Protoplast Isolation:

- Protoplasts are naked plant cells without the cell wall, but they possess plasma membrane and all other cellular components.
- Protoplasts of different species can be fused to generate a hybrid and this process is referred to as somatic hybridization (or protoplast fusion).

Historical developments:

- The term protoplast was introduced in 1880 by Hanstein.
- First isolation of protoplasts was achieved by Klercker (1892) by mechanical method.
- In 1960 by Cocking who used an enzymatic method for the removal of cell wall.
- Rakabe and his associates (1971) were successful to achieve the regeneration of whole tobacco plant from protoplasts.

Isolation of Protoplasts:

Protoplasts are isolated by two techniques

1. **Mechanical method**
2. **Enzymatic method**

Mechanical Method:

- In this method, large and highly vacuolated cell of storage tissue such as onion bulb scales, radish root and beet tissue could be used for isolation
- The cells are plasmolysed in an iso-osmotic solution resulting in withdrawal of contents in centre of cell.
- Then, cell/ tissue is dissected and deplasmolyesed to release the protoplast

Disadvantages of Mechanical Method

i. Yield of protoplasts and their viability is low.

ii. It is restricted to certain tissues with vacuolated cells.

iii. The method is laborious and tedious.

iv. Viability of protoplast is very low

Note: However, some workers prefer mechanical methods if the cell wall degrading enzymes (of enzymatic method) cause deleterious effects to protoplasts.

Enzymatic methods

- Protoplasts can be isolated from a wide variety of tissues and organs that include leaves, roots, shoot apices, fruits, embryos and microspores.
- In addition, callus and suspension cultures also serve as good sources for protoplast isolation.

Enzymes for protoplast isolation:

- The enzymes that can digest the cell walls are required for protoplast isolation.
- Chemically, the plant cell wall is mainly composed of cellulose, hemicellulose and pectin which can be respectively degraded by the enzymes cellulose, hemicellulose and pectinase.
- Various enzymes for protoplast isolation are commercially available. The enzymes are usually used at a pH 4.5 to 6.0, temperature 25-30°C with a wide variation in incubation period that may range from half an hour to 20 hours.

Enzymatic isolation of protoplasts can be carried out by two approaches:

- **Two step or sequential method:**

The tissue is first treated with pectinase (macerozyme) to separate cells by degrading middle lamella. These free cells are then exposed to cellulose to release protoplasts. Pectinase breaks up the cell aggregates into individual cells while cellulose removes the cell wall proper.

- **One step or simultaneous method:**

The tissue is subjected to a mixture of enzyme in one step recation which includes both macerozyme and cellulose. This is the preferred method for protoplast isolation.

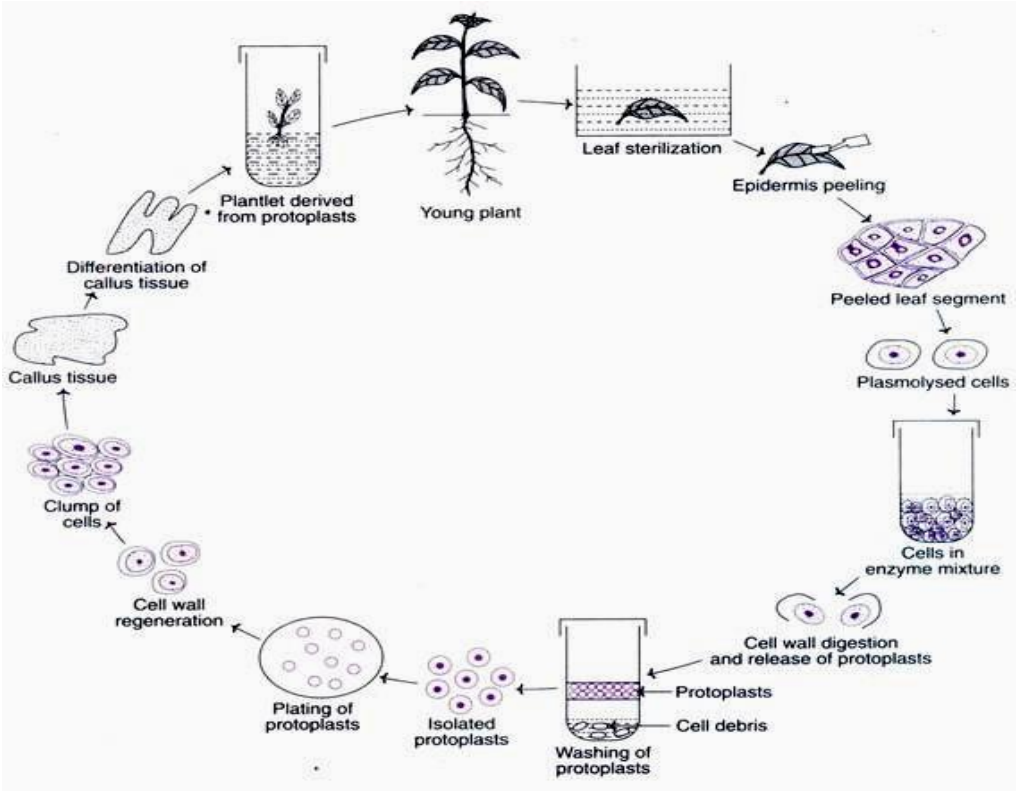

Fig. 44.2 : Major steps involved in protoplast isolation, culture and regeneration of plants.