DEPTH OF BIOLOGY

STUDY MATERIAL

YT-DEPTH OF BIOLOGY

INSTA- DEPTH OF BIOLOGY

TELE- DEPTH OF BIOLOGY

Unit III - Pharmacognosy and Phytochemistry I

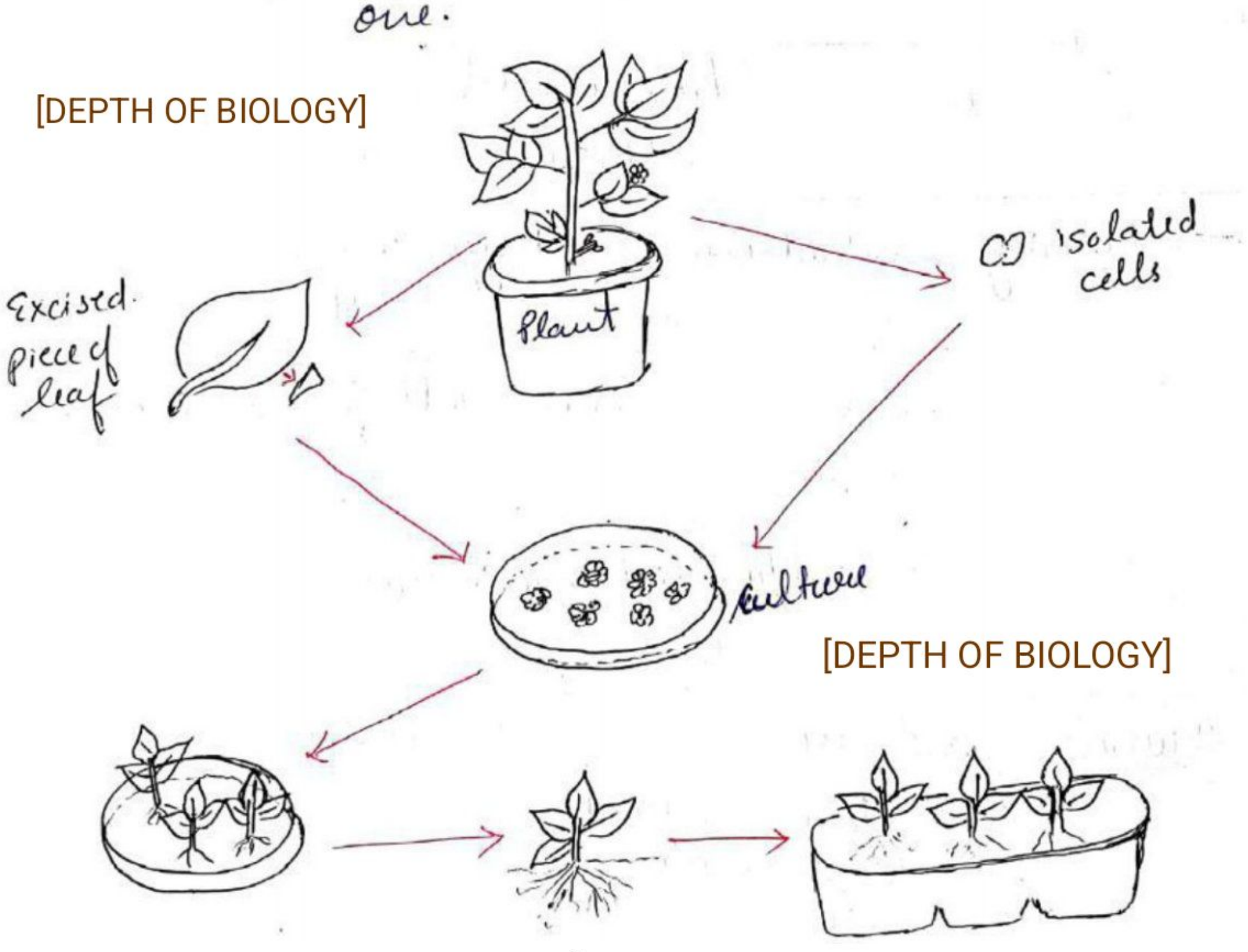
Plant Tissue Culture

In this, Plant tissues, organs or part of plants, plant cells etc ↓ used to clone the plants.

↓

to make exact copy of plant

* in vitro method. i.e. it takes place in labs - in tubes and not in natural living surroundings but in created artificial one.



I. Historical development of Plant Tissue Culture:-

* Plant tissue culture was firstly performed by Gottlieb Haberlandt → Autrian Botanist.

* He found the plant cells are Totipotent

This lead to establishment of plant tissue culture

* He is father of plant tissue culture and experimented on Mesophyll tissue in 1902

It was proposed back in 1902.

Some Important Milestones (who - what - when)

- Haberlandt firstly proposed concept of plant tissue culture in 1902
- Hanning established embryo culture for the first time in 1904
- Kusters observed fusion cell for the first time in 1909
- Robins, Kotte cultivated root tips in vitro in 1922
- White did permanent root culture for the first time in 1934. • Done in tomato plant.

- Gautheret - peerformed first callus culture using auxins and vitamin B in 1934
- observed secondary metabolity in Plant tissue culture in 1942

- Muir - developed single cell culture in 1953

- Mothes and Kala - firstly reported secondary metabolites peroduction in liquid medium in 1953.

- Vasil and Hildebrandt - regenerated plant from single cell in 1965.

- Noguchi - cultivated tobacco cells in 1977

- Simon was successful in regeneration of bulky callus, buds and roots in 1908.

How is plant tissue culture peerformed?

Based on Haberlandt observation and pointing.

Totipotency of plant is the backbone of plant tissue culture.

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tendency of plant to get fully generated by any

single cell of the same previous old plant.

Plant tissue culture — collection of techniques used to maintain and grow plant cells, tissue and organs under artificial environment of lab. which mimic the natural environment.

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Methods of Plant Tissue Culture.

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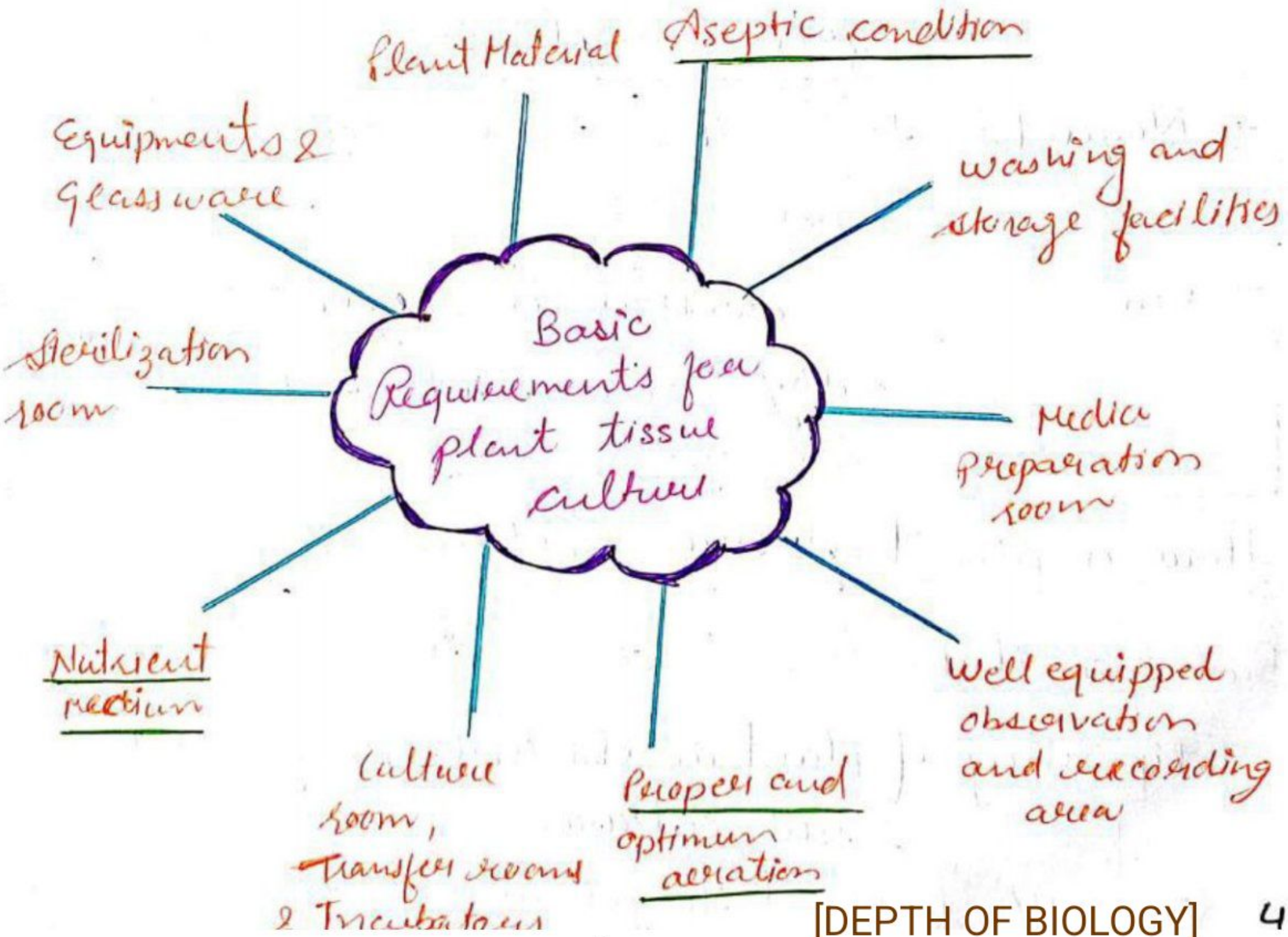
- Type of in vitro growth
  - callus and suspension cultures
- Type of explant, single cell culture, somatic embryo culture etc.



* Underlined were the important aspects in vitro culture.

1. Nutrient Medium : depending upon type of plant tissue or cell used for culture.

It usually passess –

- Inorganic salts — [macro elements, micro elements]
- Carbon source – (sucrose)
- Growth regulatores - auxins
- Specified pH – usually 5.7
- Amino acids - Arginine
- Vitamins – Eg: Pyridoxine, nicotinic acid, thiamine

2. Aseptic conditions : since, Nutrient medium contain sucrose (sugar) as carbon source → facilitates microbial growth

↓

These compete with the desired tissue and kill them.

So, aseptic conditions are necessary for culture

Sterilization is one such measure.

3. Aeration of the tissues : Proper ventilation or aeration is very important for the cultured tissue growth.

It is achieved by occasional stirring the medium by stirring manually or by automatic shaker.

4. Plant Material : It should be disease free.
- should not be old.

5. Transfer room : It is provided with the laminae flow hood where most of the work of culture initiation and subsequent sub culturing is performed. Culture re-plantation, transfer or re-initiation in a clean media, harvesting of 'ripe' cultures is also performe in this area.

6. Culture room or incubators : cultures are incubated on shelves or in incubators under specific conditions of temperature, humidity, air circulation and light as required by the plant to be cultured.
- These rooms should have both light and temperature controlled devices for whole day.
- Generally high output, cool, white fluorescent light gets preferred for the high photoperiod plants.
- These rooms should have relative humidity upto 70-75% and uniform forced air circulation.

7. Well maintained observation and recording room : Growth and maintenance of the culture is being observed and recorded to track it. All these observat-ion is done in the controlled non harming conditions to the plant. Aseptic conditions should be maintained. For microscopic examinations isolated dust free space should be kept.

8. Washing and Storage facilities : Fresh water supply and waste water management has to be done separately.

- All the requirements of the cultures like de-ionized, distilled, double distilled water has to be fullfilled.
- Proper washbasin and working area has to be made and it should be spacious enough to equip the requirement
- Dust proof covered spaces has to be available for the storage of the dried glasswares.

9. Media Preparation room: This room should be spacious enough to accomodate lab ware, culture vessels and other requirements like chemicals etc. It should have all the equipments like freezer, refrigerator and storage units for media and stock solutions earlier prepared.

10. Equipments and glassware:

All the equipments and glassware should be of high standard.

- Incubating chambers — should have UV light for aseptic transfer.
- Incubator — temp. - 36°C ± 5°C
- Autoclave - to sterilize the glassware, media etc.
- Refrigerator and freezers for storage
- Centrifuge, Water purifier, hot air oven, pH meter, burner, High power microscope, shakers, balance, shelves to store, scissors, scalpels and forceps, culture vessels, Glasswares like glass tubes, beaker, funnels etc. are required to achieve the purpose in a good sterile condition.

Basic Technique of Plant Tissue Culture:

Growth and Maintenance of plant tissue culture includes several sequential steps:

Stage 0, selection and preparation of explant and glassware required.

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Stage 1, initiation and establishment of culture

↓

Stage 2, multiplication or proliferation.

↓

Stage 3, rooting

↓

Stage 4, Acclimatization / hardening.

Steps involved —

1. Preparation of nutrient medium
2. Sterilization of glassware required.
3. Slection of explant
4. Sterilization of explant
5. Preparation of callus after incubation.
6. Inoculation of callus after incubation.
7. Regeneration.
8. Hardening and plantlet transfer.

• Preparation of Nutrient Medium : Nutrient Medium suitable to the plant need to be cultured is prepared

2. Sterilization of glassware : Glassware to be used in culture should be washed and sterilized. [DEPTH OF BIOLOGY]

These should be dipped overnight in a sulphuric acid solution or sodium dichromate solution.

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Next morning it should be washed with tap water and then distilled water.

These should be sterilized at 120°c in hot oven for 30 minutes to 1 hour.

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3. Selection of Explant : Excised part of the previous healthy plant is used eg: leaf, Bud, root, seed etc.

* Explant : part of plant which has got the regeneration potential and is capable of giving rise to a whole plant.

4. Sterilization of Explant : done using sodium hypochlorite mercuric chloride and then washed with sterilized water for atleast 6-10 times. It can also be achied using alcohol.

5. Preparation of Callus after incubation.

Explant → Nutrient Medium → Flask → kept in incubator at 25°c approx

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It is kept in light for photoperiod

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Callus is obtained within 3 - 8 days.

* Callus : undifferentiated cellular mass of plant.

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6- Inoculation of callus after incubation :

- cultures are incubated at around 25°C in humidity upto 60-70%. for 16 hrs of photoperiod.

↓

later this gets transfered to nutrient medium.
(Inoculation and preparation of callus is same)

7. Regeneration : callus is transfered to another medium and induction of roots, shoots are observed hence plantlets are regenerated.

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Plantlets are transfered to fred or green house

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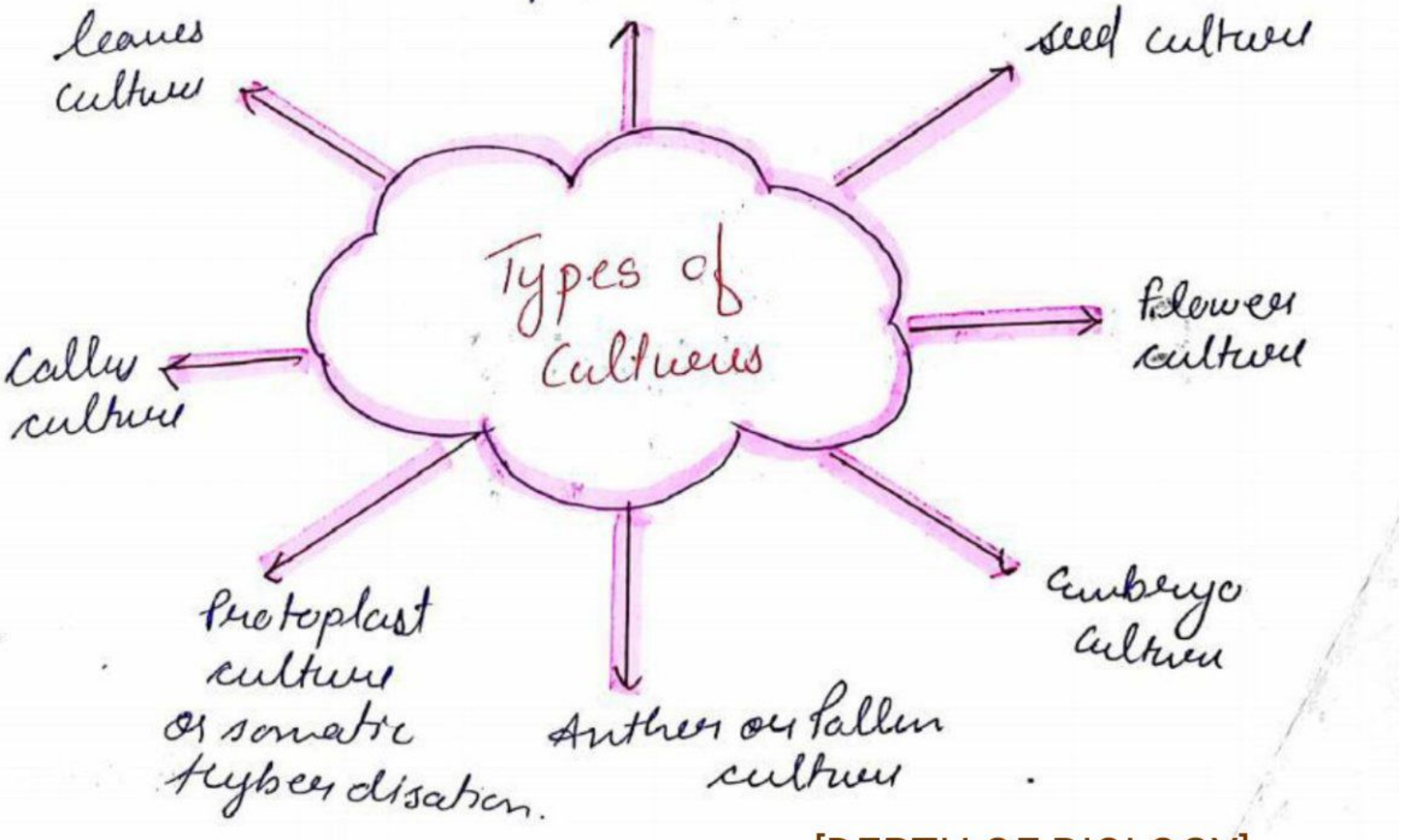
New plant is observed.



1. Protoplast culture or somatic Hyperdization:

Protoplast culture: Isolated protoplast gets cultured in liquid or semisolid agar media plates. These required somatic protection in culture medium until they generate a strong cell wall.

Isolate Protoplast

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clean it by centrifugation and decantation.

↓

Protoplast sol.^{n} is poured on sterile and cooled molten nutrient medium.

↓

Mix it quickly and gently by rotating petridish.

↓

Let it set and then seal petridishes with paraffin film and incubate

↓

Healthy protoplast leads to cell division form callus within 2-3 weeks.

↓

callus gets subcultured on fresh medium.

↓

Embryogenesis begins from callus when gets transferred to proper medium.

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Protoplast : plant cells with plasma membrane without cell wall.

Isolated by — [Mechanical Method
Enzymatic Method.

Somatic Hybridization : It is a technique of hybrid production through the fusion of isolated somatic protoplast in lab conditions.

↓

To produce hybrid plant.

New plant is obtained from the culture of an organ of the plant.

- Root tip culture – Meristematic tissue – root tip.
- Shoot tip culture – Meristematic tissue – shoot tip
- Leaf primordia culture.
- Flower culture
- Anther pollen culture
- Ovule and embryo culture.
- Ovaries culture
- Nucellus culture
- Seed culture
- Cotyledon culture
- Endosperm culture
- Fruit culture
- Plant cell culture.

→ Flower culture : New plant → flower

Flower from explant (Bud / Mature)

↓

Washed → sterilized → Rewashed

(Sterilization done by keeping it in teepol solution 5%. for 10 min.)

↓

Transfer flower → Nutrient medium within laminar air flow cabinet.

↓

Incubate for 15 hrs. at 25°C under light.

→ Leaf culture : New plant from leaf

Leaf excised from explant

↓

Sterilize and place in Nutrient Medium

↓

Cultured into New plant.

→ Anther culture : New plant from Anther

Excise anther from flower

↓

wash and sterilize it

↓

add it to nutrient medium.

↓

Embryogenesis occurs.

Plantlets are formed after 4-5 weeks of Incubation.

→ Callus Culture :- plant obtained ⟶ from Callus

Callus : undifferentiated mass obtained from explant

*Done in aseptic condition.

Explant tissue source.

↓

Source of Callus + nutrient medium.

kept at 25°C in BOD incubator

↓

Callus

- Its plant growth callus culture produce sigmoid curve (4 phases)

Lag phase | Log phase | Stationary phase | Death phase

- produced callus gets sub cultured in order to get new plant
- Best source of tissue for plant Regeneration.

→ Embryo culture : Plant from embryo

↓ obtained from

ovule | seed | Fruit

Embryo ⟶ Nutrient Media ⟶ incubate for 16 hrs at (25°C)

*After 3-4 weeks it then gets sub cultured in fresh medium

→ Seed Culture : seed ⟶ Seedlings ⟶ plants

*Increases efficiency of germination.

. Done in vitro aseptically.

> Pollen Culture : [DEPTH OF BIOLOGY]

Pollen grains are excised and gets cultured on nutrient medium in dark for 2-3 weeks.

↓

Callus is obtained (haploid)

Advantages of Plant tissue Culture

- Assure uniform supply of raw materials for the cultivation of otherwise non available medicinal plants to the industry.
  Thus, crucial for Phytopharmaceuticals.
- Best way to overcome fluctuations in the supplies and quality of the plants due to unfavourable climatic conditions. [DEPTH OF BIOLOGY]
- Helpful in Biotransformations - reactions become feasible using plant cell cultures.
  (modification of functional groups of organic cpds.
- Leads to desired, healty propagule for the large scale production.
- Propagation of plants without seeds in the controled conditions
- Improvement in the plant species.
- Desired features can be preserved in the new plants generations. [DEPTH OF BIOLOGY]

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Disadvantages of Plant Tissue Culture

- Require highly skilled experts
- Needs pure chemicals in large amounts.
- Costly or less feasible
- Slow process thus consume time and give slow results
- Direct observation is required.
- Aseptic conditions are difficult to maintain through out the process.
- Laborious task

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- Artificial medium for culture may leads to change or reduction of the usual metabolic pathways of plant.
- usually the quantity of secondary metabolites produced are less or negligible.
- Scope for the mistake or errors is less. a single mistake can leads to the failure of the whole procedure.
- less stable.

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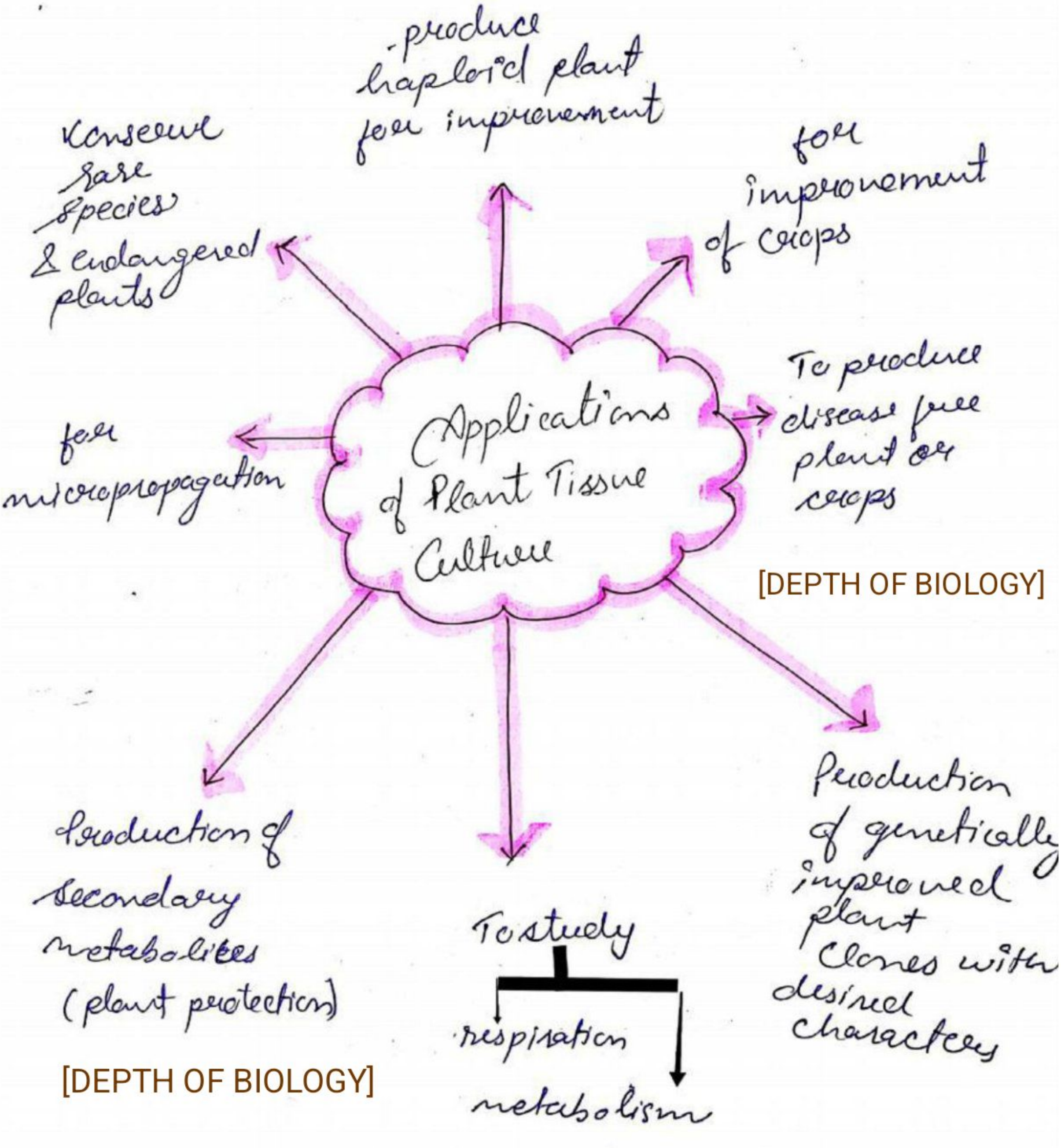

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Nutritional requirement of Plant Tissue Culture

Nutritional requirements of plants are fulfilled by culture media.

- Most comman media is MS medium (Murashige and Skoog). in lab.

Types of culture Media

- (MS) Murashige and Skoog medium
- (LS) Linsmaier & Skoog medium
- (B5) Gamborg medium
- (NN) Nitsch & Nitsch medium
- (QL) Quoirin & Lepoivre medium
- White's Medium

Main Components of Plant Tissue culture Medium

- Inorganic & Carbon → Nutrient source
  - Macro Nutrients: N, P, K, Ca, Mg, S
  - Micro Nutrients (additive): Fe, Mn, Zn, B, Cu, Mo, Co
- Organic Supplements
  - vitamins
  - Amino Acids
- Growth Regulators
  - Auxins
  - Cytokinins
  - Gibberellins (GA)

other components are— [DEPTH OF BIOLOGY]

- Solidifying agent
- Antibiotics
- PH.

- This medium contains all the vital supplements required for the plant growth so it can house the plants.

Inorganic salts → Macro nutrients req. in large amounts
→ micro nutrients required in traces

Carbon Source (Energy Source)

Glucose, Sucrose, Lactose, Galactose, Maltose, Sorbitol etc are used as external source of energy for the plant growth.

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Organic Supplements

→ vitamins : cells in culture synthesis it in less quantity so vitamins are required in small quantities externally for better growth and development.

Eg. Vit. B_1 , Vit B_3 , Vit B_6 etc.

Growth Regulators

Plant Hormones → Phytohormones
promote cellular growth.
Eg. Auxins, Cytokinins, Gibberellins, Ethylene and Abscissic acid.

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* Others - PH is adjusted b/w 5–6.
Antibiotics. → inhibits microbial growth.
- Solidifying agents used to prepare semi solid or soled tissue medium.
Eg. Agar, Gelatine, silica gel, starch etc.

Edible Vaccines

↓

Vaccines which can be eaten.

Vaccine: derived from usuage of cowpox
(Cow — vacca in latin)

It is a biological preparation used to increase immunity against the particular disease.

Vaccine contain heat killed diluted disease antigen that stimulates body to produce an antibody reaction.

↓

It is not strong enough to Produce diseases harmful effects.

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Vaccines are of 2 types

Prophylactic

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- Prevent the effect of future infection by any natural or wild pathogen.
- COVID vaccine

Infection from external source.

Therapeutic

↓

- Vaccine against cancer.
- To prevent Cancer.

Edible Vaccines :

- Transgenic plants are used as vaccine production system
- In this genes that encodes antigen of pathogens (bacterial or viral) gets expressed in plants in a form in which they retain native immunogenic properties
- Earlier considered useful only for preventing infectious diseases but now also helpful and found its application in prevention of autoimmune diseases. cancer therapies, birth control etc.

Hence, [DEPTH OF BIOLOGY]
Edible vaccines are currently being developed for a number of human and animal disease.

Why are edible vaccines are considered areas of large interest in medical sciences.

→ **Needleless** : Oral or needle free vaccines provide mucosal immunity at various sites by secreting antibodies.

→ low risk of infection : no worries about reuse, misuse or lack of sterilization.

→ **Cheap** : It requires least or no training at all in administeration.

-Estimated cost for growing antigen of Hepatitis B for 1 dose in unprocessed form is 0.005 $

Storage : They are heat stable and do not require cold-chain maintenance.

Need for transportation and distribution can be reduced or eliminated if the crop of the particular area is engineered to produce vaccine in native or local area.

Safe : It triggers immunity at the first line of defense at the mucosal surface.

- Do not need purification.

These activate both mucosal and systemic immunity against pathogen.

Mechanism of Action of Edible Vaccine

Intake of Edible Vaccine

↓ Mastication and degradation in intestine

Payer's patches - rich source of IgA producing plasma cells

↓

edible vaccine breaks down at payer's patches.

(It contains 30-40 lymphoid nodules containing follicles for development of germinal center)

↓

Antigen penetrate follicles, accumulating antigen in lymphoid structure.

Antigen contacts M cell which express MHC II molecule

↓

Pocket formation occurs which is filled with B cells, cells and macrophages

↓

M cell with antigen activates B cell within the lymphoid follicle

↓

Activated B cell leaves lymphoid follicle and reaches mucosal associated lymphoid tissue (MALT)

↓

Plasma cells are differentiated from B cells and IgA are produced

↓

IgA secreted into lumen where they interact with antigen.

Plants used for edible vaccines:

- Tobacco
- Potato
- Banana
- Tomato
- Rice
- Lettuce
- Soybean
- Alfalfa
- Muskmelon
- Carrot
- Peanuts
- Wheat
- Corn.

Edible Vaccines: are the subunit vaccines where the selected genes are introduced into the plants

↓

introduced into the plants and the transgenic plant is then induced to manufacture the encoded protiens.

Vaccines are made using plants as vectors to prevent particular disease.

* Vector Tomato is used to make vaccine for HIV virus to prevent AIDS
* Vectors Tabacco and potato are used to make vaccine against Hepatitis B virus to prevent Hepatitis B.
* Vector Tobacco is also used to make vaccine against Rabies virus to prevent Rabies.

→ **Advantages of Edible Vaccine**

- less Expensive
- Can be grown in large scale.
- Can be taken orally without injection
- Easily administered by eating
- chance of infection is low.
- Easily stored (Heat stable)
- Activates systemic and mucosal immunity.
- Eliminate refrigeration cost

Drawbacks of Edible Vaccines :-

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- Vectors used for vaccines such plants cannot be consumed raw. Part of the plant containing vaccine should be cooked well before administration. This weakens the vaccine.
- Survival of antigen is difficult in stomach due to acidic medium (low pH).
- Selection of the suitable vector is difficult and skilled task.