TOPIC : ISOLATION AND CULTURE OF PROTOPLAST

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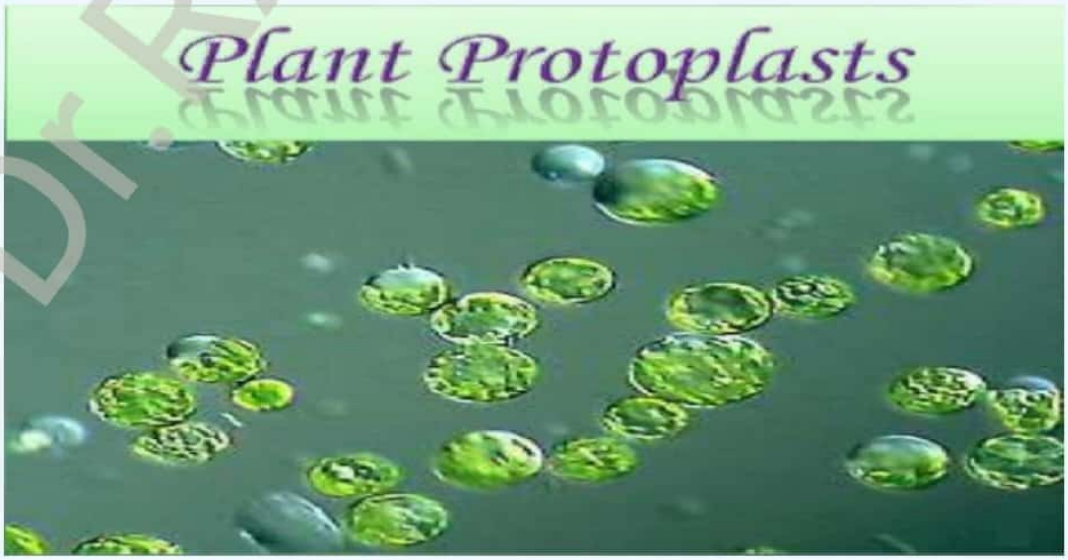
Protoplast Isolation and Culture

Protoplast culture

The culture of protoplast in an aseptic liquid media is known as protoplast culture. Protoplast is a cell without a cell wall. It is a living part of a plant cell consisting of cytoplasm and nucleus. The basic principle of **protoplast culture** is the aseptic **isolation** of large number of living **protoplasts** and **cultures** them on a suitable nutrient medium for their requisite growth and development. **Protoplast** can be isolated from variety of plant tissues but best and maximum number of protoplast can be isolated from mesophyll tissue of leaves. **Suspension culture** is a type of **culture** in which single **cells/protoplast** or small cell aggregates multiply in agitated liquid medium. The cells remain suspended in a defined aseptic media, controlled physiological condition and regulated environmental condition. It is also referred to as **cell culture** or **cell suspension culture.** Single cell suspension culture can be considered as protoplast culture. Protoplast maybe fused together to form hybrids and cybrids. Protoplast culture is used to introduce and modify genetic information inside any cell. It is used to produce transgenic plants.



Isolation of Protoplast :--

i. **Mechanical** process – non – enzymatic process
ii. **Enzymatic process --** two-step sequential process
iii. **Mixed Enzymatic** process – a simultaneous process

Mechanical isolation – It is done by cutting plasmolyzed tissue with knife and releasing the protoplast by deplasmolysis. In this method the protoplast released are very few in number.

Enzymatic isolation – A concentrated solution of cellulase enzyme is taken and the leaves are dipped in the solution. This isolates the protoplast by degrading the cell wall.

But, in higher plants a sequential or two step process for protoplast isolation is followed. In this method the plant tissues are mecerrated and then incubated with pectinase. It is then treated with the enzyme cellulase. This way, the protoplast gets isolated.

Simultaneous process – In this process two enzymes are mixed together and protoplast are isolated in a one step process.
The plant tissues are plasmolyzed in the presence of a mixture of enzymes (pectinase and cellulase). This method is **used by most of the workers** because it is **less time consuming** and **reduces the chance of microbial contamination** by excluding few steps.

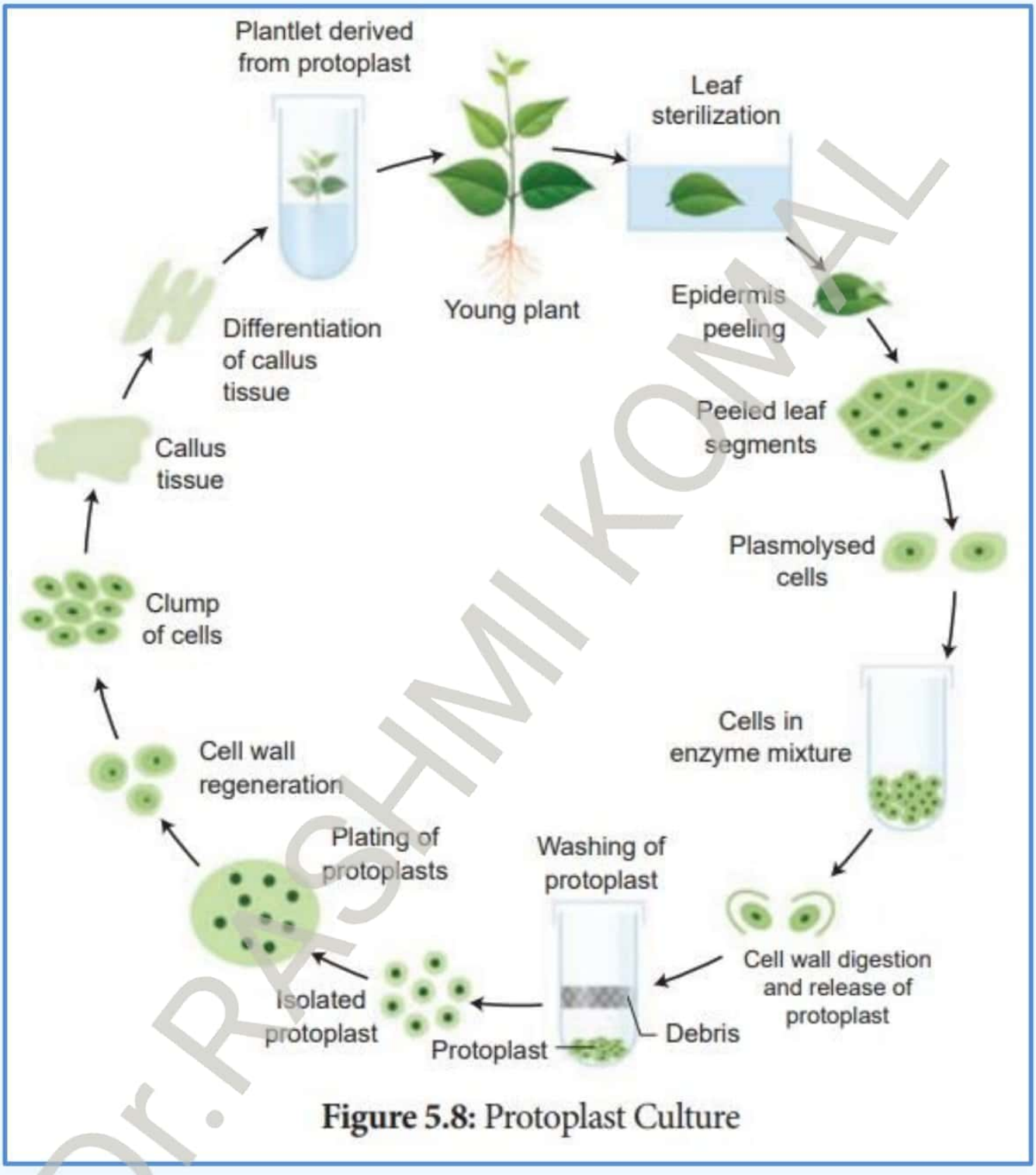


Figure 5.8: Protoplast Culture

MAJOR STEPS INVOLVED IN PROTOPLAST ISOLATION, CULTURE AND REGENERATION OF PLANTS

Source of explant

- **Leaves:** the most convenient source for isolation of plant protoplast (because it allows isolation of large number of uniform (isodiametric), mesophyll cells present in the leaves which are loosely arranged). Root, stem, anther etc. may also be used for isolation of protoplast but they give poor results.
- **Callus cultures:** young callus cultures are ideal material for obtaining large quantities for protoplast. Mature callus cultures form giant cells which have thick cell wall and they are difficult to digest. So, young callus cultures are preferred for protoplast isolation.
- **Cell suspension cultures:** cell suspension cultures provide excellent source material for protoplast isolation. The cell suspension is centrifuged and the supernatants are removed. The remaining cells are incubated in a mixture of cellulase and pectinase in a culture flask. The flask is placed in a rotatory shaker for 6 hours. This time may vary from 6-12 hours as per the requirement of the experiment.

Protoplast isolation from leaves :

It involves five basic steps:

- Sterilization of leaves
- Removal of epidermal cell layer
- Pre Enzymatic treatment
- Incubation in enzymes and
- Isolation by filtration and certification

Culture of protoplast :

The first step in the protoplast culture is the development of the cell wall around the membrane of isolated protoplasts also called the **regeneration of cell wall**. It is followed by induction of division in the protoplast. Simultaneously the cell division gives rise to small cell colonies upon giving a favorable nutritional and physiological condition. The cell colony grows into callus, which in turn regenerates into a whole plant by further sub culturing into suitable media.

For culturing protoplast in a nutrient media containing agar **Bergmann's Cell Plating Technique** is used. About 2 ml of isolated protoplast solution is mixed with an equal volume of agar nutrient medium. The temperature of the agar should be 35 to 45°C. When the agar solidifies the culture plates are sealed and kept in an inverted position at 25°C to 28°C.

Culture medium :

Nutrient components of protoplast culture media generally comprises of nutrients which are similar to those required for the callus and suspension culture.

The concentration of iron, zinc and ammonia is very high as compared to the standard plant tissue culture experiments. The MS media and the salts of B_5 are most suitable for protoplast culture.

The calcium concentration is taken 4 times than the normal concentration, because **it helps to maintain the membrane integrity as well as helps in cell wall regeneration.**

Sucrose is taken in the concentration of 2-5 %.

Vitamins used are of the same concentration as used in standard tissue culture experiment.

Osmoticum :

Osmoticum refers to the reagents/chemicals that are added to increase the osmotic pressure of a liquid. Isolation and culture of protoplast require osmotic protection until they develop a strong cell wall.

During isolation as well as culture of protoplast osmotic protection is necessary. Osmoticum is added in both isolation media and culture media. This prevents the rupture of the protoplast. If freshly isolated protoplasts are directly added to culture medium they will burst.

The most widely used osmoticum (in the protoplast culture medium as well as isolation medium) is a mixture of sorbitol, mannitol, glucose /sucrose.

Protoplasts are most stable in hypertonic solution.

Protoplast regeneration :

Cell wall formation

The process of cell wall formation may take from 2 to several days for completion. After a few hours of isolation, the cell wall starts regenerating. When the regeneration of the cell wall is complete the protoplast loses its spherical shape.

The freshly formed cell wall is composed of loosely arranged micro fibrils. For a proper cell wall to regenerate, it is necessary to supply a carbon source (sucrose) in the nutrient medium.

Protoplast with a poorly developed cell wall shows budding.

Those protoplast which are unable to regenerate cell wall don't undergo mitosis.

Development of callous/whole plant:

As soon as the cell wall is properly built around the protoplast, the cells show increase in size and cell division starts within a week. After 2-3 weeks microscopic colonies are formed. These colonies are now transferred into an osmoticum free medium which develops into a callus. These callus may either be induced for organogenesis or whole plant regeneration.

Examples of plant species that have regenerated from protoplast are *Cucumis sativas, Ipomoea batata, Beta vulgaris, Glycine max, Rosa sp.* etc.

Uses of isolated protoplast :

- Biochemical and metabolic studies
- Fusion of two different somatic cells to get somatic hybrids
- Fusion of nucleated and enucleated cells to produce sybrid
- Genetic manipulation
- Drug sensitivity