PPT Bank by MSP

1. Media Preparation
2. Basic concepts in PTC
3. Screening of Industrially imp organisms
4. Somatic Embryogenesis
5. Callus Culture

BASIC CONCEPTS IN PTC

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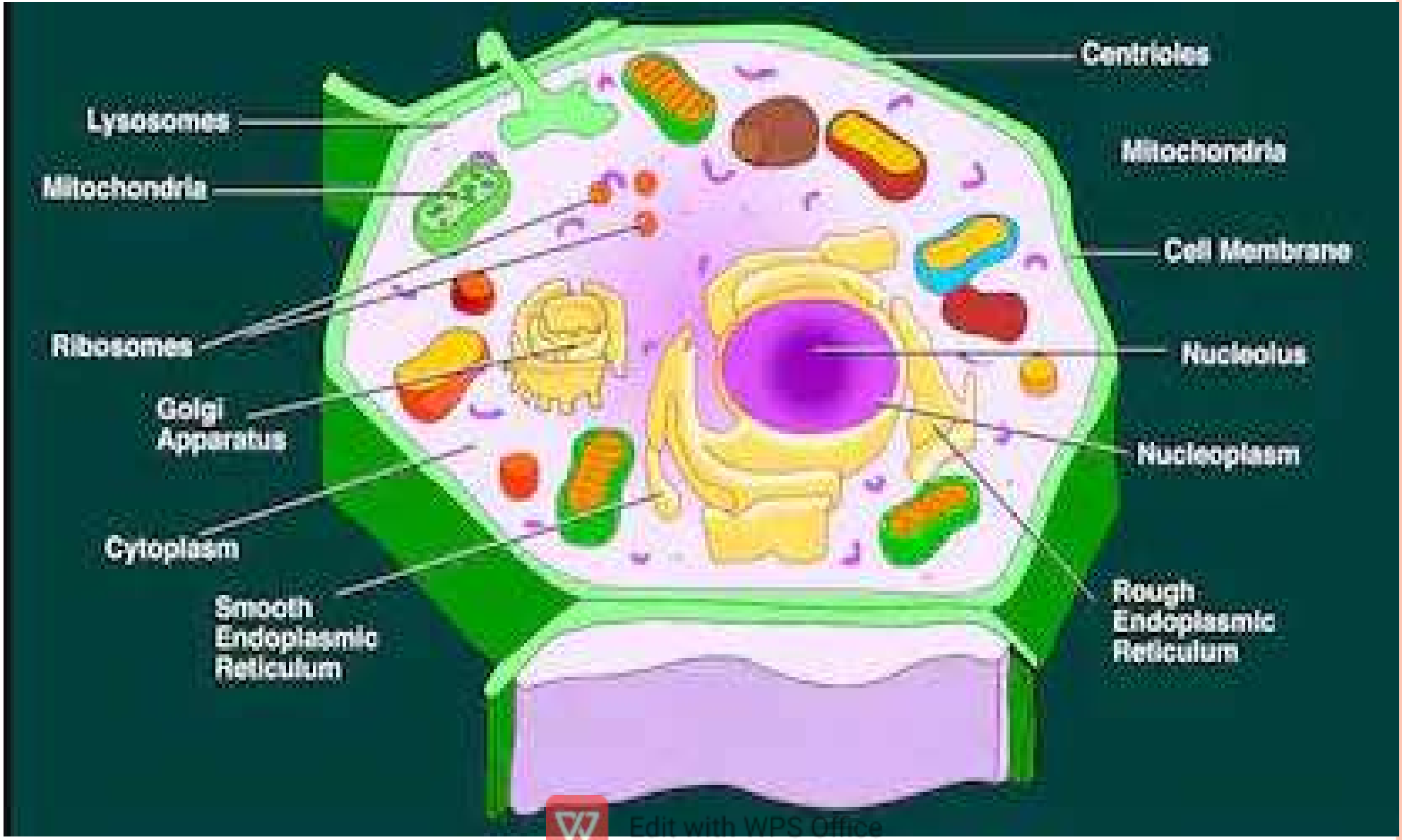
Plant Cell



CELL THEORY

- The cell theory or cell doctrine was proposed by Schleiden and Schwann in 1839.

- In 1839, Schleiden suggested that every structural part of a plant was made up of cells or the result of cells.

THREE TENETS OF CELL THEORY WERE POSTULATED;

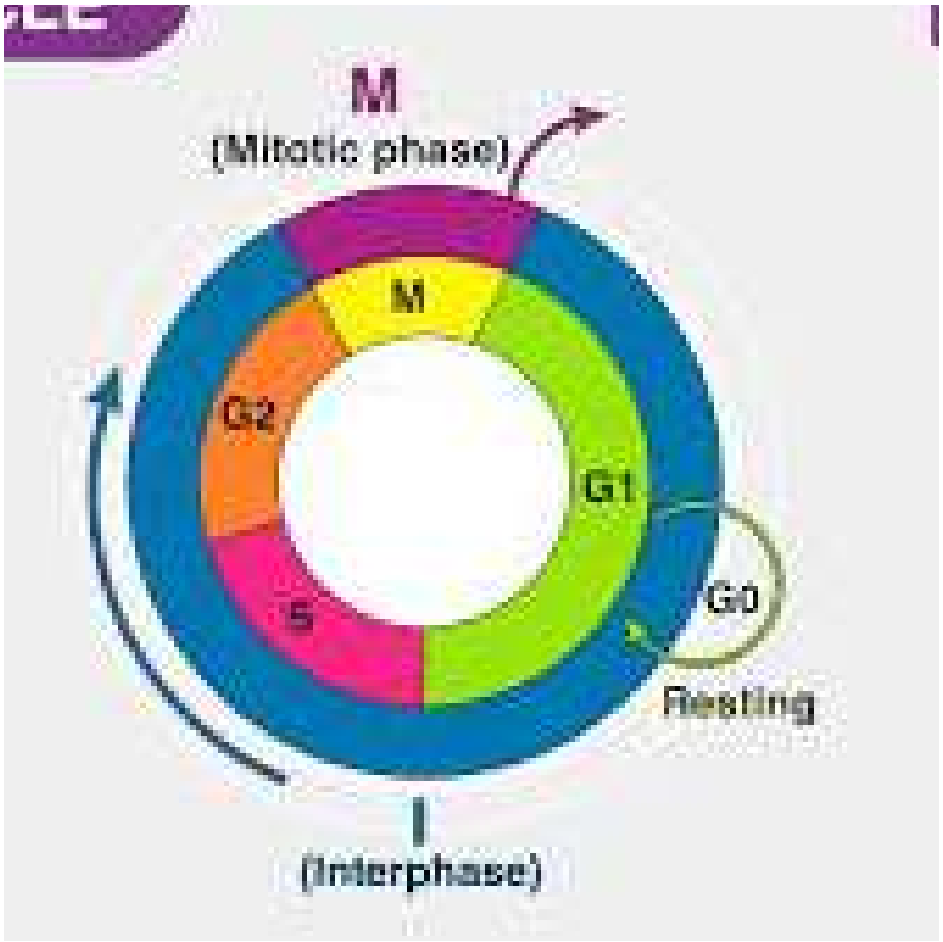
- All living organisms are composed of one or more cells
- The cell is the most basic unit of life
- In 1855, Rudolf Virchow added the third tenet to cell theory. In Latin, this tenet states *Omnis cellula e cellula*. This translated to: 'All cells arise only from pre-existing cells'

THE GENERALLY ACCEPTED PARTS OF MODERN CELL THEORY INCLUDE:

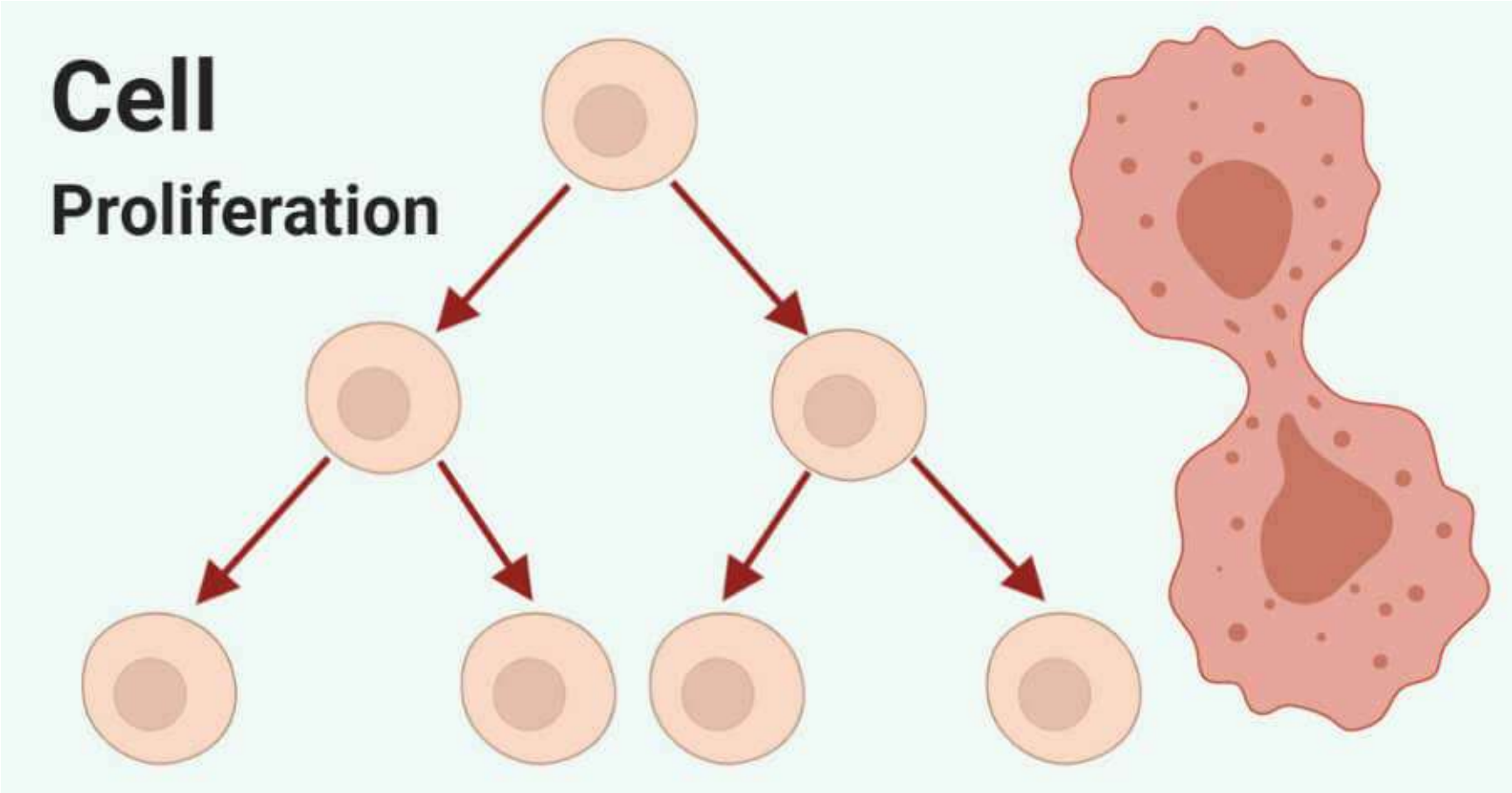
- All known living things are made up of one or more cells.
- All living cells arise from pre-existing cells by division.
- The cell is the fundamental unit of structure and function in all living organisms.
- The activity of an organism depends on the total activity of independent cells

- Energy flow (metabolism and biochemistry) occurs within cells.
- Cells contain DNA which is found specifically in the chromosome and RNA found in the cell nucleus and cytoplasm.
- All cells are basically the same in chemical composition in organisms of similar species

Cell Division



Cell proliferation



CELL DIFFERENTIATION

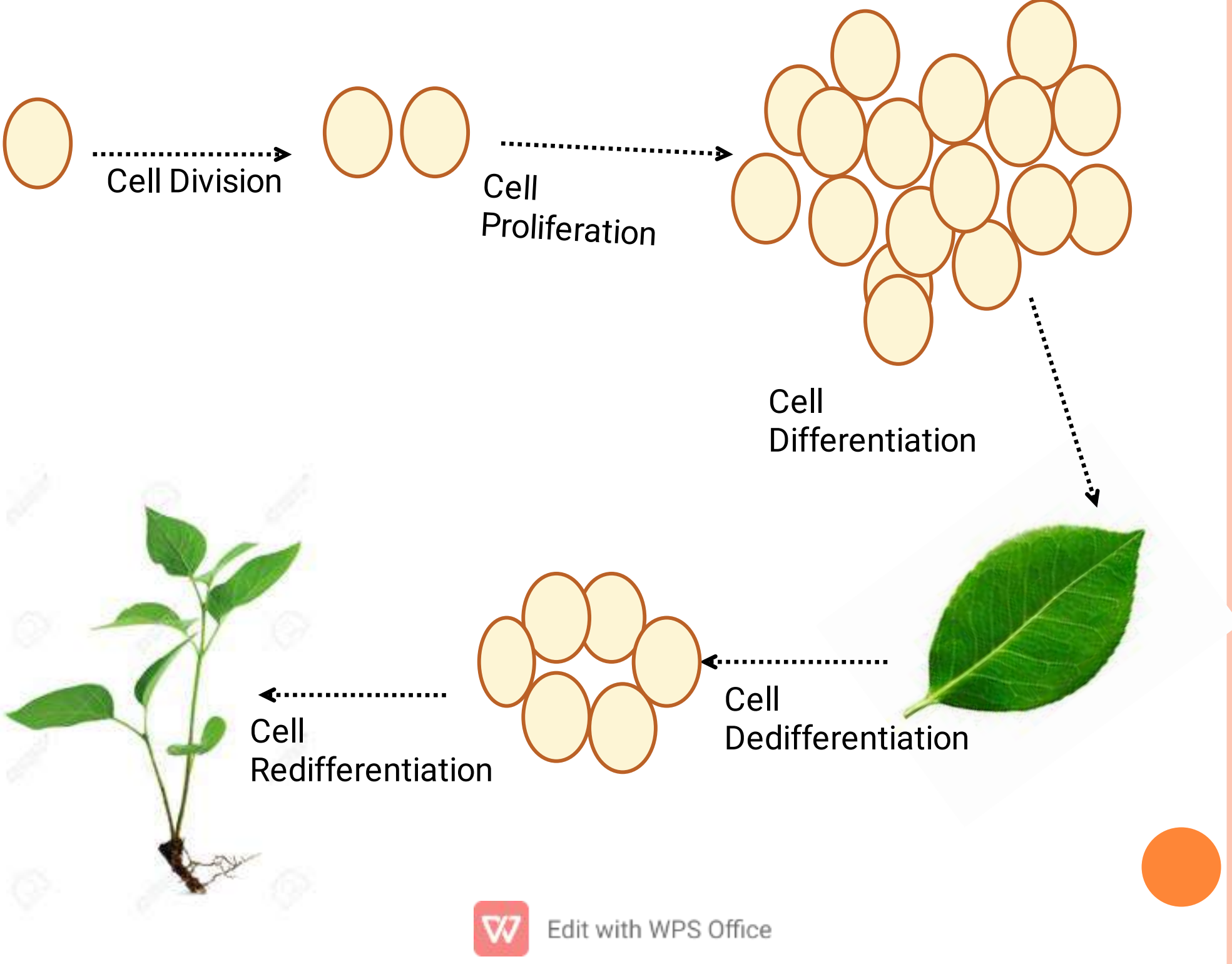
- Cell acquires specific function.

DEDIFFERENTIATION

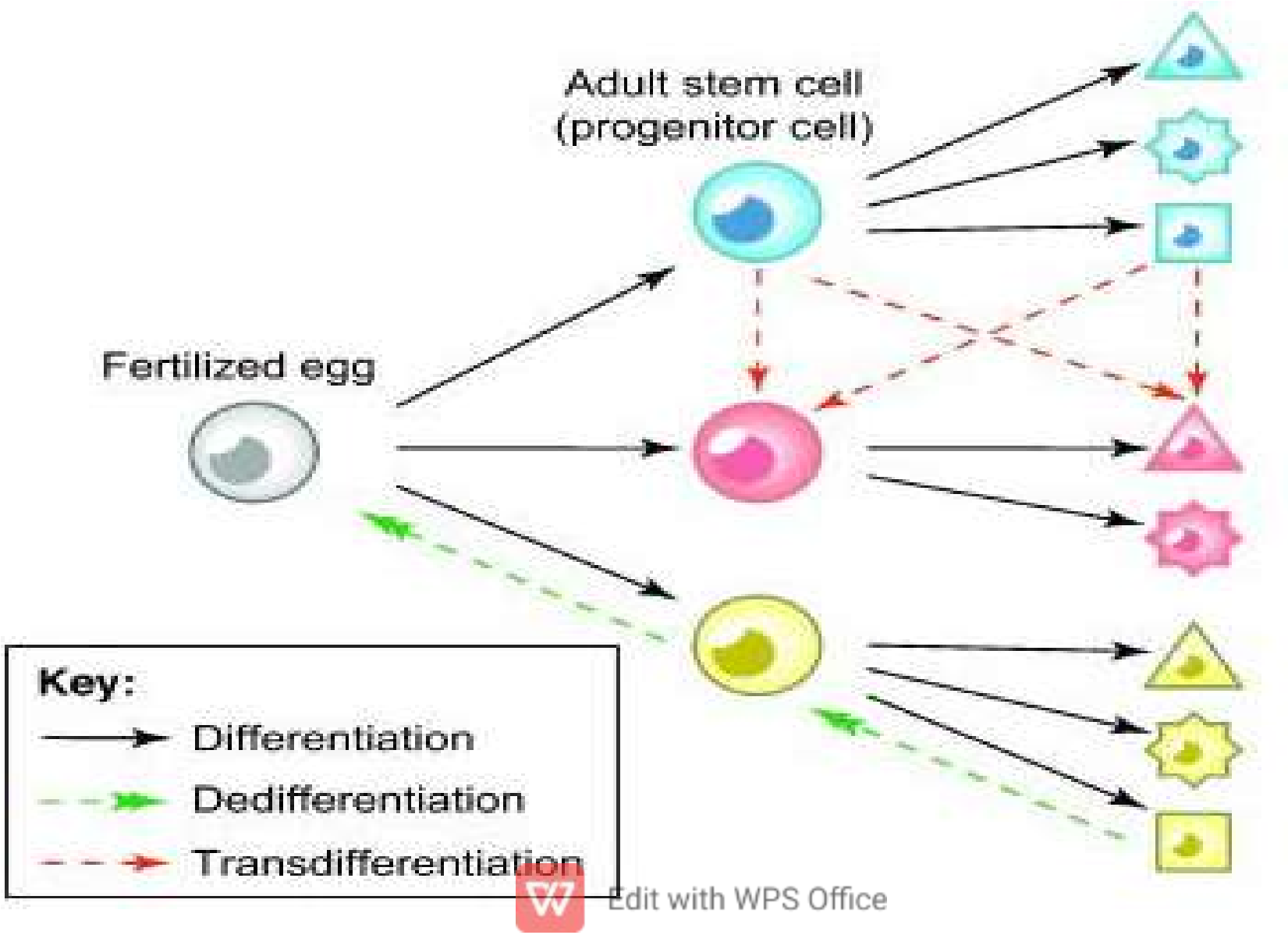
- Function removed from differentiated cell

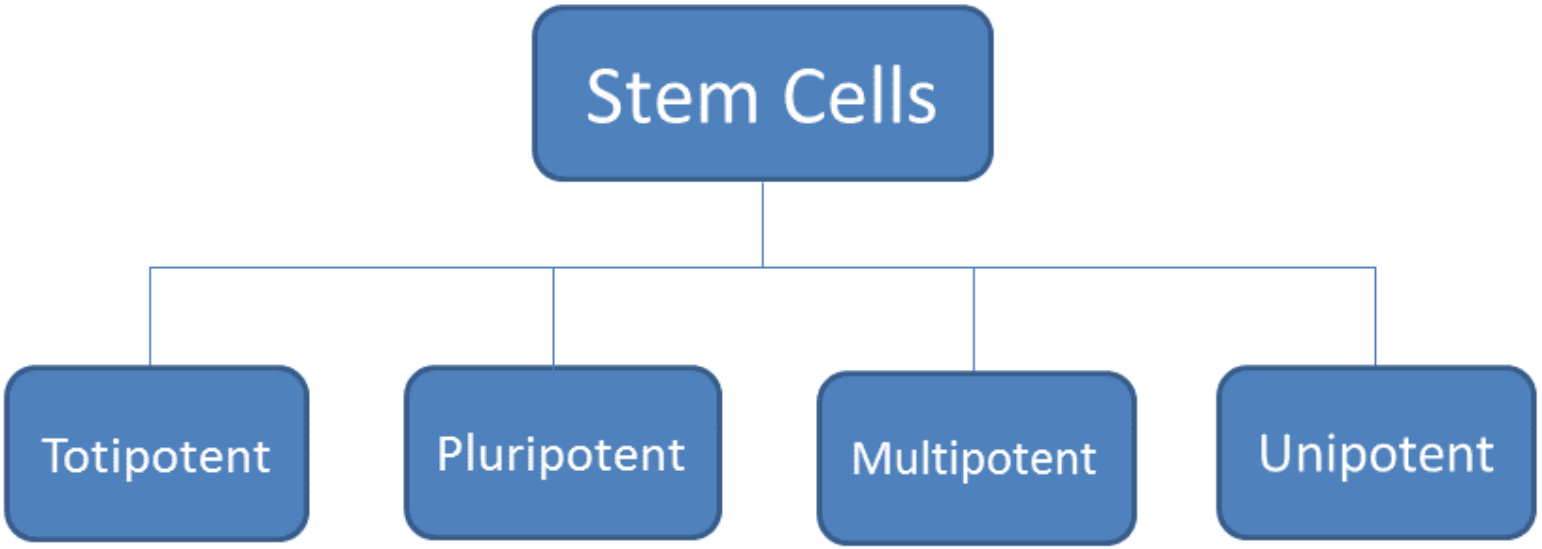
REDIFFERENTIATION

- Dedifferentiated cell again acquires function.



REGENERATION OF PLANT CELLS





THANK YOU.....

Callus Culture

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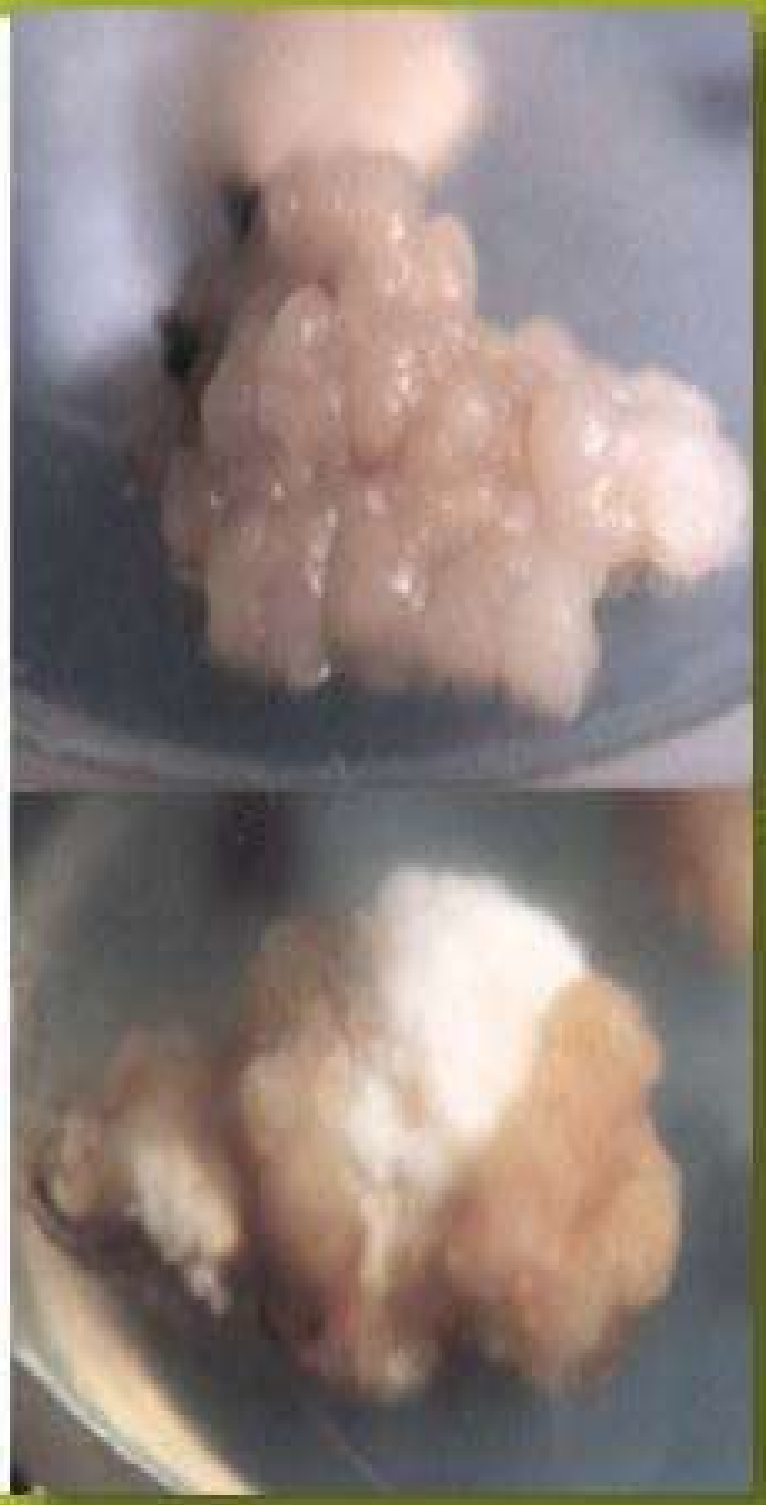
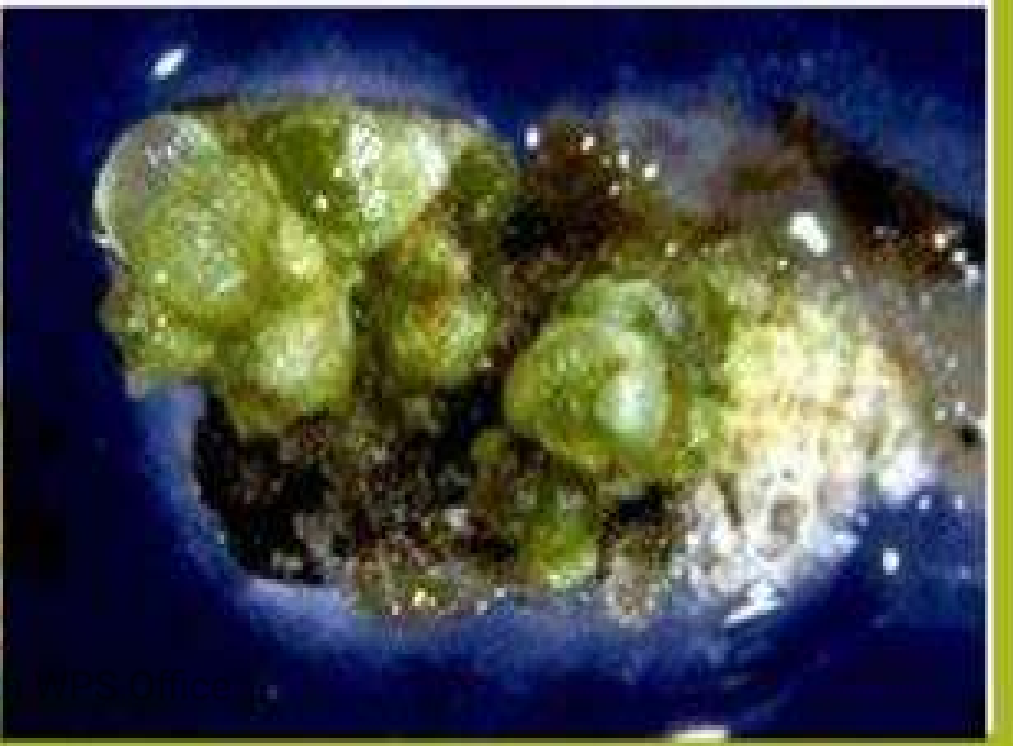
Callus

- It is an **unspecialized , unorganized, growing** and **dividing mass** of cells.
- It produced when explants are cultured on the appropriate solid medium, with both an **auxin** and a **cytokinin** in a correct conditions. 2,4-D are commonly used.
- During callus formation there is some degree of **dedifferentiation** both in morphology and metabolism, resulting in the lose the ability to photosynthesis.



- A callus is a blob of tissue – (mostly undifferentiated cells)
- A callus is naturally developed on a plant as a result of a wound
- This callus can be left to develop or can be further divided



Callus

Callus cultures may be compact or friable.

- ✓ Compact callus shows densely aggregated cells
- ✓ Friable callus shows loosely associated cells and the callus becomes soft and breaks apart easily.

- Habituation: it reduce the requirement of auxin and/or cytokinin by the culture during long-term culture.

Properties of callus culture

- It Often comes from shoot in early culturing
- It Can be maintained indefinitely
- **No- photosynthesis** and grows in dark
- It Can be used to isolate single cells with stem cell like properties (totipotent)

Three stages of callus culture

1.Induction:

Cells in explant dedifferentiate and begin to divide

2.Proliferative Stage:

Rapid cell division

3.Morphogenesis stage:

Differentiation and formation of organized structures; specifically processes that lead to plant regeneration from somatic cells

2.Division

Growth of callus over time — t
8 weeks

B. Differentiation

Organogenesis

Somatic embryogenesis

Plant morphogenesis

3.1 Organogenesis

The formation of organs (such as leaves, shoots, roots) on a plant organ, usually of a different kind

1. Enhancement of axillary bud proliferation/ development
2. Adventitious shoot formation
3. Adventitious root formation

3.2 Somatic embryogenesis

Embryo initiation and development from somatic cells

3.1 Organogenesis

- ✓ **Enhancement of axillary bud proliferation and development** - stimulation of the shoot apical meristem *in vitro* that includes proliferation of lateral buds
- ✓ **Adventitious shoot formation** - dedifferentiation and/or differentiation and development of shoots from non-meristematic cells (one or more than one) either directly or indirectly
- ✓ **Adventitious root formation** - roots are initiated adventitiously at the base of the shoot apex and a vascular continuum is established to complete plant regeneration.

3.2 Somatic Embryogenesis

- ✓ Dedifferentiation is typically minimal but a meristemoid -like tissue can be formed in the latter case
- ✓ Histogenesis of somatic embryogenesis is characterized by the formation of a bipolar structure, in contrast to adventitious organogenesis
- ✓ Single cell origin of somatic embryos makes chimerism infrequent; adventitious shoots can arise from more than one cell

MORPHOLOGY and characteristics of callus

- Callus tissue proliferate as amorphous mass of cells having no regular shape .
- So it difficult to describes its external morphology.
- But they can be distinguished on the basis of other characteristics such as Texture , Colouration , Hormone requirements etc.
- On that basis , even callus tissue initiated from explants of the same plants species may show considerable variation.

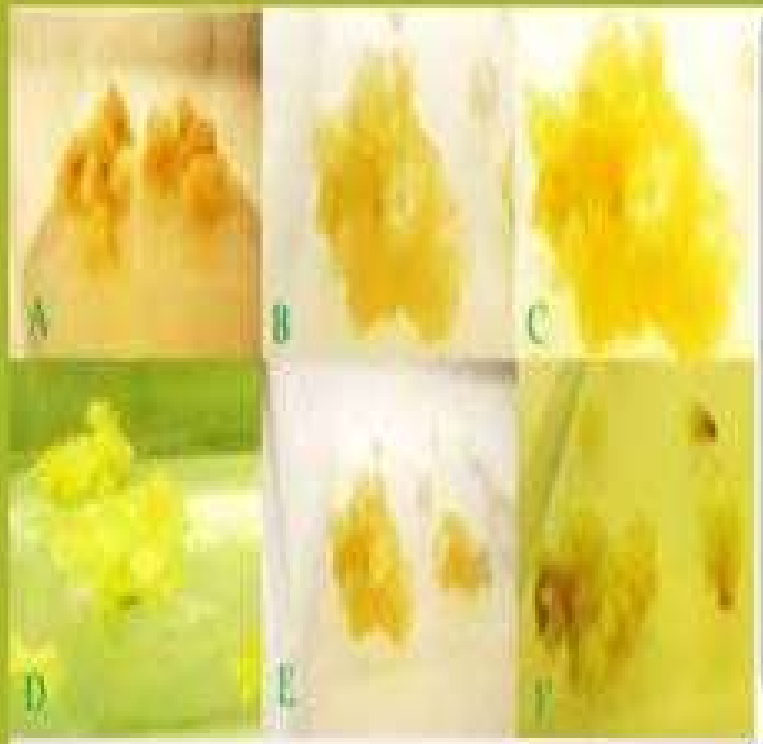
texture

- On the basis of texture callus tissue can be two categories such as ,

- **1. Soft callus** is friable in nature and is made of Heterogenous mass of cell having minimal contact.
- **2. Hard callus** consists of giant cells , tracheid like cells and closely packed cells i.e. Compact in nature .
- Hard callus may be nodular in form .

colouration

- Generally callus tissue is creamish yellow or white in colour.
- Sometime it may be Pigmented.
- Pigmentation may be uniform or patchy.
- It may be green in colour.
- Sometime white callus tissue grown under dark condition turn it into green colour after transferring in light condition.
- Some may be yellow in colour due to synthesis of Carotenoid pigments.
- In some cauliflower culture it is in purple colour due to accumulation of anthocyanin in vacuoles or due to production of oxidized form of 3,4 Dihydroxy phenylalanin (DoPA)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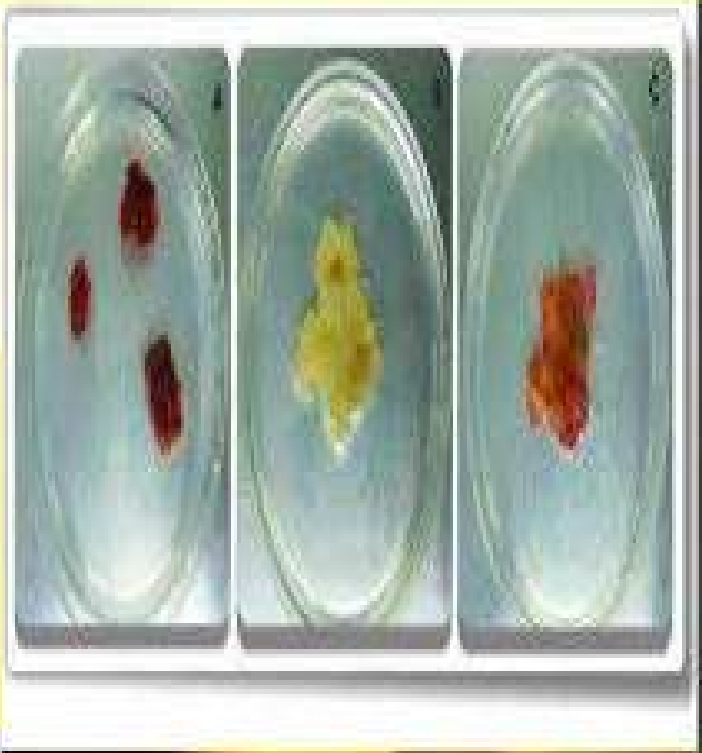


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Principle / procedure of callus culture

- There are Three criteria for callus culture are ,

1. Aseptic preparation of plant material
2. Selection of suitable nutrient medium
3. Incubation of culture under controlled physical condition

1. Aseptic preparation of plant material

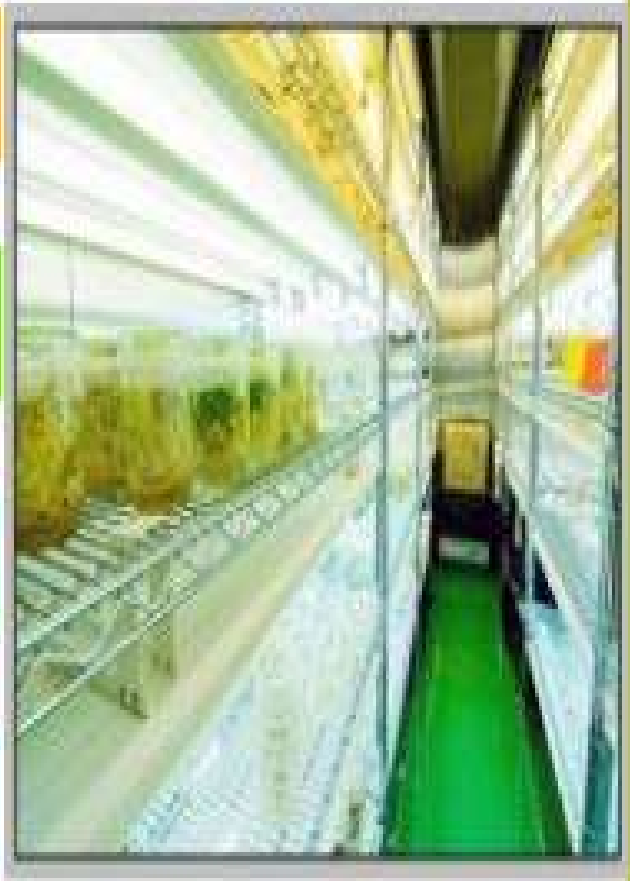
- Surface sterilization :-

2.Selection of suitable nutrient medium

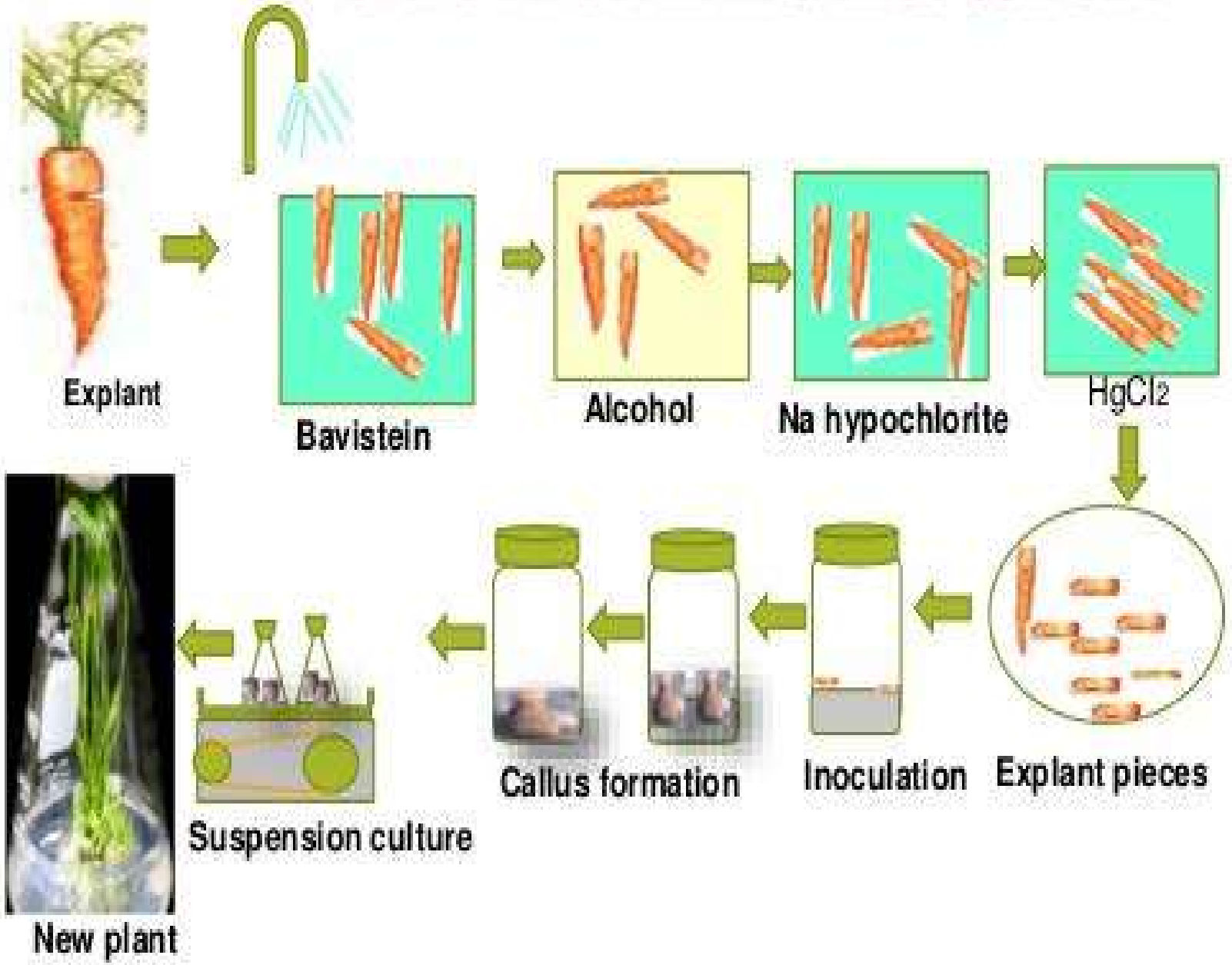
- ✓ Auxin/cytokinin 10:1-100:1 induces roots.
- ✓ 1:10-1:100 induces shoots
- ✓ Intermediate ratios around 1:1 favor callus growth .
- ✓ Agar solidified or semi – solid nutrient medium are used.
- ✓ That media are autoclaved at 15 psi pressure for 15 – 20 min at 121 $_{0}$C.

8.Incubation of culture under controlled physical condition

- Temperature : 25 ± 2 oC
- Photoperiod : 16 hr Light , 8 hr Dark
- Light intensity : 2000 – 3000 lux
- Relative Humidity : 55 % - 60 %



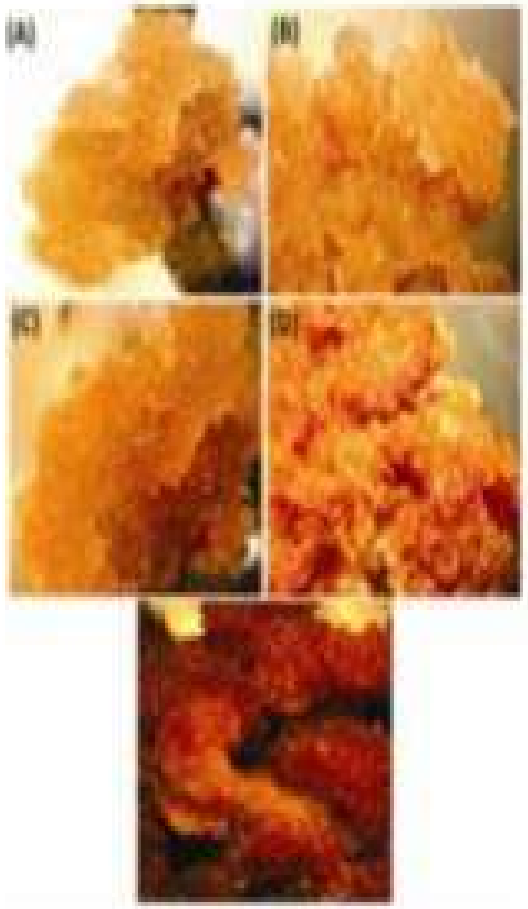
STEPS INVOLVED IN CALLUS CULTURE



Callus multiplication

Actively growing callus can be initiated on culture media with an even physiological balance of cytokinin and auxin.

After callus biomass increases two to four times (after 2–4 weeks of growth), callus can be divided and placed on fresh media.



Significances of callus culture

- Callus culture as such has no major importance unless and until it is used for other experimental objectives . Still ,callus culture got some importance

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 . This phenomenon is known as plant regeneration or organogenesis or morphogenesis .
2. Callus tissue is good source of genetic variability.
3. Cell suspension culture in moving liquid medium can be initiated from callus culture.
4. Callus culture is very useful to obtain commercially important secondary metabolites.
5. Several biochemical assay can be performed from callus culture.

Factors Affecting Callus Culture

- Explant
- The physical qualities of culture media
- The chemical composition of medium
- Temperature & Light

Thank You……..

Culture Media & its Components

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Culture Media

- Chemical requirement of tissue cultured plant is fulfilled by culture media.
- Nutritional components in media are essential for growth and development of cultured tissue.
- Various media formulations are designed by scientists for specific growth of plants.
- The choice of media depends on the type of culture (e.g. Organ Culture, Anther Culture, Somatic Embryogenesis)

Basic Components in Culture Media

- Macro inorganic nutrients
- Micro inorganic nutrients
- Iron (as chelating agent)
- Vitamins
- Carbon sources
- Organic nitrogen
- Plant growth regulators
- Agar (as gelling substance)

Classification Of Nutrients

Macro-nutrients

- Need of macro nutrients is higher.
- It is present in millimolar (mM) quantities (more than 30 ppm/1 or mg/1)
- Macro nutrients provide both anions and cations for the plant cells.
- Macro nutrients have structural and functional role in protein synthesis, cell wall synthesis enzyme Co-factors and membrane integrity.

Macro-nutrients

- Nitrogen (as NO3 and NH4)
- Phosphorus(PO4)
- Potassium(K)
- Sulphur (as SO4)
- Magnesium(Mg)
- Calcium(Ca).

Nitrogen

- In organic form used as amino acids, different organic acids and casein hydrolysate.
- In inorganic form used as Nitrate or ammonia.
- Nitrogen is major component of all plant tissue culture media.
- Nitrogen helps to synthesis complex organic molecule.

Potassium

- K ion is present in high concentration in the cytoplasm (100-200 mM) and in chloroplast(20-200 mM).
- K+ is essential for maintaining the ion balancing, activation of many enzymes.
- Maintains osmotic pressure and osmotic regulation of cells.

Calcium

- Calcium functions with different enzymes as Co-factor and bound to the cell wall and cell membrane.
- It gives strength to cell wall.
- It helps in the regulation of the cell membrane structure.
- Deficiency causes disintegration of the membrane and shoot tip necrosis.
- Important in cell and root multiplication.
- Supplied as calcium chloride and calcium nitrate.

Phosphorus

- Very important for energy metabolism.
- Essential element for DNA & RNA.
- Deficiency may cause delayed growth and dark green colour of leaves.
- Supplied as sodium hydrogen phosphate or potassium hydrogen phosphate.

Magnesium

- Essential for enzymatic reactions, energy metabolism(ATP synthesis).
- Supplied as magnesium sulphate.

Sulphur

- Important substance.
- Deficiency of Sulphur inhibits protein synthesis and decreases Chlorophyll in leaves.
- Supplied as magnesium Sulphate and Potassium Sulphate.

Micro-nutrients

- Used in less amount less than 30ppm.(mg/l).
- Concentration is always in uM.

Micro-nutrients

- Boron(B)
- Manganese(Mn)
- Zinc(Zn)
- Molybdenum(Mo)
- Copper(Cu)
- Cobalt(Co)

Zinc

- Zn plays an active role in protein synthesis and in the synthesis of tryptophan.
- Supplied as Zinc Sulphate.

Manganes

- Plays an important role in the Hill reaction of photosynthesis.
- Required in many enzymatic activities.
- Supplied as Manganese Sulphate.

Copper

- Copper plays important role in photosynthesis.
- Intermediate of the electron transport chain between photo system 1 & 2.
- Deficiency leads to decrease in photosynthesis.
- Supplied as Copper Sulphate.

_Molybdenum

- Essential for conversation of Nitrate to Ammonium.
- Supplied as Sodium molybdate.

Boron

- Involves in different enzymatic activities.
- Supplied as Boric acid.

Iron

- Important Enzyme Co-factor.
- Supplied in uM quantities.
- It is supplemented with chelators and Complex compounds due to its solubility problem.
- Supplied as Na2FeEDTA.
- Iron deficiency have severe effects on the growth and development plant cells.

Organic Nutrients
Vitamins

- Plant synthesis required vitamins.
- Essential for many biochemical reaction.
- Cultured cell are capable to produce vitamins at some level.
- They require an exogenous supply of different vitamins for optimum growth.
- Most usable vitamins are Thiamine, Pyridoxine nicotinic acid Vitamin B Complex.

Hexitols

- Most tissue culture media have this compound.
- Essential for seed germination, sugar transport, carbohydrate metabolism, membrane structure and cell wall formation.
- Mannitol and sorbitol are hexitols.

Amino Acids

- Glycine is the most common Amino Acid used in different culture media.
- It is not essential but Nitrogen containing Amino Acid enhance growth and plant regeneration.

Carbohydrate

- Cells and tissue requires exogenous supply of carbohydrates to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis.
- Supplied by adding sucrose.
- Concentration is 20-30 gm/l.

Gelling Agent
Agar – Agar

- Agar is a natural product of seaweeds.
- Since 1658 agar-agar is obtain from red algae (Gelidium gracilaria).
- With water it melts at 100’C and solidify at 45’C

Agarose

- It is highly purified agar prepared from Gelidium sp. Of seaweed.
- Agarose melt and gel at temperatures below 30’C and dissolve through boiling.
- Agarose is much more expensive agar-agar

Gelrite or Phytagel

- Gelrite is a naturally derived polymer and produced by the microbial terminatation of a bacterium Pseudomonas elodea.
- It is low cost gelling agent.
- 0.1-0.2 % concentration pen liter required.

Natural Media Constituents

- Endosperm fluid / coconut
- Coconut fruit milk
- Fruit materials
- Orange juice, Tomato juice Banana pulp
- Potato extract
- Potato
- Extracts of malts, yeast
- Malt, Yeast
- Protein hydrolysates or Casein hydrolysate Peptone

Plant growth regulators

- A plant hormone can be defined as a small organic molecule that elicits a physiological response at very low concentration PGS plays an important role in the phenotype.
- Act as messenger between environment and the genome.

Auxins

- Essential for cell division, cell elongation, cell differentiation, organogenesis and embryogenesis, callus formation .
- Natural form auxins are IAA, IBA, PAA Synthetic form of auxins are NAA, 2, 4-D.

Cytokinins

- Cytokinins promote cell division, shoot proliferation and influence the cell cycle.
- Embryogenesis and inhibit roof formation.
- Synthetic form is 2-ip which is most active Cytokinins.
- Natural forms are BAP and kinetin

Gibberellins

- It promotes stem elongation, bulb corm formation and embryo maturation but can inhibit callus growth and root induction.
- GA3 is most common gibberellins.

Abscisic acid

- It inhibits shoot growth and germination of embryo.
- It is thermostable but light sensitive

pH of tissue culture media

- pH is adjusted between 5 & 5.8 before gelling and sterilization with the help of dilute NaOH, KOH or HCL.
- pH below 5 will not gel properly.
- pH above 6 may be too hard.

Stock solution of Macronutrients (10X)

Sr no.	Stock Ingredient	Concentration in Stock (mg / lit)	Volume of Stock in medium (mg/lit)
1	MgSO4.7H20	3,700	100
2	KH2PO4	1,700	100
3	KNO3	19,000	100
4	NH4NO3	16,500	100
5	Cacl2.2H2O	4142	100

Stock solution Micronutrient(100X)

Sr no.	Stock Ingredient	Concentration in Stock (mg / lit)	Volume of Stock in medium (mg/lit)
1	H3BO3	620	10
2	MnSO4.4H2O	2230	10
3	Na2MoO4.2H2O	2.5	10
4	CuSO4.5H2O	2.5	10
5	Cacl2	2.5	10
6	KI	8.3	10
7	ZnSO4.7H2O	860	10

Stock Solution of Iron (100 X)

Sr no.	Stock Ingredient	Concentration in stock (mg/lit)	Volume of stock in Medium (mg/Lit)
1	FeSo4.7H2O	2780	10
2	Na2.EDTA	3780	10

Stock Solution of Growth Regulators (100X)

Sr No.	Growth Hormone	Solvent	Final Concentration in stock (mg/lit)
1	6 Benzyl amino purine (BAP)	1N NaOH / Water	1mg/ml
2	2,4-Dichlorophenoxy acetic acid (2,4-D)	Alcohol / 1N NaOH	1mg/ml
3	Naphthalene acetic acid (NAA)	Alcohol / 1N NaOH	1mg/ml
4	Indole Butyric acid	Water / 1N NaOH	1mg/ml
5	Indole 3 acetic acid	Alcohol / 1N NaOH	1mg/ml

Stock Solution of Vitamin (100X)

Sr no.	Stock Ingredient	Concentration in stock (mg/lit)	Volume of stock in medium (mg/ml)
1	Thymine -HCL	50	10
2	Pyridoxine - HCL	50	10
3	Nicotinic acid	50	10
4	Glycine	50	10
5	Myoinositol	50	Add fresh

Sr No	Name	Quanity
1	Sucrose	30gm/lit
2	Agar-Agar	8gm/ml
3	pH	5 to 6 (5.8)

Thank you.....

Screening Of Industrially Important Organisms

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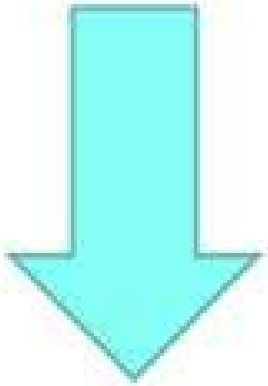
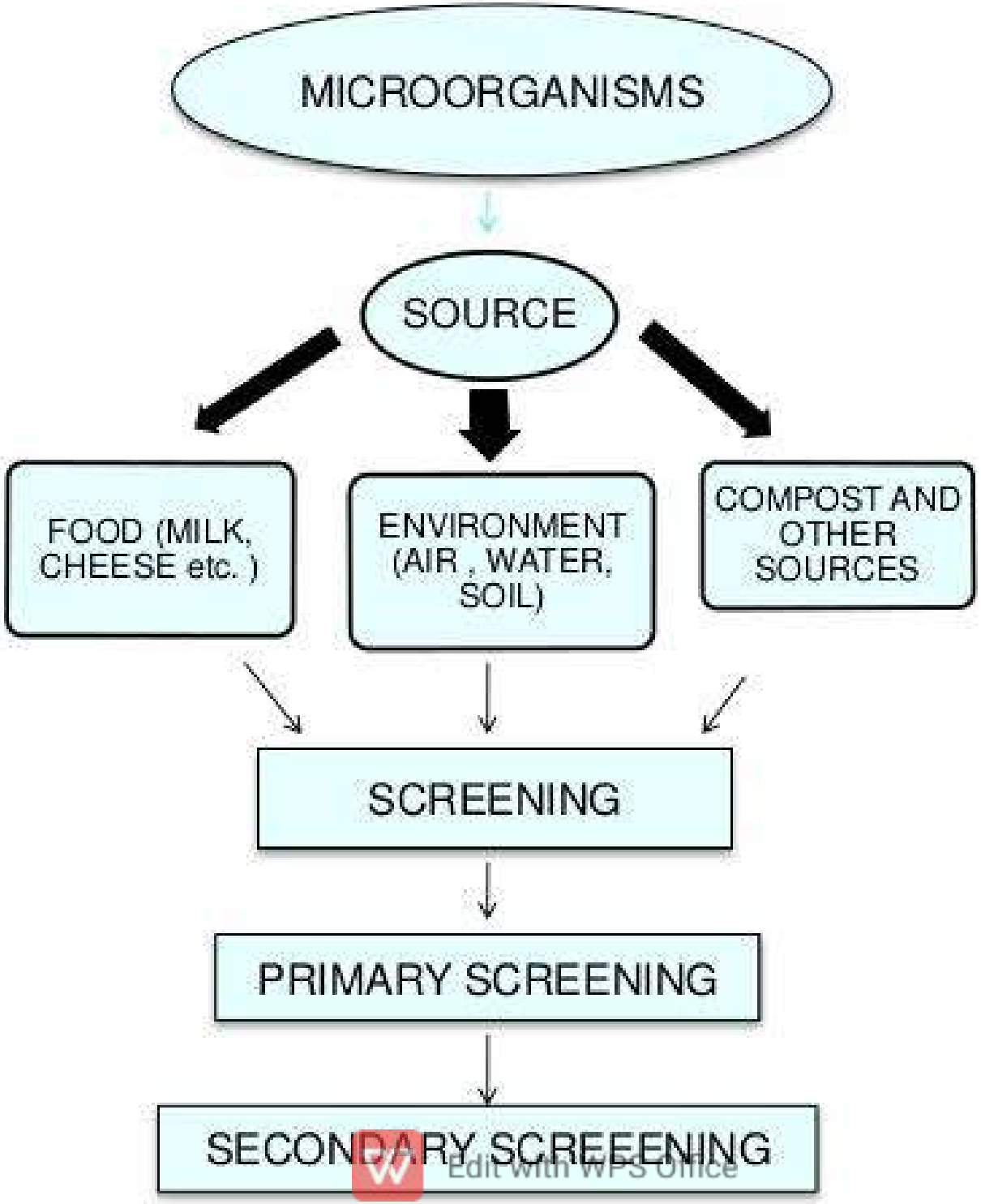
SCREENING



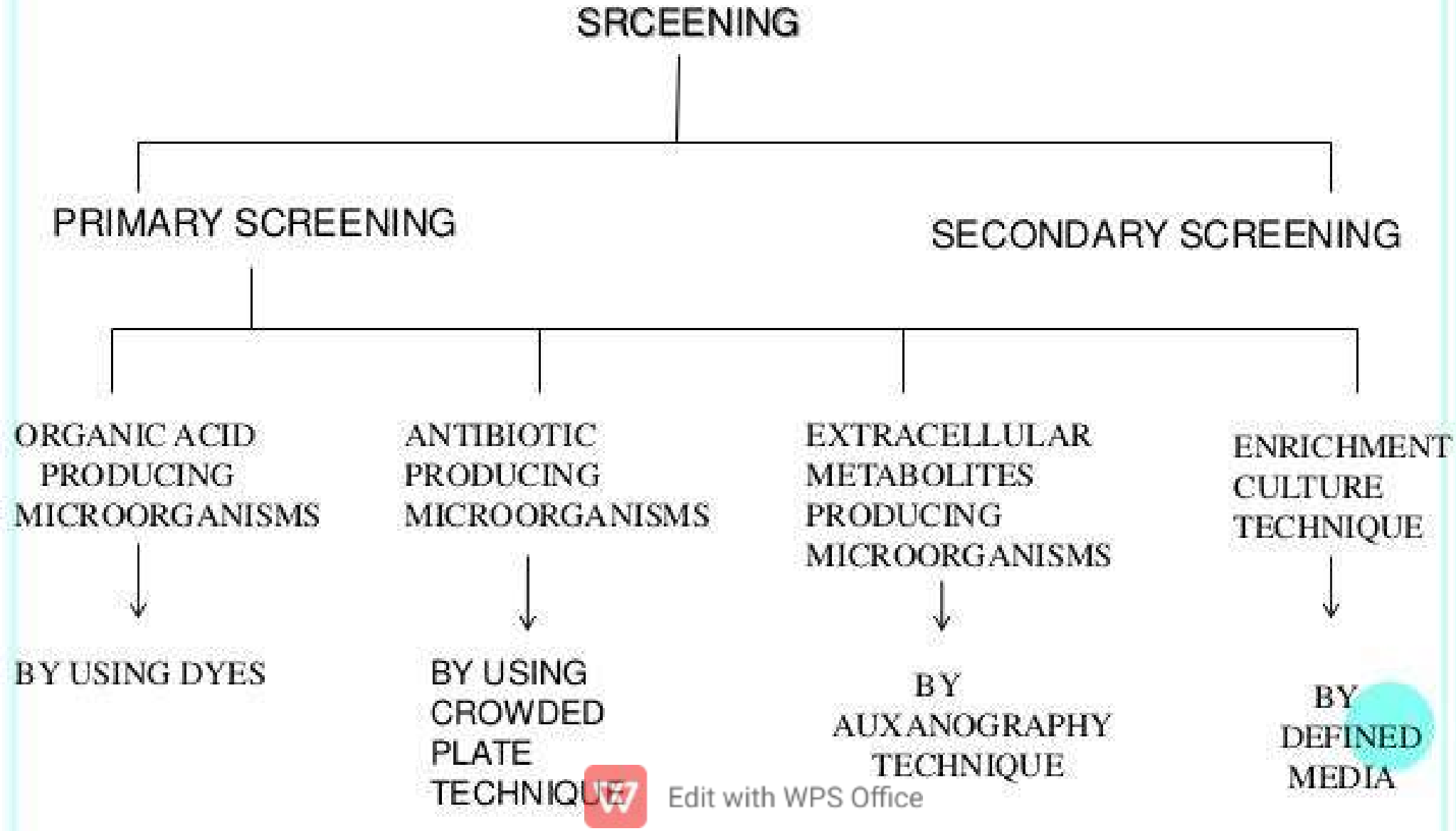
The procedure of isolation, detection , and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called SCREENING



IMPORTANT THINGS TO BE CONSIDERED WHILE SCREENING :-

- 1.) CHOICE OF SOURCE - Samples from screening is taken from soil, water, air, milk, compost etc.
- 2.) CHOICE OF SUBSTRATE -Nutrients and growth factors should be supplied for growth of desired microorganism.
- 3.) CHOICE OF DETECTION - Proper isolation and detection of desired microorganisms is important

TYPES OF SCREENING



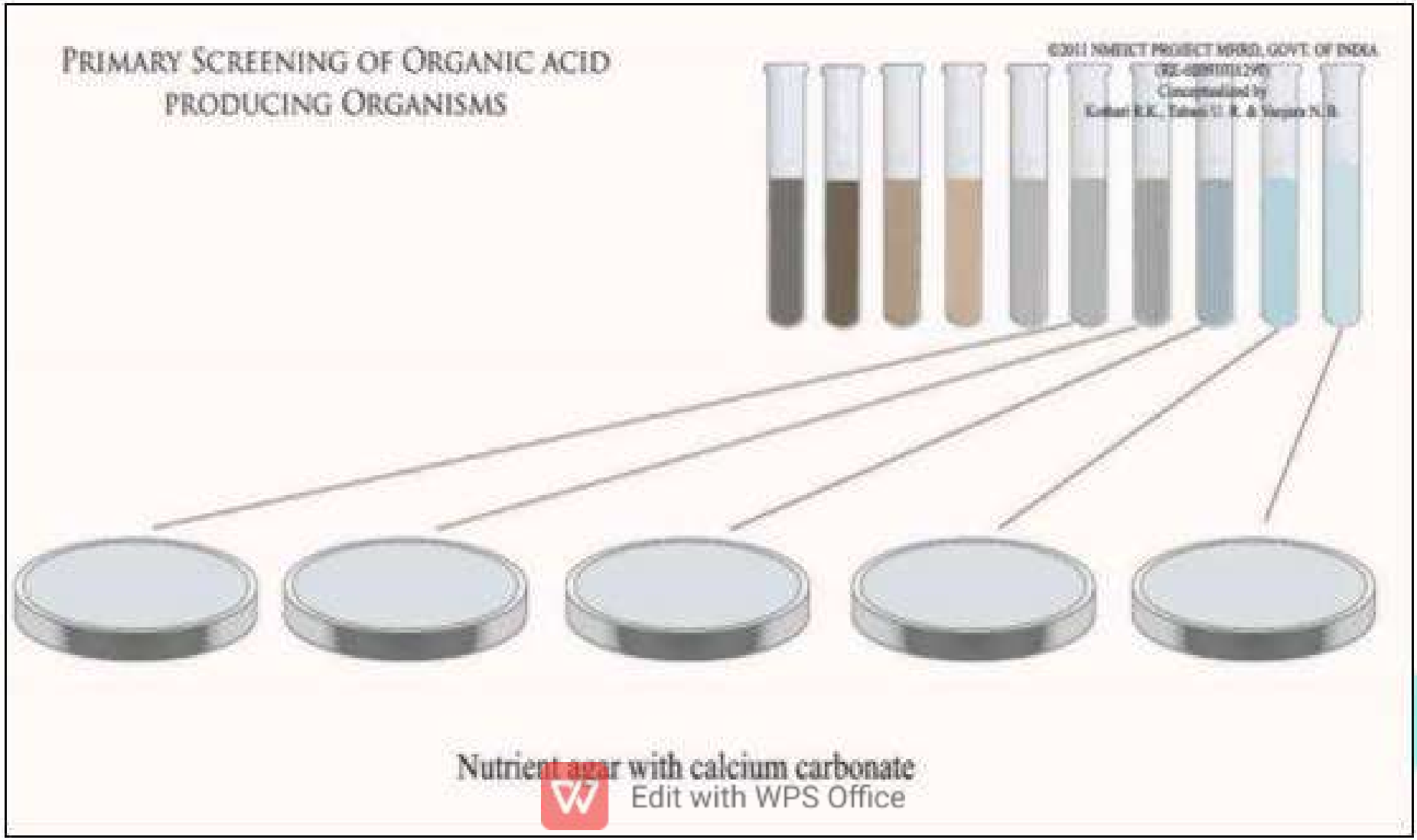
PRIMARY SCREENING

- It's a process for detection and isolation of microorganisms of our interest.
- Determines which microorganisms are able to produce a compounds.
- Does not provide much idea about the production or yield potential of microorganisms.
- It separate out only a few microorganisms, only few have commercial value while discards the valueless microorganisms .

1) PRIMARY SCREENING OF ORGANIC ACID PRODUCING MICROORGANISMS

- The ph indicating **dyes** may be used for detecting microorganism that are capable of producing organic acids.
- These dyes undergo color changes according to its ph.
- Dyes such as **Neutral red, Bromothymol blue** are added to the poorly buffered nutrient agar media .
- Colonies are subcultured to make stock culture.
- Further testing is needed since inorganic acids, bases are also metabolic products of microbial growth.

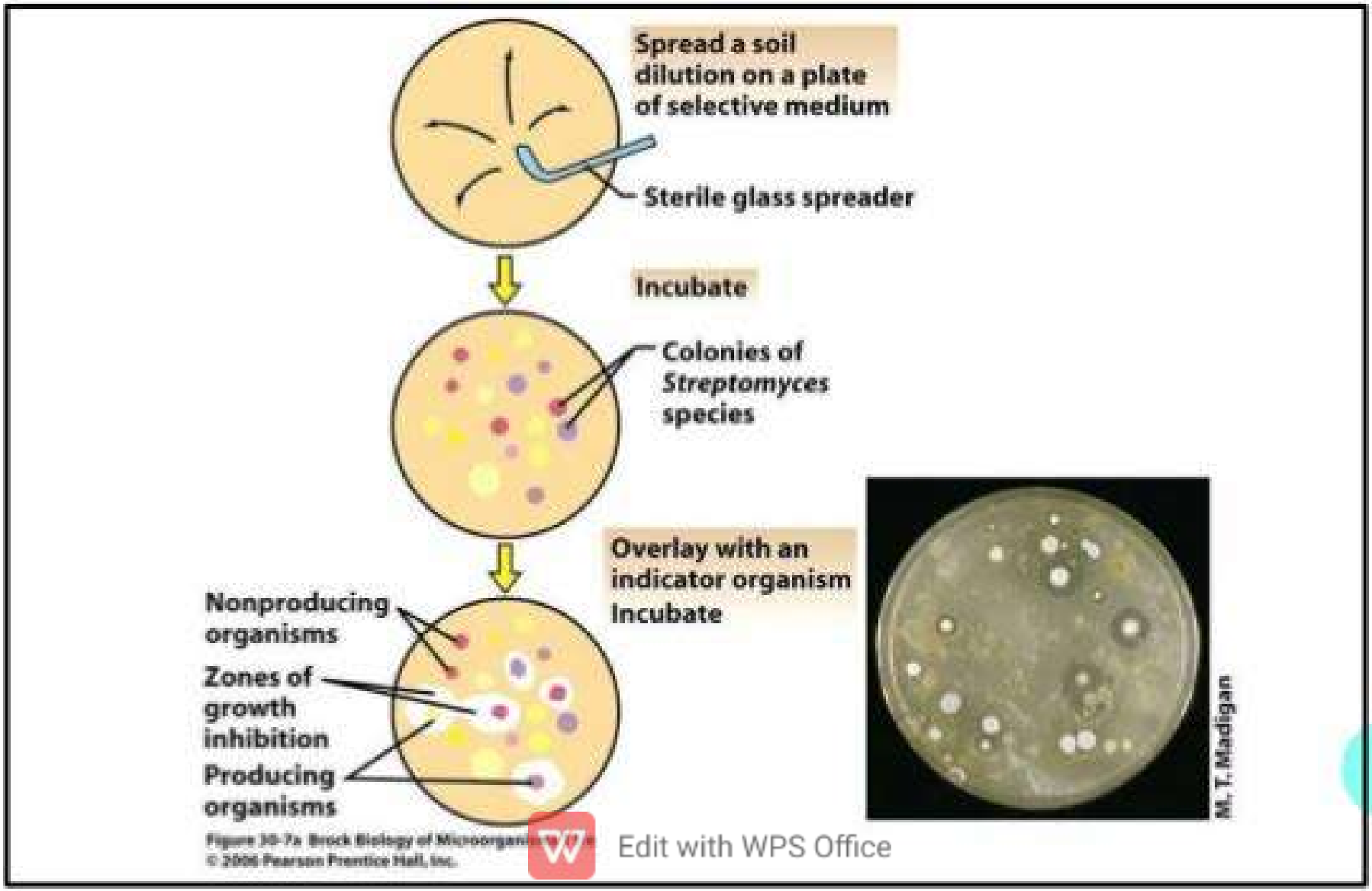
- Incorporation of $CaCO_3$ in medium is also used to screen organic acid producing microbes on basis of formation of clear zone of dissolved $CaCO_3$ around the colony.



2) PRIMARY SCREENING OF ANTIBIOTIC PRODUCING MICROORGANISMS

- Crowded plate technique is used for screening of antibiotic producing microorganisms.
- Does not give information about the sensitivity of antibiotics towards other microorganisms.
- Dilutions are made and then pouring and spreading of soil samples that give 300 to 400 or more colonies per plate.
- Colonies showing antibiotic activity are indicated by zone of inhibition around the colony .
- Such colonies are sub cultured and purified by streak before making stock cultures.

❖The purified cultures are then tested to find the Microbial Inhibition Spectrum.



3) PRIMARY SCREENING EXTRACELLULAR METABOLITE PRODUCING MICROORGANISM

- Auxanography technique is employed for detecting microorganisms able to produce growth factors , vitamins , amino acids etc. extracellularly.
- The 2 major steps are:-

A.)Preparation of first plate

- A filter paper strip is put across the bottom of petri dish.
- The nutrient agar is prepared and poured on the paper disc
- and allowed to solidify.
- Soil sample is diluted and proper dilutions are inoculated.

B.) Preparation of second plate

- A minimal media lacking the growth factors is prepared and seeded with the test organism.
- The seeded medium is poured onto fresh petri plate and the plate is allowed to set.

- The agar in first plate is then lifted and placed on the second plate without inverting.
- The growth factors produced on agar can diffuse into the lower layer containing test organism.
- The zones of stimulated growth of test organism around colonies is an indication that organism produce growth factor extracellularly.

4) ENRICHMENT CULTURE TECHNIQUE

- This was designed by Beijerinck to isolate the desired microorganism from heterogeneous microbial population.
- It consists of following steps :

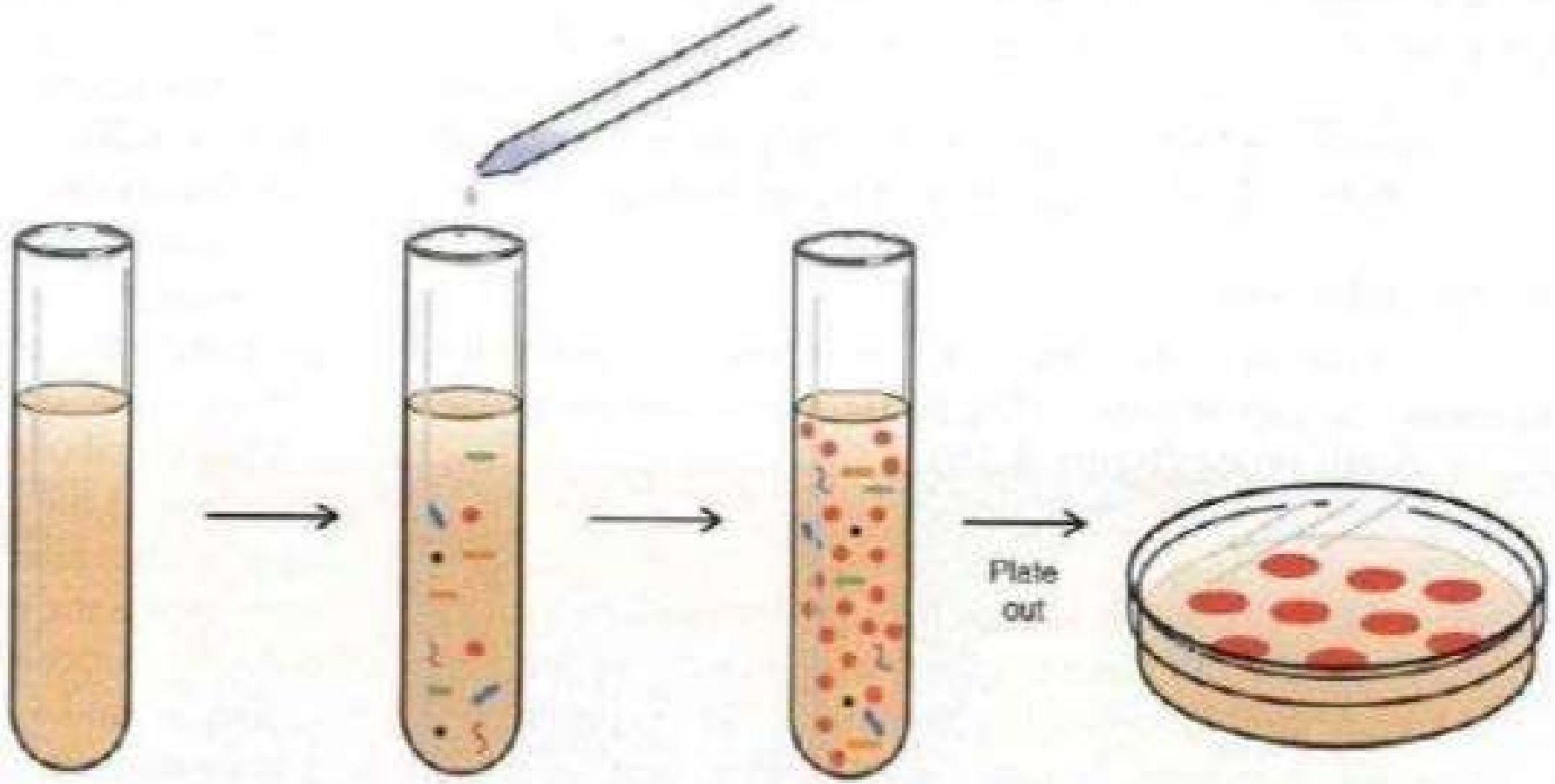
a.) Nutrient broth is inoculated with microbial source material and incubated.

b.) A small portion of all inoculums is plated onto the solid medium and well isolated colonies are obtained.

c.) Suspected colonies from the plate are sub cultured on fresh media and subjected for further testing.

Enrichment cultures

Isolating an organism from natural sources



SECONDARY SCREENING

It's a systematic screening programme intended to isolate industrially important or useful microorganisms .

SOME IMPORTANT POINTS ASSOCIATED WITH SECONDARY SCREENING ARE:-

- It is useful in sorting of microorganisms that have real commercial value. The microorganisms having poor applicability in fermentation process are discarded.
- Provides the information whether the product formed by microorganisms is new or not. This may be accomplished by paper , thin layer, chromatographic technique.

- It should show whether the product possess physical properties such as UV light absorption or fluorescence or chemical properties that can be employed to detect the compound during use of paper chromatography.
- It is conducted on agar plates, in flasks or in small fermentor containing liquid media.
- It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture.
- It helps in providing information regarding the product yield potentials of different isolates.
- It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.

- Chemical, physical and biological properties of a product are also determined during secondary screening. Moreover, it reveals whether a product produced in the culture broth occurs in more than one chemical form.
- It detects gross genetic instability in microbial cultures. This type of information is very important, since microorganisms tending to undergo mutation or alteration is some way may lose their capability for maximum accumulation of the fermentation products.
- It tells about the chemical stability of the fermentation product.
- It can be qualitative or quantitative in its approach.

EXAMPLE OF SECONDARY SCREENING – ANTIBIOTIC PRODUCNING STREPTOMYCES SPECIES

1. Streptomyces isolates are streaked as a narrow band on nutrient agar plates are incubated .
2. Test organisms are then streaked from the edge of plates without touching streptomyceal isolate and then the plates are then incubated .
3. At the end of incubation, growth inhibitory zones for each organism are measured in millimeters .
4. Such organisms are again subjected for further testing by growing the culture in sterilized liquid media and incubated at constant temperature in a mechanical shaker.

Thank You....!

Somatic Embryogenesis

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Somatic Embryogenesis:

- Somatic embryogenesis is a process by which somatic cells or tissues, including haploid cells develops into differentiated embryos and to regenerate plants.

- Stewart et al., (1958): First induced embryo through suspension culture in carrot.

- Reinert (1959): Produce embryo from callus in carrot through suspension culture.

Types of Embryos:

1. Zygotic Embryos:

- These formed by fertilized egg or the zygote.

2. Non-Zygotic Embryos:

a) Somatic Embryos:

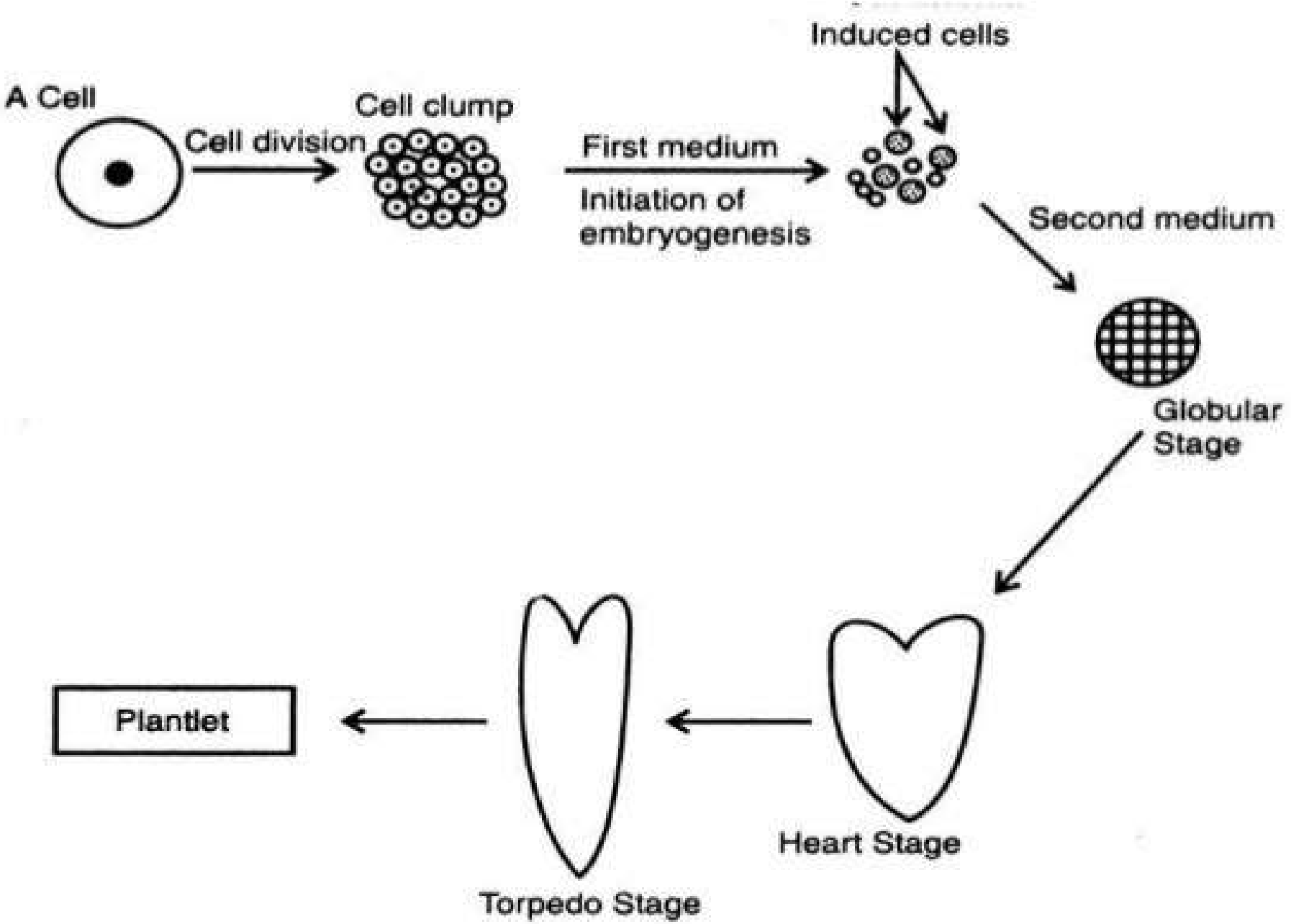
- Those formed by Sporophytic cells in in-vitro condition. Such somatic embryos arsing directly from other embryos or organs are termed adventive embryos.

b) Parthenocapic Embryos:

- Those formed by unfertilized egg.

c) Androgenic Embryos:

- Those formed by the male gametophyte.



Importance of Somatic Embryogenesis:

- Higher propagation rate.
- Suitable in Suspension culture.
- Artificial seed production.
- Labour savings.

STAGES OF SOMATIC EMBRYOGENESIS

- Somatic embryogenesis encompasses various stages such as
- 1. Callus initiation
- 2. Embryo development and maturation
- 3. Plantlet formation

Somatic Embryo Germination Media:-

- MS medium: BAP (0, 1, 2, 3, 4and 5 mg/l),
  NAA (0, 0.5, 1.0, 1.5, 2.5 and 4.0 mgL-1)

- Media were kept in the incubation room 25±2°C with 16 hrs of light provided by fluorescent bulbs and a light intensity of 16.75 µmolm^{-2}s^{-1} for eight weeks.

- Calculation: Callus induction frequency(%)
  Regeneration frequency(%).

Types of Somatic Embryogenesis:-

- Two types of somatic embryogenesis

- Direct somatic embryogenesis
  - The embryos initiate directly from explants in the absence of callus formation. Embryos are formed due to PEDCs cell.

- Indirect somatic embryogenesis
  - Callus from explants takes place from which embryos are developed. Embryos are formed due to IEDCs cells.

ADVANTAGES

- It is observable, as its various culture conditions can be controlled.
- Lack of material is not a limiting factor for experimentation.
- High propagation rate.
- Somaclonal variations.
- Germplasm conservation.
- Labour saving.
- Elimination of diseases and viruses.

DISADVANTAGES

- Confined to few species.
- The somatic embryos show very poor germination because of their physiological and biochemical immaturity.
- Instability of cultured cells in long-term cultures is a major limitation in commercial exploitation and mass propagation of SEs.

Factors Affecting Somatic Embryogenesis

- Explant
- Genotype
- Growth Regulators
- Nitrogen Source
- Polyamines

THANK YOU