RAMA UNIVERSITY

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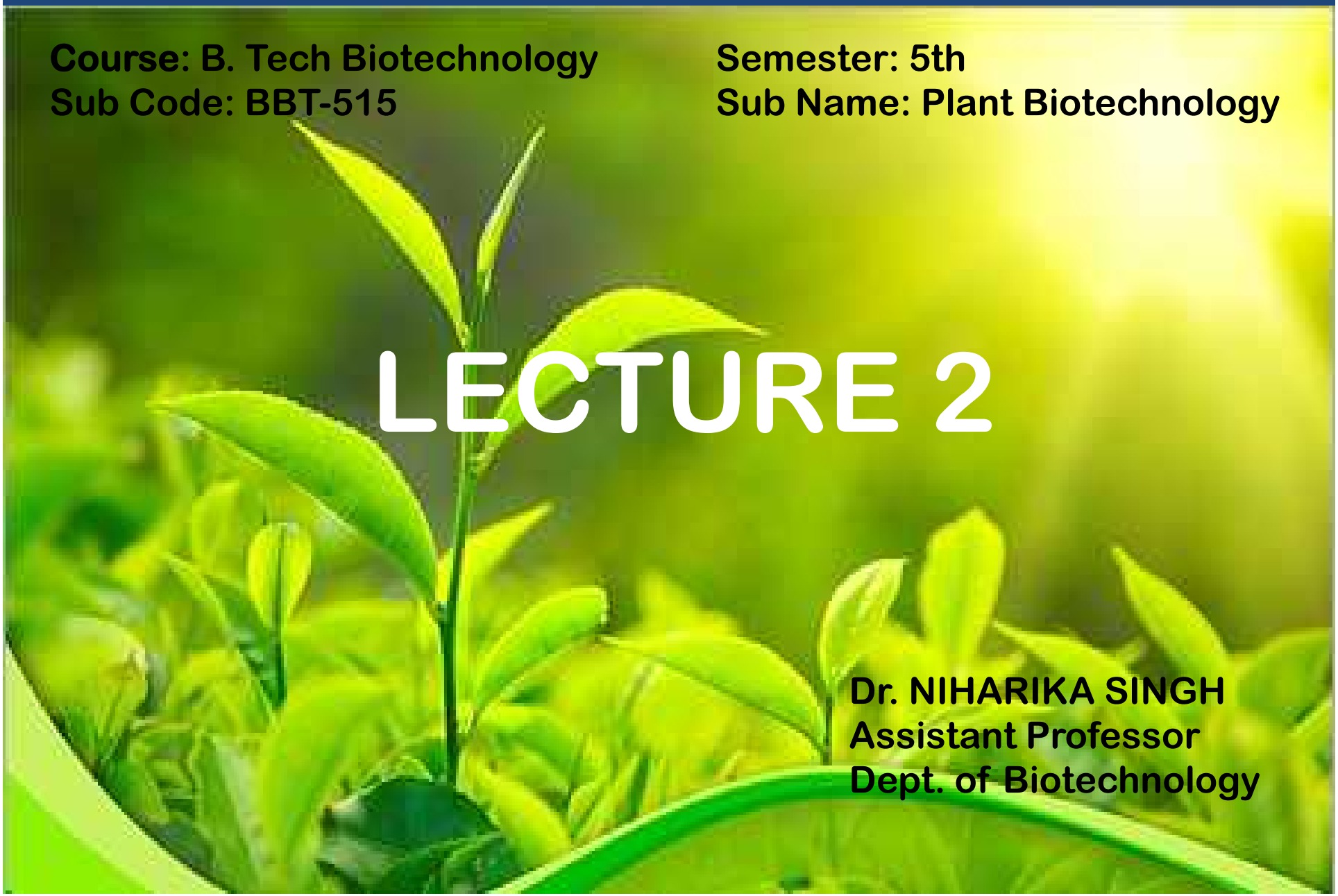
# FACULTY OF ENGINEERING & TECHNOLOGY

**Dr. NIHARIKA SINGH**
**Assistant Professor**
**Dept. of Biotechnology**

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

Semester: 5th
Sub Name: Plant Biotechnology



# LECTURE 2

Dr. NIHARIKA SINGH
Assistant Professor
Dept. of Biotechnology

# DIFFERENT METHODS OF CULTURING SINGLE CELLS

1. The Paper Raft Nurse Technique
2. The Petri Dish Platting Technique
3. The Micro Chamber Technique
4. The Nurse Callus Technique
5. The Micro Droplet Technique

# PAPER RAFT NURSE TECHNIQUE

1. Single cells are isolated from suspension cultures or a friable callus with the help of a micropipette or micro-spatula.
2. Few days before cell isolation, sterile 8 mm x 8 mm squares of filter paper are placed aseptically on the upper surface of the actively growing callus tissue of the same or different species.
3. The filter paper will be wetted by soaking the water and nutrient from the callus tissue.

4. The isolated single cell is placed aseptically on the wet filter paper raft.

5. The whole culture system is incubated under 16 hrs. cool white light (3,000 lux) or under continuous darkness at 25° C.

6. The single cell divides and re-divides and ultimately forms a small cell colony. When the cell colony reaches a suitable size, it is transferred to fresh medium where it gives rise to the single cell clone

## Summary

The callus tissue, on which the single cell is growing, is called the nurse tissue. Actually the callus tissue supplies the cell with not only the nutrients from the culture medium but some-thing more that is critical for cell division. The single cell absorbs nutrients through filter paper. The nutrients actually diffuse upward from cul-ture medium through callus tissue and filter pa-per to the single cell. A callus tissue originating from a single cell is known as a single cell clone.

- An isolated cell which generally fails to divide when plated directly on the medium used for callus cultures is able to divide under the nursing effect of the callus.



- Apparently, the callus supplies the cell with not only the nutrients from the culture medium but something more that is critical for cell division.The cell division factor(s) can diffuse through the filter paper.

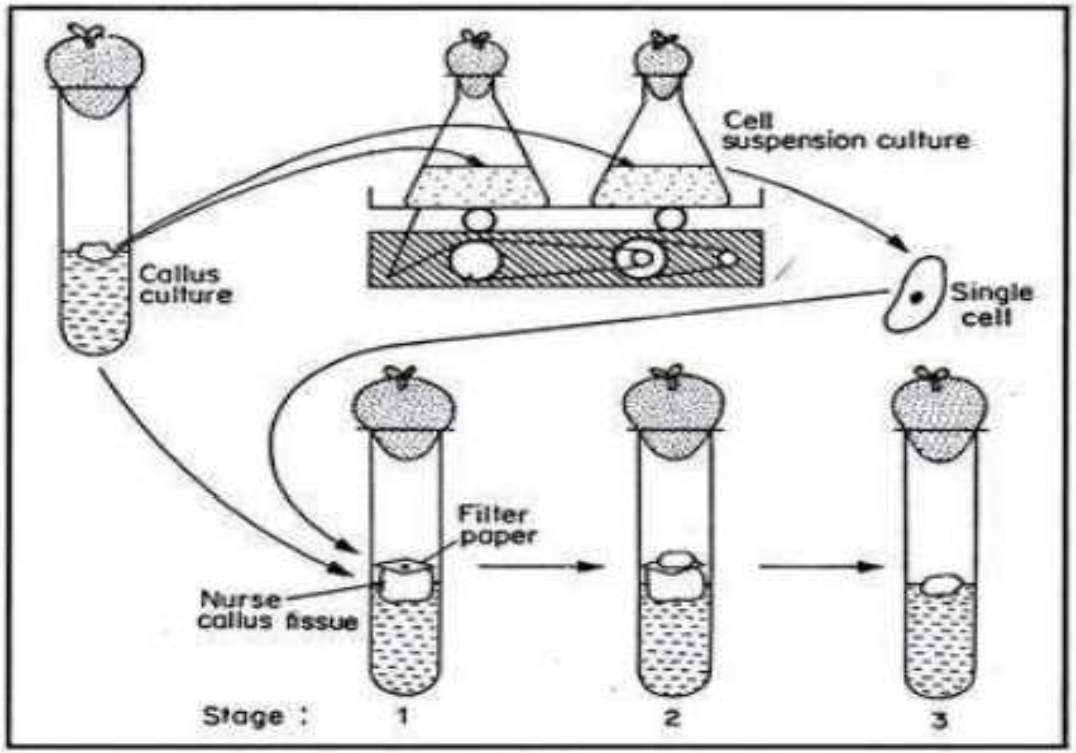



Fig 9.1

**Growth of single cells using a 'nurse' technique. Stage 1 : a single cell taken from a friable callus is placed on upper surface of filter paper which is in contact with nurse callus. Stage 2 : the single cell divides and daughter cells proliferate to form colony. Stage 3 : when colony reaches a suitable size it is transferred to fresh medium where it gives rise to a single cell clone.**

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

# QUIZ