant cells under defined conditions.
- This was made possible by the brilliant contributions from RJ. Gautheret in France and P.R. White in U.S.A. during the third and the fourth decades of 20th century.

- Most of the modern tissue culture media derive from the work of Skoog and coworkers during 1950s and 1960s.
- The first embryo culture, although crude, was done by Hanning in 1904; he cultured nearly mature embryos of certain crucifers and grew them to maturity.

- The technique was utilised by Laibach in 1925 to recover hybrid progeny from an interspecific cross in Linum.
- Subsequently, contributions from several workers led to the refinement of this technique.

- Haploid plants from pollen grains were first produced by Maheshwari and Guha in 1964 by culturing anthers of Datura.
- This marked the beginning of anther culture or pollen culture for the production of haploid plants.
- The technique was further developed by many workers, more notably by JP. Nitch, C. Nitch and coworkers.
- These workers showed that isolated microspores of tobacco produce complete plants.

- Plant protoplasts are naked cells from which cell wall has been removed. In 1960, Cocking produced large quantities of protoplasts by
using cell wall degrading enzymes.
- The techniques of protoplast production have now been considerably refined.
- It is now possible to regenerate whole plants from protoplasts and also to fuse protoplasts of different plant species.

- In 1972, Carlson and coworkers produced the first somatic hybrid plant by fusing the protoplasts of Nicotiana glauca and N. langsdorfii.
- Since then many divergent somatic hybrids have been produced.

- A successful establishment of callus cultures depended on the discovery during mid-thirties of IAA (idole-3-acetic acid), the endogenous
auxin, and of the role of B vitamins in plant growth and in root cultures.
-The first continuously growing callus cultures were established from cambium tissue in 1939 independently by Gautheret, White and
Nobecourt.

-The subsequent discovery of kinetin by Miller and coworkers in 1955 enabled the initiation of callus cultures from differentiated tissues.
-Shoot bud differentiation from tobacco pith tissues cultured in vitro was reported by Skoog in 1944, and in 1957 Skoog and Miller proposed that root-shoot differentiation in this system was regulated by auxin-cytokinin ratio.

-The first plant from a mature plant cell was regenerated by Braun in 1959. Development of somatic embryos was first reported in 1958- 1959 from carrot tissues independently by Reinert and Steward.

-Thus within a brief period, the tissue culture techniques have made a great progress.
- From the sole objective of demonstrating the totipotency of differentiated plant -cells, the technique now finds application in both basic and applied researches in a number of-fields of enquiry.

Q.2 MS Media for plant tissue culture: Murashige and Skoog medium is a plant growth media used in the laboratories for cultivation of plant cell culture. MSO was invented by plant scientistse Toshio Murahiges and Folk K. Skoog in 1962 during Murashige's search for new plant growth regulators.

- **Composition of MS Media:**

Major salts (macronutrients)

- Ammonium nitrate (NH_4NO_3) **1,650 mg/l**
- Calcium Chloride ($CaCl_2 \cdot 2H_2O$) **440 mg/l**
- Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) **370 mg/l**
- Monopotassium phosphate (KH_2PO_4) **170 mg/l**
- Potassium nitrate (KNO_3) **1,900 mg/l**.

These are major salts present in MS media .

Minor salts (micronutrients)

- Boric acid (H_3BO_3) **6.2 mg/l**
- Cobalt chloride ($CoCl_2 \cdot 6H_2O$) **0.025 mg/l**
- Cupric sulphate ($CuSO_4 \cdot 5H_2O$) **0.025 mg/l**
- Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) **27.8 mg/l**
- Manganese(II) sulphate ($MnSO_4 \cdot 4H_2O$) **22.3 mg/l**
- Potassium iodide (KI) **0.83 mg/l**
- Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) **0.25 mg/l**
- Zinc sulphate ($ZnSO_4 \cdot 7H_2O$) **8.6 mg/l**
- Ethylenediaminetetraacetic acid ferric sodium (NaFe-EDTA) constituting **5 ml/l** of a stock solution containing **5.57 g** FeSO4.7H2O and **7.45 g** Na2-EDTA per litre of water.

Vitamins and organics

- Myo-Inositol **100 mg/l**
- Nicotinic Acid **0.5 mg/l**
- Pyridoxine · HCl **0.5 mg/l**
- Thiamine · HCl **0.1 mg/l**
- Glycine **2 mg/l**
- Lactalbumin Hydrolysate (Edamin) (optional) **1 g/l**
- Indole Acetic Acid **1-30 mg/l**
- Kinetin **0.04-10 mg/l**

Q.3 Use of growth regulators:

Growth regulator may be defined as chemical substance, other than nutrient and vitamin regulate the growth of plant when applied in small quantities.

Types of Growth Regulators:

1. Auxin

2. Gibberellins

3. Cytokines

4. Ethylene

5. Inhibitors

6. Growth retardants.

1. Auxins: Dr. Kogl and his co-workers in 1933 isolated auxin –'a' from human urine and auxin 'b' from corm germ oil. Afterwards physiological roles of auxin have been reported by many scientists. These auxin who are responsible for revolutionary changes in the field of horticulture.

Action:

1. Auxin transport – pola- basi- apex to base.
2. Cell elongation.
3. Promote root initiation.

4. Inhibits root elongation.
5. Delay leaf abscission.
6. Induce callus formation.
7. Restore apical dominance.

e.g. - 1. IAA (Indole Acetic Acid)
2. IBA (Indole Butyric Acid)
3. NAA (Naphthalene Acetic Acid)
4. 2, 4-D (2, 4 – Dichlorophenxy Acetic Acid)
5. 4-CPA (4-Chloropenoxy Acetic Acid).

2. Gibberellins: In 1929 scientist 'Yabata and Hayashi' first isolated gibberellins from fungal culture. Since then number of gibberellins have been isolated from both the fungus and plants.

Action:
1. Promote growth (Specially those plants are genetically dwarf types).
2. Promote bolting and flowering.
3. Replace chilling requirements of plants and light requirements.
4. Promote seed germination and break dormancy.
5. Increase pollinations.
6. Increase cell elongation.
7. Induce maleness.

3. Cytokines: The first Cyotkininn hormone in plant was identified by 'Lethan and his co-workers' from corn seeds which can stimulate cell division.

Actions:

1. Cell division.
2. Shoot initiation.
3. Breaking dormancy: promote seed germination.
4. Retard senescence: freeness's of plants.
5. Promote hermaphrodite flower.e.g Grape.
6. Induce parthenocarpic and increase fruit size.

4. Ethylene:A synthetic chemical known as Ethrel (Ethephon -2), Chloroethyl phosphoric acid (CEPA) which reduce ethylene when applied on plant.

Actions:

1. Apical dominance arrested.
2. Stimulate of lateral growth.
3. Promote abscission of leaves, flowers, and fruit.
4. Induction of flowering.
5. Helps in fruit ripening.

6. Promote rooting.
7. Helps in chlorophyll formation.
8. Promote seed germination.
9. Increase female flowers.
10. Breaks dormancy.

5. Growth retardants:
Slow cell division and cell elongation.
e.g. i) 2,4 DNC (2,4 Dichlorobenzyl).

ii) CCC (Cycocel) and
iii) Alar.

Actions:1. Regards stem elongation.
2. Prevents cell division.
3. Accelerate flower initiation.
4. Inhibits root development.
5. Inhibitors: Suppers the growth of plants.
Synthetic inhibitors:
1. MH- Maleic hydrazide.
2. TIBA- Tri- iodobenzoic acid.

Actions:1. Accelerate the loss of chlorophyll.

2. Inhibits germination.

3. Induce flowering 9 by suppressing Vegative growth)

4. Increase the yield of tubers.

5. Induce male sterility.

Q.4 Single cell culture:

Method:Single cell culture can be done by following methods:
1. The Paper Raft Nurse Technique:1. Single cells are isolated from suspension cultures or a friable callus with the help of a micropipette or micro-spatula.

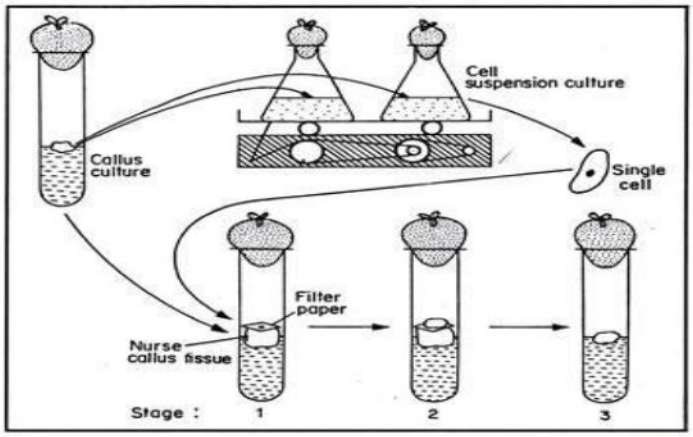
2. Few days before cell isolation, sterile 8 mm x 8 mm squares of filter paper are placed aseptically on the upper surface of the actively growing callus tissue of the same or different 3. The filter paper will be wetted by soaking the water and nutrient from the callus tissue.

4. The isolated single cell is placed aseptically on the wet filter paper raft.

5. The whole culture system is incubated under 16 hrs. cool white light (3,000 lux) or under continuous darkness at 25° C.
6. The single cell divides and re-divides and ultimately forms a small cell colony. When the cell colony reaches a suitable size, it is transferred to fresh medium where it gives rise to the callus tissue

The callus tissue, on which the single cell is growing, is called the nurse tissue. Actually the callus tissue supplies the cell with not only the nutrients from the culture medium but something more that is critical for cell division. The single cell absorbs nutrients through filter paper. The nutrients actually diffuse

upward from culture medium through callus tissue and filter paper to the single cell. A callus tissue originating from a single cell is known as a single cell clone.



❒ Fig 9.1
Growth of single cells using a 'nurse' technique. Stage 1 : a single cell taken from a friable callus is placed on upper surface of filter paper which is in contact with nurse callus. Stage 2 : the single cell divides and daughter cells proliferate to form colony. Stage 3 : when colony reaches a suitable size it is transferred to fresh medium where it gives rise to a single cell clone.

2. The Petri Dish Plating Technique:

1. A suspension of purely single cells is prepared aseptically from the stock cell suspension culture by filtering and centrifugation requisite cell density in the single cell suspension is adjusted by adding or reducing the liquid medium.

2. The solid medium (1.6% 'Difco' agar added) is melted in water bath.

3. In front of laminar air flow, the tight lid of falcon plastic petri dish is opened With the help of sterilized Pasteur pipette 1 5 ml of single cell suspension is put an equal amount of melted agar medium when it cools down at 35°C, is added in the single cell suspension .

4. The lid is quickly replaced and the whole dish is swirled gently to disperse the cell and medium mixture uniformly throughout the lower half of the petri dish.

5. The medium is allowed to solidify and the petri dish is kept at the inverted position.

6. The cultures are incubated under 16hrs light (3,000 lux, cool white) or under continuous dark at 25°C.

7. The petri dishes are observed at regular intervals under inverted microscope to see whether the cells have divided or not.

8. After certain days of incubation, when the cells start to divide, a grid is drawn on the undersurface of the petri dish to facilitate counting the number of dividing cells.

9. The dividing cells ultimately form pin-head shaped cell colonies within 21 days of incubation.

10. The plating efficiency (PE) can be calculated from the counting of cell colonies by the following formula:

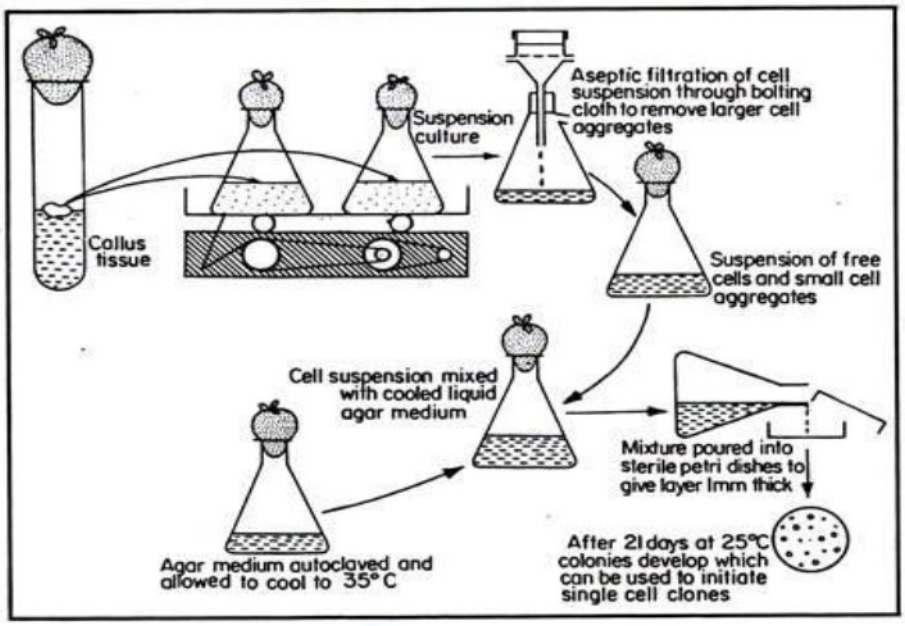


Fig 9.2

Procedure for obtaining single cell clones using a petri dish plating technique

PE = Number of colonies per plate/Number of total cell per plate x 100

11. Pin-head shaped colonies, when they reach a suitable size, are transferred to fresh medium for further growth.

3. The Micro-chamber Technique:

1. A drop of liquid nutrient medium containing single cell is first isolated aseptically from stock suspension culture with the help of long fine Pasteur pipette.

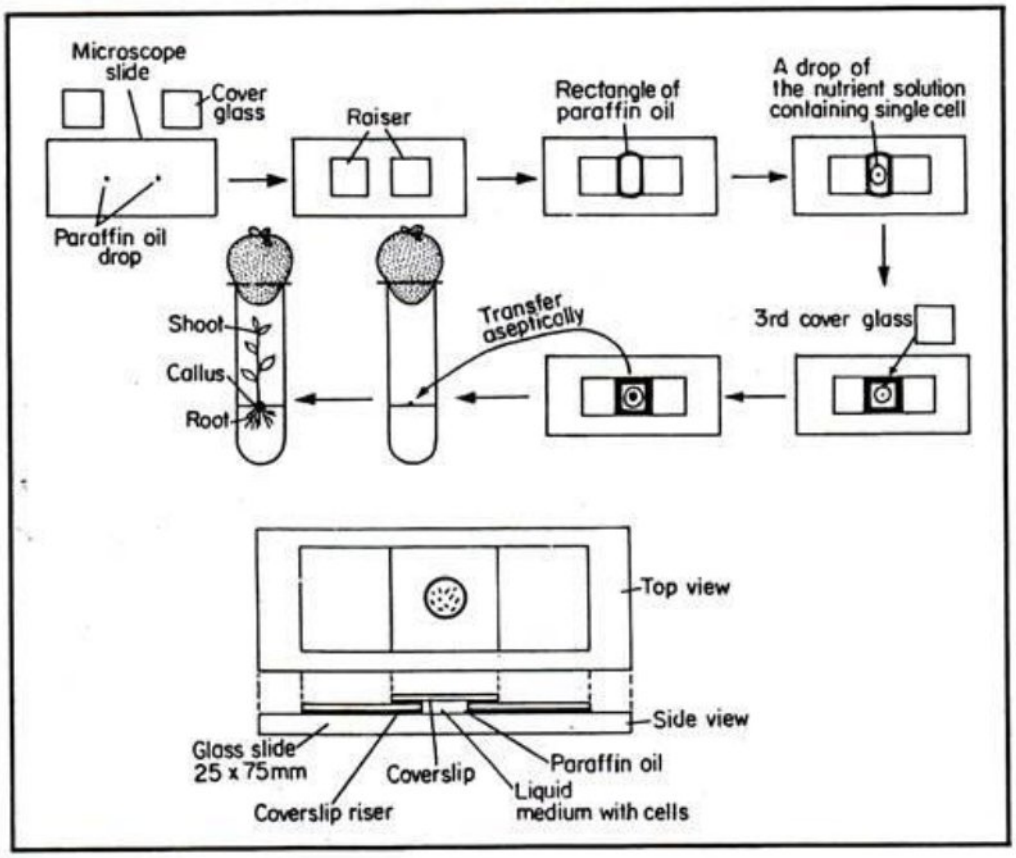
2. The culture drop is placed on the centre of a ‘ sterile microscopic slide (25 x 75 mm) and ringed with sterile paraffin oil.

3. A drop of paraffin oil is placed on either side of the culture drop and a cover-glass (called raiser) is placed on each oil drop (Fig 9.3).

4. A third cover-glass is then placed on the ‘ culture drop bridging the two raiser cover-glasses and forming a micro-chamber to enclose the single cell aseptically within the paraffin oil. The oil prevents the water loss from the culture drop but permits gaseous exchange.

5 The whole micro-chamber slide is placed in a petri-dish and is incubated under 16 hrs. white cool illumination (3,000 lux) at 25 C.

6. Cell colony derived from the single cell gives rise to single cell clone.



❒ Fig 9.3

Microchamber used to observe the growth of single cells

7. When the cell colony becomes sufficiently ‘ large, the cover-glass is removed and the tissue is transferred to fresh solid or semisolid medium.

The micro-chamber technique permits regular observation of the growing and dividing cell.

4. The Nurse Callus Technique:

This method is actually a modification of petridish plating method and the paper raft nurse culture method. In this method, single cells are plated on to agar medium in a petridish as described earlier Two or three callus masses (Nurse tissue) derived from the same plant tissue are also embedded directly along with the single cells in the same medium (Fig 9.4).

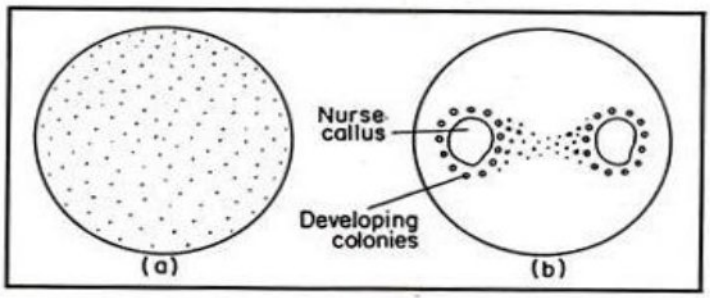


Fig 9.4

Growth of colonies from a low density cell suspension in the presence of callus tissue. A. Petri dish inoculated with low density suspension of cells—no colonies develop. B. Petri dish inoculated with low density suspension plus nurse callus—colonies grow near to nurse calluses only

Here the paper barrier between single cells and the nurse tissue is removed. Cells first begin to divide in the regions near the nurse callus indicating that the single cells closer to nurse callus in the solid medium gets the essential growth factors that are liberated from the callus mass. The developing colonies growing near to nurse callus also stimulate the division and colony formation of other cells.

5. The Micro-droplet Technique:

1. In this method, single cells are cultured in special Cuprak dishes which have two chambers—a small outer chamber and a large inner chamber. The large chamber carries numerous numbered wells each with a capacity of 0.25-25µl of nutrient medium.

2. Each well of the inner chamber is filled with a micro-drop of liquid medium containing isolated single cell. The outer chamber is filled with sterile distilled water to maintain the humidity inside the dish (Fig 9.5).

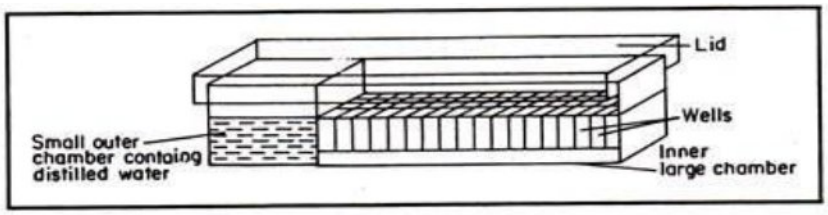


Fig 9.5

Diagrammatic view of Cuprak dish used for the microdroplet technique of single cell culture

3. After covering the dish with lid, the dish is sealed with paraffin.

4. The dish is incubated under 16hrs cool light (3,000 lux) at 25°C.

5. The cell colony derived from the single cell is transferred on to fresh solid or semisolid medium in a culture tube for further growth.

UNIT-2

Q.1 cytodifferentiation:

In plant tissue culture, during growth and maturation of the callus tissue or free cells in suspension culture, few dedifferentiated cells undergo cytoquiescence and cytosenescence and these twin phenomena are mainly associated with differentiation of vascular tissue, particularly tracheary elements.The whole developmental process is termed as Cytodifferentiation.

Protocol:

1. The hypocotyl portion of aseptically grown seedling of Vigna unguiculata can be used as initial explant.

2. The explant is cultured in Murashige and Skoog's medium supplemented with 2, 4-D (2-4 mg/L) and kinetin (0.5 mg/L) at 25°C with 16 hrs. light.

3. Cultures are maintained in a serial subcultures with 28 days passage duration.

4. Time to time, callus tissue are harvested and macerated in 4% aqueous solution of NaOH at 50°C. This treatment clears and softens the tissue

5. NaOH solution is carefully replaced by 0.04% aqueous solution of safranin.

6. After 30 minutes, the dye solution is replaced by IN HCL at 50°C.

7. After one hour, HCL is removed and glycerol is added. The acid destains the parenchyma cells but the lignified xylem retains the red dye.

8. Finally, a slide is made and observed under microscope.

Q.2 Microprapogation: Micro propagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods.

Micro propagation is used to multiply noble plants such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction.

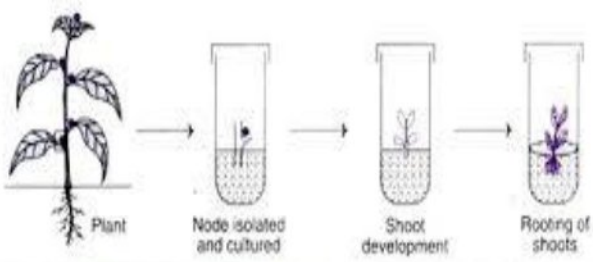


Fig. 47.3 : A diagrammatic representation of micropropagation by single node technique.

Technique of Micro propagation:

Micro propagation is a complicated process and mainly involves 3 stages (I, II and III). Some authors add two more stages (stage 0 and IV) for more comprehensive representation of micro-propagation.

Stage	Methodology involved
Stage 0	Selection of mother plant and its maintenance
↓	
Stage I	Initiation and establishment of culture
↓	
Stage II	Multiplication of shoots or rapid somatic embryo formation
↓	
Stage III	*In vitro* germination of somatic embryos and/or rooting of shoots
↓	
Stage IV	Transfer of plantlets to sterilized soil for hardening under greenhouse environment

Fig. 47.1 : Major stages involved in micropropagation.

Q.3 Organogenesis:

Organogenesis means the development of adventitious organs or primordia from undifferentiated cell mass in tissue culture by the process of differentiation.

In vitro organogenesis in the callus tissue derived from a small piece of plant tissue, isolated cells, isolated protoplasts, microspores etc. can be induced by transferring them to a suitable medium or a sequence of media that promote proliferation of shoot or root or both.

- Protocol for Organogenesis in Tobacco Callus:

This is an experiment in which mature tobacco stem is initiated to give rise to callus tissue. Under the appropriate hormonal conditions callus is induced to form either root or shoot primordia.

The protocol is given below:

1. The upper part of the stem of 3-4ft tall tobacco plants are harvested and cut into 2 cm long internode segments.

2. Surface sterilization of the tissue is done by immersing the stem pieces in 70% v/v ethanol for 30 seconds, followed by a 15 minutes incubation in sodium hypochlorite (1.0% available chlorine). Then the tissue is washed in several changes of sterile distilled water.

3. The stem explants are taken in a sterilized petri dish and cut longitudinally into two equal pieces and inoculated onto Murashige and Skoog's (1962) solid medium (MS) supplemented with 2mg/L indole acetic acid (IAA) and 0.2 mg/L kinetin. The cultures are then incubated at 25°C with an illumination of about 2,000 lux (16 hrs. photo period)

4. Callus tissue which is white/yellow in colour, begins to form in two weeks and after six weeks it should be sub cultured to fresh medium.

5. Organogenesis in callus culture can be stimulated by transferring tobacco callus onto MS medium with different auxin/cytokinin ratios. Shoot primordia develop within 3 weeks of transfer of callus to MS medium with IAA at 0.02 mg/L and kinetin at 1 mg/L (a high cytokinin/low auxin ratio). Root formation occurs within 2-3 weeks of transfer of callus to MS medium supplemented with 0.2 mg/L IAA and 0.02 mg/L kinetin (a high auxin/low cytokinin).

6. After 6 weeks, rootless shoots can be excised and placed onto the root inducing medium i.e. MS medium with 0.2 mg/L IAA and 0.02 mg/L.

7. It is possible to transplant the tobacco plantlets to soil. It should be noted that aseptic procedures are not required for the transplantation of plantlets. The plantlets are removed from

the culture vessels and care should be taken not to damage root or shoot system. The plantlets are carefully washed with tap water to remove the residual agar medium.

Individual plantlets are separated out and transplanted into pot (75 mm) containing seedling compost. The soil is watered. The pot is covered with a small inverted polythene bag. This will reduce the amount of water lost by the plantlets due to transpiration.

After 7 days, several small holes are made in the polythene bag and gradually enlarged during next 2-3 weeks. At this stage, the tobacco plantlets should be sufficiently "hardened off" to allow the complete removal of plastic bag. They can be grown to maturity in a green house.

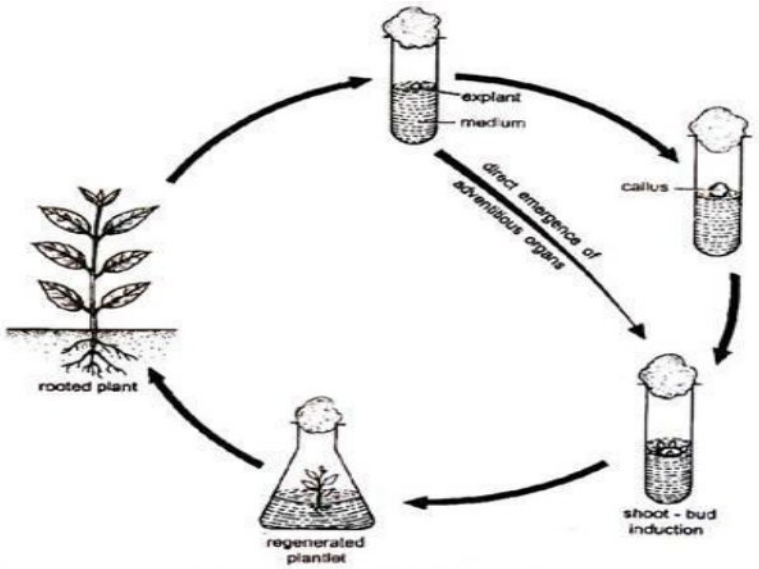


Fig. 4. Organogenic Differentiation.

Q.4 somatic embryogenesis:

Somatic embryogenesis is an artificial process in which a plant or embryo is derived from a single somatic cell or group of somatic cells. Somatic embryos are formed from plant cells that are not normally involved in the development of embryos, i.e. ordinary plant tissue.

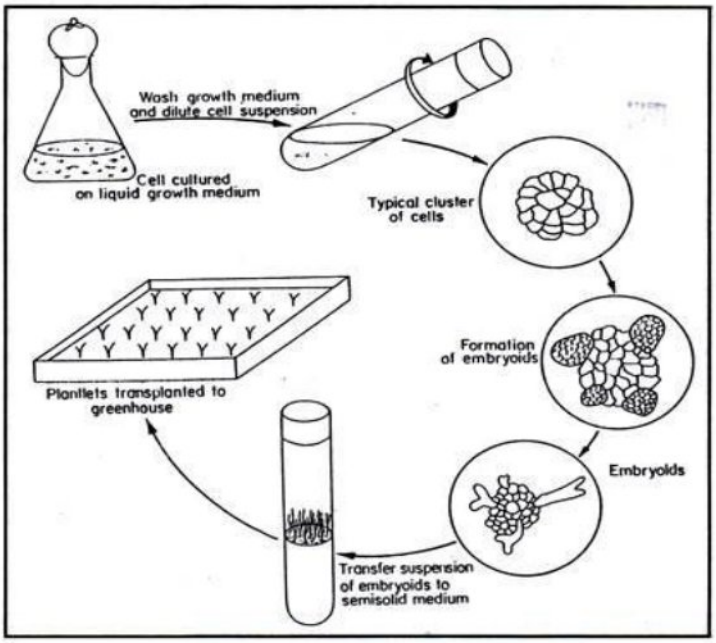


Fig 8.4

Flow diagram illustrating the protocol for inducing somatic embryogenesis in culture

Q.5 Synthetic seed:

Synthetic seed are defined as artificially encapsulated somatic embryos, shoot buds, cell aggregates, or any other tissue that can be used for sowing as a *seed* and that possess the ability to convert into a plant under in vitro or ex vitro conditions and that retain this potential also after storage.

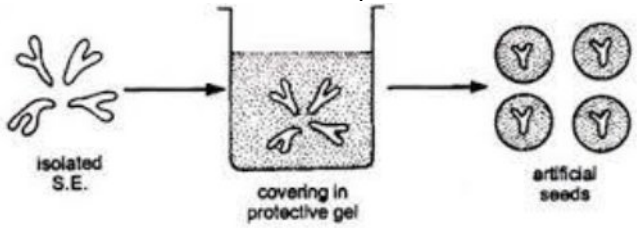


Fig. 13. Outline of preparation of synthetic seeds.

Characteristics of synthetic seeds

1. High volume. Large scale propagation method
2. Maintains genetic uniformity of plants
3. Direct delivery of propagules to the field, thus eliminating transplants
4. Lower cost per plant let
5. Rapid multiplication of plants

Advantages of synthetic seed:

1. Ease of handling while in storage
2. Easy to transport

3. Has potential for long term storage without losing viability
4. Maintains the clonal nature of the resulting plants
5. Serves as a channel for new plant lines produced through biotechnological advances to be delivered directly to the green house or field
6. Allows economical mass propagation of elite plant varieties

Application of synthetic seeds

By combining the benefits of a vegetative propagation system with the capability of long-term storage and with the clonal multiplication, synthetic seeds have many diverse applications in the field.

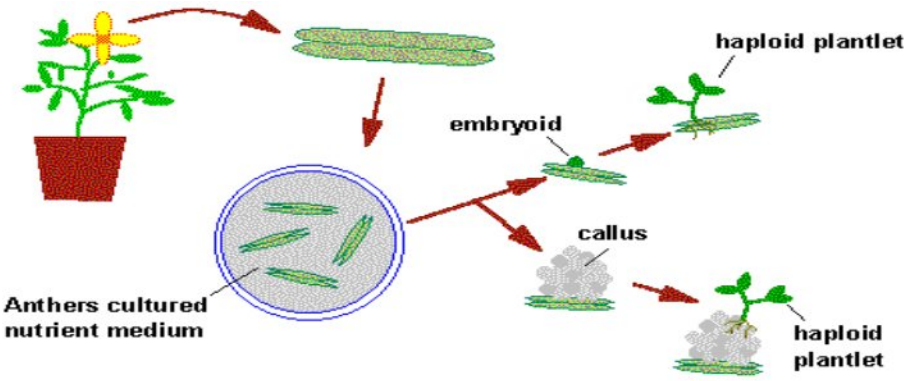
1) Multiplication of non-seed producing plants, ornamental hybrids or polyploids plants
2) Propagation of male or female sterile plants for hybrid seed production
3) Germplasm conservation of recalcitrant species
4) Multiplication of transgenic

Q.5 Haploid plants:

Haploid plants are characterized by possessing only a single set of chromosomes (gametophytic number of chromosomes i.e. n) in the sporophyte. This is in contrast to diploids which contain two sets (2n) of chromosomes. Haploid plants are of great significance for the production of homozygous lines (homozygous plants) and for the improvement of plants in plant breeding programmes.

Methods of haploid plant production:

1. **Anther Culture:** It is a technique of culturing anthers of precise and critical stage which is to be isolated from unopened flower bud and cultured on artificial medium. The microscope present in anther develops into embryoid or callus which give rise to haploid plant through the process of embryogenesis or organogenesis.



2. Ovary Culture:

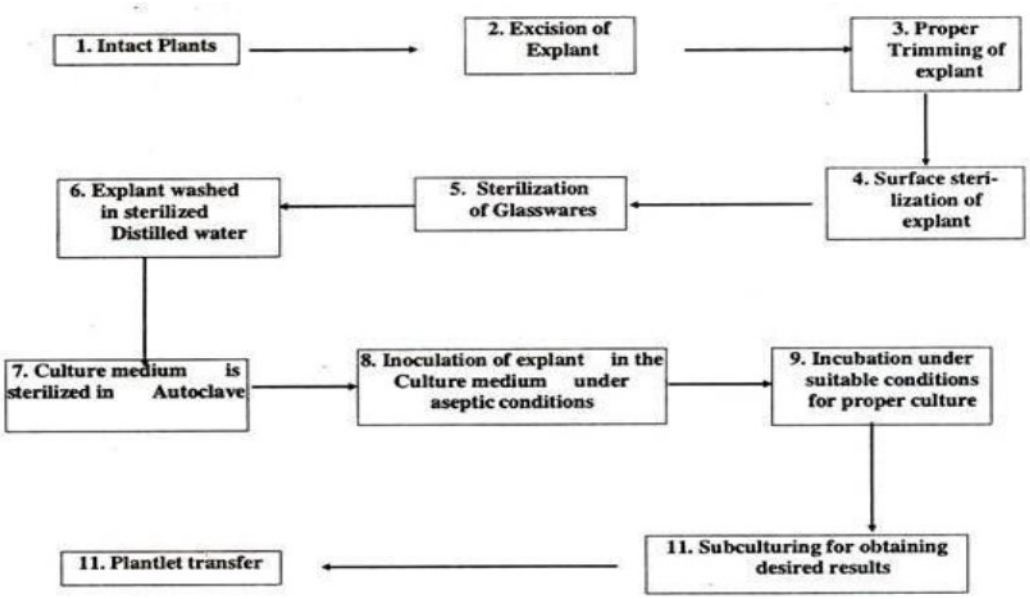


Fig. 1. Steps in general technique of Plant tissue culture.

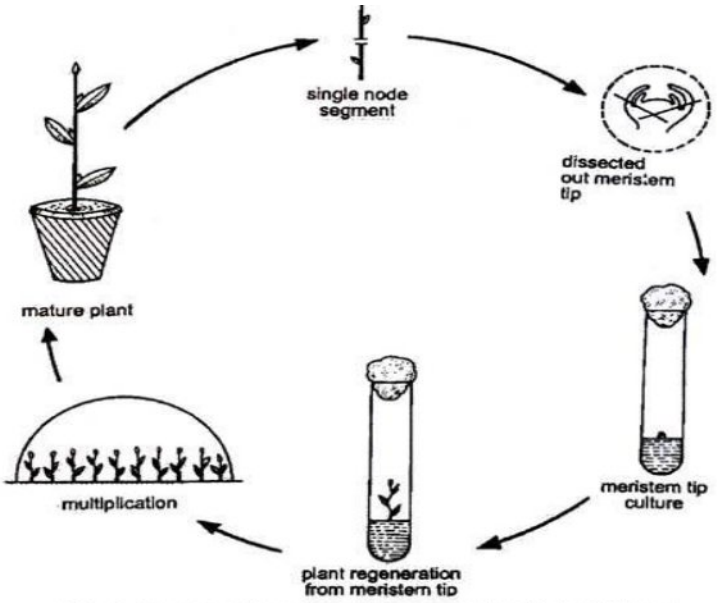


Fig. 8. Regeneration of plants through Meristem Culture.

Q.6 In vitro pollination and In vitro fertilization:

Pollination and fertilization under in vitro conditions offer an opportunity for producing hybrid embryos among plants that cannot cross by conventional methods of plant breeding. In nature, Intergeneric or Interspecific hybridization occurs less frequently. This is due to barriers hindering the growth of the pollen tube on the stigma or style. In such cases the style or part of it can be excised and pollen grains either placed on the cut surface of overy or transferred through a hole in the wall of ovary. This technique, called intraovarian pollination, has been successfully applied in such species as Papaver sommiferum , Eschscholtiza California, Argemone Mexicana and Argemone ochroleuca. Another approach to overcome the barrier to pollen tube growth is direct pollination of cultured ovules or excised ovules together with placenta. This technique was developed at university of Delhi in papaveracea and solanaceae. Various other techniques developed to overcome the prezygotic barriers to fertility include: a) Bud pollination, b) Sub pollination, c) Heat treatment of style, d) Irradication and e) Mixed pollination.

The development of seed through in vitro pollination of exposed ovules has been described as ‘test-tube fertilization’ whereas the process of seed formation following stigmatic pollination of cultured whole pistils has been referred to as ‘in vitro pollination’. Considering the fact that male gametes in plants do not float freely and are delivery by pollen tube, a general terms ‘in vitro pollination has been used for ovular pollination, overian pollination, placental pollination and stigmatic pollination under in vitro conditions.

The vitro pollination can be accomplished by procedure by following appropriate sterilization procedure, suitable nutrient medium and selection of suitable explant.

Application of in Vitro Pollination:

In plant breeding the technique of in vitro pollination has potential applications in at least three different areas, viz,

a) overcoming self-incompatibility b) overcoming cross-incompatibility, c) haploid production through parthenogenesis.