

Course code	Course Title	C	H	I	E	T
17P4BME3	BIOTECHNOLOGY	5	6	25	75	100

Objectives:

1. To impart current knowledge with recent developments in recombinant DNA technology and crop improvement of biotechnology.
2. To impetus students to give training to inculcate advanced technical skills in Plant Tissue Culture and Molecular Cloning experiments.

Learning outcome:

The significance of the course is to emphasize the students to apply the explored knowledge and skills in applied fields of biotechnology.

Unit I (15 hr)

An introduction and a brief history- Origin and Definition- Scope- Inter and Multidisciplinary approaches and avenues of biotechnology - important areas- National and International Institutions.

Plant tissue culture- Concept of Cellular Totipotency, Nutritional requirements- Media- Lab design- Cell Suspension Culture and Biotransformation- Callus Culture.

Unit II (20 hr)

Micropropagation, stages, pros and cons- Production of virus free plants- Organogenesis- Somatic embryogenesis and embryoids production- Somaclonal variations- Somatic hybridization- Anther Culture and Haploid production- Synthetic Seeds- Cryopreservation.

Unit III (20 hr)

Recombinant DNA technology- Molecular tools- Nomenclature and Characteristics of Restriction Enzymes, DNA Polymerase and DNA Ligases- Cloning Vectors Plasmids, pBR 322, and Blue script vectors- M13 vectors and Lambda vectors- YACs.

Genomic Library and cDNA Construction- Cloning of *Insulin* gene in *E. coli*- Forensic Sciences- Antisense RNA technology- Plant edible vaccines- Plantibodies- Biodegradable plastics- Gene knockouts and Gene therapy.

Unit IV (15 hr)

Cloning Methodologies, Insertion of Foreign DNA into Host Cells; Transformation- Gene isolation and characterization-Thermo Cycler and Principle reactions of PCR, Thermostable enzymes- PCR and its types, Significances- RAPD & RFLP- Primer Designing- Probe preparation and Radioactive DNA labeling - Southern, Northern and Western blotting.

Unit V (20 hr)

Biology of *Agrobacterium* species- Basis for Crown gall formation- Ti Plasmid and Ri Plasmid features- Structure of Transferred DNA- Process of T-DNA transfer, integration and expression- Disarming of *Agrobacterium*- Construction of Co-integrate vectors and salient features of Binary vectors- Use of 35S and Ubiquitin promoters, Genetic markers, Reporter genes.

Production of Transgenic plants for pest resistance (*Bt* genes) - Fungal resistance (Chitinase genes) and Herbicide resistance (*ppt* genes). Physical methods of gene delivery, Particle bombardment- Metabolic Engineering (Golden rice) - Gene Silencing and Targeting- Bioethics and Bio-safety of GM crops. Intellectual Property Rights, Patents– GATT and TRIPs.

Reference:

1. Slater, A., Scott N.W. and Fowler, M.R. 2003. Plant Biotechnology, The Genetic Manipulation of Plants. Oxford University Press, New York.
2. Gupta, P.K.1999. Elements of Biotechnology. Rastogi Publishers, India.
3. Chawla, H.S.2000. Introduction to plant biotechnology. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.
4. Gamborg, O.L and Phillips, G.C. 2005. Plant Cell Tissue & Organ Culture. Narosa Publishing House, New Delhi.
5. Kalyan Kumar, D.2008. An Intoduction to Plant Tissue Culture. New Central Book Agency, Kolkata.
6. Old, R.W and Primrose, S.B .1985. Principles of Gene Manipulation-An introduction to genetic engineering. Blackwell Scientific Publication. London.
7. Brown, T.A.2001. Gene cloning and DNA analysis. Blackwell Science Ltd. USA.
8. Reece, R.J.2004. Analysis of Genes and Genomes. John Wiley & Sons, Ltd. UK.
9. Citovsky *et al.*, 2004. *Agrobacterium* T-DNA integration molecules and models- A review. Trends in Genetics Vol. 20(8).
10. <https://WWW.bio.org>
11. <https://WWW.ncbi.nlm.nih.gov>

Practicals:

1. Isolation of Chromosomal DNA (Lysis method) & Plasmid DNA (Minipreparation method) from bacteria
2. Extraction of total genomic DNA from Plants (CTAB method).
3. Agarose Gel Electrophoresis & Staining, Visualization of DNA.
4. Transfer of pUC in to *E. coli* and selection of blue and white colonies on X-gal IPTG substrate.
5. Demonstration of Triparental mating and Cloning experiments
6. Medium preparation-Surface Sterilization and Explants Preparation- Inoculation

7. Micropropagation (Nodal and Shoot tips), Callus Culture & Somatic Embryogenesis using carrot explants.
8. Cell Suspension Cultures using Friable callus
9. Demonstration- Protoplast isolation(physical and enzymatic) and *in- vitro* cultures
10. Demonstration -Synthetic seeds production *in-vitro*
11. *Agrobacterium*- mediated genetic transformation of tobacco leaf disc (Agroinfection- Co-cultivation- GUS Histochemical assay- Molecular Characterization)
12. Demonstration of PCR, RAPD, RFLP & Blotting techniques.
13. Vector genomic maps: pBR322,pUC18, pOK 233, pCAMBIA 2300, 2301 & 1301)