PROTOPLAST ISOLATION AND FUSION

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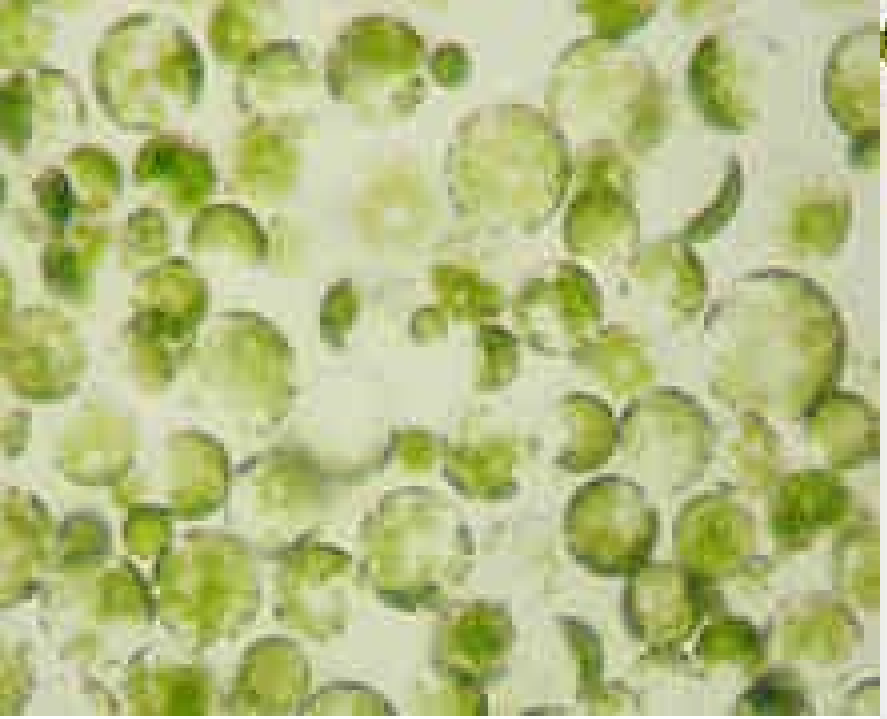
PROTOPLAST:

- Protoplast is a plant, bacterial or fungal cell that had its cell wall completely or partially removed using either mechanical or enzymatic means.

  Protoplast = cell- cell wall

- Protoplast are plant cell with the plasma membrane but without the cell wall. Protoplast allow the fusion of similar or different species and the fused product can generate into the whole plant.



Protoplast

ISOLATION OF PROTOPLAST

- In 1892, Klercker first isolate protoplast by plasmolysing and subsequently slicing the tissue.
- In 1960, Cocking demonstrate the enzymatic isolation of a large number of protoplast from cells of higher plants. (by using concentrated solution of cellulase enzyme)
- In 1968, Tkebe et al first employed the commercial preparation of the enzyme for protoplast isolation. (using macerozyme and cellulase)

- Protoplast are plant cell with the plasma membrane but without the cell wall. Protoplast allow the fusion of similar or different species and the fused product can generate into the whole plant.
- Protoplast can be isolated from almost all plant parts viz namely roots, leaves, tubers, root nodules, fruits, endosperms, pollen mother cells, pollen, pollen tetrad, embryogenic or non-embryogenic suspension culture.
- More recently viable protoplast have been isolated from male and female gametes.

MATERIAL REQUIRED FOR PROTOPLAST ISOLATION

- Plant leaves
- 13% Mannitol
- CPW salt solution
- Enzyme solutions

  Macerase and Cellulase
- Petri plate/ glass tubes

leaf including petiole

↓

70% Sodium hypochlorite solution
+ 2 drops of tween

↓

wash 3-4 times with sterile d/w

↓

peel the lower epidermis & cut into pieces

↓

leaf pieces + 13% Mannitol + inorganic salt
of CPW solution

↓

remove CPW solution with Pasteur pipette

↓

enzyme mixture in 13% Mannitol solution
(macerozyme 0.1-0.5% & cellulase 0.5-1.0%)

↓

Filter the solution and transfer the filtrate into centrifuge

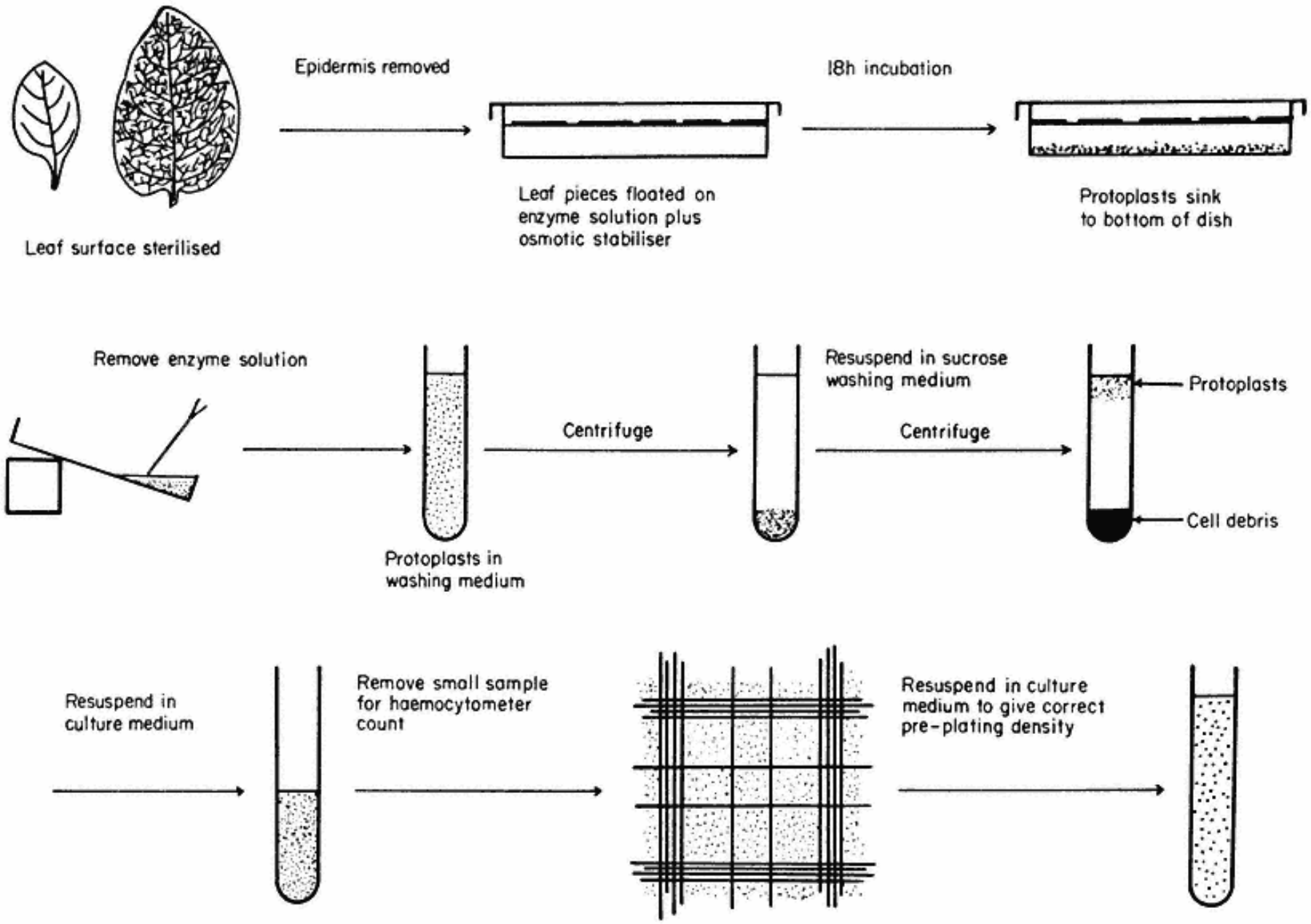
Centrifuge at 100X g for 5-10 min

remove the supernatant

Resuspend the pellet in CPW + 21% Sucrose (prepared in CPW solution)

Centrifuge again at 100X for 10 min.

protoplast float to the surface of sucrose solution


Leaf surface sterilised
Epidermis removed
Leaf pieces floated on enzyme solution plus osmotic stabiliser
18h incubation
Protoplasts sink to bottom of dish
Remove enzyme solution
Protoplasts in washing medium
Centrifuge
Resuspend in sucrose washing medium
Centrifuge
Protoplasts
Cell debris
Resuspend in culture medium
Remove small sample for haemocytometer count
Resuspend in culture medium to give correct pre-plating density

PROTOPLAST FUSION

- The fusion of protoplast from different plants to form somatic hybrid and the subsequent regeneration of plant through the callus. This pathway is an important achievement and the ultimate aim of this technique to produce hybrid which can not be produced by normal sexual means.
- This is very complicated and the fusion and aggregation of protoplast from same and different species can be achieved by fusogen like: PEG (Polyethylene Glycol)

Other fusogens are:

1. Sendai virus,
2. $NaNO_3$ Treatment,
3. High pH and Ca^{++} treatment,
4. Electrical cell fusion.

PEG (POLY ETHYLENE GLYCOL)

- PEG induced cell fusion is the simplest but most toxic, way to fuse cell. In this type of cell fusion peg act as a dehydrating agent.
- A strong affinity of PEG for water cause local membrane dehydration and increase fluidity and fuse not only plasma membrane but also intracellular membrane. This lead to cell fusion since PEG induces cell agglutination and cell to cell contact.
- Molecular weight (1500-6000) and concentration of PEG is important in fusion.
- Combination of PEG with high pH Ca^{++} Solution or the addition of DMSO concanavalin A gave rise to higher fusion frequency in comparison to treatment with PEG alone.

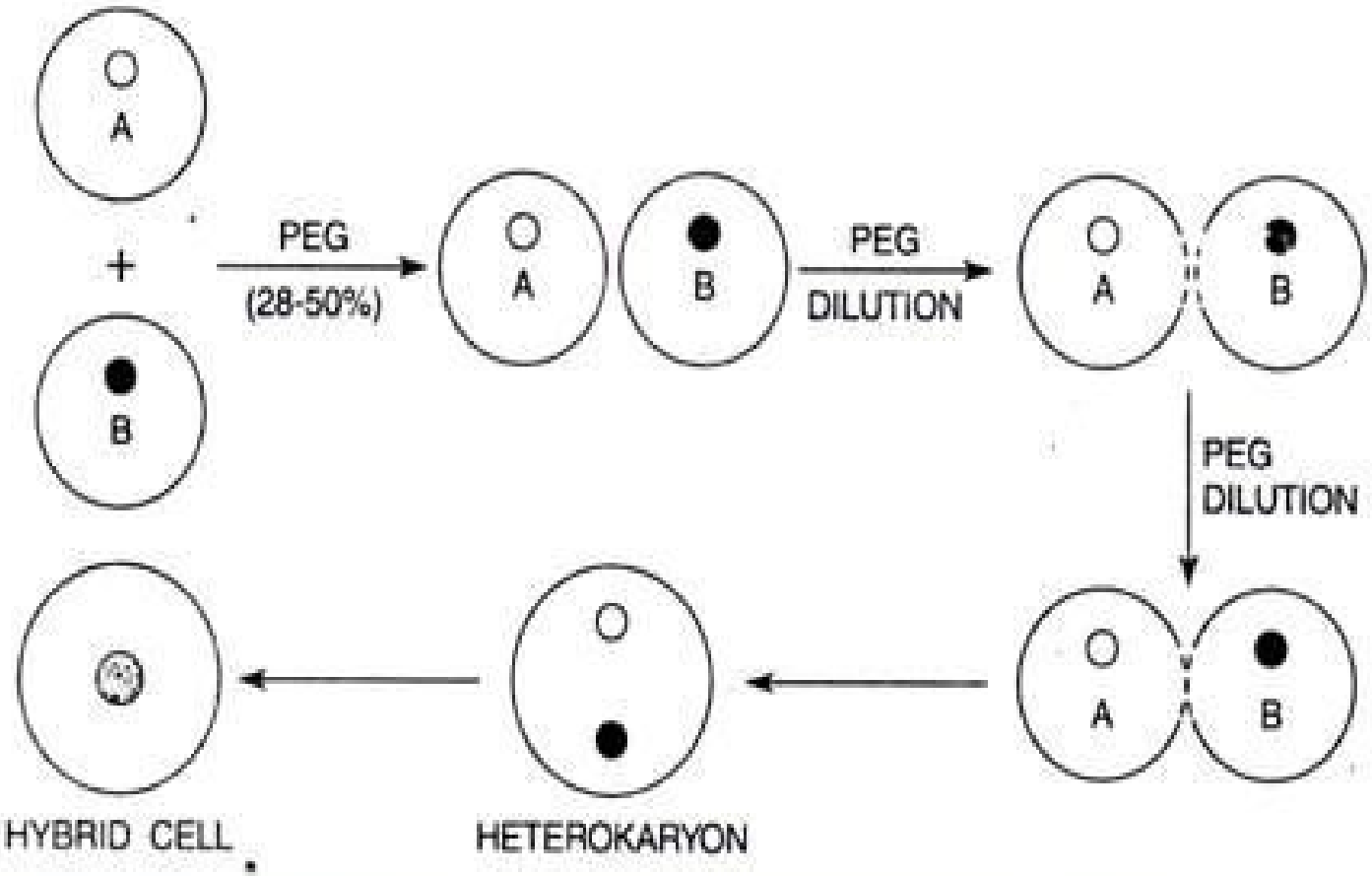


FIG. 8.11. PEG-induced protoplast fusion. Protoplasts are first brought close together (aggregation) by PEG. Fusion occurs during PEG dilution due to disturbances created in plasma membrane.

Using a Pasteur pipette a small drop of sterile silicon oil was placed at the center of Petri plant & gently covered

150 ml of protoplast suspension was pipetted out directly on the medium in the cover glass & the protoplast was allowed to sink the bottom

450 ul of PEG solution was added drop by drop into protoplast culture

Mix the suspension bye using pipette

After 20-40min at room temp. 560 ul of CPW +10% Mannitol was added drop by drop

The experiment was again repeated after 10 min and this time with 1.5 ml of CPW + 10% Mannitol

⇩

This was exactly flowed of and after 5min wash and the solution was socked off with Pasteur pipette and leaving the protoplast with a thin film of media

⇩

After this the fusion product was washed with 1 ml of washing solution

⇩

At this stage the protoplast and fusion product were set free from the cover slip & was suspended in the medium using Pasteur pipette

⇩

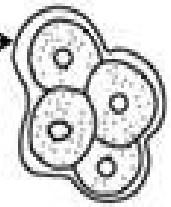
fused protoplast were inoculate into the culture medium at 25 ^{0}C for callus growth.

Explant A

Sterilized leaves

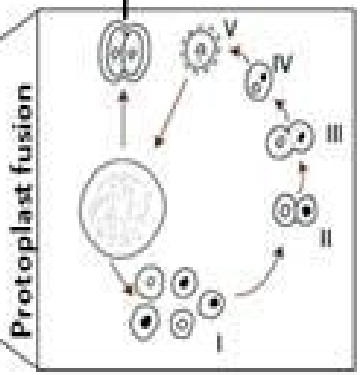
Explant B

Cell wall removal by cellulase enzyme



Micro-calli

Isolation of protoplast



Callus

Regenerated callus

Plantlet

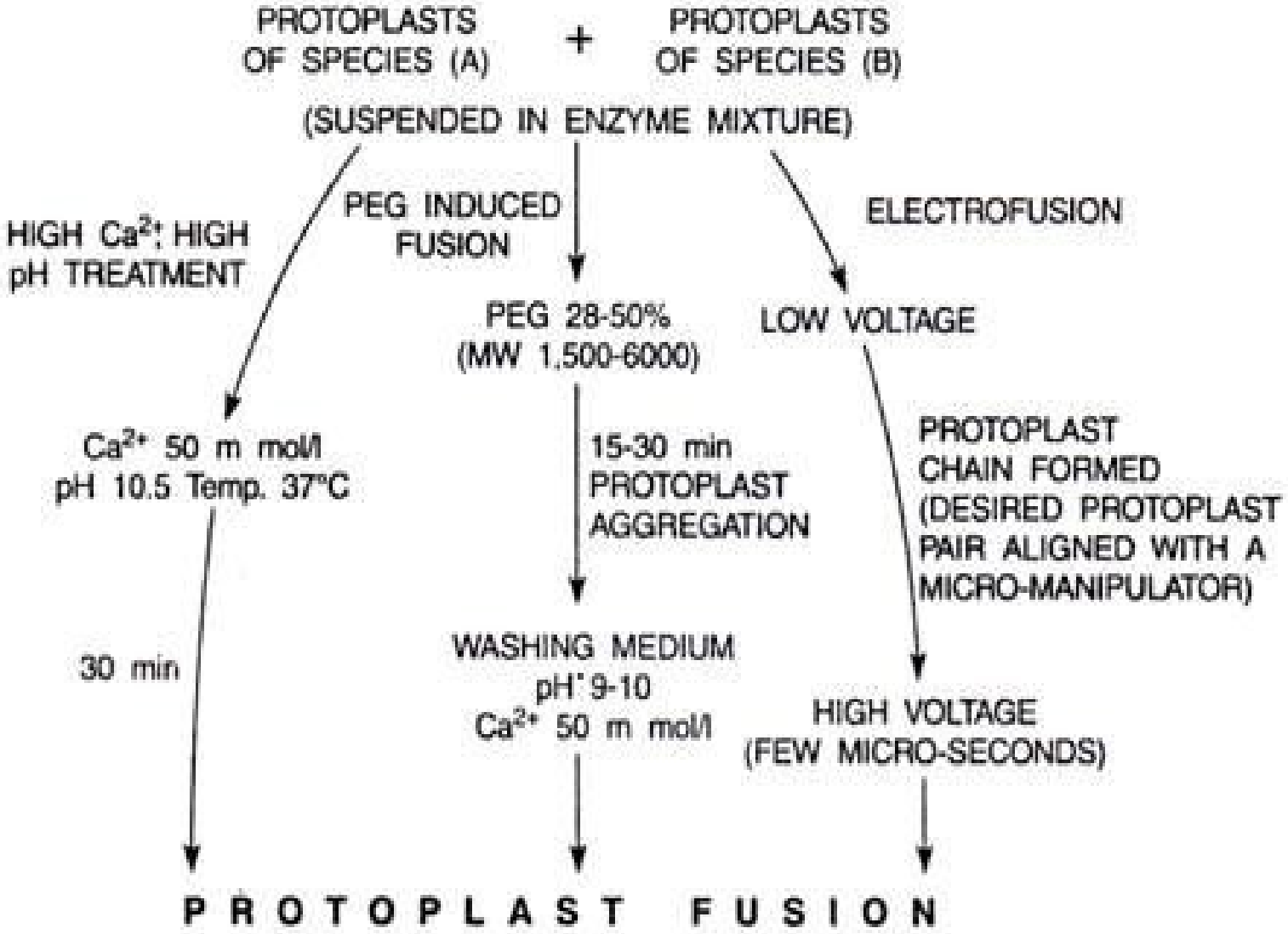


FIG. 8.10. A schematic representation of the 3 most successful protoplast fusion strategies.

APPLICATION AND SIGNIFICANCE OF PROTOPLAST FUSION

- Protoplast of sexually sterile (haploid, triploid, aneuploid) can be fused to produce fertile diploid plant.
- It overcome the sexually incompatibility barrier
- Disease resistant or insect resistant plant production
- Enhance the productivity of crops
- Monoclonal antibody production.