

S.YBSc.Credit pattern Term II BO 242
Botany paper II.Plant Biotechnology
Chapter 2- Plant Tissue culture

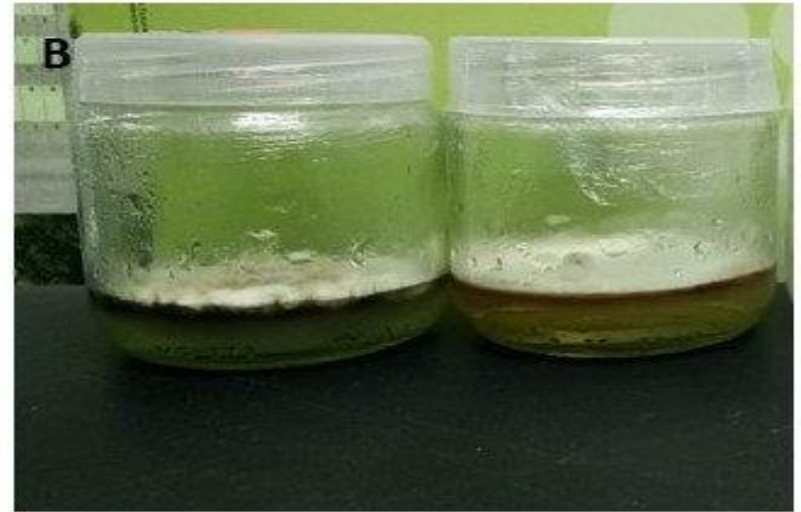
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Types of plant cultures:

- (1) Callus Culture.
- (2) Cell Suspension Culture.
- (3) Anther Culture.
- (4) Ovule Culture.
- (5) Embryo Culture.
- (6) Protoplast Culture.
- (7) Micro Propagation.



1.Seed Culture



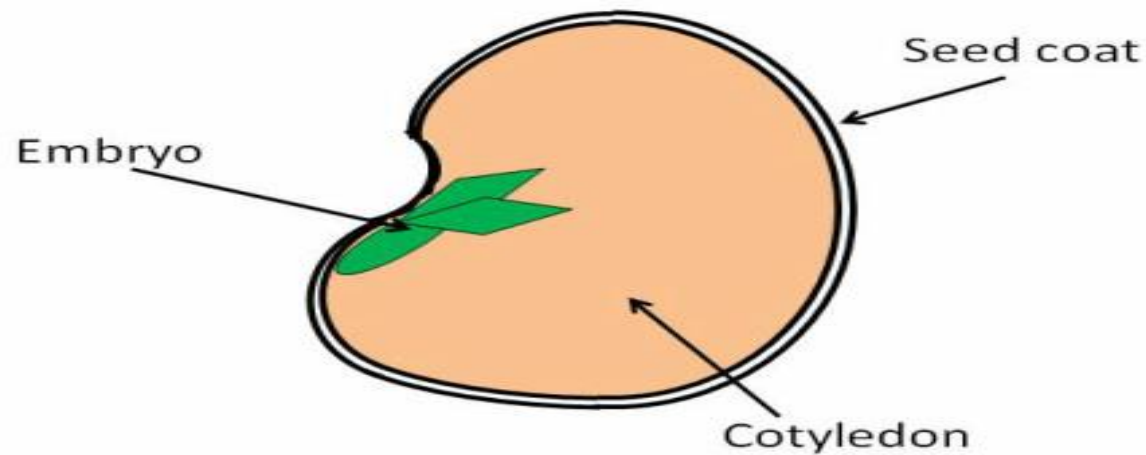
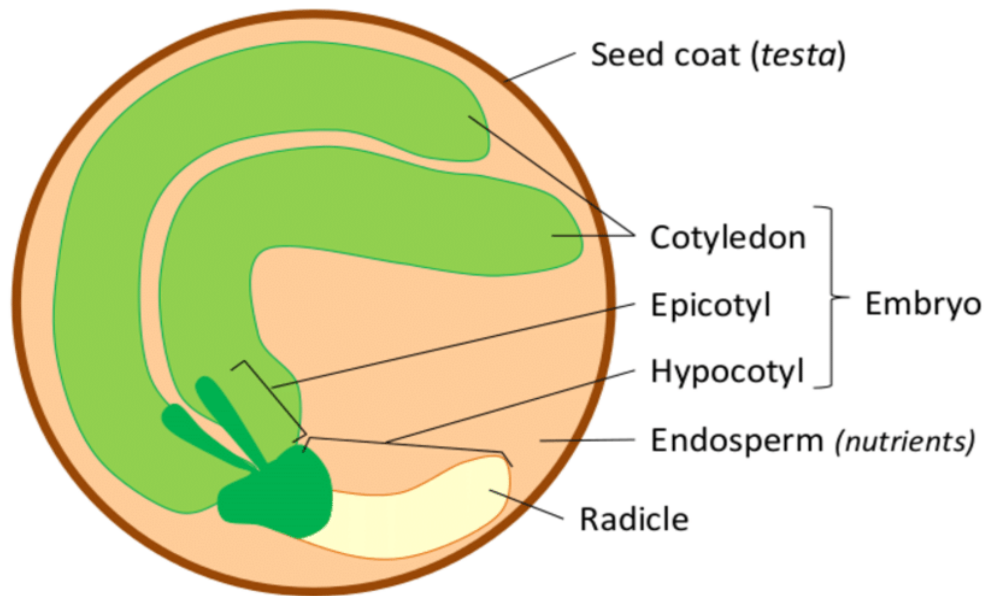
Seed culture



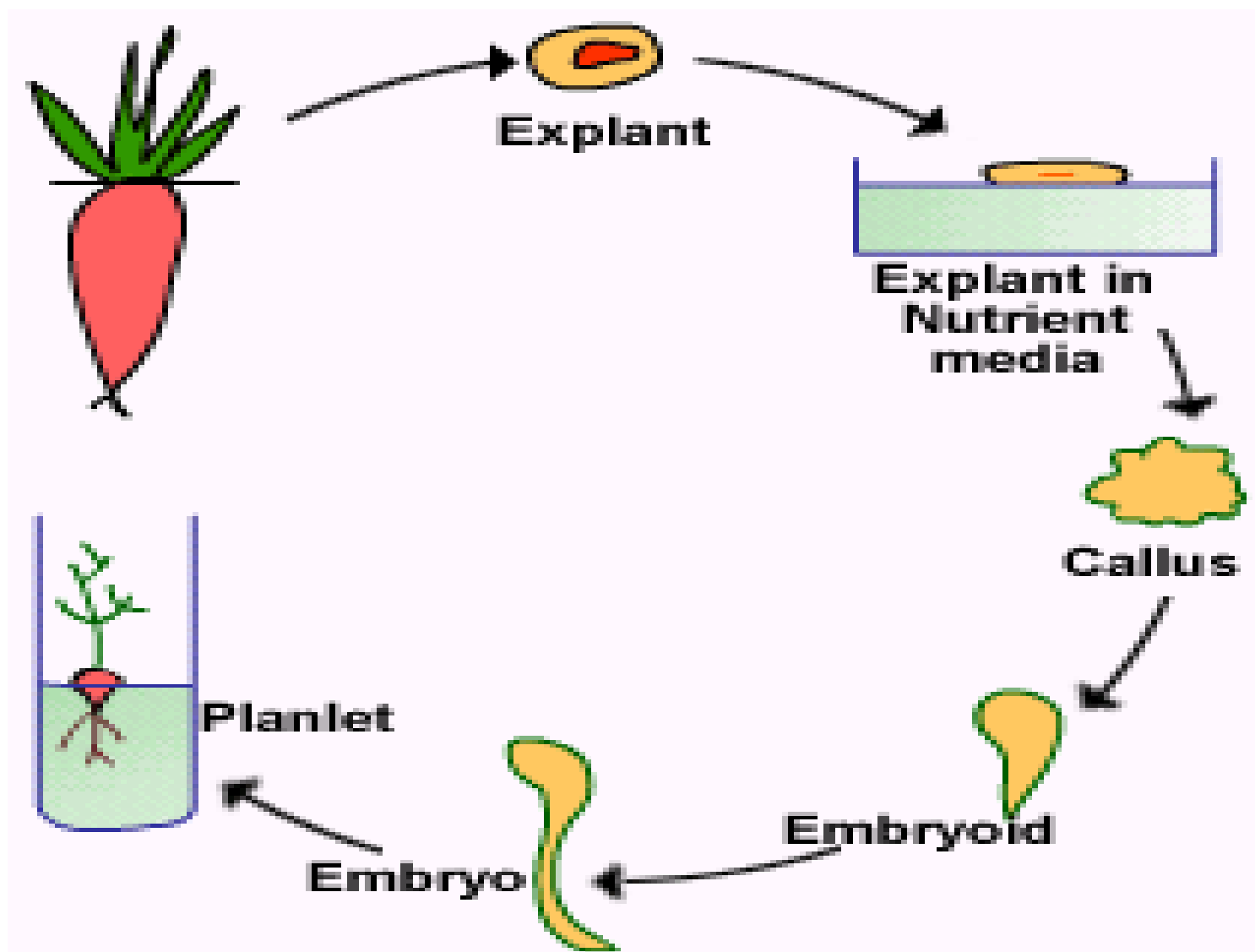
- ✓ Growing seed aseptically in vitro on artificial media
- ✓ Increasing efficiency of germination of seeds that are difficult to germinate in vivo
- ✓ it is possible to independent on asymbiotic germination. Production of clean seedlings for explants or meristem culture

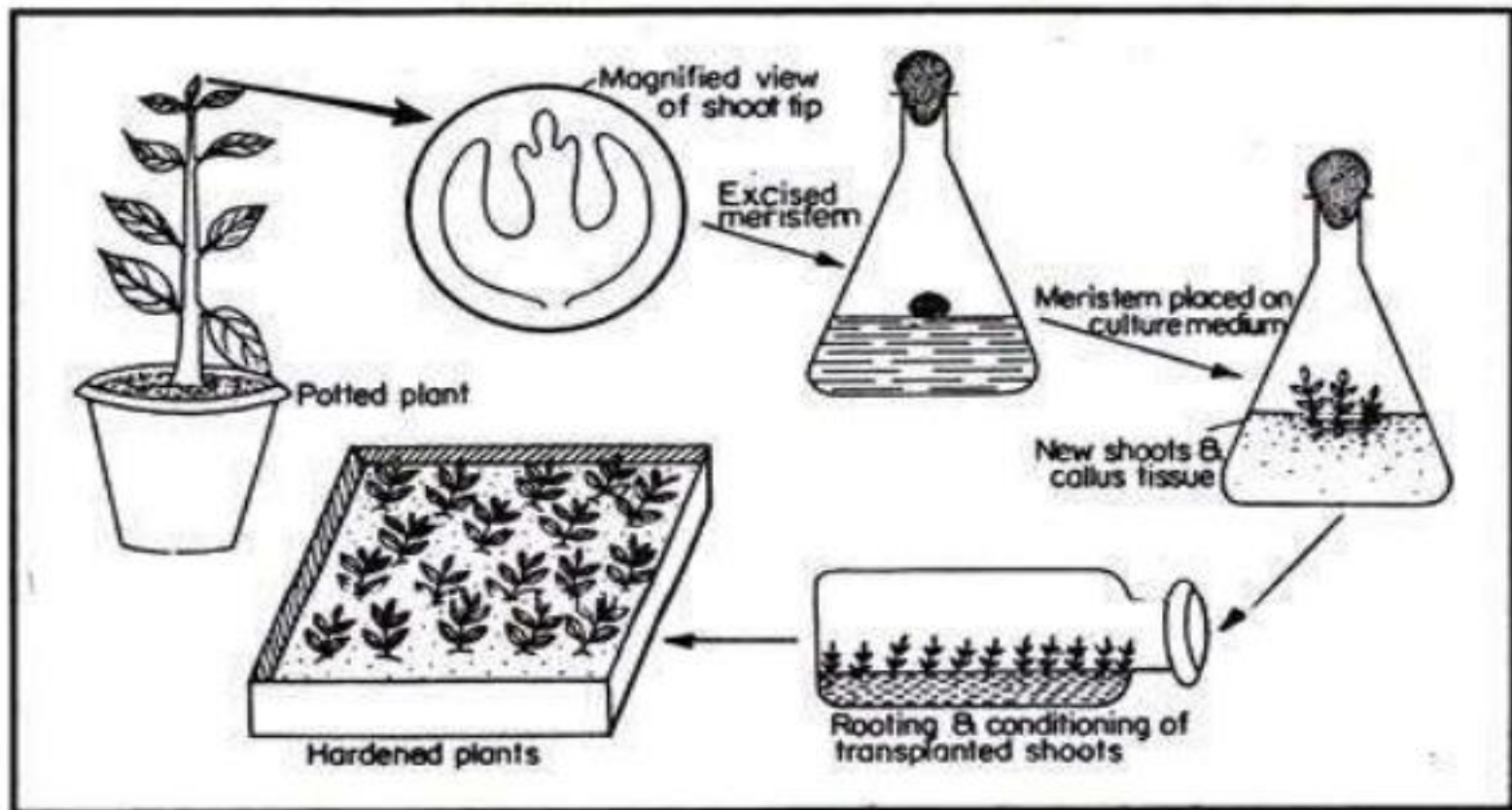
2.Embryo Culture

- Inoculation of immature or mature embryo in-vitro for obtaining of viable plant
- There may be dormancy period of embryo
- Embryo developed from hybridization between two different species may not mature fully due to incompatibility so such embryos are grown by tissue culture .It is called as **Embryo Rescue**.



If we could open your bean seeds, this is what we would see: the baby plant, or embryo, a hard seed coat to protect the embryo, and the cotyledons which will feed the embryo until it can make food for itself.





□ Fig 2.3

Flow diagram illustrating the technique of shoot tip or meristem culture

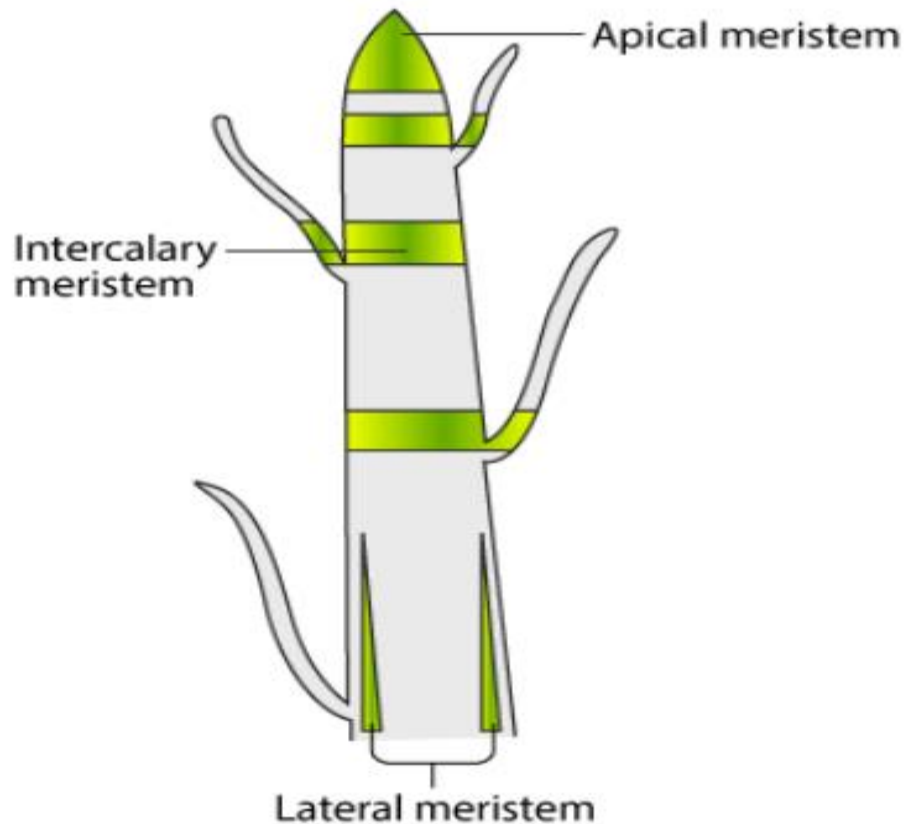


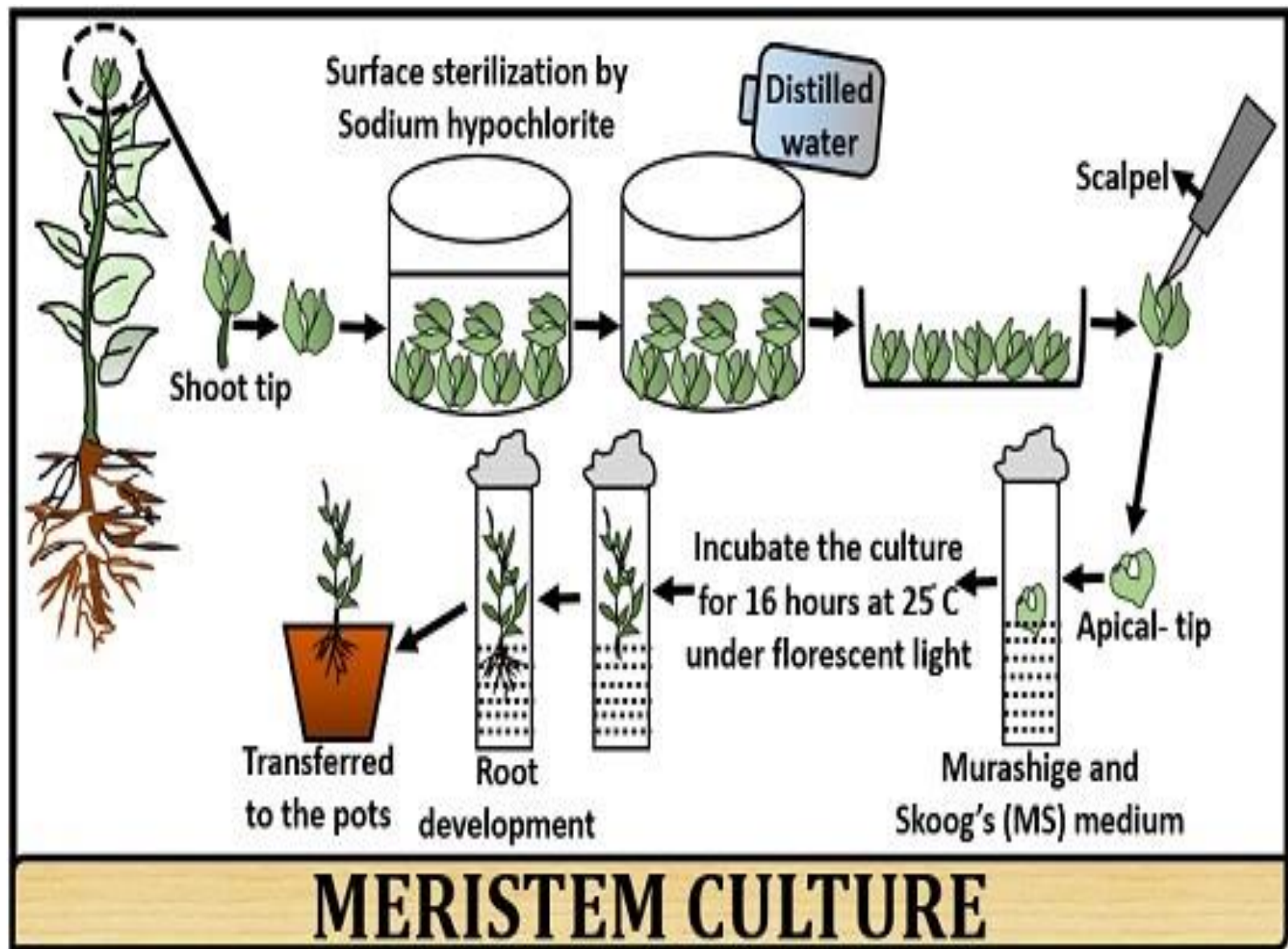
Applications of Embryo Culture

- Production of haploids
- Prevention of embryo abortion in wide crosses
- Overcoming Seed dormancy
- Shortening of breeding cycle
- Prevention of embryo abortion with early ripening stone fruits

3.Meristem Culture

- Meristem tip of 0.2-0.5mm is use to produce virus free plant.





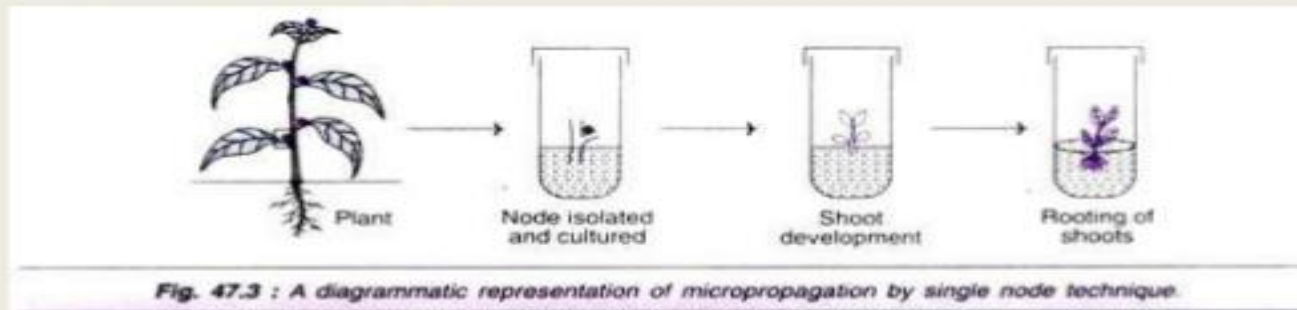
4. Bud culture

Bud is meristematic tissue

➤ **Bud cultures:** 2 types of bud cultures are used

1. Single node culture
2. Axillary bud culture

- **Single node culture:** the bud found in the axil of leaf along with a piece of stem is isolated and cultured to develop into a plantlet.
- **Axillary bud culture:** shoot tip with axillary bud is isolated.



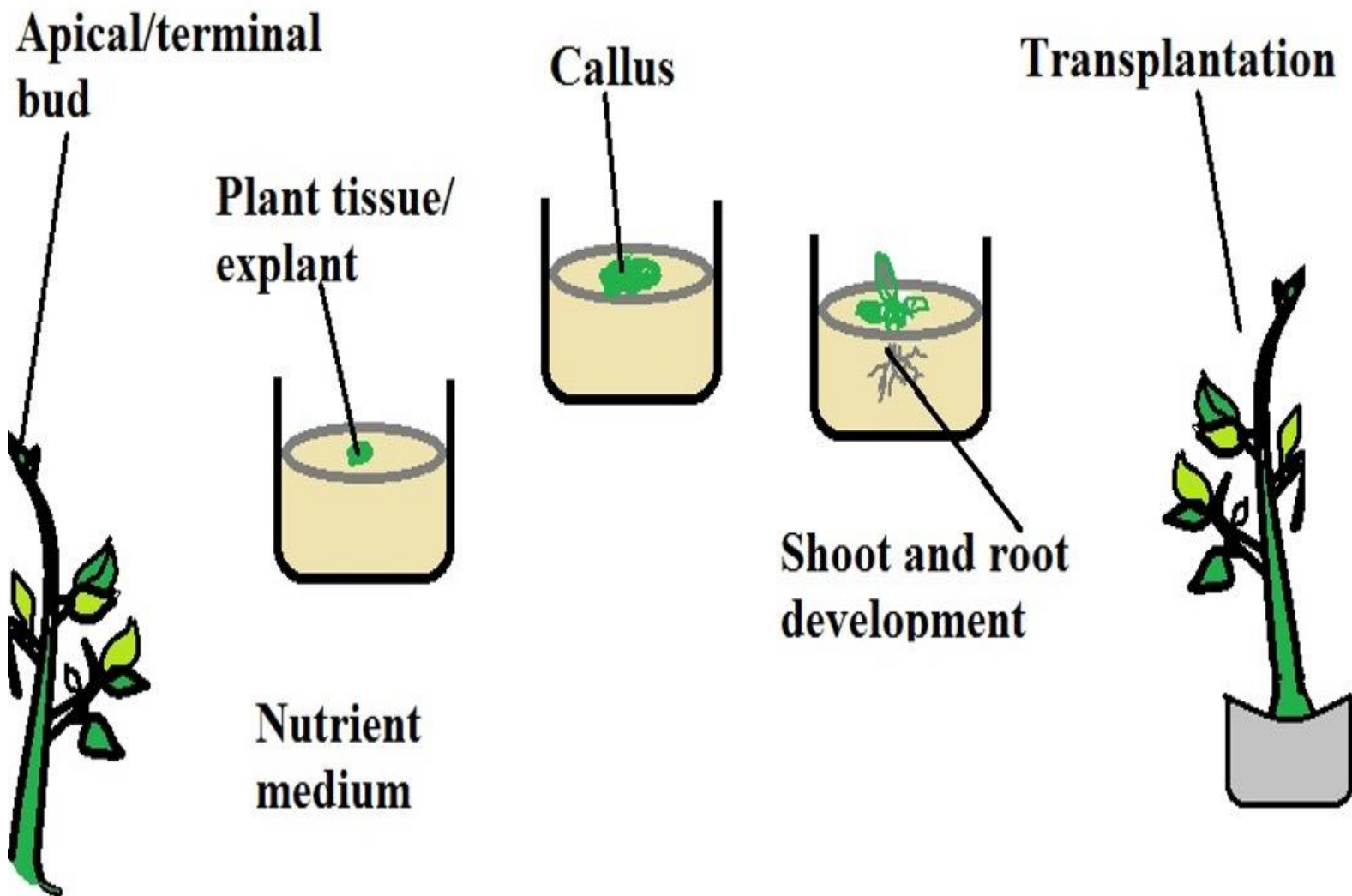
5.Callus culture

- Any small sterilised vegetative part of plant (leaf, root,stem etc.) can use for callus culture.
- That explant is inoculated on nutrient media having agar
- Cells of explant divides rapidly & form undifferentiated mass of **parenchymatous** cell called callus
- When callus subjected to new **shooting media**(high cytokinin & low auxin concentration) then it forms many shoots (morphogenesis)

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.





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.

Callus Suspension culture

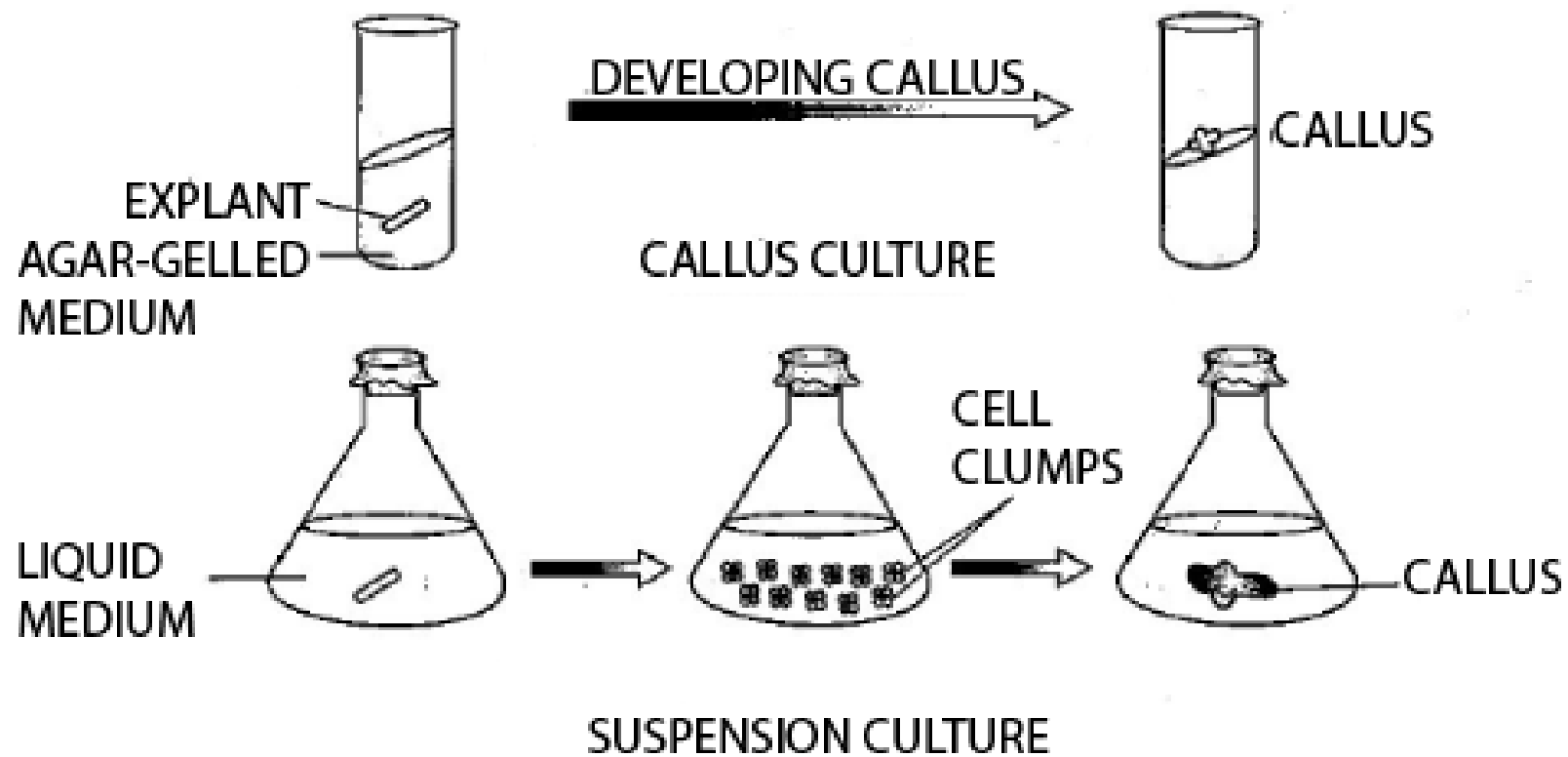
Place friable callus into a liquid medium and agitate

Single or small clumps of cells will be released into the medium, where they keep growing and dividing to eventually a cell suspension.

The size of inoculum have to be sufficient , suspension can be build up faster.

Too much cells and there is a risk that toxic products from damaged cells could accumulate to a inhibiting level.

Cell suspension cultures should also be subcultured periodically.

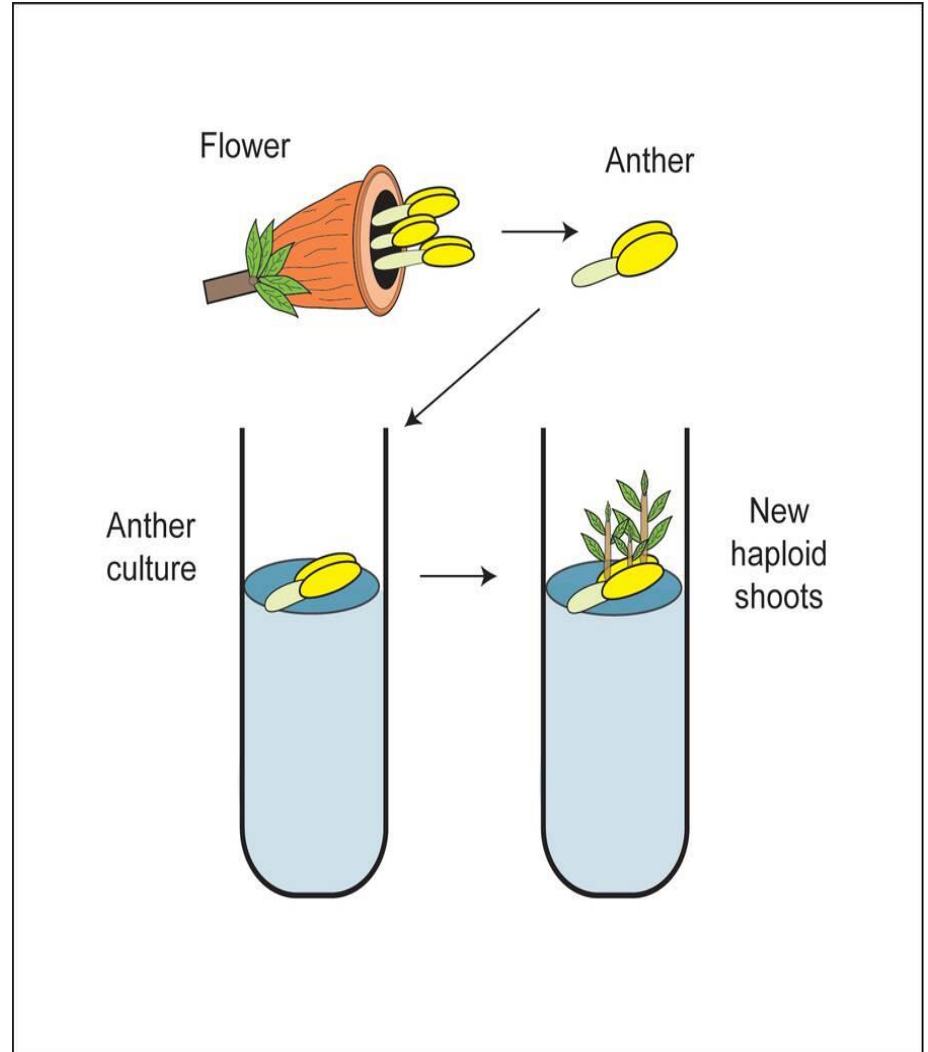


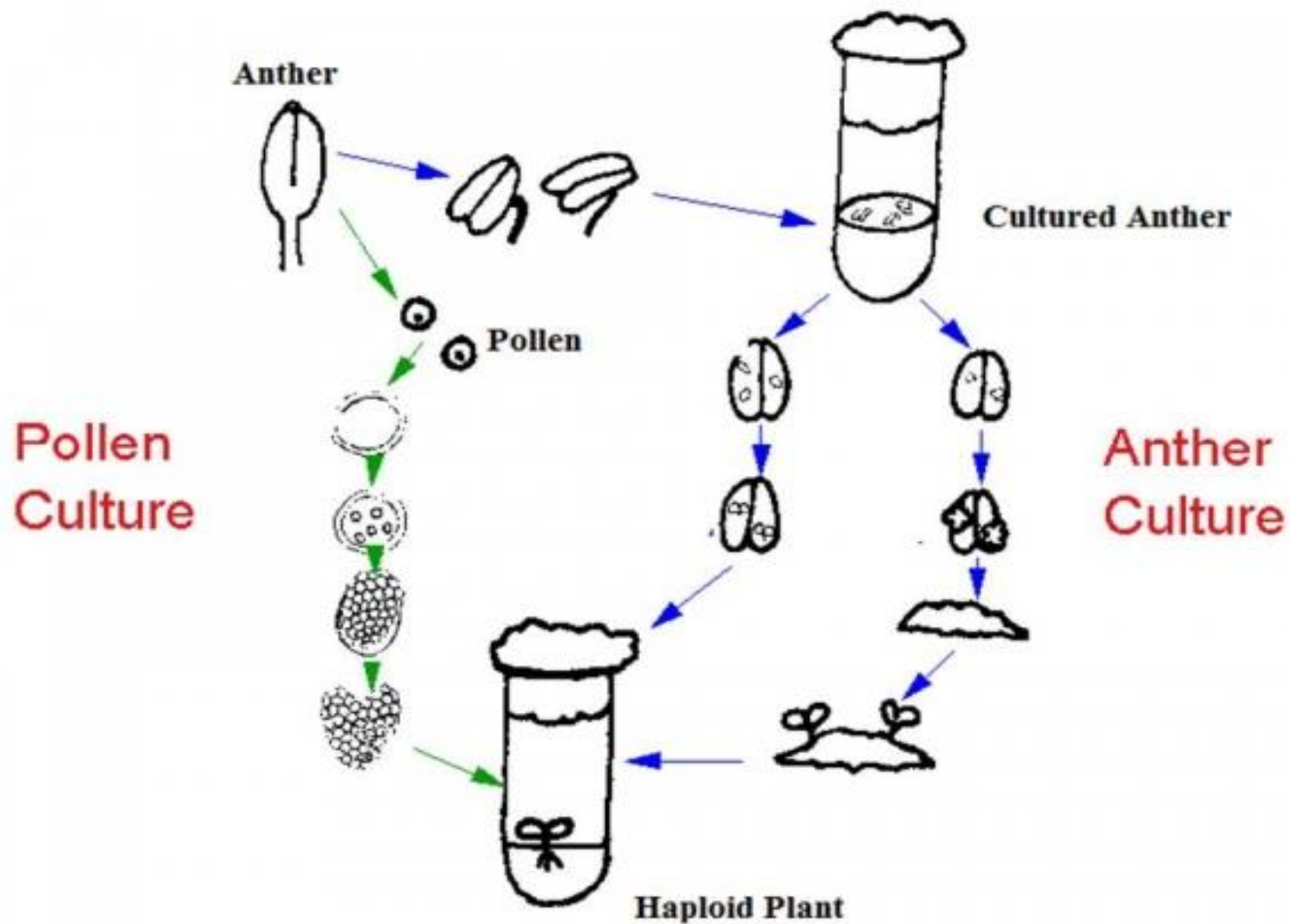


<u>Callus Culture</u>	<u>Suspension Culture</u>
1. In this culture, cell division in explant forms a callus. Callus is an irregular unorganised and undifferentiated mass of actively dividing cells.	1. It consists of single cells and small groups of cells suspended in liquid medium.
2. The culture is maintained on agar medium	2. The culture is maintained in liquid medium
3. The medium contains growth regulator the auxin such as 2, 4 -D and cytokinin like BAP.	3. The medium contains growth regulator the auxin such as 2, 4-D only
4. Callus is obtained within 2 - 3 weeks.	4. Suspension culture grows much faster than callus culture.
5. It does not need to be agitated.	5. It must be constantly agitated at 100-250 rpm (revolutions per minute).

7. Anther / pollen culture

- Uninucleated anthers are inoculated on growth medium for callus formation.
- Androgenic Callus get converted into embryos
- From embryo **haploid plants** are developed. Eg. Rice, wheat, maize mustard, pepper etc.





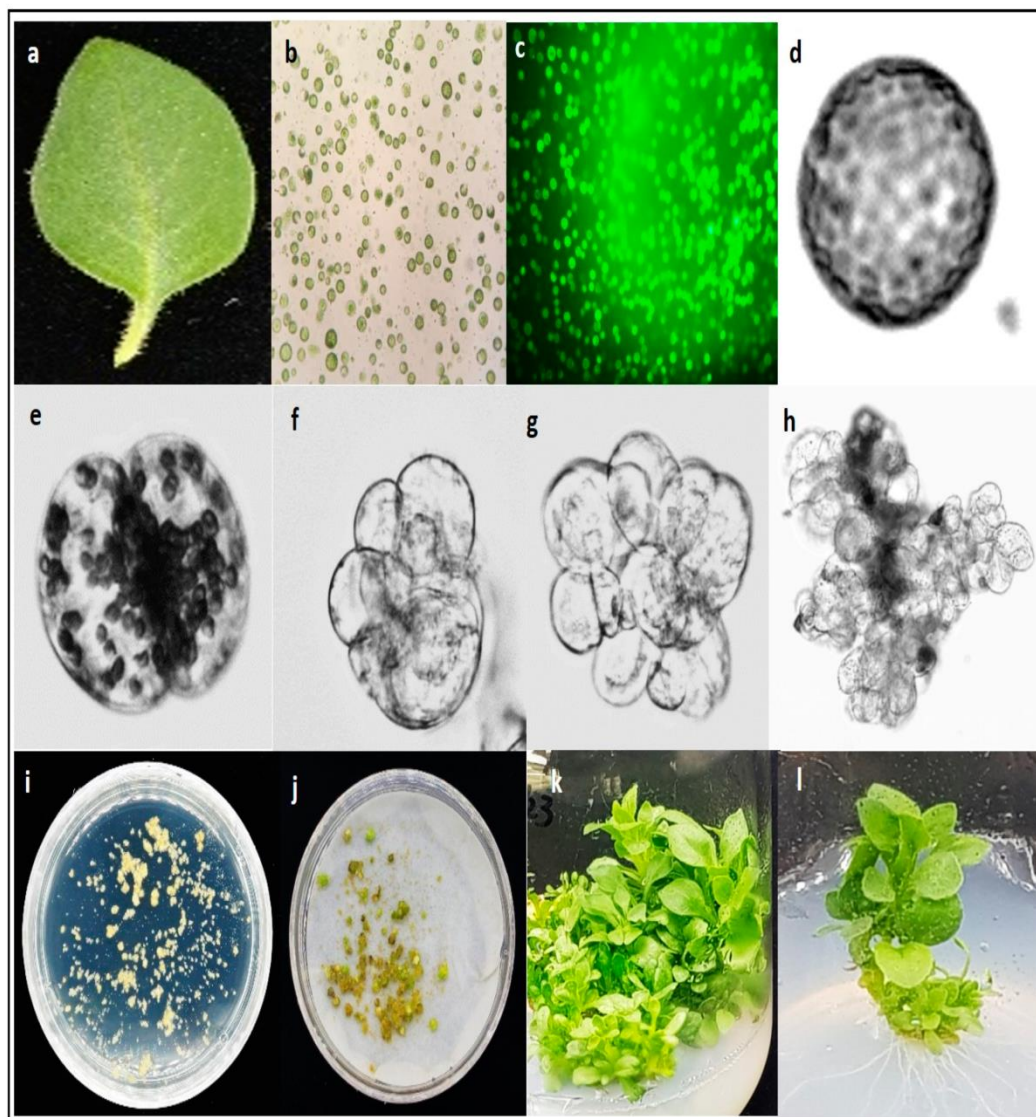
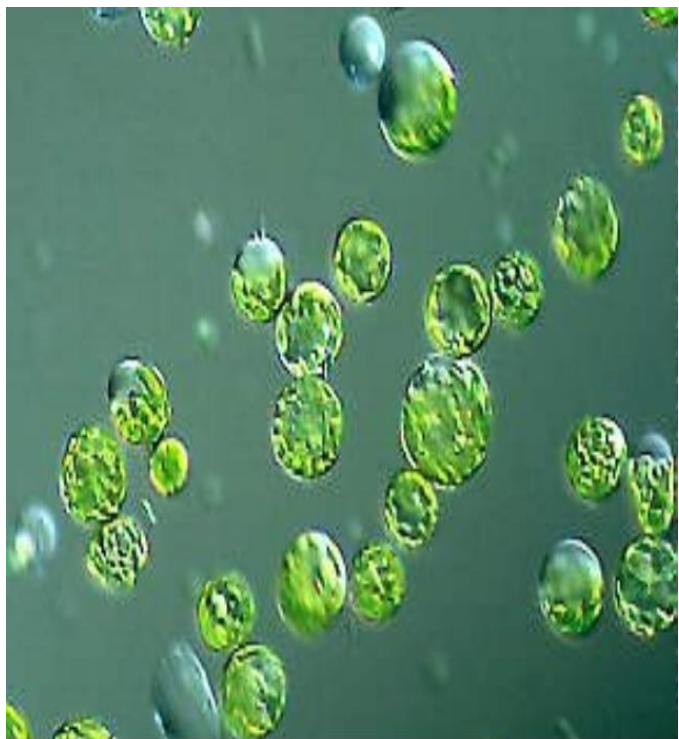
8. Protoplast culture

- Isolated **protoplasts** have been described as "naked" cells because the **cell wall has been removed** by either a mechanical or an enzymatic process.
- These viable protoplasts are purified.
- Isolated protoplast usually cultured on liquid or semi solid agar medium
- Methods of culture- 1.Hanging drop
2.microculture chamber 3.soft agar matrix

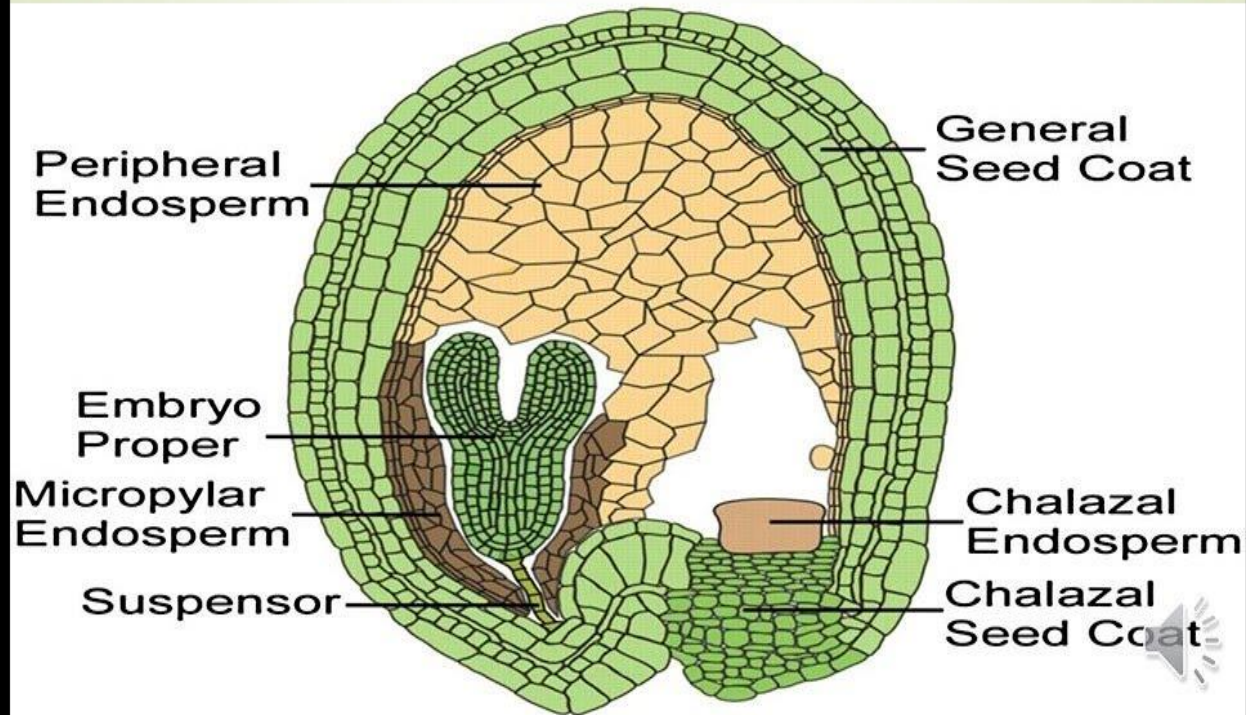
Application of Protoplast

Protoplasts can be used:

- In the production of Cybrid
- For Somatic Hybridization to overcome sexually incompatible species
- Ingesting “Foreign” material into cytoplasm
- For DNA transformation
- Used to study wall synthesis and decomposition
- Studied as Single Cell System



ENDOSPERM CULTURE AND ITS APPLICATIONS

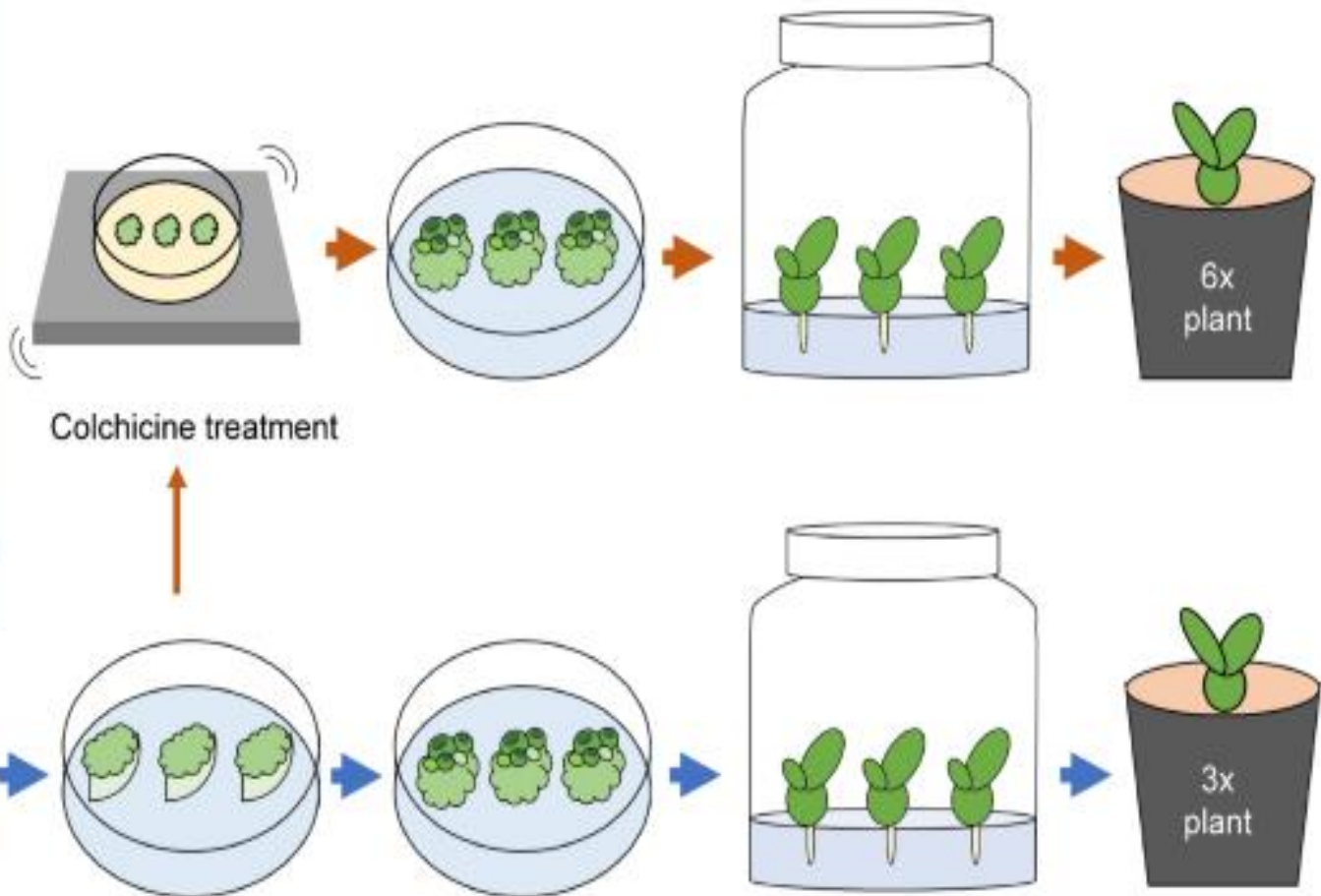
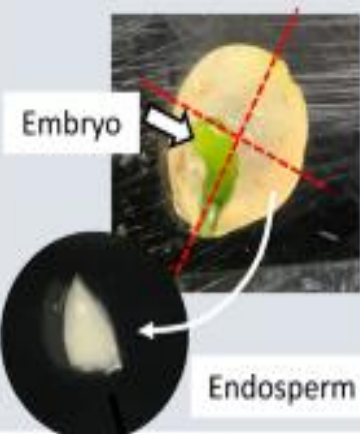


STEPS IN ENDOSPERM CULTURE

- It consist of mainly 3 steps:
- 1. The immature or mature seeds are dissected under aseptic conditions and endosperms along with embryos are excised.
- 2. Excised endosperms are cultured on a suitable medium and embryos are removed after initial stage.
- 3. Callus followed by embryogenesis or shoot bud differentiation.
- 4. Complete plant formation.



Simultaneous production of triploid and hexaploid plants by endosperm culture with colchicine treatment in diploid *Haemanthus albiflos*



Callus induction, proliferation

Somatic embryogenesis
Shoot formation

Plantlet

Acclimatization

TYPES OF ENDOSPERM CULTURE

- **1. Mature endosperm culture:** It is the in vitro development of isolated mature endosperm from ripen endospermic seed on suitable culture medium to obtain triploid plantlet is called mature endosperm culture.
- **2. Immature endosperm culture:** It is the in vitro development of isolated immature endosperm isolated at precise stage from immature seed, cultured on suitable culture medium to obtain triploid plantlet is called immature endosperm culture.

APPLICATIONS OF ENDOSPERM CULTURE

- Techniques of endosperm culture has enabled the production of triploid plants. Triploid plants are self sterile and usually seedless. The trait increases edibility of fruits and is desirable in plants such as apple, banana, grape, watermelon and mango which are commercially important.
- In timber and fuel yielding plants, triploids show better performance over their relative diploids or tetraploids. Also there is no problem of seed sterility as they can be multiplied by vegetative means.



Embryo Rescue

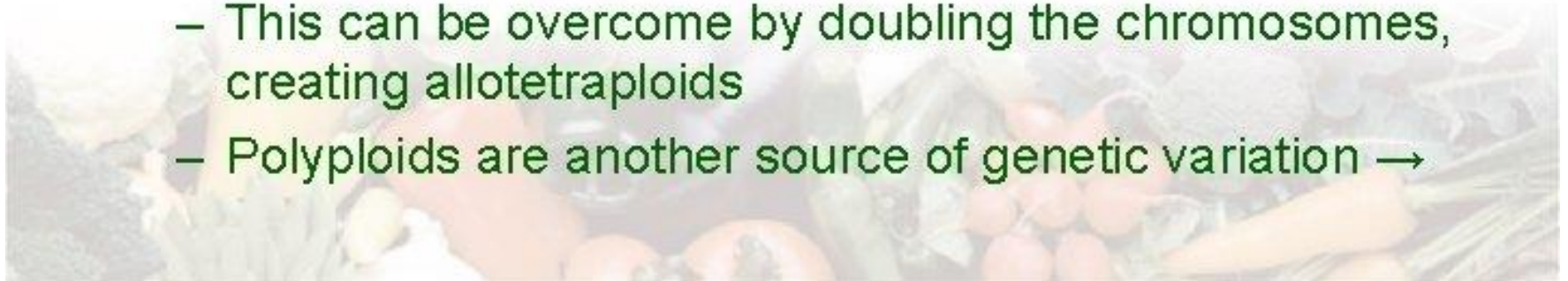
- Post fertilization barriers retard the development of the zygote after fertilization and normal development of the seed.
(due to failure of endosperm)
- This is known as embryo-endosperm incompatibility where the endosperm produces toxins that kills the embryo.

Embryo rescue :

- Embryo rescue
- When embryos fails to develop due to endosperm degeneration, embryo culture is used to recover hybrid plants; this is called hybrid rescue.
- e.g; *H. vulgare* \times *Secale cereale*.
- Embryo rescue generally used to overcome endosperm degeneration.

Embryo Rescue Process

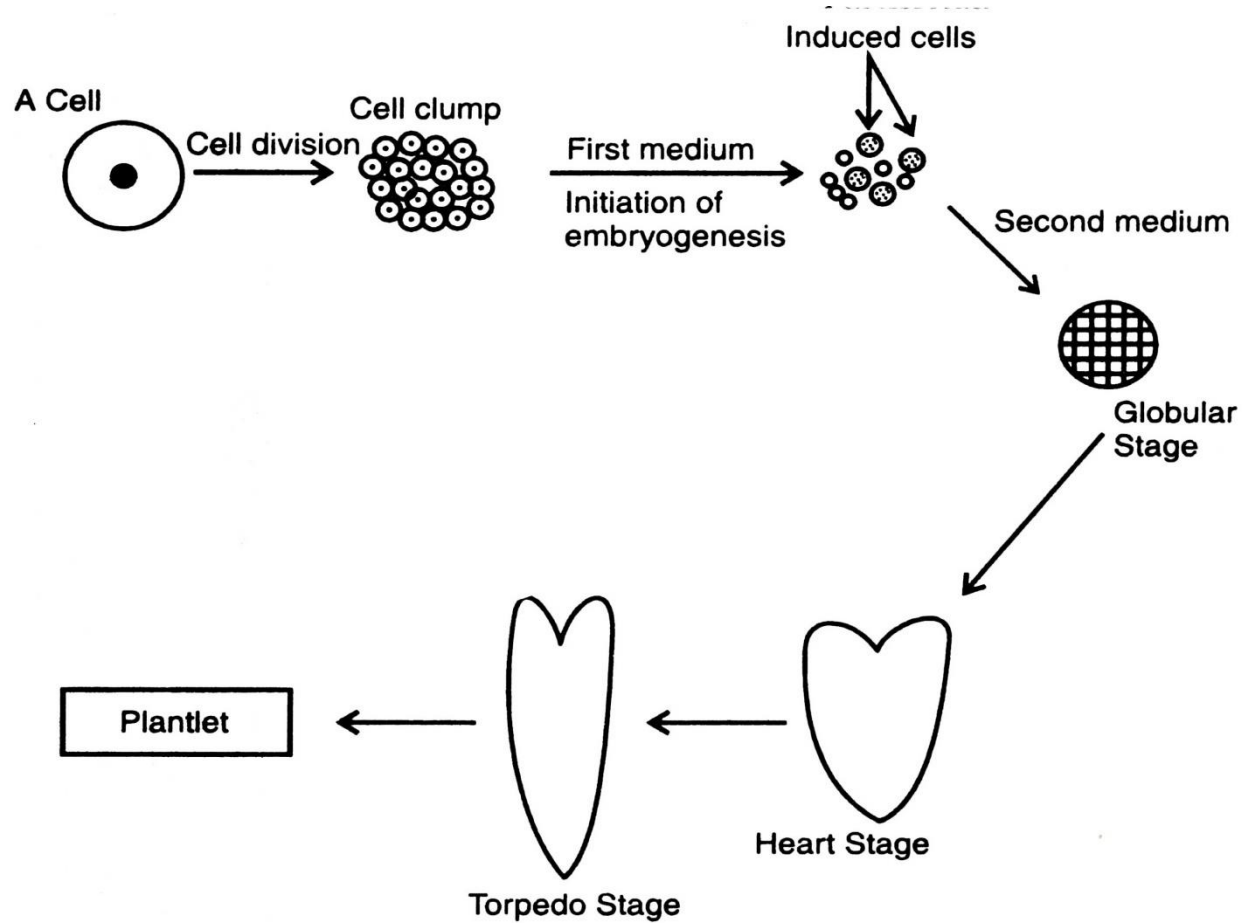
- Make cross between two species
- Dissect embryo (usually immature)
 - The younger the embryo, the more difficult to culture
- Grow on culture medium using basic tissue culture techniques, use for breeding if fertile
- Many times, resulting plants will be haploid because of lack of pairing between the chromosomes of the different species
 - This can be overcome by doubling the chromosomes, creating allotetraploids
 - Polyploids are another source of genetic variation →



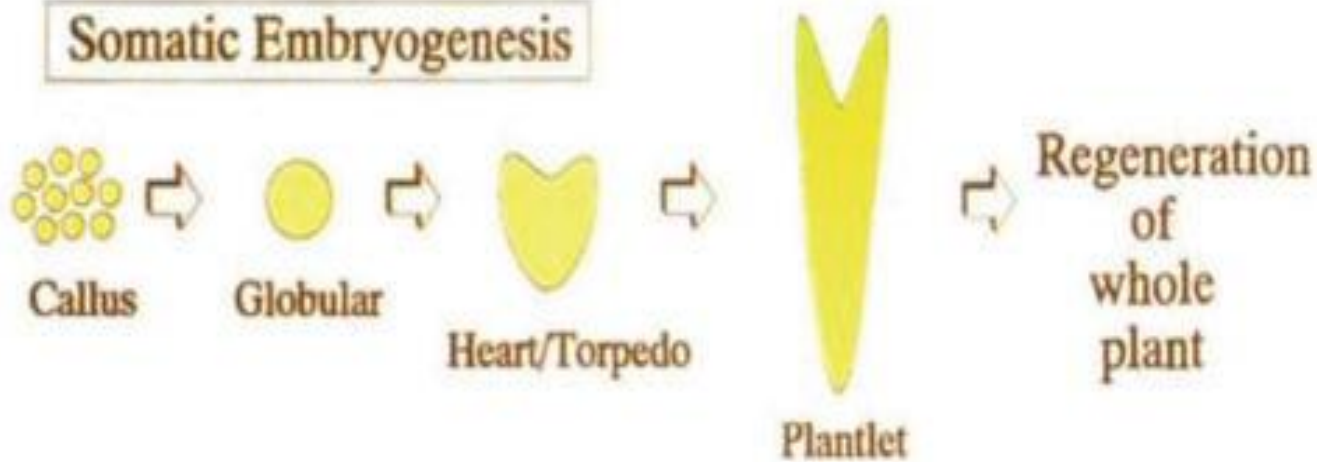
Application of Embryo Rescue

- **Overcoming dormancy-** in some fruit plants embryos require period of after ripening before germination. for example Prunus, Taxus etc.
- **Breeding cycle shortening-** seeds which takes 10-20 days for maturation places on in vitro culture to overcome maturation time.
- **Inter specific hybrids recovery-** some time distant crossed may fail cause of inability pollen to germinate, failure of pollen tube to grow, degeneration of endo-sperm, all causes fail to embryo germination so this problem overcome by embryo rescue.

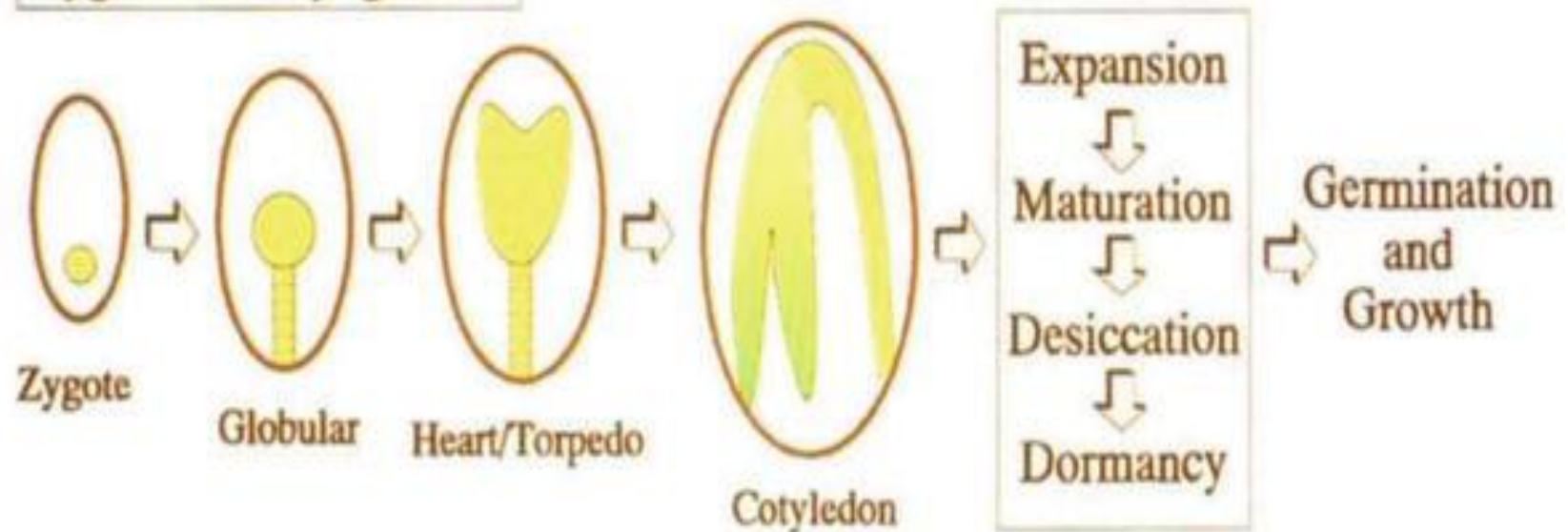
Somatic embryogenesis



Somatic Embryogenesis



Zygotic Embryogenesis



Somatic embryogenesis (Non zygotic /Non –sexual)

- Regeneration of embryos from somatic cells ,tissues,organs diretly in-vitro condition .
- Stages of embryo develop,- 1.Glogular 2. heart shape 3.Torpenodo stage.



Organ Culture

- It is the culture involving the growth of isolated plant organ such as root ,shoot, leaf and even flower as an explant in the in-vitro onditions using sutable nutrient medium.
- Infact any plant organ can serve a an explant e.g. ovary, nucellus, embryo, flower bud, microspore, etc.