Somatic hybridization

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Somatic hybridization

Development of hybrid plants by the fusion of somatic protoplast of two different species.

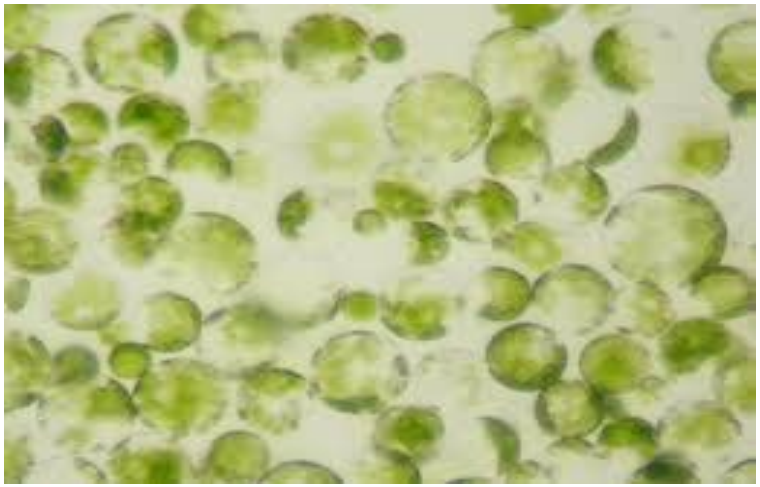
History

- In vitro somatic hybridization was first discovered by **George Barski in 1960**.

- **Hanstein** introduced the term protoplast

Protoplast

Naked plant cell without cell wall but has all cellular components and plasma membrane which is capable of cell wall regeneration



Somatic hybridization technique

1. isolation of protoplast

2. Fusion of the protoplasts of desired species/varieties

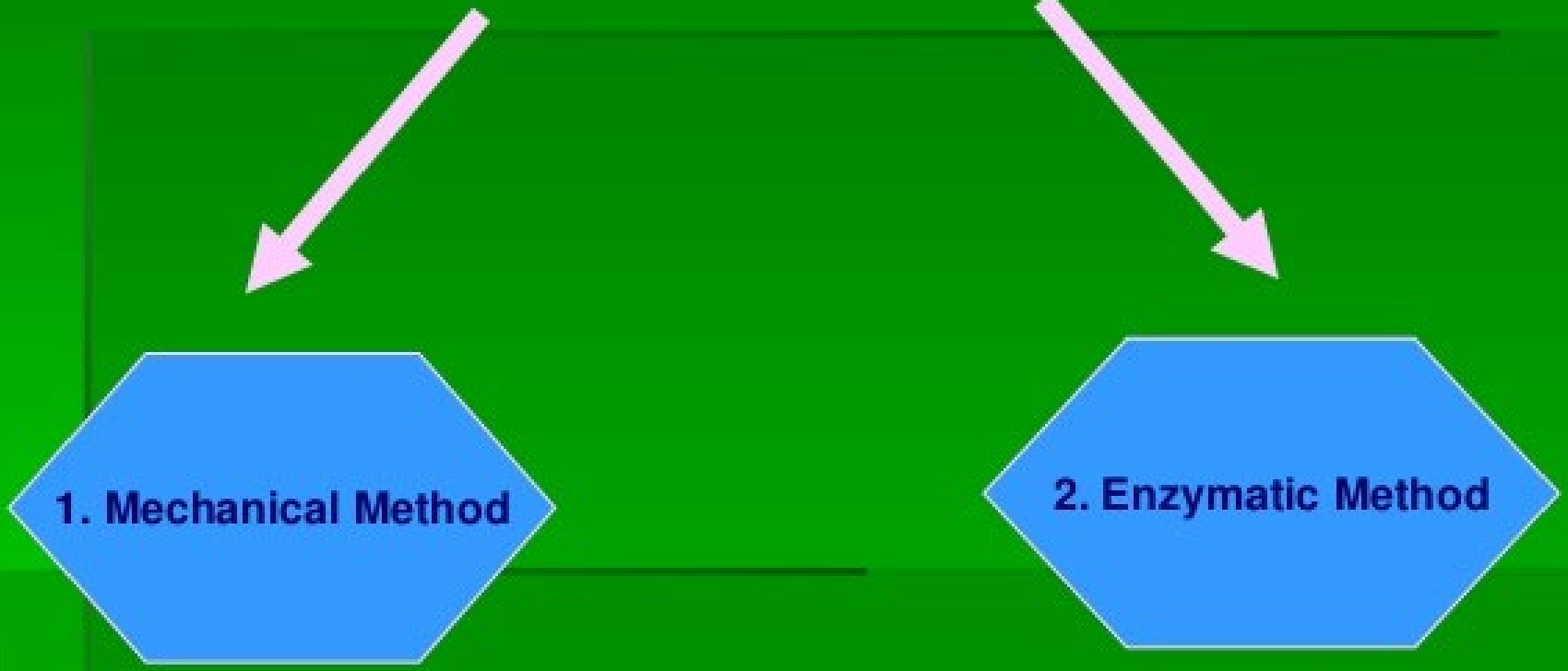
3. Identification and Selection of somatic hybrid cells

4. Culture of the hybrid cells

5. Regeneration of hybrid plants

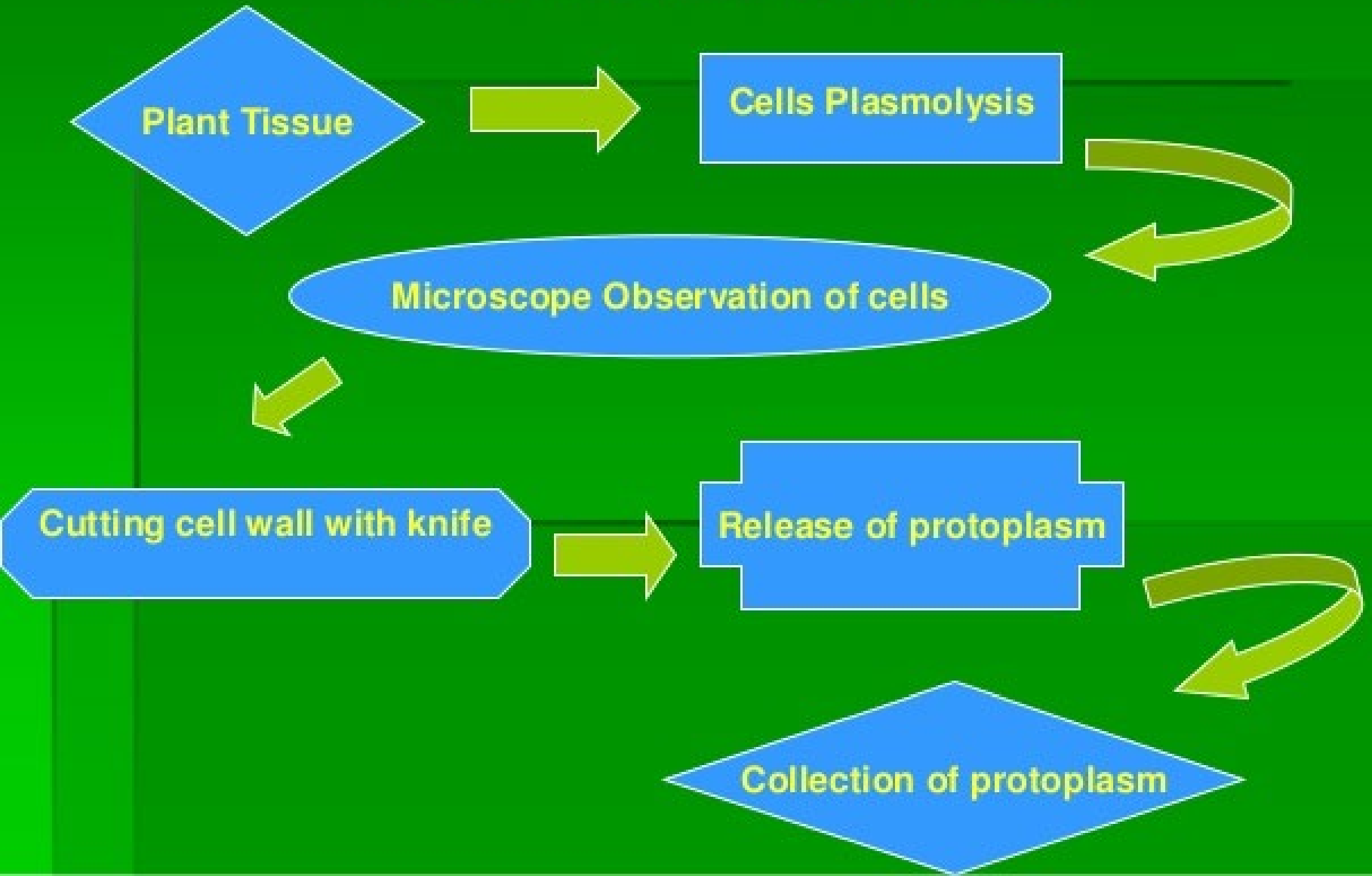
Isolation of Protoplast

(Separartion of **protoplasts** from plant tissue)

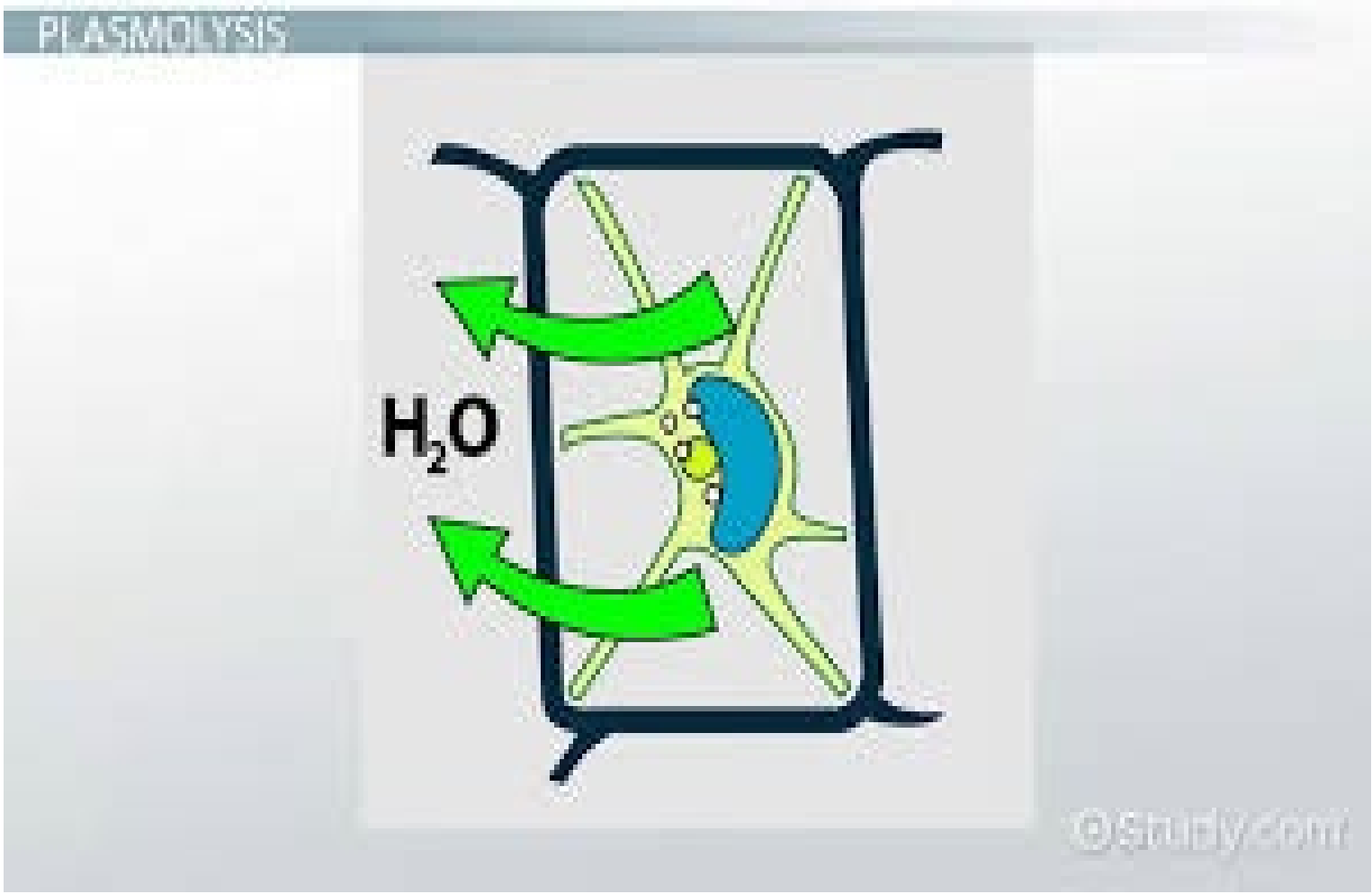


1. Mechanical Method

Klercker 1892 (cited in Cocking 1972)



Plasmolysis



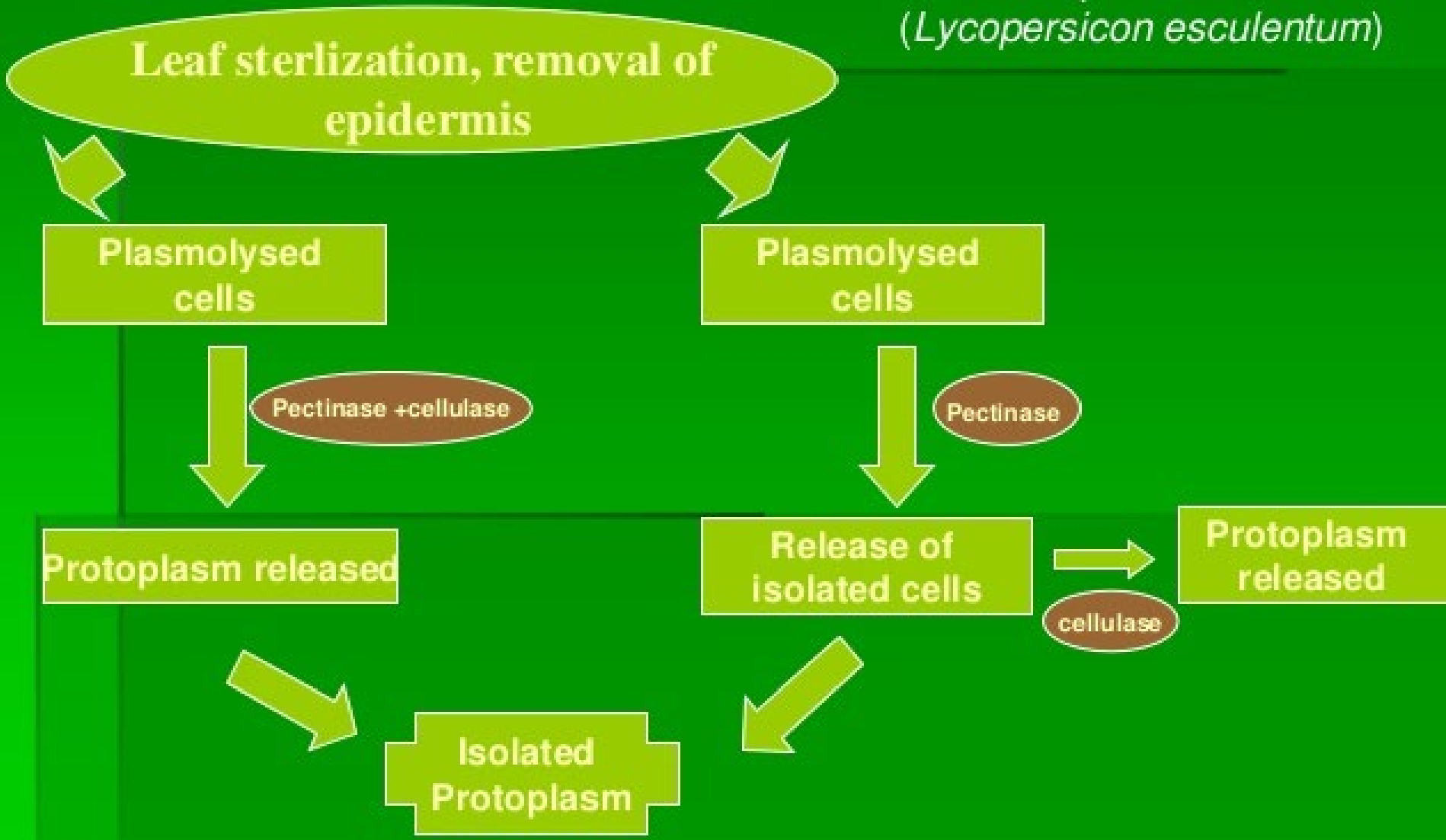
1. Mechanical Method

- Used for vacuolated cells like onion bulb scale, radish and beet root tissues
- Low yield of protoplast
- Laborious and tedious process
- Low protoplast viability
- Restricted to tissues having large vacuolated cells.

2. Enzymatic Method

(Cocking 1960)

Isolation of protoplast from root tips of tomato (*Lycopersicon esculentum*)



Two approaches

- One step or simultaneous method

- Two step or sequential method

Enzymes

- **Pectinase** (macroenzyme) –to separate cells by degrading middle lamella.
- **Cellulase** (microenzyme) – removes the cell wall.

The enzymes are used at the

Temperature 25-30◦ C

pH 4.5- 6.0

Enzymatic Method

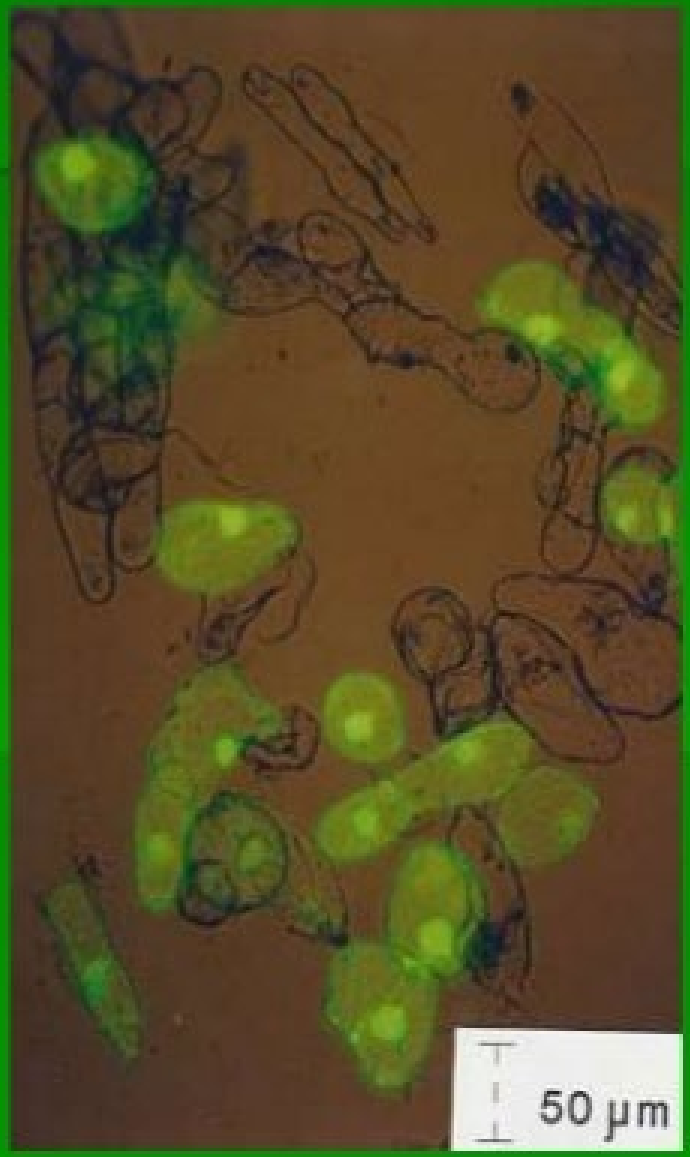
- Used for variety of tissues and organs including leaves, petioles, fruits, roots, coleoptiles, hypocotyls, stem, shoot apices, embryo microspores
- Mesophyll tissue - most suitable source
- High yield of protoplast
- Easy to perform
- More protoplast viability

Protoplast Purification

- Enzyme solution are Filtered with nylon mesh
- Filtrate centrifuged at 7oo rpm for 5 min
- Pellet contains protoplast
- Pellet is resuspended
- Pure protoplast is obtained

Checking of viability of protoplast

- **Fluorescein diacetate**: accumulates only inside the plasmalemma of viable protoplasts, can be detected with fluorescence/UV microscopy
- **Phenosafranine stainig**: used at 0.01% conc and specific for dead protoplast that turn red. Viable protoplast remain unstained
- **Evans blue**: Intact viable protoplasts, exclude the Evans blue stain. Impermeability of the cell to Evans blue indicates a living cell.



- **Calcofluor white (CFW) :** Measurement of cell wall formation is done by using CFW stain. This stain binds to the newly formed cell walls which emits fluorescence as a ring around plasma membrane.
- **Oxygen electrode:** oxygen uptake by the protoplast which measures respiratory metabolism.

Culture medium

Semi solid agar and liquid medium

- Devoid of ammonium, less iron and zinc
- High calcium – membrane stability
- High Auxin / kinetin – induce cell division
- Carbon source – glucose and sucrose
- Vitamins

Osmoticum

- 0.3-0.7 M of mannitol / sorbitol
- To increase osmotic pressure of liquid.
- To maintain protoplast from rupturing untill they develop a strong cell wall.
- Non ionic osmotica: mannitol, sorbitol, glucose, fructose, galactose and sucrose.
- Ionic osmotica: potassium chloride, calcium chloride, magnesium phosphate.

Regeneration of protoplast

- Formation of cell wall
- Development of callus/ whole plant

Formation of cell wall

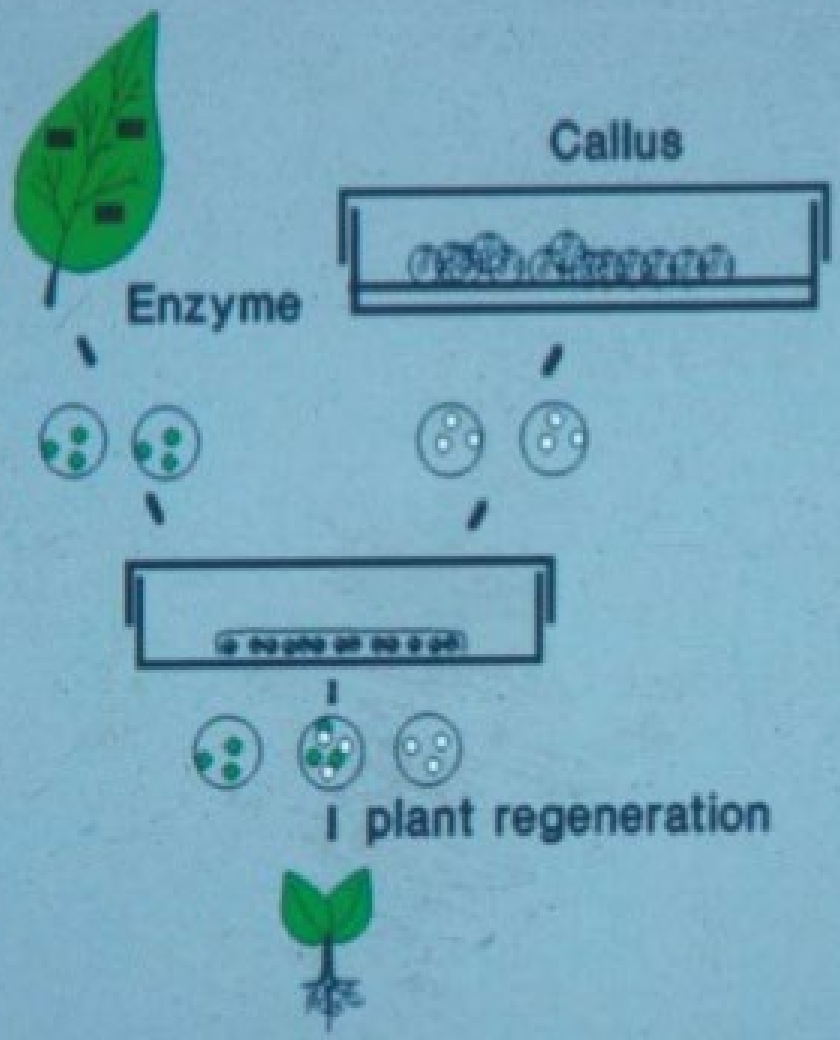
- Starts within few hours after isolaton.
- Protoplast loose their spherical shape
- CFW – to identify the new cell wall development.
- Initially the cell wall is loosely bound microfibrils later develops into an proper cell wall.

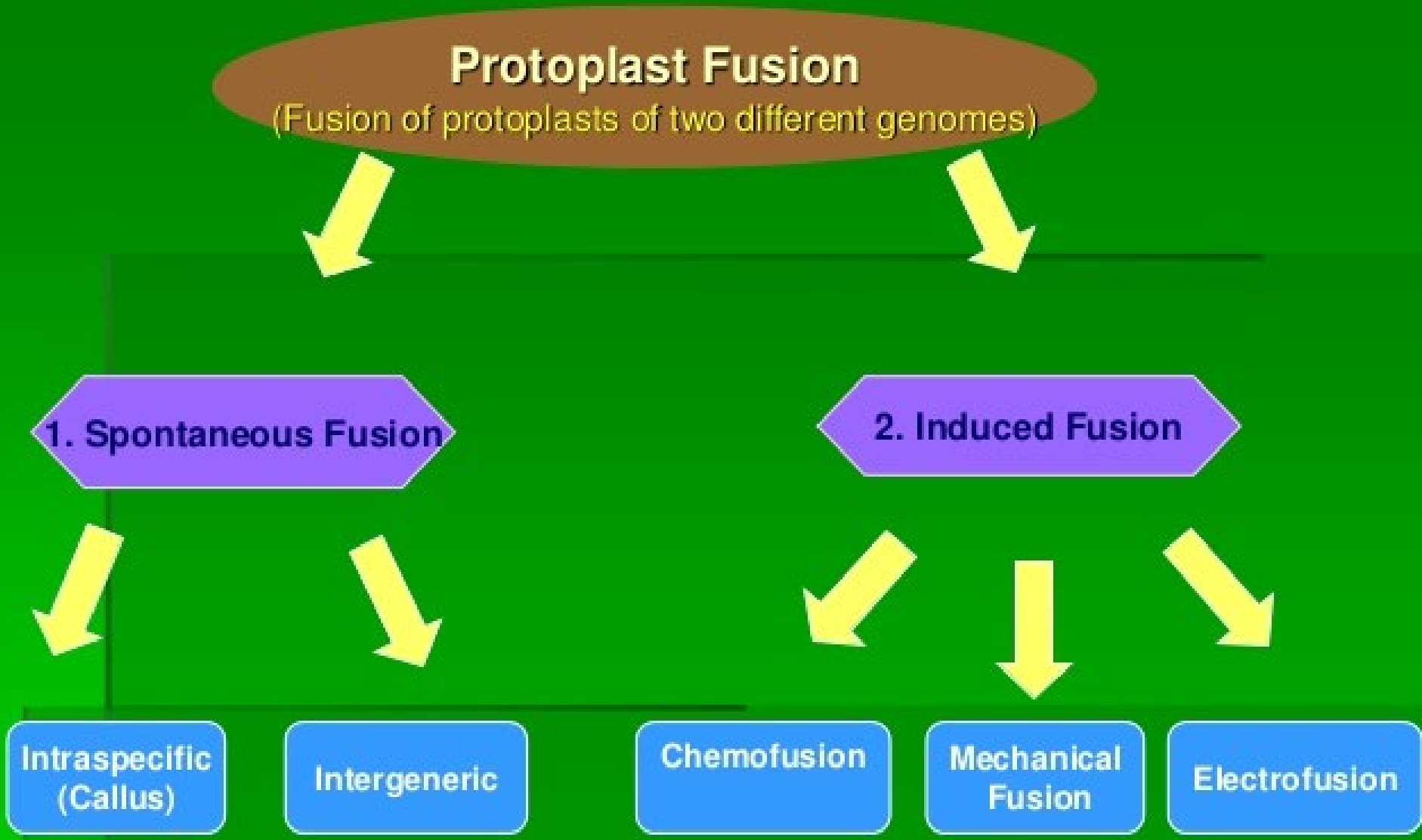
- This process requires an exogeneous supply of readily metabolised carbon source (sucrose)
- Ionic osmoticum – the cell wall development is improper.
- Protoplast with normal cell wall- undergo division
- Protoplast with improper cell wall – show budding and fail to undergo division.

Development of callus/ whole plant

- After the cell wall formation, the cell increase in size.
- First division → 2- 7 days → small colonies →3rd week visible colonies.
- Colonies transferred to osmoticum free medium
- Callus → organogenesis or embryogenic differentiation → whole plant.

Regeneration of protoplast




Protoplast Fusion
(Fusion of protoplasts of two different genomes)
1. Spontaneous Fusion
2. Induced Fusion
Intraspecific (Callus)
Intergeneric
Chemofusion
Mechanical Fusion
Electrofusion

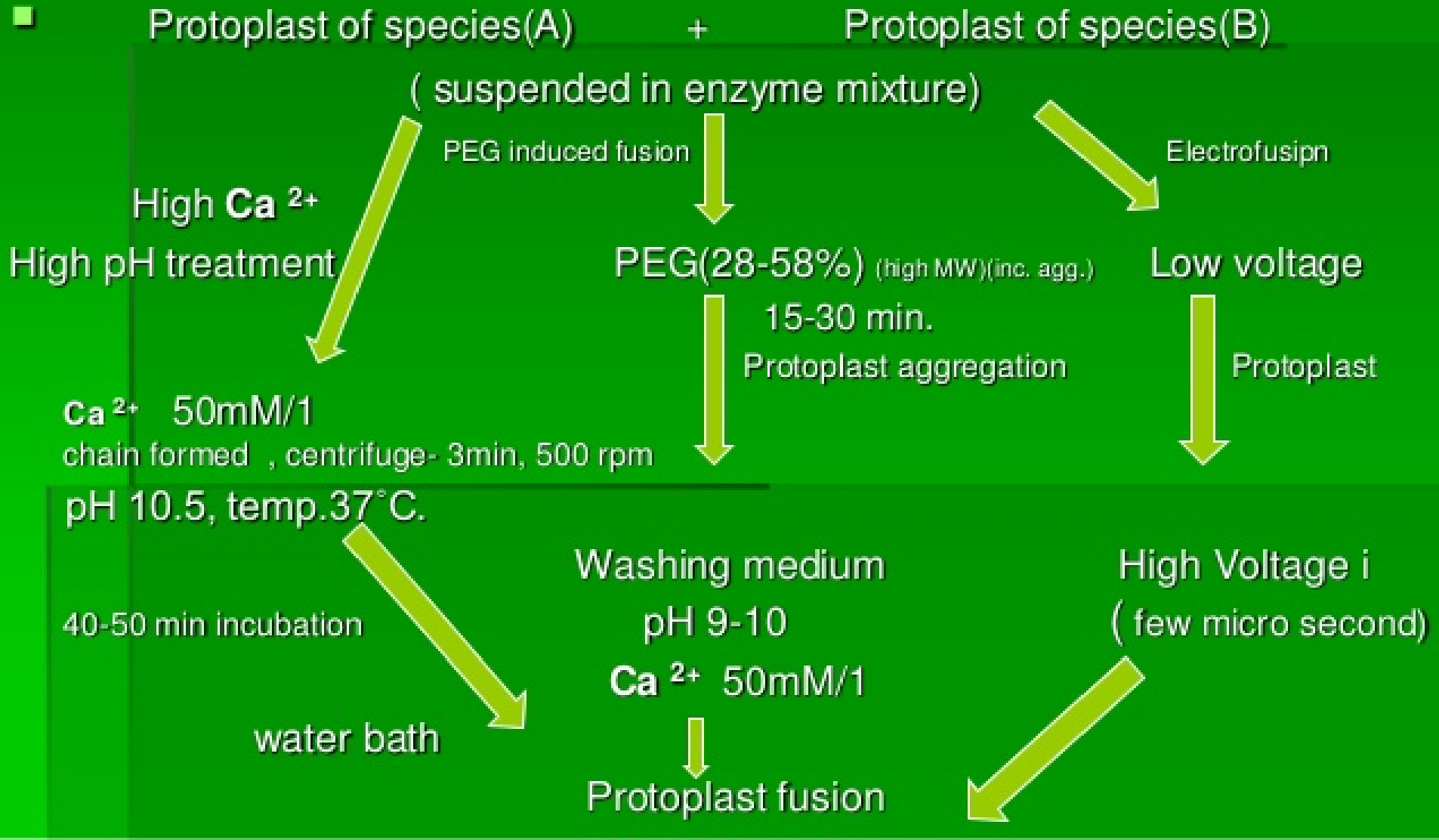
1.Spontaneous Fusion

- Protoplast fuse spontaneously during isolation process mainly due to physical contact
  - **Intraspecific produce homokaryones**
  - **Intergeneric have no importance**

Induced Fusion

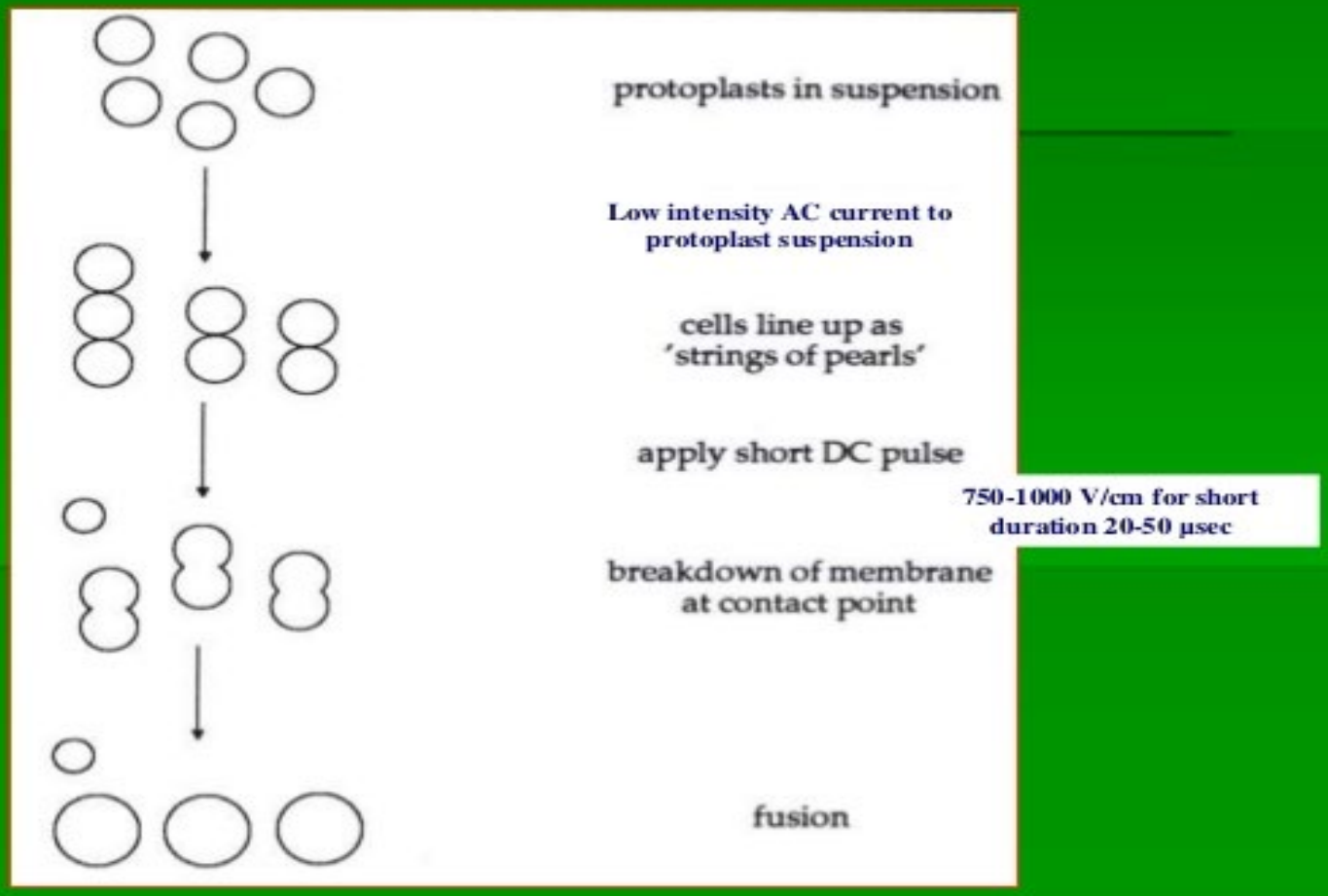
- Chemofusion
- Mechanical fusion
- Electro fusion

Schematic representation of 3 most successful protoplast fusion strategies.



Electrofusion

Protoplast fusion: Electrofusion



Selection of hybrid cells

- Protoplast suspension recovered after a treatment with a fusion inducing agent (fusogen) consists of following cell types : -
- 1) Unfused protoplast of two species / strain.
- 2) Products of fusion between two or more protoplasts of the same species(homokaryon).

3) Hybrid protoplasts produced by fusion between one (or more) protoplast (s) of each of the two species.(heterokaryon).

Therefore , a specific strategies has to be employed for their identificaton and isolation. This step is called Selection of hybrid cells.

Identification and selection of somatic hybrid cells

- **Nuclear staining:** heterokaryons are stained by carbol fuschin, aceto carmine or aceto orcein.
- **All the protoplast are cultured:** after the calli formation, based on the morphology, chromosome constitution, protein and enzyme banding pattern.

- **Fluorochromes :** FITC (fluorocein isothiocynate) and RICT (rhodamine isothiocynate) are used for labelling of hybrid cells (0.5mg/l prior incubation time)
- Presence of chloroplast.

Culture of the hybrid cells

Hybrid cells are cultured on suitable medium provided with the appropriate culture conditions.

Regeneration of hybrid plants

- Plants are induced to regenerate from hybrid calli .
- These hybrid plants must be at least partially fertile, in addition to having some useful property, to be of any use in breeding schemes.

Advantages of somatic hybridization

- Production of intergenic hybrid (pomato)
- Transfer of gene for disease resistance, abiotic stress resistance, herbicide resistance and various quality characters.
- Production of fertile diploids and polyploids
- Unique hybrids of nucleus and cytoplasm.
- Cytoplasm transfer.

limitation

- Fertile and visible seeds are not produced.
- Not successful in all plants.
- Production of unfavourable hybrids.
- Non viability of fused products.