



GUJARAT TECHNOLOGICAL UNIVERSITY

Bachelor of Engineering

Subject Code: 3170414

Tissue culture

7th Semester

Type of course: B.E. (Biotechnology)

Prerequisite: Basic Concepts of Transport Phenomena (Mass transfer and heat transfer) and Reaction kinetics for bioreactor.

Rationale:

1. At the end of this course the students will learn and systematically analyze the complexities defining regulation of various metabolic pathways. They will be able to design and learn strain-engineering strategies to alter cellular behaviour, metabolic flux, and product formation. They will also appreciate the vast industrial applications of metabolic engineering in the field of medicine, energy and environment.
2. The course intends to teach students about the traditional and new approaches used in crop improvement. This includes the conventional and marker assisted breeding approaches, plant tissue culture and its importance in generation of transgenic plants, methods to express large quantities of important proteins and modern tools for site directed mutagenesis in plants.

Teaching and Examination Scheme:

| Teaching Scheme | | | Credits C | Examination Marks | | | | Total Marks |
|-----------------|---|---|--------------|-------------------|--------|-----------------|--------|----------------|
| L | T | P | | Theory Marks | | Practical Marks | | |
| | | | | ESE (E) | PA (M) | ESE (V) | PA (I) | |
| 4 | 0 | 0 | 4 | 70 | 30 | 0 | 0 | 100 |

Course Contents:

| Sr. No. | Topics | Teaching Hours |
|---------|---|----------------|
| 1 | Animal Cell Culture: History of Animal Cell Culture, Characteristics of animal cell, metabolism, regulation and nutritional requirements, Culture Media and Growth Conditions, Development of Primary Culture and Cell Lines, Suspension Culture, Characterization and maintenance of cell lines, Cryopreservation, Common Cell Culture Contaminants, Marker Gene Characterization, Transfection and Transformation of Cells. | 8 |
| 2 | Growth and Scale Up: Need for scaling-up of cells for vaccine or antigen or | 5 |



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| | pharmaceutical protein production, Hybridoma Technology, Cell culture reactors, Scale-Up in suspension and monolayer cultures, Factors affecting cell growth, Growth Monitoring, Mass Transfer. | |
| 3 | Animal Biotechnology: Concept of transgenic animals, Methods of transgene delivery, Microinjection of recombinant DNA into fertilized eggs/stem cells, Animal Pharming, Organ Culture, Regenerative Medicine, Human Embryonic Stem Cell research | 8 |
| 4 | Crop Improvement: The need of crop improvement. Conventional methods of crop improvement, selection, mutation, polyploidy and clonal selection. Green revolution in India. Introduction to marker assisted breeding and selection | 4 |
| 5 | Plant tissue culture: History of plant tissue culture, plasticity and totipotency. Laboratory setup for a typical plant tissue culture facility. Sterilization methods used in plant tissue culture. Types of nutrient media and plant growth regulators in plant regeneration. Pathways for in vitro regeneration: organogenesis, somatic and gametic embryogenesis; protoplast isolation, culture and regeneration; culture of other explants, somatic hybridization; Haploid and triploid production and their applications. Genetic fidelity of plants raised through tissue culture. Applications of micro-propagation, meristem culture, embryo rescue, somaclonal and androclonal variations. Application of tissue culture for crop improvement. Methods for Plant Conservation, Cryopreservation, synseed production. Production of bio active secondary metabolites by plant tissue culture. | 8 |
| 6 | Principles and methods of genetic transformation: Introduction to Agrobacterium biology and biotechnology. Mechanism of T-DNA transfer to plants and Agro infection. A. rhizogenes and its application. Transplastomics and its utility. Methods for direct gene transfer, Marker and reporter genes; Promoters used in plant vectors. Plant viral vectors. Molecular techniques for analysis of transgenics (copy number, transgene stability, silencing; segregation). Marker-free transgenics and environmental, social and legal issues associated with transgenic plants. Case studies for genetic engineering in plants for traits of agronomic value, biotic, abiotic stresses and herbicide tolerance. | 8 |
| 7 | Molecular Farming: Transgenic crops for production of antibodies, viral antigens and peptide hormones in plants, Edible vaccines and Nutraceuticals. Plant Biotechnology for biofuels | 5 |



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| 8 | Genome Editing: The history of targeted mutations in plants. Use of ZFNs and TALENs as early tools for genome editing. Discovery of CRISPR-Cas system and its applications. Recent innovations in the technology and case studies where CRISPRCas has been used for plant improvement. | 6 |
|---|--|---|

Suggested Specification table with marks (Theory):

| Distribution of Theory Marks | | | | | |
|------------------------------|---------|---------|---------|---------|---------|
| R Level | U Level | A Level | N Level | E Level | C Level |
| 10 | 14 | 16 | 10 | 10 | 10 |

Legends: R= Remembrance; U= Understanding; A= Application; N = Analyze; E = Evaluate; C = Create and above Levels (**Revised Bloom's Taxonomy**)

Note: This specification table shall be treated as a general guideline for students and teachers. The actual distribution of marks in the question paper may vary slightly from above table.

Text Books:

1. Culture of Animal Cells by R.I. Freshney.
2. Animal Cell Biotechnology 2007, Humana Press by Portner R.
3. Basic Cell Culture Second Edition, Oxford University Press by Davis, J.M.
4. Principles of Plant Genetics and Breeding by George Acquaah 2007. Blackwell Publishing.
5. An introduction to Plant Tissue culture by MK Razdan. M.K. 2003. Oxford & IBH Publishing Co, New Delhi, 2003.
6. Plant Biotechnology: An Introduction to Genetic Engineering by Adrian Slater, Nigel W. Scott, Mark R. Fowler. Oxford University Press, 2008.
7. Biochemistry & Molecular Biology of Plants. Bob Buchanan, Wilhelm Gruissem, Russell Jones. John Wiley & Sons, 2002.

Course Outcomes:

| Sr. No. | CO statement | Marks % weightage |
|---------|---|-------------------|
| CO-1 | learn the techniques of animal cell culture | 15 |
| CO-2 | Strategies for scaling up animal cell cultures and its application to human disease and welfare | 25 |
| CO-3 | Learn about biotechnological application of plants | 60 |



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List of Practicals:

1. To study the various aspects of laboratory organization for plant tissue culture.
2. To collect various explants for their manipulation and surface sterilization.
3. To study the aseptic handling of explants and performing assay to identify common contaminants.
4. Preparation of Murashige-Skoog (MS) media
5. To perform induction of callus in *Solenum tuberosum*.
6. To perform callus induction in *Daucus carrota*.
7. To perform callus induction in *Zea mays*.
8. Initiation of anther culture and haploid production.
9. Establishment of primary culture of chicken liver hepatocytes.
10. Establishment of primary culture of chicken cardiac myocytes.

Open Ended Problem:

Students are free to select any area of science and technology based on Animal & Plant Biotechnology applications to define Projects. Some suggested projects are listed below:

1. To establish a desired animal cell-line
2. To analyze the difficulties faced in establishing new culture of animal cells & tissues.
3. To establish different types of cultures of plant cells and tissues.
4. To perform optimization experiments for micropropagation of explants.

List of additional study experiments:

1. Animal Cell Culture Biosafety and Ethical Concerns
2. Preparation of reagents and media for cell culture
3. Cell counting and plating
4. Maintenance of Adherent (Monolayer) and Suspension Cell culture
5. Cryopreservation of cell lines
6. Determination of common cell culture contaminants
7. Cell Viability Assay (MTT reagent)
8. Cell Cytotoxicity Assay (Trypan Blue Assay)
9. Transfection in mammalian Cell Lines
10. Preparation and sterilization of standard tissue culture media.
11. Sterilization of explants and generation of undifferentiated mass of cells. Regeneration of plants



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from undifferentiated cells.

12. Preparation of competent cells, transformation and colony PCR for confirmation of transformation in *Agrobacterium tumefaciens*.
13. *Agrobacterium* mediated transformation of plants.
14. Selection and screening of transgenic plants.
15. Evaluation of a transgenic phenotype (viz., Herbicide resistance) under contained conditions.
16. Analysis of crude extracts from medicinal plants using HPLC.
17. Use of microsatellite markers for DNA fingerprinting.

List of Open Source Software/ learning website:

Students can refer to video lectures available on the websites including NPTEL/ SWAYAM. Students can refer to the CDs which are available with some reference books. Students can refer some virtual labs for relevant practical / laboratory practices.