

TOPIC : PLANT TISSUE CULTURE

COURSE : M.Sc. BOTANY Part II

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Plant Tissue Culture

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissue or organs on a nutrient culture medium of known composition. Plant tissue culture is used as a collective term to describe all kinds of *in vitro* plant cultures. These cultures can be a cell culture, a tissue culture or an organ culture. Cultures are generally initiated from sterile pieces of plants known as explants. Explants may comprise of an organ/tissue (shoot tip, node, leaf, root or stem), a cell (protoplast, pollen, ovule or endosperm).

Plant tissue culture is widely used in plant science, forestry and horticulture. It is used in

1. clonal propagation/micro propagation,
2. in production of virus free plants
3. in germplasm conservation
4. to produce sterile plants
5. in production of sec. metabolites
6. in making artificial seed / synthetic seed
7. in production of transgenic plants
8. in production of hybrids and cybrids
9. in production of haploids, triploids and dihaploids
10. in production of polyploid plants etc.



Different types of Plant tissue cultures can be summarized as follows :

Callus culture

Callus (plural *calluses* or *calli*) is an undifferentiated and unorganized mass of parenchymatous tissue. In living plants, calluses are undifferentiated mass of cells which cover a plant wound. In biological research and biotechnology callus formation is induced in explants after surface sterilization and inoculation of explants on optimal aseptic media *in vitro* conditions. The culture medium is supplemented with a defined amount of plant growth regulators such as auxins, cytokinins and gibberellins to initiate callus formation or formation of somatic embryos. During this process cell differentiation and specialization is reversed and the explants gives rise to new tissue. The callus which is formed on an original explant is called primary callus which is compact. Secondary callus are formed by sub-culturing the primary callus. Repeated sub-culturing forms friable callus.



Compact callus



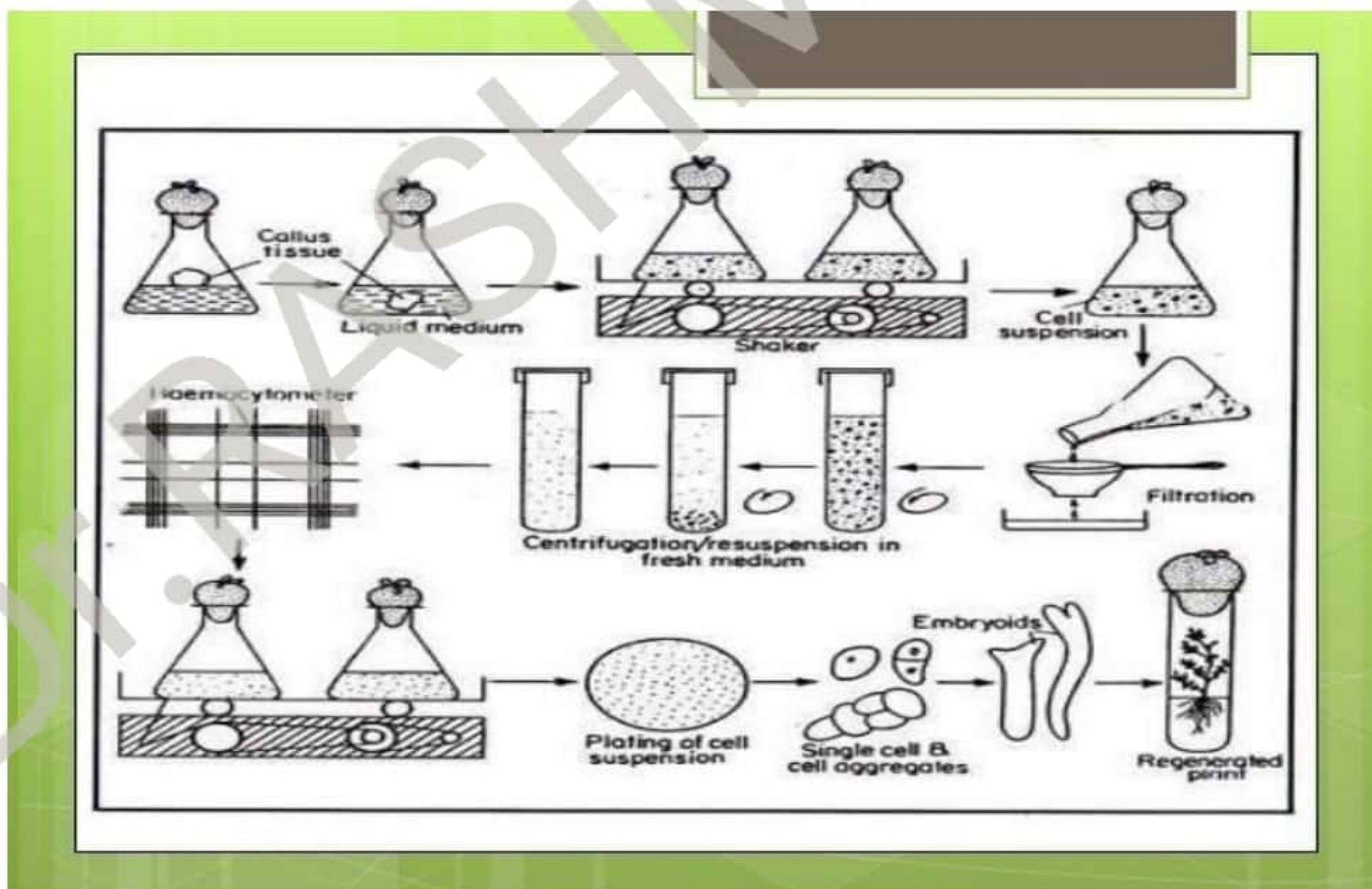
Friable callus

Suspension culture

Suspension culture is a type of culture in which single cells/protoplast or small cell aggregates multiply in agitated liquid medium. The cells remain suspended in a defined aseptic media, controlled physiological condition and regulated environmental condition. It is also referred to as cell culture or cell suspension culture. Suspension culture grows much faster than the callus culture

Several methods of suspension cultures have been developed. There are two main types of suspension cultures :

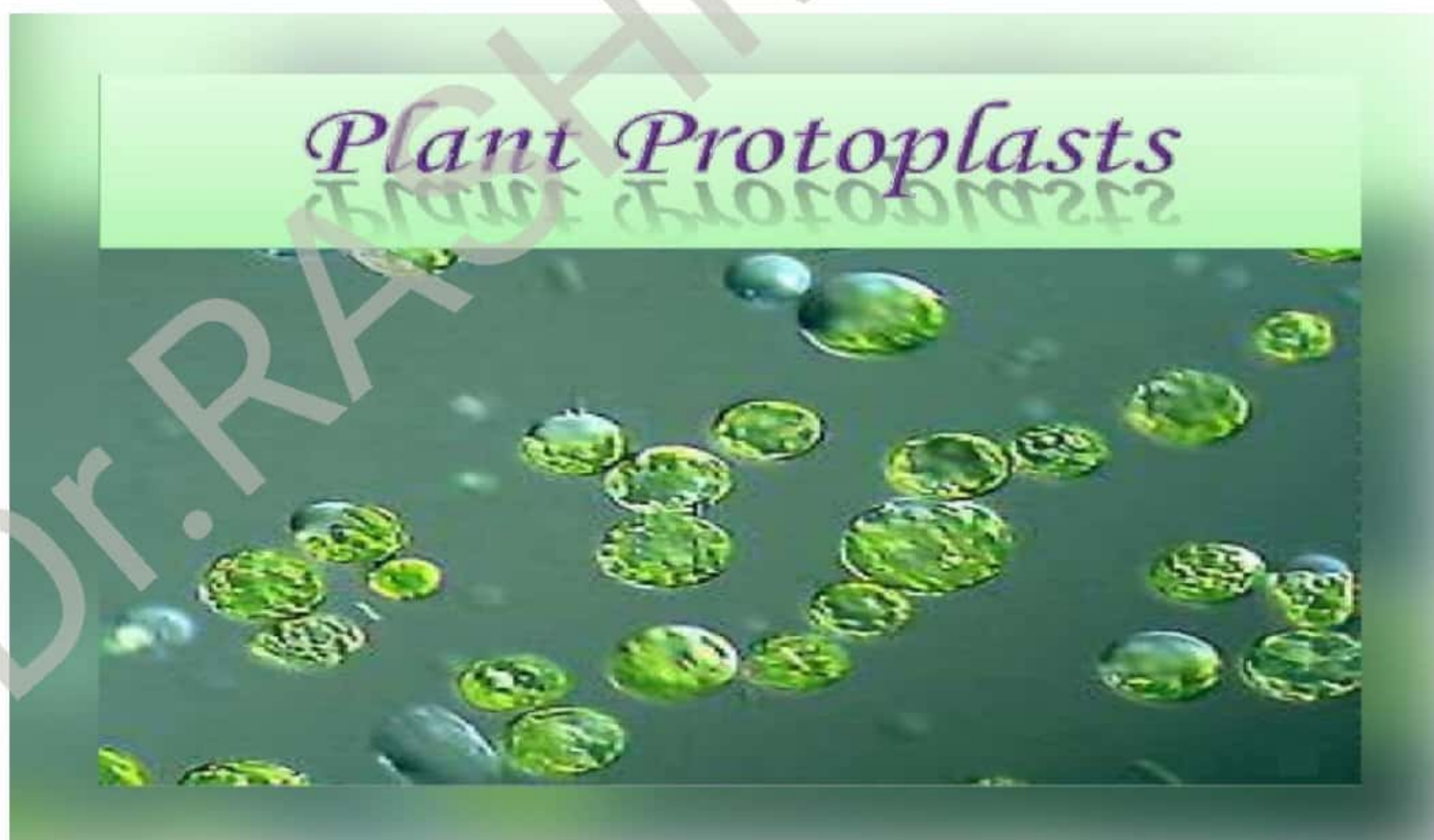
- Batch culture – The cells are nurtured in a fixed volume of medium until growth ceases. These cultures are maintained continuously by sub-culturing. These are most commonly maintained in conical flask, incubated on orbital platform shaker at the speed of 80-120 rpm. It is a closed system in which the biomass growth follows a sigmoid curve pattern of growth, constituting a lag phase, log phase and stationary phase.
- Continuous culture – The cell growth is maintained by continuous replenishment of sterile nutrient medium. 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. The liquid medium of the culture is replaced by a fresh liquid medium to stabilize the physiological states of the growing cells.



Regeneration of plants through cell suspension culture

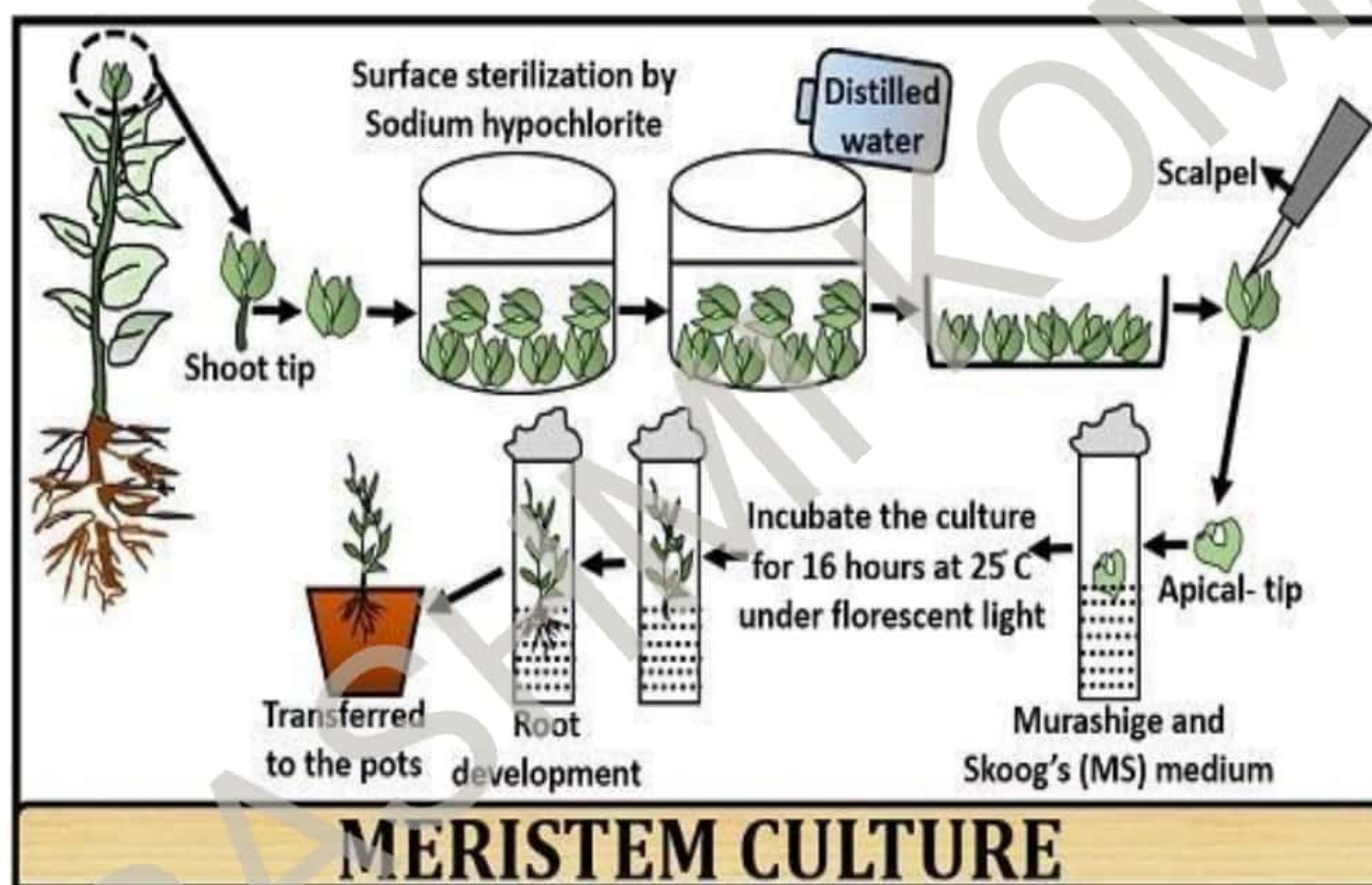
Protoplast culture

The culture of protoplast in an aseptic liquid media is known as protoplast culture. Protoplast is a cell without a cell wall. It is a living part of a plant cell consisting of cytoplasm and nucleus. The basic principle of **protoplast culture** is the aseptic **isolation** of large number of living **protoplasts** and **culturing** them on a suitable nutrient medium for their requisite growth and development. **Protoplast** can be isolated from variety of plant tissues but best and maximum number of protoplast can be isolated from mesophyll tissue of leaves. **Suspension culture** is a type of **culture** in which single **cell/protoplast** or small cell aggregates multiply in agitated liquid medium. The cells remain suspended in a defined aseptic media, controlled physiological condition and regulated environmental condition. It is also referred to as **cell culture** or **cell suspension culture**. Single cell suspension culture can be considered as protoplast culture. Protoplast maybe fused together to form hybrids and cybrids. Protoplast culture is used to introduce and modify genetic information inside any cell. It is used to produce transgenic plants.



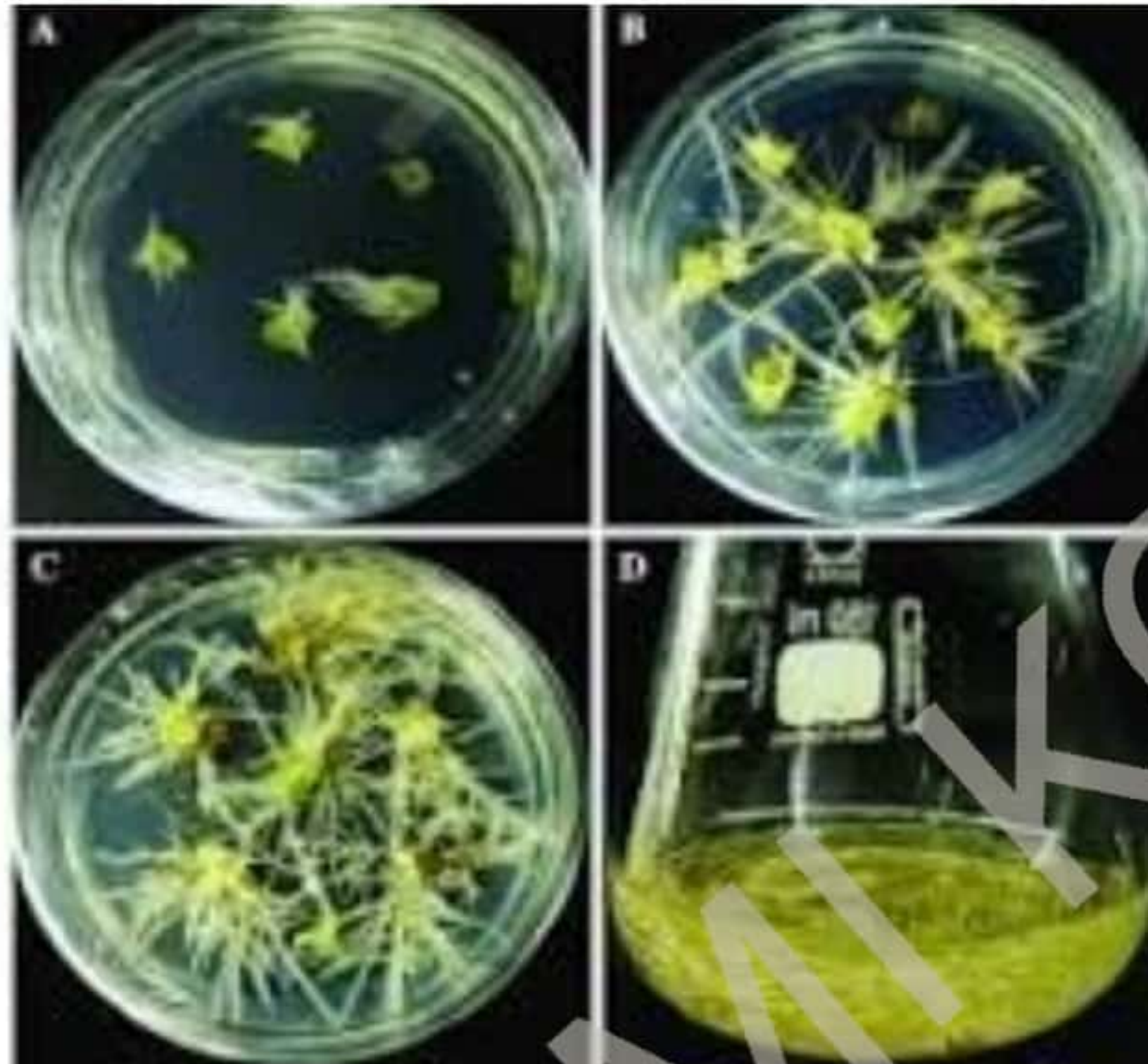
Meristem culture

Meristem culture is the culture of meristematic tissue/ shoot tip *in vitro* in a suitable culture medium. This type of culture is done mostly for the elimination of viruses and related pathogens from a large number of vegetatively propagated plants. It is the main method used in plant virus elimination programs. It is used for mass production of desirable genotypes and helpful in clonal propagation.



Root Culture

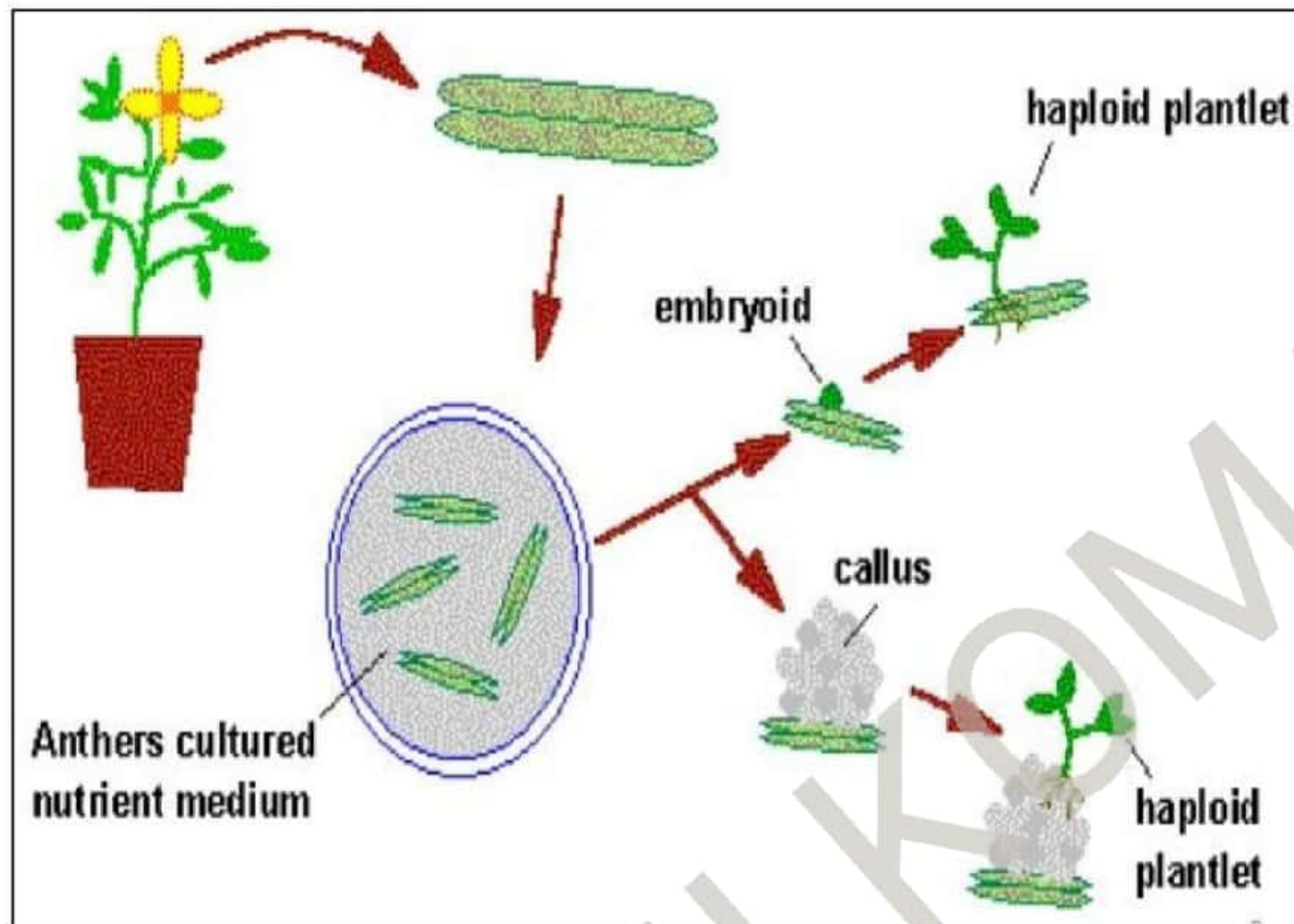
Root culture can be defined as the culture of excised radical tips of aseptically germinated seeds in a liquid medium where they are induced to grow independently under controlled conditions. It is a type of plant tissue culture that is used to study plant metabolic processes or to produce valuable secondary metabolites or recombinant proteins, often with plant genetic engineering.



Regeneration of plants from root explants in liquid medium

Haploid culture

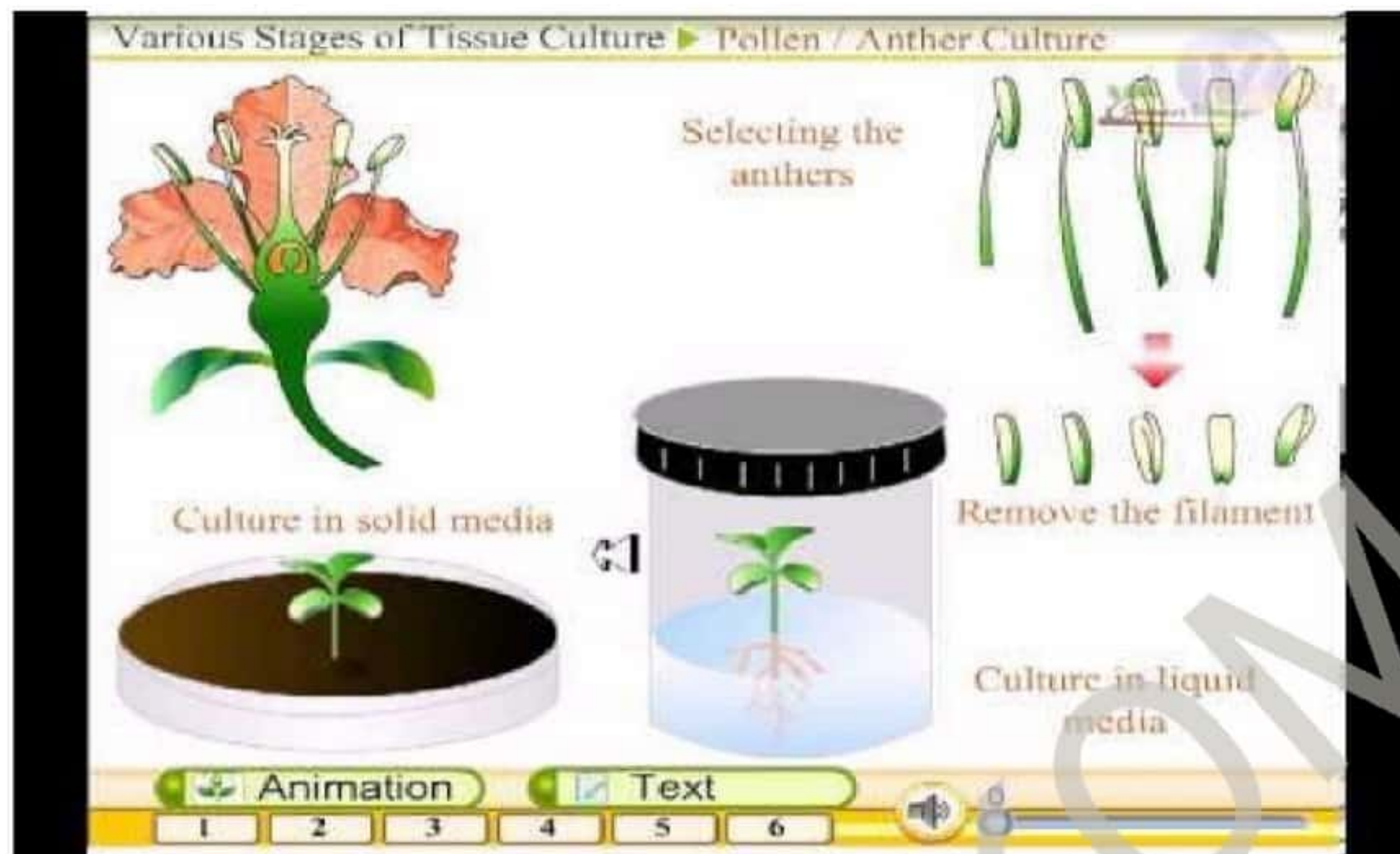
A **haploid plant** is a plant with the gametic or (n) number of chromosomes. Culture of plant tissues containing (n) number of chromosome is called haploid culture. Androgenesis is the process by which **haploid plants** develop from the male gametophyte. When anthers are cultured the procedure is called anther **culture**. When pollen is cultured it is referred as pollen culture. Ovary culture and ovule culture is referred to as the *in vitro* culture of ovary and ovule respectively



Production of haploid plants using anther tissue

Anther / pollen culture

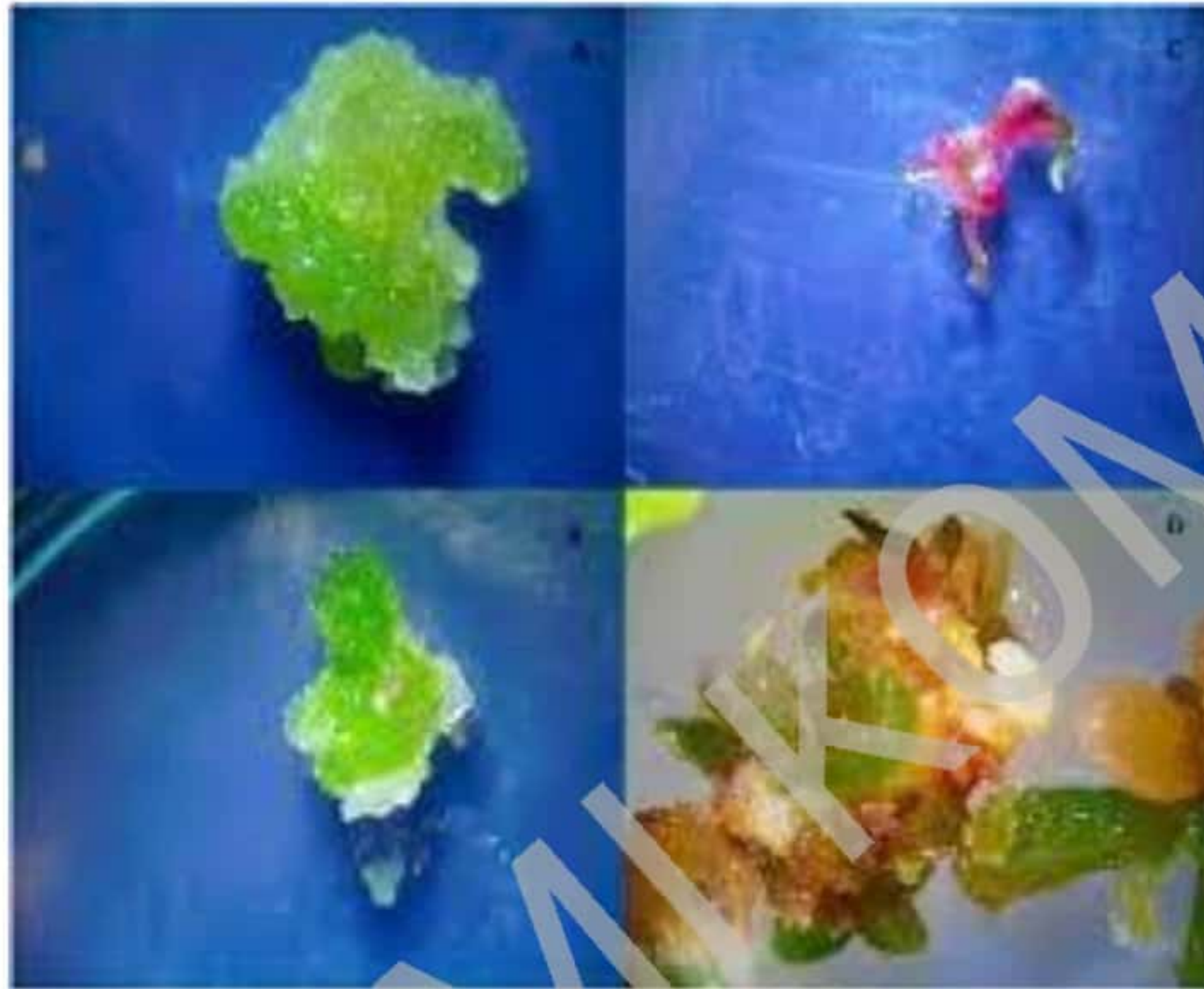
Anther culture is a technique by which immature anther is made to divide and grow into tissue (either callus or somatic embryo), primarily to produce haploid plants with (n) number of chromosome/ haploid set of chromosome. **Pollen culture** (microspore **culture**) is a technique in which haploid plants are obtained from isolated **pollen** grains. The pollen grains are dusted on a suitable culture medium and allowed to grow into callus/ somatic embryo. Plants can be regenerated through shoot and root induction in haploid callus and through culture of haploid embryos. It is possible to produce homozygous, doubled haploid, pure breeding lines through chromosome doubling treatments applied to haploid cells. Guha and Maheshwari (1964) were the first to produce plants in *Datura innoxia* through the culture of anthers containing immature pollen. This technique was discovered by Guha and Maheshwari (1964).



Anther Culture/ Pollen culture

Ovary/ovule culture

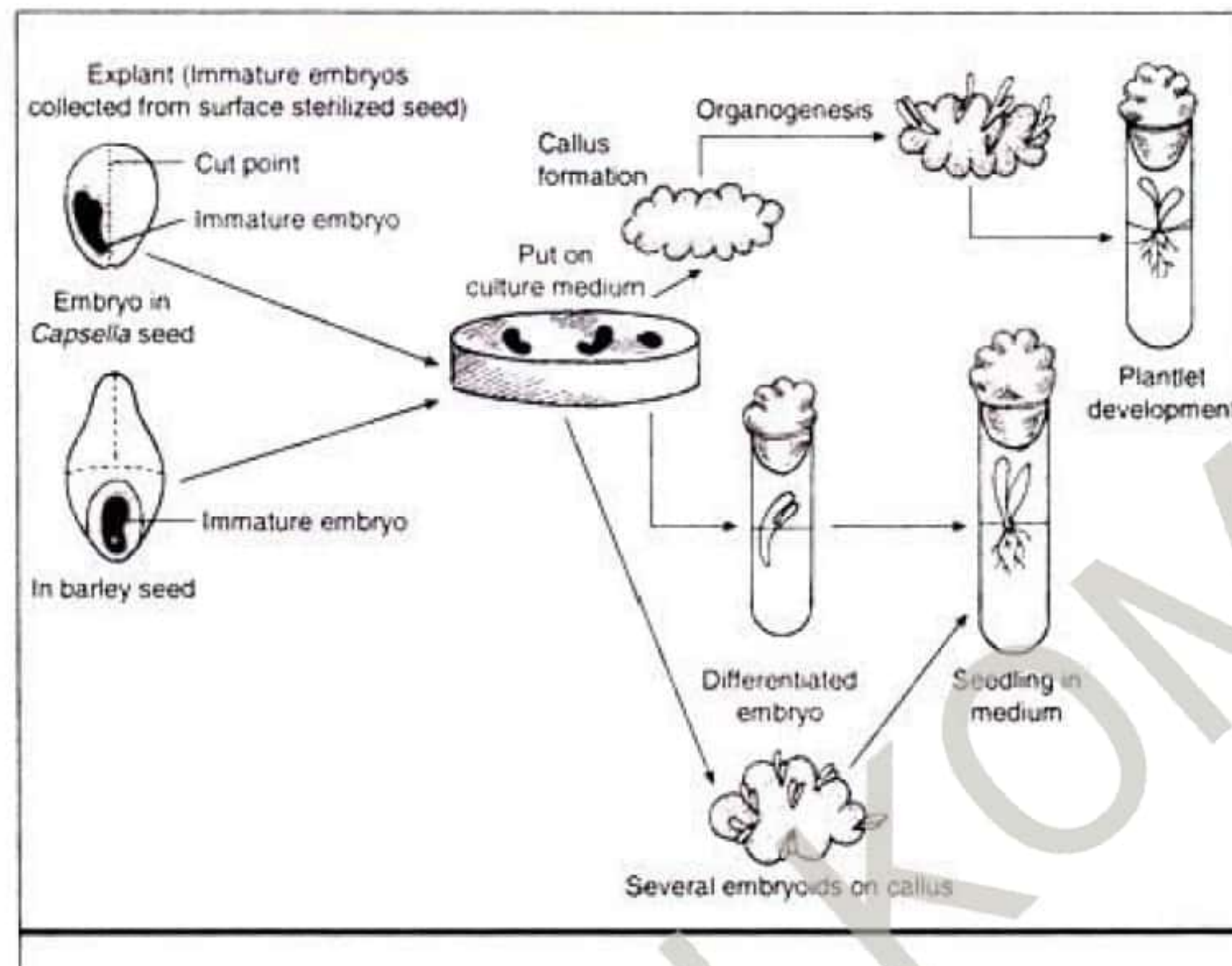
Ovule culture is an elegant experimental system by which **ovules** are aseptically isolated from the **ovary** and are grown aseptically on chemically defined nutrient medium under controlled conditions. **Ovary culture** is a technique of **culture** of **ovaries** isolated either from pollinated or un-pollinated flowers. When the tissue of pollinated and fertilized ovary is taken they produce diploid plants and when unfertilized ovary tissues are taken after it has gone meiosis, it will result in the formation of haploid plants. There is a scarcity of haploid cells within an ovule as compared to the anther so anther or microspore culture is generally used to develop haploid plants. San Noeun first reported successful ovule culture of barley. It is helpful in achieving in vitro fertilization. It can be used in the recovery of hybrid embryos which gets aborted due to incompatibility barrier.



Ovary culture/ Ovule culture

Embryo culture

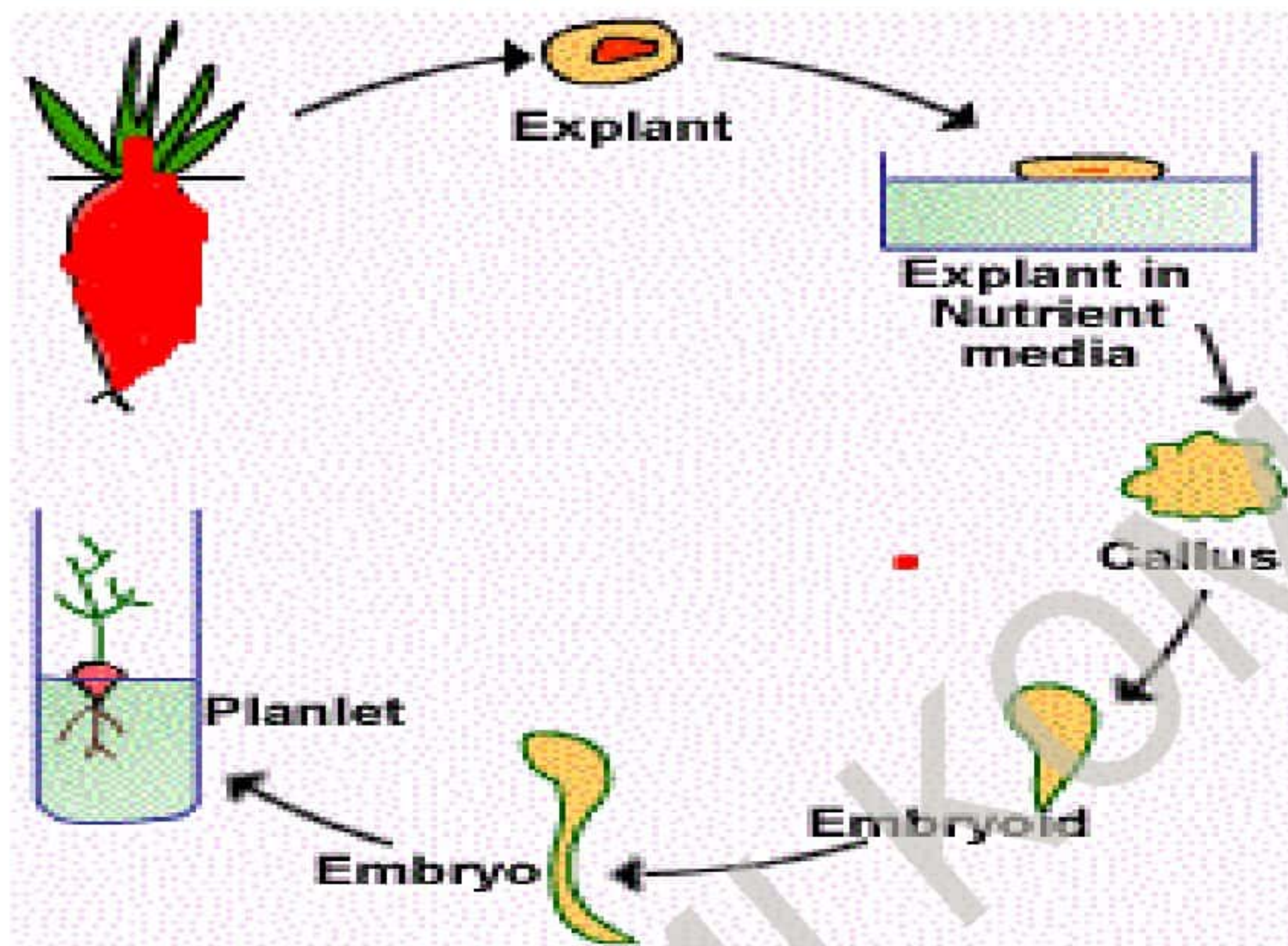
Embryo culture is the culture of isolated immature or mature embryos. Zygotic or seed embryos are often used advantageously as explants in plant tissue culture, for example, to initiate callus cultures. This embryo develops properly when nourishing tissue -- the endosperm remains present in the seed during the development. Embryo culture is applied to overcome embryo abortion due to incompatibility barrier, embryo rescue, to overcome seed dormancy and shortening breeding cycle.



Procedure of embryo culture

Somatic Embryogenesis

Embryos do not always originate from zygotes. It can originate from different types of somatic cells also. These type of embryos are known as somatic embryos and the process of formation of somatic embryo is known as somatic embryogenesis. Somatic embryos are a single cell or a group of cells capable of regeneration into a complete plant. Somatic embryos can be produced directly or indirectly. In direct method embryos are produced from cell or group of cells without forming callus. In indirect method firstly callus are produced from somatic tissues and then embryos are produced from callus tissues or from cell suspension produced from that callus. These embryos are bipolar in nature i.e. they have a radical and a plumule. They are used in synthetic seed formation, for genetic transformation, for mass multiplication of elite germplasm and can be used as materials of embryogenic protoplast.



Regeneration of plants through somatic embryo



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CONTRIBUTORS OR EMINENT SCIENTISTS TOWARDS PLANT TISSUE CULTURE



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THANK YOU