BIOTECHNOLOGY

PLANT TISSUE CULTURE

- Plant tissue culture ;
- Is the technique of invitro maintenance and growth of plant tissue , cells and organs on artificial culture medium under aseptic conditions and controlled environment.
- GOTTLIEB HABERLANDT [1854-1945] Is called FATHER OF PLANT TISSUE CULTURE.
- CELLULAR TOTIPOTENCY
- It is the ability of a somatic cell to form a complete organism.
- Proved experimentally by STEWARD et al [1957].

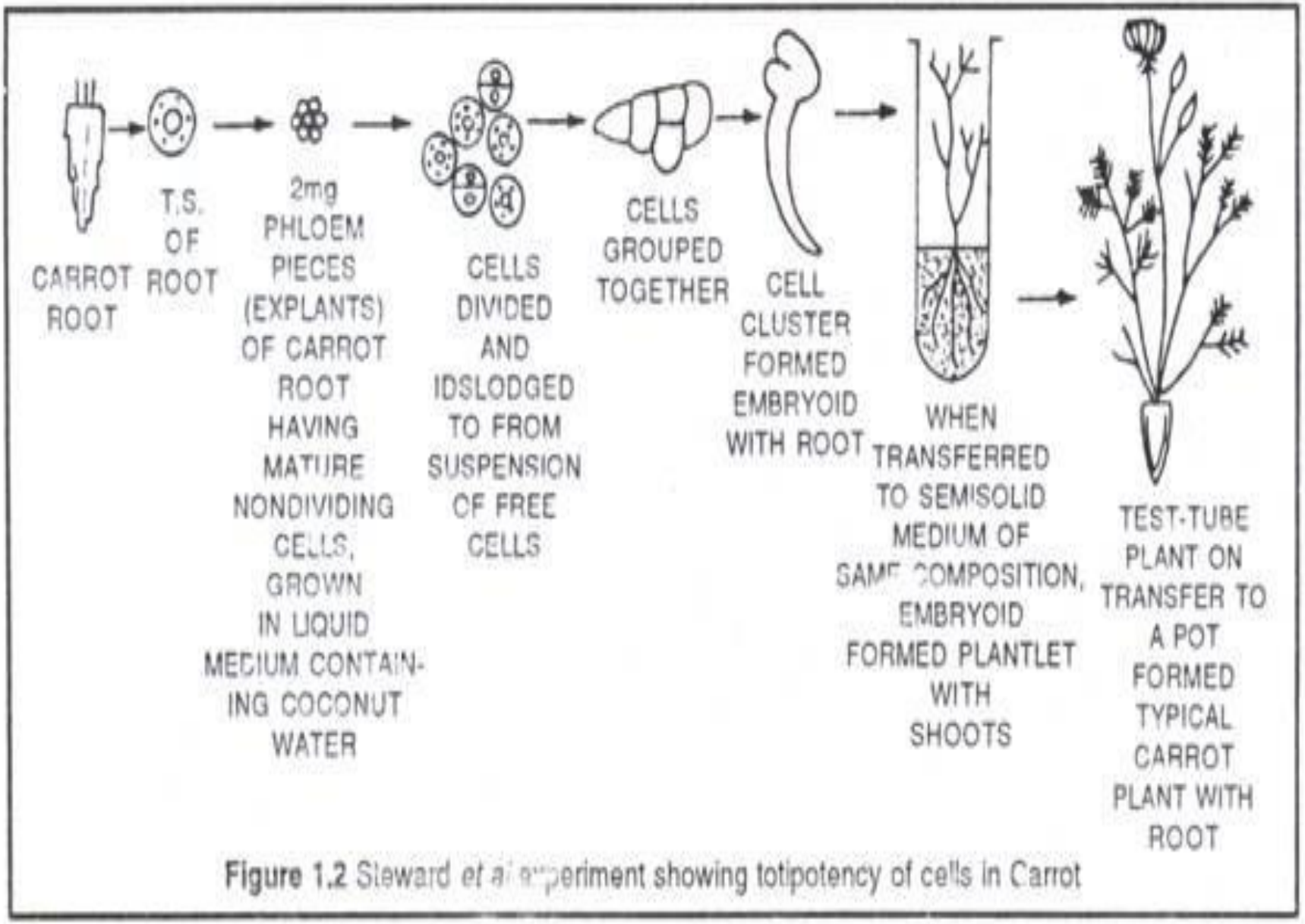
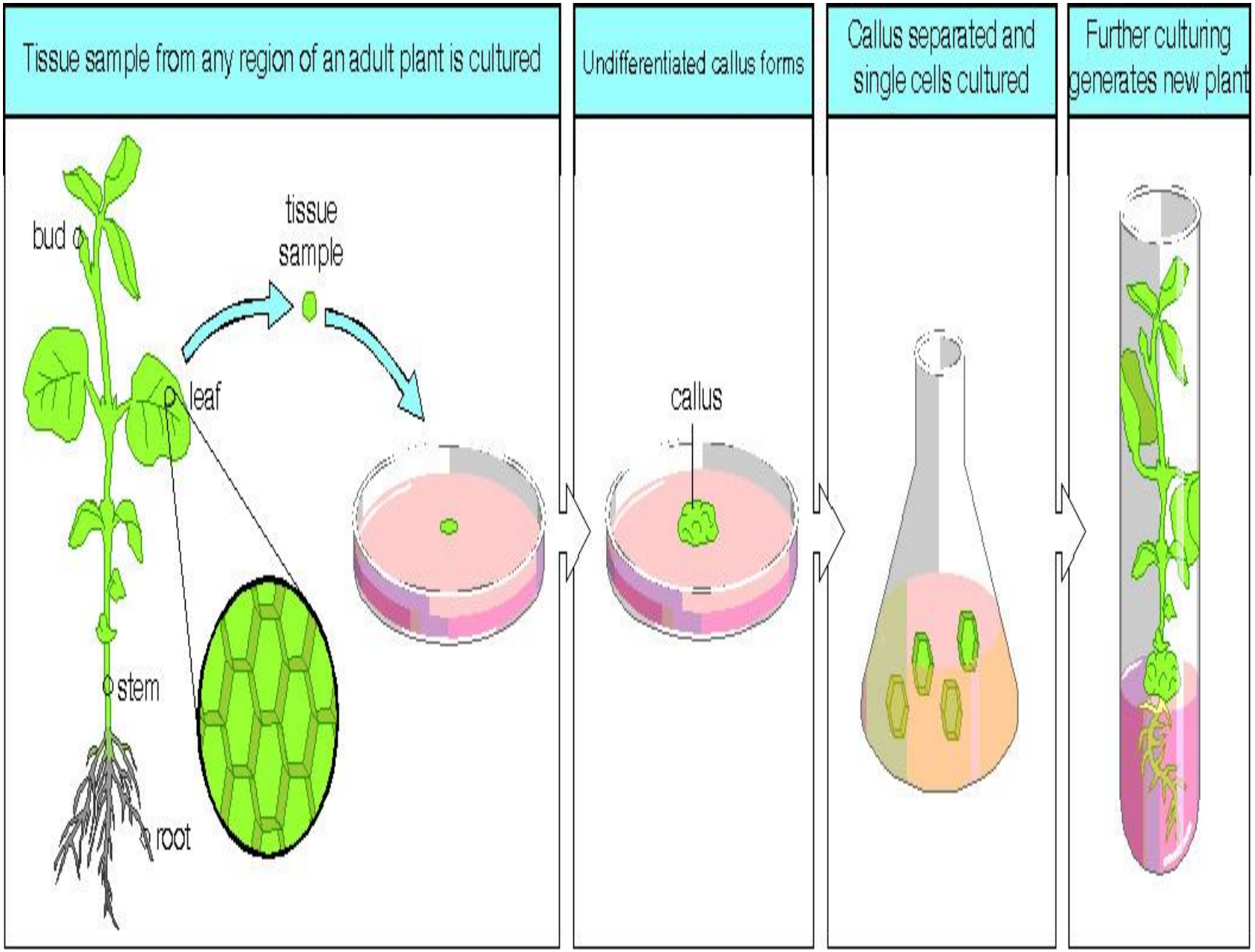


Figure 1.2 Steward *et al* experiment showing totipotency of cells in Carrot

- REQUIREMENTS FOR PLANT TISSUE CULTURE;
- EXPLANT; it is piece of plant that is used to initiate culture.
- NUTRIENT MEDIUM; it contains nutrients needed for growth and development of explant.
- The most common nutrient medium is that of MURASIGHE AND SKOOG.
- CULTURE APPARATUS.
- STERLISATION TECHNIQUES.
- PREPERATION /INOCULATION/TRANSFER AREA.
- CULTURE ROOM.
- GREEN HOUSE.


Tissue sample from any region of an adult plant is cultured
Undifferentiated callus forms
Callus separated and single cells cultured
Further culturing generates new plant
bud
tissue sample
leaf
stem
root
callus

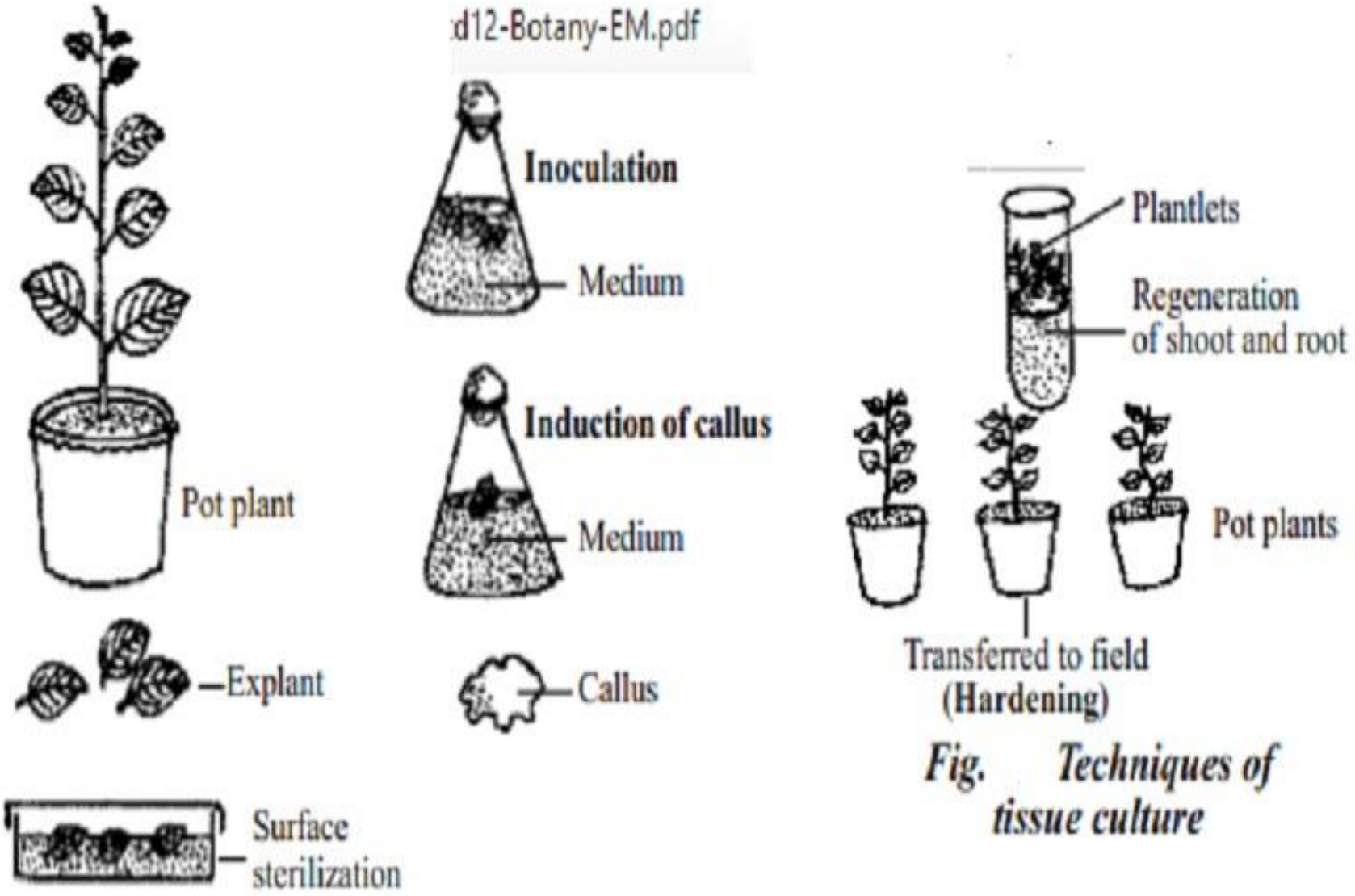


Fig. Techniques of tissue culture

- **Technique Materials sterilized**
- **Steam sterilization/Autoclaving**
- (121°C at 15 psi for 20-40 min) Nutrient media, culture vesels, glasswares and plasticwares
- **Dry heat (160-180° C for 3h) Instruments (scalpel, forceps, needles etc.), glassware, pipettes, tips and other plasticwares**
- **Flame sterilization Instruments (scalpel, forceps, needles etc.), mouth of culture vessel**
- **Filter sterilization (membrane filter**
- made of cellulose nitrate or cellulose acetate of 0.45- 0.22µm pore size) Thermolabile substances like growth factors, amino acids, vitamins and enzymes.
- **Alcohol sterilization Worker's hands, laminar flow cabinet**
- **Surface sterilization (Sodium hypochlorite, hydrogen peroxide, mercuric chloride etc) Explants**

INOCULATION ROOM

- The floor of room covered with tiles to facilitate proper cleaning.
- Room contains the laminar air flow cabinet with installation of a HEPA (high efficiency particulate air) filter for filtered air supply.
- It also requires sodium hypochlorite, mercuric chloride and 70% alcohol for surface sterilization .
- It requires forceps , scalpel, sterile bottles, sterile dishes, spirit lamp , cotton and hand care etc.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500
400
300
200
100

- THE PROCESS OF TISSUE CULTURE;
  MICROPROPAGATION
- 4 -STEPS
- 1] . INITIATION;
- It is inoculation of explant over an aseptic culture and then providing favourable invitro environment for its establishment.
- A pathogen free explant is selected .
- Its surface is disinfected with the help of 10 to 20% bleach of calcium or sodium hypochlorite to which a few drops of detergents have been added as surfactant.
- The culture vessels , instruments and culture medium are sterilized through autoclaving and filter sterilization.
- Inoculation is carried in sterilized chamber.
- Culture vessels are now placed in culture room.

- Temperature of the room is maintained between 20 to 27 C .
- Cool white light is provided.
- Higher light intensity is harmful.
- Duration of light is 12 to 16 hrs.humidity is kept between 50 to 60%.
- NUTRIENT MEDIUM contains all essential elements , vitamins , amino acids, carbon, and energy source sucrose and growth hormones with more auxin[2,4 –D] and less of cytokinin[BAP].
- With in 2-3 weeks , callus is formed .it is cut into small pieces.
- Each piece is transferred to fresh medium.
- As each piece grows in size , the same is cut again into small pieces for transfer to fresh vessels.
- The process of dividing and transferring the smaller portions of the parents culture into new culture vessels having fresh medium is called SUBCULTURING.

- In the final culture , cytokinin level is increased to 0.5 to 1.0 mg , while Auxin level is reduced .This causes development of shoot from callus.


AUTO-CLAVE

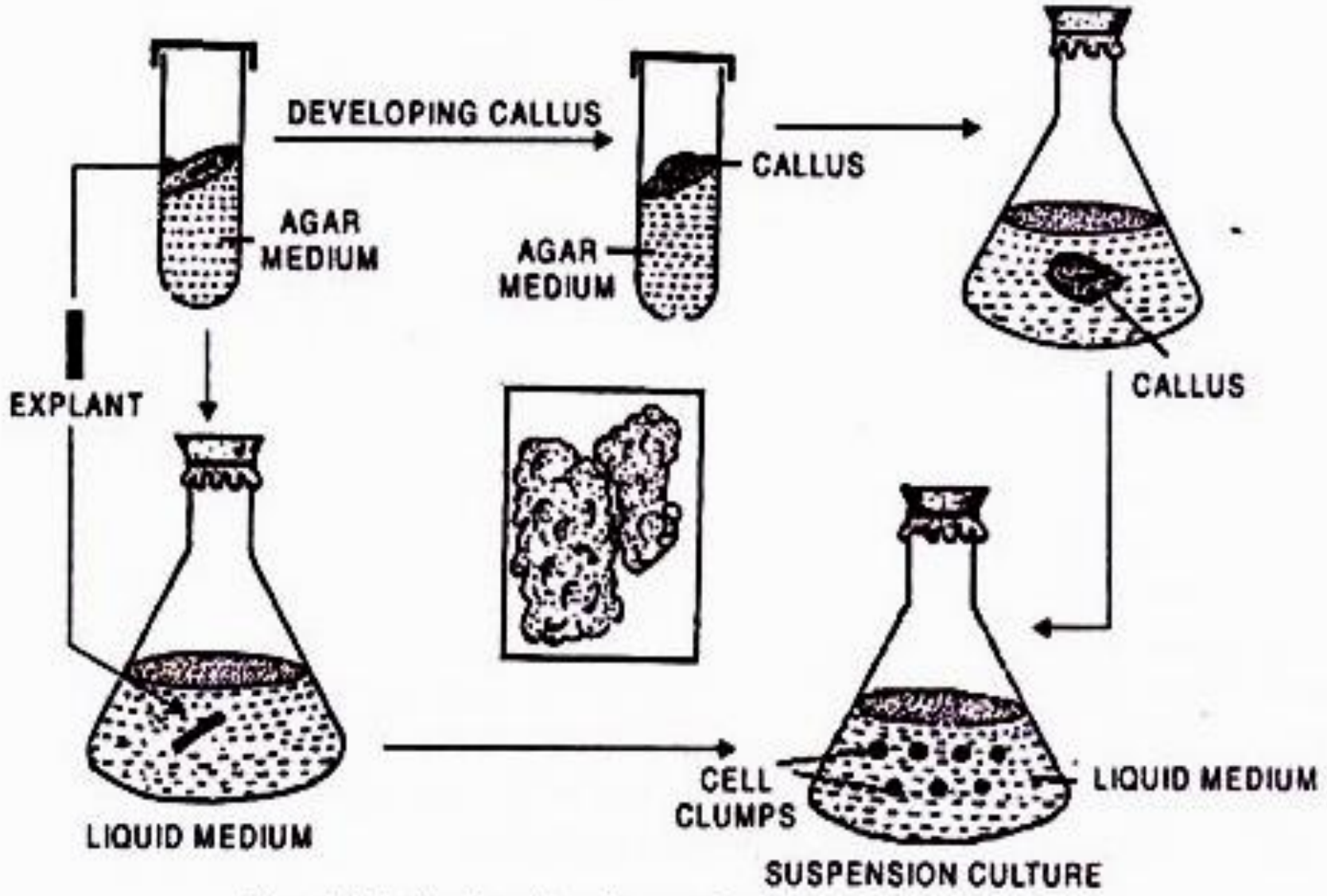


Fig. 1.8. Initiation of callus and suspension cultures

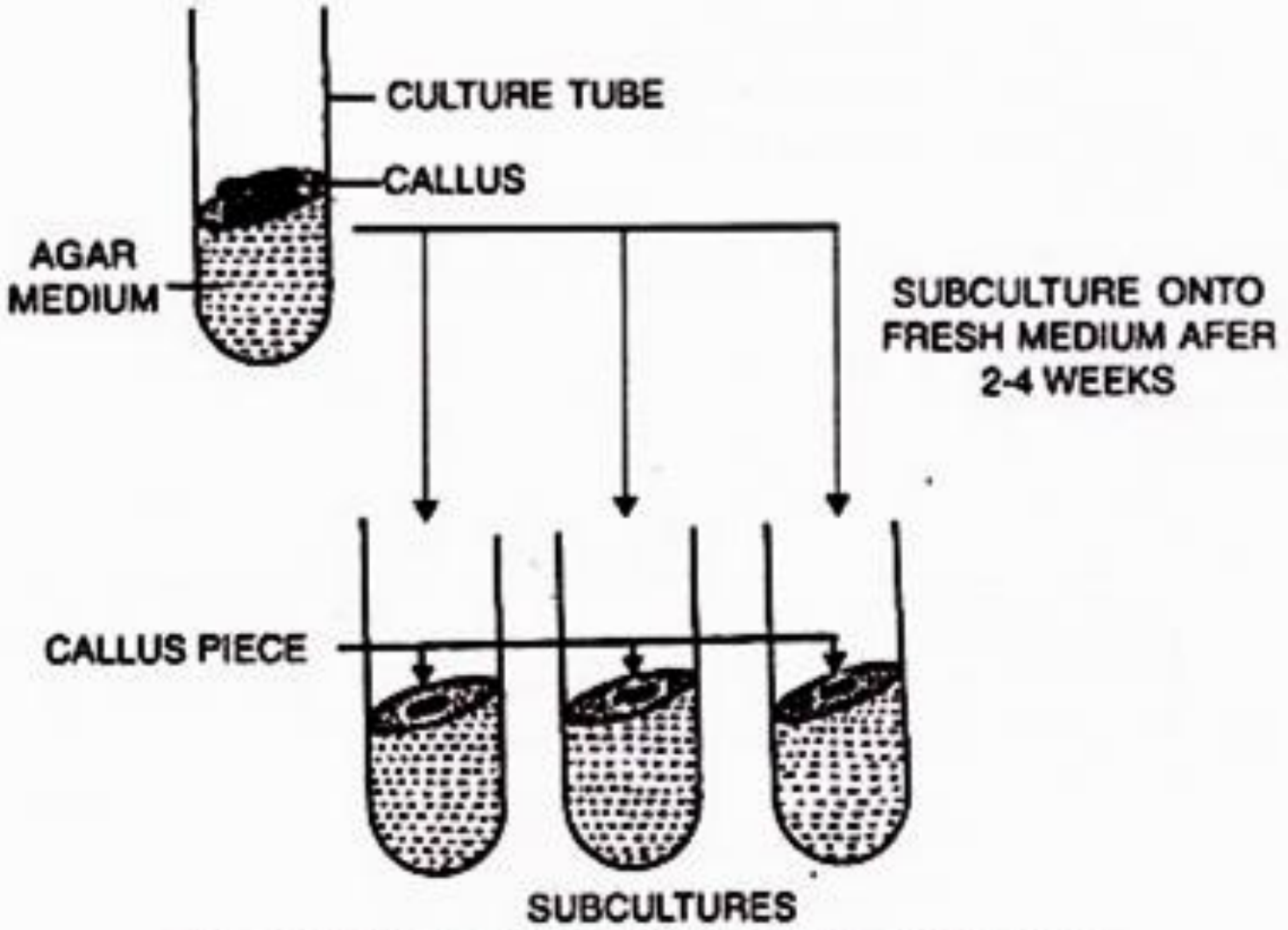
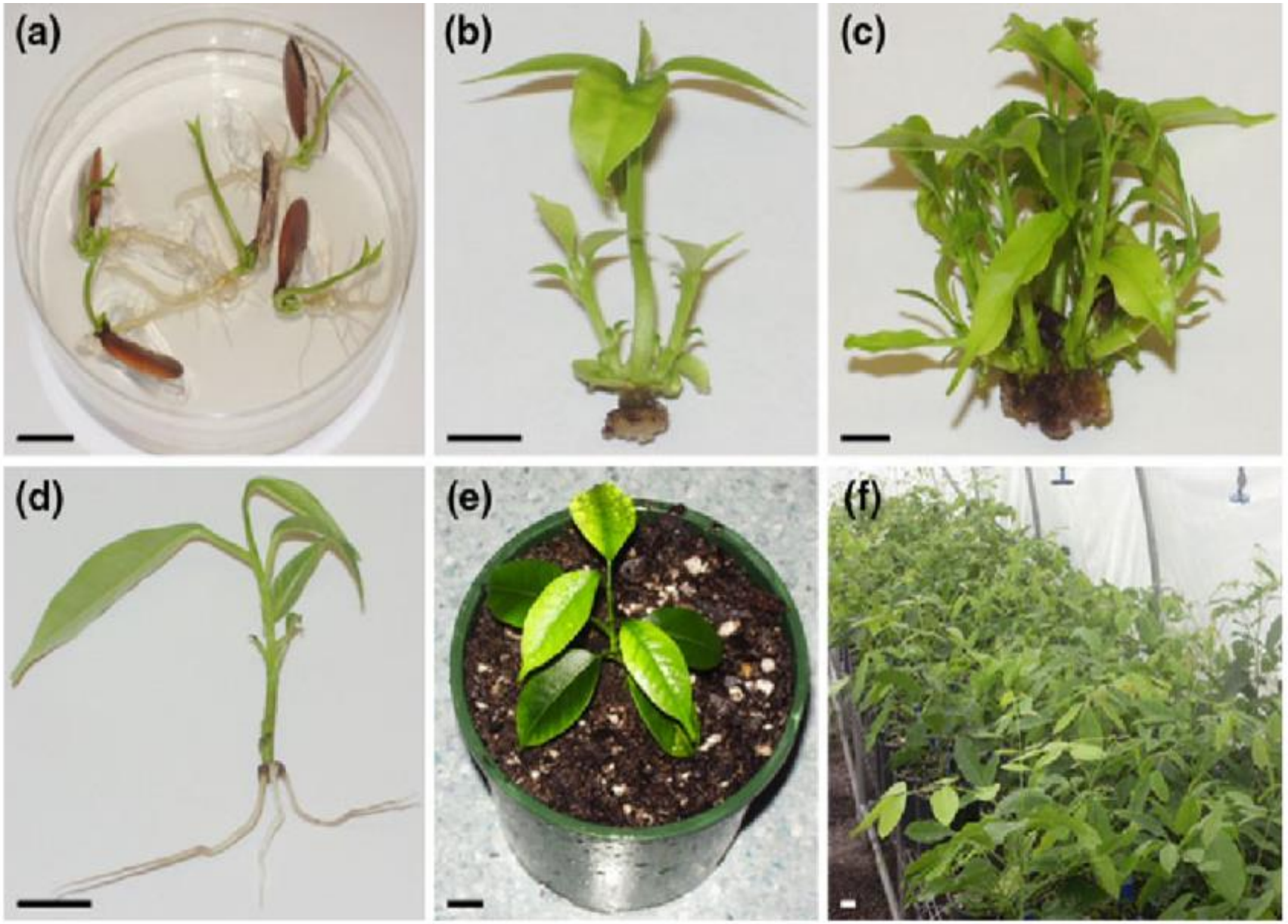


Fig. 1.7. Schematic representation of sub-culturing.

- MULTIPLICATION;
- Minerals and cytokinins are added.
- It stimulates development of a number of shoots.[5 -25],from the base ot the first microshoot.
- The culture is now divided into pieces having single shoot.
- They are transplanted into a new culture vessel.
- The phenomenon is called subculturing.
- New microshoots develop in each subculture.
- As the desired number is reached, subculturing is performed again.

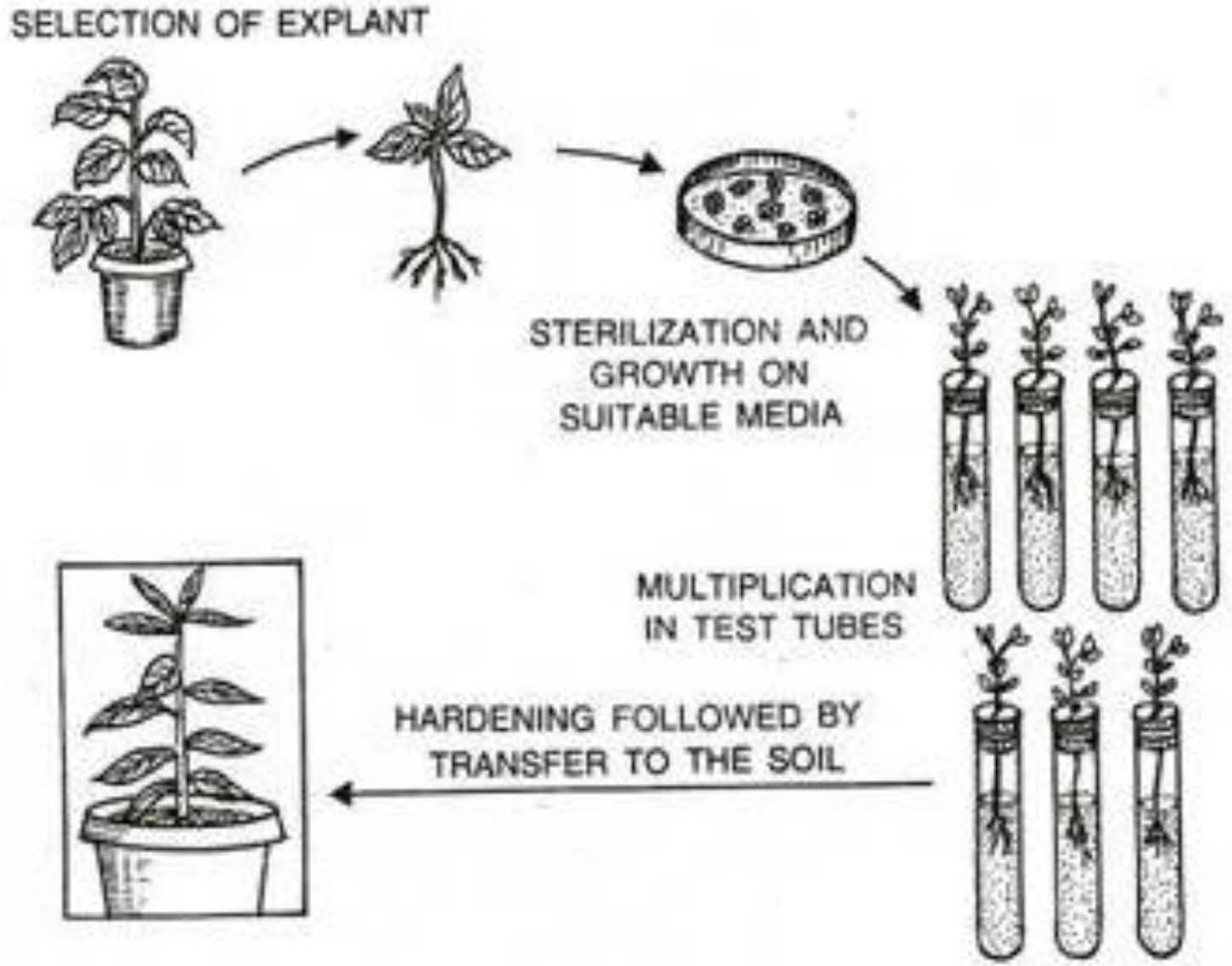

(a)
(b)
(c)
(d)
(e)
(f)

- ROOTING;
- Cultures having single shoots are transferred to fresh medium with reduced cytokinins but increased auxin content.
- Inorganic salt conc. Is also reduced slightly.
- There is slight increase in sucrose content and light intensity.
- As the roots develop , the culture is transferred to green house rooting medium.
- TRANSPLANTATION[ACCLIMATISATION]

  HARDENING

- As the plant develop good number of roots , they are removed from culture vessels.
- The roots are washed repeatedly to completely remove agar.
- They are then transplanted into a standard green house rooting medium.[2 part peat and one part vermiculite] which has been pasteurised to remove contaminations.

- Initially the plantlets are kept in shaded tents .
- They are then gradually exposed to low humidity and higher light intensity till normal roots and leaves develop and become acclematised for field transplantation.



Regeneration of whole plants using tissue culture technique.

- CULTURE SYSTEMS;
- On the basis of explant used and product formed, there are several types of culture systems ;
- MERISTEM CULTURE
- SOMATIC EMBROGENESIS
- EBMRYO CULTURE
- ARTIFICIAL SEEDS
- OVARY AND OVULE CULTURE
- ANTHER OR HAPLOID CULTURE
- TRILOID OR ENDOSPERM CULTURE
- SOMATIC HYBRIDISATION.

- MERISTEM CULTURE;
- Meristem occurs in the region of apical and axillary buds.
- Shoot apex can be removed to induce development of axillary shoots for obtaining good amount of meristem.
- Axillary meristem is also got from third and fourth nodes [from stem tip].
- These meristems are free from virus and othe infctions .
- Thermotherapy prior to removal of meristem is also helpful in obtaining virus free meristem.
- The bud is first surface sterilized by keeping them in 0.5% sodium hypochlorite for 10 min.
- They are then rinsed a few times with sterile distilld water.
- 0.1 to 1.0 mm apical part of meristem is removed with the help of fine scalpel and placed over solid culture medium under aseptic conditions.

- The medium is provided with extra cytokinin to induce shoot formation.
- There can be multiple shoots or single shoot .
- Single shoots are transferred to medium having extra auxin to promote rooting.
- The plantlets are then acclimatised or hardened for transfer to field.
- Meristem culture is used in obtaining VIRUS FREE PLANTS of potato, sugarcane ,apple, banana,orchids , etc.
- Virus free nature of the meristem is due to absence of VASCULAR CONNECTIONS.
- It is method of rapid micropropagation.
- The method is used in exchange of plant materials between institutes, states and nations.
- The technique is used in germplasm conservation.

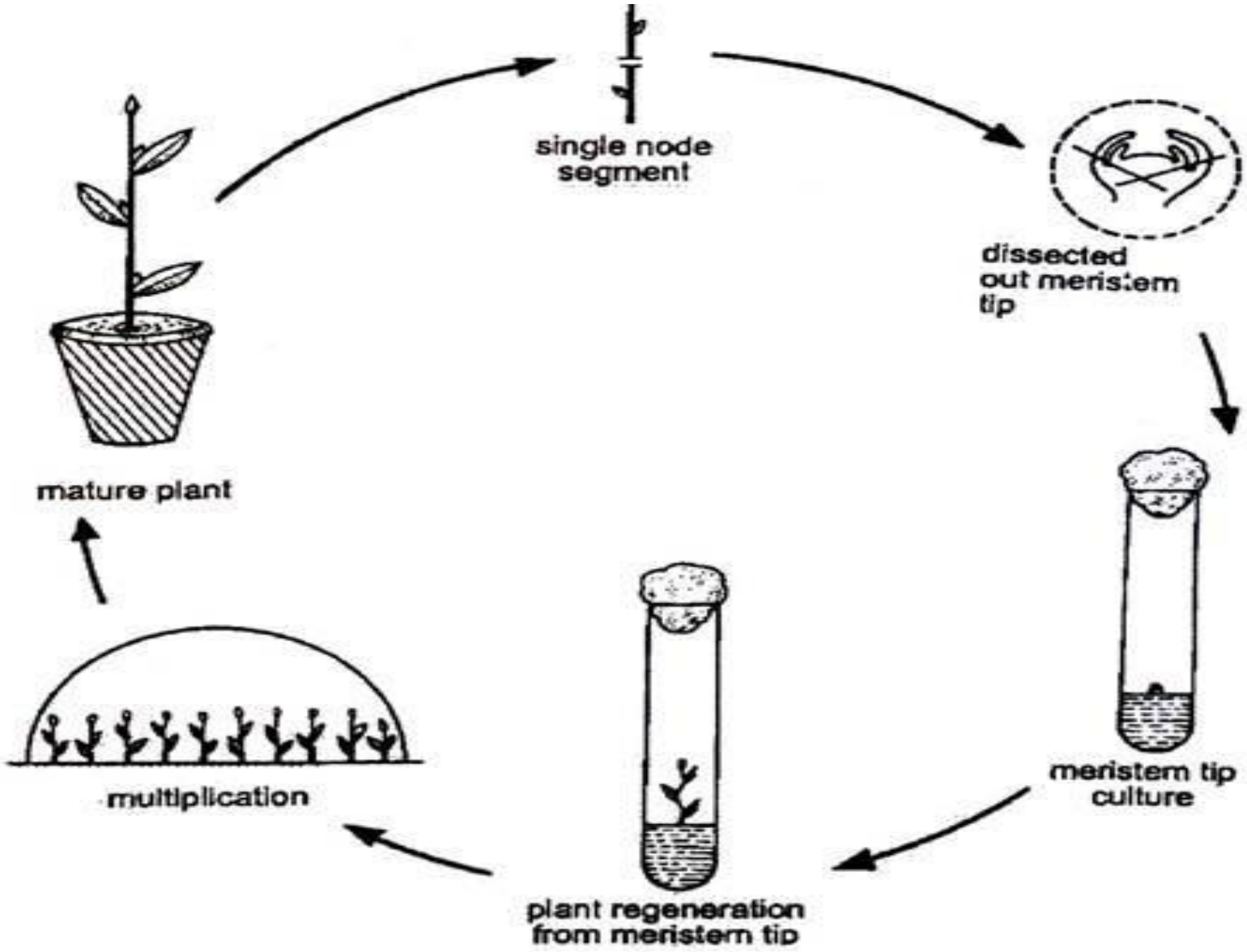
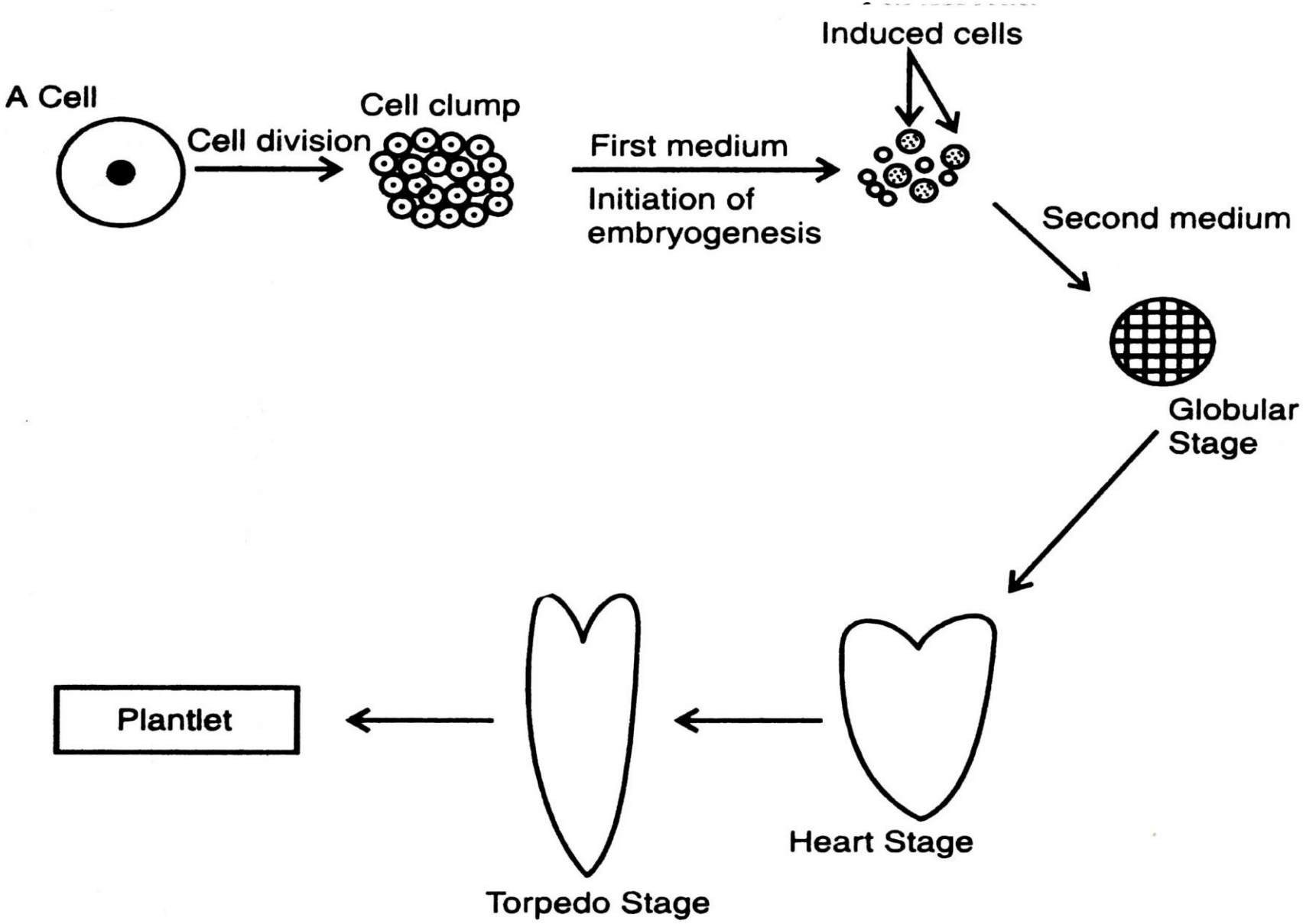


Fig. 8. Regeneration of plants through Meristem Culture.

- SOMATIC EMBRYOGENESIS;
- It is development of nonzygotic embryo like structure formed from somatic cells invitro cultures.
- Somatic embryos were discovered by Stewards et al [1958].
- They found that cultures of carrot cells having coconut milk formed clumps that differentiated into embryo like structures called EMBRYOIDS.
- Like wise embryoids are also formed on solid culture medium.
- Embryoids develop naturally from cells of callus developed from nucellus, integuments , zygotic embryo or seedlings.
- They can be induced in other somatic cells by high auxin content or coconut milk with small quantity of NAA.
- Proembryoid masses are formed , which are shifted to auxin free medium having high ammonium nitrogen.
- Somatic embryos or embryoids pass through stages similar to zygotic embryos; GLOBULAR, CORDATE , TORPEDO , COTYLEDONARY.


Induced cells
A Cell
Cell division
Cell clump
First medium
Initiation of
embryogenesis
Second medium
Globular
Stage
Plantlet
Heart Stage
Torpedo Stage

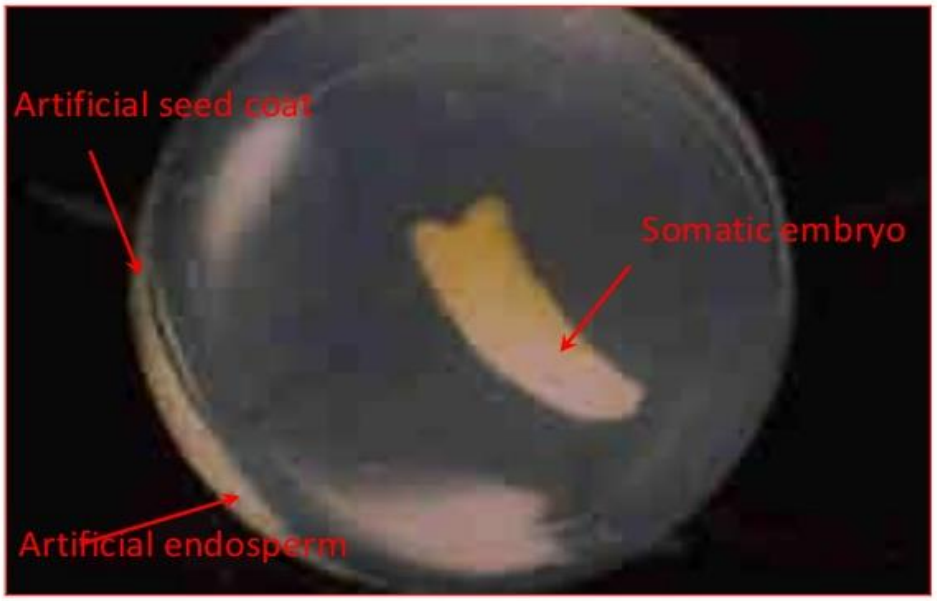


Fig 4 : The concept of Artificial Seed

SYNTHETIC SEED

- EMBRYO CULTURE;
- It is in vitro growth of embryos over culture medium for development of seedlings and young plants.
- It is performed in those cases where an embryo is unable to achieve complete development naturally, shows slow growth or undergoes dormancy.
- Fertilized ovules are picked up, surface sterilized and then excised to remove embryo.
- It is performed in;
- ORCHIDS
- STERILE SEEDS
- SEED DORMANCY
- EMBRYO RESCUE

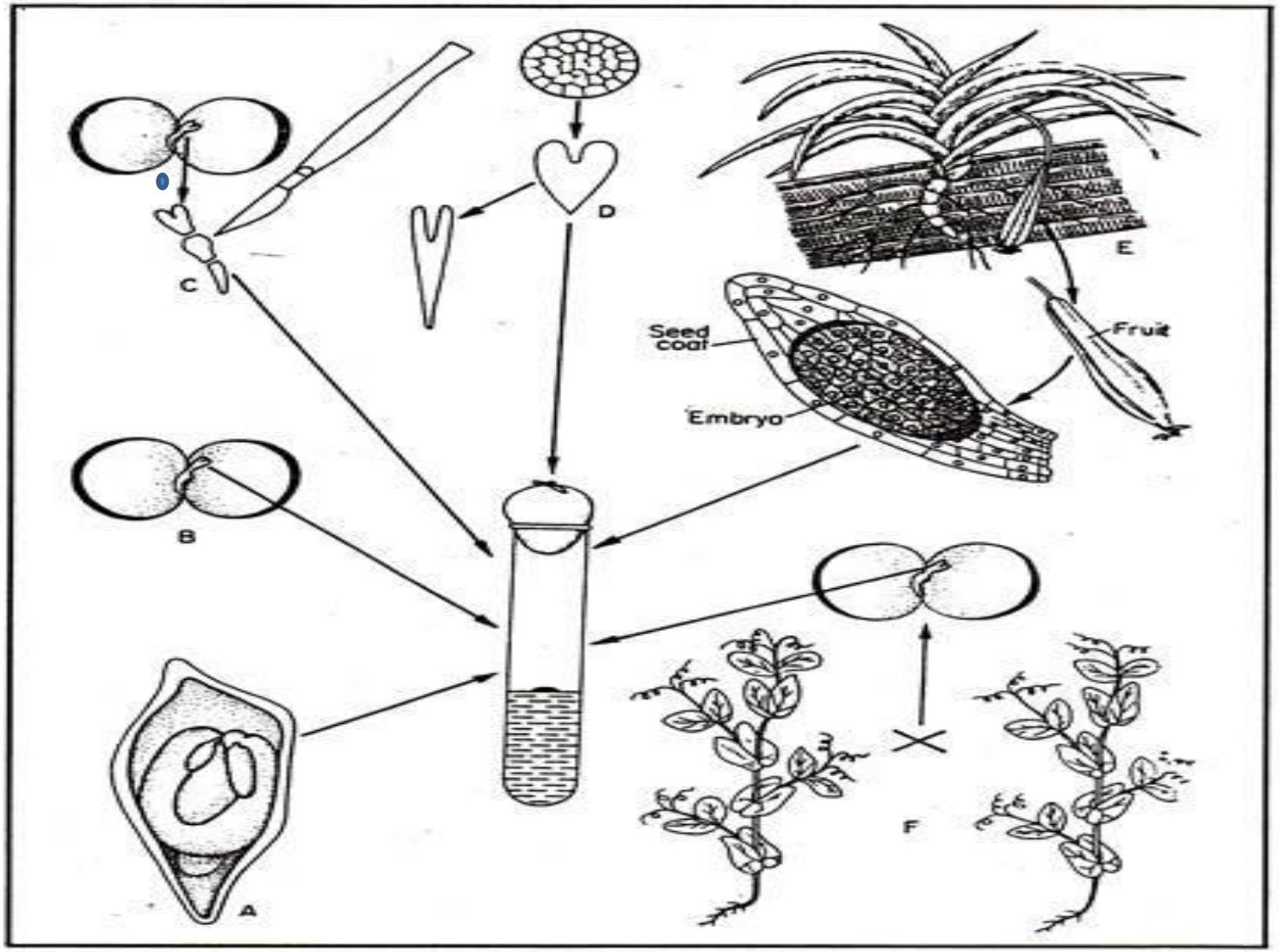


Fig 10.1

Different categories of embryo culture. A. Culture of adventive embryos from polyembryonic seeds. B. Culture of mature and intact seed embryo. C. Culture of dissected embryo. D. Culture of immature embryo. E. Culture of undifferentiated embryo of orchid. F. Culture of abortive or inviable embryos.

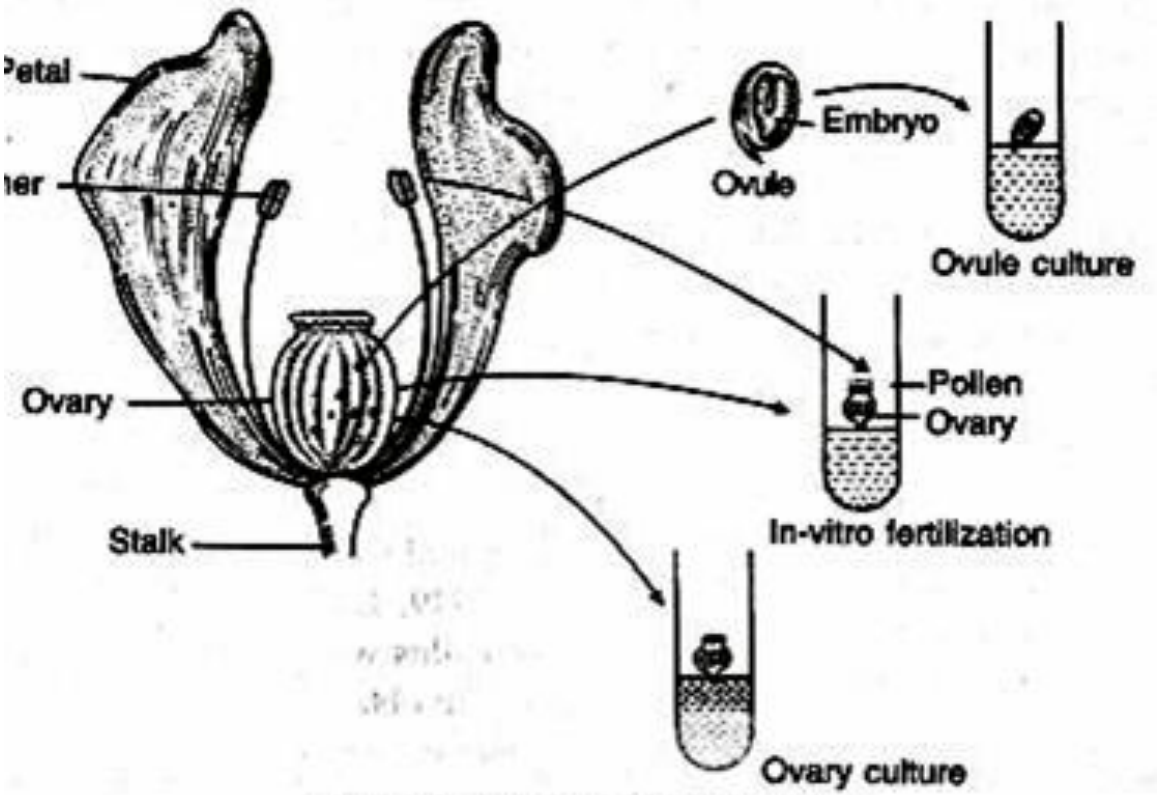
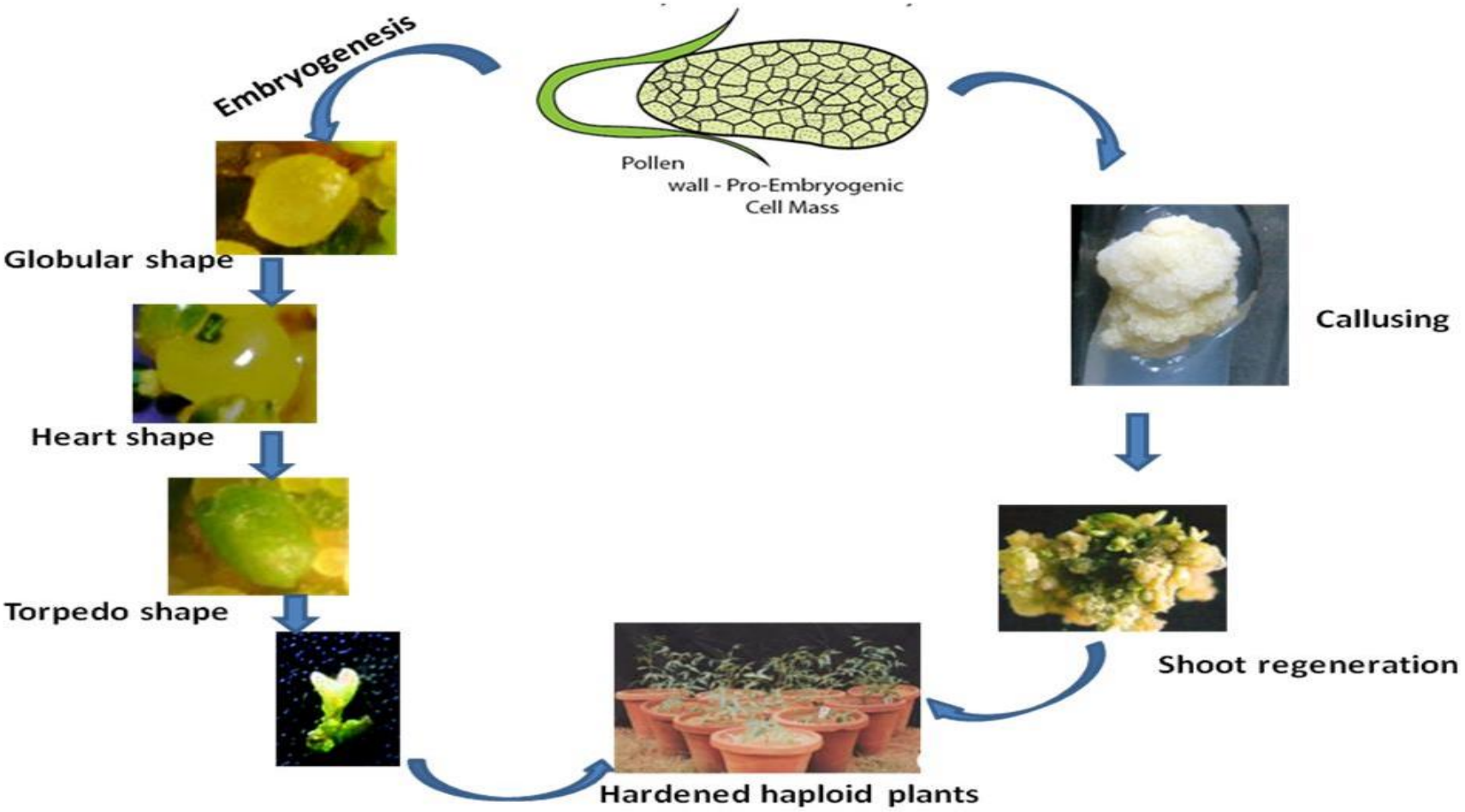
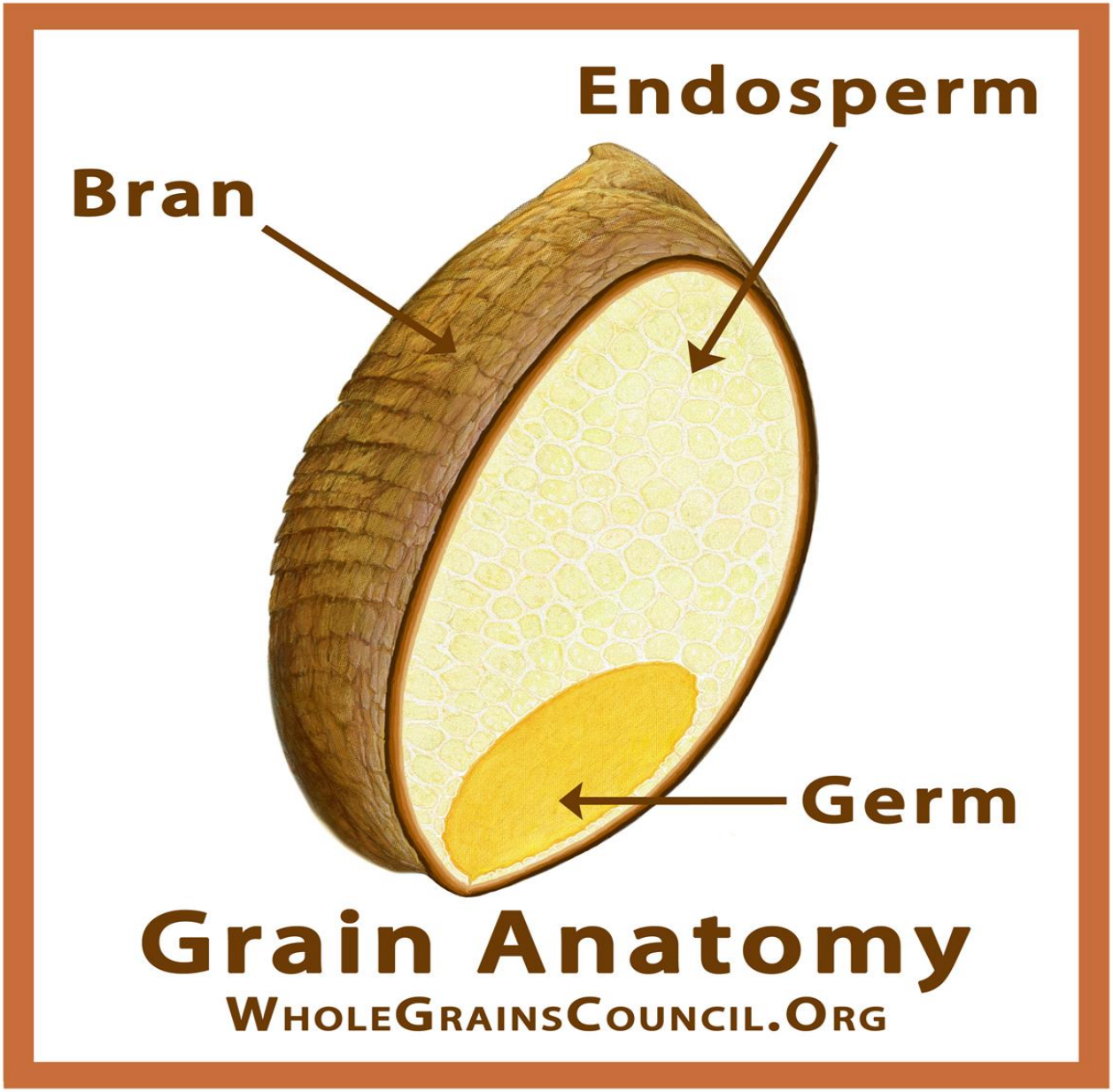


Fig. 27.1. Ovary and ovary culture.

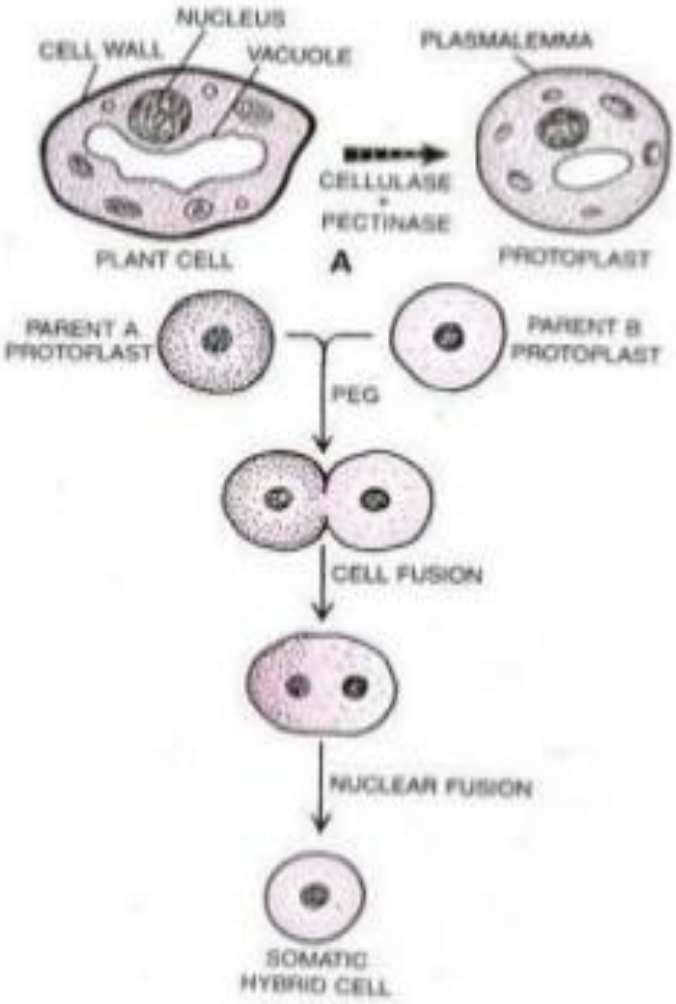
ovary and ovule culture



ANTHER OR HAPLOID CULTURE


Endosperm
Bran
Germ
Grain Anatomy
WholeGrainsCouncil.Org

Procedure



Somatic hybridisation. A, Production of protoplasts using a combination of pectinase and cellulase. B, Protoplast fusion induced by PEG ultimately yields somatic hybrid cells.

Application of tissue culture

- Crop improvement
- Horticulture
- Synthetic seeds production
- Forestry
- Propagation of rare plants
- Production of secondary metabolite
- Shortening of breeding cycle
- Production of disease-free plants

Applications of tissue culture to plant breeding

1. Haploid production (rice, wheat and barley)
2. Triploid production (fruits and poplar)
3. Embryo Rescue/ Wide hybridization (numerous examples)
4. Somatic hybridization (scientific examples, few commercial products)
5. Somaclonal Variations (Tomato with altered color, taste and texture by Fresh World Farms; Imidazolinone resistant maize, American Cyanimid; Bermuda grass (Brazos R-3) with increased resistance to fall armyworm etc.)
6. Production of disease free plants.
7. Clonal propagation
8. Secondary metabolite production (eg. Taxol production from cell cultures derived from the bark cuttings of pacific yew tree)
9. Germplasm conservation (cryopreservation)