BOTANY

PROJECT

WORK

GUIDED by

smt. pushpalatha mam

[Lecturer in Botany]

DONE By-

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III BSC [MBC]

CERTIFICATE

Certified Bonafied Record of Practical workdone by

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of III BSc (MBC) class in the Laboratories of

2020-2021 college during the year No.

of expts done and certified. 01

Lecturer Incharge

Head of the Department

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Class : III rd year

Batch : BSc (MBC)

Regd. : 718134805141

Experiment No. :

Date :

Experiment Name :

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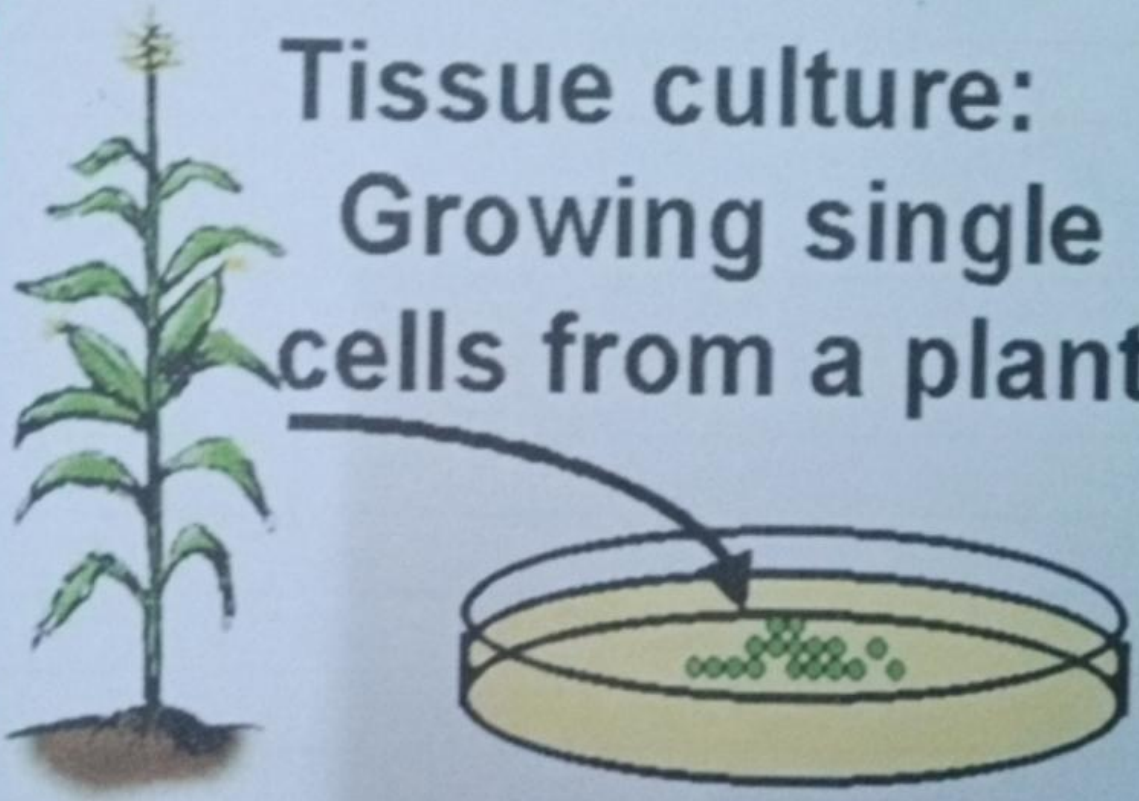
Tissue culture: Growing single cells from a plant.

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Experiment Name : TISSUE Culture

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Plant Tissue Culture:-

"It 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

Tissue Culture:- is one of the many techinques in biotechnology which can be used for the conservation of medicinal plants

Father of the tissue culture

on the basis of that 1902 address and his pioneering experiment -ation and later, Haberlandt is justifiabl recognized as the Father of plant tissue cultor 27-Jun-2007

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MILE STONE IN PLANT TISSUE CULTURE.

1902 – Haberlandt proposed the concept of invitro cell culture

1922 – Kolte and Robbins successfully culture root and shoot tips respectively

1926 – Went discovered first plant growth hormone in indole-acetic acid (IAA)

1941 – Overbeek was first to add coconut milk for cell division in Datura

1955 – Skoog and miller discovered Kinetin as cell division hormone

1957 – Skoog and miller gave concept of hormonal concept / control of organ formation

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1960 - Kanta and maheshwari developed test tube fertilization technique

1962 - murashige and skoog developed MS medium with higher salt concentration.

plant tissue culture of principle

plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency)

The controlled conditions provide the culture an environment conducive for growth and multiplication

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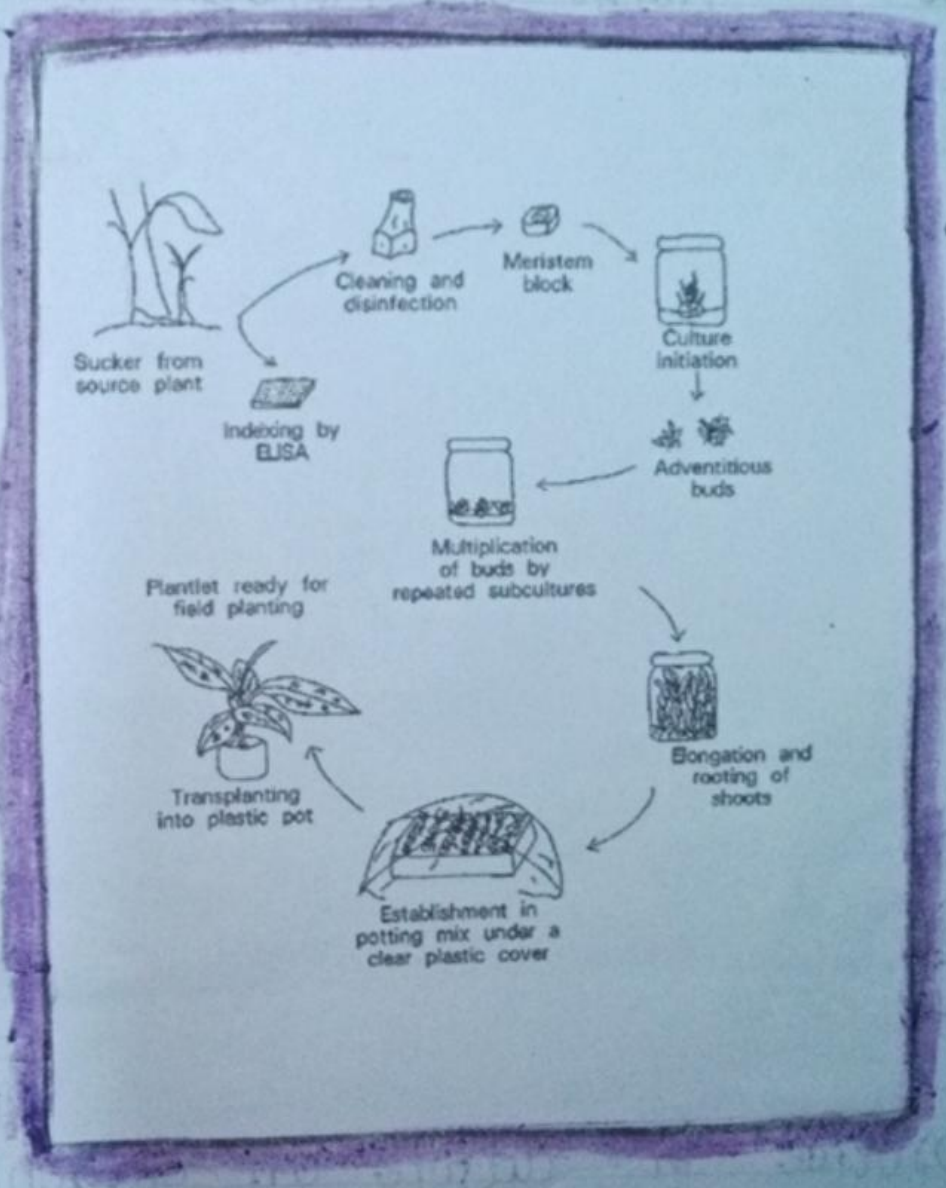
Techniques of plant tissue culture

1. micropropagation
2. preparation of donor plant
3. initiation stage
4. multiplication stage
5. Rooting stage
6. Acclimatization stage


Sucker from source plant
Cleaning and disinfection
Meristem block
Culture initiation
Indexing by ELISA
Adventitious buds
Multiplication of buds by repeated subcultures
Plantlet ready for field planting
Elongation and rooting of shoots
Transplanting into plastic pot
Establishment in potting mix under a clear plastic cover

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meant by plant tissue culture

Tissue culture involves the use of small pieces of plant tissue (explants) which are cultured in a nutrient medium under sterile conditions.

→ Using the appropriate growing conditions for each explant type plant can be induced to rapidly produce new shoots, and with the addition of suitable hormones new roots.

plant tissue culture in types

- Seed culture
- Embryo culture
- Callus culture
- Organ culture
- protoplast culture
- Anther culture

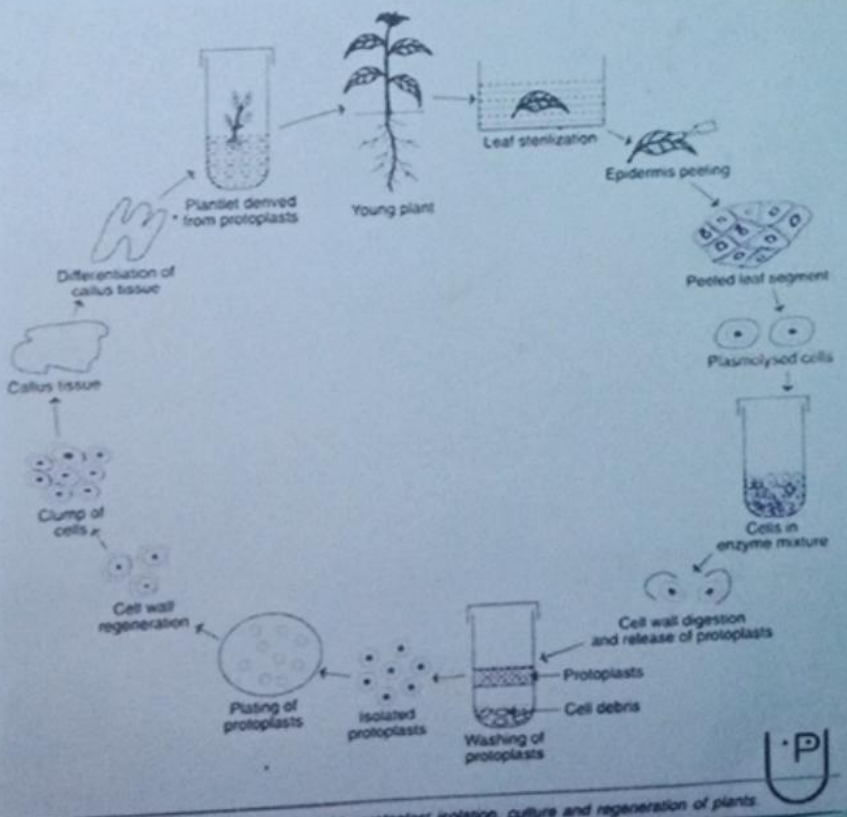
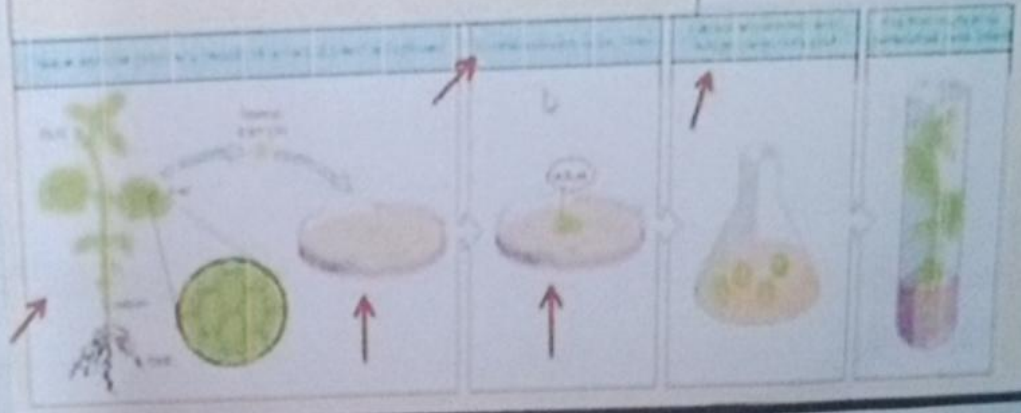


Fig. 44.2 : Major steps involved in protoplast isolation, culture and regeneration of plants

TYPES OF PLANT TISSUE CULTURE

- SEED CULTURE
- EMBRYO CULTURE
- MERISTEM CULTURE
- BUD CULTUR
- CALLUS CULTURE
- CELL SUSPENSION CULTURE
- ANTHER CULTURE/OVARY CULTURE
- PROTOPLAST CULTURE



ULTURE

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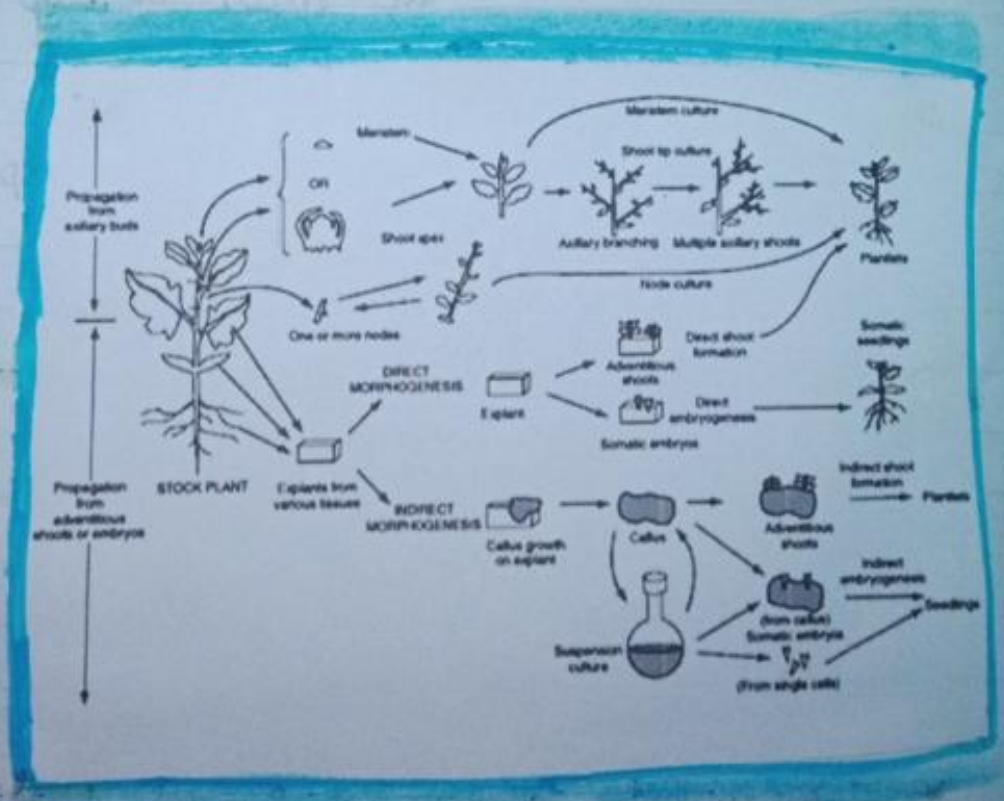
TYPES OF TISSUE CULTURE

plant tissue culture include
Two major methods

A. Type of INVITRO Growth:-
* In includes Callus and Suspension culture

B. TYPE of EXPLANT:-

* Single cell Culture
* Shoot and Root culture
* Somatic embryo culture
* meristem culture.
* Anther culture and haploid production
* protoplast culture & somatic hybridization
Embryo culture, ovule culture & ovary culture



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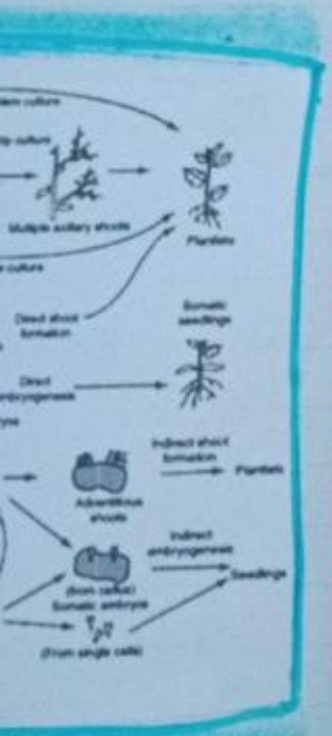

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MICROPROPAGIATION:-

→ The Regeneration of whole plant through tissue culture is called Micropropagation

→ large number of genetically identical plants can be raised from small amount of plant tissue.

→ shoot tip propagation is explored intensillely in horticulture and nurseries.

→ this method is alternative to vegetative propagation

→ It is also known as Invitro micropropaga

→ It is widely used to produce clones of a plant hence it is also known as clonal micropropagation"

Experiment No. :

Experiment Name :

M·S M

* murashi
plant
-Jes for
Culture

* Inventa
murashi
dosing
plant g

* A no
used
of t

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M·S MEDIUM :-

* murashige and Skoog medium [MSO/m
plant growth medium used in labora
-ries for the cultivation of plant cell
culture

* Invented by plant scientists Toshio
murashige and Folke K. Skoog in 1962
during murashige's search for new
plant growth regulator.

* A number behind the letters MS is
used to indicate the sucrose concentrat
of the medium.

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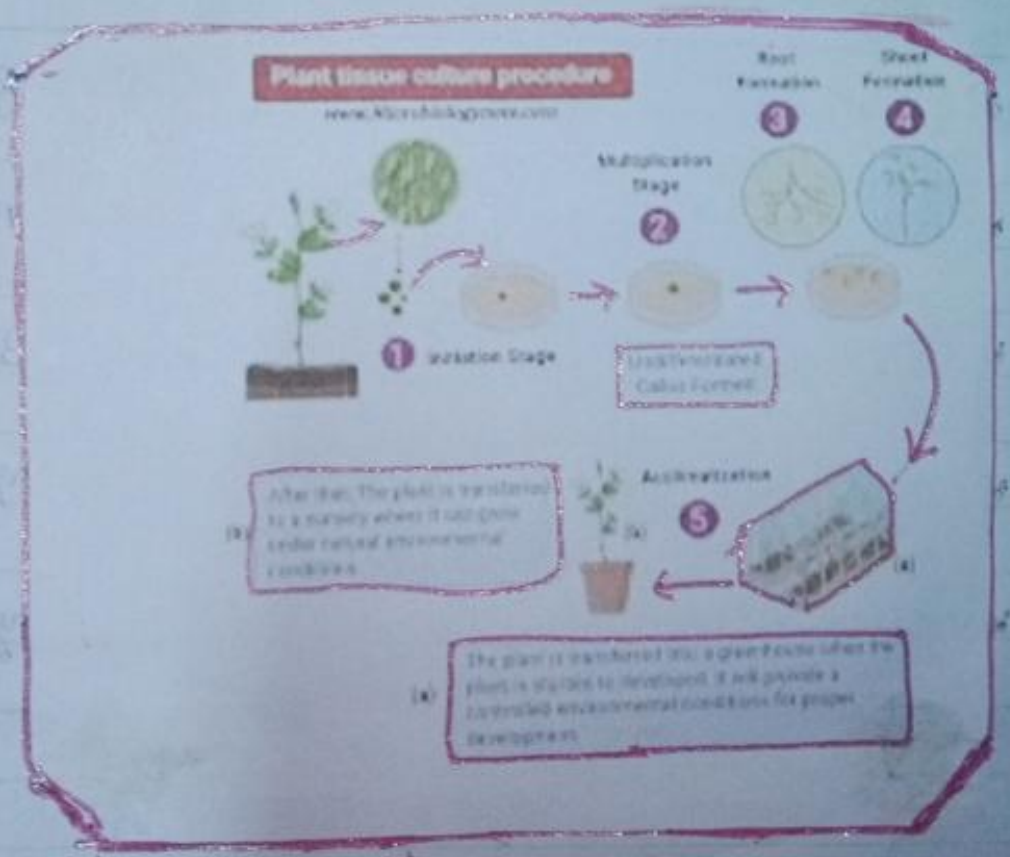
INGREDIENTS :-

MAJOR SALTS [MACRONUTRIENTS]

* Ammonium nitrate (NH_4NO_3) - 1650 mg/l
* Calcium chloride ($CaCl_2 \cdot 2H_2O$) - 440 mg/l
* magnesium sulphate ($MgSO_4 \cdot 7H_2O$) - 370 mg/l
* potassium phosphate (KH_2PO_4) - 170 mg/l
* potassium nitrate (KNO_3) - 1900 mg/l.

VITAMINS AND ORGANICS :-

* I-Inositol - 100 mg/l
* Niacin - 0.5 mg/l
* pyridoxin · HCl - 0.5 mg/l
* Glycine - 2 mg/l
* Edamine - 1 g/l.


Plant tissue culture procedure
Root Formation
Shoot Formation
3
4
Multiplication Stage
2
1
Undifferentiated Callus Formed
Acclimatization
5
(a)
(b)
(c)

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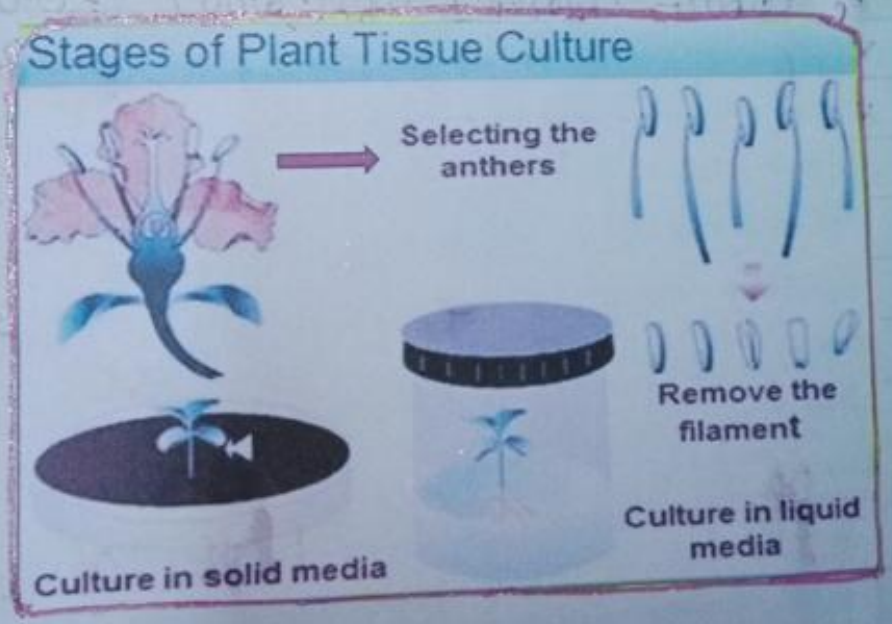
MINOR SALTS (micronutrients)

* Boric acid (H_3BO_3) — 62 mg/l
* Cobalt chloride C ($CoCl_2 \cdot 6H_2O$) — 0.025 mg/l
* Cupric sulphate ($CuSO_4 \cdot 5H_2O$) — 025 mg/l
* Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) — 27.8 mg/l
* manganese sulphate ($MnSO_4 \cdot 4H_2O$) — 22.3 mg/l
* Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) — .25 mg/l
* potassium iodide · [KI] — .83 mg/l


Stages of Plant Tissue Culture
Selecting the anthers
Remove the filament
Culture in liquid media
Culture in solid media

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STAGES OF PLANT TISSUE CULTURE

- choice of Explant
- selecting the anthers
- remove the filament
- Culture in liquid media
- Culture in solid media

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TECHNIQUE

Tissue samples from the parent plant

↓

placed in Agar growth medium containing nutrient & auxins

↓

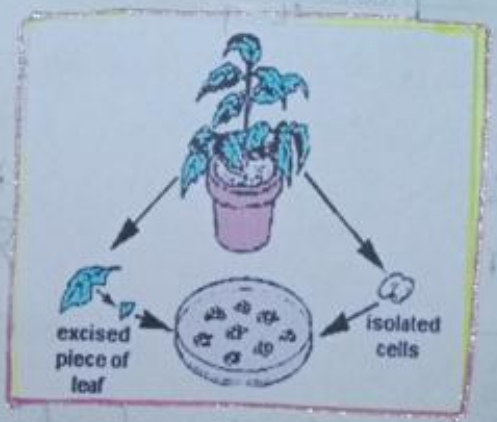
Tiny particles developed

↓

plantlet placed in compost

Experiment

Experiment



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CHOICE OF EXPLANTS

* The tissue obtained from a plant to be cultured is called an 'Explant'

* In a totipotent, explant can be collected from any part of the plant

* Explant of various organs varies rate of growth and regeneration

The choice of explant material also determines if the plantlets developed via tissue culture


A
B
C
D
E
F
G
H

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:- Techniques :-

Sterilization of Explant

↓

Explant are planted over solid / liquid media

↓

profound effect on the morphology of tissues

↓

·Cultures grow

↓

plases are typically sliced off and transferred to new media

↓

·Shoots emerge from culture

↓

may be sliced of

↓

matured one are transferred to potting soil for further growth in the green house as normal plants

REGENERATION

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REGENERATION PATHWAYS

* shoot culture:-

performed in 4 stages for mass production of plantlets through invitro vegetative multiplication

* ORGANOGENESIS

micropropagation that involves tissue regeneration of adventitious organs/ axillary buds directly (or) indirectly from the explants

* NON - ZYGOTIC EMBRYOGENESIS:-

pathways for producing somaclonal variants developing artificial seeds and synthesizing metabolites.

→ plant banana
Shade
weeks before
Sunlight

→ plants should
and banana

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Tissue cultured 🍌 in banana:-

keeping the media moist to maintain the health of the tissue - cultured seedlings -f

→ fertilize with slow-release or liquid fertilizer.

→ place banana seedling in a partially shaded area (50% shade) for 2 weeks before exposing them to full sunlight.

→ plants should be placed in a BBTV-free and banana aphid free area.

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Ans

ADVANTAGES

* production of multiples in the absence of pollinators
* Quick production Exact of mature plants
* production of Exact copies
* Reduced chances of transmitting diseases.

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~~Aa~~

ADVANTAGES

* production of multiples in the absence of pollinators
* Quick production Exact of mature plants
* production of Exact copies
* Reduced chances of transmitting diseases.

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DISADVANTAGES

* It is labour intensive and expensive process

* All plants cannot be successfully tissue cultured

It is usually because the medium of growth is not known.

DEPARTMENT OF BOTANY

PROJECT
ON
PLANT TISSUE CULTURE

Guidance By

Pushpa Latha Mam

Done By :-

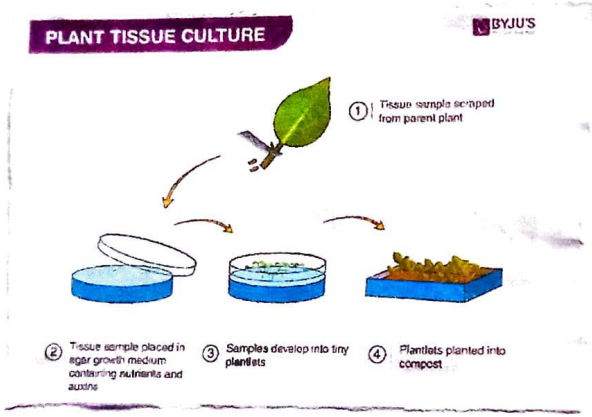
S. Ammaji

IIIrd B.Sc [MBC]

Visakha womens

Degree college

PLANT TISSUE CULTURE



Plant Tissue Culture

What is 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.

→ It is widely used to produce clones of a plant in a method known as Micropropagation

→ Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant.

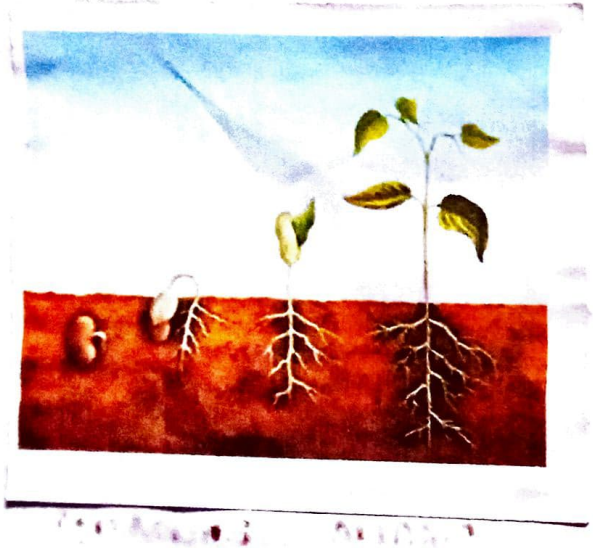
→ That is ability is called "Totipotancy"

Abstract :-

→ Plant tissue culture refers to growing & multiplication of cells, tissues & organs of plant on defined solid (&) liquid media under aseptic & controlled environment.

→ The commercial technology is primarily based on Micropropagation

→ Rapid proliferation & is achieved by system cuttings, auxillary buds & to a limited extent from "Somatic Embryo's", cell clumps & in suspension cultures & Bioreactors.



Introduction :—

→ A whole plant can be regenerated from a small tissue or plant cell in a suitable culture medium under controlled environment.

→ The plantlets so produced are called tissue - culture raised plants.

→ Tissue culture plants are characterised by disease free growth, a More fibrous, healthier root system, a bushier branching habit & mostly a High survival rate.....

→ Artificial prepared Nutrient Medium state (or) liquid is used under asceptic conditions.

HISTORY

History :—

→ plant tissue culture was proposed by the German Botanist Gottlieb Haberlandt in 1902

→ He is regarded as the father of Plant tissues culture.

→ He mainly worked on Palisade tissue & grew them on knob's salt solution with sucrose & observed the growth of cells.

→ Hanning (1904) excised matured embryo's of the cr the ueifers & successfully grow them on the mineral salt & sugar sol^n

→ The embryo culture was further developed by overback (1941)

→ This proved to be a turning point in plant tissue culture. In 1972, Carlson & other produced the first somatic between the Nicotiana glutea & Nicotiana langsdorffii by fusing their protoplast.

→ Tissue culture of first used on large scale by the orchid industry in 1950's

General procedure for plant Tissue Culture :-

a) Medium Preparation :-

The approximate medium used mostly was MS Medium

Components of MS Medium :-

Inorganic nutrients
organic nutrients
Growth harmones
Gelling agents

Preparation of stock :-

1. Micronutrient stock [100x]
→ 900 ml double - distilled water in 1 lt beaker.
→ Weigh the following salts & dissolve in water.
→ Transfer it to 1 lt solution & pipette out the 10 ml solution.

Salts	concentration 100x (mg/l)
$MnSO_4 \cdot 4H_2O$	- 2230
$ZnSO_4 \cdot 4H_2O$	- 860
H_3BO_3	- 620
KI	- 83
$Na_2MoO_4 \cdot 2H_2O$	- 25
$CuSO_4 \cdot 5H_2O$	- 2.5
$CoCl_2 \cdot 6H_2O$	- 2.5

Iron stock (20x)

→ 80 ml double - distilled water in 100 ml beaker & dissolve the components

Components	Concentration
Na_2EDTA	675 mg/100ml
$FeSO_4 \cdot 7H_2O$	556 mg/ml

→ Transfer it to 100 ml & make up the final volume.

→ Pipette 5ml of stock solution for 1 lt of MS Media.

Vitamin stock (100x) :-

→ 50 ml distilled water in 100ml beaker.

→ Weigh the vitamins given in tube.

Components	conc (100x) [mg/100ml]
Glycine	- 20
Nicotinic acid	- 5
Pyridoxine	- 15
Thiamine	- 1

Medium preparation :-

→ The appropriate mixture is mixed with distilled beaker & Sterilized.

→ Here NaOH / HCl is used to adjust the pH contents used here will depend on the plant to be cultured & no. of tissues to be cultured.

→ Agar added to mixture, heat, stirred & distilled.

→ After cooling, warm medium poured into polycarbonate tubes.

Plant Preparation :—

→ Cut the plant part into small pieces (about 1cm across). on the other hand, such parts as the African Violet Leaves can be used as whole

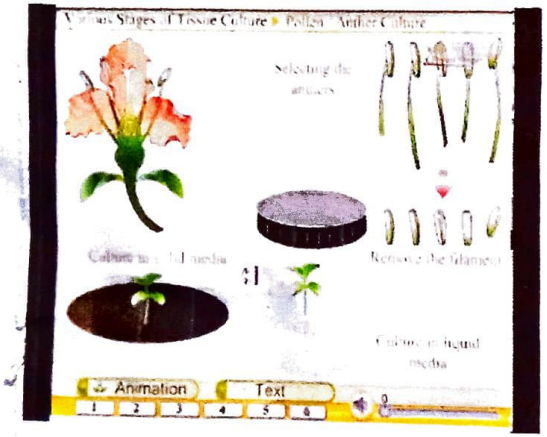
→ Using detergent & water, wash the plant part for about 20 minutes

→ Transfer the plant part in to sterilizing cloron solution and shake for a minute & leave to stock for 20 minutes

→ Using a lid, gently discard the cloron & retain the plant part in the containes & then kept the containes.

The following 4 steps (main) of Tissue culture techniques They are :-

1) Inoculation of Explant
2) Incubation of culture
3) Sub culturing
4) Transplantation of the Regenerated plant.



Stages

Stages of Tissue Culture process :-

Step - 1

Inoculation of Explant :-

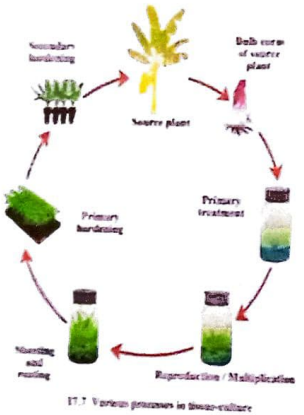
→ Successful control of contamination largely depends upon the precaution taken to prevent the entry of Microorganisms at the time of transferring the Sterilized Explants on the nutrient medium

Step - 2 :-

Incubation of culture :-

After Inoculation, the cultures are incubated in culture Room (or) in a BOD incubator at 25 ± 2°c temp. For certain plant or for some particular culture type below (or) above 25°c needed.

→ Some tissues grow well under low light condition. For regeneration Light & dark periods needed & for Regenerated plantlet well lighted condition & 16h light with 8 hours dark periods is needed.



17.7 Various processes in tissue culture

Step - 3
Sub - culturing :-

→ The growth & development of tissues cultured in vitro are generally monitored by observing the cultures at regular intervals in the culture room (or) incubator.

→ With the aid of Simple Microscope under aseptic conditions, the Explants may be required to transfer to new media.

Step - 4
Transplantation of the Regenerated plant :-

→ Plants regenerated from invitro tissue culture are transplanted to soil in pots.

→ The plants at this type develop root systems & cuticular leaf surface.

→ The process of acclimatization needs the humid chamber to make the plantlet habituated from high to normal

THE MAIN PRINCIPLE

PRINCIPLE

Principle :—

→ Plant Tissue culture relies on the fact that many plant cells have the ability to Regenerate a Whole plant called "Totipotency".

Totipotency :—

→ A Notable property that distinguishes plant from animal cells is the ability to Regenerate fertile plants.

→ This regenerating ability is not restricted only to diploid sporophytic cells.

clonal Multiplication :—

→ Shoot tip culture is being used for the clonal propagation of ferns & of ornamental & horticultural plants for several reasons, among which are the following :

1) Rapid Multiplication of hybrid cultures of proper agricultural & commercial potential.
2) Elimination of viral disease

from infected stocks.
3) vegetable propagation of species of difficult propagation.
4) clone propagation throughout the year
5) clone propagation of genetically uniform parent plants for hybrid on a commercial scale

Regeneration pathways :—

The specific differences in the regeneration potential of different organs and explants have reasons like :-

1) Differentiation in the stage of the cells in the cell cycle.
2) The availability of (or) the ability to temperature transport endogenous growth regulator.
3) The Metabolic capabilities of the cells.

→ The three common pathways of plant tissue culture regeneration are propagation from preexisting meristems are:

1) Pre-Existing Meristems
[Shoot culture / Nodal culture]
2) Organogenesis
3) Non-zygotic embryogenesis

Choice of Explant :-

→ Explants can be Taken from many different parts of a Plant including portions of shoot, leaves, stems, flowers, roots & single undifferentiated cells.

→ And also from types of mature cells provided they still contain living cytoplasm & nuclei & are able to de-differentiate and Resume cell division.

Necrosis can spoil cultured Tissues :-

→ An alternate for obtaining uncontamina-ted explants is to take explants from seedlings which are aseptically from surface-sterilized Seeds.

TYPES OF PLANT TISSUE CULTURE

Types of Plant Tissue culture :—

1) Apical Meristem culture :—

Meristem culture which uses apical meristem with 1-5 leaf primordia to prepare clones of a Plant by the ~~Vegetative~~ propagation.

This technique primarily involves the isolation of Meristem by applying a V-shape cut in the stem

2) Auxillary Bud Tissue culture :—

→ Auxillary bud culture is one of the multiple Techniques of plant invitro culture.

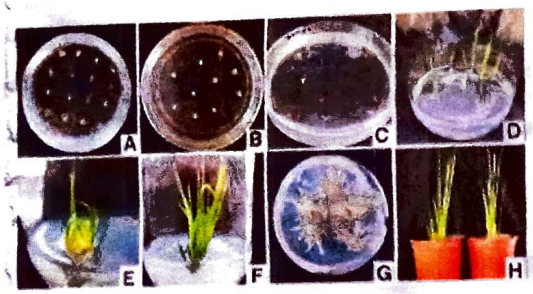
→ The auxillary bud proliferation method provides genetic stability to the plants.

3) Callus Tissue culture :—

→ A very specialised technique that involves growth of callus followed by procedures to induce organ.

→ This was proposed by R.J Gautheret.

Seed culture



4) Cell Tissue culture :-

→ Plant cell, Tissue & Organ culture is a set of techniques designed for the growth & multiplication of cells & Tissues using nutrient solutions in an asceptic & Controlled environment.

5) Seed culture :-

→ Best Method for raising the sterile seedling.

→ Seeds cultivated invitro to germinate seedlings or plants.

→ Seed culture is done to get the different kinds of explants from asceptically grown plants which help in better maintenance of Seed culture.

Embryo culture

6) Embryo culture :—

→ Sterile isolation & growth of an immature (or) mature embryo invitro with the goal of obtaining a viable plant.

→ Excision of Embryo's & culturing them in nutrient media helps in developing viable seedlings.

7) Cell Suspension Culture :—

→ The growth of individual cells that have been obtained from any kind of explant tissue (or) callus referred to as cell-suspension culture.

→ cell suspension cultures may be done in batch culture or the continuous culture system.

→ Initiated by transferring pieces of tissue explant / callus into liquid medium & then placed them on a gyratory shaker.

Protoplast culture

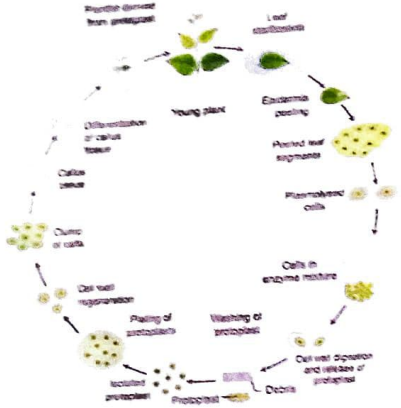


Figure 5.8: Protoplast Culture

8) Anther culture :—

→ The anthers bearing the uninucleate Microspores are selected & allowed to grow in medium to Produce callus from the Pollen-mass.

→ Then the androgenic calli is directed to produce the embryos & haploid plants are developed from these androgenic embryos.

9) Protoplast culture :—

→ culture of plant protoplasts cells devoid of cell division wall.

→ Isolated protoplasts are usually cultured in either liquid or semi solid Agar Media plates.

→ Purified & then cultured.

→ Developed mainly to genetically transformed transgenic plant protoplasts.

Applications of Plant Tissue culture

Applications of Plant Tissue culture :—

Plant Tissue culture is widely used in the plant sciences, forestry, & horticulture. Applications include :—

1) The commercial production of plant used as potting, landscape & florist subjects which uses Meristem and Shoot culture to produce large no. of identical individual
2) To ensure rare or endangered plant species.
3) Large scale growth of plant cells in liquid culture in Bioreactors for production of valuable compounds, like Plant-derived Secondary Metabolites & recombinant proteins used as Biopharmaceuticals
4) To cross distantly related species by Protoplast fusion & regeneration of the novel hybrid

5) To rapidly study the molecular basis for Physiological, biochemical & Reproductive mechanisms in plants. for Example :—
Invitro Selection for stress Tolerant plants

6) To cross pollinate distantly related species & then Tissue culture the resulting embryo which would other wise normally die — Embryo Rescue

7) As a Tissue for Transformation, followed by either short-term testing of genetic-constructs or regeneration of Transgenic plants.

8) Production of Identical sterile hybrid species can be obtained & large scale Production of artificial seeds through Somatic Embryogenesis.

9) Cryopreservation of cells and tissue - removal of these tissues & regeneration of plant through tissue culture technique are really effective in Conservation Biotechnology.

Cryopreservation involves storage of cells at a very low temperature using Liquid Nitrogen.

Cryopreservation broadly means the storage of germplasm at very low Temperatures :—

(i) over Solid Carbondioxide broadly means at -79°C

(ii) Low temp deep freezers at -80°C

(iii) In vapour phase Nitrogen at -150°C

(iv) In Liquid Nitrogen at -196°C

CONCLUSION

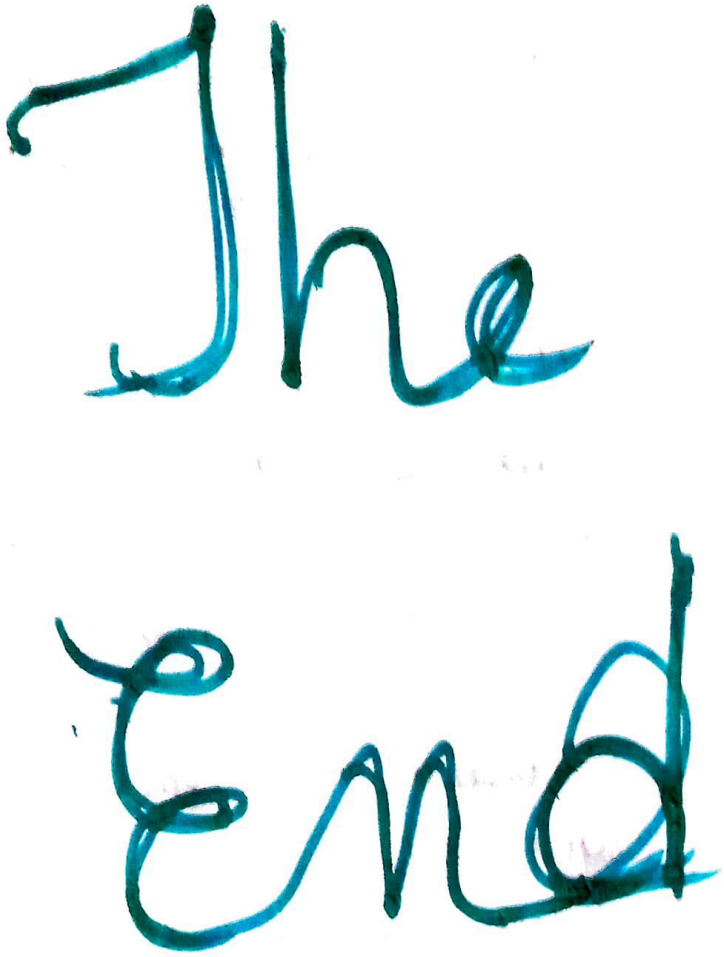
Conclusion :—

→ Both plant & Animal Tissue can be used for Tissue culture purposes animal culture it will be used for many diagnosis purposes

→ on the other hand, plant Tissue culture may be used for cloning purposes, Genetic modification of a given plant.

→ Tissue culture is therefore of great significance in biological studies due to its wide range applications.

→ contamination should be observe carefully & Tissue culture is not an experiment for everyone.


The
End