RAMA UNIVERSITY

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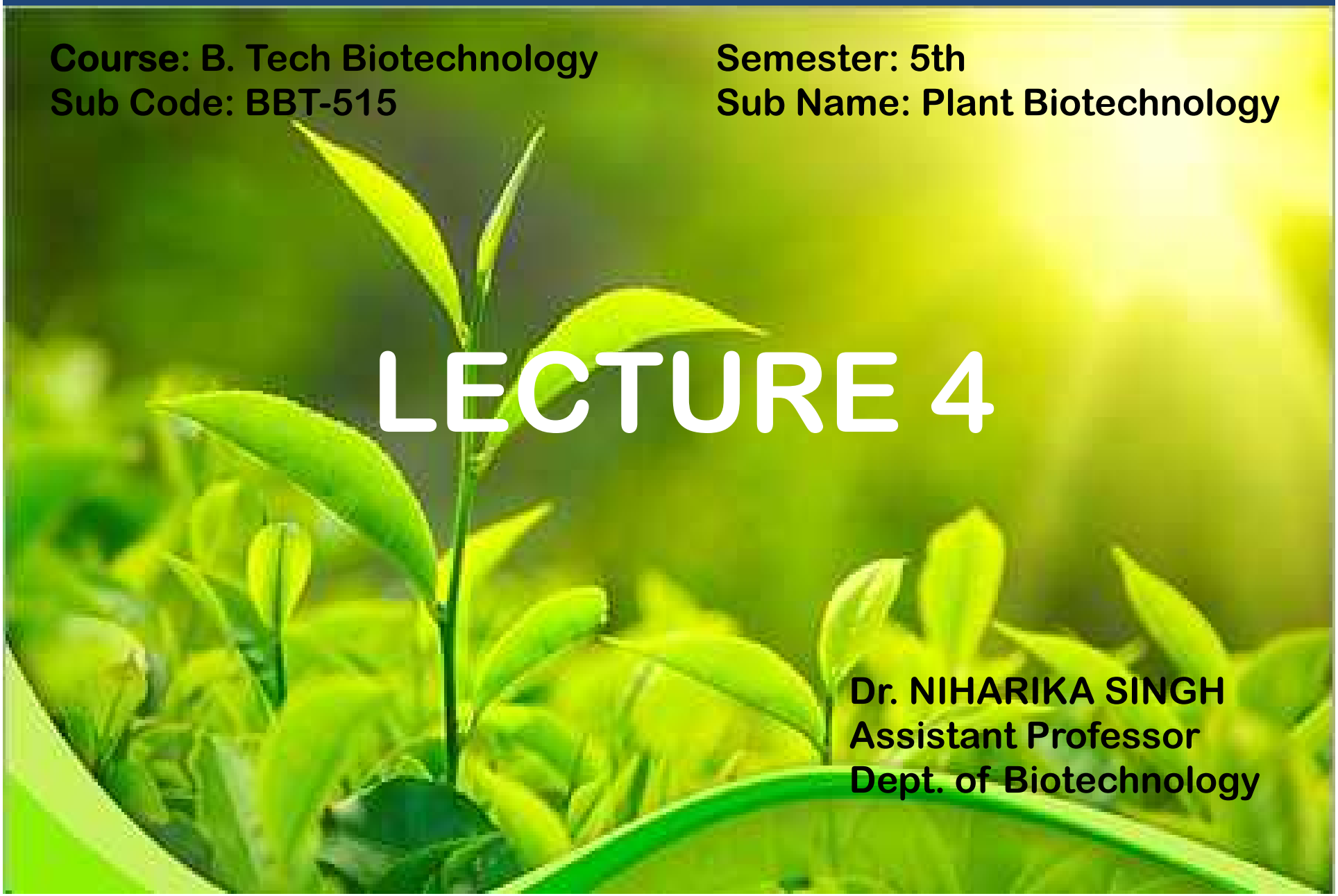
FACULTY OF ENGINEERING & TECHNOLOGY

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

Semester: 5th
Sub Name: Plant Biotechnology



LECTURE 4

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MICRO-CHAMBER TECHNIQUE

1. In this method a drop of the medium carrying a single cell is isolated from suspension cultures, placed on a sterile microscope slide and ringed with sterile mineral oil.

2. A drop of oil is placed on either side of the culture drop and a coverglass placed on each drop.

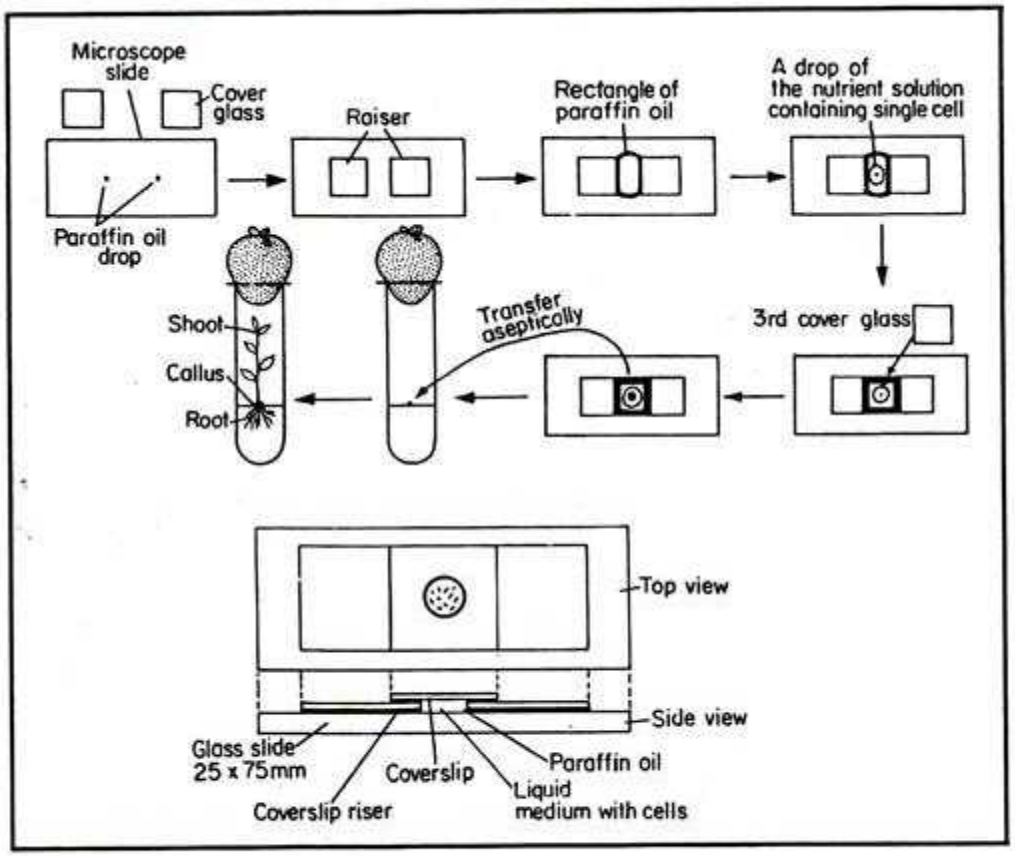
3. A third coverglass is then placed on the culture drop bridging the two coverglasses and forming a microchamber to enclose the single cell aseptically within the mineral oil.

4. The oil prevents water loss from the chamber but permits gaseous exchange.

5. The whole microchamber slide is placed in a petri-dish and incubated.

6. When the cell colony becomes sufficiently large the coverglass is removed and the tissue is transferred to fresh liquid or semi-solid medium.

7. The microchamber technique permits regular observation of the growing and dividing cell



❒ Fig 9.3

Microchamber used to observe the growth of single cells

https://www.biologydiscussion.com/plant-tissues/single-cell-culture/single-cell-culture-5-methods-with-diagram-plant-tissue-culture/14699

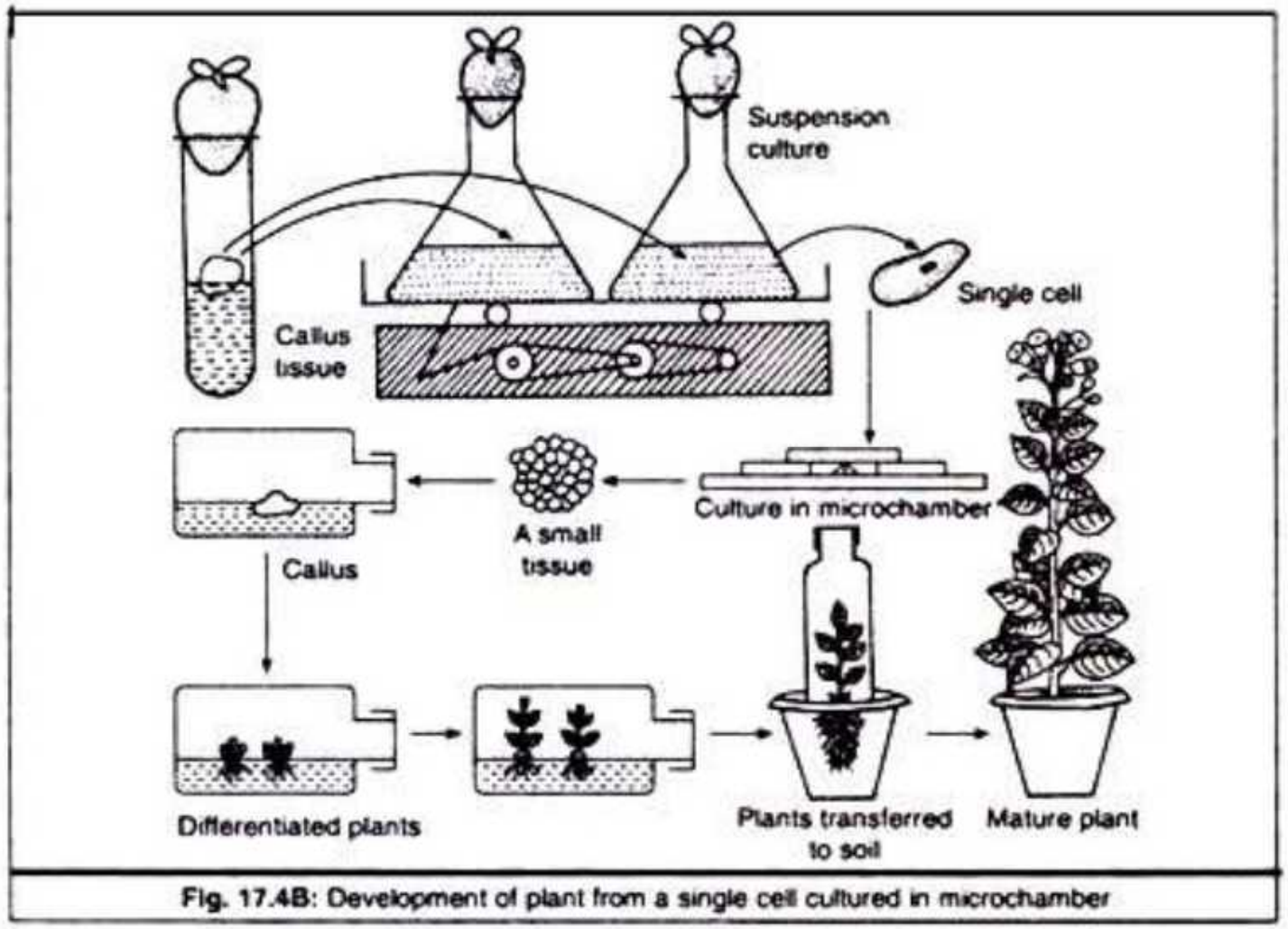


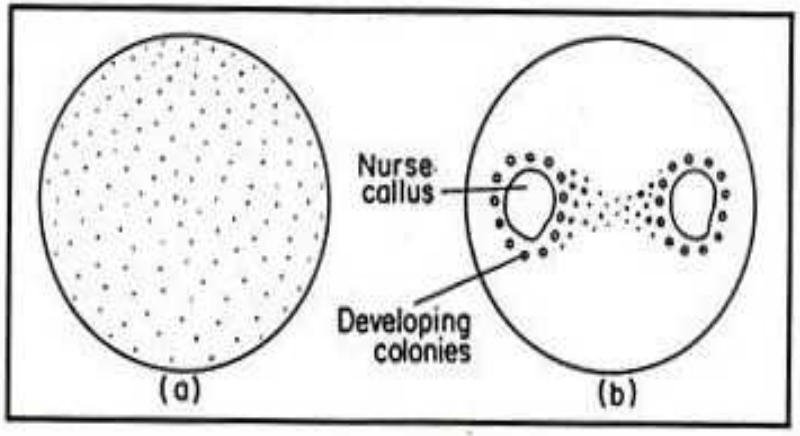
Fig. 17.4B: Development of plant from a single cell cultured in microchamber

https://www.slideshare.net/DrSureshSolleti/cell-culture-78526106

NURSE CALLUS TECHNIQUE

This method is actually a modification of petridish plating method and the paper raft nur-se culture method. In this method, single cells are plated on to agar medium in a petridish as described ear-lier Two or three callus masses (Nurse tissue) derived from the same plant tissue are also em-bedded directly along with the single cells in the same medium.

Here the paper barrier between single cells and the nurse tissue is re-moved. Cells first begin to divide in the regions near the nurse callus indicating that the single cells closer to nurse callus in the solid medium gets the essential growth factors that are lib-erated from the callus mass. The developing colonies growing near to nurse callus also stim-ulate the division and colony formation of other cells.



❒ Fig 9.4

Growth of colonies from a low density cell suspension in the presence of callus tissue. A. Petri dish inoculated with low density suspension of cells—no colonies develop. B. Petri dish inoculated with low density suspension plus nurse callus—colonies grow near to nurse calluses only

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QUIZ