REVISED CURRICULUM

M. Sc.

AGRICULTURAL BIOTECHNOLOGY

Department of Biotechnology
Ministry of Science & Technology,
Government of India

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CONTENTS

Contents for electives are given separately.

SEMESTER - I

Biochemistry - 3 Credits

Unit I

Chemical basis of life; Composition of living matter; Water – properties, pH, ionization and hydrophobicity; Emergent properties of biomolecules in water; Biomolecular hierarchy; Macromolecules; Molecular assemblies; Structure-function relationships

Amino acids – structure and functional group properties; Peptides and covalent structure of proteins; Elucidation of primary and higher order structures; Evolution of protein structure; Structure-function relationships in model proteins like ribonuclease A, myoglobin, hemoglobin, chymotrypsin etc.; Tools to characterize expressed proteins.

Unit II

Enzyme catalysis – general principles of catalysis; Quantitation of enzyme activity and efficiency; Enzyme characterization and Michaelis-Menten kinetics; Relevance of enzymes in metabolic regulation, activation, inhibition and covalent modification; Single substrate enzymes

Unit III

Sugars - mono, di, and polysaccharides; Suitability in the context of their different functions- cellular structure, energy storage, signaling; Glycosylation of other biomolecules - glycoproteins and glycolipids

Lipids - structure and properties of important members of storage and membrane lipids; lipoproteins

Unit IV

Biomembrane organization - sidedness and function; Membrane bound proteins - structure, properties and function; Transport phenomena;

Nucleosides, nucleotides, nucleic acids - structure, diversity and function; sequencing; Brief overview of central dogma

Unit V

Bioenergetics-basic principles; Equilibria and concept of free energy; Coupled processes; Glycolytic pathway; Kreb's cycle; Oxidative phosphorylation; Photosynthesis; Elucidation of metabolic pathways; Logic and integration of central metabolism; entry/ exit of various biomolecules from central pathways; Principles of metabolic regulation; Regulatory steps; Signals and second messengers.

Texts/References

1. V.Voet and J.G.Voet, Biochemistry, 3rd edition, John Wiley, New York, 2004.
2. A.L. Lehninger, Principles of Biochemistry, 4th edition, W.H Freeman and Company, 2004.
3. L. Stryer, Biochemistry, 5th edition, W.H. Freeman and Company, 2002.

Molecular Biology - 3 Credits

Unit I

Genome organization

Organization of bacterial genome; Structure of eukaryotic chromosomes; Role of nuclear matrix in chromosome organization and function; Matrix binding proteins; Heterochromatin and Euchromatin; DNA reassociation kinetics(Cot curve analysis); Repetitive and unique sequences; Satellite DNA; DNA melting and buoyant density; Nucleosome phasing; DNase I hypersensitive regions; DNA methylation & Imprinting

Unit II

DNA Structure; Replication; Repair & Recombination

Structure of DNA - A-,B-, Z- and triplex DNA; Measurement of properties-Spectrophotometric, CD, AFM and Electron microscope analysis of DNA structure; Replication: initiation, elongation and termination in prokaryotes and eukaryotes; Enzymes and accessory proteins; Fidelity; Replication of single stranded circular DNA; Gene stability and DNA repair- enzymes; Photoreactivation; Nucleotide excision repair; Mismatch correction; SOS repair; Recombination: Homologous and non-homologous; Site specific recombination; Chi sequences in prokaryotes; Gene targeting; Gene disruption; FLP/FRT and Cre/Lox recombination.

Unit III

Prokaryotic & Eukaryotic Transcription

Prokaryotic Transcription; Transcription unit; Promoters- Constitutive and Inducible; Operators; Regulatory elements; Initiation; Attenuation; Termination-Rho-dependent and independent; Anti-termination; Transcriptional regulation-Positive and negative; Operon concept-lac, trp, ara, his, and gal operons; Transcriptional control in lambda phage; Transcript processing; Processing of tRNA and rRNA

Eucaryotic transcription and regulation; RNA polymerase structure and assembly; RNA polymerase I, II, III; Eukaryotic promoters and enhancers; General Transcription factors; TATA binding proteins (TBP) and TBP associated factors (TAF); Activators and repressors; Transcriptional and post-transcriptional gene silencing

Unit IV

Post Transcriptional Modifications

Processing of hnRNA, tRNA, rRNA; 5'-Cap formation; 3'-end processing and polyadenylation; Splicing; RNA editing; Nuclear export of mRNA; mRNA stability; Catalytic RNA.

Translation & Transport

Translation machinery; Ribosomes; Composition and assembly; Universal genetic code; Degeneracy of codons; Termination codons; Isoaccepting tRNA; Wobble hypothesis; Mechanism of initiation, elongation and termination; Co- and post-translational modifications; Genetic code in mitochondria; Transport of proteins and molecular chaperones; Protein stability; Protein turnover and degradation

Unit V

Mutations; Oncogenes and Tumor suppressor genes

Nonsense, missense and point mutations; Intragenic and Intergenic suppression; Frameshift mutations; Physical, chemical and biological mutagens; Transposition - Transposable genetic elements in prokaryotes

and eukaryotes; Mechanisms of transposition; Role of transposons in mutation; Viral and cellular oncogenes; Tumor suppressor genes from humans; Structure, function and mechanism of action of pRB and p53 tumor suppressor proteins; Activation of oncogenes and dominant negative effect; Suppression of tumor suppressor genes; Oncogenes as transcriptional activators.

Text/References

1. Benjamin Lewin, Gene IX, 9th Edition, Jones and Barlett Publishers, 2007.
2. J.D. Watson, N.H. Hopkins, J.W Roberts, J. A. Seitz & A.M. Weiner; Molecular Biology of the Gene, 6th Edition, Benjamin Cummings Publishing Company Inc, 2007.
3. Alberts et al; Molecular Biology of the Cell, 4th edition, Garland, 2002.

Plant Biology - 3 Credits

Unit I

General Aspects; Novel features of plant growth and development; Concept of plasticity in plant development; Analyzing plant growth; Seed Germination and Seedling Growth; Mobilization of food reserves during seed germination; Tropisms; Hormonal control of seed germination and seedling growth.

Floral Induction and Development; Photoperiodism and its significance; Vernalization and hormonal control; Inflorescence and floral determination; Molecular genetics of floral development and floral organ differentiation; Sex determination.

Unit II

Carbon Assimilation; Light absorption and energy conversion; Calvin Cycle; Hatch-Slack pathway; Reductive pentose phosphate pathway; Carbon dioxide uptake and assimilation; Photorespiration; Glycolate metabolism.

Nitrogen Fixation — Symbiotic and non-symbiotic nitrogen fixation; Role of lectins; nod genes; nif genes; Structure, function and regulation of nitrogenase; Leghaemoglobin; Nodulins; Regulation and enhancement of nitrogen fixation.

Unit III

Long-distance Transport Mechanisms – Turgor and stomatal movements; Solute movement; Source-sink relationship; Water relations

Secondary Metabolism — Importance of Secondary Metabolites; Biosynthesis of phenolic compounds, isoprenoids, alkaloids and flavonoids.

Unit IV

Senescence and Programmed Cell Death (PCD) – Senescence and its regulation; Hormonal and environmental control of senescence; PCD in the life cycle of plants.

Signal Transduction – Basic concepts; Receptors and G-proteins; Cyclic AMP cascade; Phospholipid and Ca^{2+}-calmodulin cascade; MAP kinase cascade; Two-component sensor-regulator system; Sucrose sensing mechanism.

Unit V

Biosynthesis of Plant Hormones and Elicitors; Structure and metabolism of auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, salicylic acid, jasmonates and related compounds.

Molecular Mechanism of Hormone Action – Hormone signal perception, transduction and gene regulation; Role of mutants in understanding hormone action.
Light Control of Plant Development – Discovery of phytochromes and cryptochromes, their structure, biochemical properties and cellular distribution; Molecular mechanisms of light perception, signal transduction and gene regulation; Biological clocks and their genetic and molecular determinants.

Texts/References

1. Edited by Garry C Whitelam and Karen J Halliday, Light and Plant Development, Oxford Ames, Iowa: Blackwell Pub., 2007.
2. Esau's Plant Anatomy; Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development, 3rd Edition, John Wiley & Sons, 2006.
3. Thomas L Rost, Michael G Barbour, Terence M Murphy and C Ralph, Stocking Plant Biology (with InfoTrac), 2005.
4. Martin J Ingrouille and William Eddie, Plants: Diversity and Evolution
5. Bingru Huang, Plant-Environment Interactions, 3rd Edition, CRC Press, 2006.
6. Pamela C Ronald, Plant-Pathogen Interactions, 1st Edition, Humana Press, 2006.

Cell and Developmental Biology - 3 Credits

Unit I

Cell Theory & Methods of Study

Microscope and its modifications – Light, phase contrast and interference, Fluorescence, Confocal, Electron (TEM and SEM), Electron tunneling and Atomic Force Microscopy, etc.

Membrane Structure and Function

Structural models; Composition and dynamics; Transport of ions and macromolecules; Pumps, carriers and channels; Endo- and Exocytosis; Membrane carbohydrates and their significance in cellular recognition; Cellular junctions and adhesions; Structure and functional significance of plasmodesmata.

Unit II

Organelles

Nucleus – Structure and function of nuclear envelope, lamina and nucleolus; Macromolecular trafficking; Chromatin organization and packaging; Cell cycle and control mechanisms; Mitochondria – structure, organization of respiratory chain complexes, ATP synthase, Structure-function relationship; Mitochondrial DNA and male sterility; Origin and evolution; Chloroplast– Structure-function relationship; Chloroplast DNA and its significance; Chloroplast biogenesis; Origin and evolution.

Unit III

Endo-membrane System and Cellular Motility

Structure and function of microbodies, Golgi apparatus, Lysosomes and Endoplasmic Reticulum; Organization and role of microtubules and microfilaments; Cell shape and motility; Actin-binding proteins and their significance; Muscle organization and function; Molecular motors; Intermediate filaments; Extracellular matrix in plants and animals.

Unit IV

Cellular Movements and Pattern Formation

Laying of body axis planes; Differentiation of germ layers; Cellular polarity; Model plants like Fucus and

Volvox; Maternal gene effects; Zygotic gene effects; Homeotic gene effects in Drosophila; Embryogenesis and early pattern formation in plants; Cell lineages and developmental control genes in Caenorhabditis.

Unit V

Differentiation of Specialized Cells

Stem cell differentiation; Blood cell formation; Fibroblasts and their differentiation; Cellular basis of immunity; Differentiation of cancerous cells and role of proto-oncogenes; Phase changes in Salmonella; Mating cell types in yeast; Surface antigen changes in Trypanosomes; Heterocyst differentiation in Anabaena; Sex determination in Drosophila.

Plant Meristem Organization and Differentiation

Organization of Shoot Apical Meristem(SAM); Organization of Root Apical Meristem(RAM); Pollen germination and pollen tube guidance; Phloem differentiation; Self-incompatibility and its genetic control; Embryo and endosperm development; Heterosis and apomixis.

Texts/References

1. Lodish *et al*., Molecular cell Biology, 4th Edition, W.H. Freeman & Company, 2000.
2. Smith & Wood, Cell Biology, 2nd Edition, Chapman & Hall, London, 1996.
3. Watson *et al.*, Molecular Biology of the gene, 5th Edition, Pearson Prentice Hall. USA, 2003.
4. B. M. Turner, Chromatin & Gene regulation, 1st Edition, Wiley-Blackwell, 2002.
5. Benjamin Lewin, Gene IX, 9th Edition, Jones and Barlett Publishers, 2007.

Analytical Techniques - 3 Credits

Unit I

Basic Techniques

Buffers; Methods of cell disintegration; Enzyme assays and controls; Detergents and membrane proteins; Dialysis, Ultrafiltration and other membrane techniques

Spectroscopy Techniques

UV, Visible and Raman Spectroscopy; Theory and application of Circular Dichroism; Fluorescence; MS, NMR, PMR, ESR and Plasma Emission spectroscopy

Unit II

Chromatography Techniques

TLC and Paper chromatography; Chromatographic methods for macromolecule separation - Gel permeation, Ion exchange, Hydrophobic, Reverse-phase and Affinity chromatography; HPLC and FPLC; Criteria of protein purity

Electrophoretic techniques

Theory and application of Polyacrylamide and Agarose gel electrophoresis; Capillary electrophoresis; 2D Electrophoresis; Disc gel electrophoresis; Gradient electrophoresis; Pulsed field gel electrophoresis

Unit III

Centrifugation

Basic principles; Mathematics & theory (RCF, Sedimentation coefficient etc); Types of centrifuge -

Microcentrifuge, High speed & Ultracentrifuges; Preparative centrifugation; Differential & density gradient centrifugation; Applications (Isolation of cell components); Analytical centrifugation; Determination of molecular weight by sedimentation velocity & sedimentation equilibrium methods

Unit IV

Radioactivity

Radioactive & stable isotopes; Pattern and rate of radioactive decay; Units of radioactivity; Measurement of radioactivity; Geiger-Muller counter; Solid & Liquid scintillation counters (Basic principle, instrumentation & technique); Brief idea of radiation dosimetry; Cerenkov radiation; Autoradiography; Measurement of stable isotopes; Falling drop method; Applications of isotopes in biochemistry; Radiotracer techniques; Distribution studies; Isotope dilution technique; Metabolic studies; Clinical application; Radioimmunoassay

Unit V

Advanced Techniques

Protein crystallization; Theory and methods; API-electrospray and MALDI-TOF; Mass spectrometry; Enzyme and cell immobilization techniques; DNA & Peptide Synthesis.

Texts/References

1. Freifelder D., Physical Biochemistry, Application to Biochemistry and Molecular Biology, 2nd Edition, W.H. Freeman & Company, San Fransisco, 1982.
2. Keith Wilson and John Walker, Principles and Techniques of Practical Biochemistry, 5th Edition, Cambridge University Press, 2000.
3. D. Holme & H. Peck, Analytical Biochemistry, 3rd Edition, Longman, 1998.
4. R. Scopes, Protein Purification - Principles & Practices, 3rd Edition, Springer Verlag, 1994.
5. Selected readings from Methods in Enzymology, Academic Press.

Lab on Biochemistry & Analytical Techniques - 4 Credits

1. To prepare an Acetic-Na Acetate Buffer system and validate the Henderson-Hasselbalch equation.
2. To determine an unknown protein concentration by plotting a standard graph of BSA using UV-Vis Spectrophotometer and validating the Beer- Lambert's Law.
3. Titration of Amino Acids and separation of aliphatic, aromatic and polar amino acids by TLC.
4. AN ENZYME PURIFICATION THEME (such as E.coli Alkaline phosphatase or any enzyme of the institutions choice).

   (a) Preparation of cell-free lysates
   (b) Ammonium Sulfate precipitation
   (c) Ion-exchange Chromatography
   (d) Gel Filtration
   (e) Affinity Chromatography
   (f) Generating a Purification Table
   (g) Assessing purity by SDS-PAGE Gel Electrophoresis

(h) Assessing purity by 2-D gel Electrophoresis
(i) Enzyme Kinetic Parameters: Km, Vmax and Kcat.

5. Biophysical methods (Circular dichroism spectroscopy, fluorescence spectroscopy).
6. Determination of mass of small molecules and fragmentation patterns by Mass Spectrometry

Lab on Molecular, Cell & Plant Biology - 4 Credits

Molecular Biology

1. Plasmid DNA isolation and DNA quantitation: Plasmid minipreps, Restriction digestion, Preparation of competent cells.
2. Agarose gel electrophoresis
3. Restriction Enzyme digestion of DNA
4. Purification of DNA from an agarose gel
5. DNA Ligation
6. Transformation of E.coli with standard plasmids, Calculation of transformation efficiency
7. Cloning of genomic DNA in standard plasmid vectors
8. Confirmation of the insert, Miniprep of recombinant plasmid DNA, Restriction mapping
9. Polymerase Chain reaction by using standard 16srRNA eubacterial primers
10. RFLP analysis of the PCR product
11. Transformation of yeast *Saccharomyces cerevisiae*

Cell Biology

1. Microscopy – Bright field, dark field, phase contrast, fluorescence microscopy, visit to Electron microscope and Confocal microscope facilities.
2. Histology – Hand-sectioning of stem and leaf, saffranin and fast green staining.
3. Microtomy - fixing of tissues, dehydration, wax-embedding, sectioning and staining.
4. Mitosis – Onion root tips
5. Meiosis – Insect testes

Plant Biology

1. Plant DNA extraction, digestion of DNA with restriction enzymes, agarose gel electrophoresis.
2. Polymerase chain reaction to amplify a plant gene.
3. Homogenization of leaves, sub-cellular fractionation by differential centrifugation, chloroplast purification, SDS-PAGE analysis of chloroplast proteins.
4. RNA extraction, Agarose gel electrophoresis of RNA, RT-PCR analysis of a plant gene.

Immunology & Molecular Diagnostics - 3 Credits

Unit I

Immunology- fundamental concepts and anatomy of the immune system

Components of innate and acquired immunity; Phagocytosis, complement and inflammatory responses; Haematopoesis; Organs and cells of the immune system- primary and secondary lymphoid organs; Mucosal and Cutaneous associated Lymphoid tissue.(MALT&CALT); Antigens - immunogen,haptens; Major Histocompatibility Complex - MHC genes, MHC and immune responsiveness and disease susceptibility; HLA typing

Unit II

Immune responses generated by B and T lymphocytes

Immunoglobulins - basic structure, fine structure, class and subclasses of immunoglobulin, antigenic determinants on immunoglobulins. Multigene organization of immunoglobulin genes, B-cell receptor, immunoglobulin superfamily, generation of antibody diversity; T-cell maturation, activation and differentiation, T-cell receptors, cell-mediated immune responses, ADCC; Cytokine- properties, receptors and therapeutic uses; Antigen processing and presentation- endogenous antigens, exogenous antigens, bacterial and viral antigens and super-antigens.

Unit III

Clinical Immunology

Vaccine technology- Role and properties of adjuvants, Recombinant DNA and protein based vaccines, plant-based vaccines, reverse vaccinology; Antibody genes and antibody engineering- chimeric and hybrid monoclonal antibodies, Bacteria, viral, fungal and parasitic infections (with examples from each group)

Unit IV

Different Methods of Diagnosis

Application of restriction endonuclease analysis for identification of pathogens; Southern and Northern hybridization assays for diagnosis of animal and poultry diseases; Application of PCR for diagnosis of infectious diseases of cattle, buffalo, sheep, goats, dogs, equines, swine and poultry; Detection of pathogens in foods/feeds including vegetables, fruits, milk, meat, eggs, animal and poultry feeds; Advancements in diagnostic technology including nucleic acid and protein arrays, biosensors and nanotechnology.

Unit V

Preparation of antisera and Basic detection techniques

Antiserum production against plant hormones; Plant hormone immunoassay; Polyclonal antisera; Monoclonal antisera; Phage displayed recombinant antibodies; Purification of immunoglobulins and antibody fragments; Precipitation and agglutination reactions; Immunodiffusion tests; Immunoelectrophoresis; Labeled antibody techniques and Immunoelectron Microscopy

Texts/References

1. Kuby, RA Goldsby, Thomas J. Kindt, Barbara, A. Osborne Immunology, 6th Edition, Freeman, 2002.
2. Roitt IM, Brostoff J and Male D, Clinical Immunology, 6th Edition, Gower Medical Publishing, London, 2002.
3. Janeway et al., Immunobiology, 4th edition, Current biology publications. 1999.
4. Paul, Fundamental of Immunology, 4th edition, Lippencott Raven, 1999
5. Decker J & Reischl, U. Molecular Diagnosis of infectious diseases, Humana Press, 2004.
6. Rao JR, Fleming CC & Moore JE., Molecular Diagnostics, Horizon Bioscience, 2006.

Microbiology & Industrial Applications - 3 Credits

Unit I

Microbial Diversity & Systematics

Classical and modern methods and concepts; Domain and Kingdom concepts in classification of microorganisms; Criteria for classification; Classification of Bacteria according to Bergey's manual; Molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), Amplified rDNA Restriction Analysis and Terminal Restriction Fragment Length Polymorphism (T-RFLP) in assessing microbial diversity; 16S rDNA sequencing and Ribosomal Database Project.

Unit II

Microbial Growth & Physiology

Ultrastructure of Archaea (Methanococcus); Eubacteria (*E.coli*); Unicellular Eukaryotes (Yeast) and viruses (Bacterial, Plant, Animal and Tumor viruses); Microbial growth: Batch, fed-batch, continuous kinetics, synchronous growth, yield constants, methods of growth estimation, stringent response, death of a bacterial cell.

Microbial physiology: Physiological adoption and life style of Prokaryotes; Unicellular Eukaryotes and the Extremophiles (with classical example from each group)

Unit III

Microbial Interactions and Infection

Host–Pathogen interactions; Microbes infecting humans, veterinary animals and plants; Pathogenicity islands and their role in bacterial virulence

Unit IV

Microbes and Environment

Role of microorganisms in natural system and artificial system; Influence of Microbes on the Earth's Environment and Inhabitants; Ecological impacts of microbes; Symbiosis (Nitrogen fixation and ruminant symbiosis); Microbes and Nutrient cycles; Microbial communication system; Quorum sensing; Microbial fuel cells; Prebiotics and Probiotics; Vaccines

Unit V

Industrial Applications

Basic principles in bioprocess technology; Media Formulation; Sterilization; Thermal death kinetics; Batch and continuous sterilization systems; Primary and secondary metabolites; Extracellular enzymes; Biotechnologically important intracellular products; exopolymers; Bioprocess control and monitoring variables such as temperature, agitation, pressure, pH

Microbial processes-production, optimization, screening, strain improvement, factors affecting down stream processing and recovery; Representative examples of ethanol, organic acids, antibiotics etc.
Enzyme Technology-production, recovery, stability and formulation of bacterial and fungal enzymes-amylase, protease, penicillin acylase, glucose isomerase; Immobilised Enzyme and Cell based biotransformations-steroids, antibiotics, alkaloids, enzyme/cell electrodes.

Texts/References

1. Pelczar MJ Jr., Chan ECS and Kreig NR., Microbiology, 5th Edition, Tata McGraw Hill, 1993.
2. Maloy SR, Cronan JE Jr., and Freifelder D, Microbial Genetics, Jones Bartlett Publishers, Sudbury, Massachusetts, 2006.
3. Crueger and A Crueger, (English Ed., TDW Brock); Biotechnology: A textbook of Industrial Microbiology, Sinaeur Associates, 1990.
4. G Reed, Prescott and Dunn's, Industrial Microbiology, 4th Edition, CBS Publishers, 1987.
5. M.T. Madigan and J.M. Martinko, Biology of Microorganisms, 11th Edition, Pearson Prentice Hall, USA, 2006

Genetic Engineering - 3 Credits

Unit I

Basics Concepts

DNA Structure and properties; Restriction Enzymes; DNA ligase, Klenow enzyme, T4 DNA polymerase, Polynucleotide kinase, Alkaline phosphatase; Cohesive and blunt end ligation; Linkers; Adaptors; Homopolymeric tailing; Labeling of DNA: Nick translation, Random priming, Radioactive and non-radioactive probes, Hybridization techniques: Northern, Southern and Colony hybridization, Fluorescence in situ hybridization; Chromatin Immunoprecipitation; DNA-Protein Interactions-Electromobility Phoretic shift assay; DNaseI footprinting; Methyl interference assay

Unit II

Cloning Vectors

Plasmids; Bacteriophages; M13 mp vectors; PUC19 and Bluescript vectors, Phagemids; Lambda vectors; Insertion and Replacement vectors; EMBL; Cosmids; Artificial chromosome vectors (YACs; BACs); Animal Virus derived vectors-SV-40; vaccinia/bacculo & retroviral vectors; Expression vectors; pMal; GST; pET-based vectors; Protein purification; His-tag; GST-tag; MBP-tag etc.; Intein-based vectors; Inclusion bodies; Methodologies to reduce formation of inclusion bodies; Baculovirus and pichia vectors system, Plant based vectors, Ti and Ri as vectors, Yeast vectors, Shuttle vectors

Unit III

Cloning Methodologies

Insertion of Foreign DNA into Host Cells; Transformation; Construction of libraries; Isolation of mRNA and total RNA; cDNA and genomic libraries; cDNA and genomic cloning; Expression cloning; Jumping and hopping libraries; Southwestern and Far-western cloning; Protein-protein interactive cloning and Yeast two hybrid system; Phage display; Principles in maximizing gene expression

Unit IV

PCR and Its Applications

Primer design; Fidelity of thermostable enzymes; DNA polymerases; Types of PCR – multiplex, nested, reverse transcriptase, real time PCR, touchdown PCR, hot start PCR, colony PCR, cloning of PCR products; T-vectors; Proof reading enzymes; PCR in gene recombination; Deletion; addition; Overlap extension; and

SOEing; Site specific mutagenesis; PCR in molecular diagnostics; Viral and bacterial detection; PCR based mutagenesis, Mutation detection: SSCP, DGGE, RFLP, Oligo Ligation Assay (OLA), MCC (Mismatch Chemical Cleavage, ASA (Allele-Specific Amplification), PTT (Protein Truncation Test)

Unit V

Sequencing methods; Enzymatic DNA sequencing; Chemical sequencing of DNA; Automated DNA sequencing; RNA sequencing;

Chemical Synthesis of oligonucleotides; Introduction of DNA into mammalian cells; Transfection techniques; Gene silencing techniques; Introduction to siRNA; siRNA technology; Micro RNA; Construction of siRNA vectors; Principle and application of gene silencing; Gene knockouts and Gene Therapy; Creation of knock out mice; Disease model; Somatic and germ-line therapy- in vivo and ex-vivo; Suicide gene therapy; Gene replacement; Gene targeting; Transgenics; cDNA and intragenic arrays; Differential gene expression and protein array.

Text/References:

1. S.B. Primrose, R.M. Twyman and R.W.Old; Principles of Gene Manipulation. 6th Edition, S.B.University Press, 2001.
2. J. Sambrook and D.W. Russel; Molecular Cloning: A Laboratory Manual, Vols 1-3, CSHL, 2001.
3. Brown TA, Genomes, 3rd ed. Garland Science 2006
4. Selected papers from scientific journals.
5. Technical Literature from Stratagene, Promega, Novagen, New England Biolab etc.

Genetics - 3 Credits

Unit I

Bacterial mutants and mutations

Isolation; useful phenotypes (auxotrophic; conditional lethal; resistant); Mutation rate; Types of mutations (base pair changes; frameshift; insertions; deletions; tandem duplication); Reversion vs. suppression; Mutagenic agents; Mechanisms of mutagenesis; Assay of mutagenic agents (Ames test)

Gene transfer in bacteria

History; Transduction – generalized and specialized; Conjugation – F, F′, Hfr; F transfer; Hfr-mediated chromosome transfer; Transformation – natural and artificial transformation; Merodiploid generation; Gene mapping; Transposable genetic elements; Insertion sequences; Composite and Complex transposons; Replicative and non-replicative transposition; Genetic analysis using transposons.

Unit II

Bacteriophages and Plasmids

Bacteriophage – structure; assay; Lambda phage – genetic map, lysogenic and lytic cycles; Gene regulation; Filamentous phages such as M13; Plasmids – natural plasmids; their properties and phenotypes; Plasmid biology - copy number and its control; Incompatibility; Plasmid survival strategies; Antibiotic resistance markers on plasmids (mechanism of action and resistance); Genetic analysis using phage and plasmid

Restriction-modification systems

History; types of systems and their characteristics; Methylation-dependent restriction systems; applications.

Unit III

Mendelian Genetics

Introduction to human genetics; Background and history; Types of genetic diseases; Role of genetics in medicine; Human pedigrees; Patterns of single gene inheritance - autosomal recessive; autosomal dominant; X linked inheritance; Complicating factors - incomplete penetrance; variable expression; Multiple alleles; Co dominance; Sex influenced expression; Hemoglobinopathies - Genetic disorders of hemoglobin and their diseases.

Non Mendelian inheritance patterns

Mitochondrial inheritance; genomic imprinting; Lyon hypothesis; isodisomy. Complex inheritance - genetic and environmental variation; Heritability; Twin studies; Behavioral traits; Analysis of quantitative and qualitative traits

Unit IV

Cytogenetics

Cell division and errors in cell division; Non disjunction; Structural and numerical chromosomal abnormalities – deletion; duplication; translocation; Sex determination; Role of Y chromosome; Genetic recombination; Disorders of sex chromosomes and autosomes; Molecular cytogenetics – Fluorescence In Situ Hybridization (FISH); Comparative Genomic Hybridization (CGH).

Developmental genetics

Genes in early development; Maternal effect genes; Pattern formation genes; Homeotic genes; and Signaling and adhesion molecules.

Immunogenetics

Major histocompatibility complex; Immunoglobulin genes - tissue antigen and organ transplantation; Single gene disorders of immune system.

Unit V

Genetic variation

Mutations; kinds of mutation; agents of mutation; genome polymorphism; uses of polymorphism.

Gene maping and human genome project

Physical mapping; linkage and association

Population genetics and evolution

Phenotype; genotype; gene frequency; Hardy-Weinberg law; Factors distinguishing Hardy-Weinberg equilibrium; Mutation selection; Migration; Gene flow; Genetic drift, Human genetic diversity; Origin of major human groups.

Texts/References

1. S.R. Maloy, J.E. Cronan, D. Friefelder, Microbial Genetics, 2nd Edition, Jones and Bartlett Publishers, 1994.
2. N. Trun and J. Trempy, Fundamental Bacterial Genetics, Blackwell publishing, 2004.
3. Strachan T and Read A P, Human molecular genetics, 3rd Edition Wiley Bios, 2006.
4. Mange E J and Mange A. P., Human genetics, 2nd Edition, Sinauer Associates publications, 1999.
5. Hartl L D and Jones B, Analysis of genes and genomes, 3rd Edition, Jones and Bartlett Publishers, 1994.

Genomics and Proteomics - 3 Credits

Unit I

Introduction

Structural organization of genome in Prokaryotes and Eukaryotes; Organelle DNA-mitochondrial, chloroplast; DNA sequencing-principles and translation to large scale projects; Recognition of coding and non-coding sequences and gene annotation; Tools for genome analysis-RFLP, DNA fingerprinting, RAPD, PCR, Linkage and Pedigree analysis-physical and genetic mapping.

Unit II

Genome sequencing projects

Microbes, plants and animals; Accessing and retrieving genome project information from web; Comparative genomics, Identification and classification using molecular markers-16S rRNA typing/sequencing, EST's and SNP's.

Unit III

Proteomics

Protein analysis (includes measurement of concentration, amino-acid composition, N-terminal sequencing); 2-D electrophoresis of proteins; Microscale solution isoelectricfocusing; Peptide fingerprinting; LC/MS-MS for identification of proteins and modified proteins; MALDI-TOF; SAGE and Differential display proteomics, Protein-protein interactions, Yeast two hybrid system.

Unit IV

Pharmacogenetics

High throughput screening in genome for drug discovery-identification of gene targets, Pharmacogenetics and drug development

Unit V

Functional genomics and proteomics

Analysis of microarray data; Protein and peptide microarray-based technology; PCR-directed protein *in situ* arrays; Structural proteomics

Texts/References

1. Voet D, Voet JG & Pratt CW, Fundamentals of Biochemistry, 2nd ed. Wiley 2006
2. Brown TA, Genomes, 3rd ed. Garland Science 2006
3. Campbell AM & Heyer LJ, Discovering Genomics, Proteomics and Bioinformatics, 2nd ed. Benjamin Cummings 2007
4. Primrose S & Twyman R, Principles of Gene Manipulation and Genomics, 7th ed, Blackwell [2006].
6. Glick BR & Pasternak JJ, Molecular Biotechnology, 3rd ed, ASM Press [1998]

Lab on Immunology - 2 Credits

1. Selection of animals, Preparation of antigens, Immunization and methods of bleeding, Serum separation, Storage.

2. Antibody titre by ELISA method.
3. Double diffusion, Immuno-electrophoresis and Radial Immuno diffusion.
4. Complement fixation test.
5. Isolation and purification of IgG from serum or IgY from chicken egg.
6. SDS-PAGE, Immunoblotting, Dot blot assays
7. Blood smear identification of leucocytes by Giemsa stain
8. Separation of leucocytes by dextran method
9. Demontsration of Phagocytosis of latex beads
10. Separation of mononuclear cells by Ficoll-Hypaque
11. Flowcytometry, identification of T cells and their subsets
12. Lymphoproliferation by mitogen / antigen induced
13. Lymphnode Immunohistochemistry (direct and indirect peroxidase assay)
14. Hybridoma technology and monoclonal antibody production.
15. Immunodiagnostics using commercial kits

Lab on Microbiology - 3 Credits

1. Sterilization, disinfection, safety in microbiological laboratory.
2. Preparation of media for growth of various microorganisms.
3. Identification and culturing of various microorganisms.
4. Staining and enumeration of microorganisms.
5. Growth curve, measure of bacterial population by turbidometry and studying the effect of temperature, pH, carbon and nitrogen.
6. Assay of antibiotics production and demonstration of antibiotic resistance.
7. Isolation and screening of industrially important microorganisms.
8. Determination of thermal death point and thermal death time of microorganisms.

Lab on Genetic Engineering - 3 Credits

1. Isolation of genomic DNA from *Bacillus subtilis** genome.
2. PCR amplification of *scoC* gene and analysis by agarose gel electrophoresis
3. Preparation of plasmid, pET-28a from *E.coli* DH5α and gel analysis.

4. Restriction digestion of vector (gel analysis) and insert with NcoI and XhoI
5. a. Vector and Insert ligation
   b. Transformation in *E.coli* DH5α.
6. Plasmid isolation and confirming recombinant by PCR and RE digestion.
7. Transformation of recombinant plasmid in *E.coli* BL21 (DE3) strain
8. Induction of ScoC protein with IPTG and analysis on SDS-PAGE
9. Purification of protein on Ni-NTA column and analysis of purification by SDS-PAGE
10. a. Random Primer labeling of scoC with Dig-11-dUTP
    b. Southern hybridization of *B. subtilis* genome with probe and non-radioactive detection.

*Any other bacterial strain can be used.

Tissue Culture & Transgenic Technologies - 3 Credits

Unit I

Totipotency; Tissue culture media; Plant hormones and morphogenesis; Direct and indirect organogenesis; Direct and indirect embryogenesis; Cell suspension culture; Micropropagation – shoot tip culture, somatic embryos, artificial seeds; Applications of tissue culture; Virus elimination by shoot tip culture; Wide hybridization and embryo culture; Anther culture and dihaploids.

Unit II

Large-scale cell suspension culture; Production of alkaloids and other secondary metabolites; Protoplast culture, Plant cell wall structure and cell wall hydrolyzing enzymes; Protoplast isolation and purification; Protoplast viability test; Protoplast culture; Protoplast fusion; Somatic hybrids; Cybrids.

Unit III

Direct transformation of protoplasts using PEG; electroporation; Transformation by particle bombardment; Assembly of particle gun; Microprojectile preparation and bombardment; Chloroplast transformation by particle bombardment

Unit IV

Agrobacterium biology; Ti plasmid-based transformation; crown gall and hairy root disease, Ti and Ri plasmids, T-DNA genes, borders, overdrive, chromosomal and Ti plasmid virulence genes and their functions, *vir* gene induction, mechanism of T-DNA transfer; Ti pasmid vectors, *vir* helper plasmid, super virulence and monocot transformation, binary vector; Floral dip transformation; Promoters and polyA signals; Protein targeting signals; Plant selectable markers; Reporter genes; Positive selection; Selectable marker elimination; Transgene silencing; Strategies to avoid transgene silencing.

Unit V

Genetic engineering of crops; Commercial status of transgenic plants; Herbicide resistance, glyphosate, sulfonyl urea, phosphinothricin, atrazine; Pest resistance, *Bt* toxin, synthetic *Bt* toxin; Protease inhibitor; GNA and other lectins; α-amylase inhibitor; nematode resistance; Genetic engineering for male sterility-Barnase-Barstar; Delay of fruit ripening; polygalacturanase, ACC synthase, ACC oxidase; Improved seed storage proteins; Improving and altering the composition of starch and plant oils; Golden rice for β-carotene accumulation; Production of antibodies and pharmaceuticals in plants; Bio-safety concerns of transgenic plants.

Texts/References

1. R.H.Smith, Plant Tissue Culture: Techniques and Experiments, Academic Press, San Diego. 1992.
2. B.B. Buchanan, W. Gruissen and R.L. Jones (eds), Biochemistry and Molecular Biology of Plants, American Society of Plant Biology, Rockville, USA. 2000
3. M. J. Chrispeels and D.F. Sadava (eds), Plants, Genes and Crop Biotechnology, 2nd Edition, Jones and Barlett Press, 2003
4. J.H. Hammond, P. Mcgarvey, and V. Yusibov (eds), Plant Biotechnology, Springer Verlag, Heidelberg. 2000
5. H.K. Das (ed)., Text Book of Biotechnology, Wiley India Pvt Ltd. New Delhi, 2004

Bioinformatics and Biocomputation - 3 Credits

Unit I

Introduction theory and Biology

Definitions; History; Contentions with education; Agriculture; Medicine; Structural biology & Pharmaceutical industry; Scope of bioinformatics; Concepts of entropy; Shannon's formula; equi-probability and independence-application to DNA and protein; Introduction to computers and high performance computing (HPC)

Unit II

Databases

a. Sequence database: Concepts of database; Key features of database system; History; Database management systems; Database types; Introduction to query language; Index; Forms and Reports.

b. Structure databases: Biological databases: plant, animal, microbial, viral and organism databases and biodiversity; Sequence and structure databases; Advanced concepts and approaches in database construction and management; Knowledge discovery and data mining; Introduction to Protein Data Base (PDB); Nucleic acid databases at NCBI.

Unit III

Genetic algorithms, sequence alignment and search Introduction to genetic algorithms; Concepts and approaches; Alignment algorithms: global, linear space, map and multiple sequences; Evolutionary basis of sequence alignment; Score matrices; Statistical significance of alignment; Low-complexity regions; Repetitive elements; Motifs and patterns; Multiple and progressive alignment methods; Hidden Markov Model (HMM) and threading theory; Database similarity searching; FASTA and BLAST; Dynamic programming

Unit IV

Phylogenetic analysis: Elements of phylogenetic models; Phylogenetic data analysis; tree building and tree evolution.

Unit V

Comparative structural and functional genomics: Genome and Comparative Genome sequencing-methods and platforms; Nature of raw genome sequence data, Expressed sequence tags (ESTs), SAGE, MPSS and 454 sequence gene tags, Polymorphism, DNA chips and comparative genomics; Genome annotation-approaches and concepts; Genome-wide maps from large community of data; Use of sequences in drug design and prediction of forms; Cluster analysis; Neural networks and artificial intelligence.

Texts/References

1. David W. Mount. Bioinformatics: Sequence and Genome Analysis 2nd Edition, CSHL Press, 2004.
2. A. Baxevanis and F. B. F. Ouellette, Bioinformatics: a practical guide to the analysis of genes and proteins, 2nd Edition, John Wiley, 2001.
3. Jonathan Pevsner, Bioinformatics and Functional Genomics, 1st Edition, Wiley-Liss. 2003.
4. P. E. Bourne and H. Weissig. Structural Bioinformatics. Wiley. 2003.
5. C. Branden and J. Tooze. Introduction to Protein Structure, 2nd Edition, Garland Publishing, 1999.

Molecular Breeding - 3 Credits

Unit I

Plant Genome – Nuclear and cytoplasmic; Significance of organelle genomes; Genome size and sequence components; Modern gene concept - Gene structure, structural and functional genes.

Unit II

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

Gene flow in plants – Development of mapping population - Marker Assisted Selection (MAS), screening and validation; Trait related markers and characterization of genes involved; Mapping genes on specific chromosomes; QTL mapping; Gene pyramiding; Transcript mapping techniques. Development of ESTs

Unit IV

Molecular markers for plant genotyping and germplasm analysis; Fidelity analysis; settling IPR issues; Marker Assisted Breeding in transgenics – herbicide resistance; Pest and disease resistance; Quality enhancement etc.

Unit V

Recent advances – Non gel based techniques for plant genotyping – Homogenous assays – Qualitative/Real Time assays; DNA Chip and its technology.

Texts/References

1. Anolles, G. C. and Gresshoff, P.M., DNA markers – protocols, applications and overviews. Wiley – Liss, New York, 1997
2. Clark, D. P., Molecular Biology, Elsevier, USA, 2005.
3. Henry R. J., Plant Genotyping: The DNA fingerprinting of plants. CABI, New Delhi, 2005.
4. Patterson, Molecular dissection of complex traits, CRC Publications, Washington, 1998.
5. Purohit, S. S., Biotechnology – Fundamentals and Applications, 8th Edition, Agrobios, India, 2007.

Abiotic and Biotic Stress Biology - 3 Credits

Unit I

Review of plant cell structure and function; Review of water uptake; Introduction to plant nutrition; Mineral availability- uptake of minerals; Plant response to nutrients;– Phytohormones - Roles of auxins, cytokinins, gibberillins, abscisic acid, ethylene, jasmonates

Unit II

Introduction to light - properties and responses – Photoperiodism; Canopy response to light - Canopy closure and yield potential - Red and Far Red light – Photomorphorgeneis, Phytochrome responses, Blue light responses

Unit III

Photosynthesis and photorespiration - Review of C3, C4 and CAM photosynthesis - Stomatal mechanics and mechanisms - Plants and Clocks, Circadian rhythms, Temperature Responses, Vernalization and dormancy

Unit IV

Abiotic stress – Acclimation and crop adaptation to water stress – salinity stress – temperature stress – heat and cold – Photo oxidative stress – nutrient stress – heavy metal stress – stress signaling - metabolite engineering for abiotic stress tolerance – functional genomics of stress tolerance

Unit V

Biotic stress - plant response to pathogens and herbivores – biochemical and molecular basis of host plant resistance – toxins of fungi and bacteria – systemic and induced resistance – pathogen derived resistance – signaling - gene for gene hypothesis – genetic engineering for biotic stress resistance – gene pyramiding

Texts/References

1. U. Chakraborty, Bishwanath Chakraborty, 2005. Stress biology, Vidhyasekaran, P. 2007. Narosa Publishing House
2. Handbook of molecular technologies in crop disease management, Haworth Food & Agricultural Products Press, New York.462 p
3. Taiz and Zeiger, Plant Physiology, 3rd Edition, Panima Publishing Corporation, New Delhi, 2003.
4. Buchnan, B. B., Gruissem, W. and Jones, R. L., Biochemistry and molecular biology of plants. American Society for Plant Physiologists, Rockville, USA. 2000.
5. Gatehouse, A. M .R., Hilder, V. A. and Boulter, D., Plant Genetic manipulation for crop protection In: Biotechnology in Agriculture Series (Eds.) Vol. 7 CAB International, Wallingford, UK. 266p. 1992
6. Panda N. and G.S.Khush, Host plant resistance to insects. CAB International, Walling Ford. 431p, 1995
7. Persely, G. J. (Ed.), Biotechnology for integrated pest management. CAB International, Wallingford, UK. 475p, 1996.
8. Persely, G. J. (Ed.), Biotechnology for integrated pest management. CAB International, Wallingford, UK. 475p, 1996
9. Slater, A., Scott, N. and Fowler, M., Plant biotechnology -The genetic manipulations of plants. Oxford University press. 346p. 2003.
10. Vidhyasekaran, P., Fungal pathogenesis in plants and crops: Molecular biology and host defense mechanisms, Marcel Dekkar Inc., New York. 624p, 1997
11. Vidhyasekaran, P., Bacterial Disease Resistance in Plants: Molecular Biology and Biotechnological Applications, Haworth Food & Agricultural Products Press, New York.452p, 2005.
12. Zuckerman B.M. and Rohde, R. A. (Eds.), Plant parasitic Nematodes, Vol. III, Academic press, London 508p. 1981.
13. Pessarakli, M., Handbook of Plant and Crop stress, 2nd Edition, Marcel Dekker Inc. New York1999
14. K.V. Madhava Rao, A.S. Raghavendra and K. Janardhan Reddy, Physiology and Molecular Biology of Stress Tolerance in Plants. Springer, Netherlands. 2006
15. Satoh, K. and Murata, N., Stress responses of photosynthetic organisms, Elsevier, Amsterdam. 1998

IPR, Biosafety & Biodiversity - 3 Credits

Unit I

Introduction to Intellectual Property

Types of IP: Patents, Trademarks, Copyright & Related Rights, Industrial Design, Traditional Knowledge, Geographical Indications, Protection of New GMOs; International framework for the protection of IP

IP as a factor in R&D; IPs of relevance to Biotechnology and few Case Studies; Introduction to History of GATT, WTO, WIPO and TRIPS

Unit II

Concept of 'prior art'

Invention in context of "prior art"; Patent databases; Searching International Databases; Country-wise patent searches (USPTO, EPO, India etc.); Analysis and report formation

Basics of Patents

Types of patents; Indian Patent Act 1970; Recent Amendments; Filing of a patent application; Precautions before patenting-disclosure/non-disclosure; WIPO Treaties; Budapest Treaty; PCT and Implications; Role of a Country Patent Office; Procedure for filing a PCT application

Unit III

Patent filing and Infringement

Patent application- forms and guidelines, fee structure, time frames; Types of patent applications: provisional and complete specifications; PCT and convention patent applications; International patenting-requirement, procedures and costs; Financial assistance for patenting-introduction to existing schemes; Publication of patents-gazette of India, status in Europe and US

Patenting by research students, lecturers and scientists-University/organizational rules in India and abroad, credit sharing by workers, financial incentives

Patent infringement- meaning, scope, litigation, case studies and examples

Unit IV

Biosafety

Introduction; Historical Backround; Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels; Biosafety Levels of Specific Microorganisms; Recommended Biosafety Levels for Infectious Agents and Infected Animals; Biosafety guidelines - Government of India; Definition of GMOs & LMOs; Roles of Institutional Biosafety Committee, RCGM, GEAC etc. for GMO applications in food and agriculture; Environmental release of GMOs; Risk Analysis; Risk Assessment; Risk management and communication; Overview of National Regulations and relevant International Agreements including Cartagena Protocol.

Unit V

Biodiversity

Biodiversity Legislation in India; Indian Biodiversity Act and provisions on crop genetic resources. Convention on Biological Diversity (CBD) and Cartagena protocol on Biosafety; Biodiversity Act 2002; Agricultural biodiversity; International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA); Conservation strategies for seed gene bank; Climate change and conservation of plant genetic resources; Global efforts for management of crop genetic resources; Strategies on PVFR and Biodiversity Acts; Impact of GE crops on Biodiversity. Functions of International union for the protection of new varieties of plants (UPOV); International treaties relating to Biodiversity; Tutorials shall comprise of Seminars, Group Discussions based on recent case studies.

Important Links

http://www.w3.org/IPR/

http://www.wipo.int/portal/index.html.en

http://www.ipr.co.uk/IP_conventions/patent_cooperation_treaty.html

www.patentoffice.nic.in

www.iprlawindia.org/ - 31k - Cached - Similar page

http://www.cbd.int/biosafety/background.shtml

http://www.cdc.gov/OD/ohs/symp5/jyrtext.htm

http://web.princeton.edu/sites/ehs/biosafety/biosafetypage/section3.html

Lab I - 3 Credits

Plant Tissue Culture, Transgenic Technologies and Molecular Breeding

1. Preparation of Murashige and Skoog medium, stocks of macronutrients, micronutrients, vitamins and hormones, autoclaving, filter sterilization of hormones and antibiotics.
2. Surface-sterilization of seeds, establishment of axenic plants, acclimatization of tissue culture plants and establishment in greenhouse.
3. Callus induction in tobacco leaf discs, regeneration of shoots, root induction, role of hormones in morphogenesis.
4. Isolation of plasmids with reporter (*gus*) gene, preparation of microprojectiles, transformation using a particle gun, GUS staining.
5. Leaf disc transformation using *Agrobacterium*, establishment of transgenic plants, and GUS staining or GFP viewing.
6. DNA extraction from transgenic plants, DNA estimation, PCR analysis, Southern blot analysis to prove T-DNA integration, RT-PCR to study transgene expression, western blotting to study the accumulation of transgene-encoded protein.
7. DNA finger printing methods, RAPD, SSR.

Lab II - 3 Credits

Bioinformatics & Biocomputation

Post sequence analysis, introduction to genomic data and data organization, comparative genome organization, comparative genome analysis and post-genomic biology. Homologous sequences and scoring matrices. Sequence comparison and multiple sequence alignments. Homologous search algorithms: BLAST, FASTA and SENSEI. Sequence to trees, metabolic reconstruction. Integration of sequence and metabolic data. Homologous modeling-protein structure prediction. Related software packages

Biotic & Abiotic Stress Biology

1. Laboratory techniques to measure water and nutrient uptake in plants.
2. Methods to measure various physiological processes (photosynthesis, transpiration, gas exchange, stomatal conductance, epicuticular wax, Chlorophyll stability index, cell membrane stability) in plants – methods to quantify endogenous hormones (auxin, ABA etc.,) and proline in plants
3. Rapid screening tests for abiotic stress tolerance (drought, salinity - PEG, Mannitol & NaCl)
4. Estimation of antioxidants and antioxidant enzymes - Ascorbate, Superoxide dismutase, Catalase, and Peroxidase
5. Major insect, nematode pests and diseases of crop plants – study of phytotoxaemia and other categories of insect damage in crop plants
6. Toxin – production, extraction, purification, selection of toxin resistant calli, assay of toxins to pathogens, bioassay for PR protein, culturing and isolation of *Bt* - bioassay techniques