Student Training Course
Classical and Modern Approaches in Crop Breeding
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TISSUE CULTURE IN PLANT BREEDING

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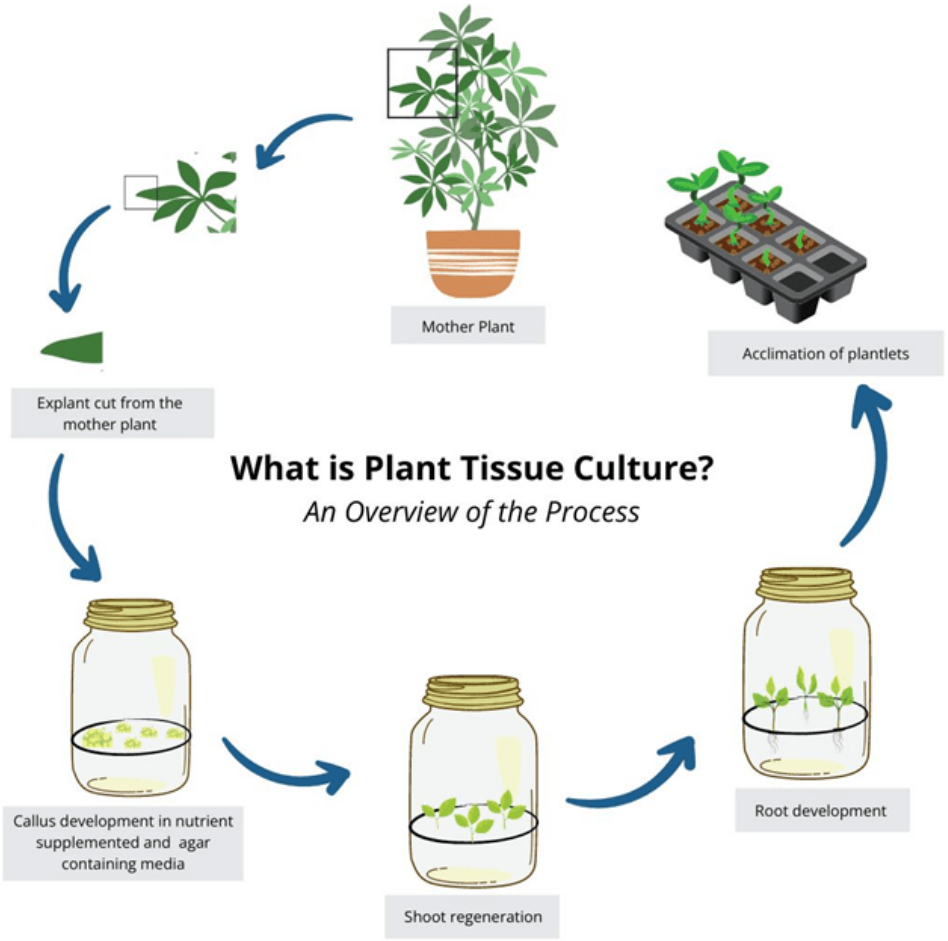
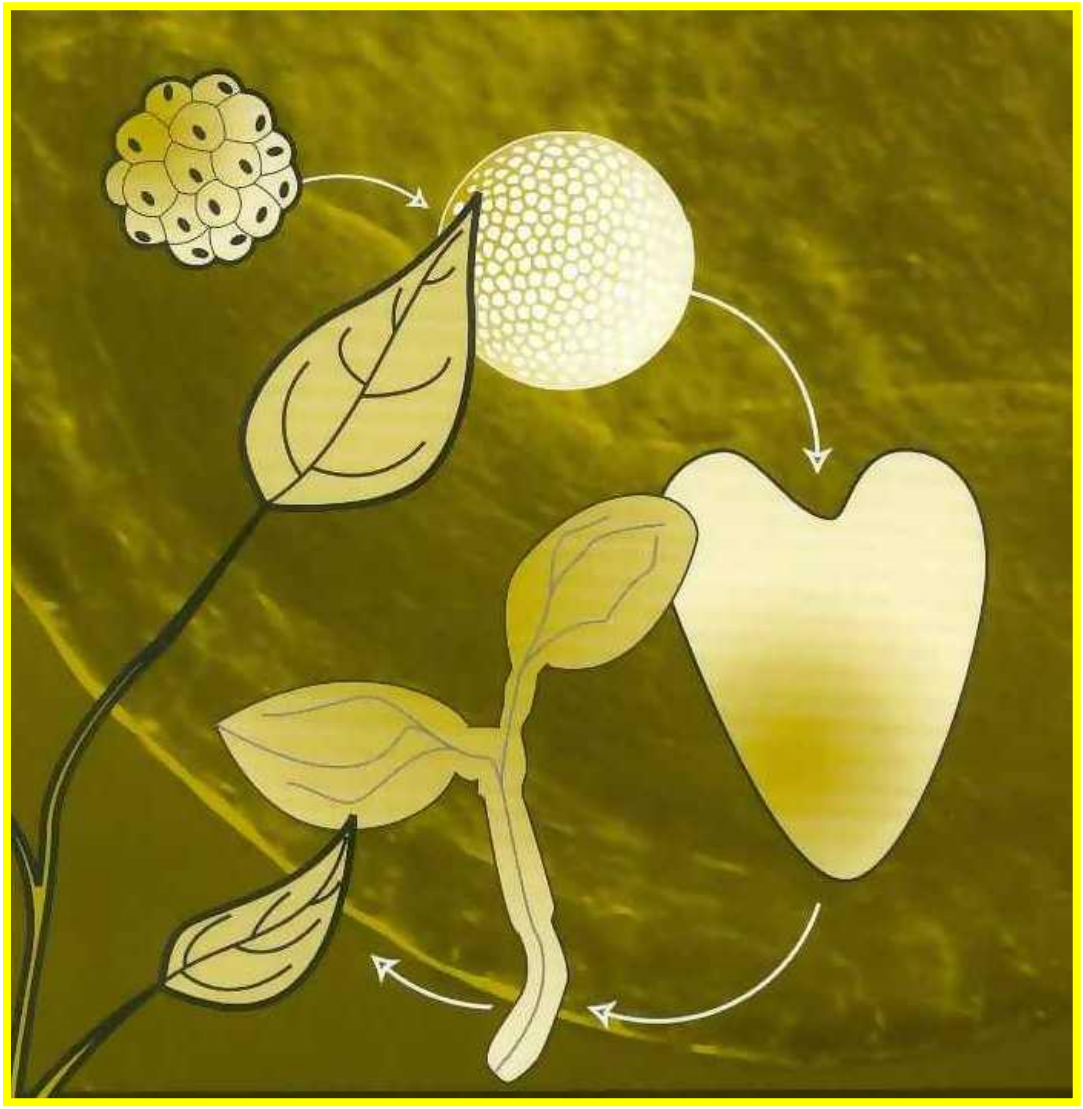
Definition

- Technique that involves cultivating plant cells, tissues, or organs in a sterile, nutrient-rich artificial medium outside the plant's natural environment, known as *in vitro*.
- The Latin term in vitro means "in glass," referring to laboratory processes conducted under sterile, controlled conditions
- This process relies on the totipotency of plant cells



Principle of Totipotency

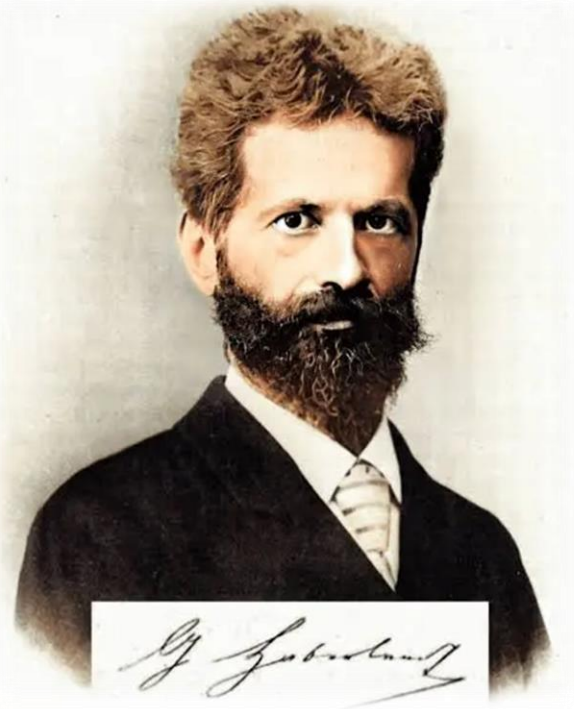
- Every living plant cell has the genetic potential to develop into a complete plant.
- Basis of all tissue culture techniques.



Importance

- A significant technique in plant research
- Foundation for plant biotechnology
- Enables rapid multiplication of superior genotypes
- Critical for modern plant breeding programs

History



1902
Gottlieb Haberlandt proposed totipotency of plant cells

1930s–1940s
First callus cultures developed

1950s
Successful plant regeneration from callus

1950s
Successful plant regeneration from callus

1960s–1970s
Micropropagaation and anther culture introduced

1980s–1990s
Protoplast fusion, somatic hybridization

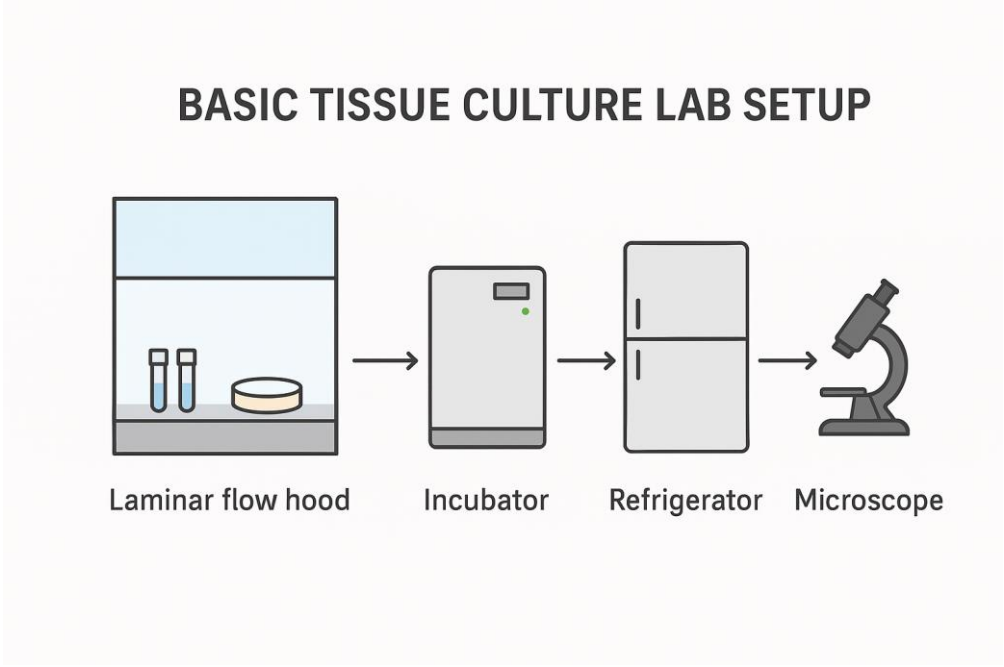
2000s–Present
Integration with molecular tools & CRISPR

General requirements

1. LABORATORY ORGANIZATION

- Room for preparing, sterilizing and storing
- Facilities to wash labware and explants
- Culture rooms or incubators

(control temperature, humidity and light)



2. CULTURE MEDIUM

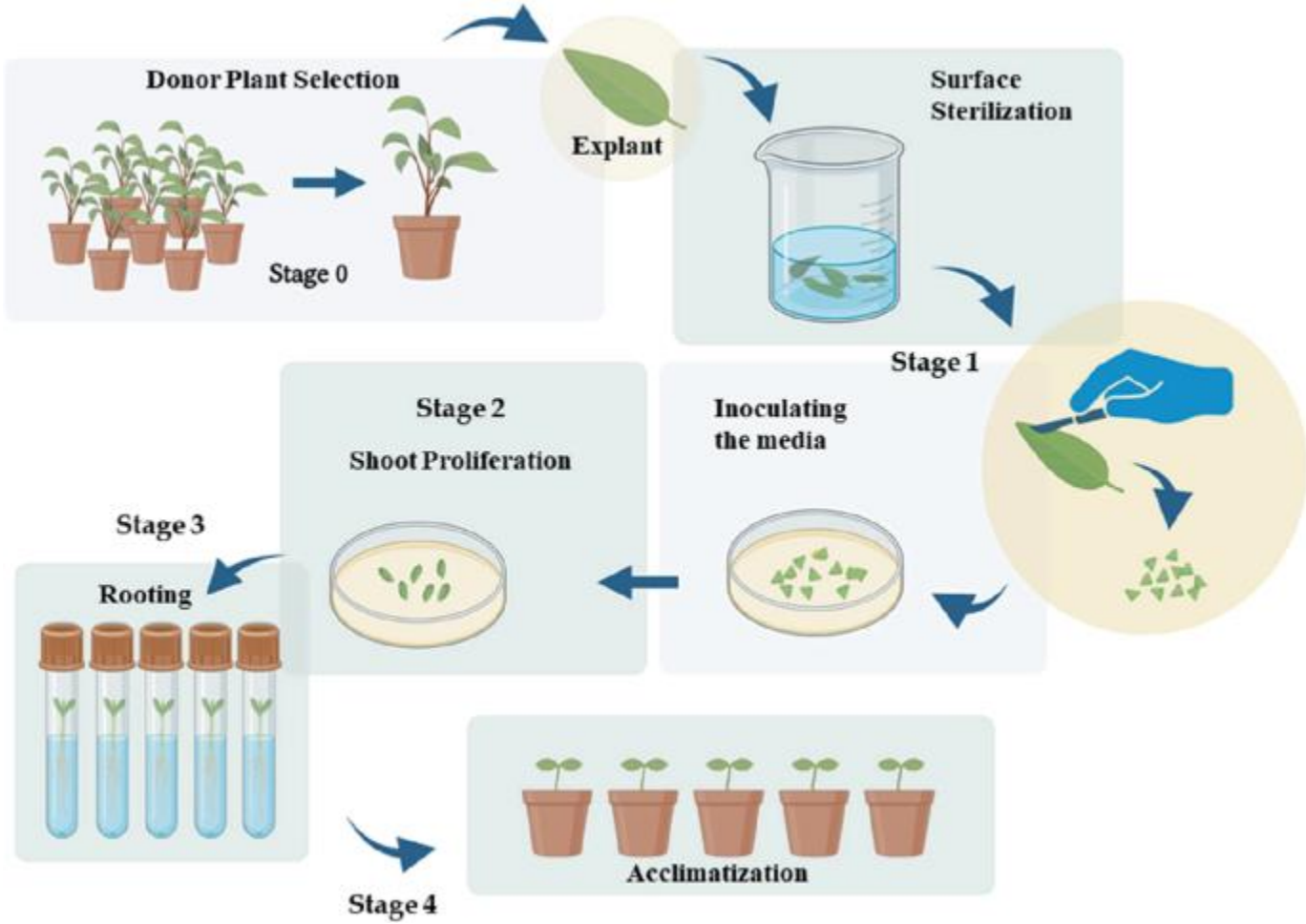
- Macronutrients & Micronutrients
- Carbon source (sucrose)
- Vitamins
- Plant growth regulators (auxins, cytokinins)
- Different plants and organs need specific media types like: MS, LS, B5 or White's medium.
- Solid or liquid

3. ASEPTIC CONDITIONS

- Vital and challenging
- Conditions free from microorganisms
- Fungi and bacteria – most common
- Use of laminar airflow cabinets
- Sterilization of media and instruments

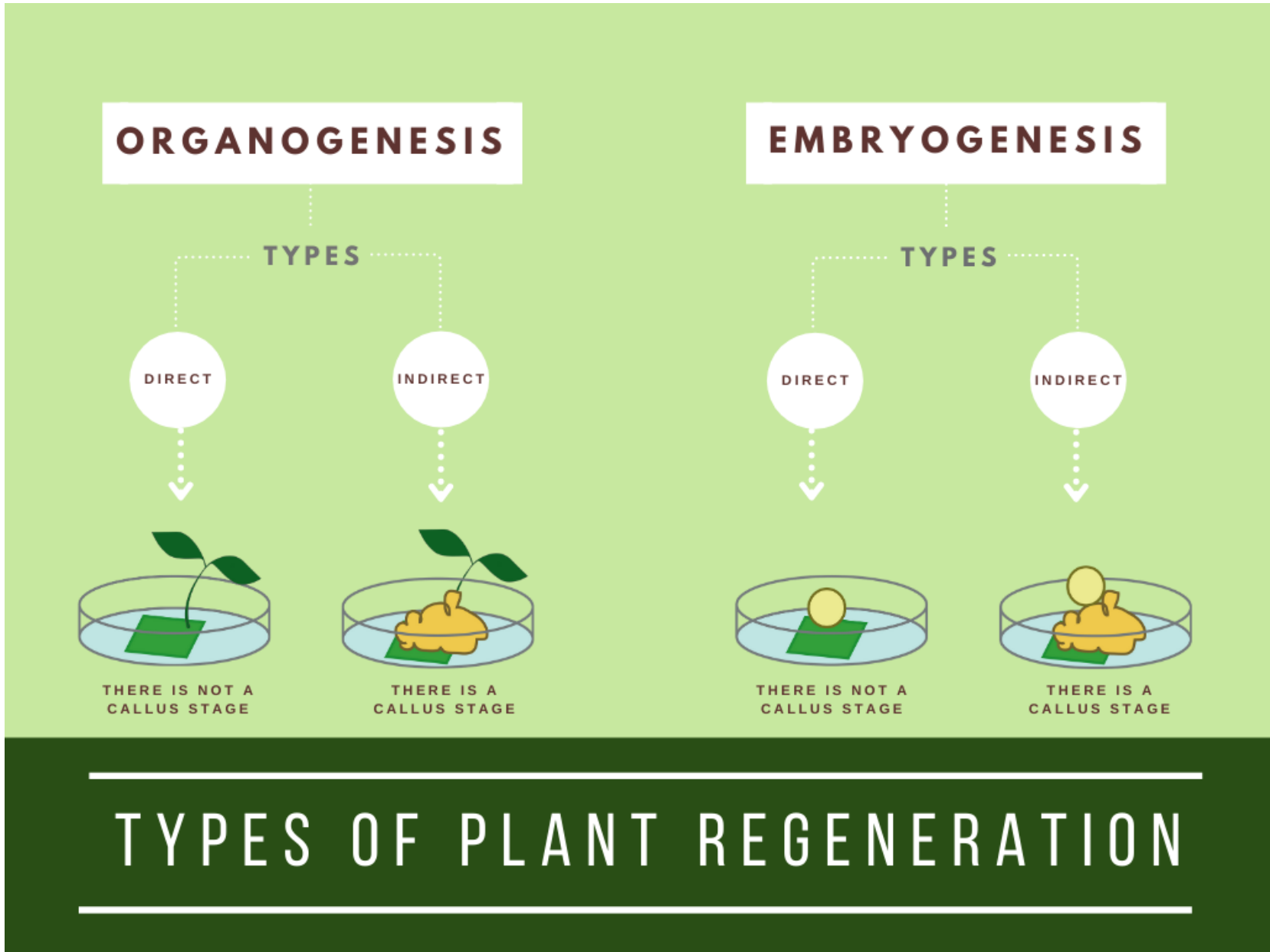
Stages (general)

0. Explant:
A piece of plant tissue (e.g., immature cotyledons, immature flowers, hypocotyls) is taken from the parent plant.
1. Inoculation on nutrient medium
2. Germination and Plantlet Formation:
3. Rooting
4. Maturation and Acclimation:
The plantlet eventually grows into a complete plant, which is then acclimated to ex vitro conditions for further growth and development.



Regeneration

Shoot regeneration in plant tissue culture is the process of creating new shoots and whole plants from explant tissues, a phenomenon rooted in the concept of plant totipotency. This complex process requires specific environmental conditions and relies on the careful manipulation of plant hormones, particularly cytokinins, to promote cell division and differentiation into organs like shoots. Factors like the basal medium composition, explant genotype, and controlled physical conditions are also crucial for successful shoot regeneration in vitro.



Organogenesis

This is a major path of regeneration that involves the differentiation of culture cells or callus tissue into organs such as shoot and roots. Organogenesis can occur directly from the explants depending on the hormonal combination of the medium and the physiological state of the explants. Medium supplemented with relatively high auxin concentration will promote root formation on the explants and high cytokinin concentration will promote shoot differentiation. In tissue culture practices there may be three types of medium in relative combinations of auxins and cytokinins, which promote either the shoot formation or root formation or both simultaneously. In the latter case, you can get the complete plantlets, having both shoot and roots, which can be directly transferred to the pots in the greenhouse. Whereas in other cases, after the formation of shoots, individual shoots are transferred to the rooting medium, which promote root formation. The rooted plantlets can be transferred to a greenhouse for acclimatization. Plant regeneration through organogenesis is commonly used for mass multiplication, for micropropagation, and for conservation of germplasm at either normal or subzero temperatures (cryopreservation).

Embryogenesis

Embryos have been classified into two categories: zygotic embryos and non-zygotic embryos.

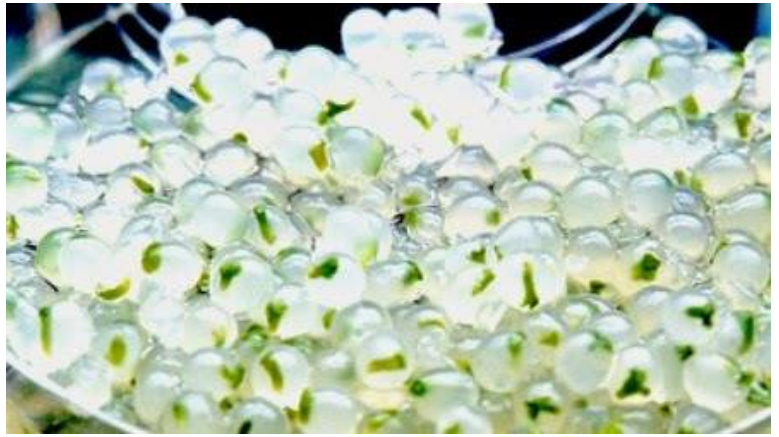
Zygotic embryogenesis

Embryos developing from zygotes (resulting from regular fusion of egg) are called as zygotic embryos or often simply embryos.

Non-zygotic embryogenesis

Usually non-zygotic embryos are formed by cells other than the zygote. Parthenogenetic embryos - formed from unfertilized eggs or a fertilized egg without karyogamy. Androgenetic embryos – formed from microspores, micro-gametophytes or sperm. Somatic embryo is an embryo derived from a somatic cell, other than zygote, usually on in vitro culture. The process of somatic embryo development is called as **somatic embryogenesis**.

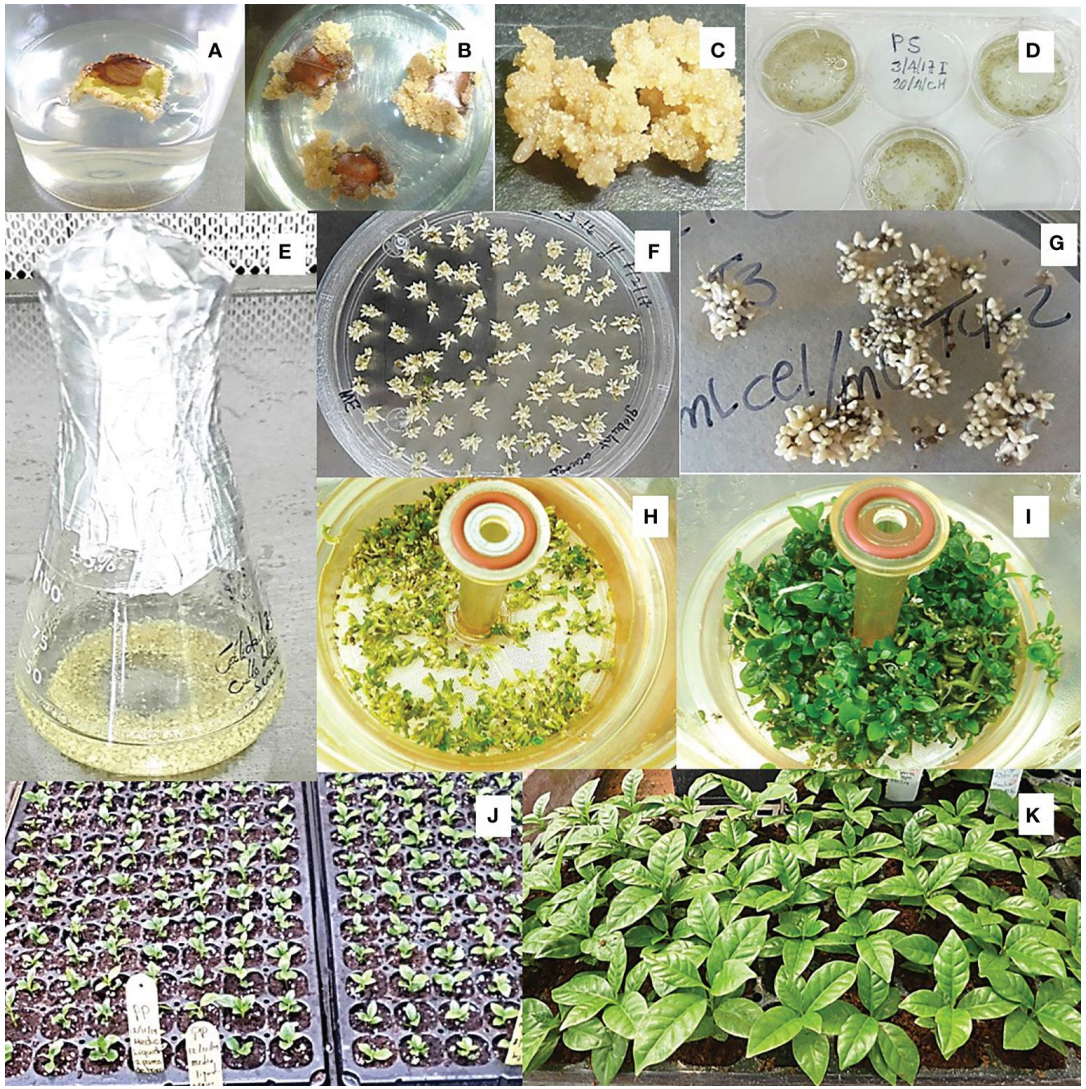
Somatic embryogenesis



This is another major path of regeneration and development of plantlets for micropropagation or mass multiplication of specific plants. The cells, under a particular hormonal combination, change into the physiological state similar to zygotes (somatic zygotes) and follow an embryonic path of development to form somatic embryos. These somatic embryos are similar to normal embryos (seed embryos) developed from zygotes formed by sexual fertilization. The somatic embryos can develop into a complete plant. Because of that they can be entrapped in certain inert polymers such as calcium alginate and used as artificial seeds. Since the production of artificial seed is amenable to mechanization and for bioreactors, it can be produced in large numbers.

Case Study: Coffee Somatic Embryogenesis

- Large-scale production of elite coffee varieties
- Ensures genetic uniformity and high yield



Types of culture

- Organ cultures
- Tissue or explant culture
- Callus Culture
- Cell suspension culture
- Protoplast culture

ORGAN CULTURE

Culturing isolated organs or tissues such as roots, stem, or leaf in an artificial media under controlled conditions are known as organ culture. Depending on the type of organs used for establishing the culture, organ cultures are named accordingly. The following are the various types of organ culture:

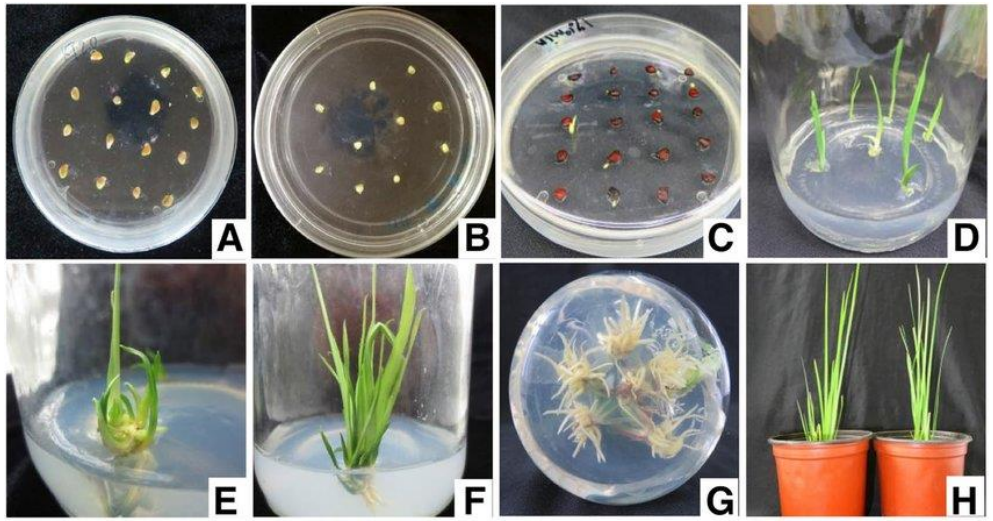
- Seed culture
- Embryo Culture
- Ovule or Ovary Culture
- Anther and Microspore Culture

Seed Culture

- A technique to grow plants from seeds in a sterile, artificial environment on nutrient-rich media.
- To produce healthy seedlings or complete plants, especially for species with poor seed germination in vivo or for mass propagation.
- Seeds are surface-sterilized, then placed on a pre-sterilized nutrient medium for germination and development into a whole plant or seedling.

Seed Culture - Applications

- Increasing the efficiency of germination of seeds that are difficult to germinate in vivo,
- Precocious germination by application of plant-growth regulators,
- For orchids, to eliminate viruses and improve overall germination rates



Embryo Culture

Laboratory technique that involves isolating and growing an embryo in a special nutrient medium under sterile conditions to promote its development into a viable plant.

Classification

On the basis of stage of the isolated embryo explants, embryo culture is classified into mature embryo culture and immature embryo culture (embryo rescue). Whereas, considering the histological origin of the isolated embryo explants, embryo culture has been classified into zygotic and somatic embryo culture. Those four broad classes of embryo culture rather overlap when the approaches adopted in different research initiatives are studied.

Embryo Culture - Application

- **Embryo Rescue:**

Immature embryos are excised and cultured on a specific nutrient medium to prevent their abortion in crosses where the endosperm (nutrient tissue) fails to develop properly.

- **Overcoming Dormancy:**

Culturing mature embryos can help bypass seed dormancy, which is caused by various factors like inhibitors or specific light/temperature requirements, allowing for faster germination and shorter breeding cycles.

- **Genetic Variability:**

Embryo culture helps preserve the genome of a hybrid embryo and can induce genetic variability for crop improvement.

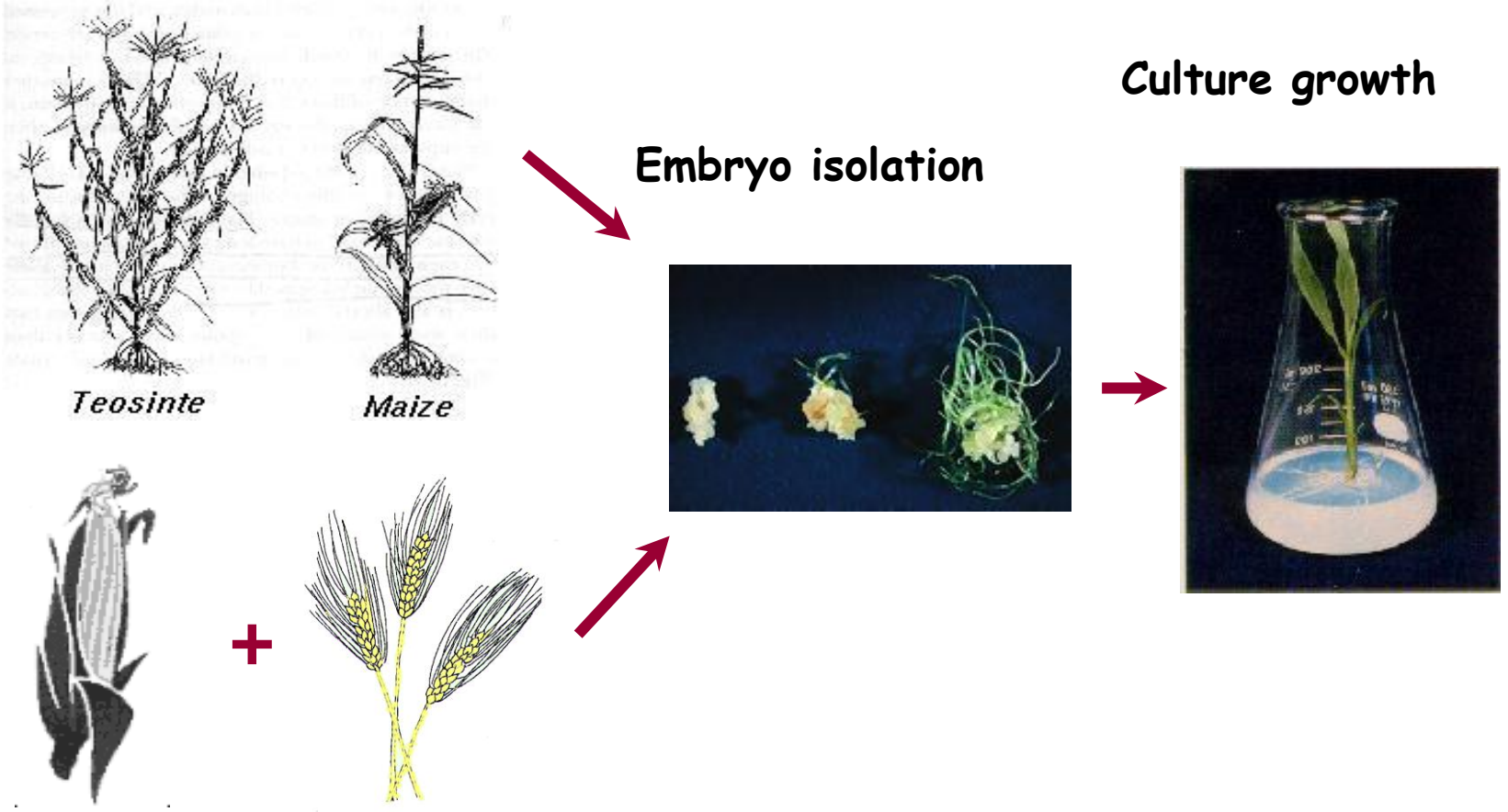
- **Stress Tolerance Screening**

Cultures can be grown on media containing salt, heavy metals, or drought-mimicking agents. Surviving plantlets often exhibit improved tolerance, which can be transferred into breeding pipelines.

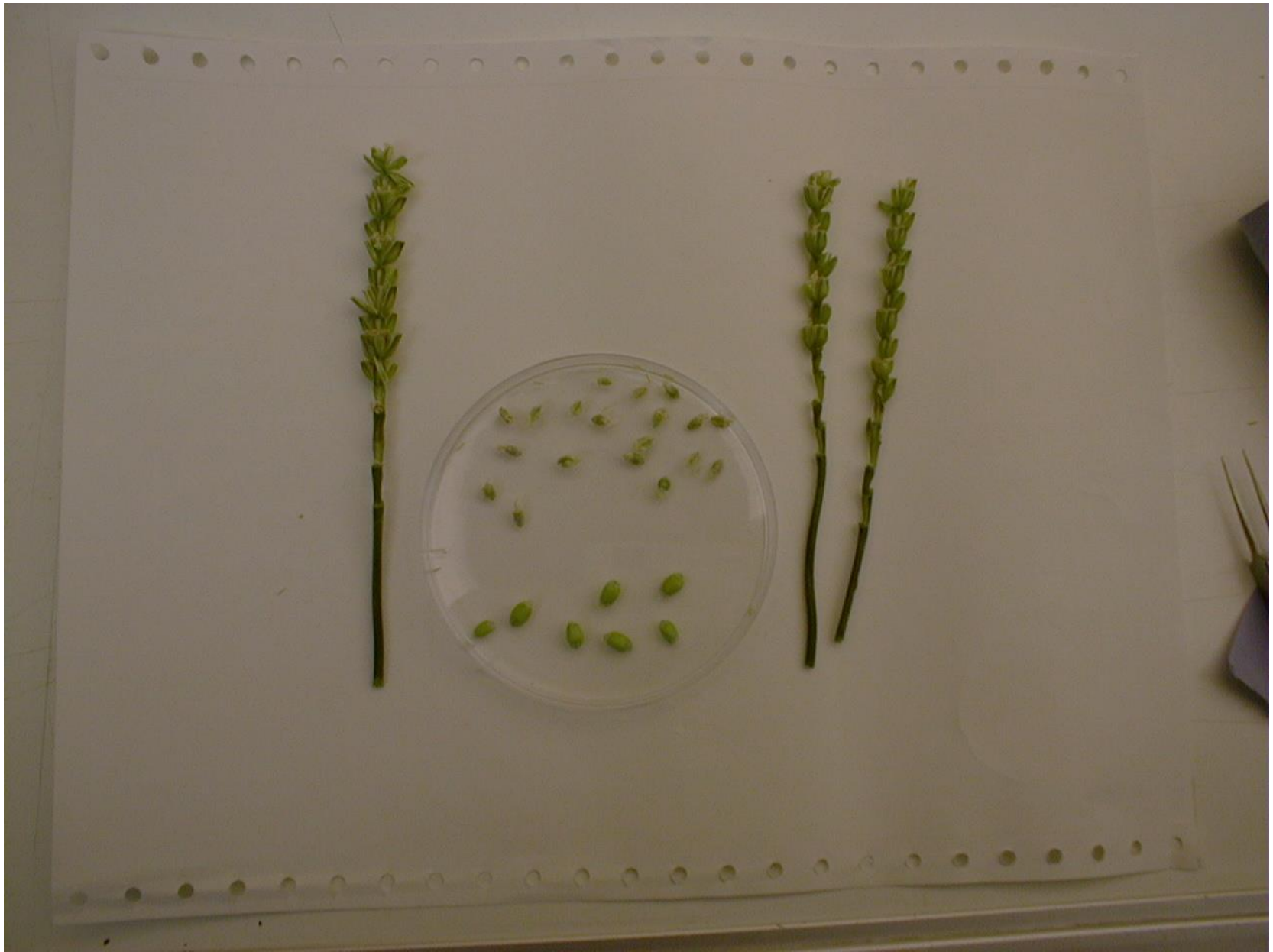
Case study - herbicides

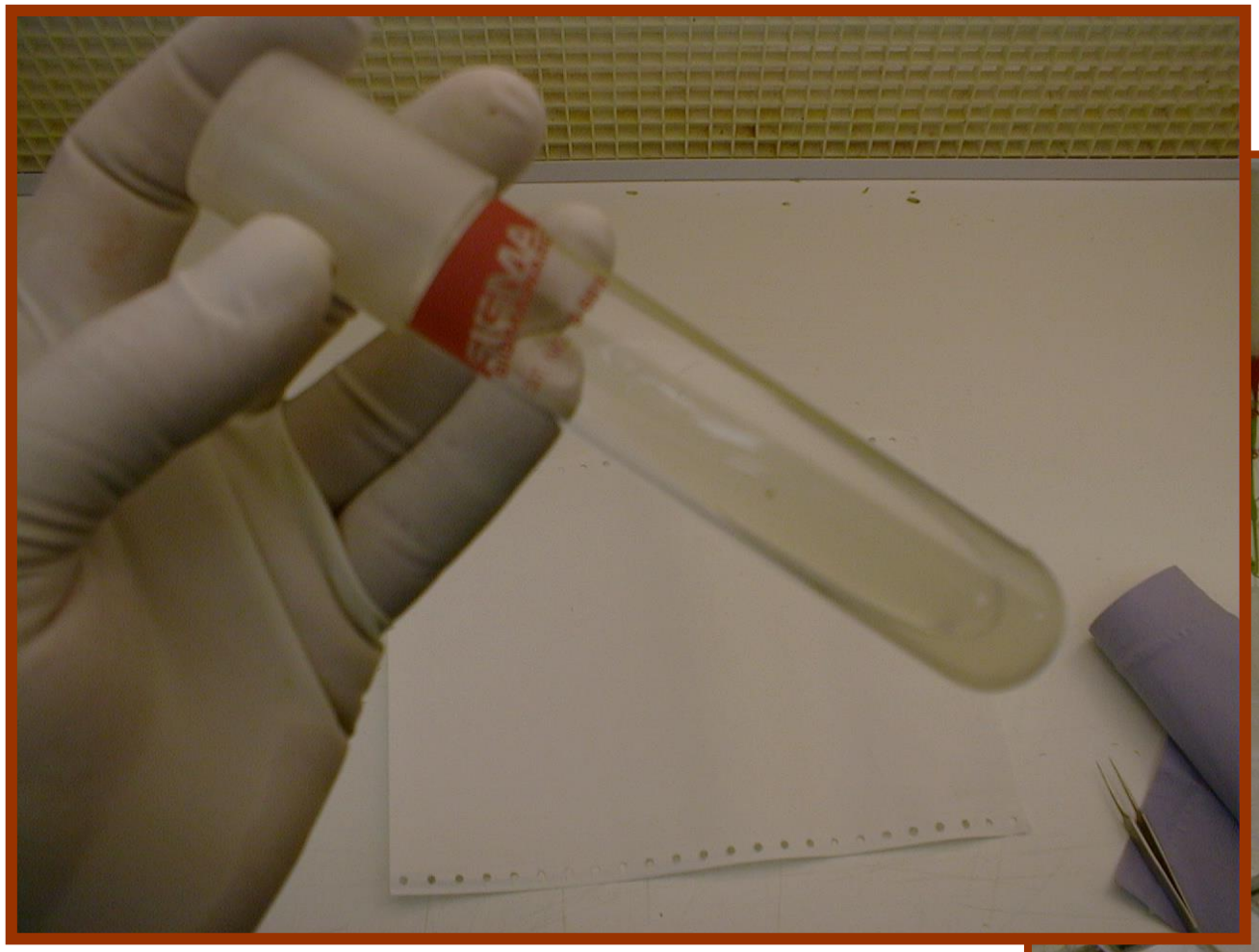
Case study - lead

Embryo rescue – haploid production



Case study – wheat x maize hybridization





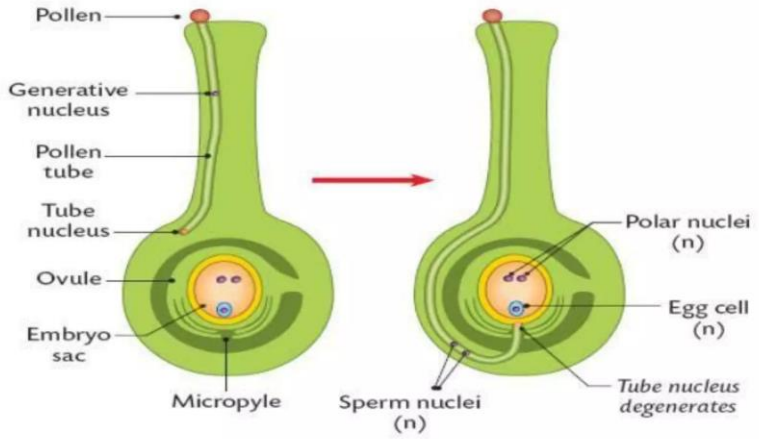
DH population

Ovary or ovule culture

- Ovary and ovule culture are in vitro plant tissue culture techniques where unfertilized ovaries or individual ovules are cultured on a nutrient medium to induce plant regeneration. Ovary culture involves culturing the entire ovary, which can produce haploid or doubled-haploid plants via embryogenesis from the ovary wall. Ovule culture isolates the ovule, containing the megaspore or egg cell, and requires more precise techniques but is a more efficient method for producing haploids and studying early embryo development.

(Note:-A pollinated flower is cultured into the simple nutrient medium whereas the un-pollinated flower is cultured on the special medium added with synthetic auxins and sucrose.)

Diagram of ovary (stigma , style , then ovary):-

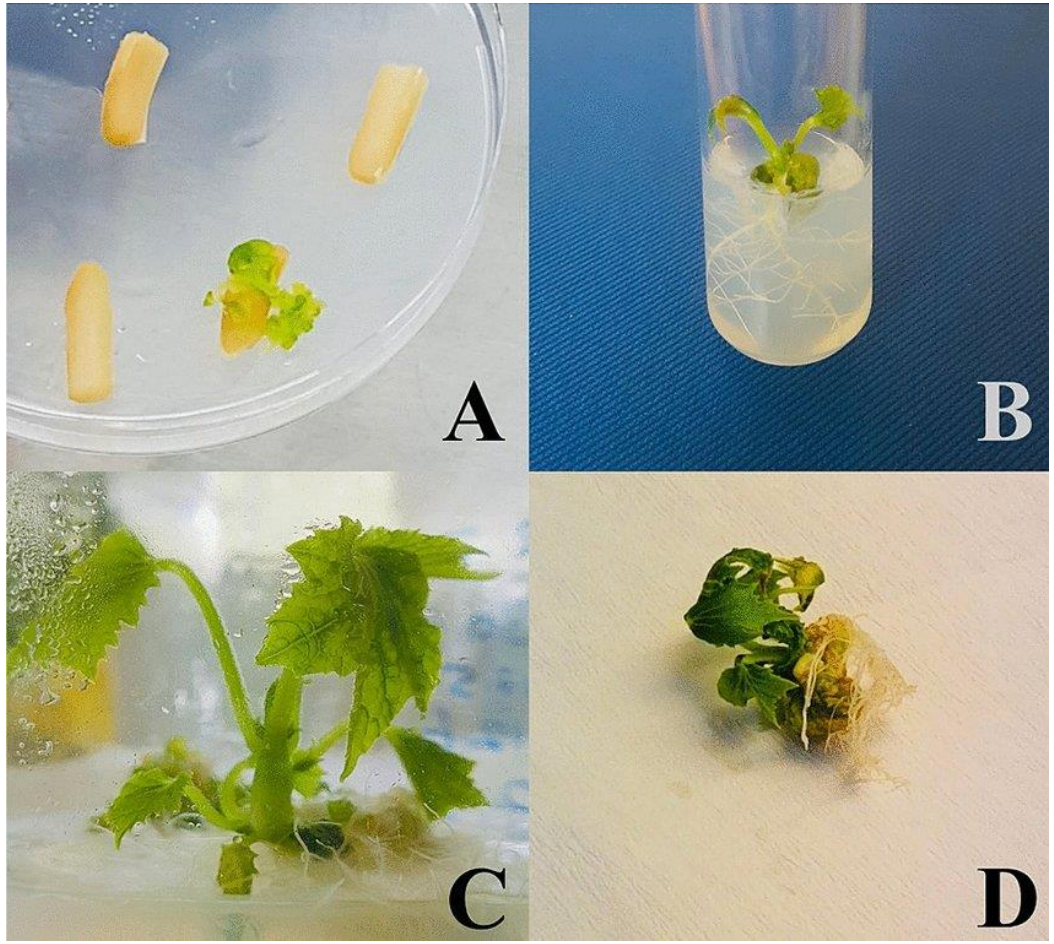


PRINCIPLE:-

1. The principle of gynogenesis is based on the Regeneration principle, where an ovary can regenerate into a fully differentiated plant.
2. In the ovary culture, the flowers are excised from the plant either in pollinated or non-pollinated

Ovary or ovule culture - applications

- Production of haploid plants,
- Overcoming abortion of embryos of wide hybrids at very early stages of development due to incompatibility barriers,
- *In vitro* fertilization for the production of distant hybrids.

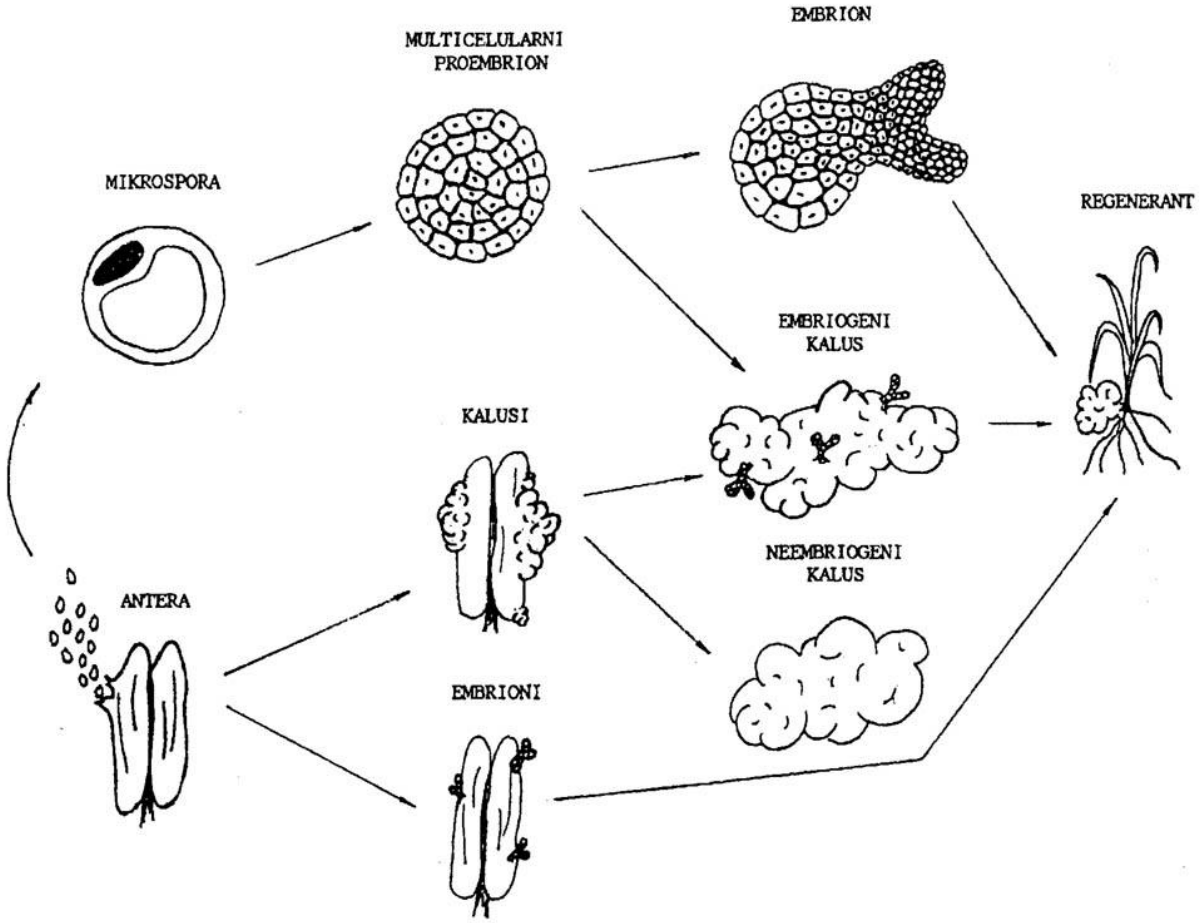


Anther and Microspore Culture

In vitro plant tissue culture techniques that induce haploid microspores (immature pollen) to develop into haploid plants, which can then be converted into homozygous, double haploid (DH) plants. Anther culture involves culturing the entire anther, while isolated microspore culture involves isolating and culturing the microspores separately. Both methods use nutrient media and apply stress, such as temperature or osmotic shocks, to trigger the microspores' normal gametophytic pathway to a sporophytic one, allowing for rapid production of uniform, fertile homozygous lines for plant breeding

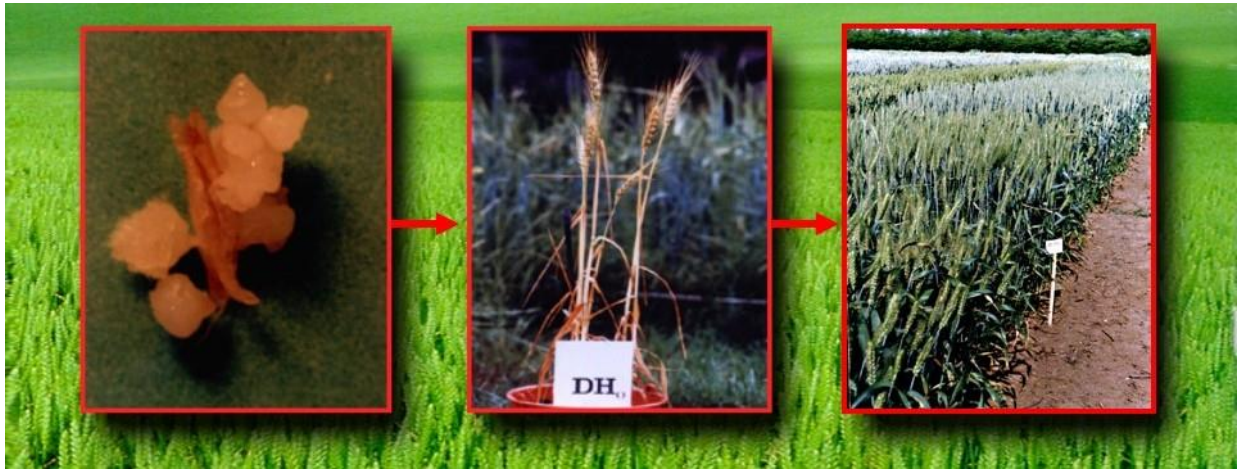
Anther and Microspore Culture - Applications

- Production of haploid plants,
- Production of homozygous diploid lines through chromosome doubling,
- Reducing the time required to produce inbred lines,
- Uncovering mutations or recessive phenotypes



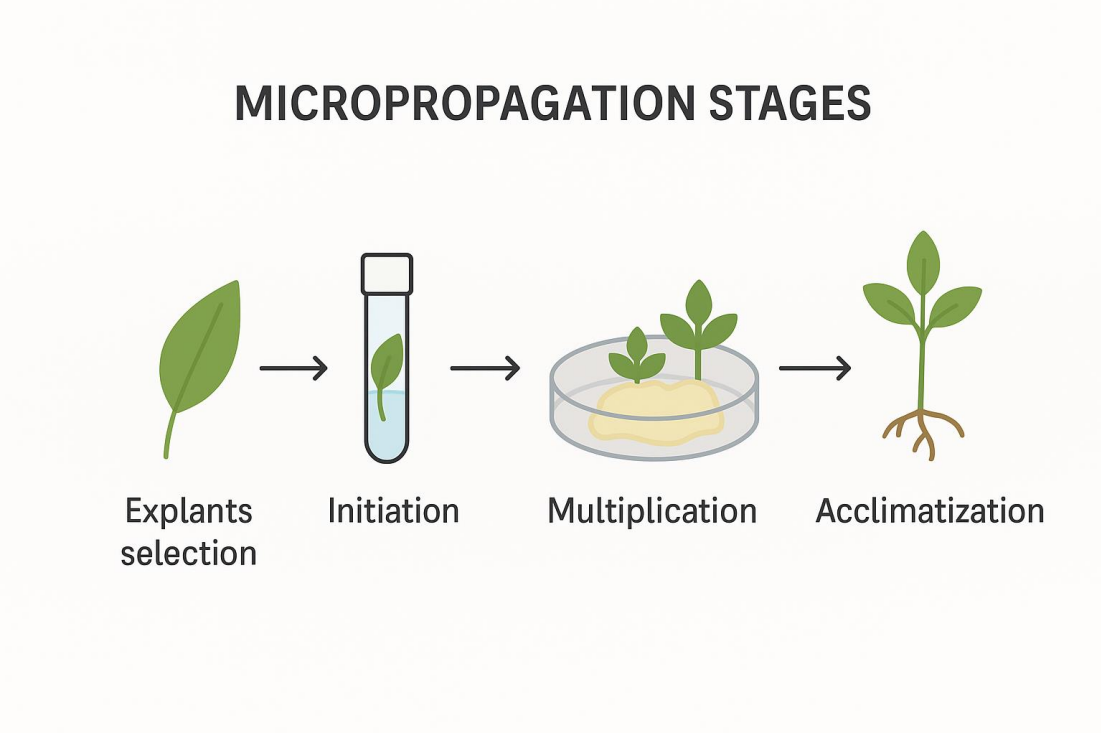
Case Study: Wheat Haploids

- Anther and microspore culture used to develop doubled haploids (DH)
- Commercial DH wheat varieties were registred in China, Hungary, France, Canada etc.
- Accelerated breeding of rust-resistant wheat



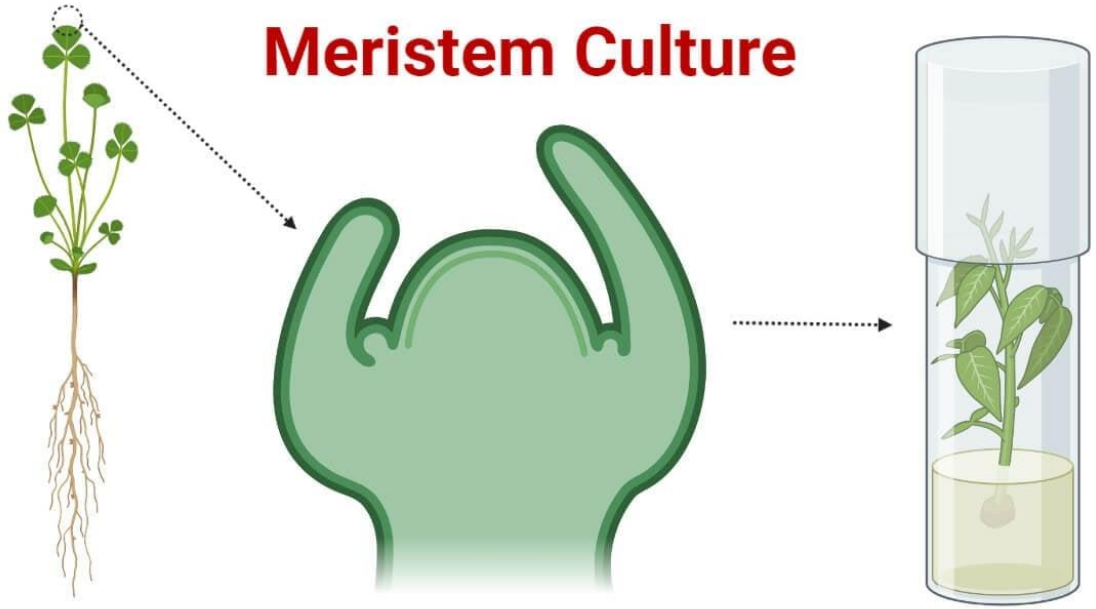
TISSUE OR EXPLANT CULTURE

Technique that involve growing different plant tissues in an artificial, nutrient-rich medium under sterile conditions to produce whole plants or to propagate large quantities of genetically uniform plant material.



Tissue culture - Applications

- Production of virus-free germplasm or plantlets,
- Mass production of desirable genotypes - micropropagation,
- Cryopreservation (cold storage at -196°C) or in vitro long-term preservation of germplasm without genetic change, etc.



Case Study: Banana Improvement

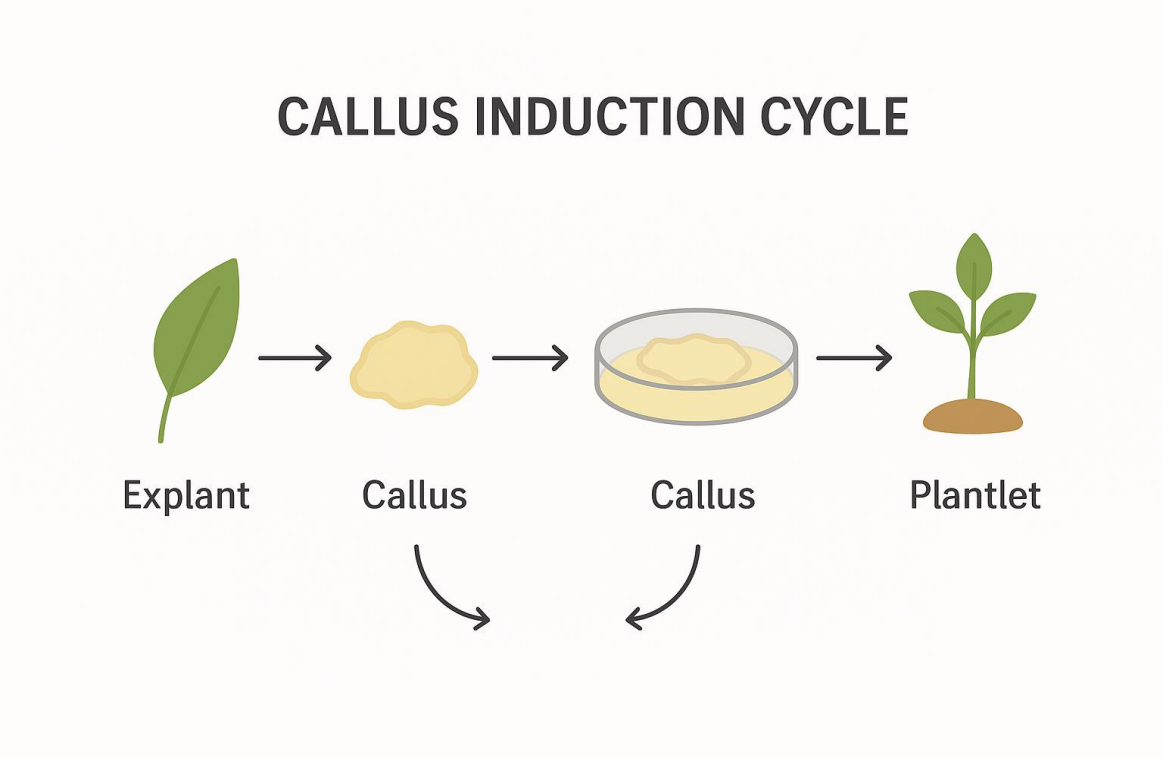
- Tissue culture used to mass-produce disease-free banana plants
- Resistance to Panama disease improved through somaclonal variants

Case Study: Potato Seed Production

- Micropropagation + aeroponic systems
- Production of virus-free seed potatoes at commercial scale

CALLUS CULTURE

Callus represents an unorganized or undifferentiated mass of cells. They are generally composed of parenchymatous cells and usually undergo division. When an explant is cultured in a medium supplemented with sufficient amount of auxins, it starts producing mass of cells from the surface of the explant.



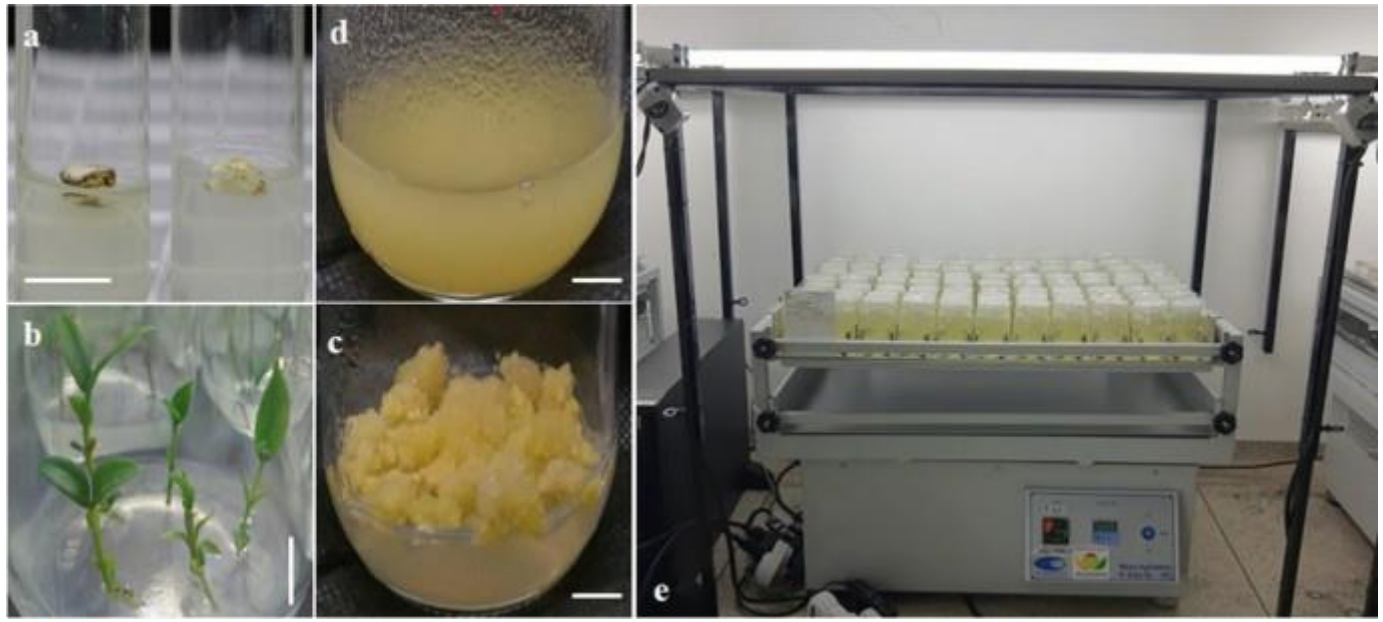
Callus culture - Applications

Can be maintained for a very long time by intermittent sub-culturing to a fresh medium and manipulated for different purposes:

- Regeneration of plantlets,
- Preparation of single cells or suspension cultures,
- Protoplasts preparation,
- Genetic transformation studies,
- Generation of useful somaclonal variants (genetic or epigenetic)
- In vitro selection of cells and tissue variants.

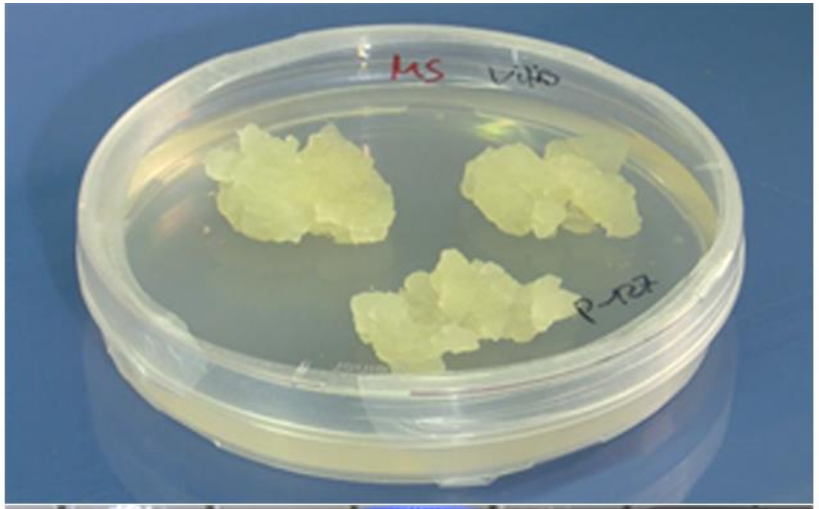
CELL SUSPENSION CULTURE

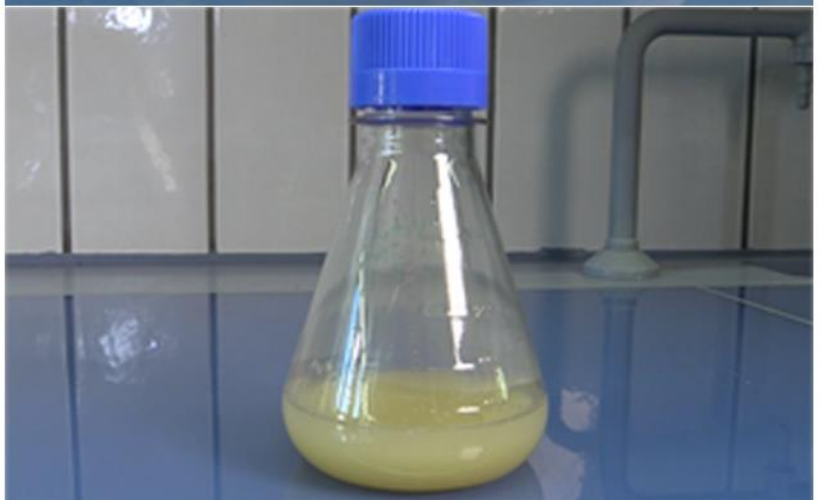
Single-cell cultures and suspension cultures can be established from callus cultures by transferring a piece of callus tissue into liquid medium and subjecting it to continuous shaking. The growth rate of the suspension-cultured cells is generally higher than that of the solid culture. By subculturing for several generations, a fine cell suspension culture containing small-cell aggregates and single cells is established. The time required to establish the cell-suspension culture varies greatly and depends on the tissue of the plant species and the medium composition.



Enzymatic method for cell-suspension culture

This is based on the use of certain pectin digesting enzymes in the culture medium, such as pectinase or macerozyme. These enzymes act on the pectin, which joins two adjacent cells in plant tissues, so that the cells become independent and grow freely as single cells.





Cell-suspension culture - Applications

- Inducing somatic embryogenesis
- Preparation of artificial seeds,
- Induction of somatic mutation – somaclonal variations
- Selection of mutants by screening the cells
- Genetic transformation experiments to produce transgenic plants
- Production of alkaloids, flavors, fragrances

Cell-suspension cultures and bioreactors

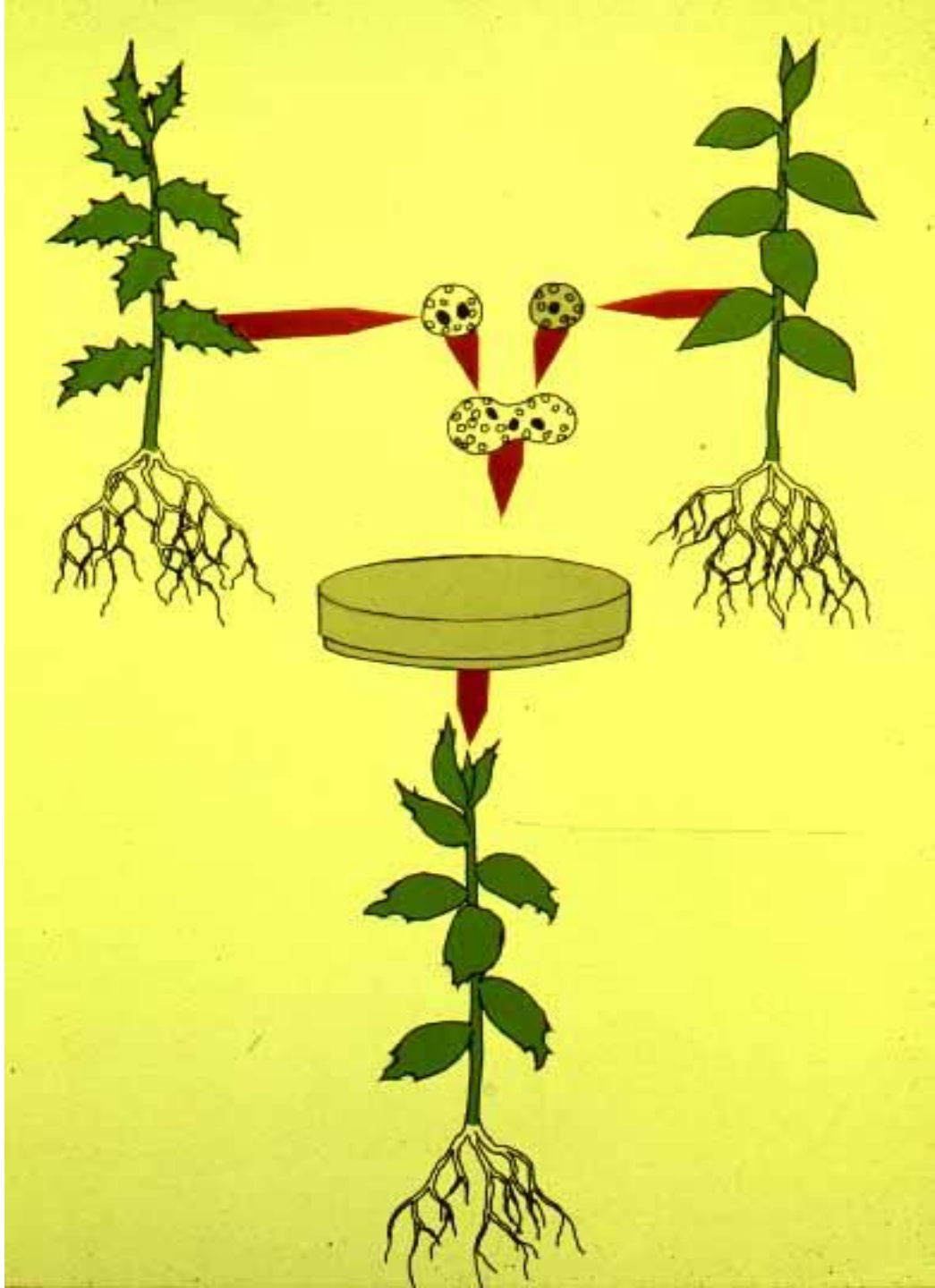
The main application of plant cell-suspension cultures is for the bioproduction of certain important phytochemicals or secondary metabolites by applying the principle of biochemical engineering. The suspension cultures can be cultivated in specially designed bioreactors known as airlift bioreactors for the mass-cell cultures for the production of plant secondary metabolites on industrial scale. Normal bioreactors with mechanical stirrer cannot be used in plant-cell cultures because it can result in the breaking of cells and thereby the cell viability can be drastically reduced. Whereas the airlift bioreactor can provide both stirring and air inflow to meet the high demand of oxygen.

PROTOPLAST CULTURE

Protoplasts are plant cells without cell walls. The cell wall can be removed with an enzymatic method. The cells may be from the leaf tissue or from any other part of the plant or may be the cells from the suspension cultures. These cells are incubated in an enzyme mixture consisting of cellulase, hemicellulase, and pectinase for a specific period of time. The enzyme mixture acts on the cell wall and is completely digested, so that the underlying cell membrane is exposed. This protoplast on culturing in a proper medium will regenerate its cell wall and becomes a normal cell and then can regenerate into a whole plant.

Protoplast culture - Applications

The plant protoplasts can be used for various biochemical and metabolic studies and it can be used for the somatic cell fusion to create somatic hybrids. Fusion of aenucleated and nucleated protoplasts can result in a special type of somatic hybrids known as cybrids, in which there is no fusion of nucleus, but fusion of the cytoplasm. Protoplasts can also be used for genetic transformation studies with biolistic methods, electroporation techniques, by PEG-mediated DNA transfer or by direct injection of DNA into the nucleus of the protoplast using micro-syringes.



Integration with Molecular Breeding

- Production of mapping populations (DH lines) for molecular markers identification
- Marker-assisted selection combined with tissue culture
- Regeneration of genetically modified organisms.
- CRISPR/Cas genome editing through callus transformation.

ADVANTAGES OF IN VITRO CULTURE

DISADVANTAGES OF IN VITRO CULTURE

1. Faster Trait Selection
2. Species-Specific Insights
3. Controlled and Simplified Environment
4. Year-Round Experimentation
5. Direct Study of Plant Responses

1. High cost and technical expertise required
2. Risk of somaclonal variation
3. Contamination sensitivity
4. Limited field relevance
5. Labor-intensive and time-consuming

Economic Importance

While initial setup and labor costs can be high, tissue culture ultimately saves time and resources by ensuring high survival rates, uniform crops, and faster variety development.

- Large-scale production of ornamentals
- Conservation of endangered plants
- Support for commercial agriculture:
  - shortens breeding cycles and
  - facilitates gene introgression from wild relatives,
  - speeding up the release of improved varieties.

Ethics and Biosafety

Tissue culture itself is safe, but when combined with genetic engineering and genome editing, regulatory oversight is required. It’s important to address public concerns about GM and GE crops.

Conclusion

- Tissue culture is indispensable for modern plant breeding.
- Provides speed, precision, and genetic diversity.
- Future trends integrate biotechnology and automation for sustainable agriculture.

Thank you for your attention!

Any questions?