## M.Sc. (Agri) – Plant Molecular Biology and Biotechnology (GGPB21)

### 1. Programme Specific Outcome

**To provide**

1. Design, conduct experiments, analyze and interpret data for investigating problems in Biotechnological manipulation.
2. Gaining insight into the most significant molecular methods used today to expand our understanding of biology by utilizing modern equipments and instruments .
3. Comprehensive understanding in formulation and design Tissue culture protocols based on active principle production and confirmation for different agriculturally economic plants and accessibility of different agricultural plant species for genetic transformation
4. Understanding the physiological processes to understand source sink relationship in different groups of plants and also hormonal, environmental and stress physiology in crop plants
5. Suggest and outline solution to theoretical and experimental problems in Genomics and Proteomics fields.
6. Justify the impact of biotechnological innovations on environment and their implementation for finding sustainable solution to issues pertaining to environment agriculture.
7. Entrepreneurship ventures such as Establishment of Environmental units, crop production units, consultancy and training centers ,national bio-resource development firms

### COURSE OBJECTIVES AND OUTCOMES

### GGPB21 611 - PRINCIPLES OF BIOTECHNOLOGY (2+1)

**Learning Objective**

- To familiarize the students with the fundamental principles of biotechnology, various developments and their applications and scope.

**Theory**

**UNIT I : DNA science**

History, scope and importance of biotechnology - Nucleic acid structure and its function-Modes of DNA replication- Genetic code - Central dogma of life – Transcription - Translation.

**UNIT II : rDNA technology**

Recombinant DNA technology - DNA modifying enzymes –Cloning Vectors – Plasmids-cosmids-phagemids-Shuttlevectors-BAC-YAC-HAC-applications – Gene libraries – Genomic DNA and cDNA; Applications - Nucleic acid hybridization; Methods and Uses, Gene cloning and its applications in basic and applied research.

**UNIT III : Molecular markers & genome editing**

Variants of PCR, Molecular markers-PCR and Restriction based markers-applications of molecular markers- DNA sequencing- Sanger-Gilbert techniques-Omics-Genomics- transcriptomics-proteomics and phenomics – Genome editing technologies – Meganucleases, ZFM, TALEN, CRISPR Cas9, MAGE – Applications and Limitations.

**UNIT IV : Gene transfer & M.A.S.**

Gene transfer methods – *Agrobacterium* - mediated gene transfer, direct gene transfer, gene silencing – Principles of QTL and Marker Assisted Selection (MAS) – Achievements - Transgenic plants – Achievements – Current trends.

**UNIT V : IPR in biotechnology**

Intellectual property rights (IPR) in biotechnology. Bio-safety and bioethics issues - Public perception of biotechnology - Application of biotechnology in Agriculture, Medicine, Animal husbandry, Environmental remediation, Energy production and Forensics.

**Practical**

Gel electrophoresis techniques-Restriction enzyme digestion, ligation, transformation and screening of transformants- PCR and molecular marker analysis-Plant tissue culture: media preparation, cell and explant culture-regeneration and transformation.

**Theory schedule**

1. History, scope and importance
2. Nucleic acid structure and its function
3. Modes of DNA replication
4. Central dogma of life,
5. Genetic code & Transcription
6. Translation
7. DNA modifying enzymes
8. Cloning vectors
9. Artificial chromosomes as cloning vectors
10. Gene libraries
11. CDNA libraries
12. Nucleic acid hybridization
13. Plant cell and tissue culture techniques and their applications.

14. Molecular markers and their applications
15. PCR amplification and variants
16. DNA sequencing methods
17. **Mid-semester examination**
18. Applications of gene cloning in basic and applied research
19. Genetic engineering and transgenics; Genomics, Transcriptomics
20. Proteomics and Phenomics
21. Genome editing tools, applications and limitations.
22. Agro bacterium-mediated gene transfer
23. Direct gene transfer,
24. Introduction to QTL
25. MAS
26. Transgenic plants: insect resistance,
27. Genetic engineering for virus resistance,
28. Genetic engineering for to fungal / bacterial diseases,
29. Genetic engineering for longer shelf life
30. Intellectual property rights in biotechnology
31. General application of biotechnology in Agriculture
32. Public perception, Bio-safety and bioethics issues
33. Energy production and Forensics
34. Applications of biotechnology

**Practical schedule**

1. Laboratory equipment handling and safety guidelines
2. Preparation of buffers, reagents and media etc
3. Isolation and characterization of genomic DNA for *E.coli*
4. Cutting of DNA and cleanup of DNA for ligation
5. Demonstration of PCR
6. Analysis of amplified product
7. Minipreparation & digestion of plasmid DNA
8. Demonstration of DNA sequencing
9. Casting sequencing gel
10. Gel electrophoresis
11. Autoradiography
12. Agrobacterium-mediated gene transfer
13. Direct gene transfer
14. Demonstration of RFLP, RAPD and AFLP
15. Plant tissue culture media preparation
16. Micropropagation and its stages
17. **Practical examination**

**References**


1. Brown TA. Gene Cloning and DNA Analysis. 2006. 5th Ed. Blackwell Publishing.
2. Brown CM, Campbell I and Priest FG. 2005. Introduction to Biotechnology. Panima Publications
3. Bhojwani and Dantu, 2013. Plant tissue culture: An introductory text, Springer, New Delhi.
4. Chawla, H.S. 2008. Introduction to Plant Biotechnology, 3rd Ed. Oxford IBH, India.
5. Dale, J.W. and Von Schantz, M. 2002. From Genes to Genomes: Concepts and Applications of DNA Technology. John Wiley & Sons, New york, USA.
6. Nigel W. Scott, Mark R. Fowler and Adrian Slater. 2008. Plant Biotechnology: The genetic manipulation of Plants. 2nd Ed. Oxford University Press.
7. Singh BD. 2012. Biotechnology: Expanding Horizons, 4th Ed. Kalyani Publishers, New Delhi.


**Outcomes**

- Ability to apply the concepts and principles of plant tissue culture techniques on research problems pertinent to crop improvement
- Dissemination of skills on usage of the acquired knowledge on practical biotechnology tools to augment need based research.
- Technical knowhow and exhibition of contemporary knowledge in Biotechnology for economic utilization.
- Compile and interpret results applying tools of biotechnology research.
- Applying learned process to undertake sustainable exploitation of plant and microbial resources in an environmentally-sensitive manner.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
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| CO3 | | | | | | 2 | |
| CO4 | 4 | 3 | | | | | |
| CO5 | | | | | | | 4 |

## GGPB21 612 - FUNDAMENTALS OF MOLECULAR BIOLOGY (2+1)

**Learning Objective**

- To familiarize the student with basic structure and functions of macromolecules in a cell.
- To provide the students about the various cellular process mediated by the macromolecules

**Theory**

**UNIT I : Structure of DNA**

Historical developments of molecular biology- its importance – central dogma of molecular biology – Constituents of a cell - Small Organic molecules – Chemistry and Structure - Carbohydrates, Lipids, Nucleic acids, Proteins - Nucleic acids as genetic material – DNA content – chemistry – Purines and Pyrimidines – nucleosides and nucleotides – structure of DNA and RNA – Primary, secondary and tertiary structure.

**UNIT II : DNA replication**

Non-coding DNA sequence, Extra genic sequence, gene families – DNA Packaging – viral DNA, Bacterial DNA, Eukaryotic DNA, Chromatin organization- Organelle Genome – Mitochondrial genome, Chloroplast genome- DNA replication – features – replication in prokaryotes- initiation, elongation, maturation of Okazaki fragments – Replication in Eukaryotes – Termination and regulation of replication.

**UNIT III : Endonucleases and DNA modification**

DNA modification enzymes – Polymerases, Ligases, Phosphatases, Polynucleotide kinases – DNA degrading enzymes – Nucleases – Endo and Exo nucleases, Restriction endonucleases – Types of DNA Damages and repair – Nucleotide excision repair, base excision repair, mismatch excision repair, double stand break repair – DNA recombination and events.

**UNIT IV : Transcription and post transcription changes**

Transcription in Prokaryotes – Initiation, elongation, termination, regulation – Transcription in Eukaryotes – Promoters of polymerases, transcriptional factors, transcription activators, chromatin and transcription – Post transcriptional events – splicing, RNA editing, processing of mRNA at 3'end and 5' end – production of mature rRNA, tRNA -Translation and post-translational modifications - - Lac operon concept - trp operon.

**UNIT V : Protein synthesis**

Protein synthesis in prokaryotes –Components of protein synthesis – Messenger RNA, transfer RNA, Ribosome – Mechanism – Initiation, elongation, termination – Regulation of protein synthesis – global regulation, mRNA specific regulation – protein folding – protein modifications – glycosylation, attachment of lips and glycolipids, protein phosphorylation – protein degradation – Lysosomal pathway, Ubiquitin-proteasome pathway.

**Practical**

Laboratory safety measure - extraction of proteins - quantification - Isoenzymes - SDS PAGE - Western blotting - Isoelectric focusing - Genomic DNA, total RNA, mRNA extraction - quality and quantity check - Northern blotting- Cell free system and protein synthesis.

**Theory Schedule**

1. Historical developments of molecular biology and its importance
2. Central dogma of molecular biology
3. Constituents of a cell
4. Small Organic molecules
5. Chemistry and Structure of Carbohydrates, Lipids, Nucleic acids, Proteins
6. Nucleic acids as genetic material
7. Structure of DNA and RNA and its properties
8. Non-coding DNA sequence, Extra genic sequence and gene families
9. DNA Packaging for viral DNA and Bacterial DNA
10. Eukaryotic DNA, Chromatin organization
11. Organelle Genome, Mitochondrial genome and Chloroplast genome
12. DNA replication
13. Features and replication in prokaryotes- initiation, elongation, maturation of Okazaki fragments
14. Features and Replication in Eukaryotes – Termination and regulation of replication.
15. DNA modification enzymes like Polymerases, Ligases, Phosphatases, Polynucleotide kinases
16. DNA degrading enzymes like Nucleases, Endo and Exo nucleases.
17. **Mid-semester examination**
18. Restriction endonucleases
19. Types of DNA Damages and repair
20. Nucleotide excision repair, base excision repair, mismatch excision repair, double stand break repair
21. DNA recombination and events.
22. Transcription in Prokaryotes, Initiation, elongation, termination, regulation
23. Transcription in Eukaryotes, Promoters of polymerases, transcriptional factors, transcription activators, chromatin and transcription

24. Post transcriptional events like splicing, RNA editing, processing of mRNA at 3'end and 5' end and production of mature rRNA, tRNA
25. Translation and post-translational modifications
26. Lac operon concept - trp operon, Protein synthesis in prokaryotes .
27. Components of protein synthesis
28. Messenger RNA, transfer RNA, Ribosome
29. Mechanism – Initiation, elongation, termination
30. Regulation of protein synthesis and global regulation, mRNA specific regulation
31. Protein folding and protein modifications
32. Glycosylation, attachment of lips and glycolipids, protein phosphorylation
33. Protein degradation
34. Lysosomal pathway, Ubiquitin-proteasome pathway.

**Practical Schedule**

1. Laboratory safety guidelines.
2. Extraction of proteins.
3. Quantification by Lowry's and Bradford method.
4. Polyacrylamide gel electrophoresis - Isoenzymes.
5. Electrophoretic separation of proteins by SDS - PAGE.
6. Western blotting.
7. Isoelectric focusing - I.
8. Isoelectric focusing - II.
9. Extraction of DNA.
10. Quality and quantity check of the DNA.
11. Extraction of total RNA & Purification of RNA.
12. Northern blotting - I.
13. Northern blotting - II.
14. Cell- free system of protein synthesis - I.
15. Cell- free system of protein synthesis - II.
16. Electrophoresis, staining, destaining and documentation.
17. **Final Practical Examination**.

**References**


1. Benjamin Lewin. 2007. Genes IX. Jones and Bartlett publishers, Inc., 892p Brown, T. A. 2007.
2. Genome 3. Garland Science Publishing. 713p Malacinski, G.M. 2007. Essentials of Molecular
3. Biology **(**IV edn.) Jones and Bartlett Publishers, Inc., 491p Watson, J. D., T. A. Baker, S. P. Bell, A.
4. Gann, M. Levine, R. Losic. 2006. (V edn.) Molecular Biology of the Gene. Pearson Education. 732p.

Campbell, M. K., S. O. Farrel. 2007. Biochemistry. (V edn.) Baba Barkha Nath Printers. Delhi. 689p.


**Outcomes**

- Understand and apply the principles and techniques of Molecular biology.
- Comprehensive understanding on Nucleic acids that provides insight into cellular and molecular mechanisms.
- The knowledge on DNA control mechanism in terms of replication and recombination to design and execute gene manipulation research underlying social and environmental ventures.
- The ability to synthesize, evaluate and understand molecular marker based data.

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| CO4 | | 2 | | | | | |

## GGPB21 613 TECHNIQUES IN MOLECULAR BIOLOGY (2+1)

**Learning Objective**

- To provide hands on training on basic molecular biology techniques
- To provide the knowledge of various technology in field of molecular biology

**Theory**

**UNIT I : Quantification of macro-molecules**

Good lab practices-Preparation of buffers and reagents, Principle of centrifugation-analytical and preparative – Differential Centrifugation-Chromatographic techniques (TLC, Gel Filtration Chromatography, Ion exchange Chromatography, Affinity Chromatography). Electron microscopy-preparation of specimens-TEM and SEM-UV and spectrophotometric techniques

**UNIT II : Gel electrophoresis**

Extraction of Nucleic acid-CTAB-Delaporta- Electrophoreis of nucleic acids- agarose gel electrophoresis, DNA sequencing gels, pulse field gel electrophoresis. Electrophoresis of proteins- SDS-PAGE-Native gels, gradient gels, isoelectric focusing, 2-D PAGE. Cellulose acetate electrophoresis, Detection, estimation and recovery of proteins in gels, Autoradiography.

**UNIT III : Molecular techniques**

PCR- principle and applications-Primer designing-Modified PCR techniques-Reverse transcriptase PCR and Real time PCR. DNA Sequencing- chemical and enzymatic methods. Blotting techniques-Southern, Northern, Western and alternative blotting techniques. Preparation of probes. DNA fingerprinting.

**UNIT IV: Gene cloning**

Recombinant DNA technology-YAC, BAC and cosmid library construction-Genomic and cDNA libraries–screening using heterologous and homologous probes - differential screening – expression library screening-functional complementation

**UNIT V : Immunological techniques**

Dot blot analysis-ELISA- Immunoelectrophoresis, RIA, immunoblotting.

**Practical**

Centrifugation techniques- Chromatography-Electron microscopy-Electrophoresis of DNA and proteins-PCR- Primer designing-blotting techniques- DNA sequencing methods- DNA fingerprinting- YAC, BAC libraries- cDNA libraries–screening using heterologous and homologous probes - screening-functional complementation

**Lecture schedule**

**Theory**

1. Good lab practices
2. Preparation of buffers and reagents,
3. Principle of centrifugation
4. Analytical and preparative centrifugation
5. Principle involved in Chromatography
6. UV and Nano drop spectrophotometer
7. Electron microscopy-preparation of specimens-TEM and SEM.
8. Agarose gel electrophoresis
9. Electrophoreis of nucleic acids
10. DNA sequencing gels, pulse field gel electrophoresis.
11. Electrophoresis of proteins- SDS-PAGE.
12. Native gels, gradient gels, isoelectric focusing, 2-D PAGE.
13. Cellulose acetate electrophoresis.
14. Detection, estimation and recovery of proteins in gels,
15. Autoradiography
16. PCR- principle and applications.
17. **Mid- semester examination**
18. Primer designing
19. Modified PCR techniques
20. Reverse transcriptase PCR and Real time PCR
21. DNA Sequencing- chemical and enzymatic methods.
22. Blotting techniques: Southern
23. Northern blotting techniques
24. Western and alternative blotting techniques.
25. DNA fingerprinting.
26. Recombinant DNA technology
27. YAC and BAC library construction
28. Cosmid library construction
29. Genomic and cDNA libraries
30. Screening using heterologous and homologous probes
31. Differential screening
32. Expression library screening
33. Functional complementation
34. Immunoelectrophoresis., RIA, dot blot, Immunoblotting

**Practical schedule**

1. Preparation of stock solutions and reagents.
2. Extraction of plant genomic DNA by Dellaporta method.
3. Extraction of plant genomic DNA by CTAB method.
4. Centrifugation technique
5. Chromatography technique
6. UV- spectrophotometer
7. Restriction digestion of DNA.
8. Southern transfer, labelling of DNA, Southern hybridization.
9. Northern and western blotting procedure
10. Autoradiography.
11. Amplification of DNA with thermocycler with random primers.
12. Analysis of PCR products through agarose gel eletrophoresis and gel scanning.
13. Primer designing

14. DNA sequencing.
15. Genomic library construction
16. ELISA
17. **Final Practical Examination**.

**Suggested Readings**


1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K. 2002. Short Protocols in Molecular Biology. John Wiley, USA.
2. Sambrook J, Russell DW. 2001. Molecular Cloning: A laboratory manual, 3rd edition, Cold Spring Harbor Laboratory Press, New York.
3. Joseph Sambrook and David Russell. 2006. The Condensed Protocols From Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
4. Old, R.W. and Primrose, S.B. 1989. Principles of gene manipulation. An introduction to genetic engineering, 4th edition, Blackwell Scientific Publications, Oxford, England.
5. Good man, R.N., Z.Kiraly and K.R. Wood. 1986. The Biochemistry and physiology of Plant Diseases, Univ. of Missouri Press, Columbia, M.O.


**Outcomes**

- Conceptual knowledge on creation of new genetic variation and significance of genetically modified organisms.
- Gain insight into the most significant molecular methods used today to expand our understanding of biology.
- The knowledge required to design, execute, and analyze the results of Molecular markers in gene manipulation systems.
- Prepares students for further education employment in teaching, basic research or agricultural professions.
- Present hypotheses and select, adapt and conduct molecular and cell-based research program to either confirm or reject the hypotheses.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
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| CO5 | | | | | | | 2 |

## GGPB21 621 MOLECULAR CELL BIOLOGY (2+1)

**Learning Objective**

- To familiarize the students with the cell biology at molecular level
- To enrich the students with genomic organization of organelles in the cell

**Theory**

**UNIT I : Cell structure & cell organelles**

Cell theory, Structure of prokaryotic and eukaryotic cells- Similarities and distinction between plant and animal cells; Structure and function of major organelles: Nucleus, Chloroplasts, Mitochondria, Ribosomes, Lysosomes, Peroxisomes, Endoplasmic reticulum, Microbodies, Golgi apparatus, Vacuoles, etc.

**UNIT II : Cell physiology**

Cell division and regulation of cell cycle; Membrane transport; Transport of water, ion and biomolecules; Diffusion, osmosis, ion channels, active transport, ion pumps, mechanism of protein sorting and regulation of intracellular transport, cell communication and cell signaling ; cell junctions- gap junctions, extracellular matrix, integrins, actin filaments, actin-binding proteins, fibroin and muscle, Protein targeting.

**UNIT III : Genome organisation**

Organization of bacterial genome-Plant genome-Choloroplast genome-mitochondrial genome-Structure of eukaryotic chromosomes; Role of nuclear matrix in chromosome organization and function; Matrix binding proteins; Heterochromatin and Euchromatin Genome organization of *Arabidopsis thaliana*

**UNIT IV : Microbial genetics**

Genome size and evolutionary complexity; Microbial genetics: plasmids, conjugation, transduction and transformation in bacteria. Bacteriophages-Lytic and lysogenic phases of phage, Genetic recombination and its molecular mechanism.

**UNIT V : Cell signaling in plants**

Cellular responses to environmental signals in plants and animals: mechanisms of signal transduction (Rhizobium legume symbiosis, steroids, protein/peptides).

**Practical**

Microscopy - light, fluorescent, phase contrast - electron and scanning microscopes. Fractionation of tissues and cells - Methods of separating whole cells - Quantification of cells - Cellular micrometry - Microscopic preparations - fixatives, differential action of fixatives, pretreatment of specimen, staining procedures, microtomy. Mitosis and meiosis - identification stages.

**Lecture Schedule**

**Theory**

1. Cell theory
2. Structure of prokaryotic
3. structure of eukaryotic cells
4. Similarities and distinction between plant and animal cells
5. Structure and function of major organelles
6. Nucleus Chloroplasts, Mitochondria, Ribosomes
7. Lysosomes, Peroxisomes
8. Endoplasmic reticulum
9. Microbodies, Golgi apparatus, Vacuoles
10. Cell division
11. regulation of cell cycle
12. Membrane transport
13. Transport of water molecules-Aquaporin
14. Transport of ion
15. Transport of biomolecules
16. Diffusion, osmosis, ion channels, active transport, ion pumps, mechanism of protein sorting and regulation of intracellular transport, cell communication and cell signaling
17. **Mid Semester examinations**
18. Cell junctions- gap junctions, extracellular matrix, integrins, actin filaments, actin-binding proteins, fibroin and muscle; Protein targeting.
19. Organization of bacterial genome
20. Plant genome-Choloroplast genome
21. Plant genome-Mitochondrial genome
22. Genome organization of *Arabidopsis thaliana*
23. Structure of eukaryotic chromosomes
24. Role of nuclear matrix in chromosome organization and function
25. Matrix binding proteins
26. Heterochromatin and Euchromatin
27. Genome size and evolutionary complexity
28. Microbial genetics: plasmids, conjugation
29. transduction and transformation in bacteria
30. Bacteriophages and their genetic systems
31. Lytic and lysogenic phases of l phage
32. Genetic recombination and its molecular mechanism
33. Cellular responses to environmental signals in plants
34. Mechanisms of signal transduction (Rhizobium legume symbiosis, steroids, protein/peptides).

**Practical**

1. Cell staining techniques
2. Microscopy: Bright field and dark field
3. Phase contrast Microscopy
4. Fluorescence Microscopy
5. Electron microscopy
6. Microtomy & Histochemical techniques
7. Demonstration of Mitosis
8. Demonstration of Meiosis
9. Bacterial conjugation,
10. Bacterial transduction and transformation,
11. Isolation of bacterial genome
12. Nuclear genome isolation
13. Chloroplast genome isolation
14. Mitochondrial genome isolation
15. Agarose gel electrophoresis
16. Gel-documentation –Autoradiography
17. **Final practical examination**

**Suggested Readings**


1. Gupta PK. 2003. Cell and Molecular Biology. 2nd Ed. Rastogi Publication, meerut, UP, India
2. Benjamin Lewin, 2007. Gene IX, 9th Edition, Jones and Bartlett Publishers international, London.

3. Harvey Lodish, Arnold Beck, Chris A. Kaiser, Monty Krieger, Anthony Bretscher, HiddePloegh, Angelika Amon, Matthew P. Scott. 2012. Molecular Cell Biology, 7th edition, W. H. Freeman and Company, USA.
4. Watson JD, Hopkins NH, Roberts JW, Seitz JA and Weiner AM. 2007. Molecular Biology of the Gene, 6th Edition, Benjamin Cummings Publishing Company Inc, USA.
5. Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2002. Molecular Biology of the Cell, 4th edition, Garland Science; New York, USA.
6. Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts, and James D Watson. 2007. Molecular Biology of the Cell Fifth Edition. Garland Science New York

**Outcomes**

- Implant Knowledge on energy utilization and generation in cells
- Insinuate causal relationships between molecule and cell level phenomena and organism-level patterns of heredity
- Understand the structure and function of prokaryotic and eukaryotic cells, as whole entities and in terms of their sub cellular processes.
- Link the rapid advances in cell and molecular biology to better understanding of diseases including cancer
- Demonstrate advanced laboratory bench skills, lab notebook record keeping, and team work.
- To exhibit clear and concise communication of scientific data.

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| CO4 | 1 | 2 | | 1 | | 1 | |
| CO5 | | | | | | | 3 |

**GGPB21 622 - PLANT TISSUE CULTURE AND GENETIC TRANSFORMATION (2+1)**

**Learning Objective**

- To familiarize the students and provide hands on training on various techniques of plant tissue culture.
- The students will learn how the genes can be cut and pastes from one organism to another and what are its implications

**Theory**

**UNIT I : Introduction to plant tissue culture**

History of plant cell and tissue culture; Culture media-Laboratory organisation - sterile techniques - Nutrition of plant cells - Media composition- callus differentiation- Techniques in Micropropagation- stages- Organogenesis-somatic embryogenesis.

**UNIT II : Types of *invitro* culture**

Embryo rescue techniques-artificial seeds-Somatic hybridization: protoplast fusion, cybrids- Meristem and virus elimination –Haploid production &diplodization- Somaclonal variation- Secondary metabolites in cell suspension culture-*In vitro* germplasm conservation- Application of plant cell culture in crop improvement.

**UNIT III : Plant genetic engineering**

Plant transformation vectors and transgene design-Promoters and Marker genes-scorable and reporter genes- Plant transformation methods -Vector mediated methods of transformation-*Agrobacterium* biology and genetic engineering-Indirect gene transfer methods-Biolistic gun - protoplast mediated transformation - microinjection techniques-Organellar transformation - chromosome Engineering

**UNIT IV : Transgenics in crop improvement**

Molecular pharming-Analysis of transgenic plants- Application of genetic engineering in crop improvement and crop productivity - resistance to disease - herbicides - stress conditions -quality characters - Plant genetic engineering - current status - problems and strategies for practical applications.

**UNIT V : Gene silencing and genome editing**

Gene knockout technologies-Cas9-Cre-Lox recombination system- Genome editing using CRISPR -Terminator gene technology-Development of marker-free plants-Identification of gene integration site - Advance methods-cisgenesis, intragenesis.

**Practical**

PCR- Variation in PCR- RT - PCR - PCR - based analysis of tranformants – Primer designing-Induction and analysis of crown gall tumour in intact plant - Isolation of Ti-Plasmid. Isolation of DNA and organelle DNA - *Agrobacterium* mediated transfer

**Lecture Schedule**

**Theory**

1. Laboratory organization-sterile techniques
2. Nutrition of plant cells-media composition.
3. Establishment and maintenance of calluses and suspension culture - cellular differentiation and regulation of morphogenesis.
4. Somatic embryogenesis - molecular aspects - control of organogenesis and embryogenesis - single cell methods - cytology of callus.
5. Haploid production : Androgenesis - anther and microspore culture.
6. Diplodization and double haploids
7. Gynogenesis - embryo culture and rescue in agricultural and horticultural crops.
8. *In vitro* pollination and fertilization.
9. Protoplast isolation - culture - regeneration.
10. Somatic hybrids - cybrids.
11. *In vitro* genetic conservation.
12. Somatic emryogenesis and artificial seeds.
13. Meristem culture and virus elimination - shoot tip culture.
14. Somaclonal variation in i*n vitro* cultures
15. Secondary metabolites in cell culture - essential oils - scented varieties
16. Application of various techniques for crop improvement in agriculture, horticulture and forestry.
17. **Mid semester examination**
18. Methods of plant transformation
19. *Agrobacterium* biology and genetic engineering
20. Biolistic method - protoplast mediated transformation
21. Microinjection techniques
22. Terminator gene technology
23. Chromosome Engineering
24. organellar transformation
25. Molecular pharming
26. Genetic and molecular analyses of transgenics
27. Genetic engineering for resistance to insect, disease and herbicides
28. Genetic engineering for quality characters
29. Gene knockout technologies
30. Cas9-Cre-Lox recombination
31. Genome editing using CRISPR Cas9
32. Development of marker-free plants-
33. Identification of gene integration site
34. Advance methods-cisgenesis, intragenesis

**Practical**

1. Laboratory set-up.
2. Preparation of nutrient media; handling and sterilization of plant
3. Explant inoculation, subculturing and plant regeneration.
4. Anther and pollen culture.
5. Embryo rescue.
6. Suspension cultures and production of secondary metabolites.
7. Protoplast isolation, culture and fusion.
8. Gene cloning and vector construction.
9. Isolation of plasmids with reporter (*gus*) gene,
10. Preparation of microprojectiles, transformation using a particle gun, GUS staining.
11. Leaf disc transformation using *Agrobacterium*, establishment of transgenic plants, and
12. GUS staining or GFP viewing.
13. DNA extraction from transgenic plants, DNA estimation, PCR analysis,
14. Southern blot analysis to prove T-DNA integration,
15. RT-PCR to study transgene expression
16. Western blotting to study the accumulation of transgene-encoded protein.
17. **Final Practical Examination**.

**Suggested Readings**


1. Bhojwani SS. 1983. *Plant Tissue Culture: Theory and Practice*. Elsevier
2. Gamborg OL and. Philips GC. 1995. Plant Cell, Tissue and organ culture. Fundamental Methods, Narosa Publishing House, New Delhi.
3. Potrykus F and Spangenberg. 1995. Gene Transfer to Plants, Springar Verlag, Germany.
4. Brown T A. 2010. Gene Cloning and DNA Analysis: An Introduction, 6th Edition, Blackwell publications, USA.
5. Christou P & Klee H. 2004. *Handbook of Plant Biotechnology*. John Wiley & Sons.
6. Singh BD. 2007. *Biotechnology: Expanding Horiozon.* Kalyani.

7. Lewin‘s Genes XI 2012. Jones and Bartlett Learning, USA
8. U. Satyanarayana. Biotechnology, Book and allied (P), Ltd, 2013.

**Outcomes**

- standardize protocols for the *in vitro* propagation from *ex vitro* explants
- To optimize the culture conditions for rapid propagation and regeneration of agriculturally important plants.
- Biochemical monitoring of explants proliferation and regeneration
- Optimization of medium and culture conditions for the enhancement of active principle production
- Biochemical characterization of regeneration and genetic transformation for economic utilization.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
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## GGPB21 623 GENOMICS AND PROTEOMICS (2+1)

**Learning Objective**

- To familiarize the students with recent tools used for genome analysis and their applications and to provide knowledge on analysis of genome and proteome.

**Theory**

**Unit I : Structural genomics**

Structural genomic: Organization of genomes: main features of bacterial and eukaryotic genome organization – Gene library and mapping - Strategies for genome sequencing: Next Generation Sequencing, pyrosequencing, Illumina Sequencing, Sequence assembly - Clone contig and shotgun approaches. Model plant genome project and its applications. Locating the genes: ORF scanning, homology searches. Identification and classification using molecular markers - 16S rRNA typing/sequencing, EST’s and SNP’s.

**Unit II : Functional genomics**

Functional genomics: Determination of the functions of genes, candidate gene identification in crop plants, gene inactivation (knock-out, anti-sense and RNA interference) and gene over expression. Approaches to analyze global gene expression: transcriptome, Serial Analysis of Gene Expression (SAGE), Expressed Sequence Tags (ESTs), Massively Parallel Signature Sequencing (MPSS), microarray and its applications, gene tagging; Metagenomics.

**Unit III : Proteomics**

Proteomics – introduction, Expressional Proteomics, Functional Proteomics, Structural Proteomics-Techniques in Proteomics, Protein separation techniques - Strategies in protein identification, 2D Gel electrophoresis, Isoelectric Focusing (IEF). Mass spectrometry in proteomics – Principle, techniques (MALDI-TOF) and analysis, SAGE, applications. Protein- Protein interactions- experimental and computational- Differential display proteomics, Protein sequence analysis - N-terminal determination methods- Protein modification – Yeast two hybrid system.

**UNIT IV : Structural proteomics**

Structural proteomics: protein structure determination, prediction and threading, software and data analysis/ management, etc. - DNA chips and their use in transcriptome analysis; Metabolomics and ionomics for elucidating metabolic pathways, etc. Application of metabolomics in elucidating metabolic pathways, metabolic pathways resources: KEGG, Biocarta etc., Nutrigenomics and metabolic health

**Unit V : Proteome analysis and application**

Protein Biomarker - Discovery and Validation – Emerging technologies: Microfluidics. Analysis of microarray data; Protein and peptide microarray-based technology; PCR-directed protein *in situ* arrays; Applications of genomics and proteomics in agriculture, human health and Industry.

**Practical**

Isolation of genomic DNA and proteins- RAPD-RFLP-AFLP-SNPs-2-D electrophoresis of proteins; isoelectricfocusing; Peptide fingerprinting; LC/MS-MS for identification of MALDI-TOF; SAGE and Differential display proteomics, Protein-protein interactions, Yeast two hybrid system.

**Theory schedule**

1. Structural genomics
2. Organisation of genome
3. Genomic libraries and physical mapping of genomes
4. Strategies for genome sequencing
5. Clone contigs and Shotgun approaches
6. Plant genome projects; locating the genes
7. Identification and classification using molecular markers-16S rRNA typing/sequencing, EST's and SNP's
8. Functional genomics
9. Gene inactivation and over expression
10. SAGE, EST, MPSS
11. Microarray and applications
12. Gene tagging, Metagenomics
13. Proteomics, types
14. Protein separation techniques
15. Protein identification
16. Protein analysis (amino-acid composition, N-terminal sequencing);
17. **Mid- semester examination**
18. 2-D electrophoresis of proteins &Isoelectricfocusing
19. Peptide fingerprinting,
20. Mass spectroscopy, principles
21. LC/MS-MS for identification of proteins and modified proteins;
22. MALDI-TOF and SAGE
23. Differential display proteomics
24. Protein-protein interactions and N-terminal determination methods
25. Yeast two hybrid system.
26. Protein structure determination
27. DNA chips in transcriptome analysis
28. Metabolomics and ionomics
29. Elucidating metabolomic pathways
30. KEGG, Biocarta, Nurtigenomics
31. Protein biomarkers
32. Microfluidics
33. Analysis of microarray data
34. Applications of genomics and proteomics in agriculture, human health and Industry.

**Practical schedule**

1. Physical mapping of genome
2. Genetic mapping
3. Linkage mapping
4. Molecular mapping using RFLP
5. Molecular mapping using RAPD
6. Molecular mapping using AFLP
7. Molecular mapping using SNP
8. Gene prediction and annotation using database
9. Database for Comparative Genomics
10. DNA microarrays technology
11. DNA chips technology
12. Protein microarray
13. Peptide microarray
14. 2-D electrophoresis of proteins
15. LC/MS-MS for identification of proteins and modified proteins
16. MALDI-TOF and SAGE for protein-protein interaction
17. **Practical examination**

**References**


1. Campbell, A.M. and Heyer, L.J. 2007. Discovering Genomics, Proteomics and Bioinformatics, 2nd edition, Benjamin Cummings, UK.
2. Liebler, D.C. 2002. Introduction to Proteomics – Tools for the New Biolog, 1st Edition, Humana Press Inc, New Jersey, USA.
3. Orengo, C.A., Jones, D.T. and Thornton, J.M. 2003. Bioinformatics – Genes, Proteins and Computers, 1st Edition, BIOS Scientific Publishers Limited, Oxford, UK.
4. Primrose, S.B. and Twyman, R.M. 2003. Principles of Genome Analysis and Genomics, 3rd edition, Blackwell Publishing Company, Oxford, UK.
5. Twyman, R.M. Principles of Proteomics. BIOS Scientific Publisher, New York. 2004.

6. Dubitzky W., Granzow M., Berrar D.P. (2007) Fundamentals of Data Mining in Genomics and Proteomics. Springer Science- Business Media.
7. Lovric J. (2011) Introducing Proteomics: From concepts to sample separation, mass spectroscopy and data analysis. John Willey and Sons Ltd.
8. Mine Y., Miyashita K., Shahidi F. (2009) Nutrigenomics and Proteomics in Health and Disease: Food Factors and Gene Interaction. Wiley Blackwell

**Outcomes**

- Identify and use bioinformatics tools to solve problems in molecular biology and plant breeding.
- Identify and describe the different components in prokaryotic and eukaryotic genomes and proteomes.
- Tools commonly used in genome sequencing, assembly and annotation.
- Use the different methodologies, techniques commonly used in proteomics and metabolomics.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
|---|---|---|---|---|---|---|---|
| CO1 | 2 | | | | 2 | | |
| CO2 | 1 | 4 | | | 1 | | |
| CO3 | | | | 1 | | 2 | |
| CO4 | 3 | | | | 3 | | |

## GGPB21 624 MOLECULAR PLANT BREEDING (2+0)

**Learning Objective**

- To familiarize the students about the use of molecular biology tools in plant breeding.
- To provide the knowledge of various recent advances in agriculture

**Theory**

**UNIT I : Methods in plant breeding**

Principles of plant breeding; Breeding methods for self and cross pollinated crops; Heterosis breeding; Limitations of conventional breeding; Aspects of molecular breeding.

**UNIT II: Molecular markers**

Molecular markers – History of Molecular markers- Restriction based and PCR based; DNA profiling using different assays- RFLP, RAPD, AFLP, ISSR, SNP etc. Development of SCAR and SSR markers.

**UNIT III: QTL mappings**

Linkage disequilibrium- Linkage mapping- QTL analysis- QTL mapping; Strategies for QTL mapping - desired populations for QTL mapping - statistical methods in QTL mapping - QTL mapping in Genetic analysis; -Gene pyramiding; Transcript mapping techniques. Development of ESTs-AB-QTL analysis; Association mapping of QTL; Fine mapping of genes/QTL; Map based gene/QTL isolation and development of gene based markers; Allele mining by TILLING and Eco-TILLING

**UNIT IV: Marker Assisted Selection**

Use of markers in plant breeding. Marker assisted selection (MAS) - Approaches to apply MAS in Plant breeding - selection based on marker - simultaneous selection based on marker and phenotype - factors influencing MAS. Marker Assisted Selection (MAS), screening and validation; Marker assisted selection (MAS) in backcross and heterosis breeding- Mapping genes on specific chromosomes-Transgenic breeding; Foreground and background selection; MAS for gene introgression and pyramiding: MAS for specific traits with examples.

**UNIT V:Techniques in DNA analysis**

Recent advances – Non gel based techniques for plant genotyping – Homogenous assays – Qualitative/Real Time assays; DNA Chip and its technology. Phenomics- Application of high-throughput phenotyping platforms in plant breeding

**Theory schedule**

1. Principles of plant breeding
2. Breeding methods for self and cross pollinated crops
3. Heterosis breeding
4. Limitations of conventional breeding
5. Aspects of molecular breeding.
6. Molecular markers

7. Restriction based and PCR based
8. DNA profiling using different assays
9. RFLP, RAPD,AFLP
10. ISSR, SNP
11. Development of SCAR and SSR markers
12. Marker Assisted Selection (MAS)
13. Screening and validation
14. Marker assisted selection (MAS) in backcross
15. Marker assisted selection in heterosis breeding
16. Mapping genes on specific chromosomes
17. **Mid semester examination**
18. Transgenic breeding; Foreground and background selection;
19. MAS for gene introgression and pyramiding:
20. MAS for specific traits with examples.
21. Linkage disequilibrium
22. QTL mapping using structured populations
23. Gene pyramiding; Transcript mapping techniques.
24. Development of ESTs-AB-QTL analysis
25. Association mapping of QTL
26. Fine mapping of genes/QTL
27. Map based gene/QTL isolation and development of gene based markers;
28. Allele mining by TILLING and Eco-TILLING
29. Use of markers in plant breeding.
30. Non gel based techniques for plant genotyping
31. Homogenous assays
32. Qualitative/Real Time assays;
33. DNA Chip and its technology
34. high-throughput phenotyping platforms

**References**


1. Chittaranjan, K. 2006-07. Genome Mapping and Molecular Breeding in Plants. Vols. I-VII. Springer -Verlag, USA.
2. Henry, R.J. 2005. Plant Genotyping: The DNA fingerprinting of plants. CABI, New Delhi
3. Newbury, H.J. 2003. Plant Molecular Breeding. Blackwell Publication, Oxford, UK.
4. Weising K., Nybom, H., Wolff, K. and Kahl, G. 2005. DNA Fingerprinting in Plants: Principles, Methods and Applications. Taylor & Francis, London.
5. Nagata, T., Lorz, H. and Widholm, J. M. (2005) Molecular Marker Systems in Plant Breeding and Crop Improvement. Springer-Verlag Berlin, German
6. Kang, M. S. (2002) Quantitative Genetics, genomics and Plant Breeding. CABI, USA.
7. Srivastava, P.S., Narula A., Srivastava Sh.(2005). Plant biotechnology and molecular markers. Anamaya Publishers, New Delhi, India
8. Kang MS. (2003). Handbook of Formulas and Software for Plant Geneticists and Breeders. Haworth Press Inc, New York, USA


**Outcomes**

- Conceptual understanding of plant breeding and the molecular techniques
- Deliniate molecular techniques as tools for conventional plant breeding
- Explore practical applications and impacts of molecular breeding tools in crop improvement programs
- Learn how to use molecular techniques data analysis software including mapping software programs

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
|---|---|---|---|---|---|---|---|
| CO1 | 2 | 1 | | | | | |
| CO2 | 1 | 4 | | | | | |
| CO3 | 3 | 3 | | | | 2 | |
| CO4 | 3 | 4 | | | | | 2 |

**OPC- GGPB21 621 CONCEPTS OF CROP PHYSIOLOGY (2+1)**

**Learning Objective**

- To impart knowledge in understanding the physiological processes taking place during growth and development of plants.
- To understand source sink relationship in different groups of plants and also hormonal, environmental and stress physiology in crop plants.

**Theory**

**Unit I : Photo physiology**

Role of physiology in different branches of agriculture. Physiological processes on productivity – Photosynthesis – Mechanism of light interaction. Physiological processes influenced by radiation. Light and phytochrome mediated processes. – $CO_2$ reduction – utilization of assimilatory power and carbohydrate synthesis - $C_3$, $C_4$ and CAM mechanisms – Major differences.

**Unit II : Growth and Development**

Growth Vs Development. Dry Matter Accumulation and Harvest Index – components of Dry Matter Accumulation and Harvest Index and their role in productivity. Growth analysis. Photorespiration and dark respiration.

**Unit III : Source sink relationship**

LAI and its components –interception of solar energy. Photosynthates partitioning – source – sink relationship – mode of partitioning at different stages in different species. Role of growth regulators in monitoring source and sink.

**Unit IV : Environmental physiology**

Green house effect and Global warming. Ozone layer depletion - Causes, effects. $CO_2$ enrichment and plant productivity. Physiology of crops under high altitude and flooding – air pollution and plant growth – effect of effluent on plant growth.

**Unit V : Stress physiology**

Mechanisms of drought, salt, cold, heat and UV radiation stress tolerance – adaptation of crop plants – crop management practices under unfavourable situations – Importance of selection indices for crop productivity – recent advances in physiological research.

**Practical**

Leaf Area measurement – measurement of leaf angle and interception of solar radiation – light transmission ratio – measurement of photosynthesis – difference in the photosynthetic rate between the leaves at different position – photosynthetic efficiency of $C_3$ and $C_4$ plants – estimation of chlorophyll – RuBP case and PEP case – Measurement of respiration – Growth regulation – response to source and sink relationship – Measurement of water potential and its component. Measurement of leaf temperature, diffusive resistance and transpiration rate – use of antitranspirants – yield component analysis – study of selection indices.

**Lecture Schedule**

**Theory**

1. Role of physiology in different branches of agriculture
2. Physiological processes on productivity
3. Photosynthesis – Mechanism of light interaction
4. Photo Physiology
5. Physiological processes influenced by radiation
6. Light and phytochrome mediated processes
7. Utilization of assimilatory power and $CH_2O$ synthesis
8. $C_3$-$C_4$ and CAM mechanisms and major differences
9. Photosynthetic measurements
10. Germination, growth and development
11. DMA and HI. Components of DMA and HI.
12. Role of DMA, LAI and HI in crop productivity
13. Growth analysis
14. Photorespiration and dark respiration
15. Oxidative phosphorylation.
16. Release and utilization of energy for various metabolisms.
17. **MID-SEMESTER EXAMINATION**
18. Interception of solar energy
19. Source-sink relationship
20. Photosynthate partitioning
21. Mode of partitioning at different stages and different species
22. Role of growth regulators in monitoring source-sink relationship
23. Growth regulators – auxins, gibberellins and cytokinins, biosynthesis, functions and agricultural role.
24. Abscisic acid and ethylene. Biosynthesis, functions and agricultural role.
25. Growth retardants. Role in agricultural and horticultural crops
26. Green house effect and plant productivity.
27. $CO_2$ enrichment and plant productivity.
28. Water stress, effect of water stress on various physiological processes
29. Mechanisms of adaptation to stress condition.
30. Salt stress, classifications and its effects on physiological processes of plant
31. Temperature stress – cold tolerance – adaptation
32. Heat stress – Heat shock proteins – heat tolerance – adaptation.

33. Physiology of crops under high altitude flooding, air and water pollution
34. Recent advances in physiological research

**Practical Schedule**

1. Leaf area index measurement. Measurement of leaf angle and interception of solar radiation
2. Measurement of photosynthesis
3. Determination of Photosynthetic efficiency of various crop plants
4. Estimation of soluble protein content
5. Estimation of chlorophyll contents
6. Estimation of water potential
7. Determination of chlorophyll stability index
8. Estimation of relative water content
9. Estimation of leaf proline content
10. Measurement of leaf temperature, diffusive resistance and transpiration
11. Growth analysis of field crops
12. Determination of nitrate reductase activity
13. Determination of IAA oxidase activity
14. Estimation of total phenolics
15. Estimation of peroxidase activity
16. Estimation of catalase activity
17. **FINAL PRACTICAL EXAMINATION**

**References**


1. Devlin, B. 1983. Plant Physiology. Narosa Publishing House, New Delhi.
2. Franklin P. Gardner, R. Brent Pearce and Roger L. Mitchell,1988. Physiology of crop plants. Scientific Publishers, Jodhpur.
3. Gupta, U.S. 1988. Progress in Crop Physiology. Oxford IBH Publishing Co. Pvt., Ltd., New Delhi.
4. Kumar, A. and S.S. Purohit. 1996. Plant Physiology. Agro Botanical Publishers, Bikaner.
5. Lincoln Taiz, Eduardo Zeiger. 2002. Plant Physiology 2nd Edition. Replica press Pvt. Ltd., Delhi.
6. Noggle, G.R. and G.J. Fritz. 1986. Introductory Plant Physiology. Prentice Hall of India Ltd., New Delhi.
7. Panday, S.N. and B.K.Sinha. 1972. Plant Physiology. Vikas Publishing House Pvt. Ltd., New Delhi.
8. Price, C.A. 1974. Molecular approaches to plant physiology. Tata MCGraw Hill Publishing Co. Ltd., New Delhi.
9. Purohit, S.S. 2005, Plant Physiology. Student Edition Agrobios, Jodhpur.
10. Purohit, S.S., Q.J. Shammi, and A.K. Agrawal, 2005. A Text book of Environmental sciences, Student Edition, Agrobios, Jodhpur.
11. Salisbury, F.B. and C.M.Ross. 2004. Plant Physiology. Thomson and Wadsworth publications, Belmont, California.


**Outcomes**

- Students able to identify different physiological process like imbibitions, diffusion and ascent of sap
- Will be able to identify C3, C4 and CAM plants
- Will be able to identify and rectify the various stresses

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
|---|---|---|---|---|---|---|---|
| CO1 | | | | 4 | | | |
| CO2 | | | | 3 | | | |
| CO3 | | | | 3 | | 2 | |

## OPC- GGPB21 711 BIO-INSTRUMENTATION (2+1)

**Learning Objective**

- To provide hands on training on basic molecular biology techniques
- To provide the knowledge of various technology in field of molecular biology

**Theory**

**UNIT I : Spectroscopy & Microscopy**

Spectroscopy-Principle, instrumentation and applications of UV – visible spectrophotometry and spectrofluorimetry-luminometry-Atomic spectroscopy- Microscopy-SEM and TEM.

**UNIT II : Centrifugation**

Basic principles of sedimentation-Clinical Bench Centrifuges-High Speed Refrigerated Centrifuges-Continuous flow Centrifuges-Ultracentrifuges-Analytical ultracentrifuge -instrumentation and applications-Preparative ultracentrifuge

**UNIT III : Chromatography**

Principle of chromatography-Types- Column Chromatography-Paper Chromatography-Thin Layer Chromatography-Gas Chromatography-High Performance Liquid Chromatography-Affinity Chromatography-Ion-Exchange Chromatography

**UNIT IV : PCR and Electrohoresis**

PCR-principles. RT-PCR. Real time PCR-DNA/RNA-Agarose gel electrophoresis-Principles-Protein electrophoresis-principles-SDS and Native PAGE, 2D-gel electrophoresis.

**UNIT V: Blotting techniques**

Blotting techniques-Southern-Northern-Western. DNA sequencing techniques, Dot blot analysis-ELISA- Immunoelectrophoresis, RIA, immunoblotting

**Practical**

Centrifugation techniques- Chromatography-Electron microscopy-Electrophoresis of DNA and proteins-PCR-blotting techniques-DNA sequencing techniques.

**Lecture schedule**

**Theory**

1. Good lab practices
2. Preparation of buffers and reagents,
3. Principle of centrifugation
4. Analytical and preparative centrifugation
5. Principle involved in Chromatography
6. UV and Nano drop spectrophotometer
7. Ion exchange spectroscopy
8. Atomic absorption spectroscopy
9. Electron microscopy
10. TEM and SEM.
11. Agarose gel electrophoresis
12. Electrophoresis of proteins-principles
13. Native and SDS PAGE
14. Gradient gel
15. Isoelectric focusing
16. 2-D PAGE.
17. **Mid- semester examination**
18. Detection, estimation of proteins
19. Recovery of proteins in gels,
20. Autoradiography
21. PCR- principle and applications
22. Mid semester examination
23. Modified PCR techniques
24. Reverse transcriptase PCR
25. Real time PCR
26. DNA Sequencing
27. Chemical method
28. Enzymatic method
29. Blotting techniques: Southern
30. Northern blotting techniques
31. Western blotting techniques.
32. Immunoelectrophoresis
33. RIA
34. Dot blot technique and immunoblotting.

**Practical schedule**

1. Preparation of stock solutions and reagents.
2. Extraction of plant genomic DNA by Dellaporta method.
3. Extraction of plant genomic DNA by CTAB method.
4. Centrifugation technique
5. Chromatography technique
6. UV- spectrophotometer
7. Restriction digestion of DNA.
8. Southern transfer, labelling of DNA, Southern hybridization.
9. Northern and western blotting procedure
10. Autoradiography.
11. Amplification of DNA with thermocycler with random primers.
12. Analysis of PCR products through agarose gel eletrophoresis and gel scanning.
13. Primer designing
14. DNA sequencing.
15. Genomic library construction
16. ELISA
17. **Final Practical Examination**

**Suggested Readings**

1) Wilson and Walker. A biologists guide to principles and techniques of practicalbiochemistry. 5th ed. Cambridge University Press 2000.
2) Boyer, R. Modern Experimental Biochemistry. 3rd ed. Addison WesleryLongman, 2000.
3) Upadhyay, Upadhyay and Nath. Biophysical Chemistry Principles andTechniques. Himalaya Publ. 1997.
4) Simpson CFA &Whittacker, M. Electrophoretic techniques.
5) Sambrook. Molecular Cloning. Cold Spring Harbor Laboratory, 2001.
6) Friefelder and Friefelder. Physical Biochemistry – Applications to Biochemistryand Molecular Biology. WH Freeman & Co. 1994.
7) Pavia et al. Introduction to Spectroscopy. 3rd ed. Brooks/Cole Pub Co., 2000.

**Outcomes**

- Ability to understand diagnosis and repair of related equipments
- Understanding the problem and ability to identify the necessity of an equipment to a specific problem
- Ability to take measurements involved in some agricultural equipments.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
|---|---|---|---|---|---|---|---|
| CO1 | | 1 | | | | | |
| CO2 | | 4 | | | | | |
| CO3 | | 2 | | | | | |

## OPC- GGPB21 712 PLANT TISSUE CULTURE (2+1)

**Learning Objective**

- To familiarize the students and provide hands on training on various techniques of plant tissue culture.
- The students will learn how the genes can be cut and pastes from one organism to another and what are its implications

**Theory**

**UNIT I : Basic principles**

History of plant cell and tissue culture; Culture media- sterile techniques - Media in plant tissue culture-Plant Growth Regulators-Components of a Plant Tissue Culture Medium-Explants-callus-totipotency-Basic concepts Plant tissue culture.

**UNIT II : Micropropagation method**

Basic techniques in plant tissue culture-Techniques in Micropropagation- stages-Organogenesis-somatic embryogenesis-Virus free plants production

**UNIT III : In vitro culture techniques**

Callus culture- Suspension culture- Single cell culture- Organ culture- Seed, embryo, endosperm, nucellus, shoot, root, leaf, anther and ovary. Protoplast culture-somatic hybridization-cybrids.

**UNIT IV : Haploids production**

Embryo rescue techniques-artificial seeds-Haploid production &diplodization-Somaclonal variation- *In vitro* germplasm conservation- Application of plant cell culture in crop improvement.

**UNIT V : Genetic engineering**

Plant transformation methods- *Agrobacterium*-Biolistic gun- Analysis of transgenic plants- Application of genetic engineering in crop improvement and crop productivity - resistance to disease – herbicides-quality characters.

**Practical**

PCR- Variation in PCR- RT - PCR - PCR - based analysis of tranformants – Primer designing-Induction and analysis of crown gall tumour in intact plant - Isolation of Ti-Plasmid. Isolation of DNA and organelle DNA - *Agrobacterium* mediated transfer

**Lecture Schedule**

**Theory**

1. Laboratory organization-sterile techniques
2. Nutrition of plant cells-media composition.
3. History of plant cell and tissue culture
4. Culture media-Sterile techniques
5. Media in plant tissue culture
6. Plant Growth Regulators

7. Components of a Plant Tissue Culture Medium
8. Explants-callus-totipotency
9. Basic concepts Plant tissue culture.
10. Basic techniques in plant tissue culture
11. Micropropagation stages-Organogenesis-Somatic embryogenesis
12. Virus free plants production
13. Callus culture
14. Midterm examination
15. Suspension culture
16. Single cell culture.
17. **Mid-semester examination**
18. Organ culture
19. Seed, embryo,endosperm, nucellus
20. Shoot, root, leaf culture
21. Protoplast culture
22. Somatic hybridization-cybrids.
23. Embryo rescue techniques
24. Artificial seeds
25. Haploid production-diplodization
26. Somaclonal variation
27. *In vitro* germplasm conservation
28. Application of plant cell culture in crop improvement
29. Plant transformation methods
30. *Agrobacterium* mediated gene transfer
31. Biolistic gun
32. Genetic and molecular analyses of transgenics
33. Genetic engineering for resistance to insect pests
34. Genetic engineering for resistance to herbicides and quality characters.

**Practical**

1. Laboratory set-up.
2. Preparation of nutrient media; handling and sterilization of plant
3. Explant inoculation, subculturing and plant regeneration.
4. Anther and pollen culture.
5. Embryo rescue.
6. Suspension cultures and production of secondary metabolites.
7. Protoplast isolation, culture and fusion.
8. Preparation of microprojectiles, transformation using a particle gun, GUS staining.
9. Leaf disc transformation using *Agrobacterium*, establishment of transgenic plants, and
10. DNA extraction from transgenic plants, DNA estimation
11. Protein extraction
12. Agarose and PAGE electrophoresis
13. Southern blot analysis to prove T-DNA integration
14. PCR
15. RT-PCR to study transgene expression
16. Western blotting to study the accumulation of transgene-encoded protein.
17. **Final Practical Examination.**

**Suggested Readings**


1. Bhojwani SS. 1983. *Plant Tissue Culture: Theory and Practice*. Elsevier
2. Gamborg OL and. Philips GC. 1995. Plant Cell, Tissue and organ culture. Fundamental Methods, Narosa Publishing House, New Delhi.
3. Potrykus F and Spangenberg. 1995. Gene Transfer to Plants, Springar Verlag, Germany.
4. Brown T A. 2010. Gene Cloning and DNA Analysis: An Introduction, 6th Edition, Blackwell publications, USA.
5. Christou P & Klee H. 2004. *Handbook of Plant Biotechnology*. John Wiley & Sons.
6. Singh BD. 2007. *Biotechnology: Expanding Horiozon.* Kalyani.
7. Lewin's Genes XI 2012. Jones and Bartlett Learning, USA
8. U. Satyanarayana. Biotechnology, Book and allied (P), Ltd, 2013.


**Outcomes**

- standardize protocols for the in vitro propagation from ex vitro explants
- To optimize the culture conditions for rapid propagation and regeneration of agriculturally important plants.
- Biochemical monitoring of explants proliferation and regeneration
- Optimization of medium and culture conditions for the enhancement of active principle production
- Biochemical characterization of regeneration and genetic transformation using Agrobacterium.

| | PSO 1 | PSO 2 | PSO 3 | PSO 4 | PSO 5 | PSO 6 | PSO 7 |
|---|---|---|---|---|---|---|---|
| CO1 | | | 4 | | | | |
| CO2 | | | 4 | | | | |
| CO3 | | | 4 | | | | |
| CO4 | | | 4 | | 1 | | |
| CO5 | | | | | | | 1 |