RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY

Course: B. Tech Biotechnology
Sub Code: BBT-515

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Sub Name: Plant Biotechnology

LECTURE 6

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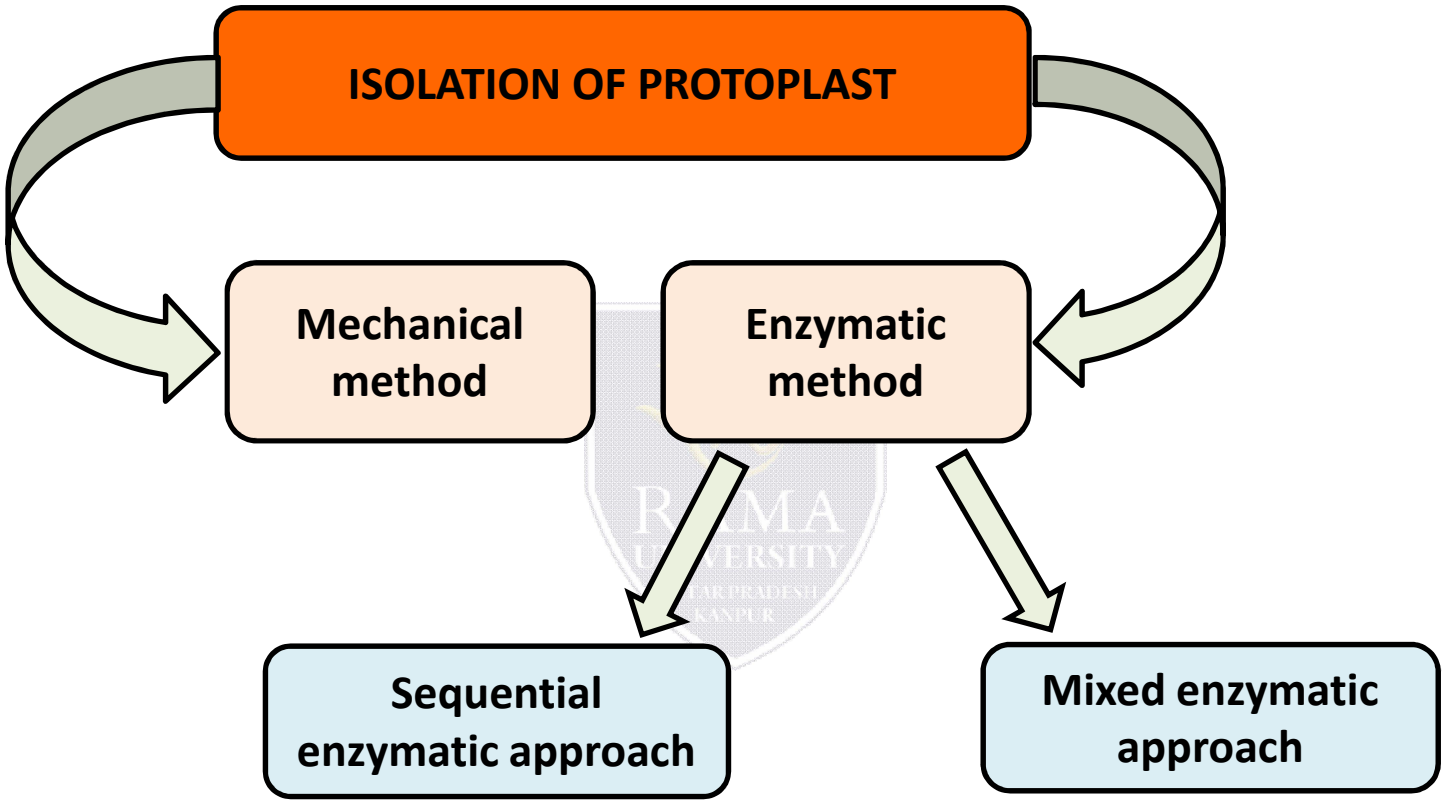
PROTOPLAST ISOLATION, FUSION & CULTURE

- The word “PROTOPLAST” was coined by “Hanstein” in 1880 for the living matter surrounded by the plasma membrane but without cell wall.

- Provide the starting point for many of the technique of genetic manipulator of plants (induction of somaclonal variation, somatic hybridization and genetic transfer).

- Cultivated in liquid as well as on solid media.



Protoplasts can be isolated from almost all plant parts i.e., roots, leaves, fruits, tubers, root nodules, endosperm, pollen cells, and cells of callus tissue.

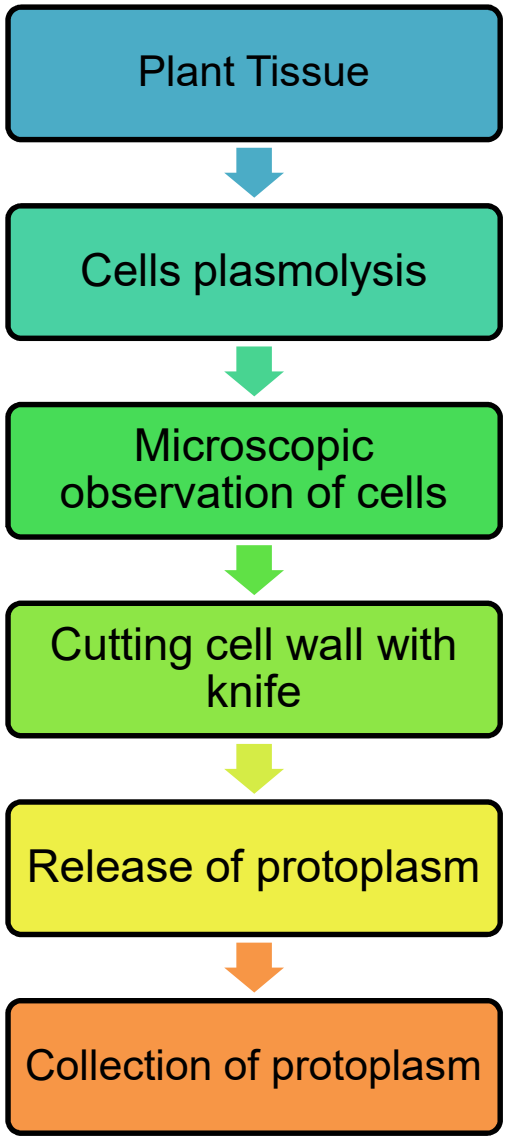
1. Mechanical method (Non-enzymatic)

- ✓ The plant tissue were kept in a suitable plasmolyticum (lysis of plasma membrane) and then cut with a sharp knife.
- ✓ Then these pieces are deplasmolysed by using dilute solution to release the protoplasts.

Advantage: Unknown effets of enzymes on protolast eliminated

Disadvantage:

- ✓ Low yield of protoplasts
- ✓ Cells may be broken



2. Enzymatic method

Commercial preparations of purified cell wall degrading enzymes such as macroezyme, cellulase and hemicellulose became available that gave further progress to enzymatic isolation of protoplasts.

Advantages:

- ✓ Yield of protoplast is high.
- ✓ Cells remain intact and not damaged
- ✓ Osmotic shrinkage of protoplast is minimum.

Disadvantage:

- ✓ Unknown effects of enzymes on protoplast

Enzymatic method of protoplast isolation can be classified into two groups.

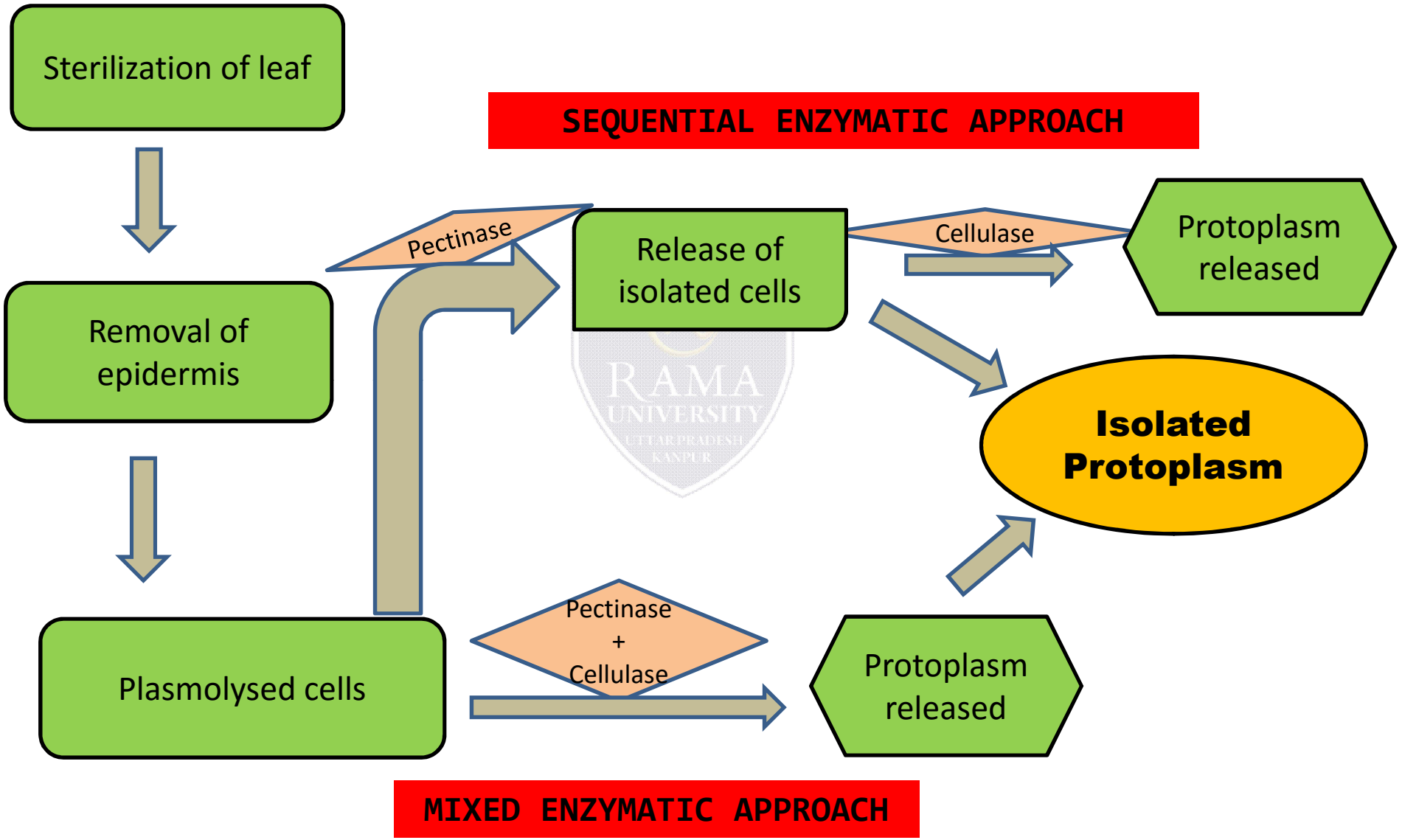
❖ Sequential enzymatic (Two-step treatment)

This involves two steps where macerated plant tissues was first incubated with pectinase (degrade pectin cell wall) to get single cells followed by cellulase (degrade cellulosic cell wall) treatment for release of protoplast.

❖ Mixed enzymatic (Simultaneous process treatment)

Protoplasts can be isolated by treating cells, with a suitable mixture of cell wall degrading enzymes. The mixture of Pectinase or Macerozyme (0.1-1.9%) and Cellulase (1-2%) is suitable for majority of plant parts.

ENZYMATIC METHOD



QUIZ