CELL AND TISSUE CULTURE IN PLANTS

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Learning outcomes:

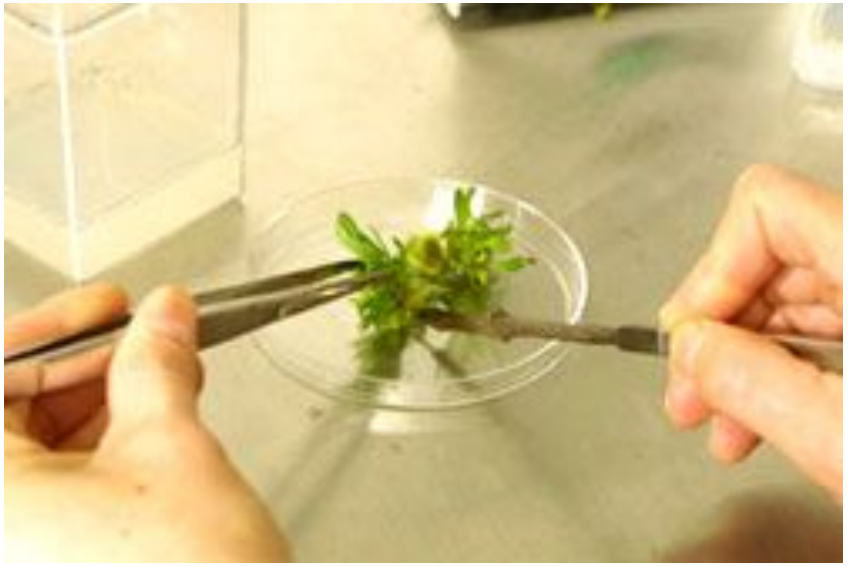
You will be able to understand

- Cell and tissue culture in plants
- Totipotency
- Callus culture
- Micropropagation

PLANT CELL AND TISSUE CULTURE:

Plant cell/tissue culture is also referred to as in vitro, axenic, or sterile culture.

Plant tissue culture is a technique of maintaining and growing plant cells, tissues or organs on a nutrient culture medium of known composition in suitable containers under sterile and controlled environmental conditions.



TECHNIQUE OF PLANT TISSUE CULTURE

A

- Preparation of culture medium
- Sterilization of culture medium

B

- Selection of Explant
- Surface sterilization of explant

C

- Inoculation of explant in the medium
- Maintenance and growth of culture

A. PLANT TISSUE CULTURE MEDIA:

Plant tissue culture media should contain

1. Macronutrients (C ,H, O , N, PK,, Ca, Mg and S)
2. Micronutrients (Fe, Mn, Zn, B, Cu and Mo)
3. Vitamins (Thiamine,Nicotinic acid and Pyridoxine)
4. Amino acids or nitrogen supplements (Casein hydrolysate,L glycine, L glutamine, L asparagine,
   L arginine, L cysteine and L tyrosine)
5. Source of Carbon (Sucrose)
6. Growth regulators (Auxin, Cytokinin, Gibberellins) &
7. Solidifying agents (Agar/ Agarose)

Basic Media

Basic media that are frequently used in plant tissue culture are -

To induce organogenesis and regeneration of plants in cultured tissues Murashige and Skoog (MS) media,1962 and Linsmaier and Skoog (LS) media contain the desired salt composition and are widely used.

Gamborg (B5) medium,1968 originally designed for cell suspension or callus cultures, with modifications proved valuable for protoplast culture and regeneration of protoplast –derived plants.

White"s (W) medium, 1953 was developed for root culture.

Nitsch and Nitsch (NN) medium,1969 is frequently used for anther culture.

Chu (N6) media was formulated by Chu, 1978 and is used for cereal anther culture.The medium helps in the initiation, growth and differentiation of callus from the rice pollen cultures.

Composition of commonly used culture medium

Components	Amount (mg l^{-1})				
	White's	Murashige and Skoog (MS)	Gamborg (B5)	Chu(N6)	Nitsch's
Macronutrients					
$MgSO_4.7H_2O$	750	370	250	185	185
KH_2PO_4	—	170	—	400	68
$NaH_2PO_4.H_2O$	19	—	150	—	—
KNO_3	80	1900	2500	2830	950
NH_4NO_3	—	1650	—	—	720
$CaCl_2.2H_2O$	—	440	150	166	—
$(NH_4)_2.SO_4$	—	—	134	463	—
Micronutrients					
H_3BO_3	1.5	6.2	3	1.6	—
$MnSO_4.4H_2O$	5	22.3	—	4.4	25
$MnSO_4.H_2O$	—	—	10	3.3	—
$ZnSO_4.7H_2O$	3	8.6	2	1.5	10
$Na_2MoO_4.2H_2O$	—	0.25	0.25	—	0.25
$CuSO_4.5H_2O$	0.01	0.025	0.025	—	0.025
$CoCl_2.6H_2O$	—	0.025	0.025	—	0.025
KI	0.75	0.83	0.75	0.8	—
$FeSO_4.7H_2O$	—	27.8	—	27.8	27.8
$Na_2EDTA.2H_2O$	—	37.3	—	37.3	37.3
Sucrose (g)	20	30	20	50	20
Organic supplements					
Vitamins					
Thiamine HCl	0.01	0.5	10	1	0.5
Pyridoxine (HCl)	0.01	0.5	1	0.5	0.5
Nicotinic acid	0.05	0.5	1	0.5	5
Myoinositol	—	100	100	—	100
Others					
Glycine	3	2	—	—	2
Folic acid	—	—	—	—	0.5
Biotin	—	—	—	—	0.05
pH	5.8	5.8	5.5	5.8	5.8

Murashige and Skoog Medium (MS) was originally formulated by Murashige and Skoog in 1962.

It is widely used for micro propagation, organ culture, callus culture and suspension culture.

Stock solution of MS basal medium

Constituent	Concentration in MS medium (mg/l)	Concentration in the stock solution (mg/l)	Volume to be taken/litre of medium
Macronutrients (10x) Stock solution I			
NH_4NO_3	1650	16500	100 ml
KNO_3	1900	19000	
$MgSO_4 . 7H_2O$	370	3700	
KH_2PO_4	170	1700	
Macronutrient (10x) Stock solution II			
$CaCl_2\ 2H_2O$	440	4400	100 ml
Micronutrients (100x) Stock solution III			
H_3BO_3	6.2	620	10 ml
$MnSO_4 . 4H_2O$	22.3	2230	
$ZnSO_4 . 7H_2O$	8.6	860	
Kl	0.83	83	
$Na_2MoO_4.2H_2O$	0.25	25	
$CuSO_45H_2O$	0.025	2.5	
$CoCl_2 . 6H_2O$	0.025	2.5	
Iron source			
Fe EDTA Na salt	40	Added fresh	
Vitamins			
Nicotinic acid	0.5	50 mg/100 ml	1 ml
Thiamine HCl	0.1	50 mg/100 ml	0.2 ml
Pyridoxine HCl	0.5	50 mg/100 ml	1 ml
Myo-inositol	100	Added fresh	
Others			
Glycine	2.0	50 mg/100 ml	4 ml
Sucrose	30,000	Added fresh	
Agar	8000	Added fresh	
pH 5.8			

Constituents	**Stock solution (conc.)**	**Quantity required for 1 L**	**Quantity required for volume of medium under preparation (e.g. 500ml)**
Macro stock solution I	10x	100ml	50 ml
Macro stock solution II ($CaCl_2$)	10x	100 ml	50 ml
Micro stock solution III	100x	10 ml	5 ml
Iron-EDTA Na salt	Added fresh	40 mg	20 mg
Vitamins			
Nicotinic acid	50 mg/100 ml	0.5 mg/l = 1 ml	0.5 ml
Thiamine HCl	50 mg/100 ml	0.1 mg/l = 0.2ml	0.1 ml
Pyridoxine HCl	50 mg/100 ml	0.5 mg/l = 1ml	0.5 ml
Myo-inositol	Added fresh	100 mg	50 mg
Others			
Glycine	50 mg/100 ml	2 mg/l = 4ml	2.0 ml
Growth regulators			
Sucrose	Added fresh	30 g	15 g
Agar	Added fresh	8 g	4 g
pH			

Directions for making media :

- Stock solutions are measured as directed in the basal media.
- Agar is separately dissolved in distilled water by heating on a water bath.
- Mix all the ingredients and agar and make up the final volume with distilled water.
- Adjust the pH of the medium to 5.75 ± 0.5 using 0.1N NaOH/ 0.1 N HCl.
- Dispense the medium in sterile flask or test tube (only one third volume should be filled). Plug the flask/ tube with cotton plugs.
- Sterilize the medium by autoclaving at 15 psi and 121°C for 15-20 min.

- Cool the autoclaved medium to about 45°C before adding heat labile supplements.

- pH of solutions of vitamins and growth regulators is adjusted using 0.1N NaOH or 0.1 N HCl.

- Vitamins and growth regulators are sterilised by filtering through a microfilter of pore size 0.22-0.45 μm and added in the autoclaved media under laminar air flow cabinet (sterile conditions).

pH of the plant tissue culture media is adjusted between 5-6.

Media is sterilized by autoclaving at 120°C for 20 minutes

pH meter

Pressure cooker

Horizontal autoclave

Vertical Autoclave

B. SELECTION OF EXPLANT :

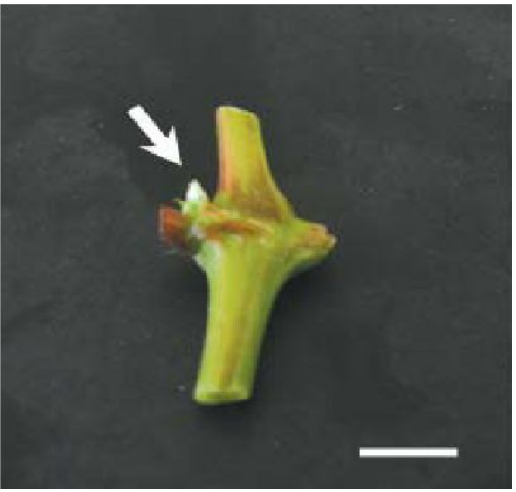
Explants are small pieces of plant parts or tissues that are aseptically cut and inoculated in culture medium to grow.

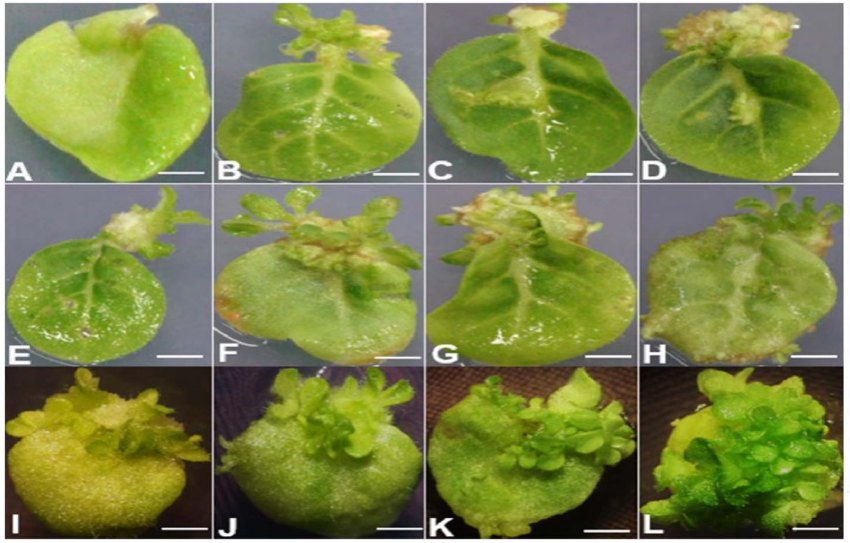
Explants can be taken from many different part of the plant such as :

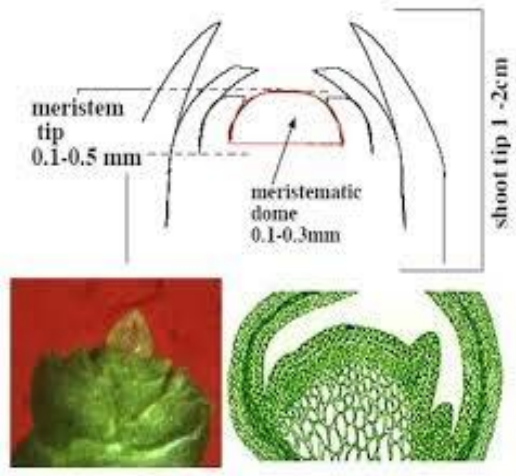
- stem segments
- leaves
- roots
- apical meristem
- bud, ovule, anther, pollens
- Cotyledon
- hypocotyl
- embryo
- endosperm

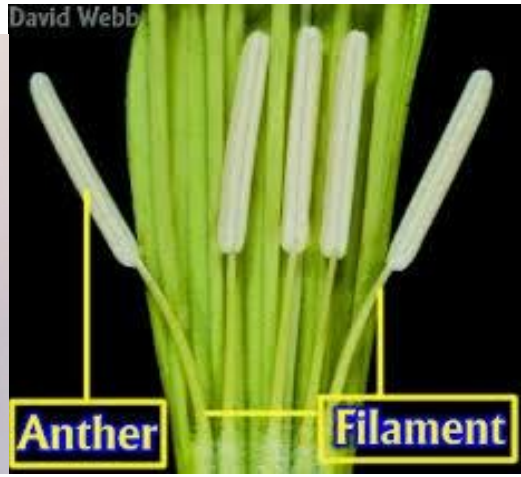
EXPLANT :

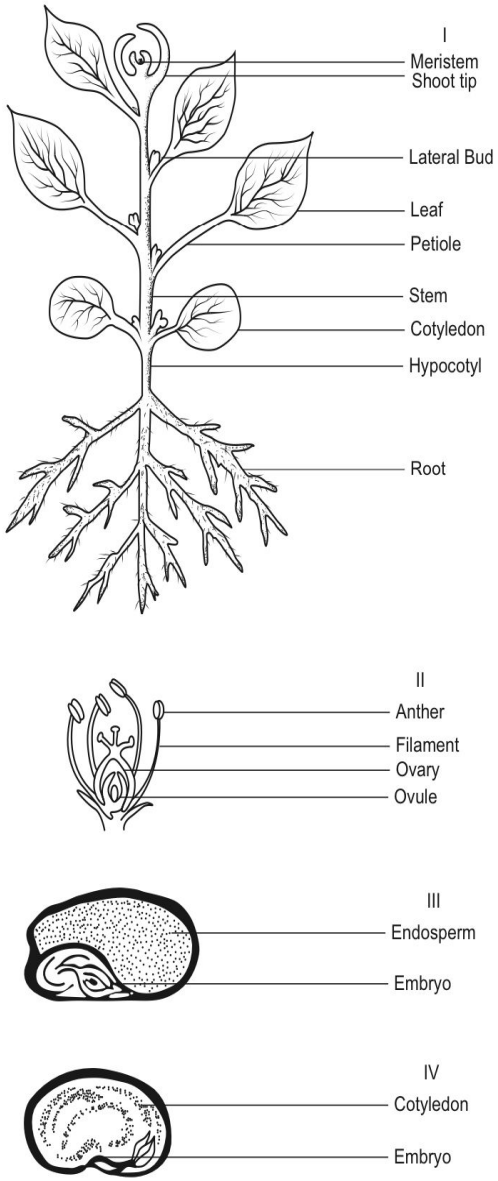
Explants can be taken from many different part of the plant such as : stem segments, leaves, roots, apical meristem, bud, ovule, anther etc.











Potential explant tissues (ref: Plant Tissue Culture Techniques and Experiments,Roberta H. Smith)

SURFACE STERILIZATION OF EXPLANT:

Commonly used disinfectants are:

- Calcium hypochlorite – 9-10% for 5-30 minutes
- Sodium hypochlorite – 0.5 – 5% for 5-30 minutes
- Hydrogen peroxide – 3-12% for 5-15 minutes
- Ethyl alcohol – 70-95% for 0.1 – 5 minutes
- Silver nitrate – 1% for 5-30 minutes
- Mercuric chloride – 0.1- 1.0% for 2-10 minutes
- Benzalkonium chloride- 0.01- 0.1% for 5-20 minutes

TECHNIQUE OF PLANT TISSUE CULTURE:

- Preparation and sterilization of medium
- Selection and surface sterilization of explant
- Inoculation of explant in culture medium
- Plant tissue culture work is performed under aseptic conditions under HEPA filtered air provided by a laminar flow cabinet.
- Plant tissue is grown in sterile containers such as petri dish, flask or test tubes in a growth room with controlled temperature and light intensity.

Laminar air flow cabinet

Inoculation room

Growth room

CELLULAR TOTIPOTENCY

CELLULAR TOTIPOTENCY :

- The genetic potential of a differentiated plant cell to give rise to a whole plant (like the zygote) is described as cellular totipotency.
- The development of an adult organism from a single cell is the result of the integration of cell division and cell differentiation.
- The potential of a cell to grow and develop a multicellular organism indicates that all genes responsible for differentiation are present within individual cells which are expressed under adequate culture conditions.

CELLULAR TOTIPOTENCY :

The term totipotency was coined by T.H. Morgan (1901).

Concept of cellular totipotency was introduced by the famous German plant physiologist, Gottlieb Haberlandt (1902), he suggested that the differentiated plant cells containing the entire complement of chromosomes, should be capable of regenerating whole plants.

To prove his hypothesis, he isolated single cells from highly differentiated tissues of the plant body and cultured in nutrient medium.

Although he did not succeed in his experiments due to technical limitations at that time, his idea attracted the attention of many scientists to pursue this line of investigation.

Gottlieb Haberlandt (1902) is known as " Father of Plant Tissue Culture"

CELLULAR TOTIPOTENCY :

Even highly mature and differentiated cells retain the ability to regenerate to a meristematic state.

To express totipotency, the differentiated cells first undergo dedifferentiation and then redifferentiation

The phenomenon of a mature cell reverting to the meristematic state and forming undifferentiated callus tissue is termed **"dedifferentiation"**.

The phenomenon of conversion of component cells of callus tissue to whole plant or plant organs is called **" redifferentiation".**

Differentiation of one organ directly from another organ, such as shoot from root explants of Arabidopsis, is referred to as **transdifferentiation** .

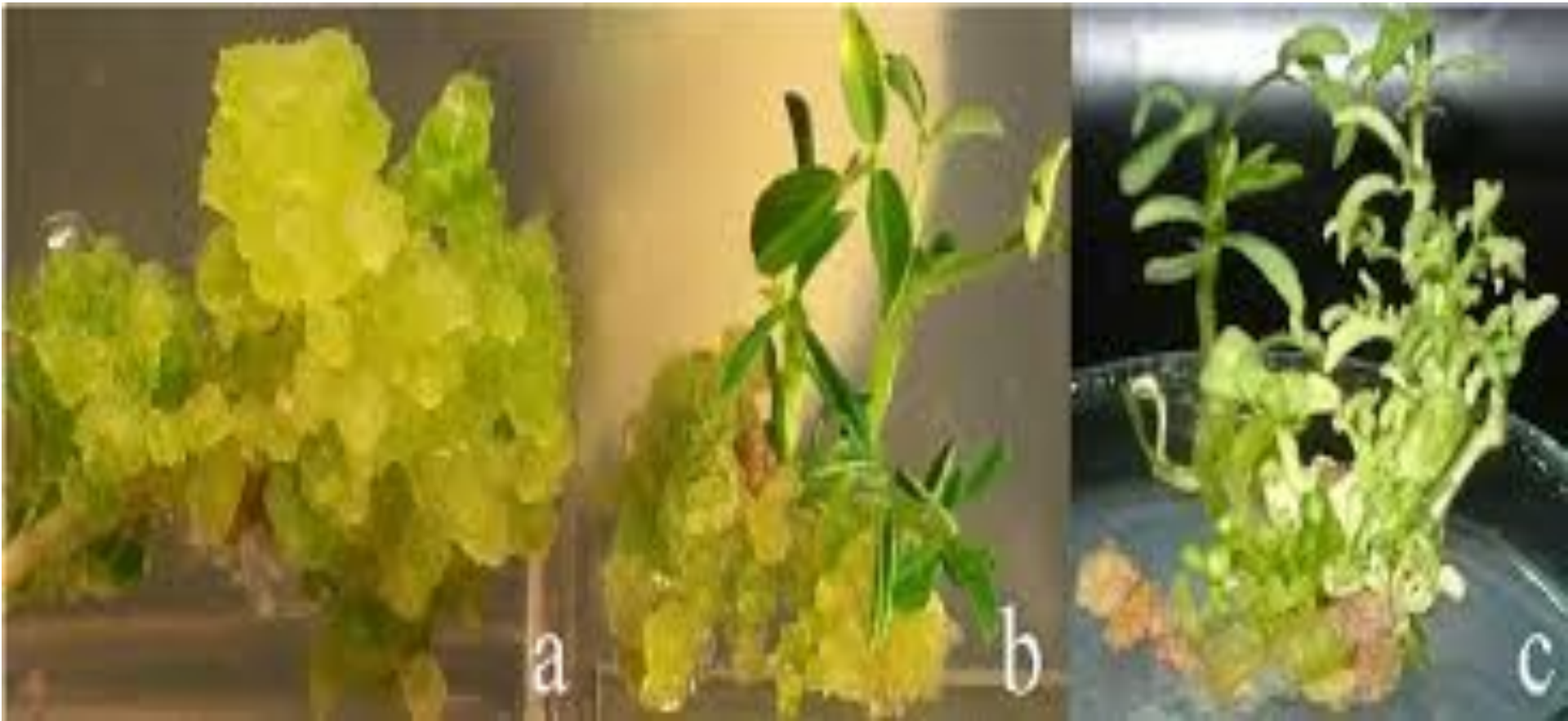
Mature cell reverts to meristematic state

Dedifferentiation

Callus

Redifferentiation

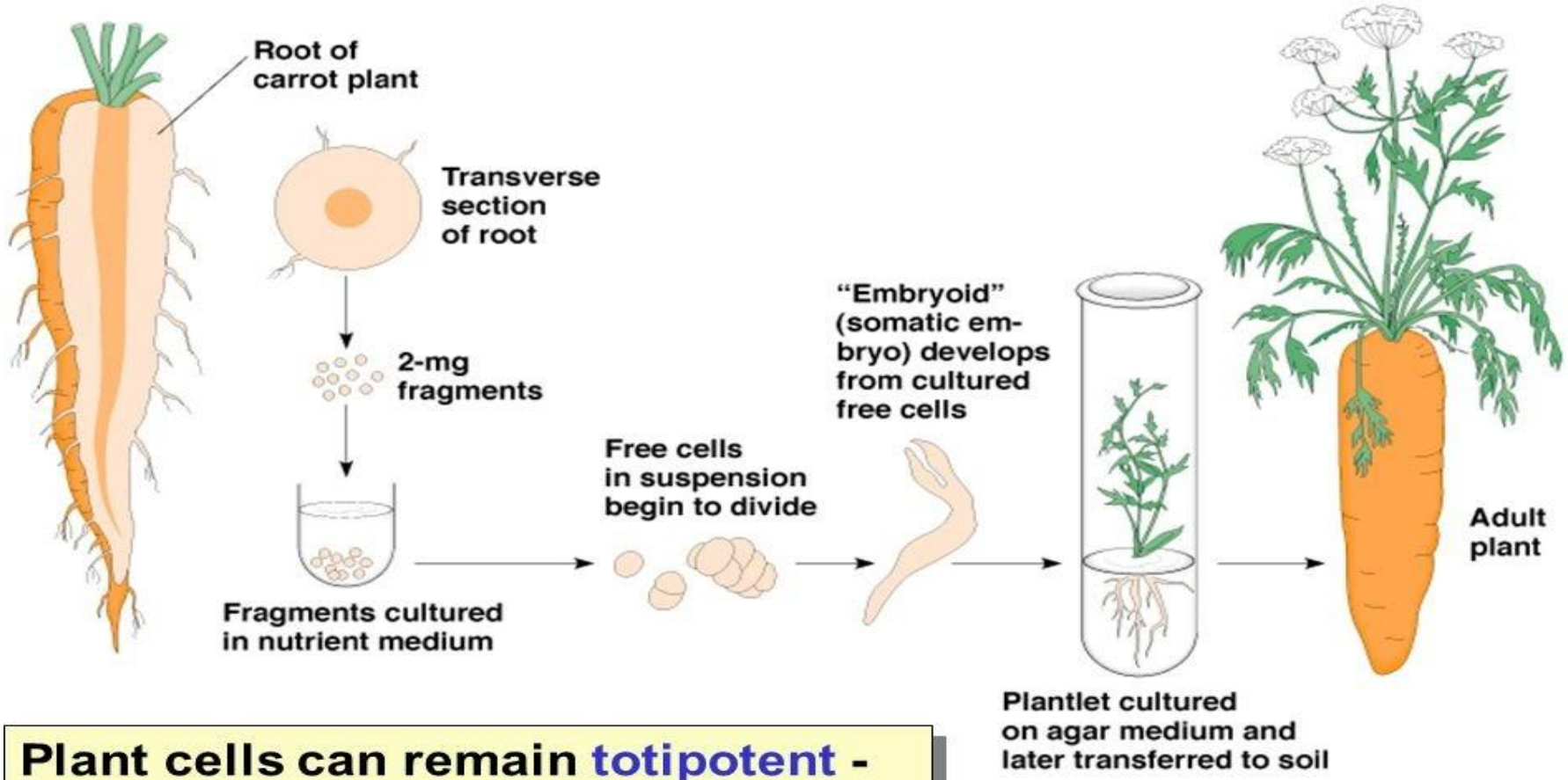
Whole plant



Proof of totipotency of plant cells

https://www.gf.org/wp-content/uploads/2014/07/F.-C.-Steward-Botany-1963_250x250.jpg

- F.C. Steward and his colleagues (1958) devised a method for growing callus tissue from secondary phloem tissue of carrot root in a liquid medium containing coconut milk.
- Single cells loosened from the surface of growing tissue and developed into somatic embryos .
- These somatic embryos developed into complete plants.



Plant cells can remain totipotent - they can retain the potential to form all parts of the plant

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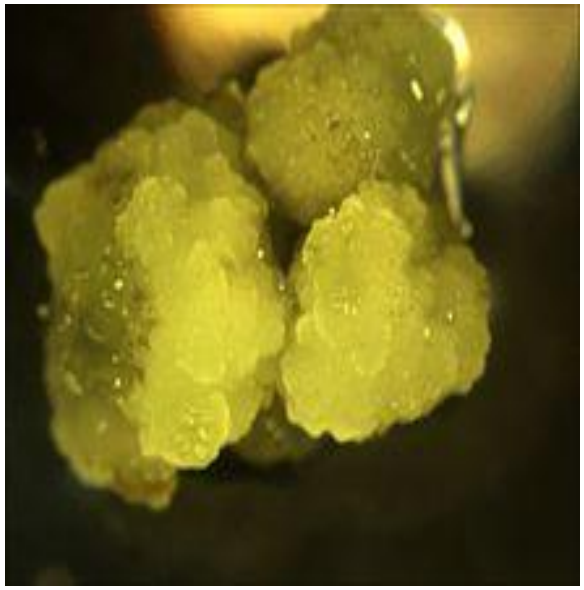
CALLUS CULTURE

Callus

Callus is an irregular mass of parenchymatous meristematic tissue.

It lacks any organized structure but often shows cellular differentiation, mostly tracheidal elements.

A well-established callus comprised different types of cells. The cellular heterogeneity of the callus is derived from the multicellular explants used to initiate callus cultures and/or induced by the culture conditions.



Brief History of Callus Culture:

R. J. Gautheret (France) (1934-1937) succeeded in promoting the development of callus from excised cambial tissue of Salix and other woody species in a nutrient medium supplemented with three vitamins (thiamine, pyridoxine and nicotinic acid) and indole-3-acetic acid (IAA)

P. Nobecourt (France) (1939):

He was first established the callus culture of tap root explant of *Daucus carota* capable of potentially unlimited growth on semisolid agar medium by transferring portions of the callus to fresh medium at regular interval of four to six weeks.

Van Overbeck, M. E. Conklin and A. F. Blakeslee (1941):

They first reported the importance of coconut milk in callus culture.

S. M. Caplin and F. C. Steward (1948):
used coconut milk in combination with synthetic auxin 2,4- D (2, 4-dichlorophenoxy acetic acid) in medium and were able to induce the callus formation.

F. Skoog (1954-1955):
discovered kinetin from autoclaved sample of herring sperm DNA. Addition of kinetin in culture medium renewed the growth of tobacco stem callus.

F. Skoog and C. O. Miller (1957):
They first put forward the **concept of hormonal control of organ formation** from callus tissue. They also suggested that equal concentration of auxin and kinetin induced the continuous growth of callus tissue. The inclusion of kinetin in culture media has made it possible to produce callus culture from a large number of plant species.

Callus

An explant can develop a callus as a wound response that consists of unorganized, dividing cells,or

A callus can be produced without wounding by germinating seeds on a medium containing 2,4-D.

Callus cells vary in size, shape, pigmentation, and sometimes in genetic expression.

Callus cells have a large central vacuole and the nucleus is to the side.

This is in contrast to undifferentiated, meristematic cells that are isodiametric, small, lack a prominent vacuole, and have a large central nucleus.

These meristematic cells are sometimes initiated in callus masses and are referred to as meristemoid regions.

Meristemoids can give rise to adventitious roots, shoots, or somatic embryos.

Types of Callus

The calli from the same explant may show considerable variation with regard to color, texture, compactness, amount of water content, and chemosynthetic and morphogenic potential.

The calli may be-

- compact or friable,
- dry or wet,
- light, or dark colored

These features may also change with passage of time in cultures due to genetic or epigenetic changes or due to change of culture medium.

Callus culture

The calli can be multiplied as unorganized tissue for unlimited period through periodic subcultures on fresh medium or

induced to differentiate organized structures (roots, shoots, embryos) by manipulating the culture medium.

In nature, several plants develop irregular unorganized structures due to disturbance in the endogenous levels of growth regulators caused by infection,

by insects (insect galls),

microorganisms (crown gall), or

specific genetic recombinations (genetic tumours).

Callus culture : Explant

For callus induction,

- pieces of the cotyledon,
- hypocotyl,
- stem,
- leaf, or
- embryo are usually used.

Excellent explants for callus induction are **seedling tissues** from aseptically germinated seeds or immature inflorescences.

To produce haploid plants or callus, the anther or pollen is cultured.

Factors controlling callus formation

- source of the explant
- individual plant genotype,
- age and nutritional status of the source plant
- The level of plant growth regulators (auxin, cytokinins, gibberellins, ethylene, etc.).
- Culture conditions (temperature, light, etc.) are also important in callus formation and development.

Use of Callus Cultures

Once established, callus cultures may be used for a variety of experiments -

- protoplast isolation,
- somatic embryogenesis,
- organogenesis, and
- secondary product production
- regenerable callus is useful as a target for genetic transformation.

CALLUS CULTURE FROM EXCISED TAP ROOT OF CARROT

1. A fresh tap root of carrot is taken and washed thoroughly under running tap water.

2. The tap root is then dipped into 5% 'Teepol' for 10 minutes and then the root is washed.

3. The carrot root, sterilized forceps, scalpels, other instruments, autoclaved nutrient medium petri dishes are then transferred to laminar air flow or inoculation chamber.

4. Throughout the manipulation sequence forceps, scalpels must be kept in 95% ethanol and flamed thoroughly before use.

5. The tap root is surface sterilized by immersing in 70% v/v ethanol for 60 seconds, followed by 20-25 minutes in sodium hypochlorite (0.8% available chlorine).

6. The root is washed three times with sterile distilled water to remove completely the hypochlorite.

7.The carrot is then transferred to a sterilized petri dish containing a filter paper. A series of transverse slice 1 mm in thickness is cut from the tap root using a sharp scalpel.

8. An area of $4mm^2$ across the cambium is cut from each piece so that each small piece contains part of the phloem, cambium and xylem. Size and thickness of the explants should be uniform.

9. The cotton plug from a culture tube is removed and flamed the uppermost 20 mm of the open end. While holding the tube at an angle of 45°, an explants is transferred using forceps onto the surface of the agarified nutrient medium. Nutrient medium is Gamborg's B_5 or MS medium supplemented with 0.5 mg/L 2, 4-D.

10. The cotton plug is immediately placed on the open mouth of each tube. The forceps are always flamed before and after use. Date, medium and name of the plant are written on the culture tube by a glass marking pen or pencil.

11. Culture tubes after inoculation are taken to the culture room where they are placed in the racks. Cultures are incubated in dark at 25°C.

12. Usually, after 4 weeks in culture the explants form a substantial callus. The whole callus mass is taken out aseptically on a sterile petri dish and divided into two or three pieces.

13. Each piece of callus tissue is transferred to a tube containing fresh same medium.

Habituated callus cultures

Some callus cultures, which initially require an auxin for growth, acquire an irreversible capacity to synthesize excess quantities of auxin, and consequently become autotrophic for auxin. Such callus cultures, also called habituated, can grow independent of an exogenous auxin (Gautheret 1946).

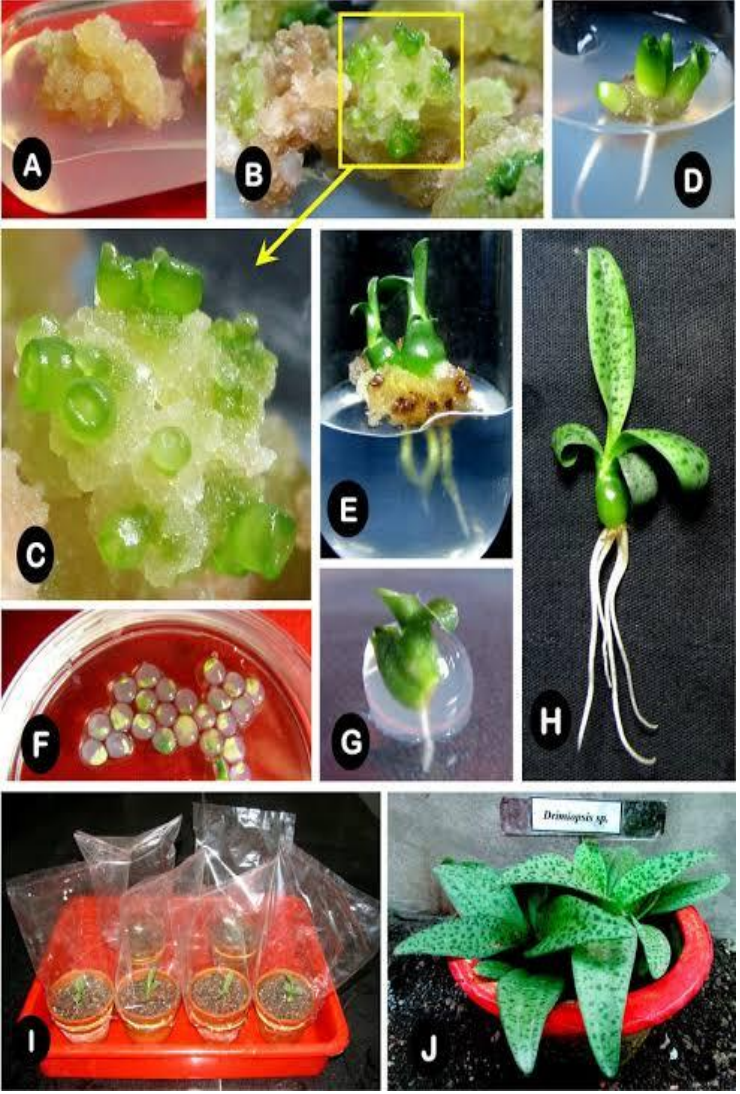
Similar habituation of cultured tissues with respect to cytokinin requirement has also been observed.

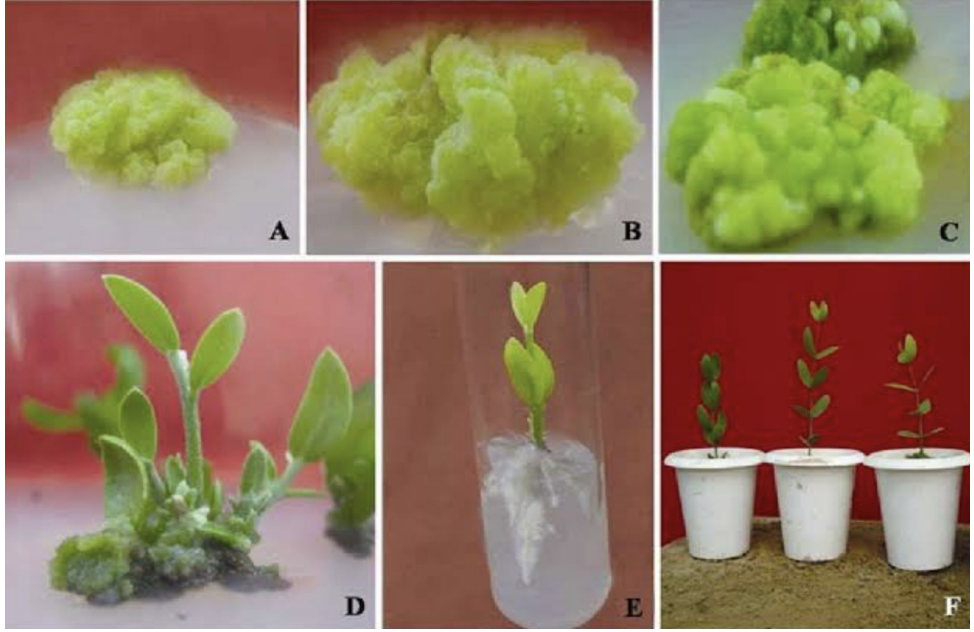
Significance of Callus Culture:

1. The whole plant can be regenerated in large number from callus tissue through manipulation of the nutrient and hormonal constituents in the culture medium.

2. Similarly, by manipulation of nutrient and hormonal constituents, somatic embryos can be obtained directly from the somatic cells of callus tissue. This phenomenon is known as somatic embryogenesis. Somatic embryo directly gives rise the whole plant.

3. Callus tissue is good source of genetic or karyotype variability, so it may be possible to regenerate a plant from genetically variable cells of the callus tissue and generate useful somaclonal variations.





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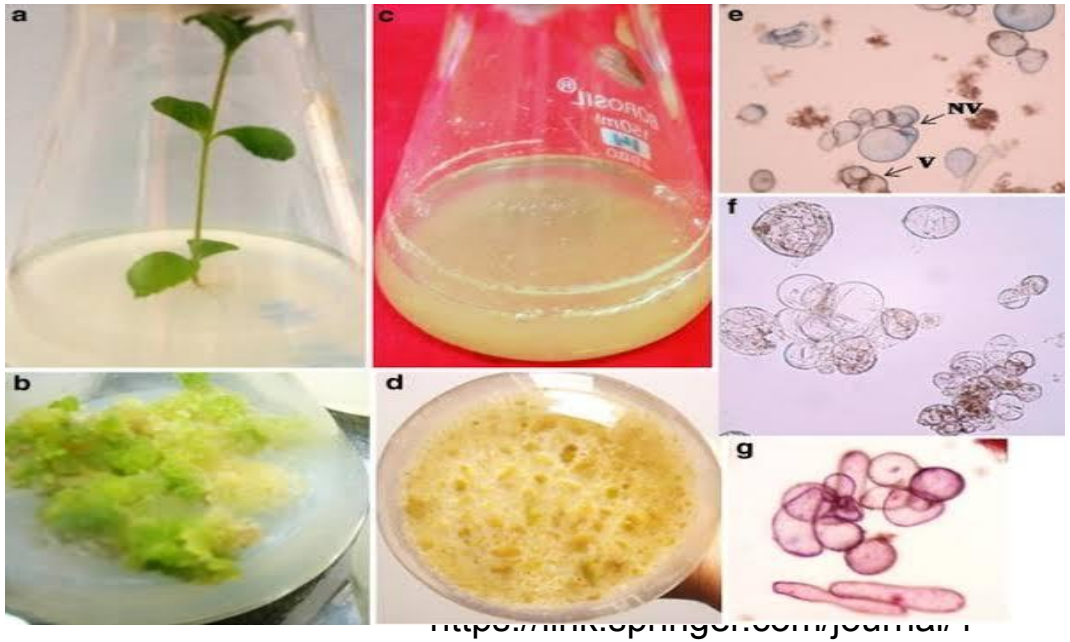

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4. Callus cultures serve as the starting material to initiate single cell and suspension cultures in liquid medium.

5. Secondary metabolites or drugs can be directly extracted from the callus tissue without sacrificing the whole plant. So, this alternative technique helps the conservation of medicinal plants in nature.

6. Several biochemical assays can be performed on callus culture.

Suspension cultures from callus

- To initiate cell suspension cultures, pieces of undifferentiated, friable calli are transferred to liquid medium in flasks.
- the medium is continuously agitated (30–100 rpm) by fixing on a gyratory/orbital shaker .
- Agitation of the medium exerts a mild pressure on the tissue, breaking it into smaller cell aggregates and single cells.
- It also maintains uniform distribution of cells and cell clumps in the medium, and helps aeration of the cells.
- Suspension cultures consist of both single cells and small cell aggregates.

MICROPROPAGATION

MICROPROPAGATION

- Multiplication of genetically identical copies of a plant by asexual reproduction is called clonal propagation.
- **Clonal propagation through tissue culture is called micropropagation.**
- Use of tissue culture technique for micro propagation was first started by Morel (1960) for propagation of orchids, and is now applied to several plants.
- Murashige developed a protocol for gerbera clonal propagation.

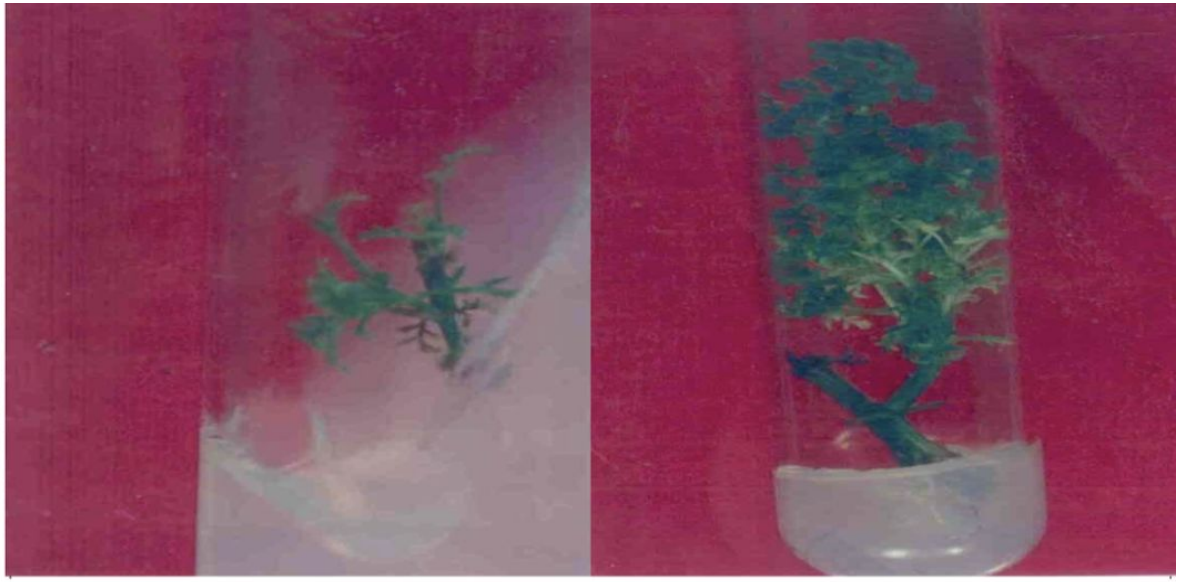
Micro propagation mostly involves in vitro clonal propagation by two approaches:

1.Multiplication by axillary buds-

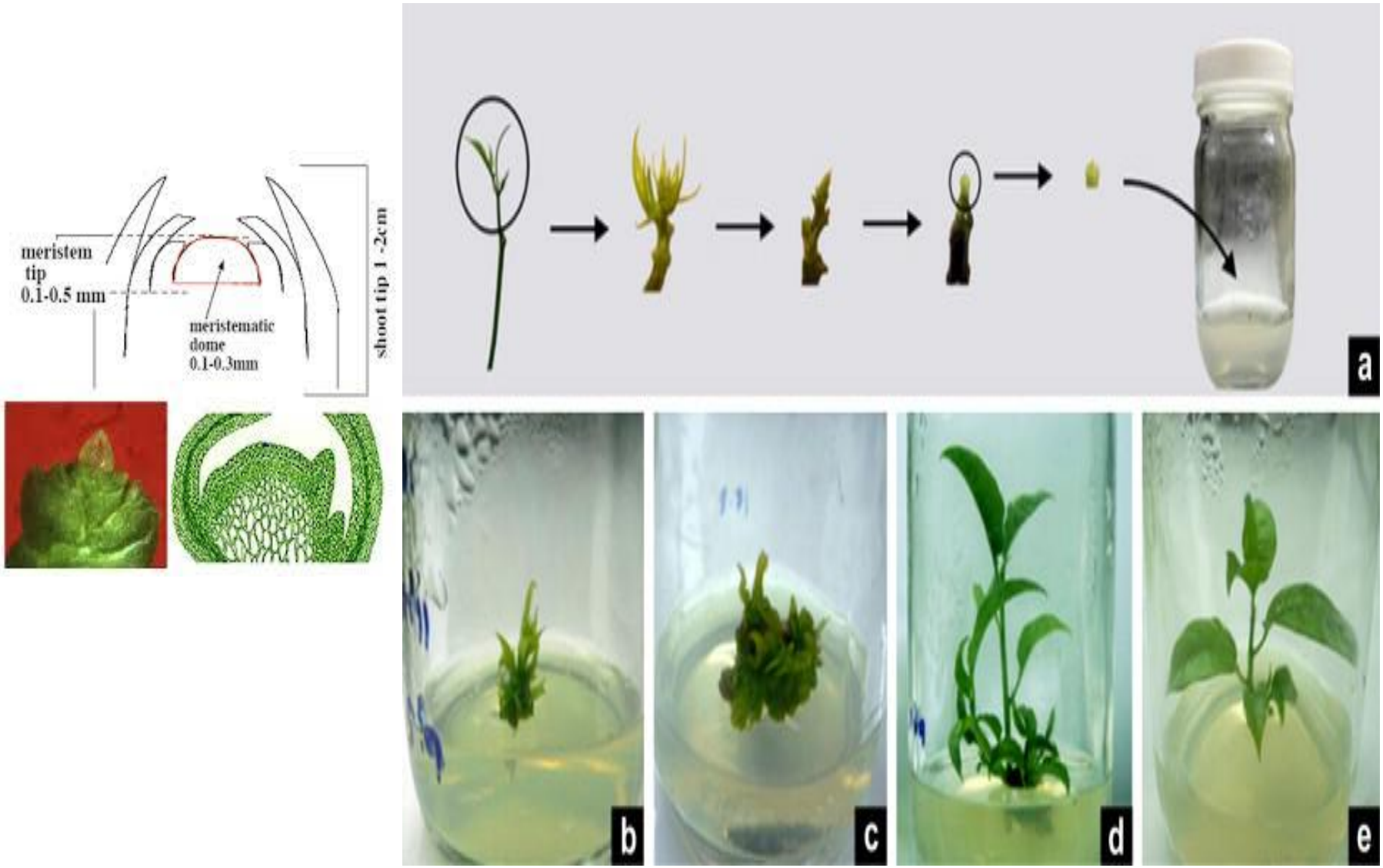
- The axillary bud along with a piece of stem is isolated and cultured to develop into a plantlet.
- The cultures are carried out with high cytokinin concentration. As a result of this, apical dominance stops and axillary buds develop.
- Closed buds are used to reduce the chances of infections.

2. Meristem and Shoot Tip Cultures:

- Apical meristem is a dome of tissue located at the extreme tip of a shoot.
- The apical meristem along with the young leaf primordia constitutes the shoot apex.
- For the development of disease-free plants, meristem tips should be cultured.



Micropropagation of Artemisia annua from nodal explants containing axillary buds (Dr. Sugandha Tiwari)



Meristem tip culture is used to obtain disease free plants.

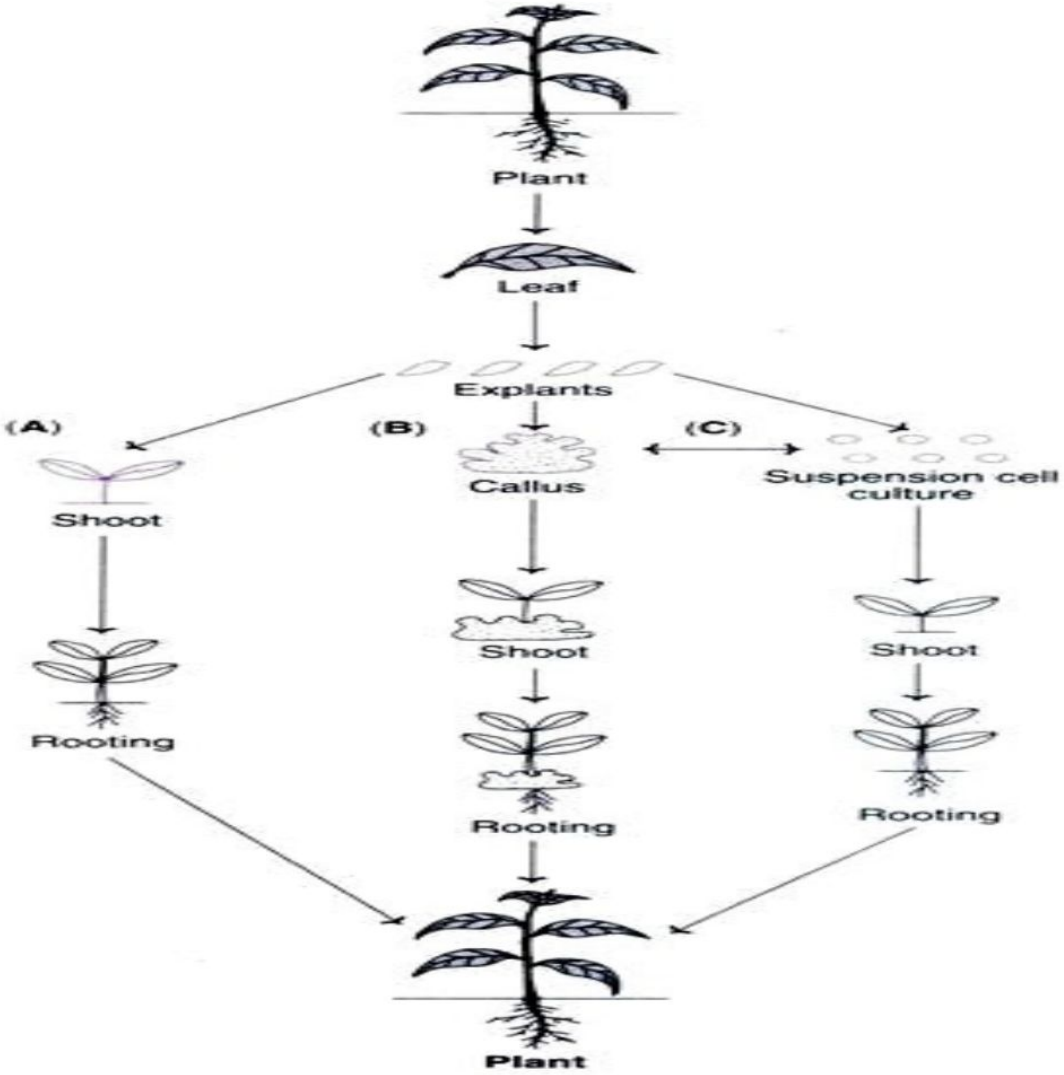
Organogenesis:

Organogenesis is the process of **morphogenesis** involving the **formation of plant organs** i.e. shoots, roots, flowers, buds from explant or cultured plant tissues.

It is of two types —

1. Direct organogenesis
   - Tissues from leaves, stems, roots and inflorescences can be directly cultured to produce plant organs.
   - In direct organogenesis, the tissue undergoes morphogenesis without going through a callus or suspension cell culture stage.

2. Indirect organogenesis
   - When the organogenesis occurs through callus or suspension cell culture formation, it is regarded as indirect organogenesis.



Micropropagation of plants by organogenesis (A) Direct organogenesis

(B) Indirect organogenesis through callus

(C) Indirect organogenesis through cell suspension culture

By varying the concentrations of auxins and cytokinins, in vitro organogenesis can be manipulated:

a. Low auxin and low cytokinin concentration will induce callus formation.

b. Low auxin and high cytokinin concentration will promote shoot organogenesis from callus.

c. High auxin and low cytokinin concentration will induce root formation.

General Micropropagation Technique

Murashige (1974) identified major stages in the in vitro propagation process.

Micropropagation comprises of five steps, each with its specific requirements and problems :

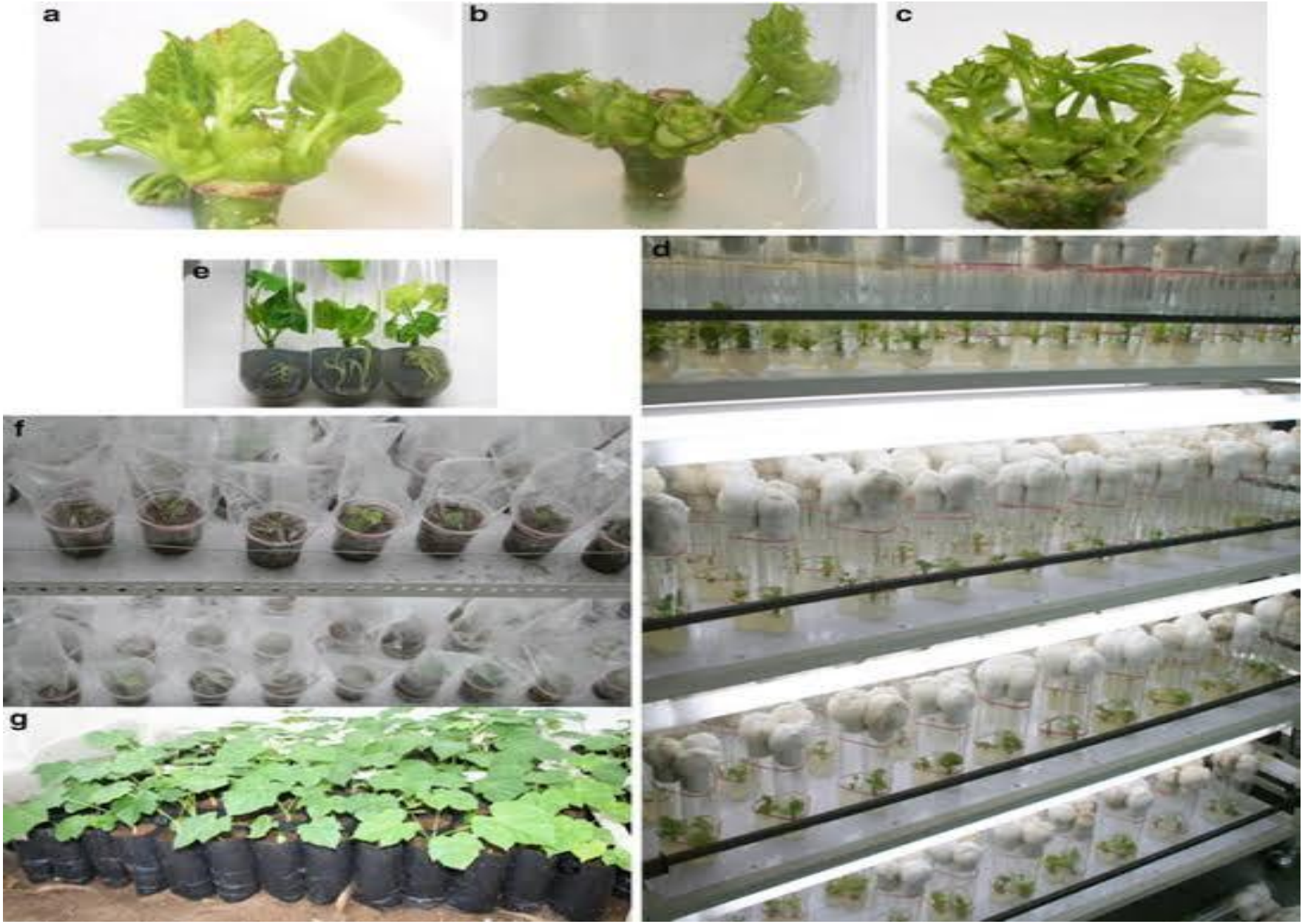
Stage 0: It is the preparatory stage to provide quality explants;

Stage 1: Initiation of aseptic cultures;

Stage 2: Multiplication;

Stage 3: Rooting of in vitro formed shoots; and

Stage 4: Transfer of plants to greenhouse or field conditions (transplantation).



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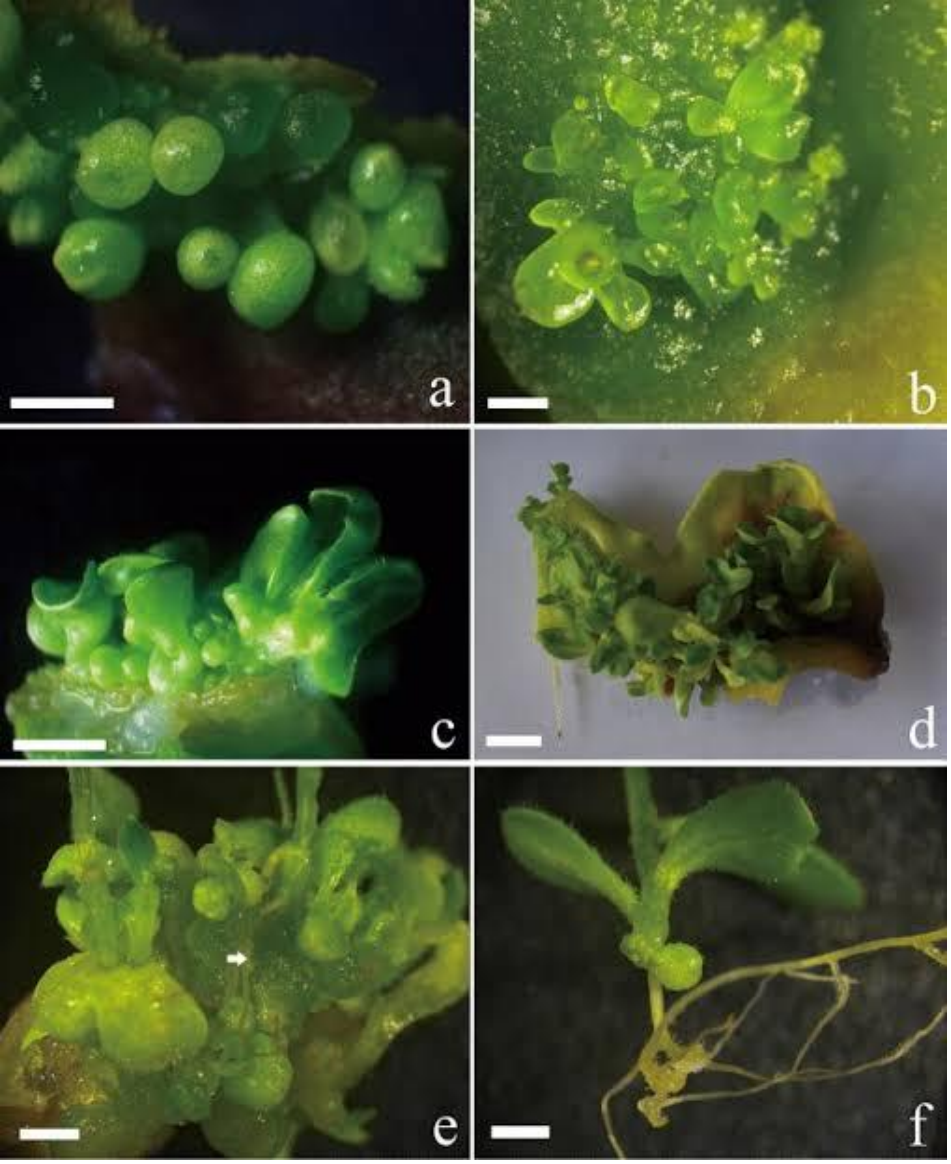
Major stages involved in Micropropagation

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



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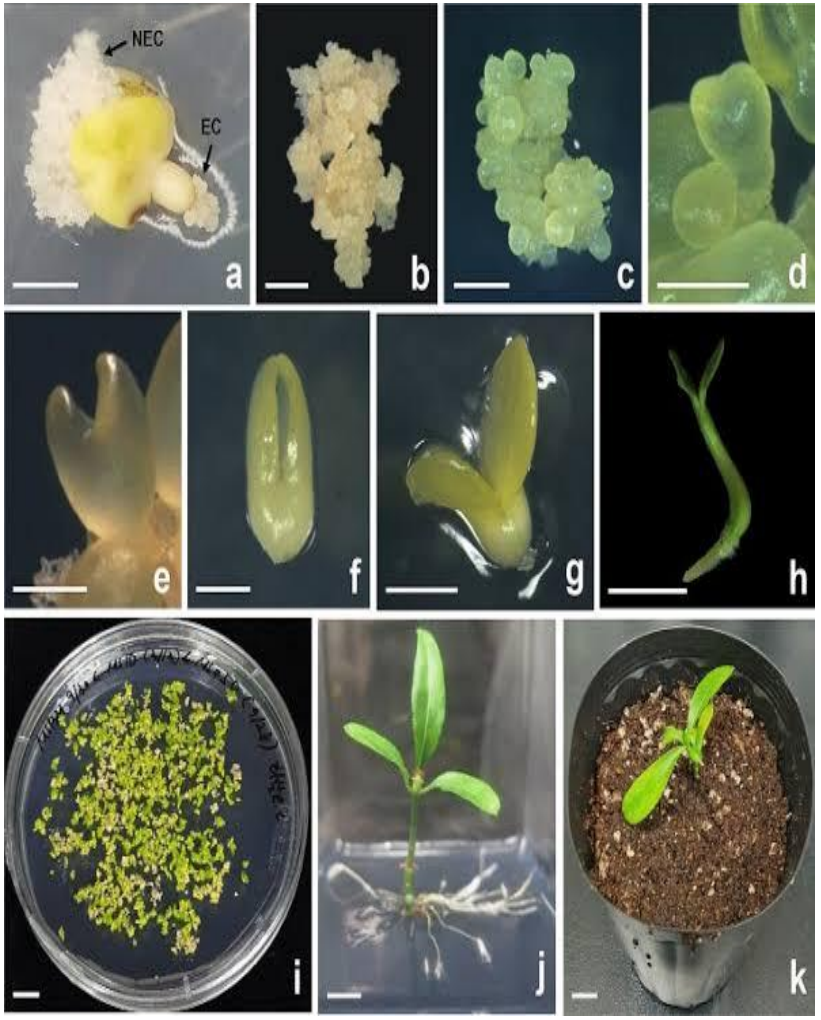
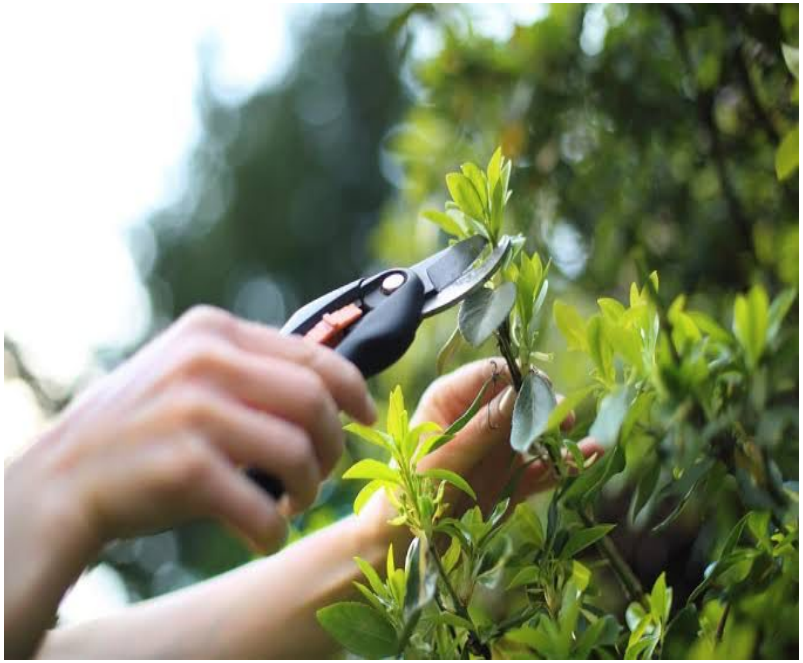


Woo et al. Efficient plant regeneration from embryogenic cell suspension cultures of Euonymus alatus. Sci Rep 11, 15120 (2021). https://doi.org/10.1038/s41598-021-94597-4

Major stages in micropropagation:

Stage 0:

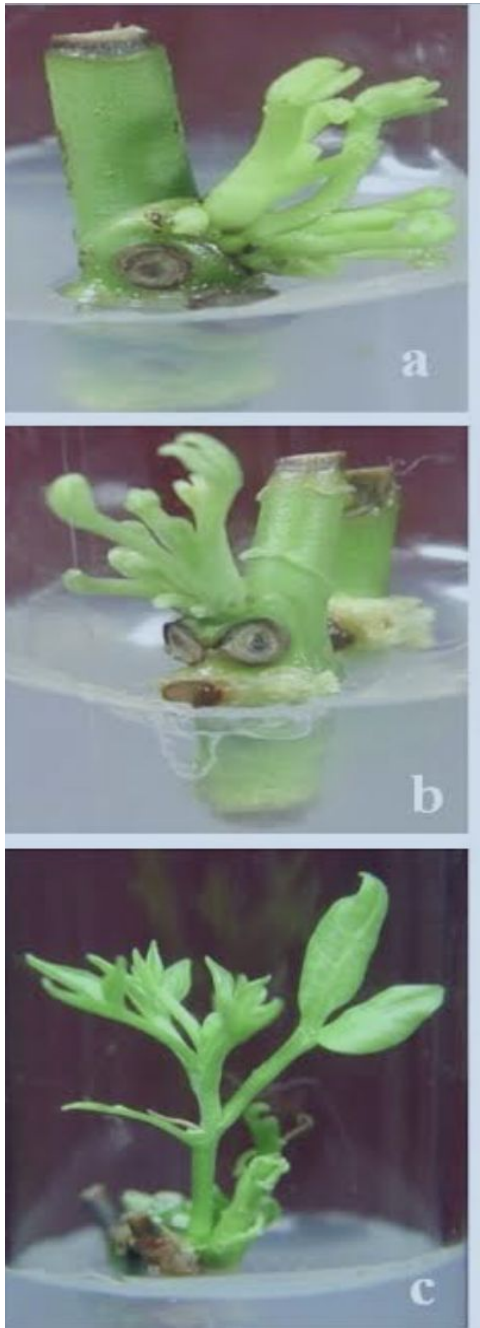
- This stage involves the selection and growth of stock plants under controlled conditions.
- Measures taken to improve the quality of the parent plant, involving its hygiene or physiological status, are included in Stage 0.



Major stages in micropropagation:

Stage I:

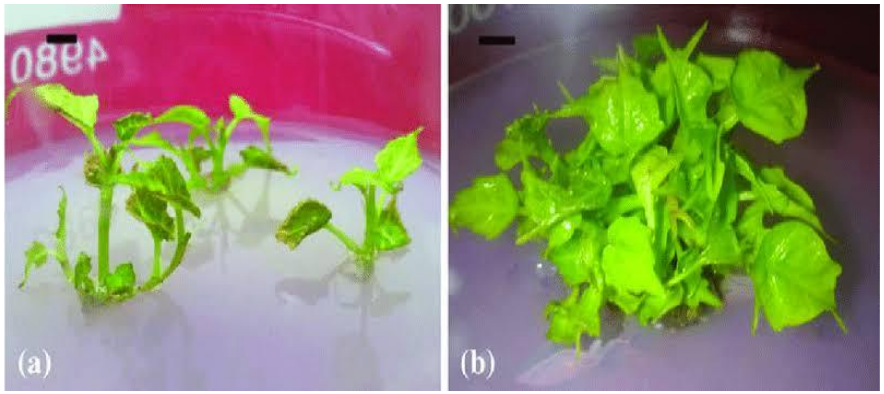
- Stage I is the establishment of aseptic cultures.
- The most commonly used explants are stem segments with axillary buds or shoot tips.
- The chosen explant is surface sterilized and washed before use.
- Explant is inoculated in the culture medium aseptically.
- This stage can often be difficult due to contamination and production of phenolic compounds by the explant.
- However, in this first stage, the goal is to optimize a surface disinfestation protocol and nutrient medium for survival and growth of the explant.

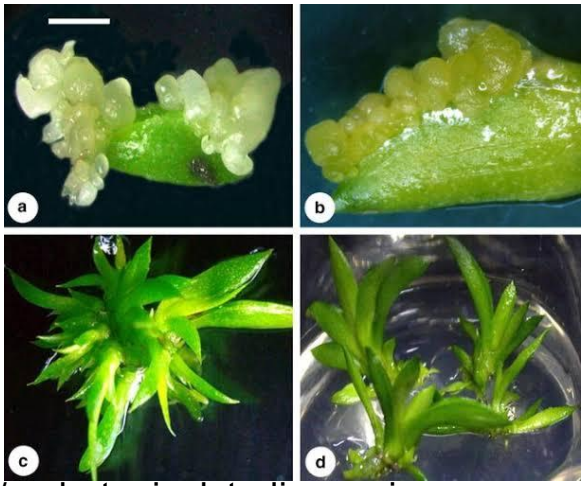


Major stages in micropropagation:

Stage II:

- Stage II involves multiplication of shoots from the explant.
- Generally, a cytokinin enhances multiple-shoot production from preexisting axillary buds or multiplication is achieved by adventitious bud formation from leaf, stem, or petiole explants.
- Somatic embryo formation can also result in a high multiplication rate. **Stage II cultures can be subcultured three to six times.**
- If callus is involved in this stage, the occurrence of aberrant plants or somaclonal variation is a problem.





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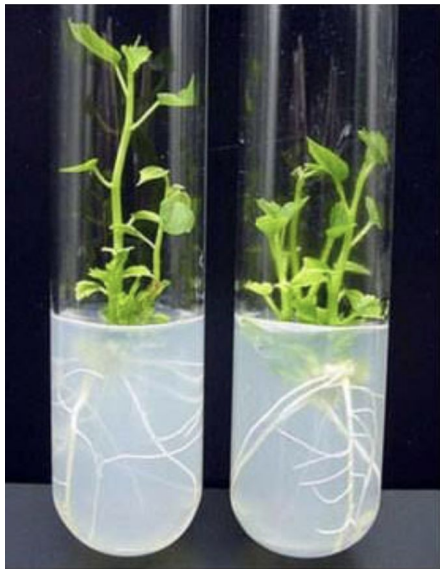
Major stages in micropropagation:

Stage III:



- Stage III prepares plants for transfer to soil.
- This stage involves the transfer of shoots to a rooting medium for rapid development of roots.
- Generally an auxin is included for rooting of individual shoots.
- For many plants, this stage can take place in the greenhouse in potting soil. Sometimes, the shoots are directly planted in soil to develop roots.

Stage IV:

This stage involves the establishment of plantlets in soil. The process is known as acclimatization/ hardening.

The rooted cuttings are removed from the culture tube, and the agar is gently washed from the root system.

The plants are placed in a potting mix and kept in high humidity and shade.

Usually after 2 weeks, the plants have been conditioned and can tolerate more light and lower humidity of the field condition

Factors Affecting Micro propagation:

1. Genotype of the plant:

Plants with vigorous germination and branching capacity are more suitable for micropropagation.

2. Physiological status of the explants:

Explants (plant materials) from young parts of plants are more effective than those from older regions.

3. Culture media:

Addition of growth regulators (auxins and cytokinins) and alterations in mineral composition are required for different plant species and type of explant.

4. Culture environment:

Light:

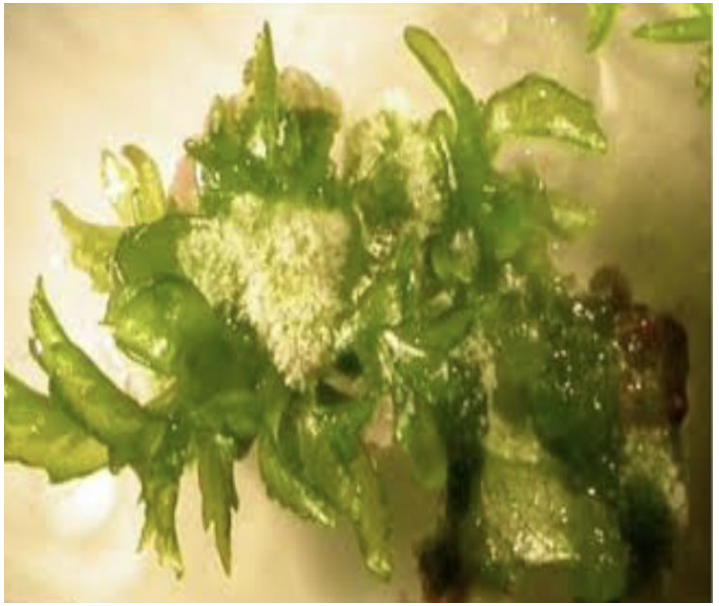
An illumination of 16 hours day and 8 hours night is satisfactory for shoot proliferation.

Temperature:

Majority of the culture for micropropagation requires an optimal temperature around 25°C.

Vitrification or Hyperhydricity

- Vitrification or hyperhydricity can be a problem in micropropagation.
- Shoots that are in this condition are difficult to establish as plants.
- Vitrified plants have a glassy appearance, their stems and leaves are enlarged, thick, translucent and brittle.
- The morphological malformations are associated with chlorophyll deficiency, poor lignification and excessive hydratation of tissues.
- The reasons for the occurrence of vitrification in tissue-culture plants is not clear.



Advantages of Micropropagation

1. The major benefit is clonal propagation, resulting in genetically identical copies of a cultivar.
2. Germplasm conservation of threatened or endangered plants.
3. More rapid propagation,
4. Meristem tip cultures to develop pathogen-free plants .
5. Enhanced axillary branching of in vitro-derived plants (resulting in fuller foliage),
6. Production of a uniform crop,
7. Year-round production,
8. Hastening of a new crop introduction, and

Applications of Micro propagation:

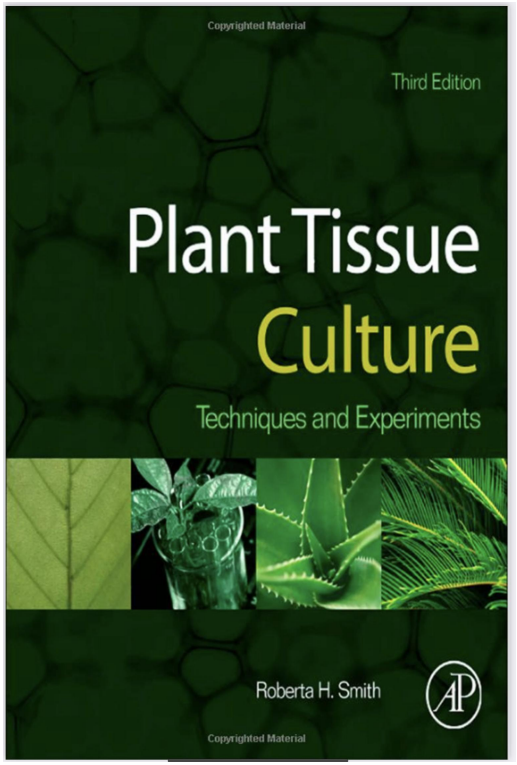
9. Cloning of only the desirable female plants (date palm) or male plants (asparagus).

10. Minimum growing space (millions of plant species can be maintained inside culture vials in a small room in a nursery).

11. The small sized propagules obtained in micro propagation can be easily stored for many years (germplasm storage), and transported across international boundaries.

12. Automated micro propagation using bioreactors for large scale multi plication of shoots and bulbs

References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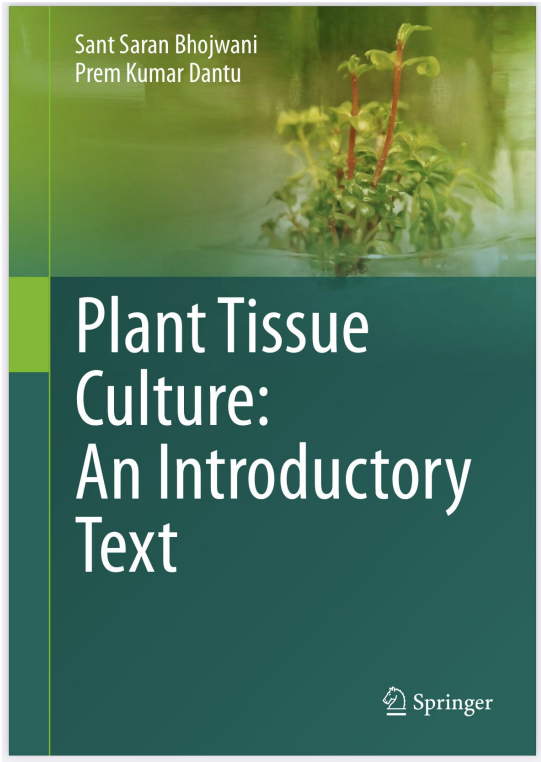
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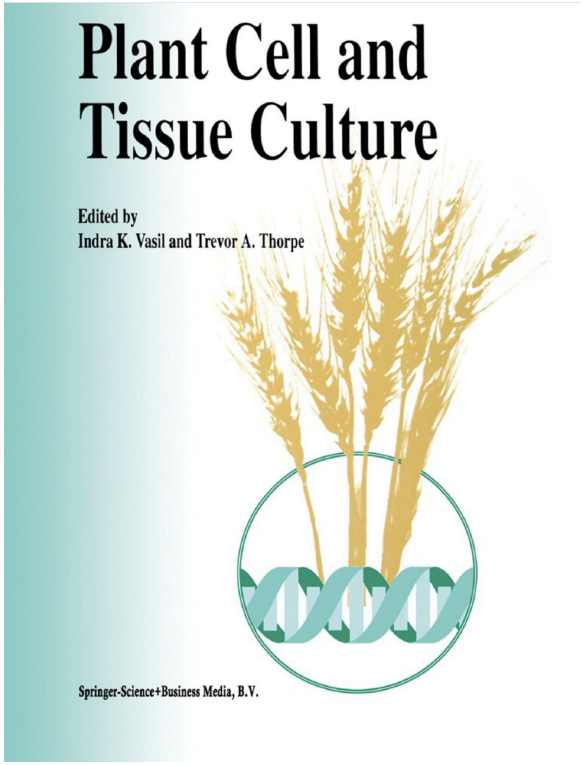
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Thank you all for listening

Any questions ??