RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF ENGINEERING & TECHNOLOGY

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

Semester: 5th
Sub Name: Plant Biotechnology

LECTURE 2

Dr. NIHARIKA SINGH
Assistant Professor
Dept. of Biotechnology

Flower culture

- Flowers (2days after pollination) are excised, sterilized by immersion in 5% calcium hypochloride, washed with sterilized water.
- Transfer this to culture tubes containing an agar medium.
- Fruits, which develop are smaller than their natural counterpart, size can be increases by supplementing the medium with appropriate combination of growth hormones.

Anther Culture

- Young flower buds are removed from the plant & surface sterilized.
- The anthers are then excised and transferred to an appropriate nutrient medium.
- The plantlet are formed after 4-5 weeks of inoculation.
- Many plantlets are produced from the single anther.

Pollens culture

- Pollen grains are removed from the anther.
- Anthers are placed in a 5ml liquid medium in petri dish.
- Petri dishes containing the pollen grains in the culture media are sealed with parafilm & incubated at 28ºC in dark for 14 days.
- 3-4 weeks may be required to obtain haploid plantlets.

The regenerability of an explant is influenced by several factors:

1. Organ from which it is derived.
2. The physiological state of explant
3. Size of the explant
4. Orientation of the explant on the medium and
5. Its inoculation density.

Estimation of Growth

The growth of cell suspension cultures may be monitored by measurement of one or more of the following parameters:

1. Cell number is the most informative measure of cell growth. This measurement is applicable to only suspension cultures and even there cell aggregates must be treated with pectinase, to dissociate them into single cells before counting the cell number in a haemocytometer

2.Packed cell volume of suspension cultures is easily determined by pipetting a known volume in to a 15 ml graduated centrifuge tube, spinning at 2000 rpm for 5 minutes and reading the volume of cell pellet which is expressed as ml cells/l of culture.

3.Culture fresh and dry weights are the most commonly used measures of growth of both suspension and callus cultures. In case of callus cultures, the cell mass is placed on a preweighed dry filter paper or nylon filter and weighed to determine fresh weight. Cells from a suspension culture are filtered on to a filter paper or nylon filter, washed with distilled water, excess water removed under vacuum and weighed along with the filter; the filter is preweighed in wet condition. For dry weight determination, the cells and the filter are dried in an oven at 60°C for 12 hr and weighed. The filter is preweighed in dry condition. Cell fresh and dry weights may either be expressed as 'per ml or per culture'.

4. Medium conductivity- A Conductivity change of the culture medium is inversely proportional to cell fresh weight.

5. Cell viability- In addition to microscopic examination for protoplast streaming and the presence of an intact nucleus, cell viability may also be assessed by use of vital staining of intact living cells eg. Evan's blue (0.025% w/v) or colored salts (Tetrazolium) or Fluorescein diacetate - which are metabolized in living cells to give fluorescent products.

6. Mitotic Index is the ratio between Number of Nuclei in mitosis and Total number of nuclei examined in the sample

QUIZ