

TYPES OF TISSUE CULTURE

TO,
FIFTH SEMESTER STUDENTS

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TYPES OF TISSUE CULTURE

1. Callus culture
2. Suspension culture
3. Cell culture
4. Meristem culture
5. Root tip culture
6. Leaf or leaf primordial culture
7. Shoot tip culture
8. Complete flower culture
9. Anther & pollen culture
10. Ovule & embryo culture
11. Bud culture
12. Seed culture
13. Organ culture



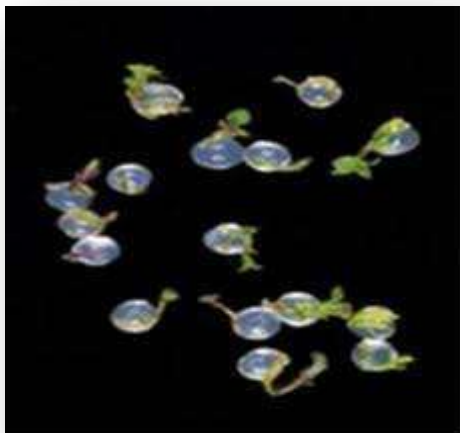
Callus culture



Ovule culture



Protoplast culture



Suspension culture



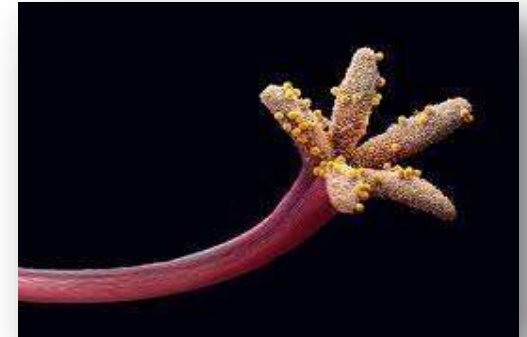
Root tip culture



Leaf primordial culture



Pollen culture



Shoot tip culture



Flower culture



Callus Culture

- In Callus culture, cell division in explant forms a callus.
- Callus is **irregular unorganized and undifferentiated mass of actively dividing cells.**
- Darkness & solid medium gelled by agar stimulates callus formation.
- The medium contains the auxins and BAP (Benzyl amino purines). Both are growth regulators (Hormones).
- This stimulates cell division in explant.
- Callus is obtained within **2-3 weeks.**

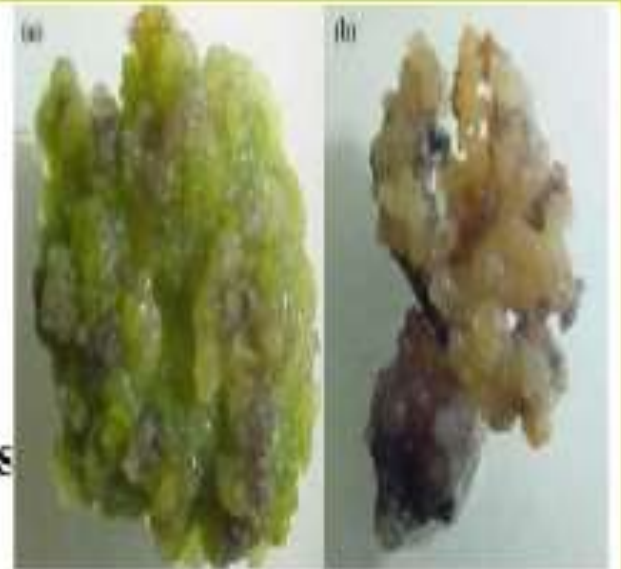
Callus

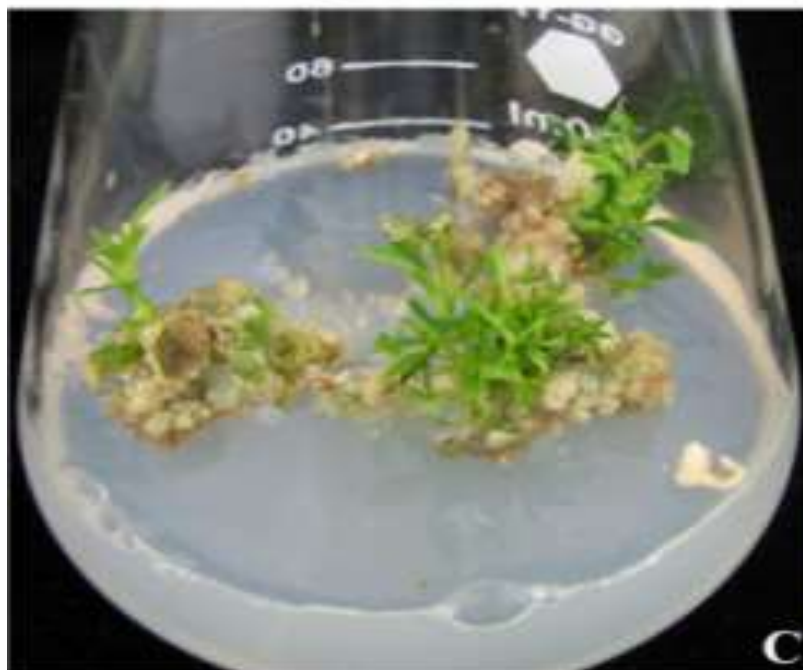
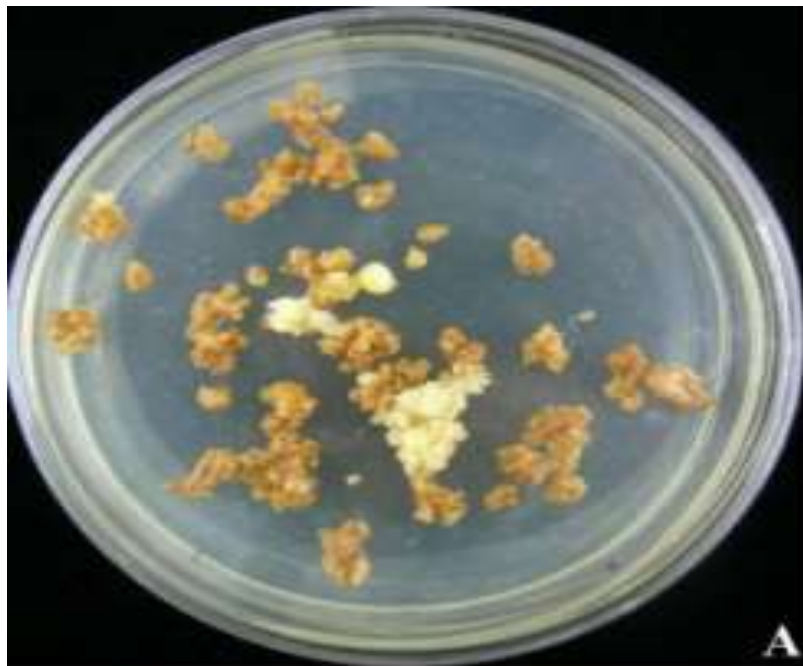
Callus cultures may be compact or friable.

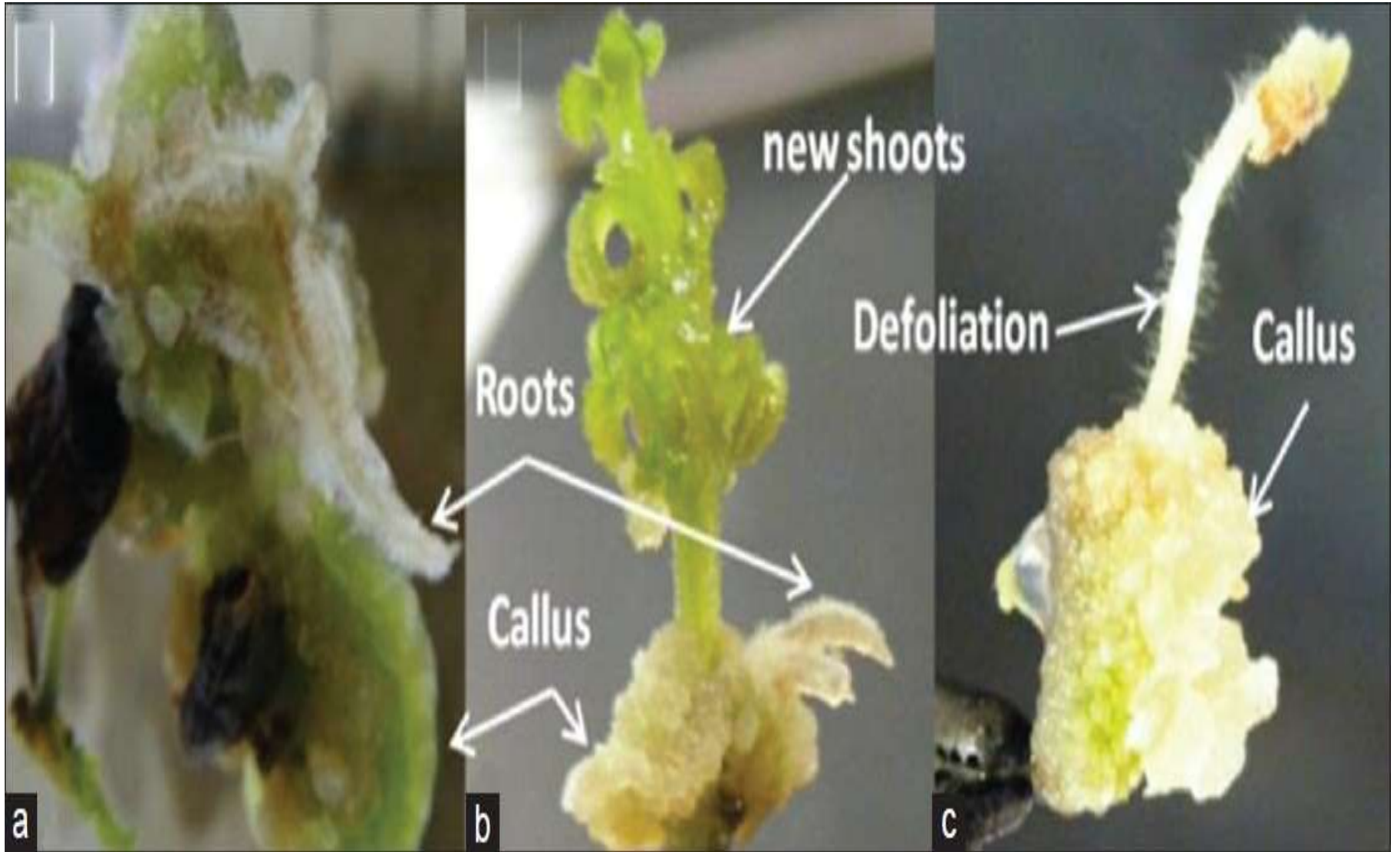
✓ **Compact callus** shows densely aggregated cells

✓ **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.

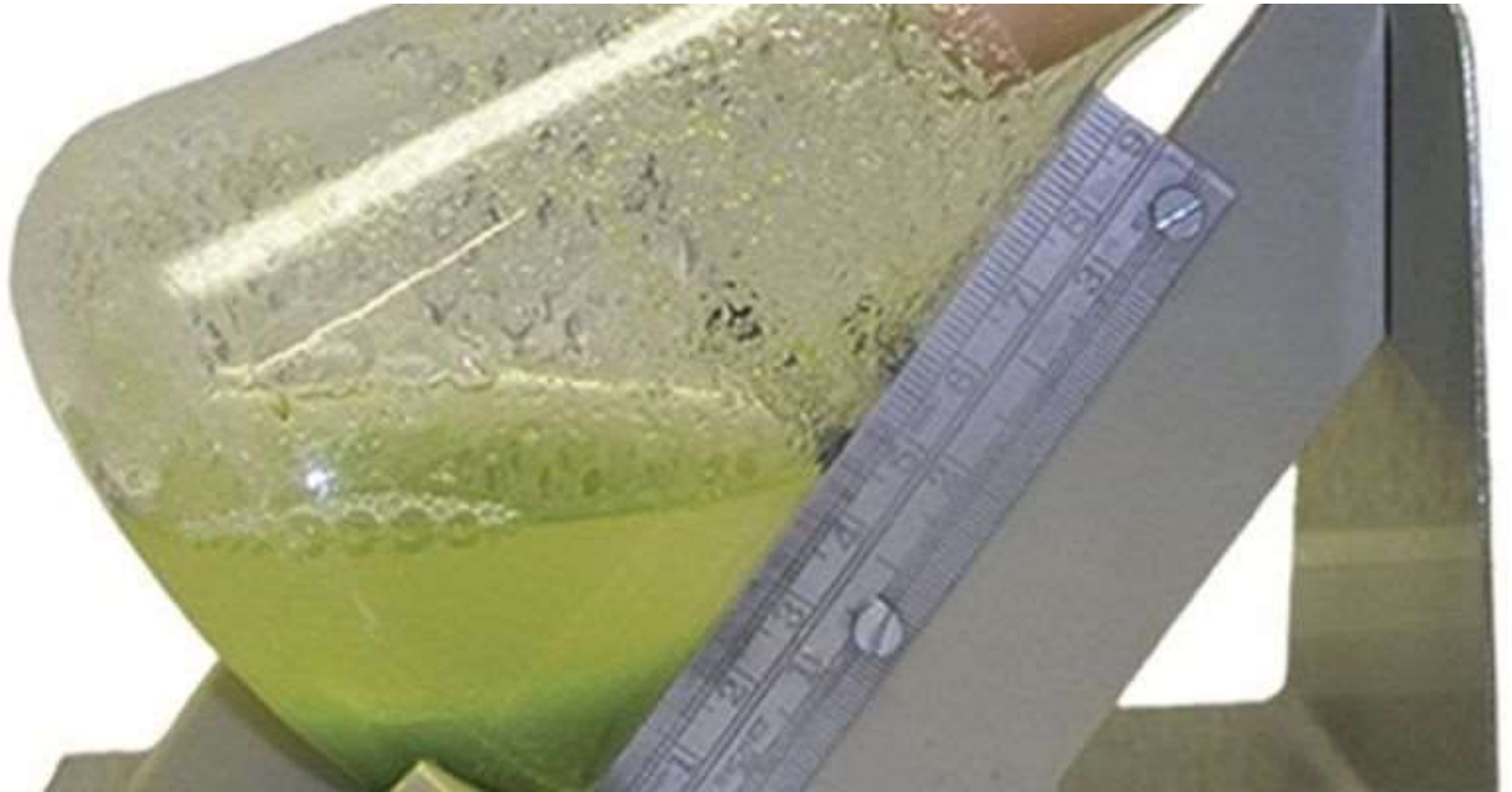






SUSPENSION CULTURE

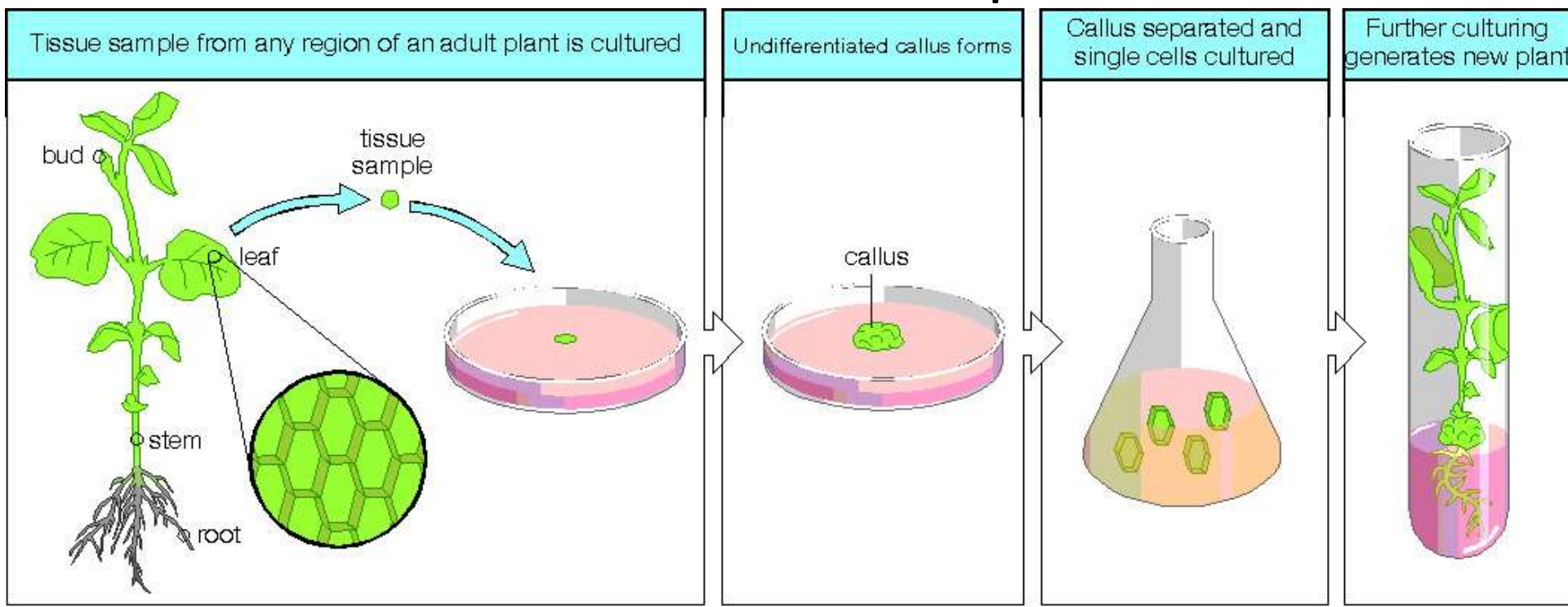
- It involves active proliferation of callus as undivided unit suspended or submerged in a **liquid medium**.
- The nutrient medium in this case is in constant **agitation** so as to prevent the cells from settling or aggregating in to clumps.
- Suspension cultures are normally initiated by transferring pieces of undifferentiated **callus** to a liquid medium which is agitated during incubation.





CELL CULTURE

- Culture of isolated individual cells, explant tissues or calluses.
- Carried out in a liquid medium in which isolated cells remain suspended or dispersed.
- cell culture also called cell suspension culture



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.

PROTOPLAST CULTURE

- Culture of isolated protoplast.
- They are cultivated in liquid as well as on solid media.
- Protoplast are cells devoid of cell walls.

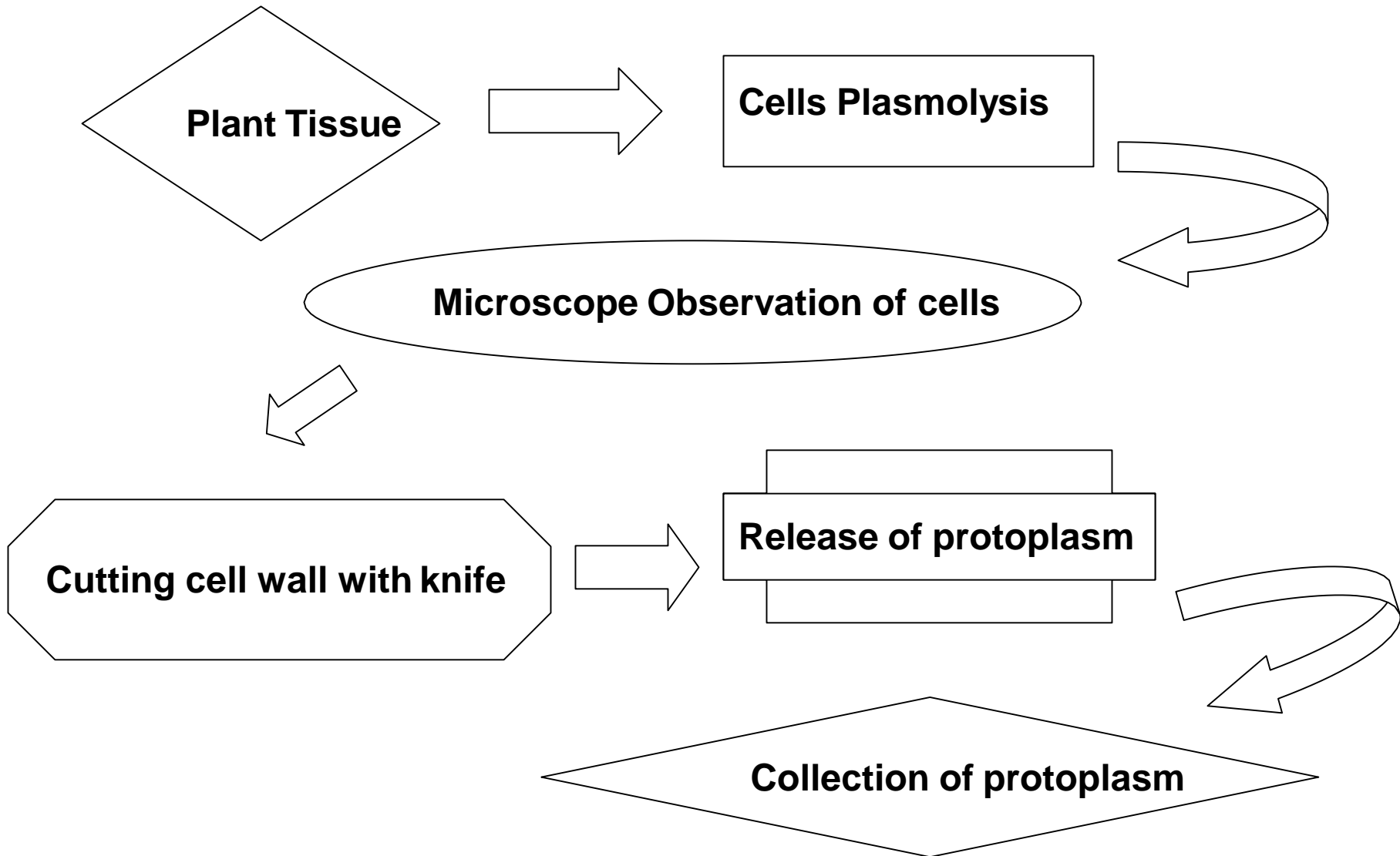
Isolation of Protoplasts is by two methods.

Protoplasts can be isolated from almost all plant parts i.e., roots, leaves, fruits, tubers, root nodules, endosperm, pollen cells, and cells of callus tissue.

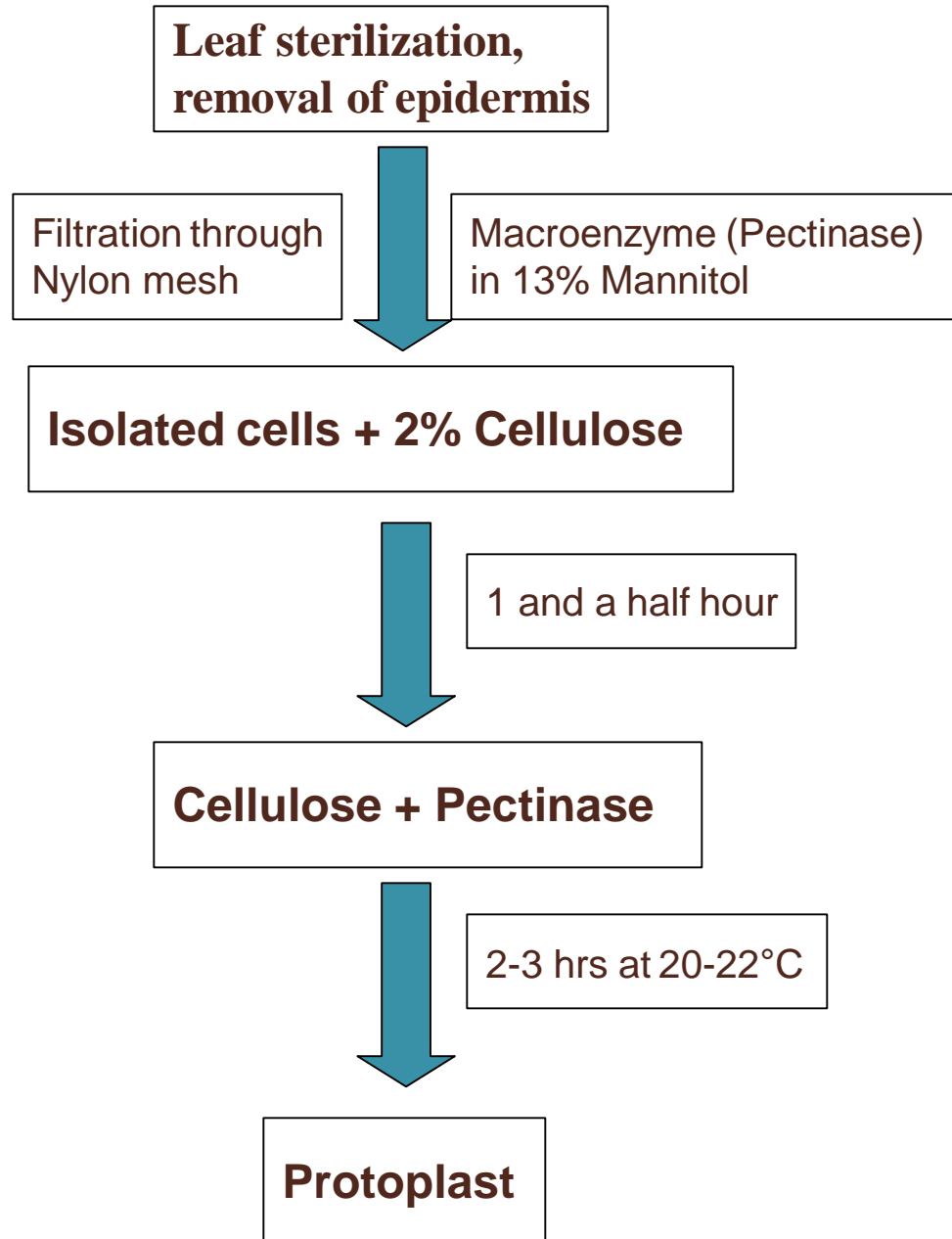
1. Mechanical method

2. Enzymatic method

1. Mechanical Method



2. Enzymatic Method



ISOLATION OF PROTOPLASTS in brief

Isolation, mainly from leaves & pollen

Mechanical

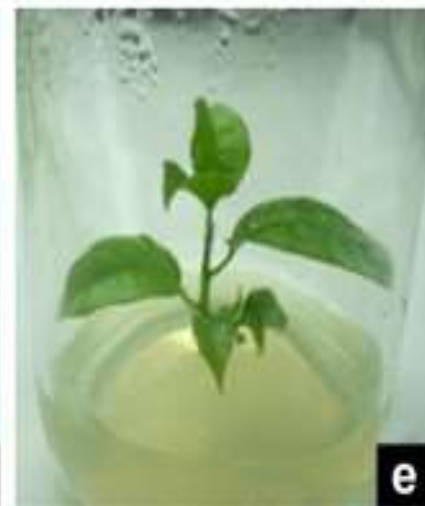
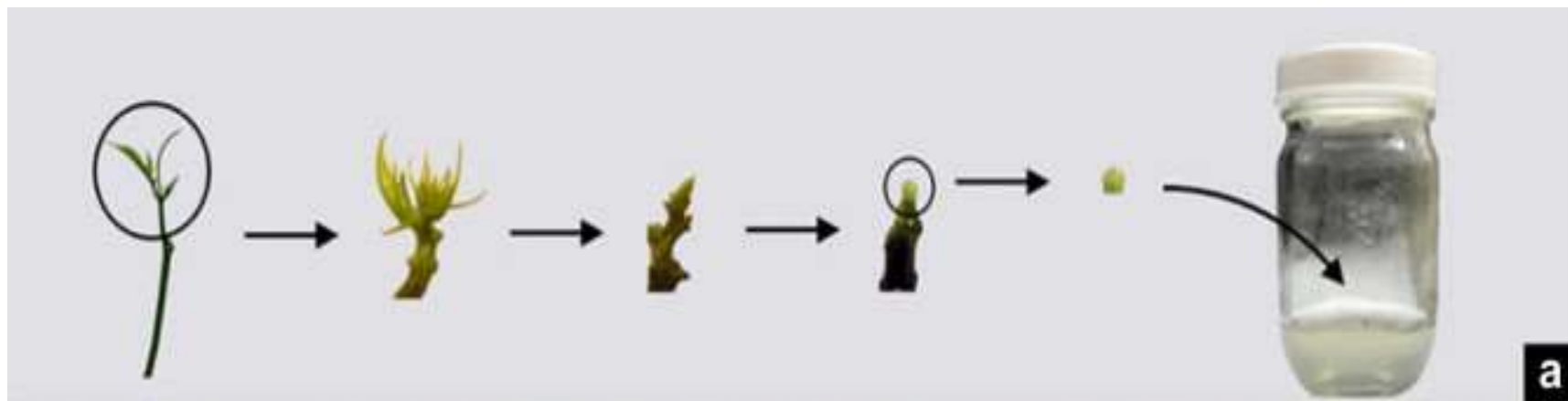
- Used occasionally
- **Advantage:**
Unknown effects of enzymes on protoplast eliminated
- **Disadvantage:**
Cells may be broken

Enzymatic

- Generally used
- **Advantages:**
 1. Large quantity of protoplasts obtained
 2. Cells not broken
 3. Osmotic shrinkage is much less
- **Disadvantage:**
Unknown effects of enzymes on protoplast

MERISTEM CULTURE

- Culture of an actively dividing meristematic tissue of shoot tip, root tip, vegetative bud etc..
- It involves the regeneration of an entire plant from a meristematic tissue.
- They mostly produce virus-free plants.
- More successful in herbaceous plants than in woody plants.



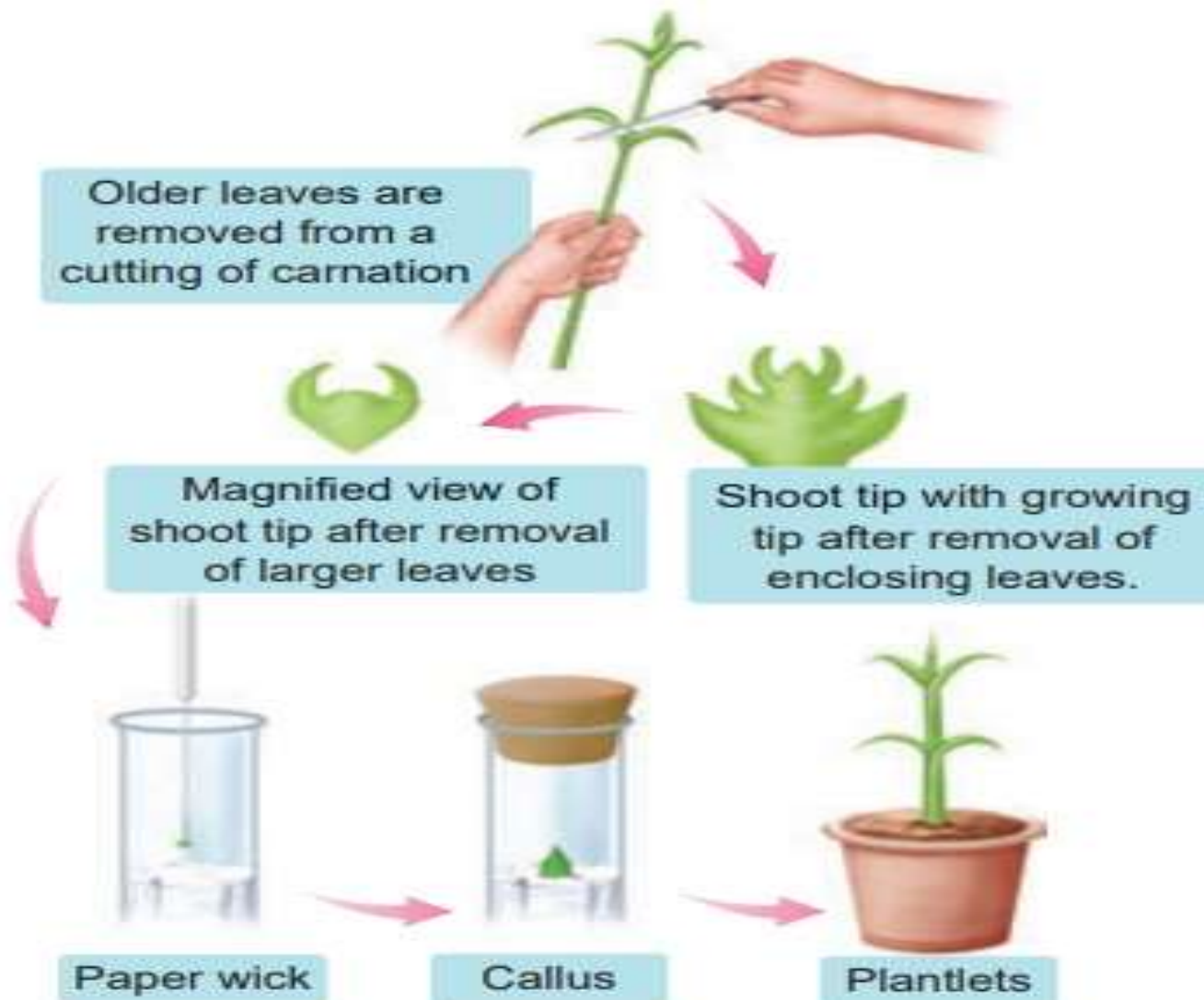


Figure 5.7: Meristem Culture

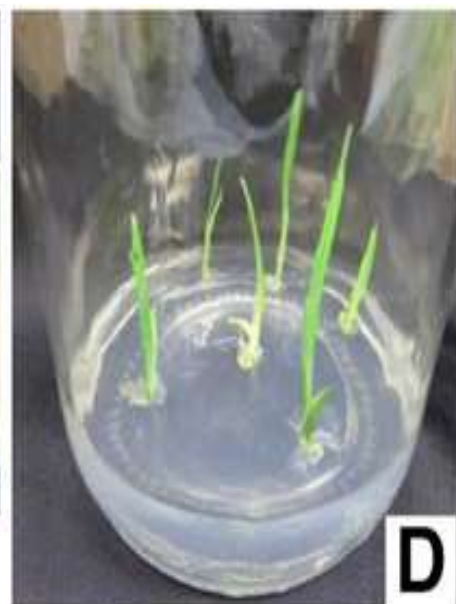
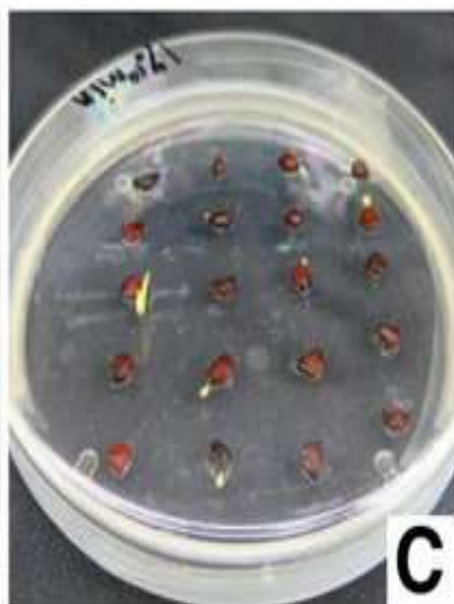
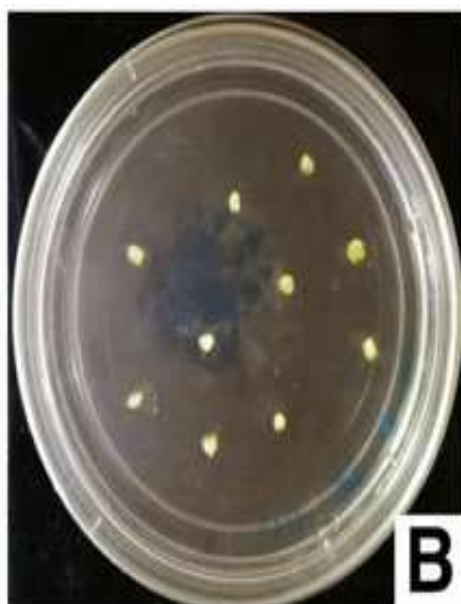
BUD CULTURE

- Culture of isolated apical or axillary vegetative buds.
- Buds contain quiescent or active meristems which can develop to shoots.



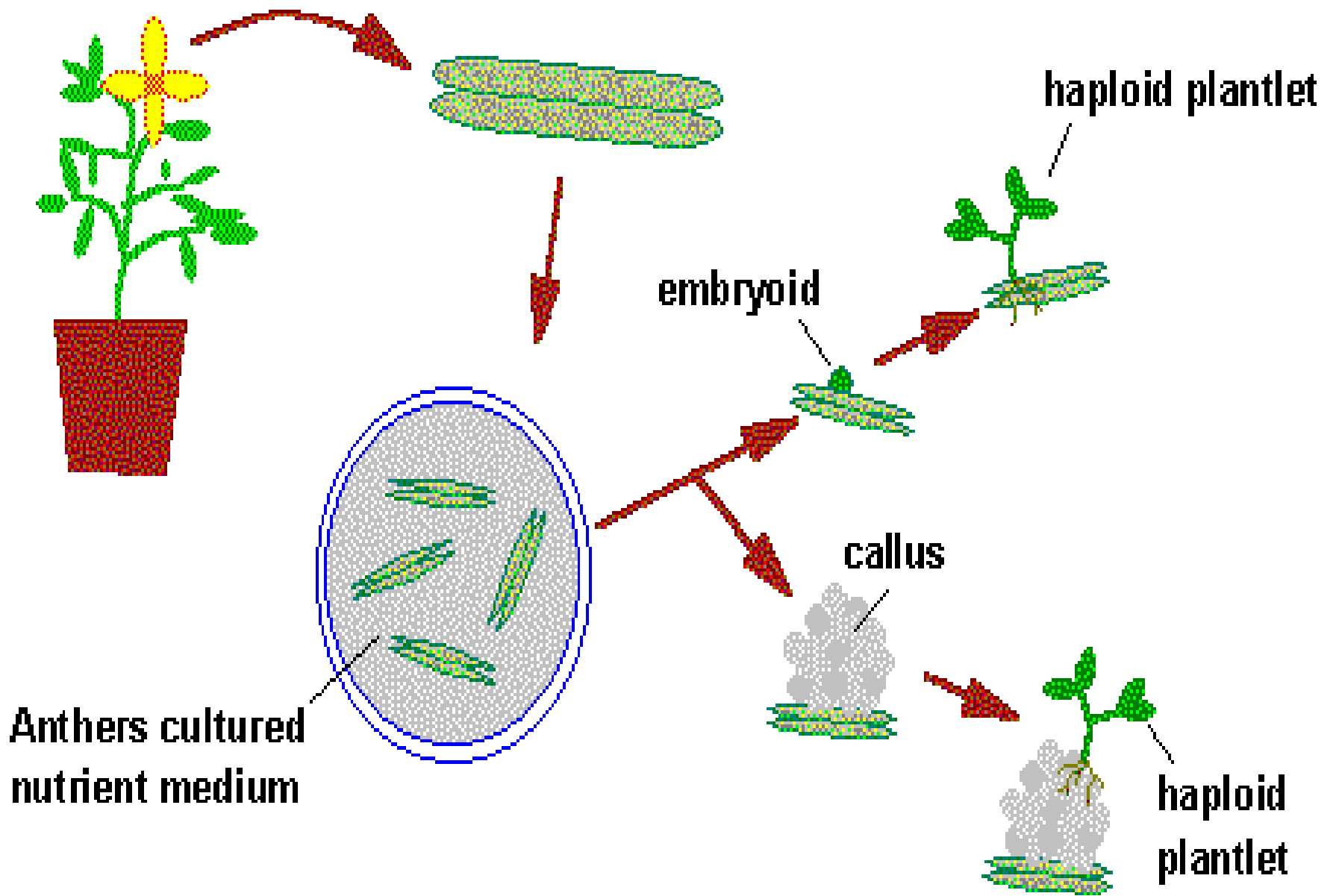
SEED CULTURE

- In-vitro culture of seeds to **produce seedlings or plantlets**.
- Best method for **raising sterile seedlings**.
- In **orchids**, seed culture is the commonest method.
- The seeds are treated with **70% alcohol** for about two minutes, washed with **sterile distilled water**, treated with **surface sterilizing agent** for specific period.
- Once again rinsed with sterilized distilled water and kept for germination by placing them on **double layers of pre-sterilized filter paper**, placed in **petri-dish moistened with sterilized distilled water** or placed on moistened cotton swab in petri-dish.
- The seeds are germinated in dark at 25-28°C and small part of the seedling is utilized for the initiation of callus.



Anther culture

- Culture method in the production of Haploids.
- First successfully carried out by Guha and Maheshwari in *Datura*.
- Anthers bearing uninucleate microspores are isolated, selected and cultured on a suitable solid medium to form androgenic calluses from the pollen mass through dedifferentiation.
- Calluses undergo re-differentiation and give rise to embryos and haploid plants.
- Rice, Wheat, Maize, mustard, Pepper



Embryo culture

- Culture of **isolated immature or mature embryos** for producing viable plants.
- Mature embryos are excised from ripened ovule/seeds and cultured mainly **to avoid inhibition in the seed for germination**. Very small globular embryos require a delicate balance of the hormones.
- Embryo is dissected from the ovule/seed and put into culture media.
- This type of culture is **relatively easy as the embryos require a simple nutrient medium containing mineral salts, sugar and agar for growth and development**.
- Also, multicellular immature embryos are dissected out and cultured aseptically to obtain **viable hybrids**. Once the embryo is rescued, two genomes are needed to be combined together to produce a fertile plant.

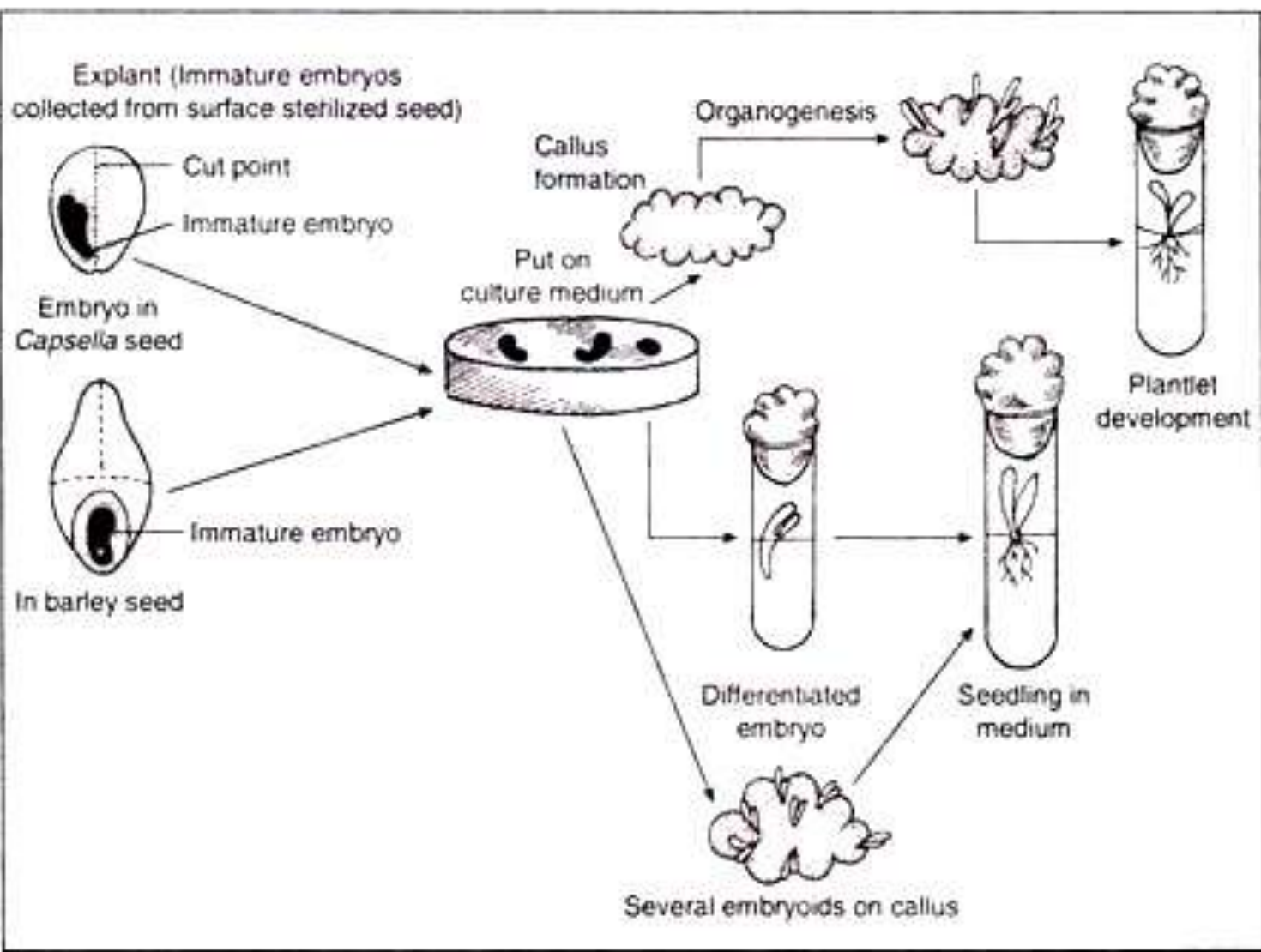


Fig. 19.1: Procedure of Embryo Culture

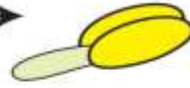
Embryo Culture Uses

- Rescuing interspecific and intergeneric hybrids
 - wide hybrids often suffer from early spontaneous abortion
 - cause is embryo-endosperm failure
 - Gossypium, Brassica, Linum, Lilium
- Production of monoploids
 - useful for obtaining "haploids" of barley, wheat, other cereals
 - the barley system uses *Hordeum bulbosum* as a pollen parent

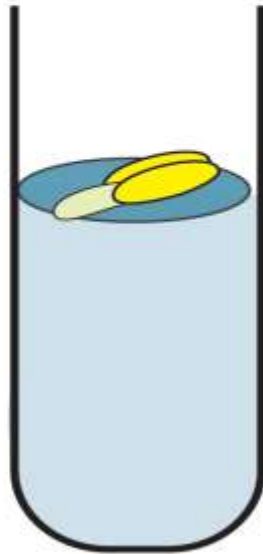
Flower



Anther



Anther culture



New haploid shoots

