RAMAIAH
College of Arts, Science & Commerce

M.S..RAMAIAH COLLEGE OF ARTS, SCIENCE AND COMMERCE

MSRIT Post, M.S.R. Nagar, Bengaluru-560054.
(Re-Accredited with "A" by NAAC, Recognised by GOK & AICTE, rank.
Permanently affiliated to Bangalore University

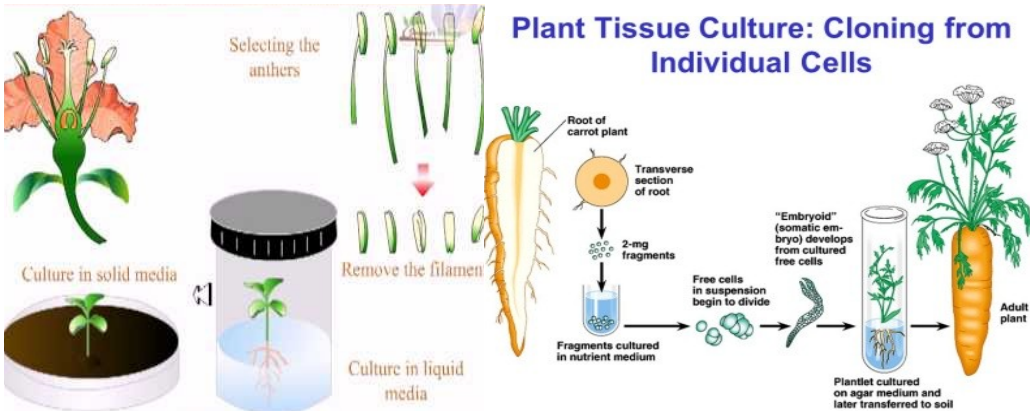
STUDY MATERIAL

Plant Tissue Culture

Value Added Program

FOR

Undergraduates



A Research Approach

Organised By

Department of Biotechnology/Genetics

Plant Tissue Culture

Experiment : 1

Introduction , Instrumentation of Plant Tissue Culture 03 Hour

PLANT TISSUE CULTURE

Definition

Tissue culture is in vitro cultivation of plant cell or tissue under aseptic and controlled environmental conditions, in liquid or on semisolid well defined nutrient medium for the production of primary and secondary metabolites or to regenerate plant.

Tissue culture relies on three fundamental abilities of plant there are:

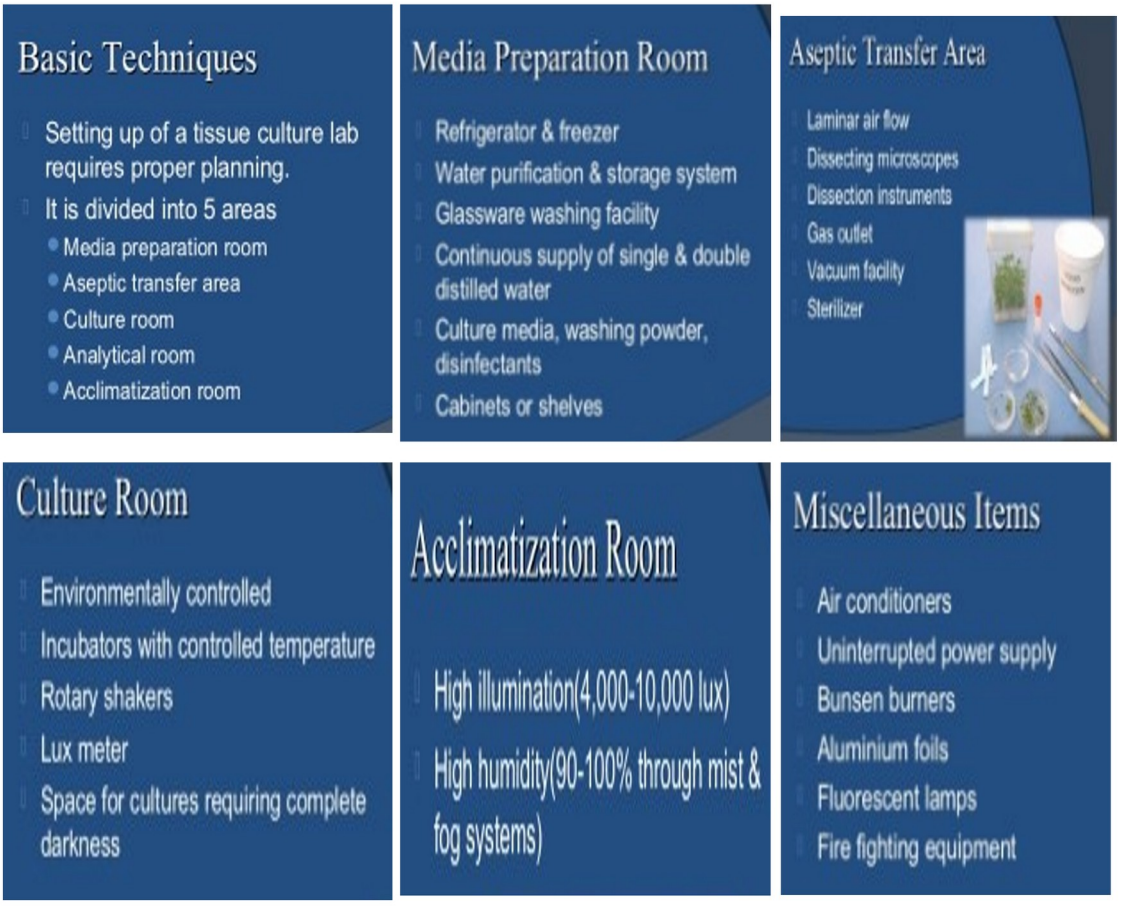
Totipotency
Dedifferentiation
competency

Tissue culture is a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function

❖ BASIC REQUIREMENT FOR A TISSUE CULTURE LABORATORY

For the successful achievement, the following general basic facilities are required:

- ❑ Equipment & apparatus
- ❑ Washing and storage facilities
- ❑ Media preparation room
- ❑ Sterilization room
- ❑ Aseptic chamber for culture
- ❑ Culture rooms or incubators fully equipped with temperature, light and humidity control devices
- ❑ Observation or recording area well equipped with computer for data processing



Experiment: 2

Aseptic techniques

Washing of Glass wares and sterilization techniques 03 Hours

❖ STERILISATION OF MEDIA

•The prepared media should be sterilized by ISI mark Autoclave(for large amounts) at 121° Domestic pressure cookers(for small amounts)

•For the sterilization of glassware and metallic equipments Hot air oven with adjustable tray is required.

Incubator

Hot air oven

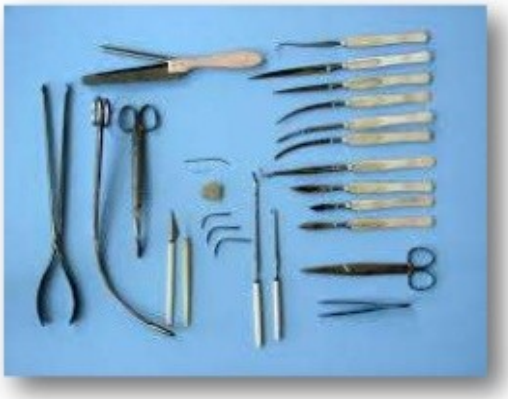
EQUIPMENT & APPARATUS

❖ VESSELS & GLASS WARE :

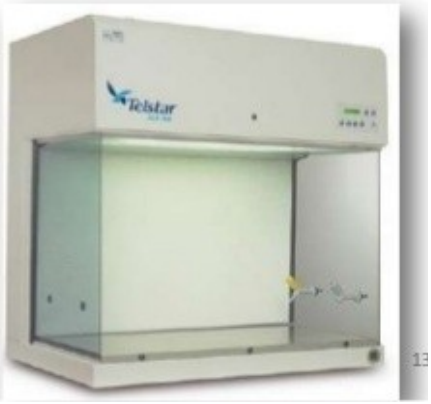
- All the glassware should be of Pyrex.
- Large test tubes,flasks,graduated pipettes etc.. are used.

❖ EQUIPMENT :

- Scissors,scapels,foreceps are used for explants preparation.
- A spirit burner for flame sterilization.
- Hot air oven.
- A PH meter.
- A BOD incubator.
- Laminar air flow chamber.
- A balance to weigh nutrients.
- Data collection and recording room.



Laminar air flow chamber



Experiment: 3

Media and Culture Preparation **03 Hour**

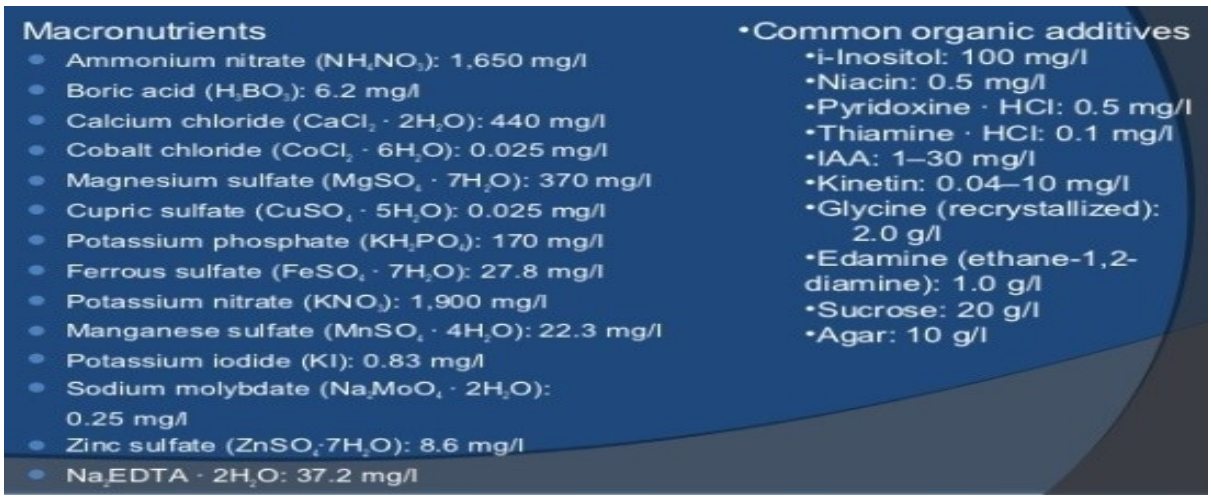


TABLE 43.1 Composition of commonly used plant tissue culture media

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					
Thlamine 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

Nutrient Requirements

INORGANIC & ORGANIC SUPLLEMENTS

COUMPOUNDS	Mg/Ml
NH4NO3	1,650.00
KNO3	1,900.00
CaCl2 (anhyd)	332.20
MgSO4 (anhyd)	180.70
KH2PO4	170.00
Na2EDTA	37.25
FeSO4.7H2O	27.80
H3BO3	6.20
MnSO4.H2O	16.90
ZnSO4.H2O	5.37
KI	0.83
Na2Mo4.2H2O	0.25
Sucrose	30,000.00
i-Inositol	100.00
Thiamine.HCl	0.40

➢ **Antibiotics :**

Stertomycin,kanamycin

Activated charcoal

➢ **Other organic supplements :**

Protein, coconut milk,yest,malt extract, orange juice, and tomato juice

➢ **Growth regulators :**

Auxins,cytokinins

➢ **Water :**

Demineralized or distilled water

➢ **Solidifying agents :**

Agar, gelatin.

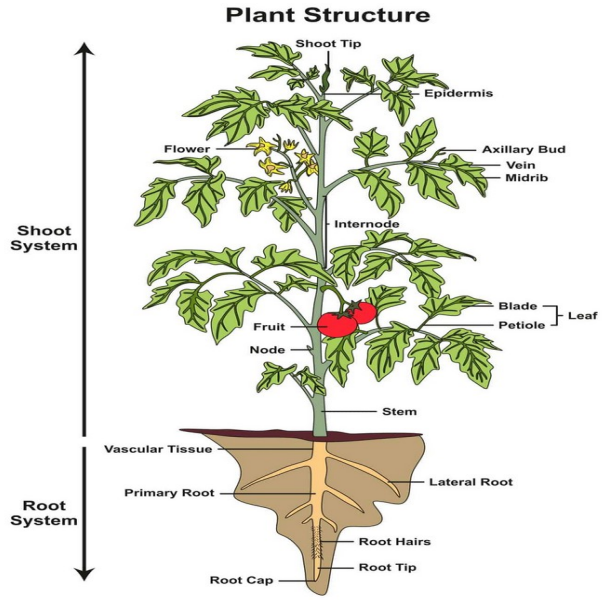
➢ **pH adjusters :**

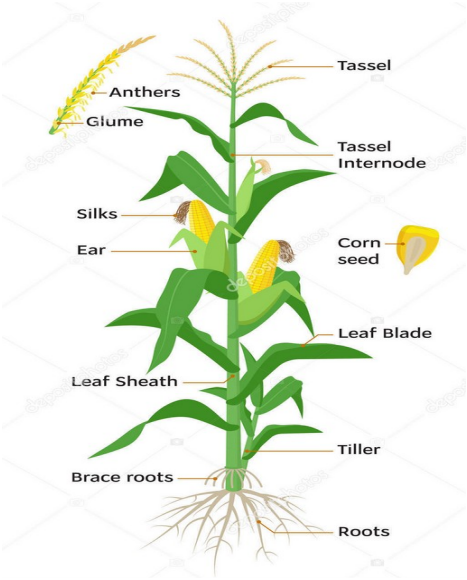
5 - 6 it is considered to be optimum.

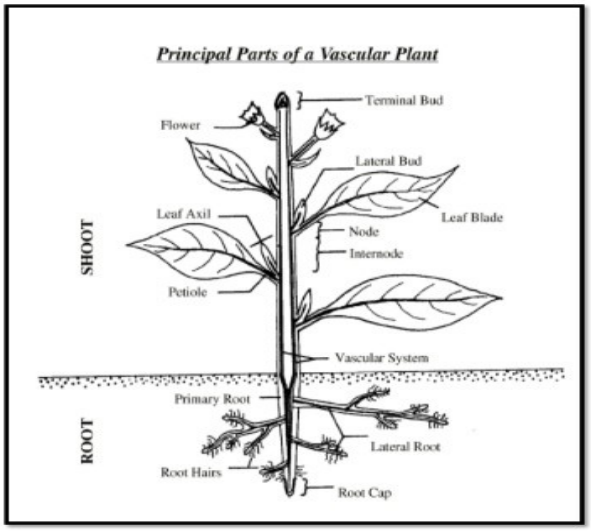
Experiment: 4

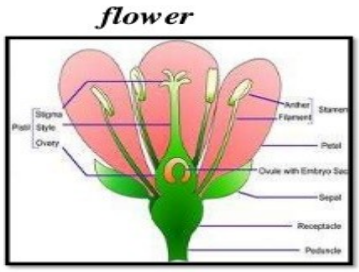
Monocot and Dicot Seed cultures for the establishment of organ cultures

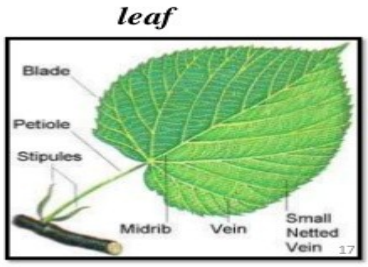
03 Hours

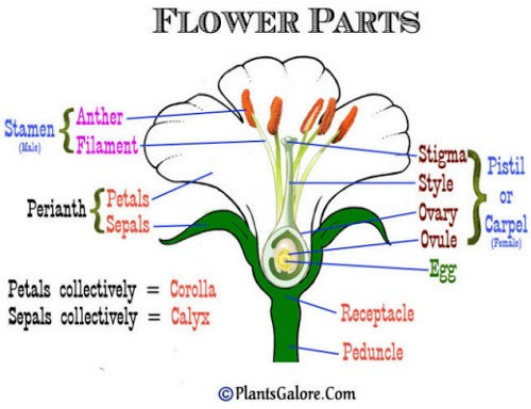


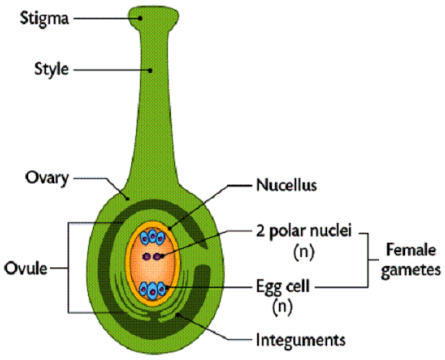






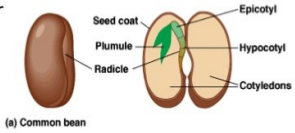


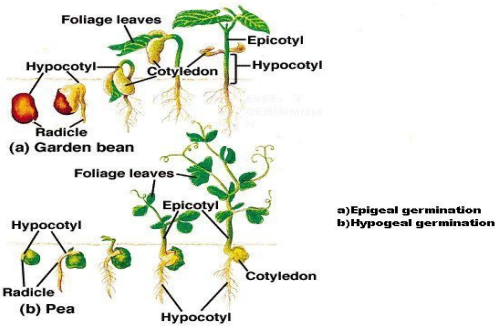




SEED STRUCTURE

- External
  - Seed coat (*testa*)
  - Hilum
- Embryo
  - Cotyledor
  - Epicotyl /
  - Pumule
  - Radical





Experiment: 5

Culture Techniques **03 Hours**

❖ EXPLANT PREPARATION

EXPLANT : It is defined as a portion of plant body, which has been taken from the plant to establish a culture

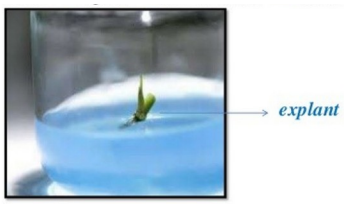
- Explant may be taken from any part of the plant like root,stem,leaf,or meristematic tissue like cambium, floral parts like anthers, stamens etc..
- Age of the explant.
- Homozygous plants are preferred.

❖ SURFACE STERILISATION OF EXPLANT

For surface sterilization chromic acid, Hgcl(0.11%),calcium hypochlorite, sodium hypochlorite(1-2%),alcohal(70%) are used.

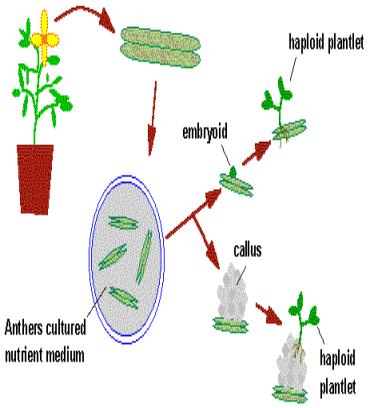
Process depends on the type of explant.

- ❖SEED : absolute ethyl alcohol → calcium hypochlorite →bromine water → sterile water
- ❖FRUIT : ethyl alcohol → sodium hypochlorite → sterile water
- ❖STEM : running water → sodium hypochlorite → sterile water
- ❖LEAF : surface clean →Hgcl2 → sterile water →dried



❖ TYPES OF CULTURE

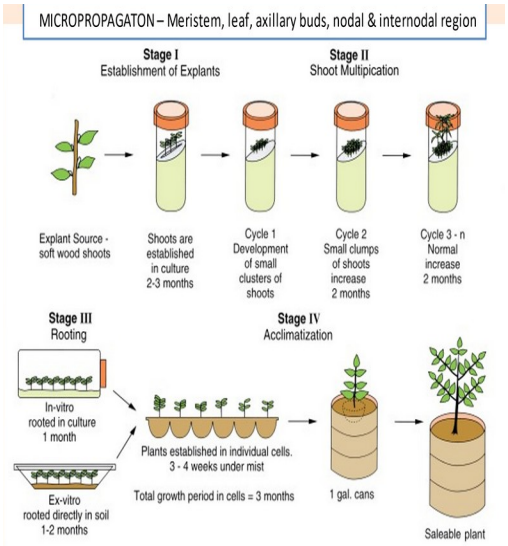
- ➢Callus culture
- ➢Suspension culture
- ➢Root tip culture
- ➢Leaf or leaf primordial culture
- ➢Shoot tip culture
- ➢Complete flower culture
- ➢Anther & pollen culture
- ➢Ovule & embryo culture
- ➢Protoplast culture



Experiment: 6

Micropropagation

Micropropagation or Clonal propagation is a field dealing with the ability to regenerate plant directlyfrom explants or from explants or from a single individual by asec8al reproduction, constitute a clone.



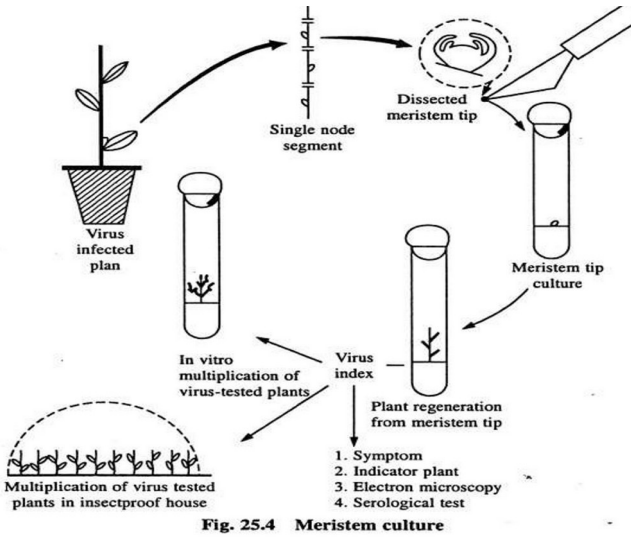


Fig. 25.4 Meristem culture

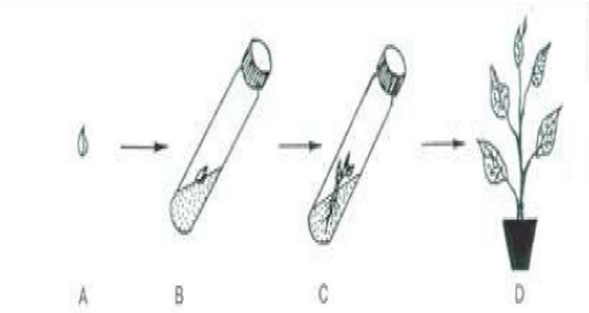


FIG. 8.4. Embryo culture. (A) Proembryo dissected 3 to 5 days after pollination. (B) Proembryo cultured on solid agar medium. (C) Plantlet developing from embryo. (D) Plantlet transplanted into soil.

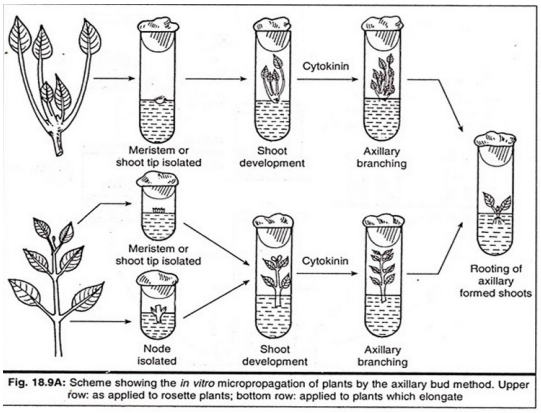
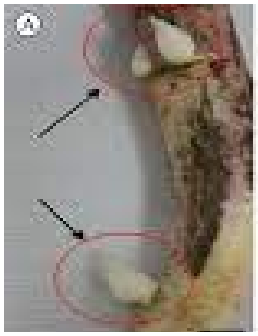


Fig. 18.9A: Scheme showing the *in vitro* micropropagation of plants by the axillary bud method. Upper row: as applied to rosette plants; bottom row: applied to plants which elongate

Potato Stem Eyes



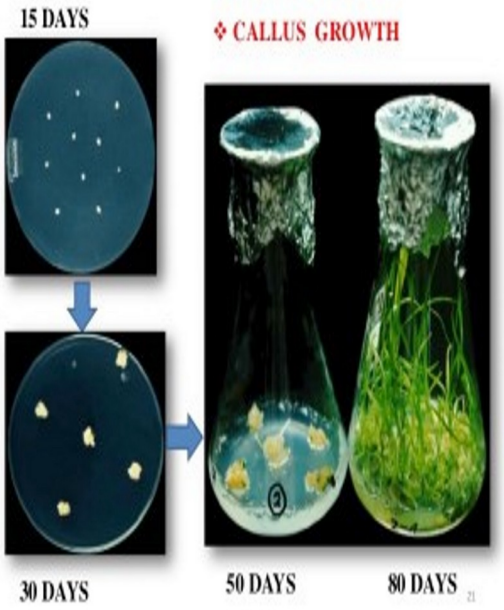
Bulbils of Bellodona Lily

Experiment: 7

Cult Culture & Establishment 03 Hours

❖ PRODUCTION OF CALLUS FROM EXPLANT

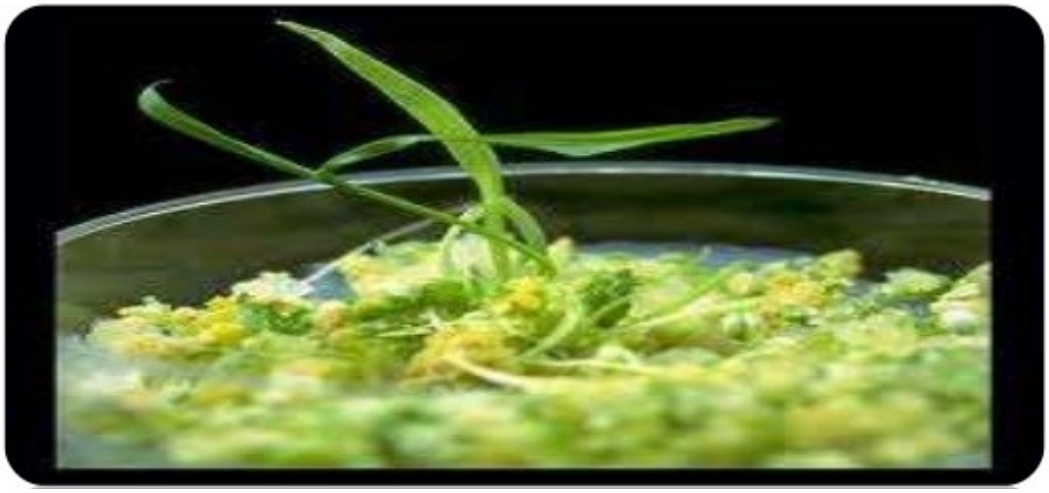
- Sterilized explant is transferred aseptically onto defined medium.
- Transfer to BOD incubator.
- Temperature (25 2°) and light is necessary for callus production.
- Callus produced with in 3-8 days.



❖ PROLIFERATION OF CULTURE

• if callus is well developed, it should cut into small pieces & transferred to another fresh medium containing hormones, which supports growth.

•The medium used for production of more amount of callus is called ***proliferation medium***.



Experiment: 8

Single cell culture **03 Hours**

❖ GROWTH PROFILE OF PLANT TISSUE CULTURE

They are classified as :

Single cell culture
Callus culture

SINGLE CELL CULTURE

The single cell culture exhibits various stages of growth.

a) **Lag phase**: Tissue starts to grow.

b) **Exponential phase**: This phase is characterized by rapid cell multiplication.

c) **Linear phase**: The growth follows a linear pattern with respect to time

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❖ GROWTH PROFILE OF PLANT TISSUE CULTURE

They are classified as :

Single cell culture
Callus culture

SINGLE CELL CULTURE

The single cell culture exhibits various stages of growth.

a) Lag phase: Tissue starts to grow.

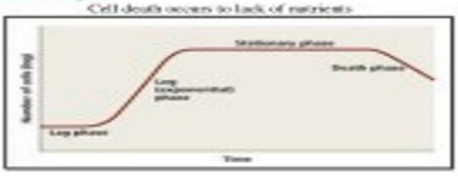
b) Exponential phase: This phase is characterized by rapid cell multiplication.

c) Linear phase: The growth follows a linear pattern with respect to time

d) Progressive declaration phase:
Aging of culture increase ——→ cell division decreases

e) Stationary phase:
No growth of cells occur
Rate of production of cells = rate of their death

f) Senescent phase:
Cell death occurs to lack of nutrients



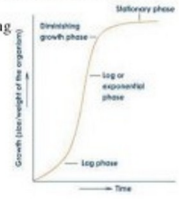
❖ IN CALLUS CULTURE

a) **Lag phase**:
In this phase cell trying to adjust the new environment condition.

b) **Exponential phase :**
By utilizing nutrients rapid multiplication occurs.

c) **Decline phase :**
Due to starvation some cells leads to decline in the callus culture.

d) **Stationary phase :**
No growth is evident, requires sub culturing

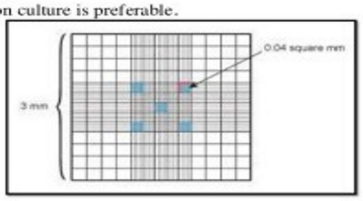


❖ GROWTH DETERMINATION

Methods used to determine are..

❖ CELL NUMBER

• By counting the cell number in haemocytometer under a microscope.
•Suspension culture is preferable.



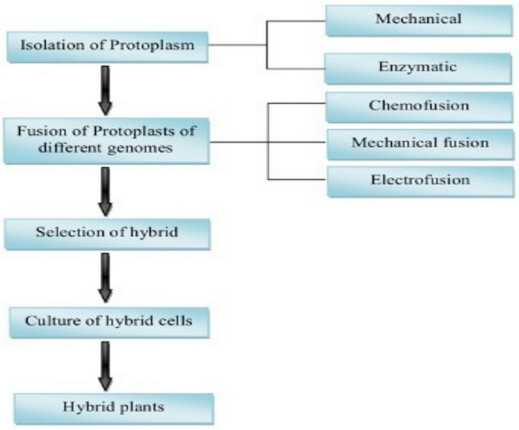
❖ PACKED CELL VOLUME

•Cell suspension is transfer to graduated centrifuge.
•Centrifuged at 2000 rpm for 5mints.
•Cell will form pellets called biomass volume, expressed by ml-1

❖ FRESH CELL WEIGHT

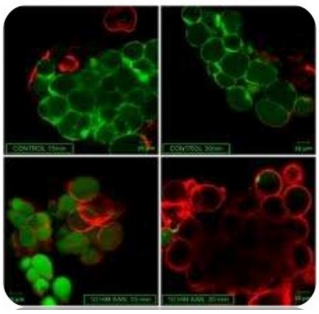
•When cells increase in number, the liquid will be turbid.
•As a result optical density altered, detected by colorimeter.

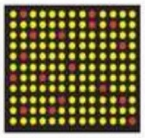
Protoplast Culture



❖ **VIABLE CELL TEST**

•The staining method such as fluorescein di-acetate is used for accessing the cell viability.
•Dead cells appear as fluorescein red.



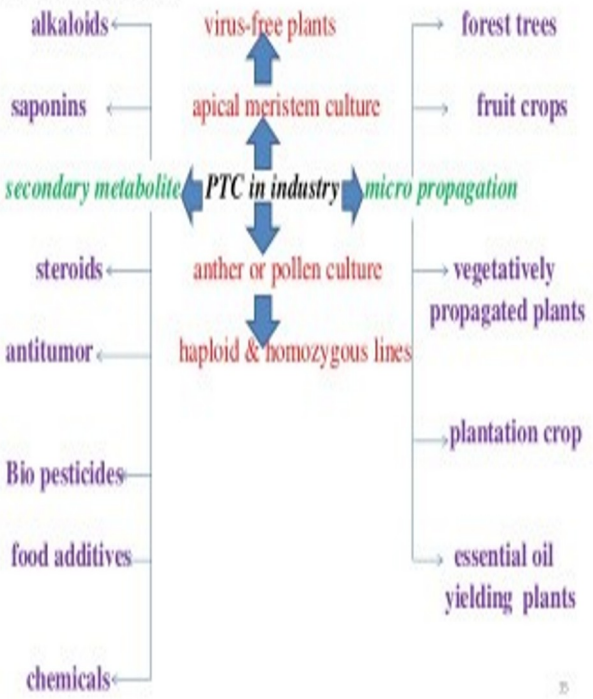


Basic research

Advantages of tissue culture :

- Reduce time to propagate plant
- Save time for crop improvement selection
- Can create potential disease-free plants
- Save space and reduce cost for land use
- Has pharmaceutical properties
- Can conserve endangered species
- Can be use to manage genetic resources
- Is not limited by seasonal change

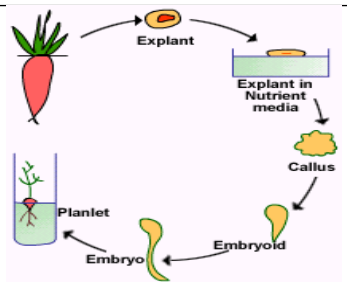
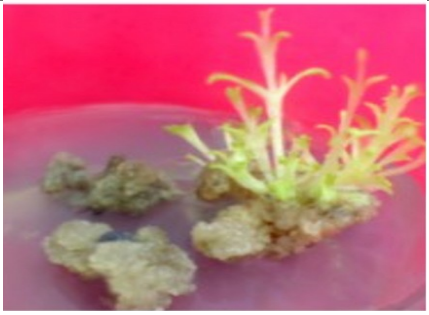
❖ *APPLICATIONS*



Experiment: 09

Propagation of horticultural, floricultural, medicinal and aromatic plants

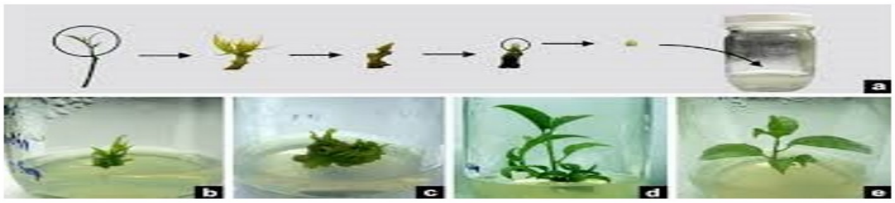
03 hours

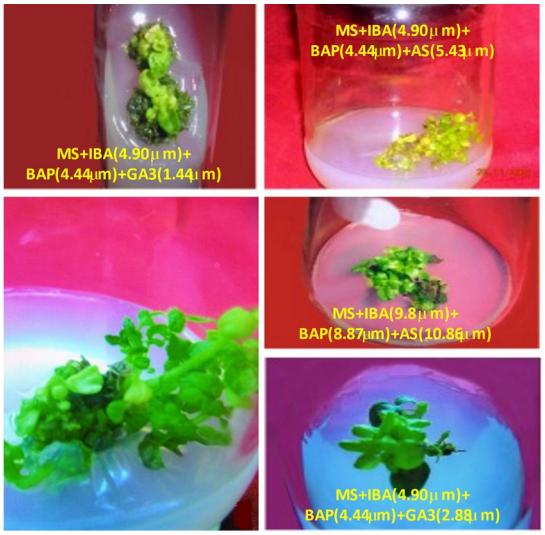
Horticulture **Carrot** **Banana**	***Daucus carota*** **Umbelliferae** **Musa species** **Musaceae**	 	 
Floriculture **Petunia**	**Petunia species** **Solanaceae**		
Medicinal **Ginger**	***Zingiber officinalis*** **Zingiberaceae**		
Aromatic **Lavender**	***Lavendula Angustifula*** **Lamiaceae**		

Experiment:10

Hardening methods and utility of plants **03 Hours**

Sweet Leaf: Stevia rebaudiana





Calamus huegelianus

Cane , Calamus, Arecaceae

Introduction:

Calamus belongs to the family Arecaceae, and it is considered as a non timber forest product. *C. heugelianus* is a high climbing, clustering cane with a diameter of about 3 to 5cm with sheath and 2.5 cm without sheath.

L2+BAP

L2 + KIN

BAP + IAA

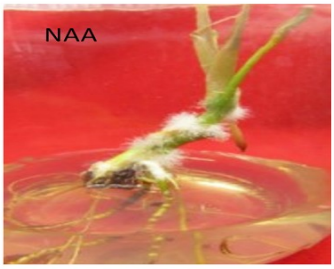
BAP + IBA

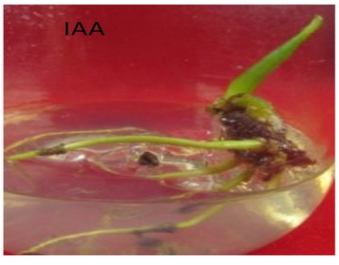
L2 + IAA

L2 + NAA

BAP + NAA

BAP + 2,4-D





Hardening

Rough Work