

STEPS INVOLVED IN PTC

1. SELECTION OF EXPLANT

Explant:

- It is defined as a portion of plant body, which has been taken from the plant to establish a culture
- Explant may be taken from any part of the plant like root, stem, leaf, or meristematic tissue like cambium, floral parts like anthers, stamens etc.

2. SURFACE STERILIZATION OF EXPLANT

For surface sterilization chromic acid, Hgcl(0.11%), calcium hypochlorite, sodium hypochlorite(1-2%), alcohol(70%) are used. Process depends on the type of explant.

❖ **SEED** : absolute ethyl alcohol → calcium hypochlorite → bromine water → sterile water

❖ **FRUIT** : ethyl alcohol → sodium hypochlorite → sterile water

❖ **STEM** : running water → sodium hypochlorite → sterile water

❖ **LEAF** : surface clean → Hgcl₂ → sterile water → dried

3. PROLIFERATION OF CULTURE

If callus is well developed, it should cut into small pieces & transferred to another fresh medium containing hormones, which supports growth. The medium used for production of more amount of callus is called *proliferation medium*.

4. SUBCULTURING OF CALLUS

- After sufficient growth of callus it should be periodically transferred to fresh medium to maintain viability of cells.
- This subculture will be done at the interval of 4-6 weeks.
- After a maximum growth transfer into a soil under required condition.

TYPES OF CULTURE

- Root tip culture
- Leaf or leaf primordial culture
- Shoot tip culture
- Anther & pollen culture
- Ovule & embryo culture
- **Protoplast culture**
- **Callus culture**
- **Suspension culture**

} **Most important methods**

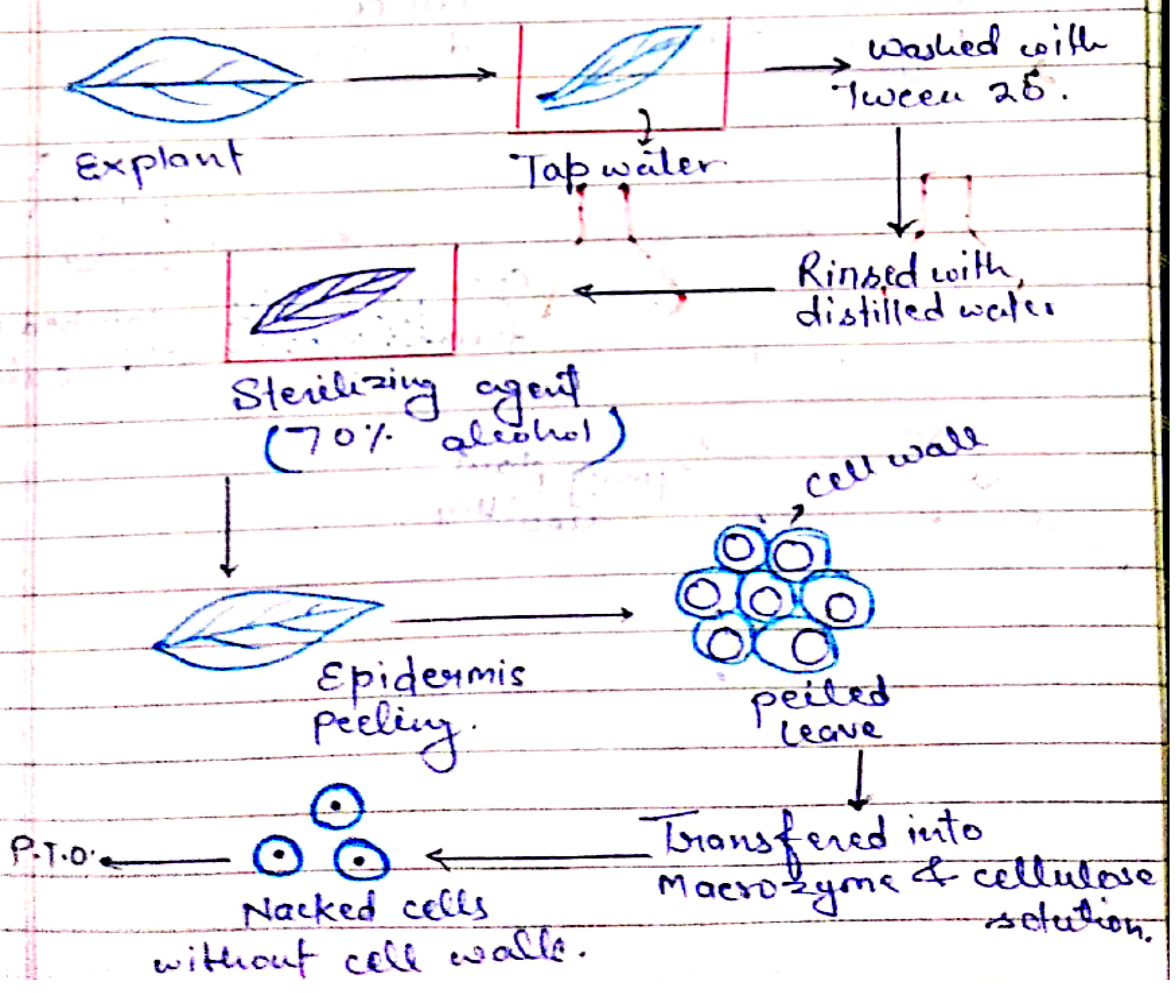
PROTOPLAST CULTURE

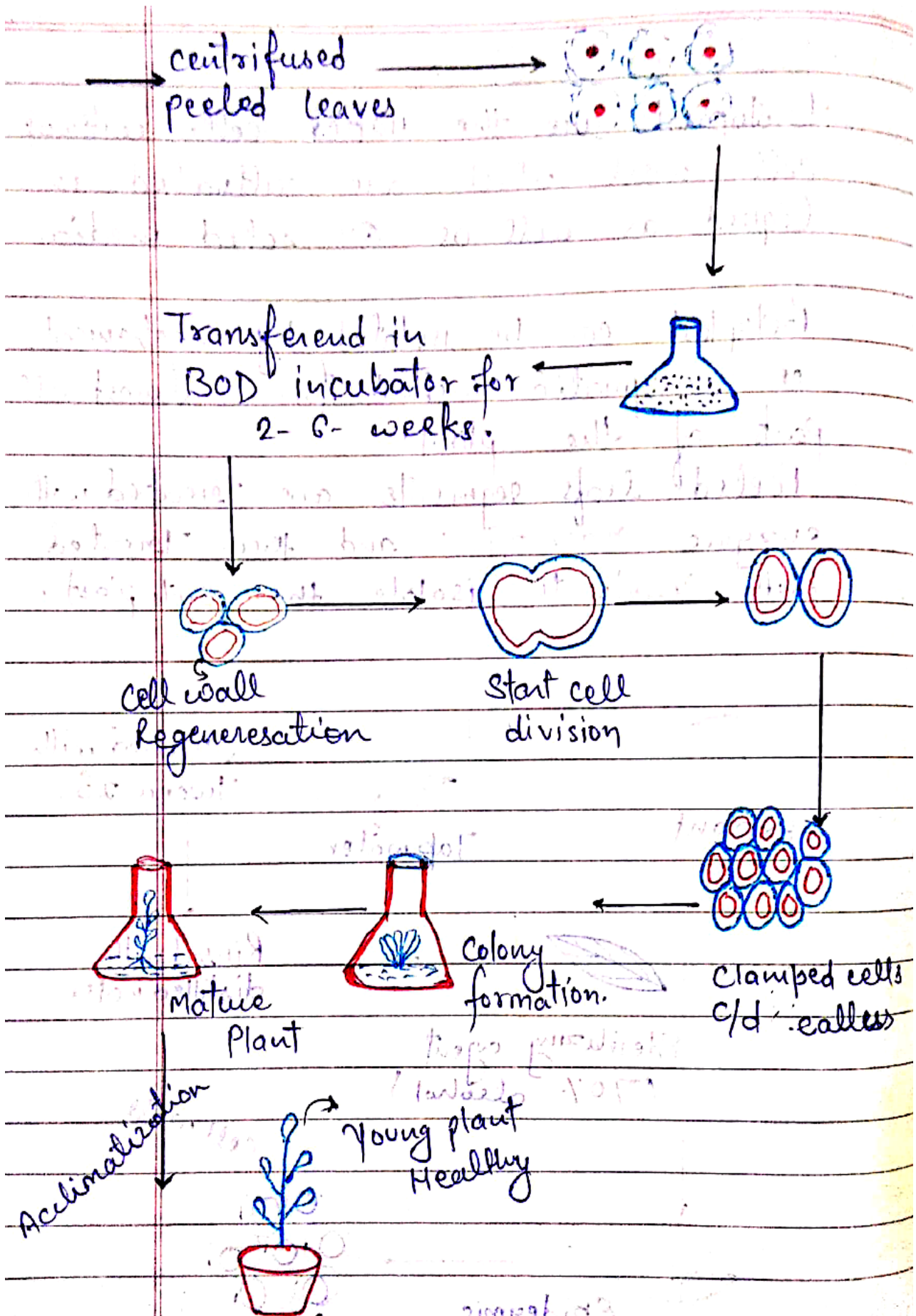
⑥. Protoplast Culture -

• Protoplast are the naked cells without cell wall which are cultivated in liquid as well as on solid media.

• Protoplast can be isolated by mechanical or enzymatic method from almost all parts of the plant.

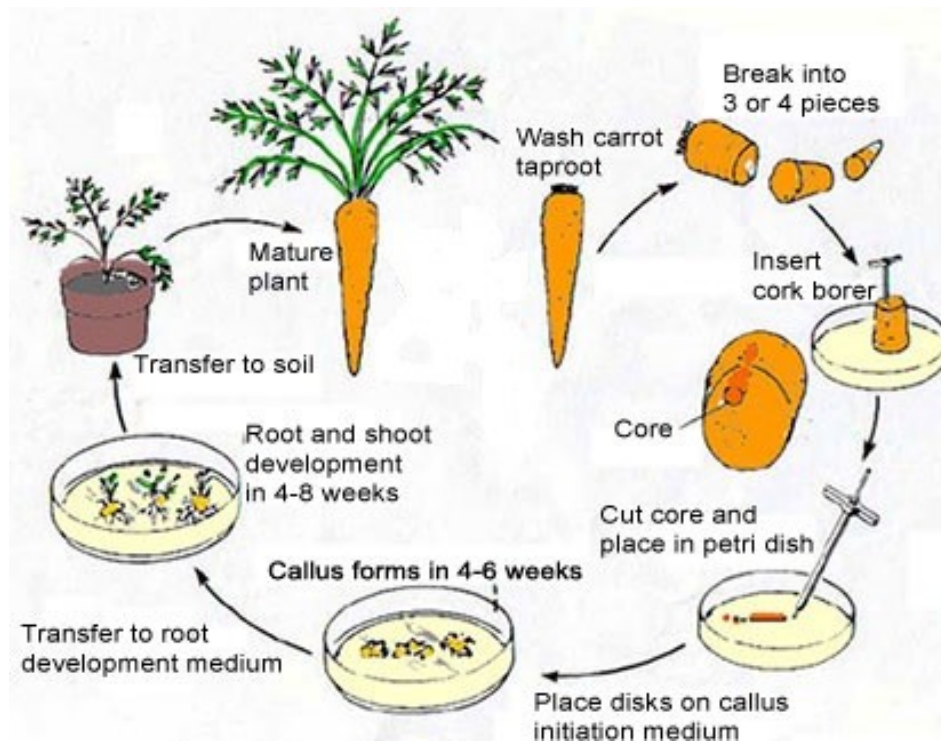
Peeled leaf segments are treated with enzyme Macrozyme and then treated with Cellulose to isolate the protoplast.





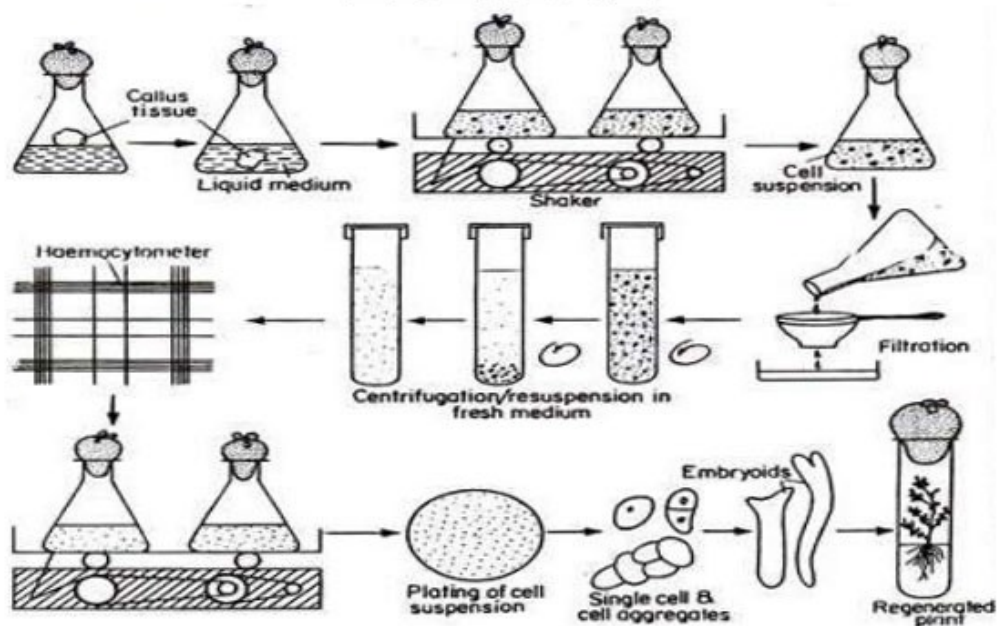
CALLUS CULTURE

Callus is an undifferentiated, unspecialized, unorganized, loosely binding growing and dividing mass of cells.



SUSPENSION CULTURE

CELL SUSPENSION CULTURE



⑦. Suspension Culture -

↓ • Suspension culture contains a uniform suspension of separate cells in liquid medium. For the preparation of suspension culture, callus fragments is transferred to liquid medium (without Agar),

which is aerated continuously to keep the cells separate.

• The culture maintain medium homogeneous by stirrer speed (50-150 rpm).

• After sufficient no. of cells are produced and ~~sub~~-culturing can be done & freshly prepared ~~directly~~ solid medium.

↓
• Callus fragments

↓
Transferred in liquid medium
without Agar

↓
Aerated continuously in BOD
incubator to keep the cells separate
(speed = 50-150 rpm)

↓
After sufficient no. of cells are produced &
to form a homogeneous mixture of cells.

↓
Start Sub-culturing / in freshly prepared solid medium i.e. the single cell is inoculated in fresh medium.

↓
Multiply

↓
Growth of cells.

↓
Organogenous

↓
whole plant.