Protoplast Culture : Culture & Regeneration

Lecture III

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Culture

Isolated protoplasts are either cultured in liquid or agar gelled media.

Prior to culture viability of the protoplast must tested

Sometimes the protoplast is first grown in liquid media and then transferred into the agar media plates.

Culture density should be kept $1 \times 10^4 - 1 \times 10^5$ Protoplast/ml

Culture Medium

Physical State: Liquid or Agar gelled

Composition: Nutrient and growth regulator concentration should be optimized as per protoplast requirement

Osmoticum: Slightly hypertonic than internal cytoplasmic concentration

Maintained by some non ionic chemicals such as mannitol, sorbitol etc.

Low Number Culture

Methods to culture protoplast in low number are

(i) Feeder layer

(ii) Co-culturing

(iii) Microdrop culture technique

Feeder Layer

A feeder layer of X-ray irradiated non-dividing but metabolically active protoplasts after washing are plated on agar gelled medium. The protoplasts of the same species or different species can be used as a feeder layer. Non-irradiated protoplasts of low density are plated over this feeder layer.

Co-culturing

- This method involves co-culture of protoplasts from two different species to promote their growth or that of the hybrid cells.

- The co-culture methods is generally used where calli arising from two types of protoplasts can be morphologically distinguished. For example, protoplasts isolated from albino plants and green plants are easily distinguishable based on color where albino protoplast will develop non green colonies.

- Metabolically active and dividing protoplasts of two types - slow and fast growing are cultured together, the fast growing protoplast provide other species with diffusible chemicals and growth factors which helps in cell wall formation and cell division.

Microdrop Method

In this method, protoplasts are cultured in special Cuprak dishes which have two chambers—a small outer chamber and a large inner chamber. The large chamber carries numerous numbered wells each with a capacity of 0.25-25µl of nutrient medium.

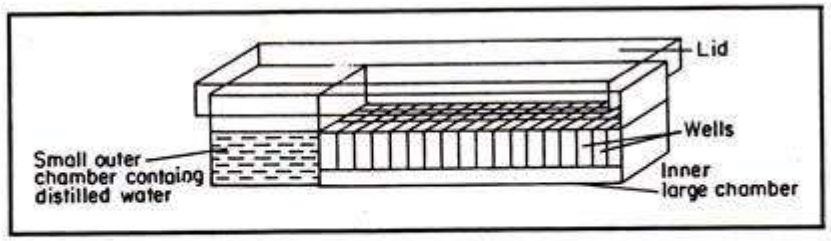

Fig 9.5

Diagrammatic view of Cuprak dish used for the microdroplet technique of single cell culture

Fig. source:https://www.biologydiscussion.com › plant-tissues › sin...

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- **Each well of the inner chamber is filled with a micro-drop of liquid medium containing isolated single protoplast. The outer chamber is filled with sterile distilled water to maintain the humidity inside the dish**

- **After covering the dish with lid, the dish is sealed with paraffin and incubated in culture room.**

- **The cell colony derived from the single cell is transferred on to fresh solid or semisolid medium in a culture tube for further growth**

Protoplast Regeneration

Protoplast starts to regenerate a cell wall within few days (2-4 days) of culture.

during this process, protoplasts lose their characteristic spherical shape which has been taken as an indication of new wall regeneration.

Cell wall regeneration can be confirmed by Calcofluor White staining method.

There is direct relationship between wall formation and cell division.

Once the cell wall formation is completed, cells undergo division resulting in increase size of cells.

After an interval of 3 weeks, small cell colonies appear, these colonies are transferred to an osmotic-free callus induction medium.

This is followed by introduction into organogenic or embryogenic medium leading to plantlet development.

Calcofluor White Staining

- **This staining method assures protoplast viability by detecting onset of cell wall formation.**
- **Calcofluor binds to beta linked glucosides in newly synthesized cell wall which can be observed as a fluorescent ring around the membrane.**
- **Optimum staining is achieved when 0.1 ml of protoplast is mixed with 5.0 µl of 0.1% w/v solution of CFW.**

Reference

https://www.biologydiscussion.com › plant-tissues › sin...

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