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Course: B. Tech Biotechnology
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LECTURE 1

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WHAT IS SINGLE CELL CULTURE METHOD???

Single cell culture is a method of growing isolated single cell aseptically on a nutrient medium under controlled condition

PRINCIPLE

- The basic principle of single cell culture is the isolation of large number of intact living cells and cultures them on a suitable nutrient medium for their requisite growth and development.
- Single cells can be isolated from a variety of tissue and organ of green plant as well as from callus tissue and cell suspension.
- Single cells from the intact plant tissue (leaf, stem, root cladode etc.) are isolated either mechanically or enzymatically.

- Single cell culture could be used successfully to obtain single cell clones.
- Plants could be regenerated from the callus tissue derived from the single cell clones.
- Single cell culture are very important in relation to crop improvement programmes.
- Single cell culture is an ideal system for the study of biotransformation.
- single cell culture in large-scale could become a valuable technique for industrial production of such important natural compound.

ISOLATION OF SINGLE CELLS

This can be achieved from:

1. Intact organs

2. Callus

From intact organ: Mechanical and enzymatic methods are used to isolate the cells from intact organs.

- ✓ The plant part preferably leaf tissue is subjected for grinding in a mortar with pestle along with addition of grinding medium consisting of sucrose 20 µmol . MgCl2 10 µmol, Tris Hydrochloride buffer 20 µmol (pH 7-8).The resulting mixture is subjected for centrifugation and cells are taken up into a liquid media.

- ✓ The plant tissue is treated with an enzyme known as macerozyme along with 1% potassium dextran sulphate, which causes the digestion of middle lamella there by liberating the cells.

- ✓ The enzyme also causes the weakening of cell wall thus creating the problems associated with osmosis. Hence the cells are provided with osmotic protection with a substance known as osmoticum.

- ✓ Commonly used osmoticums are sorbitol 450-800 mmol/l, KCl 335 mmol/l, Magnesium sulphate 40 mmol/l.

From Callus:

- ✓ Callus preferably friable callus upon agitation in suitable liquid media results in the dissociation of the cells from callus.

- ✓ The agitation will also helps in uniform distribution, breaking up of lumps and gaseous exchange between culture air and atmosphere.

FACTORS AFFECTING SINGLE CELL CULTURE

1. The composition of the medium for the growth of single cell culture is generally more complex than callus and cell suspen-sion culture. For example, Convolvulus cells require a cytokinin and amino acids that are not necessary for the callus culture of that species.

2. Induction of division of single cells using pa-per raft technique indicates that isolated cells get the exact essential nutrient from the callus mass. It has been suggested that the callus mass leaches out the essential nu-trient through plasma membrane of the cells.

3. In case of petri dish plating technique the initial plating cell density is very critical.

IMPORTANCE OF SINGLE CELL CULTURE

Single cell culture technique is very impor-tant for the fundamental and mutation studies and it has a wide industrial application.

1. Single cell culture could be used successfully to obtain single cell clones.

2. Plants could be regenerated from the cal-lus tissue derived from the single cell clones.

3. The occurrence of high degree of spontaneous variability in the cultured tissue and their exploitation through single cell culture are very important in relation to crop im-provement programmes.

4. One of the major problems of mutation breeding in higher plants is the formation of chimeras following the mutagenic treat-ment of multi-cellular organism. In this re-spect single cell culture method are more ef-ficient. Isolated single cells can be handled as a microbial system for the treatment of mutagens and for mutant selection.

In prac-tice, single cells are grown on a medium con-taining the mutagenic compounds and the proliferating cell lines are isolated. The mu-tant nature of the selected cell lines can be confirmed by regenerating the plants and comparing their phenotypes with a normal plant. Many cell lines resistant to ammo acid analogues, antibiotics, herbicides, fun-gal toxins etc. have been selected by this simplest method.

5. Many plants synthesize various important natural compounds in the form of alkaloids, steroids etc. Some of these natural com-pounds are highly medicinally important. Several workers have reported the synthesis of several times higher amounts of alka-loid by cell culture than the alkaloid con-tent in the intact plant. So, from the com-mercial point of view, single cell culture in large-scale could become a valuable tech-nique for industrial production of such im-portant natural compound.

6. Biotransformation means the cellular con-version of an exogenously supplied subs-trate compound not available in the cell or the precursor of a particular cellular com-pound to a new compound or the known compounds in higher amounts.

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