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UNIT-IV

Plant Tissue Culture

OR

In vitro culture

OR

Sterile culture or Aseptic

Defination

Plant tissue culture is a method or technique who isolated parts (protoplasm cells, tissue & organ) and grow them on Controlled medium under aseptic condition in a controlled space so that it tissue can grow and developed into complete plant

OR

Plant tissue culture is a collection of technique used to maintain or growth of plant cell tissue or organ on a nutrient medium of known composition under sterile condition es

OR

Tissue is invitro cultivation of plant cell or disease tissue under aseptic condition and controlled environment condition in liquid or semisolid well defined culture medium for the production of 1° & 2° metabolites OR Regenerate a new plant.

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Advantages-

→ Availability of raw material
→ Quickly produces mature plant
→ Reproduction of disease free plants
→ Crop improvement by Somatic Hybridization or production of hibrid.
→ The Regeneration of whole plants from plant cell that have been modified
→ Immobilization of cells
→ Easy purification of the compounds
→ Tissue culture can be used for proving the biosynthetic pathway of secondary metabolites by using labelled precursor in the culture medium
→ Patent rights

Explant → Parts of plant

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Historical Development of Plant Tissue Culture

Year	Name/Author	Results
1902	Haberlandt (Father of Plant Tissue culture)	Ist cultivation experiment with isolated plant cell, Cell growth but no cell division obtained
1904	Hanning	Established of the embro. culture of the first time
1922	Kotte & Rabbiris	In vitro cultivation of Root tips, No Permant culture obtained
1934	White	Succesful culture of Tomato roots in a long time he replaced the yeast extract in a medium contain inorganic salt and sucrose with 3 vitemine

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1934	Gautheret	fist perament callus culture using Vitamine B and Oxgin
1946	Ball	Micro-propogration, Develoment by stem tips and branches
1941	Van overbeek	The use of coconut water for the young embryo culture in datura plant
1954	Muiretal	first suspension culture of Singal cell or callus
1955	Mathur & Kala	fist reports of Secondary metabolites production in liquid medium
1957	Skoog & Miller	Proposed the concept of hormonal control of orginal production

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1958	Maheshwari & Rangaswamy	Regeneration of somatic embryo of citrus ovules
1960	Bergmann	Cell colony (exact copy) obtained from single culture cell plated in an agar medium
1960	Reinert steward	growth and development of carrot suspension culture, enzymatic degradation of cell wall via somatic embryogenesis
1967	Guha & Maheshwari	The discovery first Hypoid plant by immature pollination in Datura plant
1970	Power etal	Demostrated intra and inter specific fusion between the protoplast of different plant roots
1978	Melchar's etal	first enter generic somatic hybridization between tomato and Potato plant

Basic requirement for a tissue culture of Laboratory -

- For the successful achivement of ay type of tissue culture laboratory should have the following basic facilities-

(1). Equipment & Apparatus -

- Main different kinds of vessels may be used for growing culture.
For the successful growth of any tissue it requires wide mouth conical flask (Erlen-meyer flask). In addition to the culture vessels, Glasswares such as graduated pipettes, measuring Cylinder, Beake, funnel & petridishs are all so required for making preparation.

- A spirit burner or Gas micro burner for flame sterilization are also used.
- Autoclave for sterilizing culture media, Hot air oven for sterilizing glaswares are also used.
- Scissors & forceps are also used for the preparation of explant.
- An equivation chamber, or laminar air flow with U.V. fitted light are also used for aseptic transfers of explant into culture medium.

② Washing & storage facilities -
A good PTC laboratory should have fresh water supply & disposal of the waste water.

③. Media preparation Room -
Media preparation room should have sufficient space to accomodate chemical, labwares, chemical vessels & equipment required for weighing & mixing, hot plate, PH meter, water bath, micro wave oven, autoclave & domestic pressure cooker.

④. Aseptic Chamber -
- For the transfere of culture into sterilized media, contaminent environment is mainitory.
- Know a days mordern laboratory have laminar air flow cabinate having vertical or horizontal air flow.
- The air coming out of the fine filter (0.3 μm ~~HEPA~~ filter) is ultra clean & having adiquate velocity to prevent micro-contamination of the working area.

⑤ Incubation room -
Air conditionor or air room theater required to the maintain temp of

25±c.

- Light is adjusted in the term of photo-period duration.

Important

Culture Media – Nutrient Media –

Nutrition requirement for optimal growth of a tissue culture may vary with the species. Even tissue from different parts of a plant may have different requirement for the favorable growth of plant cell.

(a) Media Composition –

To maintain the vital function of a culture the basic medium contains inorganic nutrients, organic components, growth regulators & utilizible carbon sources & a gelling agents.

(b). Inorganic Nutrient –

Mineral elements play very important role in the growth of a plant.

ex - Mg^{2+}, is a part of chlorophyll.

Ca^{2+}, is a component of cell wall.

N, is an important element of amino acid.

Iron, Zinc, Molebednum are parts of certain enzyme.

Essentially about 15 elements are required for the favorable growth of a plant tissue in a culture media.

- Elements required in the life of a plant greater than 0.5 m.mol/lit or refered as micro-nutrient ~~is~~ those less than 0.5 m.mol/lit as micro nutrient.

- The macro-nutrient elements includes 6-major elements, Nitrogen, P, Ca, Mg, S, K

- The micro-nutrients are required in small quantities but essential for proper growth of plant cell or tissue these Nutrient are B, Co, Fe, Mn, Zn & Mo.

(c) Organic Nutrients -

Most cultured plant cells for capable of synthesizing essential vitamins but not in sufficient amount.

- To achive best growth it requires essential vitamins such as -
  Vit - B_1 - (Theamine).
  Vit - B_6 - (Pyrodoxine).
  Vit - B_5 - (Pentothenate).

Carbon sources -

It is essential to suppliment the tissue culture media with utilizable source of Carbon.

- The most commonly used carbon source is sucrose at concn 2-5%.
  Glucose & fructose also known to be used for growth of some tissues.
- In general dicotiledenous roots grow better with sucrose where as monocots do best with glucose.

(d). Growth Hormones -

In addition to the nutrients it is generally necessary to add one or more growth hormone.

Examples -

- Auxins
  - Natural - IAA
  - Synthetic - IBA, NAA, 2,4-D.
- Cytokinin
  - Natural - Zeatin
  - Synthetic - Kinetin,
- Gibrellins (GA)

- Auxin are use elongation of stem and internode and apical dominance and rooting etc.
- In tissue culture auxin induces all division and stimulate route formation
  - IAA - Indole-3-acetic acid.
  - IBA - Indole-3-butyric acid.
  - NAA - Napthalene acetic acid.
  - 2,4-D - 2,4-Dichloro phenoxy acetic acid.

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Cytokinin -

It is a phytohormone naturally in plant which are to start cell division.

Cytokinin { Natural - Zeatin.
Synthetic - kinetin.

Gibrellins - (GA)

- GA are GA-3 (Gibrellic acetic acid-3) is the most common form of GA.
- It is used for parthinocarpy and apical dominant in plant.

Internodal gation of genitically plant is the most important property of GA.

- ## Solidifying Agents

Due to improved O_2 supply & support to the culture growth solid media are prefered for proper growth of cell. common solidifying agents are - Agar, Gelatin, alginate, Carriageenan, are used in culture media.

Types of plant tissue Culture -

1. Root tip culture.
2. Leaves tip culture.
3. Shoot tip culture.

4. Anther & pollen grain culture.
5. Ovary & Embryo culture.
6. Protoplast culture.
7. Callus culture.
8. Suspension culture.

1. Root Tip culture -
Tip of the lateral root of sterilized excised & transfer to fresh medium. The lateral root continue to grow ~~aerobites~~ provides several roots after few days it becomes a mature plants.

2. Leaves Culture -
Leaves may be detached / cut from shoots, surface sterilized and placed on a semi-solid medium where they will remain in a healthy condition for a long period.

3. Shoot tip culture -
The excised shoot tips of many plant species can be cultured on a simple nutrient medium under sterile condition.

4. Anther and pollen culture -
Young flower buds are removed from the plants and surface sterilize. The anther are carefully excised and transferred to an appropriate nutrient medium.

⑤ Ovule and Embryo culture -

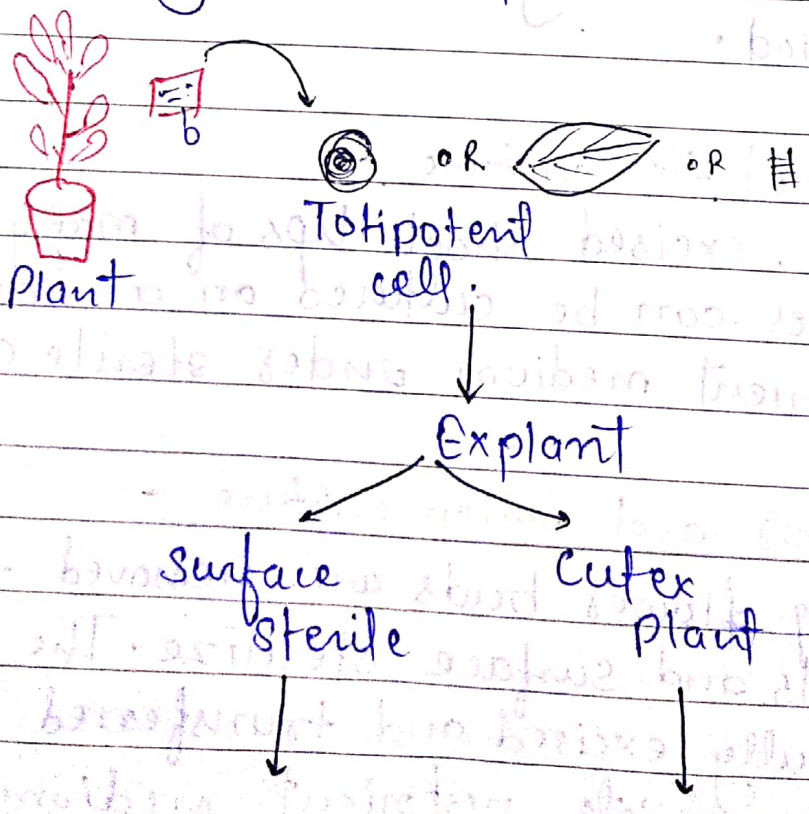
- Embryo is dissected from the ovule and put into the culture medium.
- The seeds are treated with 70% alcohol for about 2 min. washed with sterile distilled water followed by surface sterilizing agent for specific period of time.

Exp -

Systemic procedure of Plant tissue culture/ Technique :-

Explant - Any part of the plant body which are used to establish a culture.

- Explant should be disease free, young, healthy and totipotent.



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Sterilizing Agent
eg- Bromine water.
- Benzal konium chloride.
- H_2O_2
- $AgNO_3$.
- 70% Alcohol.

Cutex plant → Surface Sterilization

Sterilized explant.

Transfer

Callus ← Inoculation of explant into sterilized culture media.

into BOD for at least one week → Incubation (Added phyto-hormone if required before incubation.)

Organogenesis.

Rooted plant lets

Hardening

(Acclimatization) grow in soil

Transfer in Polyhouse or green-house.

New plant.

Composition of MS Media - (Murashige Skoog Media)

Macro element

Ingredients	Quantity (mg / lit)
NH_4NO_3	1650
KNO_3	1900
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	170

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Miro Element

Ingredient	Quantity (mg/lit)
KI	0.83
H_2BO_3	6.20
$MnSO_4 \cdot 4H_2O$	22.30
$NaMoO_4 \cdot 2H_2O$	0.25
$ZnSO_4 \cdot 4H_2O$	8.60
$CaSO_4 \cdot 5H_2O$	0.025
$Na_2EDTA \cdot 2H_2O$	37.30
$FeSO_4 \cdot 7H_2O$	27.80

Amino acid & vitamine

Ingredient	Quantity
Ionositol	100
Glycine	2
Thiamine	0.1
Pyridoxin	0.1
Nicotinic Acid	0.5

Phytohormones

Ingredients	Quantity
Kinetin	0.4-10
IAA	0.30

Carbon Source

Ingredients	Quantity
Source	30
Agar	

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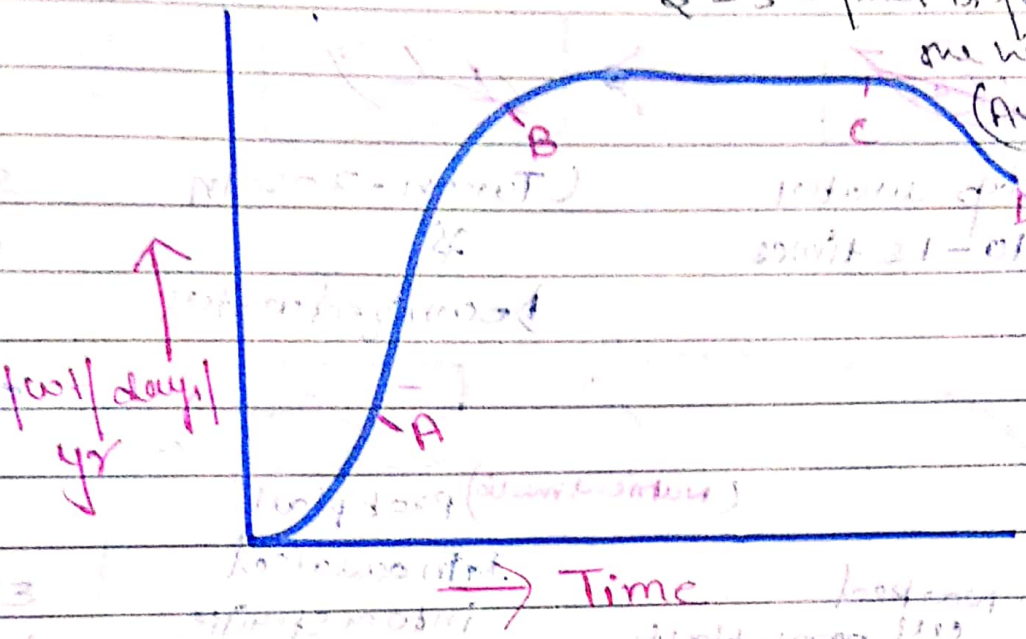
Growth Profile Measurement

S - shaped is formed with the help of (Auxanometer)



O - A = Lag Phase
A - B = Log Phase / exponential
B - C = steady state phase.
C - D = Decline phase OR senescence

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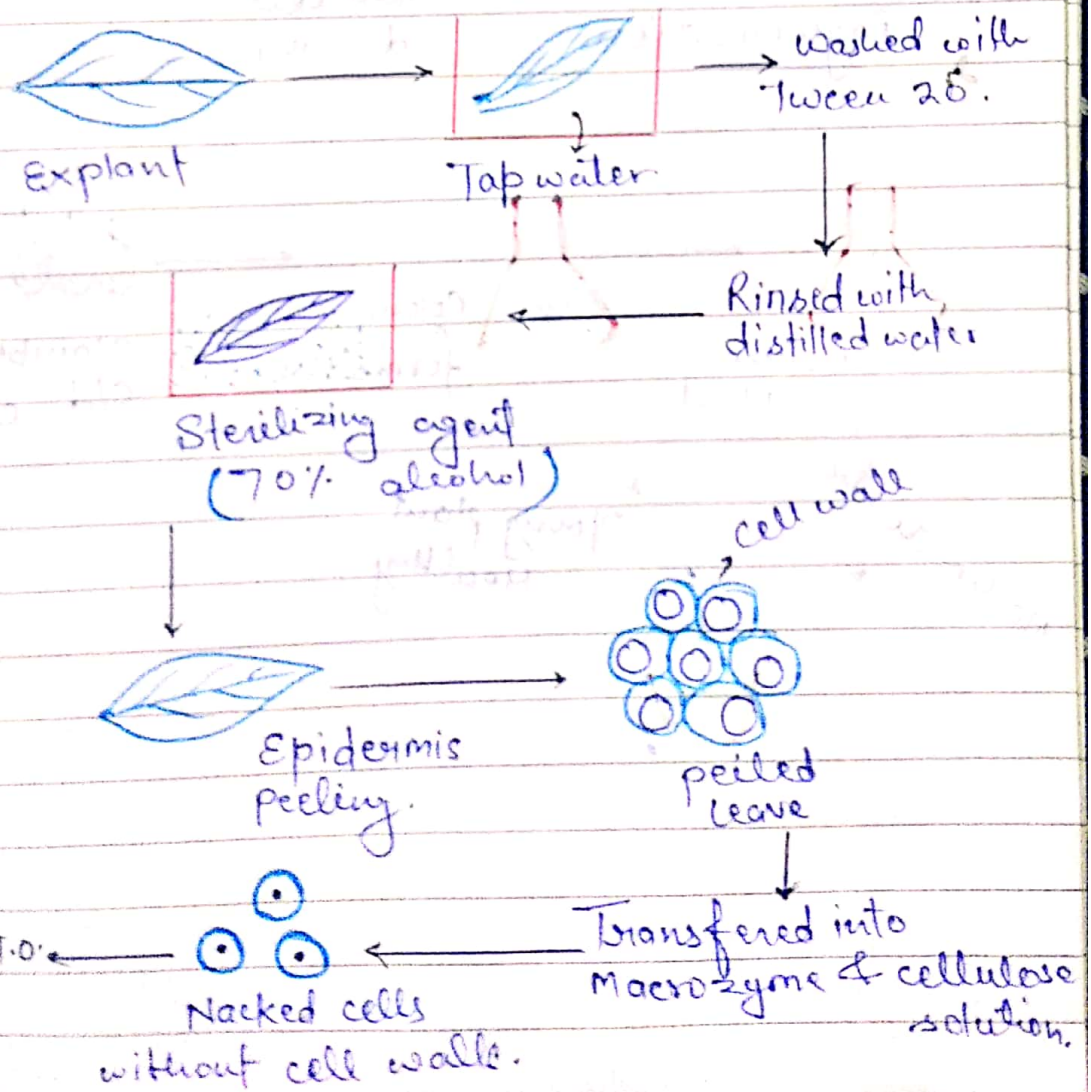
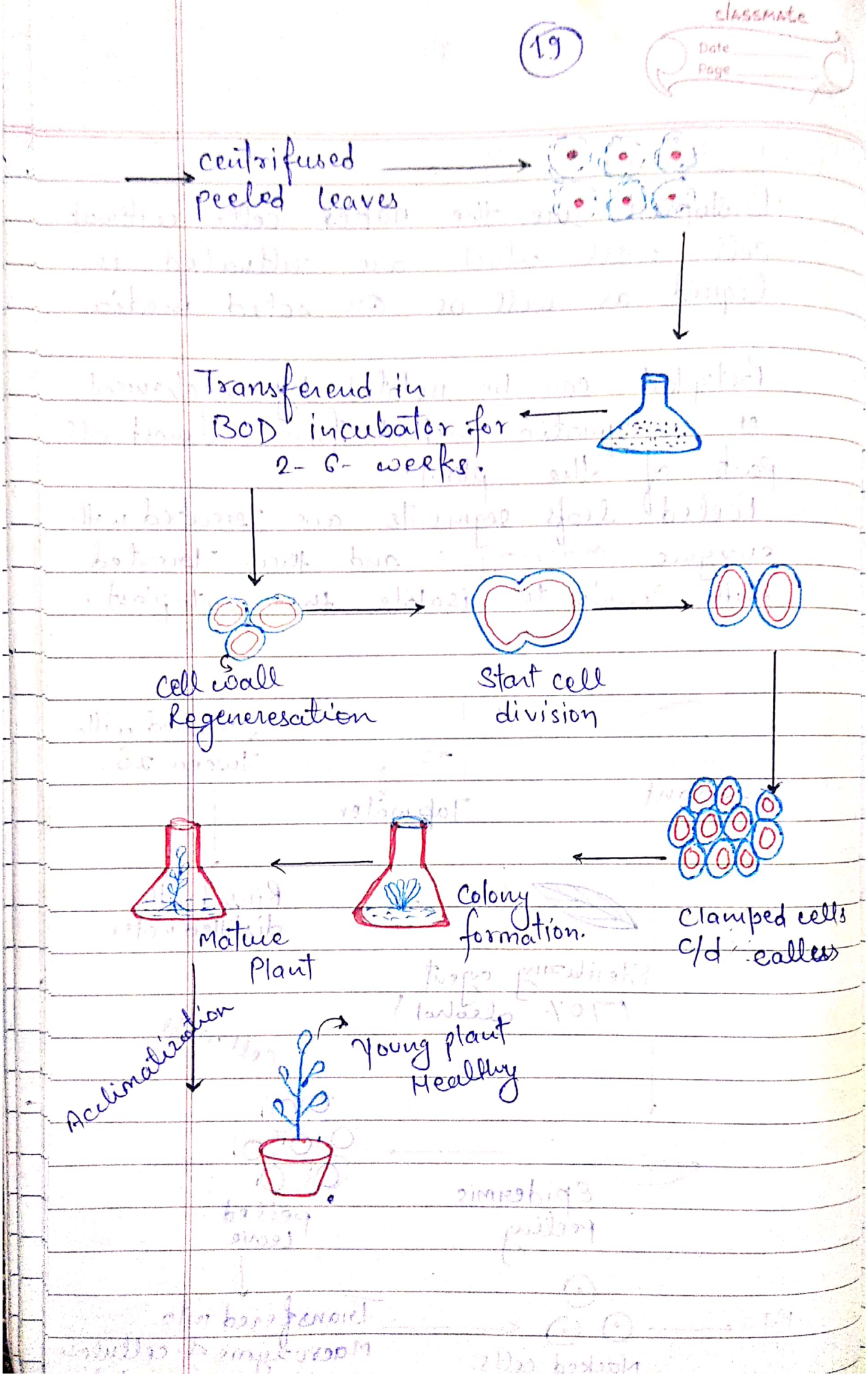
⑥. Protoplast Culture -

- Protoplast are the naked cells without cell wall which are cultivated in liquid as well as on solid media.

- Protoplast can be isolated by mechanical or enzymatic method from almost all parts of the plant.
Peeled leafs segments are treated with enzyme Macrozyme and then treated with Cellulose to isolate the protoplast.





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① Suspension Culture -

- Suspension culture contains a uniform suspension of separates cells in liquid medium. For the preparation of suspension culture, calles fregments is transfered to liquid medium (without Agar), which is kestitated continously to keep the cells separate.
- The culture maintain medium thomogenious by Azitator speed (50-150rpm).
- After sufficient no. of cells are produced and ~~scale~~ - culturing can be done in freshly prepared ~~directly~~ solid medium.

↓

Callus fregments

↓

Transfered in liquid medium without Agar

↓

Azitated continuosly in BOD incubator to keep the cells separate (speed = 50 - 150 rpm)

↓

After sufficient no. of cells are produce to form a thomogenous mixture of cells.

↓

Start Sub-culturing / in freshly prepared solid medium i.e. The single cell is inoculated in fresh medium.

↓

Multiply.

↓

Growth of cells.

↓

Organogenous.

↓

whole plant.

⑧. Callus Culture -

Callus is an amorphous aggrigate of loosely arranged parenchymatous cells which proliferate from mother cells.

Cultivation of callus usually on solidified neutrient medium under sterile condition is known as Callus culture.

1. Application of plant tissue culture -

Plant tissue culture technique has been used in almost all the field of bioscience. Major Megaa application are the listed below.

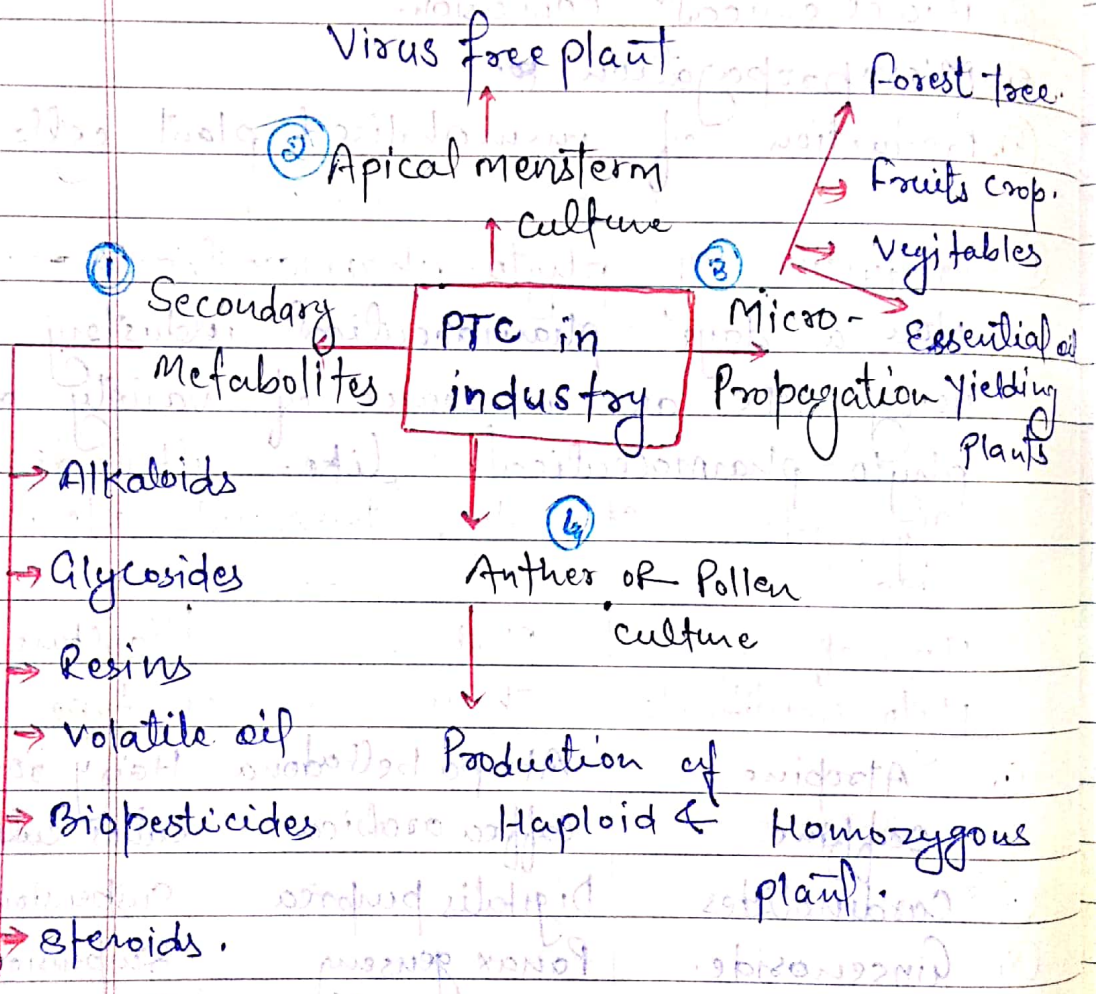
1. Production of phyto-pharmaceuticals.
2. Bio chemical conversion.
3. Micropropagation
4. Production of immobilised plant cells.

(1). Production of phyto-pharmaceuticals -

Now a days pharmaceutical industry is using PTC as a source of variety of phyto-pharmaceuticals like- alkaloids, Glycosides, steroids, terpenoids etc.

Name of Phyto-constituents	Plant Species	culture type
(1) Atropine	Atropa belladona	Hairy root.
(2) Caffine	Coffea arabica	callus culture.
(3) Cardinolides	Digitalis purprea	Suspension " .
(4) Ginsenoside.	Panax geuseng	Suspension " .
(5) Morphine	Papaver Somniferum	Suspension " .
(6) Nicotine	Nicotiana tobacom	Suspension " .
(7) Quinine & (8) Quinoline	Cinchona officinalis	callus & Suspension " .
(9) Resespine	Rauwolfia serpentina	Suspension " .

① Industrial production of 2° Metabolites -
Majority of noble medicinal agent are produced by plant tissue culture technique from various type of medicinal plant.



② Biochemical conversions - or Biotransformations -
The conversion of small part of a chemical molecule by means of biological System is termed as Biotransformation.

Eg- Podophyllum peltatum in semi continuous culture can produce anticancer drug by biotransformation of Synthetic Dibenzyl butanolide to lignan suitable for conversion to Etoposide

③. Micropropagation - OR
Clonal propagation
Clonal propagation is the technique for rapid production of a large no. of identical clones within a short duration in a limited spaces

④. Immobilization of plant cells -
Immobilization of plant cells or enzymes has increase the utilization of plant cell biotechnology for the production of pharmaceuticals.
The plant cells can we immobilized by using alginates. Polyacralamide, Polyurethiane.
Such methodology may be implimented in case of enzymes which are used as solid support for plant cells.

5 Other application of plant tissue culture

1) Production of genetically variable plant
2) Virus eradication (apical meristem are generally virus free or very low conc. virus.
3) The apical meristem is the only way to obtained a colon of various disease free plant
4) Study of crown gall by plant tissue culture
5) Importance of tissue culture in biotechnology
6) production of transgenic plant
7) Production genetically variable plants

Factor Influence In PTC

→ Size of explant

→ PTC is generally depended upon the size of explant the large explant is consist parenchyma have greater regenerative than small explant

→ Source of explant

The source of explant counted in important in determining the potential of organogenesis explant may taken any part of plant root stem and leaves meristematic tissue compare culture condition

→ Age of explant

The physiological age of explant is another factor which often plant and important role in organogenesis ~~And~~ young tissue are more suitable than mature tissue

→ Seasonal variation

→ Quality and Intensity of light

The quality and intensity of incidance light one culture may play ~~and~~ effective role in the promotion of organogenesis

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Normally plant tissue culture take place in illumination about 2000 to 3000 lux for photo period of 16 hr day and 8 hr night.

→ Temperature

Most tissue culture are grown successfully and temperature around 25°C in a no. of bulbus ~~parts~~ species, the optimum temp may ~~such~~ lower than 15°C

→ Phytohormones

When the conc. of cytokein are high relative to ~~skin~~ auxin, shoot are induced. When the conc. of cytokein are low relative to auxin roots are induced

→ Culture Medium

The essential compound of plant tissue culture is the macronut along with vitamins amino acid carbohydrate are also required growth and development of plant cell with high conc. agar the nutrient medium become ~~brown~~ which does not allow the diffusion of the growing tissue, so the conc. of agar also play a vital role in organogenesis.

→ **PH of the Medium.**

The PH of culture medium is generally adjusted b/w 5.6 to 5.7 before sterilization. The ph is another factor that may have a determining role in organogenesis

→ **Age of culture medium**

The young and newly prepared culture medium is generally used for the favourable growth of cell or tissue