PPT bank by Supriya Potdar

1. Suspension culture
2. Nucleic acid database
3. Lab organization Plant Tissue culture
4. Introduction to ptc
5. Clonal Propagation

Clonal propagation (mocropropagation)

Ms . Supriya D. Potdar
Assistant Professor
Department of Biotechnology
Vivekanand College (Autonomous),
Kolhapur

Micropropagation

- **Plant Tissue Culture technique:**

Increasingly popular alternative means --→ Plant vegetative propagation

- Plants can be propagated by sexual (through generation of seeds) or asexual (through multiplication of vegetative parts).

- **Plant tissue culture** involves –
  - Asexual method of propagation
  - Primary goal --→ crop improvement

- *In-vitro* selection, genetic manipulation techniques --→ Higher plant

Clonal Propagation

Clonal propagation -------→ *In-vitro* selection **Micropropagation**

Greek word ---→ Clone ---→ Twig ----→ Identical copies

- The process of multiplication of genetically identical copies of individual plants by asexual reproduction is called **Clonal Propagation**
- The term clone is used to represent a plant population derived from a single individual by asexual reproduction.

Process --→ Asexual reproduction ---→ Multiplication occur -----→ Individual plants genetically identical copies

- Multiplication of genetically, identically individual by asexual reproduction
- Apomixes (seed development without meiosis and fertilization) and vegetative propagation (regeneration of new plants from vegetative parts)
- Short time

- Rapid process --→ Commeralization of improvement plants.

Eg: Apple, pear etc.

- Asexual reproduction through multiplication of vegetative parts is the only method for the *in-vivo* propagation of certain plants, as they do not produce viable seeds.

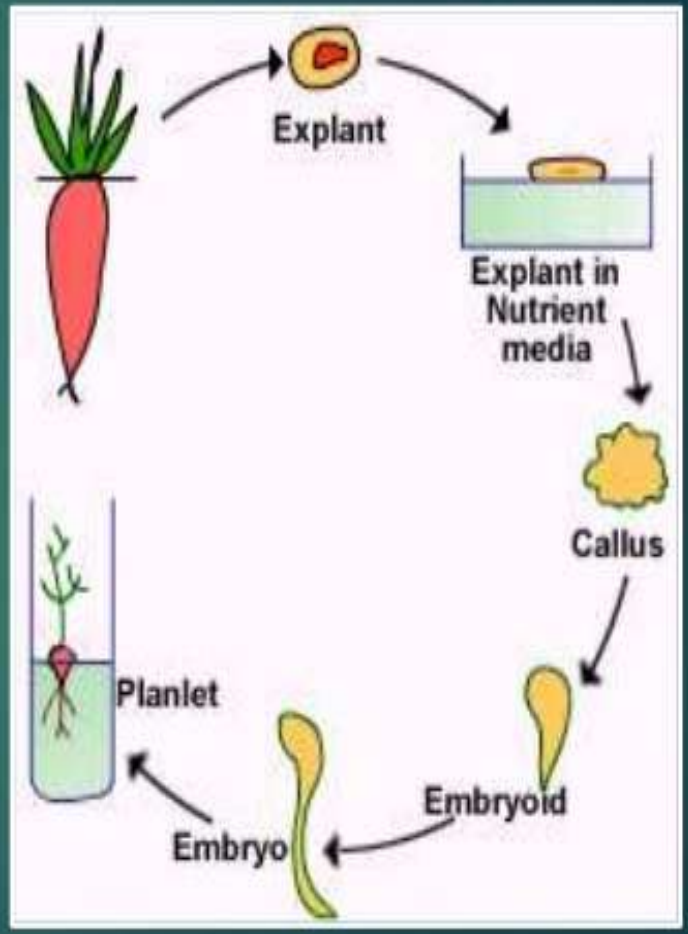
Eg: Banana, Grape, Fig and Chrysanthemum.

- Clonal propagation has been successfully applied for the propagaton.

Eg: Apple, Potato, Tuberous and several Ornamental plants

Micropropagation

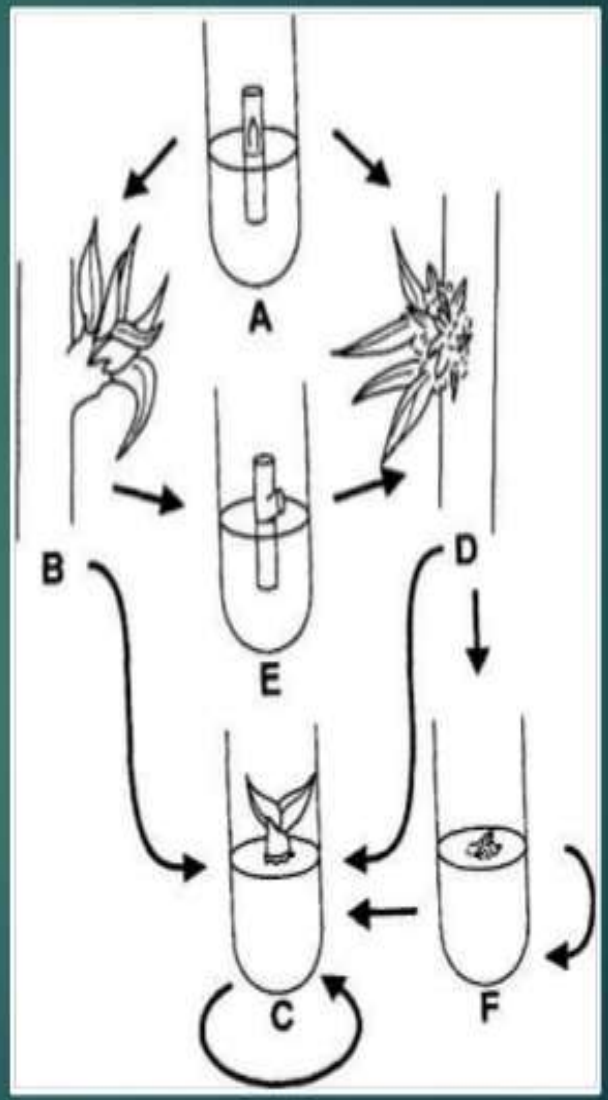
- Aseptic method of clonal propagation is called as **Micropropagation**
- **Synonyms --→ tissue culture**
- Rapid multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods
- Widely used for ***Orchids, Ferns, many interior foliage plants etc.***



In-vitro Clonal Propagation

Use of tissue culture technique for micropropagation was first started by Morel (1960) for propagation of orchids, and is now applied to several plants.

Micropropagation is a handy technique for rapid multiplication of plants



Selection of Plant Material

- Part of plant
- Genotype
- Physiological condition
- Season
- Position on plant
- Size of explants

Stages of Micropropagation

Stage 0: Preparation of the mother plant

Stage 1: Initiation and establishment of cultures

Stage 2: Multiplication [shoot or rapid somatic embryo formation]

Stage 3: *In-vitro g*ermination of somatic embryo and rooting of shoots

Stage 4: Transfer of plantlets to sterilized soil for Hardening under greenhouse/ field conditions (transplantation)

Stages of Micropropagation

Stage 0: Preparatory stage

Involves the preparation of mother plants to provide quality explants for better establishment of aseptic cultures

- Maintained light, temperature and moisture regimes under which the mother plants
- Loosely covering growing branches with polythene
- Application of growth regulators
- Watering of plant from the base

Stages of Micropropagation

Stage 1: Initiation of Cultures

- Explant selection
- Sterilization treatment
- Choice of growth medium

Stage 2: Multiplication

Through Callusing

Initiation of culture on high Auxin media

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Development of unorganized mass of cells called callus

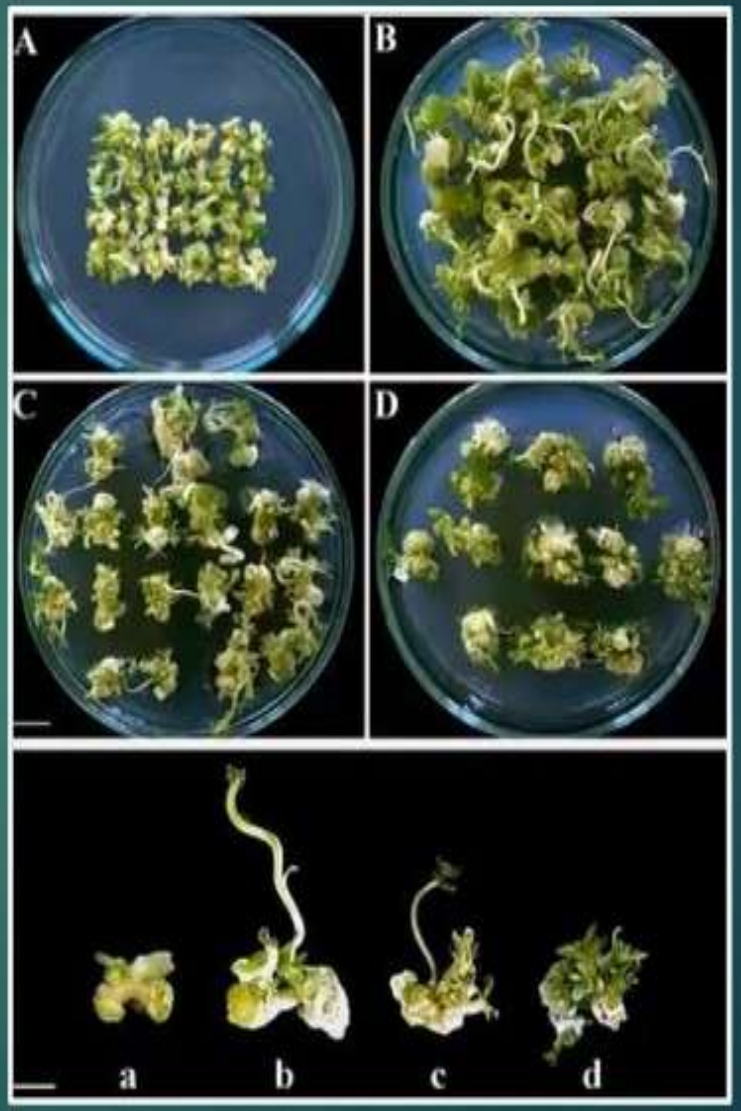
↓

Transfer small piece of callus on regeneration media

↙ ↘

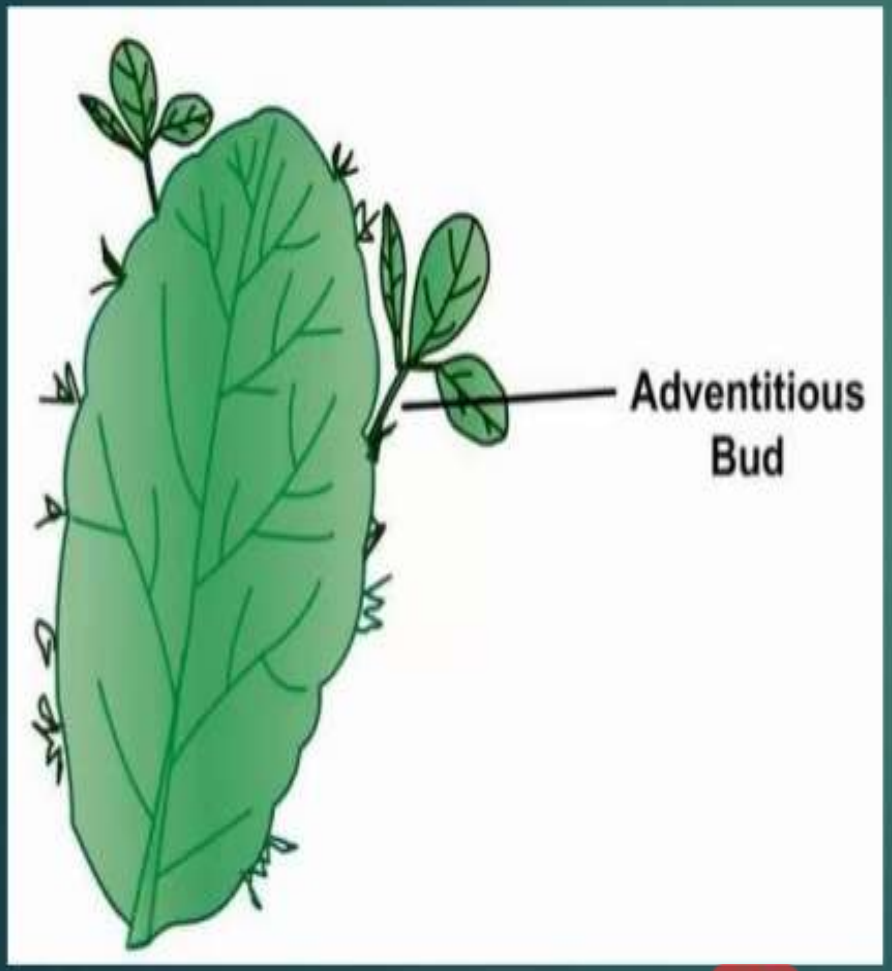
Shoot bud regeneration

Somatic embryo



Stage 2: Multiplication

Adventitious Bud Formation

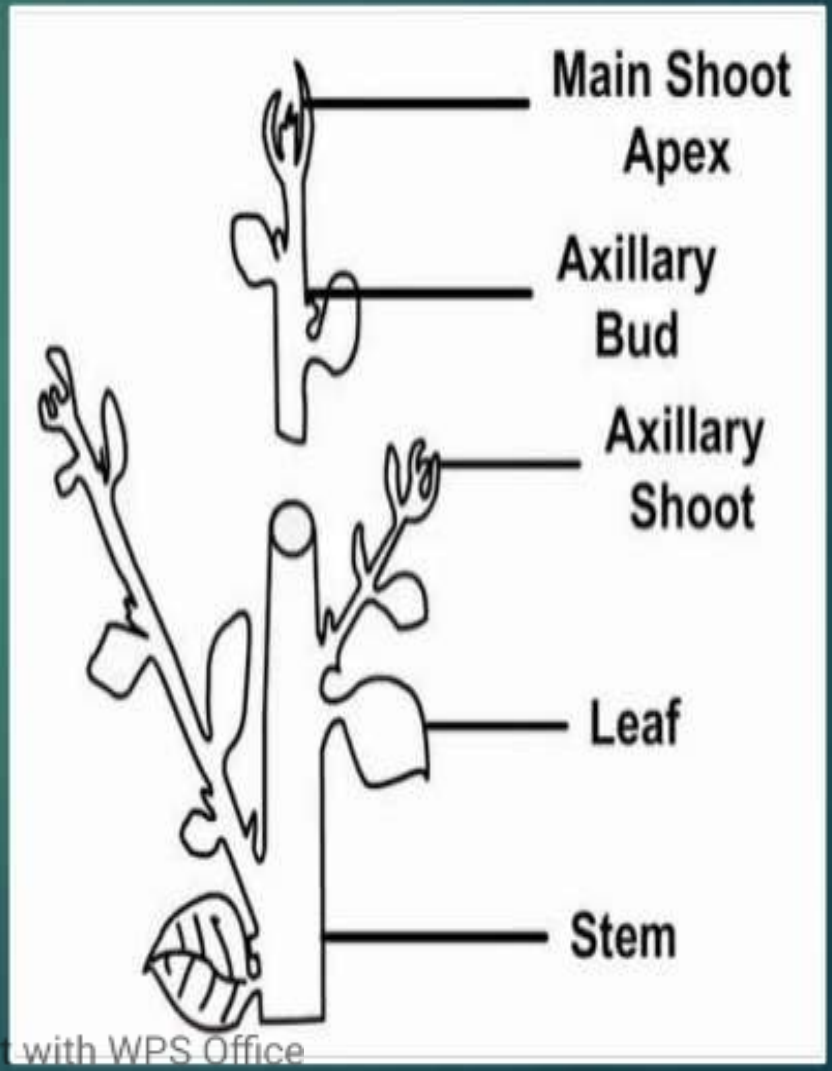


- Transfer to proliferation media
- Shoots can be constantly divided

Stage 2: Multiplication

Enhanced Axillary Branching

- Small bud present
- Apical dominance is removed
- Cytokinins
- Auxins
- Cluster formation
- Off types



Stage 3: Rooting of shoots

Rooting & Hardening

- Transfer of shoots to a medium for rapid development into shoots
- Sometimes, the shoots are directly planted in soil to develop roots
- *In-vitro* of shoots is preferred while simultaneously handling a large number of species

Stage 3: Rooting of shoots

Rooting & Hardening

- Somatic embryos carry a preformed radical and may develop directly into plantlet.
- Embryos sow very poor conversion into plantlets, especially under *in-vitro* conditions
- Require an additional step of maturation to acquire the capability for normal germination
- Adventitious and axillary shoots developed in cultures in the presence of a cytokinins generally lack roots
- Obtain full plants the shoots must be transferred to a rooting medium which is different from the shoot multiplication medium, particularly in its hormonal and salt compositions
- For rooting, individual shoots measuring 2 cm in length are excised and transferred to the rooting medium

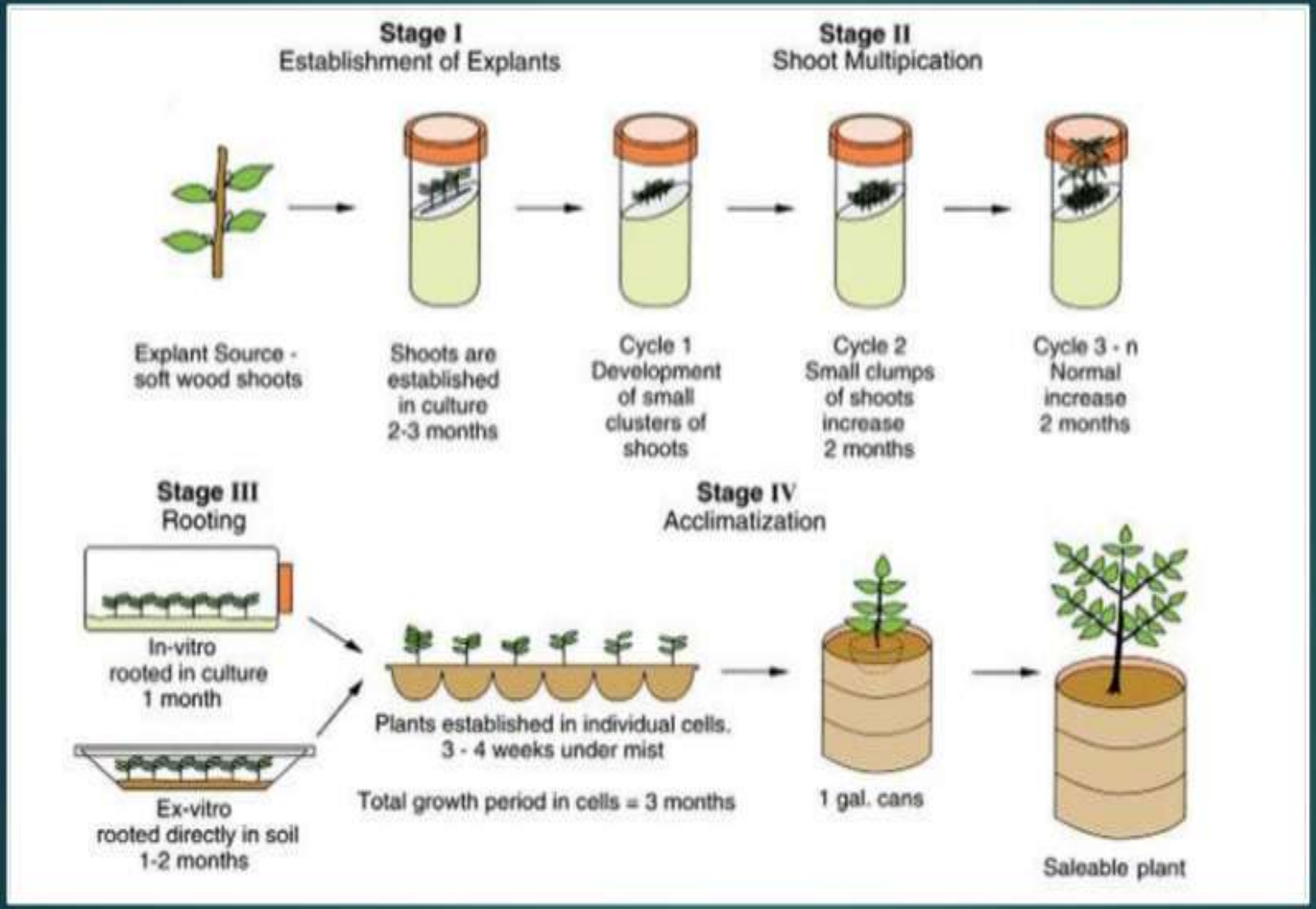
Stage 4: Transplantation & Accilimitization

Transferring the plantlets of stage 3 from the laboratory to the environment f green house.

Some plant species, stage 3 skipped, and unrooted stage 2 shoots are planted in pots or in suitable compost mixture

Tissue culture raised plants are characterized by:

- Low photosynthetic rates
- Non-functional stomata
- Low cuticular wax
- Abnormal leaf morphology
- Accilimitization
- Green house
- 2 weeks
- Temperature (±10º)



Stages of Micropropagation

Methods of Micropropagation

- ***Organogenesis***
  - Organogenesis via callus formation
  - Direct adventitious organ formation
- ***Embryogenesis***
  - Direct embryogenesis
  - Indirect embryogenesis
- ***Microcutting***
  - Meristem culture (Mericloning)
  - Bud culture

Applications of Clonal Propagation

- Production of disease and virus free plantlets
- Production of seeds in some crops
- High rate of plant propagation
- Cost effective process
- Automated micropropagation

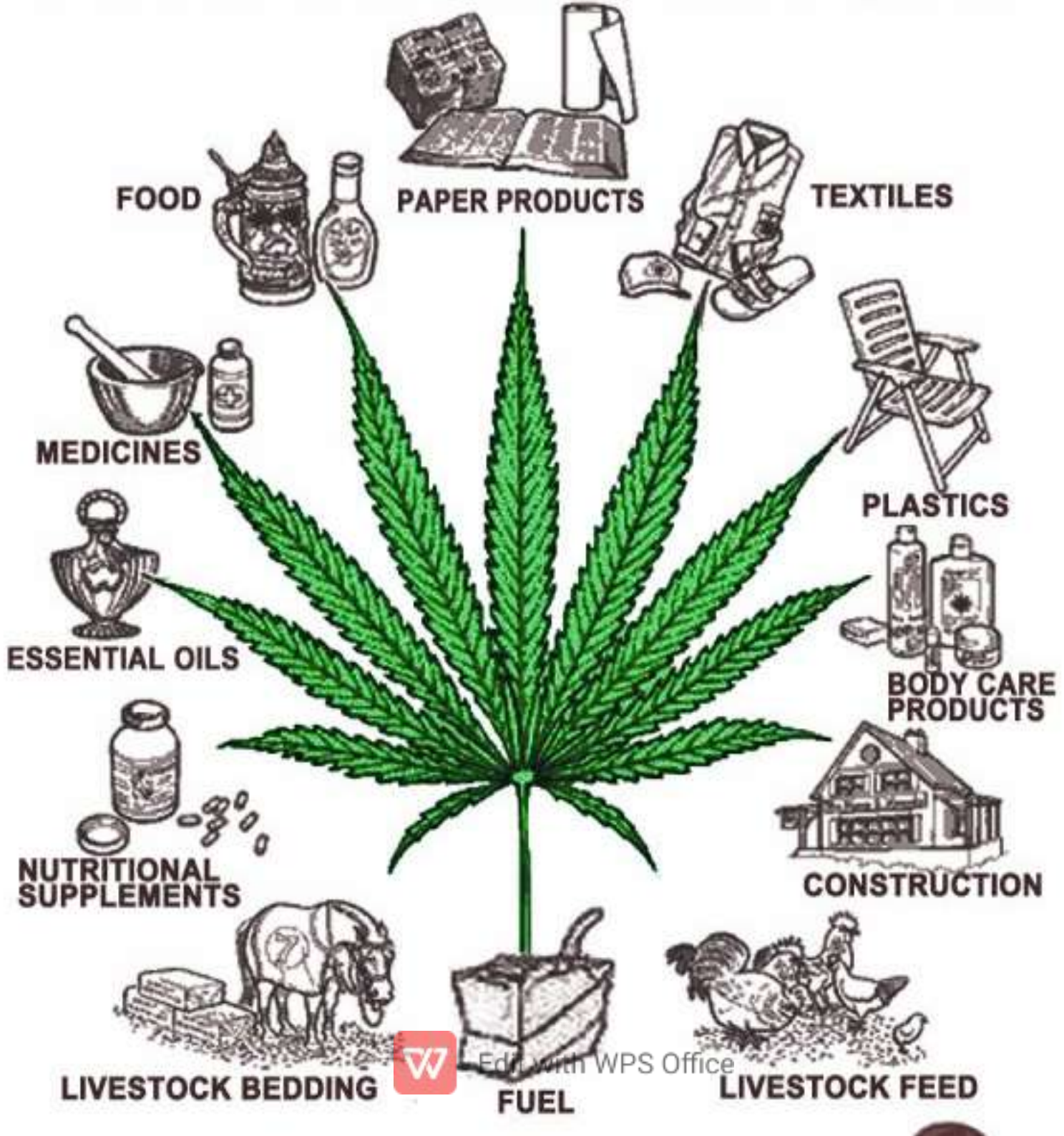
Artificial Seeds from Somatic Embryo

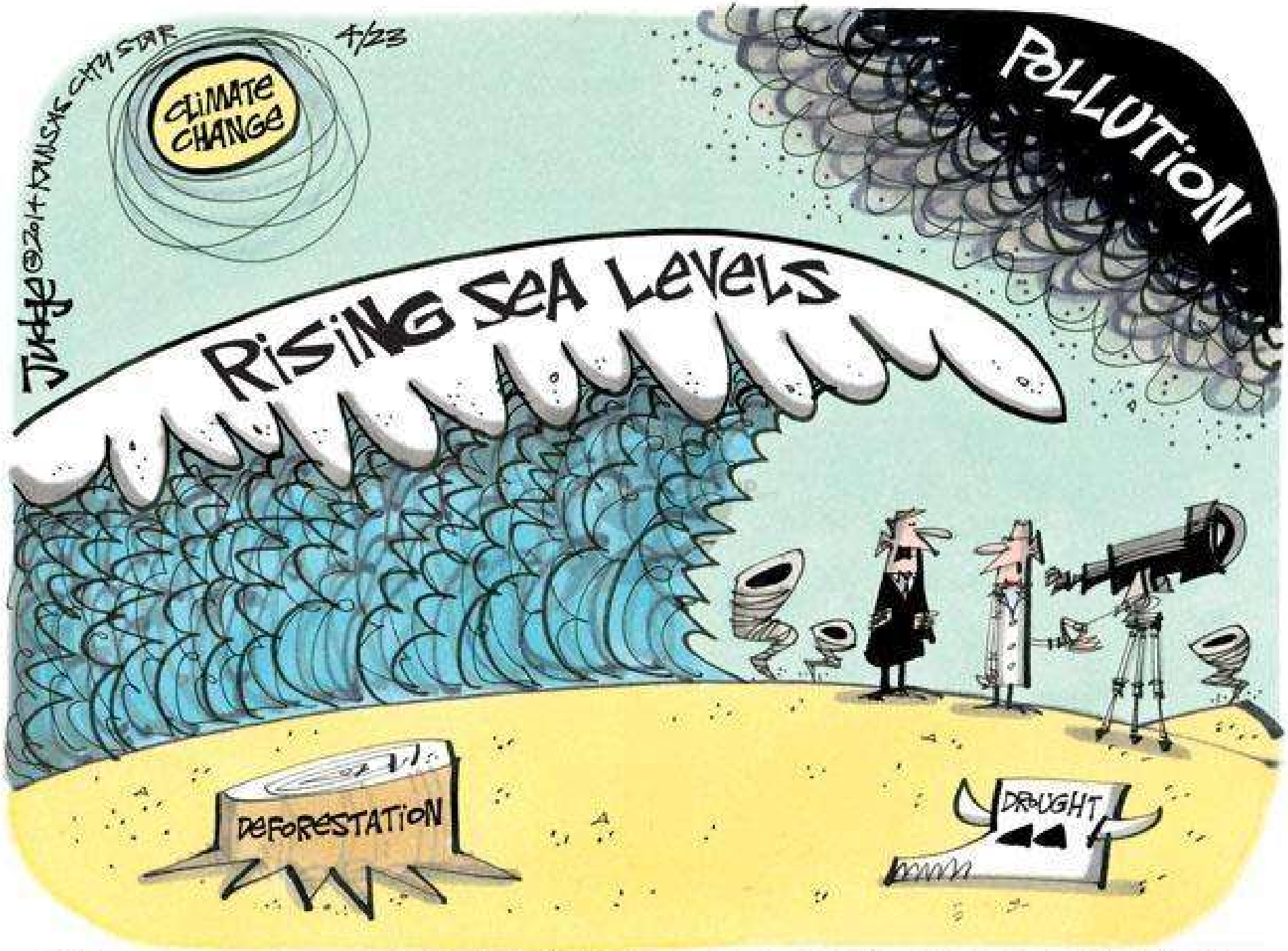
- Artificial seeds can be made by encapsulation of somatic embryos.
- The embryos, coated with sodium alginate and nutrient solution, are dipped in calcium chloride solution.
- The calcium ions induce rapid cross-linking of sodium alginate to produce small gel beads, each containing an encapsulated embryo.
- These artificial seeds (encapsulated embryos) can be maintained in a viable state till they are planted

THANK YOU……!

Introduction to Plant tissue culture

Ms. Supriya D. Potdar
Assistant Professor
Vivekanand College, Kolhapur (Autonomous)





"WHY ALL THIS INTEREST IN FINDING A HABITABLE PLANET?"

IS THEIR ANY SOLUTION TO THIS ?

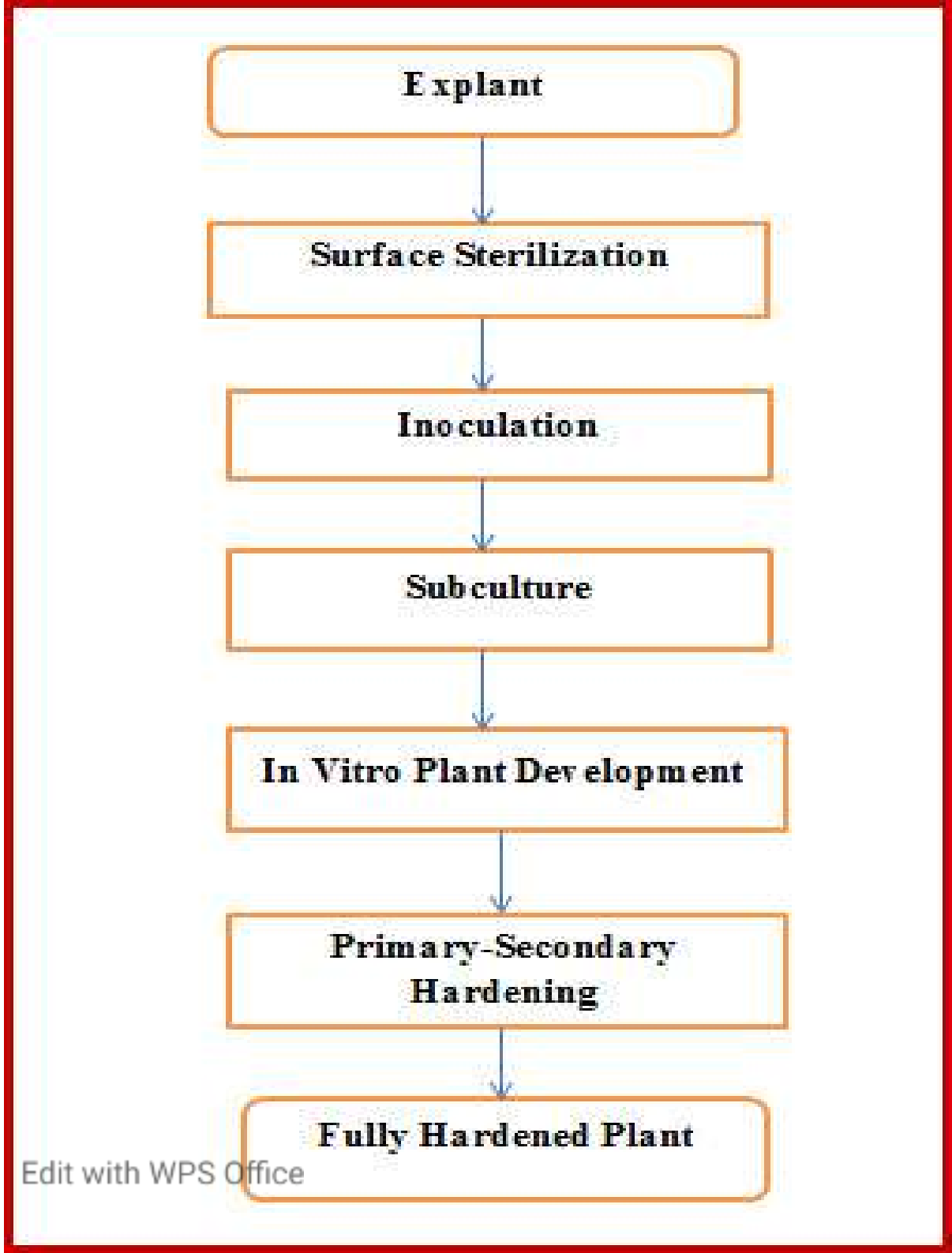
IS THEIR ANY ALTERNATIVE ?

PLANT TISSUE CULTURE

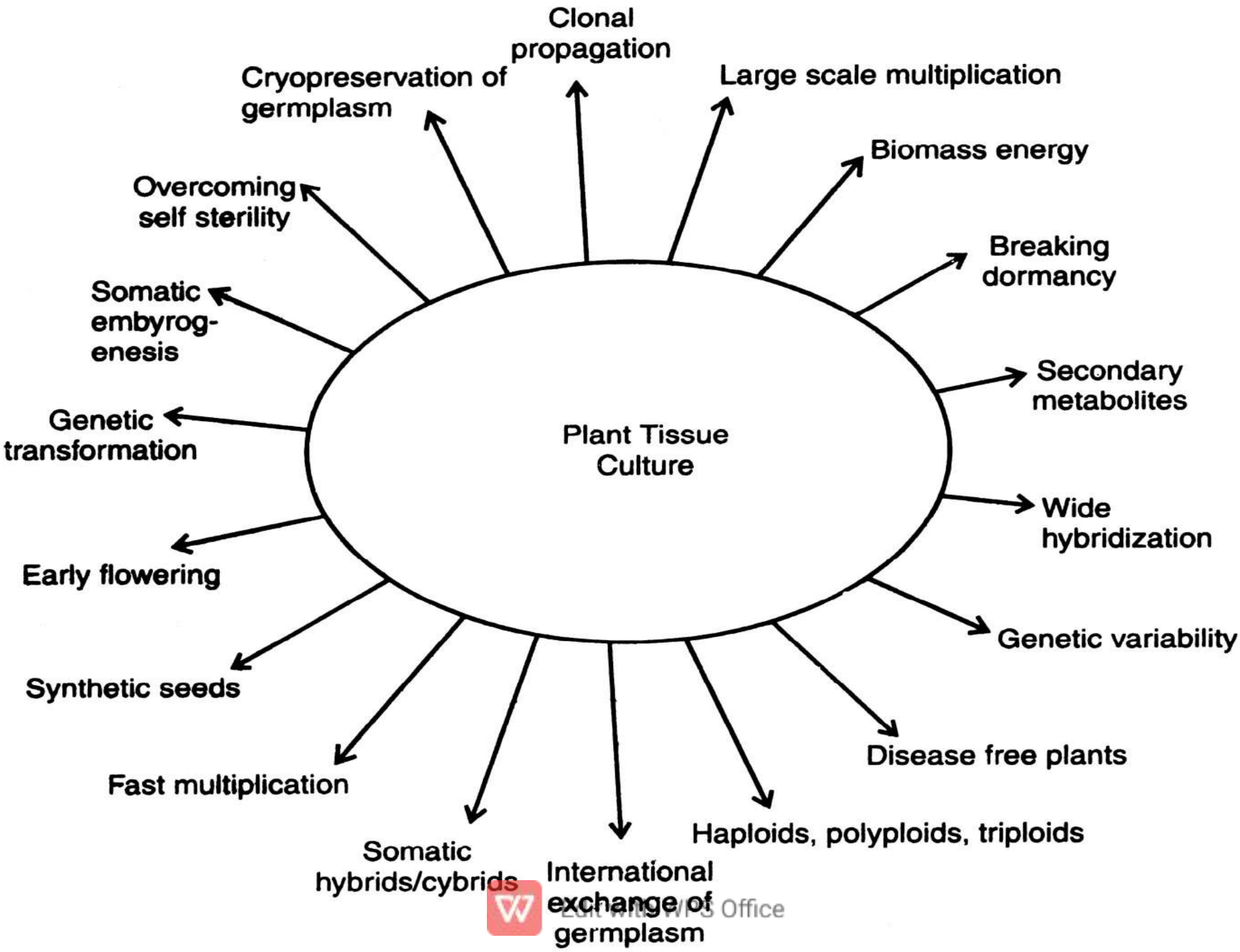
WHAT IS PTC?

- **Plant tissue culture** is **culturing plant** seeds, organs, explants, tissues, cells, or protoplasts on a chemically **defined** synthetic nutrient media under sterile and controlled conditions of light, temperature, and humidity.

How it works?



WHAT ARE THE APPLICATIONS ?



LABORATORY ORGANIZATION

Ms. S.D.Potdar
Vivekanand College(Autonomous),
Kolhapur

Any laboratory, in which tissue culture techniques are performed, regardless of the specific purpose, must contain a number of basic facilities. These includes:

- A general washing area
- A media preparation, sterilization, and storage area
- An aseptic transfer area
- culture rooms, incubation area
- Acclimatization & Hardening

WASHING AREA

- large sinks(lead-lined to resist acids & alkalis)
- Tap water (Hot & Cold)
- Brushes of various sizes
- Space for drying & draining- boards or Racks
- Storage (cupboards)
- Distillation Unit

MEDIA PREPARATION, STERILIZATION, AND STORAGE AREA

- Cupboard for chemicals
- culture vessels and closures & glassware
- Bench space for hot plates/stirrers, pH meters, balances, water baths, and media-dispensing equipment.
- Bunsen burners with a gas source
- refrigerators and freezers
- a microwave or a convection oven, and an autoclave or domestic pressure cooker

ASEPTIC TRANSFER AREA

- Under very clean and dry conditions, tissue culture techniques can be successfully performed on an open laboratory bench. However, it is advisable that a laminar flow hood or sterile transfer room be utilized for making transfers. Within the transfer area there should be a source of electricity, gas, compressed air.

LAMINAR AIR FLOW CABINET

CULTURE ROOM

- Typically, the culture room for growth of plant tissue cultures should have a temperature25 ±3, with a temperature fluctuation of less than ±0.5°C.
- Humidity almost 100%
- The culture room should have enough fluorescent lighting to reach the 10,000 lux; the lighting should be adjustable in terms of quantity and photoperiod duration.

ACCLIMATIZATION & HARDENING

- Adaptation of plants to natural environment.
- Polyhouses for acclimatization.
- Introduction of natural soil environment to tissue cultured plant.

THANK YOU.....

Nucleic Acid Databases

MISS.S. D. POTDAR
ASST. PROF
DEPT. OF BIOTECHNOLOGY

Content

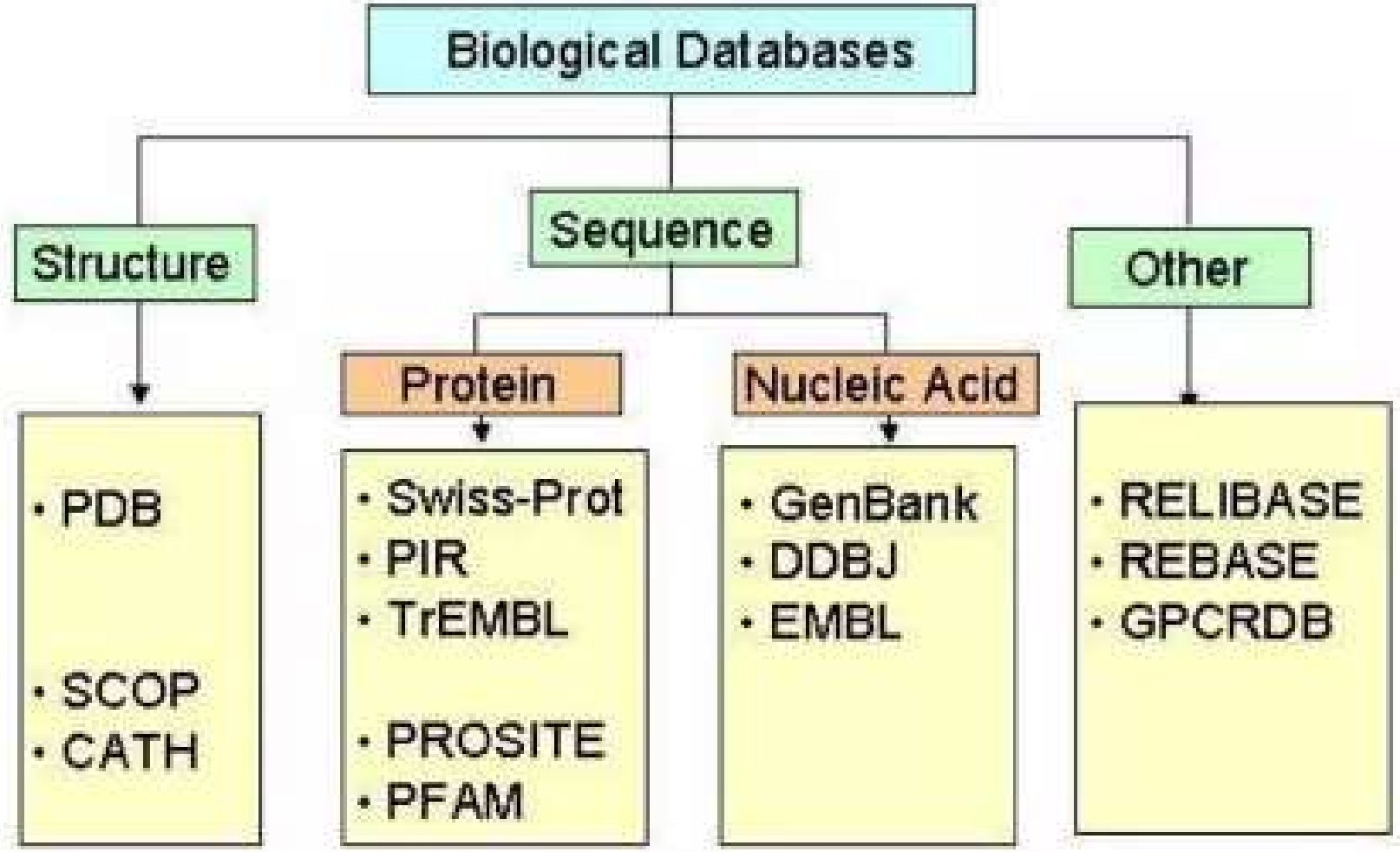
- ✓ INTRODUCTION: BIOLOGICAL DATABASES
- ✓ NUCLEIC ACID SEQUENCE DATABASES
  - GENBANK
  - EMBL
  - DDBJ
- ✓ CONCLUSION

BIOLOGICAL DATABASES:

- libraries of life sciences information
- collected from scientific experiments
- published literature
- high-throughput experiment technology
- computational analyses

- INFORMATIONS including genomics, proteomics, metabolomics, microarray gene expression, and phylogenetics.

- Biological database design, development, and long-term management is a core area of the discipline of bioinformatics.



Nucleic Acid Sequence databases

- There are three major sites for finding information about nucleic acids (DNA and/or RNA sequences) on the Web, and all of them contain basically the same information.

- The methods and databases that you will want to use will depend mainly on how much data you want and in what form.

- The databases EMBL, GenBank, and DDBJ are the **three primary nucleotide sequence databases**:

- They include sequences submitted directly by scientists and genome sequencing group, and sequences taken from literature and patents.

- There is comparatively little error checking and there is a fair amount of redundancy

- The entries in the EMBL, GenBank and DDBJ databases are **synchronized** on a daily basis, and the accession numbers are managed in a consistent manner between these three centers.

- The nucleotide databases have reached such large sizes that they are available in **subdivisions** that allow searches or downloads that are more limited, and hence less time-consuming.

- For example, GenBank has currently 17 divisions.

- There are **no legal restrictions** on the use of the data in these databases. However, there are patented sequences in the databases.

GENBANK OR NCBI:

- For most sequence searches, GenBank is your best bet.

- It offers a daily exchange of information with other major sequence databases, has a variety of user interfaces, fairly detailed online help (with e-mail addresses for more information if what is already available is not sufficient), and a speedy interface.

- Because of its popularity, however, GenBank can also be very slow during peak research hours.

- Very detailed searches or searches with massive amounts of output might be completed more quickly after hours.

- Established by the National Center for Biotechnology Information (NCBI), GenBank is a collection of all known DNA sequences from scientists around the world.

- As of July 1, 1996, approximately 286,000,000 bases and 352,400 sequences are stored in GenBank, and many more are added each day.

- **Searching GenBank** is fairly straightforward and can be done with a variety of search tools.

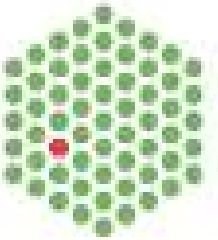
- if you have never used GenBank before, you will probably want to start your search with a general query.

- Other means of searching GenBank include:
  - BLAST (Basic Local Alignment Search Tool) Searches
  - dbEST (Database of Expressed Sequence Tags)
  - dbSTS (Database of Sequence Tagged Sites)

- **Submitting sequences to Gen Bank** is also very easy and is required by most journals before articles pertaining to the sequence are published (this provides easy access to the information for the journal's readers).

- You can submit sequences via the WWW with BankIt.

EMBL:



- EMBL is good to use when you need a limited amount of data and when you are not trying to identify a gene by sequence analysis.

- However, because EMBL and all of its mirror sites are located in Europe, your connection will be slow more often than not.

- All of the information submitted to EMBL is mirrored daily in both GenBank and DDBJ, so searching elsewhere might provide the same amount of information in less time.

- EMBL is the database for the European Molecular Biology Laboratory.

- It is a flat-file database that is searched by a multitude of various search engines.

- EMBL sequences are stored in a form corresponding to the biological state of the information in vivo.

- Thus, cDNA sequences are stored in the database as RNA sequences, even though they usually appear in the literature as DNA.

DDBJ:

DDBJ
DNA Data Bank of Japan

- Because DDBJ mirrors its information daily with GenBank and EMBL, beginning sequence searchers might want to try a database with a friendlier searching interface.

- However, DDBJ also offers all of its pages in Japanese as well, so if you are more comfortable reading the Japanese versions of the pages, it can be very useful.

- DDBJ, the DNA Data Bank of Japan, was established in 1986 to be one of the major international DNA Databases (with GenBank and EMBL).

- It is certified to collect information from researchers and assign accession numbers to submitted entries.

- SEARCHING DDBJ is some what awkward as the only way to access most of the data is by its accession number via anonymous FTP.

Conclusion:

- Biological databases are an important tool in assisting scientists to understand and explain a host of biological phenomena from the structure of biomolecules and their interaction, to the whole metabolism of organisms and to understanding the evolution of species.

- This knowledge helps facilitate the fight against diseases, assists in the development of medications and in discovering basic relationships amongst species in the history of life.

Thank you....!!!

Suspension Culture

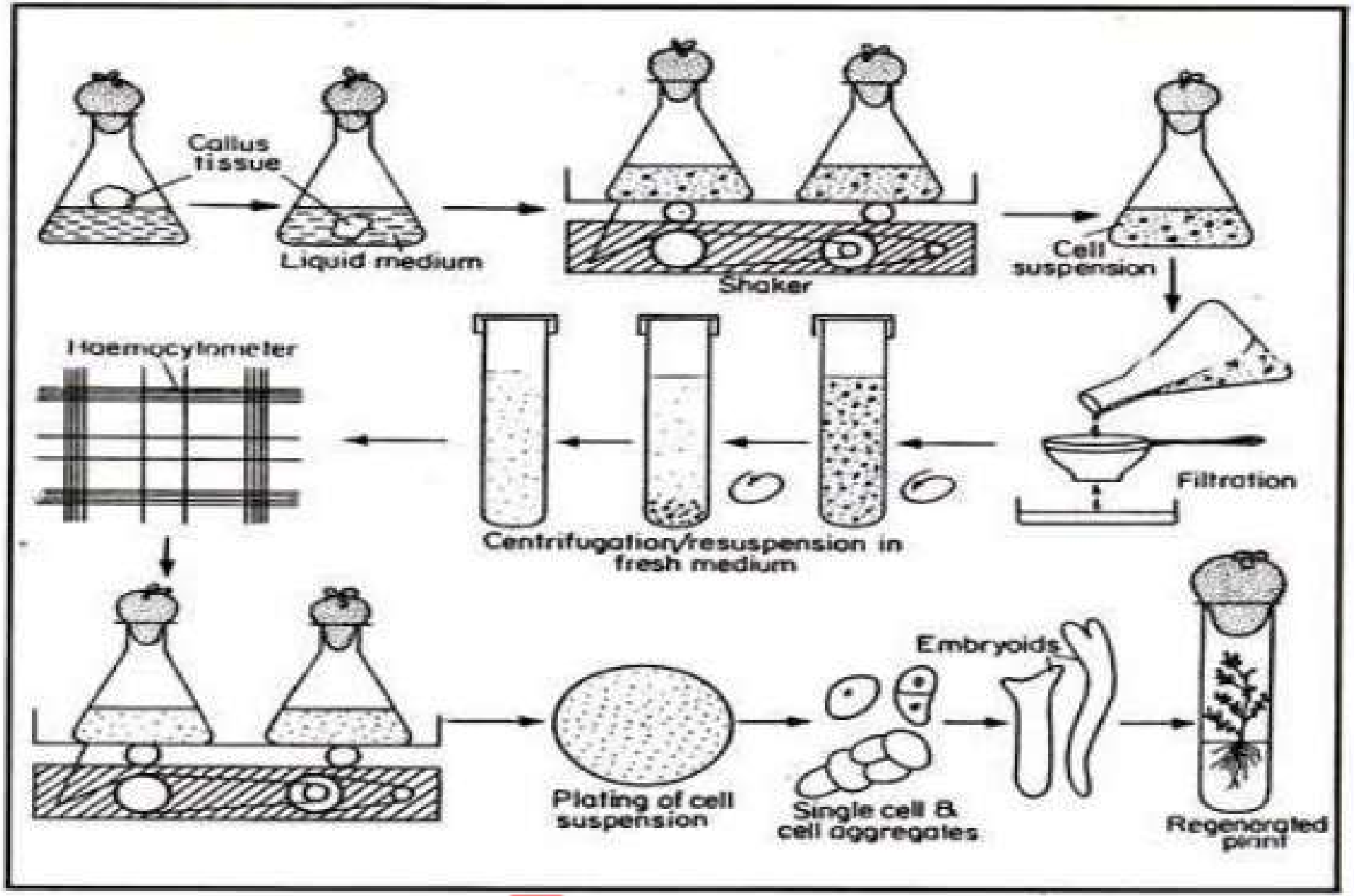
Ms . Supriya D. Potdar
Assistant Professor
Department of Biotechnology
Vivekanand College
(Autonomous),
Kolhapur

Introduction

- **Plant Tissue Culture (PTC)** is defined as a collection of experimental methods of growing plant cells, tissues and organs in an artificially prepared nutrient medium static or liquid, under aseptic conditions.
- It is also referred to as micropropagation.
- It was introduced by G. Haberlandt.
- The basic key used in plant tissue culture is the totipotency of plant cells, meaning that each plant cell has the potential to regenerate into a complete plant.
- With this characteristic, plant tissue culture is used to produce genetically identical plants (clones) in the absence of fertilization, pollination or seeds.

Cell suspension Culture

- The **cell suspension culture** also called as the **plant cell culture** is a system for production of fine chemicals.
- It can be defined as "The culture of tissue and cells cultured in liquid nutrient medium, producing a suspension of single cells and cell clumps."
- Cell suspension culture is the primary route for studying plant cell secondary metabolism.
- The cell suspension culture requires optimization of the cell line, the cultivation media, and the bioreactor system.



Types Of Cell Suspension Cultures

- There are two types of cell suspension cultures :

A. **Batch culture**

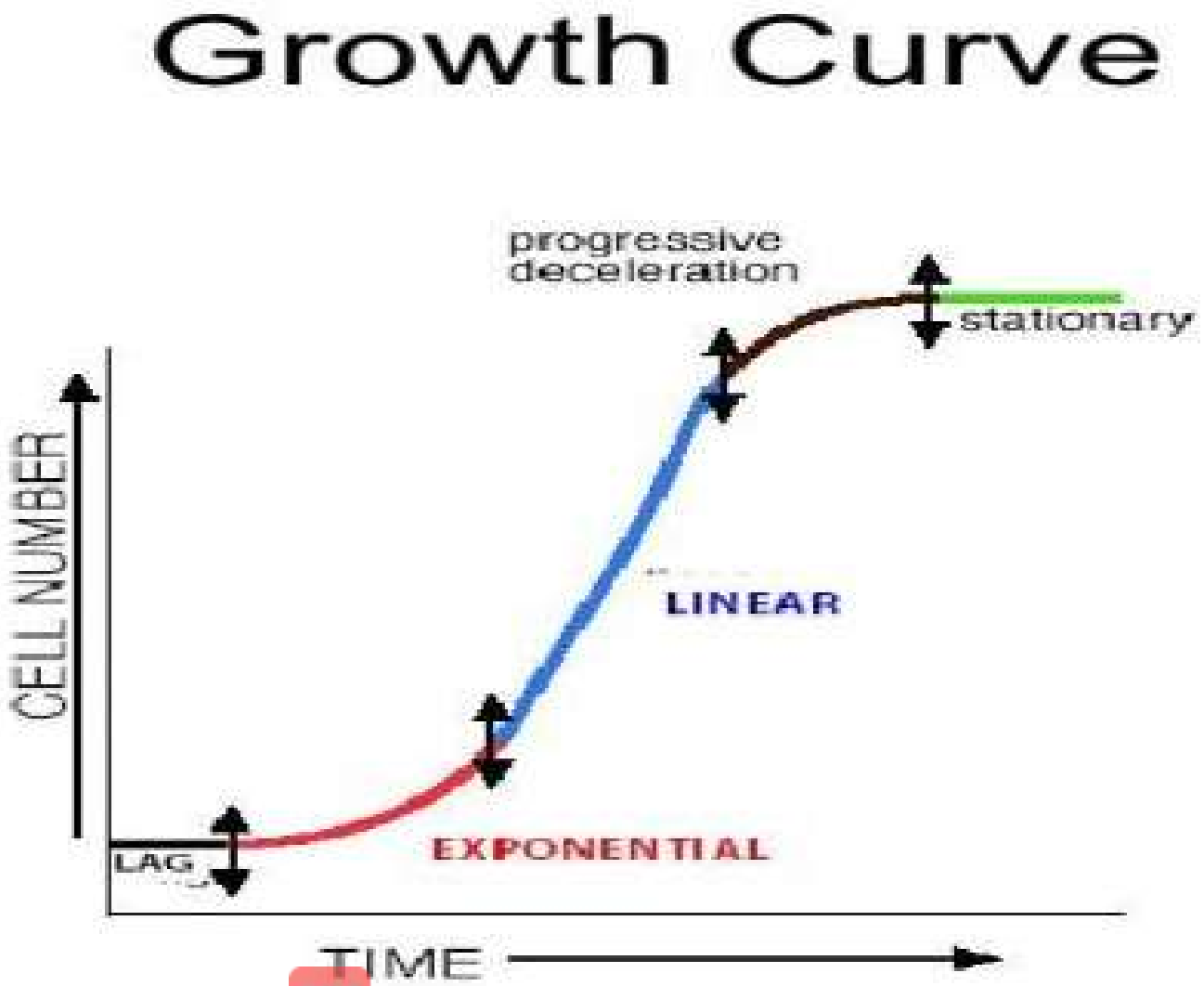
B. **Continuous culture**

- Each of these cultures have its own advantage and all types are being used in practice.

A. Batch Culture

- Batch culture is a type of cell suspension where the cell material grows in a finite volume of agitated liquid medium.
- These cultures are maintained continuously by sub culturing.
- Batch cultures are most commonly maintained in conical flasks incubated on orbital platform shakers at the speed of 80-120 rpm.
- It is a closed system, with no additions or removal of nutrient and waste products during the period of incubation.

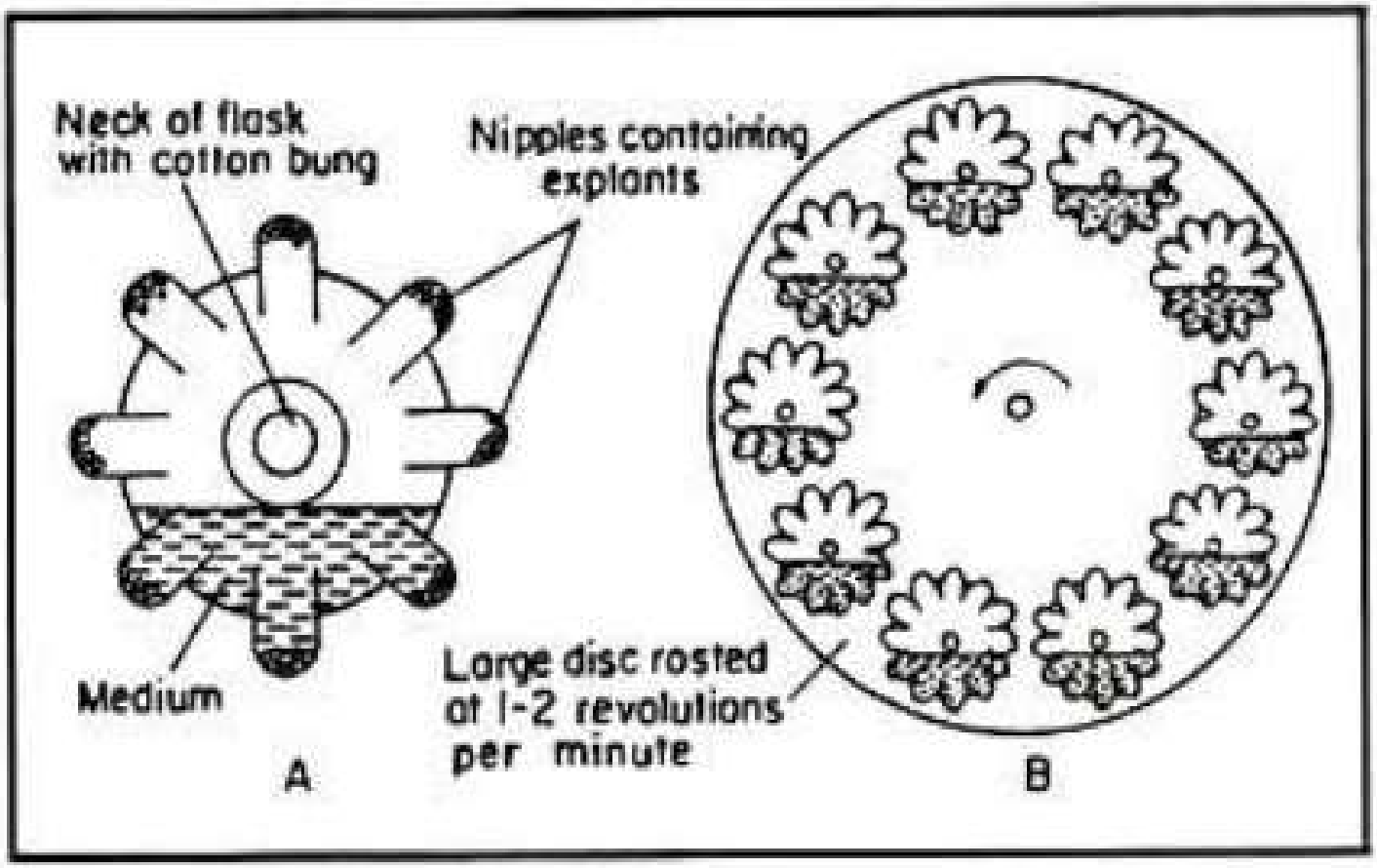
Growth curve in batch culture



Types

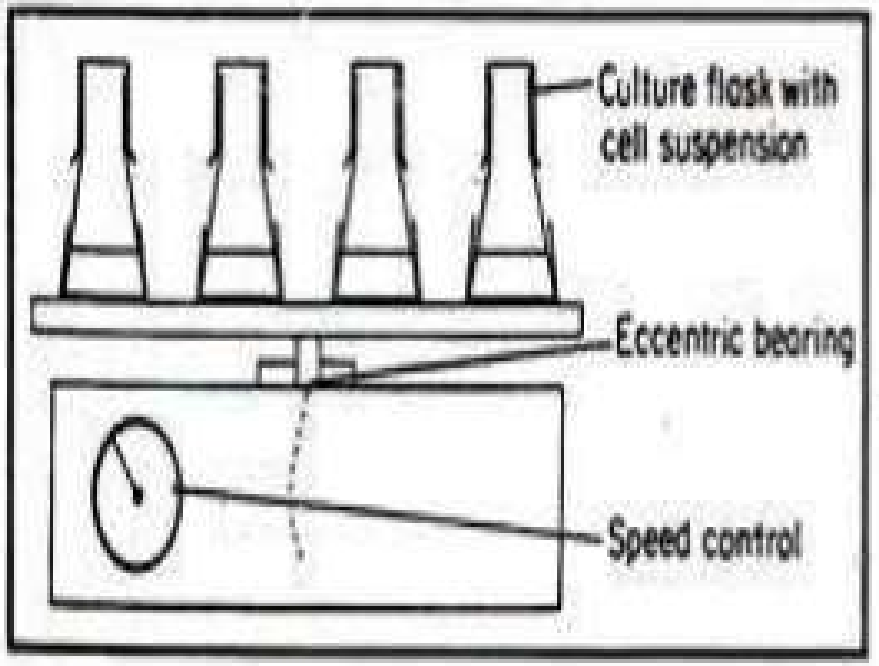
1. Slow rotating cultures :

- In this culture, single cells and cell aggregates are grown in a specially designed flask, *the nipple flask*.
- Each nipple flask possesses eight nipple like projections, having a capacity of 250ml.
- They are loaded in a circular manner on the large flat disc of vertical shaker.
- When the flat disc rotates at a speed of 1-2rpm, the cells within each nipple of the flask are alternatively bathed in the culture medium and exposed to air.



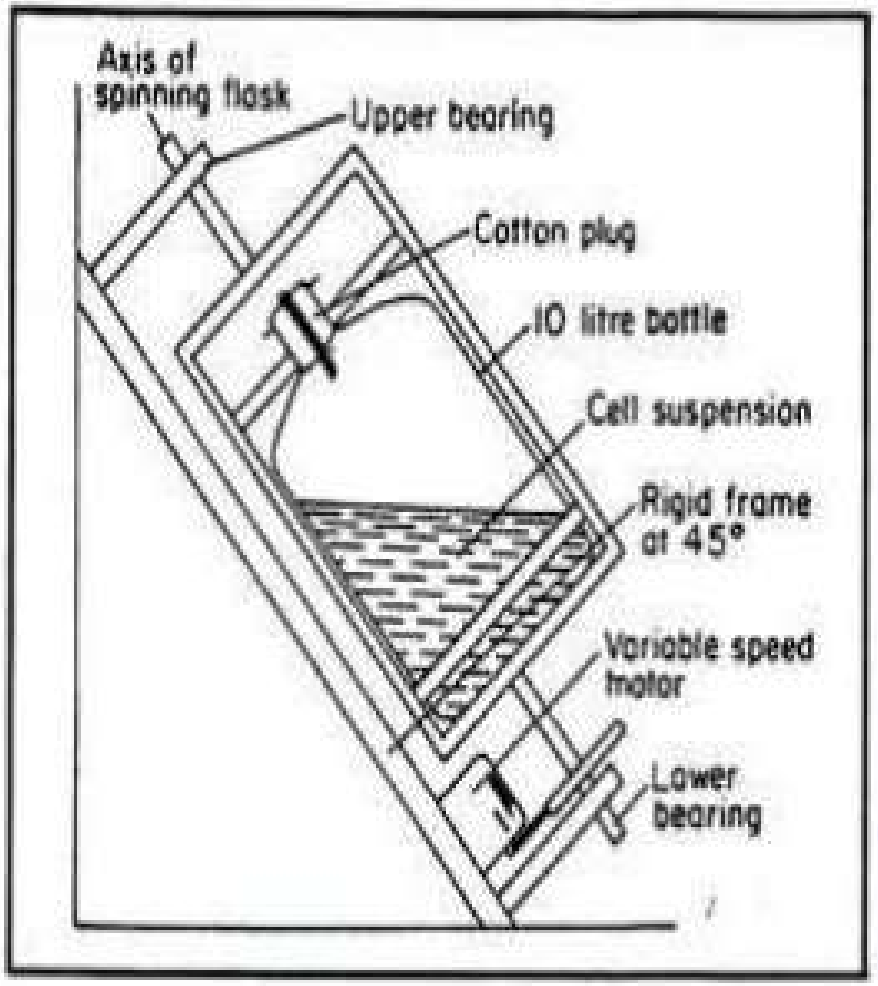
2. Shaker cultures :

- It is very and effective system.
- In this method, single cells and cell aggregates in fixed volume of liquid medium are placed in conical flasks.
- These flasks are then mounted with the help of clips on a horizontal large square plate of an orbital platform shaker.
- The square plate moves in a circular motion at the speed of 60-180 rpm.



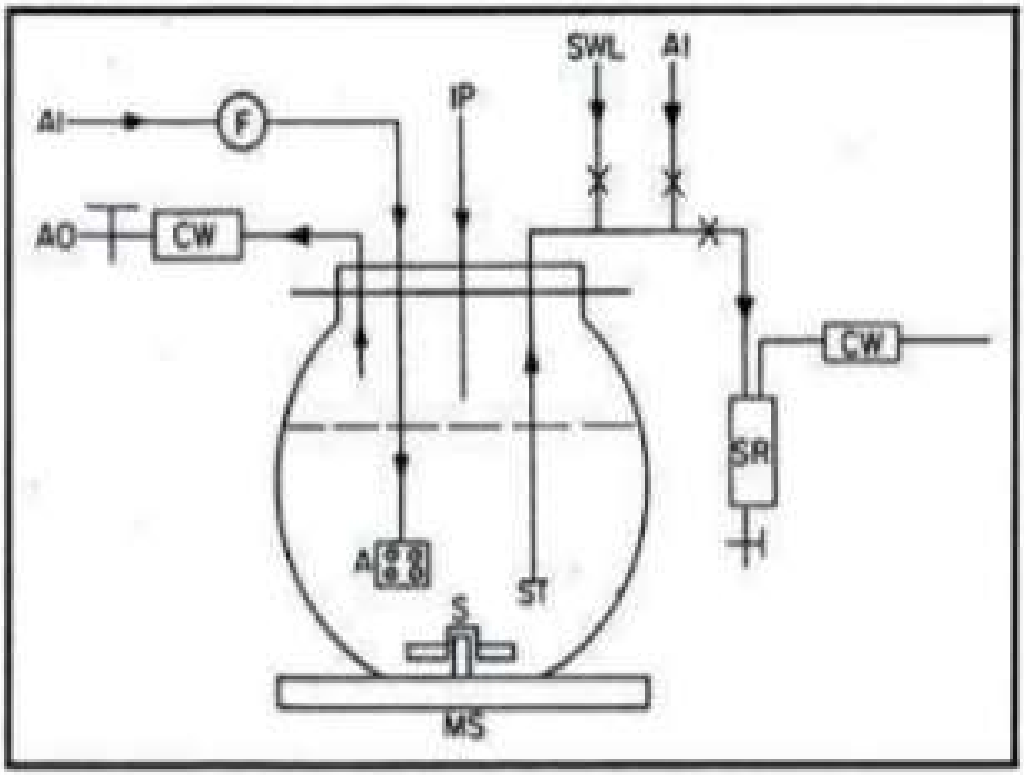
3. Spinning Cultures :

- In this culture system, large bottles are used, usually bottles with the capacity of 10L.
- Large volumes of cell suspension is cultured in 10L bottles, with the bottles spinning in a spinner at 120 rpm at an angle of 45° .



4. Stirred Culture :

- This system is used for large scale batch culture.
- In this method, the large culture vessel (round-bottom flask) is not rotated but the cell suspension inside the vessel is kept dispersed continuously by bubbling sterile air through the culture medium.
- Internal magnetic stirrer is used to agitate the culture medium safely.
- The magnetic stirrer revolves at 200-600 rpm.



❒ Fig 4.6

Stirred batch culture unit. Arrow indicate direction of flow of air; AI = air input; F = sterilizing glass-fibre air filter; AO = air outlet; CW = cotton wool; IP = inoculation port; A = aerator; S = stirrer magnet; ST = sample tube; MS = magnetic stirrer; SWL = sterile water line; SR = sample receiver; (Diagram after Dr. P. King)

B. Continuous Culture

- In continuous culture system, the old liquid medium is replaced continuously by the fresh liquid medium to stabilize the physiological states of the growing cells.
- In this system, nutrient depletion does not occur due to the continuous flow of nutrients and the cells always remain in the steady growth phase.
- Continuous culture is further divided into two types :

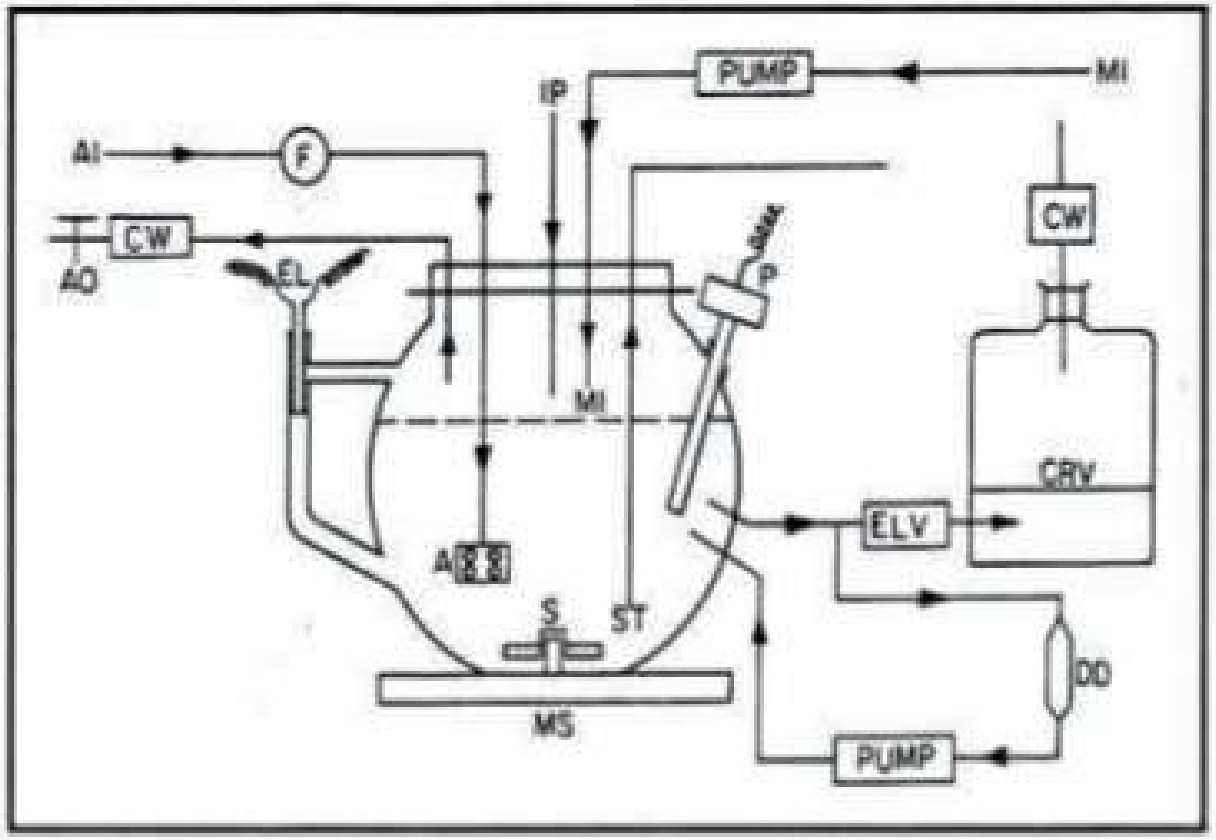
1. In closed type, the used medium is replaced with the fresh medium, hence, the cells from used medium are mechanically retrieved and added back to the culture and thus, the cell biomass keeps increasing.

2. In open type, both the cells and used medium are replaced with fresh medium thus maintaining culture at constant and submaximal growth rate.

- Open continuous cell suspension culture is of two types :

i. **Chemostat :**

- In this system, culture vessels are usually cylindrical or circular in shape and possess inlet and outlet pores for aeration and the introduction and removal of cells and medium.
- Such a system are maintained in a steady state.
- Thus in a steady state condition the density, growth rate, chemical composition and metabolic activity of the cells all remain constant.
- Such continuous cultures are ideal for studying growth kinetics and the regulation of metabolic activity in higher plants.



❒ Fig 4.7

Chemostat culture. Arrows indicate direction of flow of liquid; AI = air input; F = sterilizing glass-fibre; AO = air output; CW = cottol wool; EL = volume-sensing electrodes; ELV = volume controlling outlet valve; MI = medium input; S = stirrer magnet; ST = sample tube; P = probe for oxygen tension; DD = density detector, CRV = culture receiving vessel; MS = magnetic stirrer; IP = inoculation port (Diagram after Dr. P. King)

ii. **Turbidostats** :

- A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed.
- In this system, the cells are allowed to grow upto a certain turbidity, when the predetermined volume of culture is replaced by fresh culture.
- The turbidity is measured by the changes of optical density of medium
- An automatic monitoring unit is connected with the culture vessel and such unit adjusts the medium flow in such a way as to maintain the optical density or PH at chosen, present level.

Importance of cell suspension culture

- Such systems are capable of contributing significant information about cell physiology, biochemistry, metabolic events, etc.
- It is important to build up an understanding of an organ/embryoid formation starting from a single cell.
- Mutagenesis studies maybe facilitated by cell suspension culture to produce mutant cell clone from which mutant plants can be raised.

Advantages :

- The nutrients can be continually adjusted.
- This system can be scaled for large scale production of the cells.
- A whole plant can be regenerated from a single plant cell.

Disadvantages :

- The productivity of suspension cultures decreases over extended subculture periods.
- Slow growth and low productivity of plant cells.
- Cells may get damaged by shear conditions.

THANK YOU