TOPIC : CELL SUSPENSION CULTURE

COURSE : M.Sc. Botany Part II

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(CYTOGENETICS AND PLANT IMPROVEMENT / PLANT RESOURCE UTILIZATION AND CONSERVATION)

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Establishment of single cell culture provides an excellent opportunity to investigate the properties and potentiality of a plant cell. The early experiments of suspension culture were done by Haberlandt in 1902. When we obtain plants through tissue culture or organ culture, we get a variety of plants. Cell cultures have a great potential for crop improvement. Free cells in a culture permit or withdraw diverse chemicals by making them easy targets for mutation. The individual cells within a population of cultured cells invariably show cytogenetical and metabolic variation. In this way the cell line selection technique can be used to produce high yielding cultures as well as plants with superior agronomic traits.

Isolation of single cell from plant organs:

Most suitable material for isolation of single cell is the leaf tissue; this is because the leaf has homogeneous population of mesophyll cells. These are isodiametric and loosely arranged and can be obtained easily. From such intact plant organs like leaf tissues single cells can be isolated by using two methods:

- Mechanical method
- Enzymatic method

Mechanical method:

Gnanam and Kulandaivelu (1969) developed a process which has been used successfully to isolate mesophyll cells (which are active in photosynthesis and respiration) from mature leaves of several species of dicots and monocots.

The procedure involves mild maceration of 10 gram leaves in 40 ml of grinding medium (20 µmol sucrose, 10 µmol $MgCl_2$, 20 µmol Tris HCL buffer with PH 7.8) with a mortar and pestle. The homogenate is passed through two layers of muslin cloth and the cells thus released are washed by centrifugation at low speed using the same medium.

Enzymatic method:

This was first done by Takebe and his co-workers in 1968. In this method the leaf tissues are dissolved in a mixture of enzymes pectinase, cellulase/ hemicellulase. Sometimes a mixture of enzymes is taken to dissolve cellulose, hemicellulose and middle lamella. Mecerozyme can also be used to dissolve middle lamella. A proper osmoticum is added before putting the tissues in the solution of enzymes to prevent plasmolysis. The middle lamella and the cell wall get dissolved and a large number of metabolically active cells are obtained.

Isolation of single cells by enzymatic method is more convenient because a high yield of single cells is obtained from spongy parenchyma with minimum damage or injury to the cells. A Friable callus is necessary for getting a fine cell suspension in a liquid medium. However, it is difficult to obtain single cells of cereals with this method because the mesophyll cells of cereals are elongated with interlocking constructions which prevent their isolation.

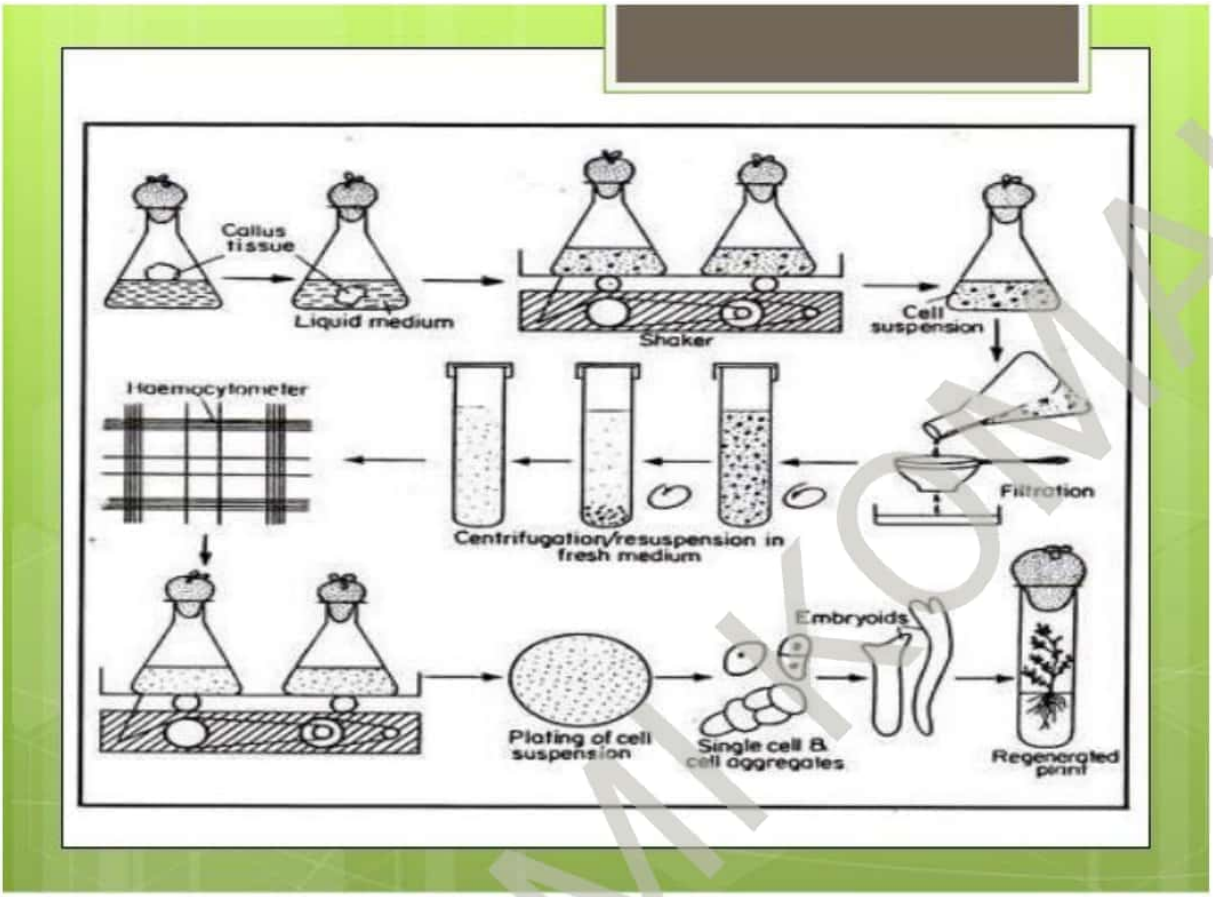
Isolation from cultured tissues :

Freshly cut pieces of surface sterilized plant organs are simply placed on suitable culture medium to initiate calluses. The callus is transferred to a fresh medium to build a mass of tissues. Repeated subcultures make the callus friable. A piece of friable callus is then transferred in a continuously agitated liquid medium. Agitation is done by placing the culture flask on an Orbital Platform Shaker. Movement of the culture medium exerts mild pressure on small pieces of tissue breaking them into free cells or small cell aggregates.

Growth and subculture of suspension culture :

During the incubation period the biomass of the suspension culture increases due to cell division and cell enlargement. Due to continuous cell division and enlargement some factors may get exhausted or there may be accumulation of toxic substances in the medium. This is the time when the cell suspension is transferred to a fresh liquid medium of the same composition. The normal incubation time of a stock culture is 21 to 28 days after which it is subcultured. The incubation time may vary by ± 3 days. The incubation period from culture initiation to the stationary phase can be determined by:

a. Initial Cell density
b. Duration of lag phase
c. Growth rate of cell line



Types of suspension culture:

A. Batch culture

These cultures are maintained by continuously propagating a small amount of the liquid of the inoculum in a moving liquid medium and transferring it into a fresh medium, at regular intervals. Cell suspensions are generally grown in100 to 250 ml flask. Each culture flask contains 20-75 ml of the culture media. The flask containing suspension culture is allowed to stand still for a few seconds after which, the large colonies settle down. The suspension

is then taken from the upper part of the culture with the help of a pipette or syringe and transferred to a fresh medium.

The biomass growth in batch culture follows a fixed pattern :

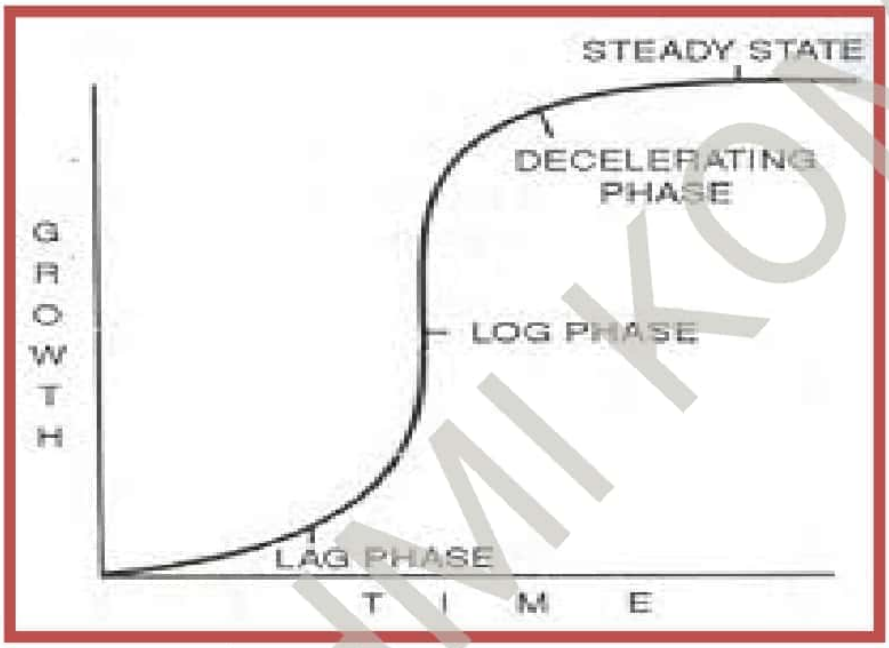


Fig 1: Graph showing different growth phase in a Batch culture

When **the cell number in a suspension culture is plotted against the time of incubation**, a growth curve is obtained. The curve reveals that initially the culture passes through a log phage followed by a brief exponential phase (the most fertile period for active cell division). The growth declines after 3 to 4 generation. This denotes that the culture has entered a stationary phase.

Batch culture shows a constant change in cell growth and metabolism so they are **not considered** to be an ideal system for the study of cellular behaviour.

B. Continuous culture:

The large-scale culture grown under steady state for long periods by adding fresh medium and draining out the used medium in a number of specially designed culture vessels are known as continuous or mass culture. Continuous cultures are of two types:

- Close type
- Open type

In close type, the addition of fresh medium is balanced by the outflow of the old medium. The cells passing through the outgoing medium are separated and reintroduced in the culture. With the proceeding growth, the cell biomass continues to increase.

In open type the inflow of the medium is done by balancing harvest of an equal volume of the culture medium and cells. This allows the indefinite maintenance of cultures at a constant and submaximal growth rate. Basically there are two major types of (continuous) cultures. Example: chemostat and turbidostat.

Besides commercial applications, continuous cultures have certain other advantages:

a. ease of maintaining sterile over a period of time

b. less detrimental effects during mechanical failures

c. a degree of automation

d. versatility with regard to growth conditions like temperature, aeration, stirring speed, illumination, nutrient and growth regulator levels.

Culture medium for cell suspensions :

The basic requirement of a fine suspension culture is a friable callus. Manipulation in media and frequent subcultures lead to the formation of a friable callus. M.S basal media along with some growth regulators can be used for a suspension culture. Sometimes small amount of hydrolytic enzymes (cellulase and pectinase) or yeast extract are used for dissociation of callus cells. Some culture results are bettered by adding 2,4-D in a small quantity with vitamins and casein hydrolysate. B5 and ER (Ericsson 1965) media are specially cultures of higher plants. pH is adjusted according to the type plants used for experiment and their requirements.

Importance Of Cell Suspension Culture :

- Cabable of providing information about cell physiology, biochemistry and metabolic events.
- Mutagenesis studies maybe facilitated by cell suspension culture to produce mutant cell clone, from which mutant plants can be raised.

THANKYOU