BSC. (HONS.) BOTANY
SEMESTER - VI
Category-I
Botany (H) Courses for Undergraduate Programme of study with Botany as a Single Core Discipline

DISCIPLINE SPECIFIC CORE COURSE - 16: Plant Biotechnology

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Plant Biotechnology **DSC-16**	4	**2**	**0**	**2**	Class XII pass with Biology/ Biotechnology	Nil

Learning objective:

- to provide knowledge of techniques used in plant biotechnology and their application.

Learning outcomes: At the end of the course the students will be able to:

14. understand basic concepts, principles and methods in plant biotechnology.
15. explain the use of acquired knowledge in biotechnological, pharmaceutical, medical, ecological and agricultural applications.

Unit 1: Introduction to Biotechnology **02 Hours**

Historical timeline; sectors of Biotechnology, brief overview of techniques and methods in Biotechnology.

Unit 2: Plant Tissue Culture **08 Hours**

Historical perspective (Major contributions of Haberlandt, Laibach, White, Reinert and Steward, Murashige and Skoog, Cocking, Guha and Maheshwari, Bhojwani, Morel and Martin); types and composition of media: roles of nutrients (major and minor), vitamins, hormones and others (coconut water, activated charcoal); plasticity and totipotency; regeneration: organogenesis (direct and indirect) and embryogenesis (somatic and zygotic); protoplast isolation, culture and fusion; tissue culture applications (micropropagation, androgenesis, haploids, triploids, cybrids, production of virus-free plants).

Unit 3: Recombinant DNA technology **07 Hours**

Restriction Endonucleases (History, Types I - IV, biological roles and applications); modifying enzymes and their applications (nucleases, ligases, alkaline phosphatase, polynucleotide kinase), introduction to prokaryotic and eukaryotic cloning vectors: pBR322, pUC18, pUC19, BACs, Lambda phage, YACs. Gene Cloning: Restriction digestion of DNA, elution of DNA from agarose gels, ligation, bacterial transformation and selection of

recombinant clones (alpha complementation, antibiotic selection, restriction enzyme based selection)

Unit 4: Genetic transformation of Plants **05 Hours**

Methods of gene transfer to plants: *Agrobacterium*-mediated transformation (Ti plasmids, development of binary vectors), Direct gene transfer by Electroporation, Microinjection, Microprojectile bombardment; selection of transgenic plants: selectable marker genes (Positive selection markers – antibiotic- and herbicide-resistance conferring genes) and reporter genes (Luciferase, GUS, GFP); Introduction to genome editing.

Unit 5: Applications **08 Hours**

Pest resistant (Bt-cotton) and herbicide resistant plants (RoundUp Ready™ soybean); Transgenic crops with improved quality traits (Flavr Savr™ tomato. Golden™ rice); Improved horticultural varieties (Moondust carnations); Bioremediation (Superbug); Edible vaccines; Biosafety of transgenic plants.

Practicals **60 hours**

4. Preparation of Murashige & Skoog's (MS) medium.
5. Initiation of axenic cultures- seed sterilisation and inoculation
6. Micropropagation (shoot induction) using leaf and/or nodal explants of tobacco/*Datura*/ *Brassica* etc.
7. Study of anther culture, embryo and endosperm culture, somatic embryogenesis usingdigital resources.
8. Preparation of artificial seeds.
9. Induction of callus and analysis of effects of growth regulators (Auxin and Cytokinin) on *in vitro* regeneration using tobacco leaf explant.
10. Preparation of chemically competent cells of *E. coli*.
11. Transformation of *E. coli* with plasmid DNA by heat shock method.
12. Restriction digestion and gel electrophoresis of plasmid DNA.
13. Construction of restriction map of circular and linear DNA from the data provided.
14. Visit to a research laboratory.

Suggested Readings:

5. Slater, A., Scott, N. W. & Fowler, M. R. (2010) Plant Biotechnology: The Genetic Manipulation of Plants. 2nd edn. New York, USA: Oxford University Press Inc.
6. Snustad, D.P., Simmons, M.J. (2010) Principles of Genetics, 5th edition. Chichester, England: John Wiley and Sons.
7. Brown, T. A. (2020) Gene Cloning & DNA Analysis: An Introduction. 8th edn. UK: Wiley Blackwell.
8. Primrose, S. B. & Twyman, R.M. (2006). Principles of Gene Manipulation and Genomics. 7th edn. Victoria, Australia: Blackwell Publishing.
9. Bhojwani, S.S., Razdan, M.K., (1996). Plant Tissue Culture: Theory and Practice. Amsterdam, Netherlands: Elsevier Science.

Additional Resources:

9. Bhojwani, S.S. and Dantu, P.K. (2013). Plant Tissue Culture: An Introductory Text. Springer New Delhi Heidelberg New York Dordrecht London

10. Glick, B.R., & Patten C. (2022). Molecular Biotechnology: Principles and Applications. 6thedn. Washington, U.S.: ASM Press.
11. Bhojwani, S.S., Bhatnagar, S.P. (2011). The Embryology of Angiosperms, 5th edition. New Delhi, Delhi: Vikas Publication House Pvt. Ltd.
12. Stewart, C.N. Jr. (2008). Plant Biotechnology and Genetics: Principles, Techniques and Applications. New Jersey, U.S.: John Wiley & Sons Inc.
13. Glick, B.R., Pasternak, J. J. & Patten C. (2010). Molecular Biotechnology: Principles and Applications. 4thedn. Washington, U.S.: ASM Press.
14. Glick, B.R., & Patten C. (2017). Molecular Biotechnology: Principles and Applications. 5thedn. Washington, U.S.: ASM Press.

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DISCIPLINE SPECIFIC CORE COURSE – 17: Plant Biochemistry and Metabolism

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Plant Biochemistry and Metabolism **DSC - 17**	**4**	**2**	**0**	**2**	Class XII pass with Biology/ Biotechnology	**Nil**

Learning Objectives:

- To understand different pathways of metabolism in plant cells.
- To understand how various metabolic pathways work in a synchronized manner.

Learning Outcomes: At the end of the course the student will:

6. know the details of carbon assimilation, oxidation, synthesis of ATP- the energy currency of the cell, nitrogen fixation and lipid metabolism.
7. understand the role of enzymes in regulating metabolic pathways for molecules like carbohydrates, lipids and proteins.
8. understand the coordination of various biochemical reactions with reference to cell requirement and its economy.

Unit 1:Concepts in Metabolism **01 Hour**

Introduction, anabolic and catabolic pathways, coupled reactions

Unit 2: **Enzymes** **04 Hours**

Structure, classification and mechanism of action, Michaelis-Menten equation (no derivation), enzyme inhibition (competitive, non-competitive and uncompetitive), allosteric regulation and covalent modulation, factors affecting enzyme activity.

Unit 3:Carbon Assimilation **07 Hours**

Concept of light, absorption and action spectra, photosynthetic pigments (no structural details), PSI, PSII antenna molecules and reaction centres, LHC, photochemical reaction, photosynthetic electron transport, photophosphorylation (cyclic and non-cyclic)

Dark reactions: CO_2 reduction in C3, C4 pathways and CAM, photorespiration

Unit 4: Carbohydrate Metabolism **02 Hours**

Metabolite pool and exchange of metabolites, synthesis and degradation of sucrose and starch (no structural details)

Unit 5:Carbon Oxidation **06 Hours**

Glycolysis, fate of pyruvate- aerobic, anaerobic respiration and fermentation, regulation of glycolysis, oxidative pentose phosphate pathway, oxidative decarboxylation of pyruvate, Krebs cycle and its regulation, amphibolic role of Krebs cycle, mitochondrial electron transport, oxidative phosphorylation, cyanide-resistant respiration

Unit 6: ATP Synthesis **02 Hours**

Mechanism of ATP synthesis-substrate level phosphorylation, oxidative and photophosphorylation, chemiosmosis, ATP synthase

Unit 7: Lipid Metabolism **04 Hours**

Triglycerides: synthesis, degradation through alpha and beta -oxidation, glyoxylate cycle

Unit 8: **Nitrogen Metabolism** **04 Hours**

Nitrate assimilation (NR and NiR), biological nitrogen fixation in legumes (nodulation and role of dinitrogenase) Ammonia assimilation: GS-GOGAT, reductive amination and transamination.

Practicals **60 Hours**

1. Study the activity of urease and the effect of substrate concentration on its activity.
2. Study the effect of pH on the activity of catalase enzyme.
3. Chemical separation of photosynthetic pigments (liquid-liquid partitioning).
15. Study Hill reaction by dye reduction method.
16. Study the law of limiting factors.
17. Compare the rate of respiration in three different parts of a plant.
18. Study the activity of Nitrate reductase in leaves of two different plants.
19. To study the activity of lipases in germinating oil seeds and explain mobilization of lipids during germination.
20. To study the fluorescence in isolated chlorophyll pigments.
21. To study the absorption spectrum of photosynthetic pigments.
22. To study respiratory quotient (RQ).

Suggested Readings:

14. Nelson, D.L., Cox, M.M. (2017). Lehninger Principle of Biochemistry, 7th edition. NewYork, NY: W.H. Freeman, Macmillan learning.
15. Taiz, L., Zeiger, E., Moller, I. M. & Murphy, A. 2018. Plant Physiology and Development, International 6thedn, Oxford University Press, Sinauer Associates, New York, USA.

16. Hopkins, W.G., Huner, N. (2008). Introduction of Plant Physiology, 4th edition. New Jearsey, U.S.: John Wiley and sons.
17. Jones, R., Ougham, H., Thomas, H., Waaland, S. (2013). The molecular life of plants. Chichester, England: Wiley-Blackwell.

Additional Resources:

19. Buchanan, B.B., Gruissem, W. and Jones, R.L. (2015). Biochemistry and Molecular Biology of Plants, 2nd edition. New Jearsey, U.S.: Wiley Blackwell.
20. Kochhar, S.L. & Gujral, S.K. 2020. Plant Physiology: Theory and Applications, 2nd Edition. Cambridge University Press, UK.
21. Bhatla, S.C., Lal, M.A. (2018). Plant Physiology, Development and Metabolism. Singapore: Springer.

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DISCIPLINE SPECIFIC CORE COURSE – 18: Advanced tools & Analytical Techniques in Plant Biology

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Advanced tools & Analytical Techniques in Plant Biology **DSC- 18**	**4**	**2**	**0**	**2**	Class XII pass with Biology/ Biotechnology	**Nil**

Learning Objectives:

- To gain the knowledge on various techniques and instruments used for the study of plant biology

Learning Outcomes: At the end of this course, students will be:

- competent in the basic principles of major techniques used in study of plants
- understand principles and uses of light, confocal, transmission and electron microscopy, centrifugation, spectrophotometry, chromatography, x-ray diffraction technique and chromatography techniques

Unit 1: Imaging and related techniques **06 Hours**

Electron microscopy: Transmission and Scanning electron microscopy, cryofixation, negative staining, shadow casting, freeze-fracture, freeze-etching; Chromosome banding, FISH, GISH, chromosome painting.

Unit 2: Fractionation methods **04 Hours**

Centrifugation: types of rotors, differential and density gradient centrifugation, sucrose density gradient, ultracentrifugation, caesium chloride gradient; marker enzymes for analysis of cellular fractions.

Unit 3: Radioisotopes **04 Hours**

Types of radioisotopes; types of emissions (alpha, beta, gamma radiations); half-life; use of radioisotopes in biological research; auto-radiography; pulse-chase experiment; Biosafety measures and disposal of radioactive material

Unit 4: Spectrophotometry **02 Hours**

Principles and applications of UV, Visible and IR spectrophotometry

Unit 5: Chromatography **05 Hours**

Principles and applications of Paper chromatography, Column chromatography, TLC, GLC,

HPLC, Ion-exchange chromatography, Molecular sieve chromatography, Affinity chromatography.

Unit 6: Techniques for detection and analysis of nucleic acids and proteins 09 Hours
PCR – design of PCR primers, enzymes used for PCR, cloning of PCR products; DNA polymorphism and its applications (RFLP, AFLP, SSR, SNPs); RNA isolation and analysis, cDNA synthesis and qRT-PCR; Extraction of proteins, PAGE (Native and denaturing); Blotting and hybridization techniques: Southern (Radioactive and Non-radioactive), Northern and Western; DNA sequencing – Sanger's dideoxy sequencing; ELISA.

Practicals 60 hours

1. Study of microscopic techniques using digital resources (freeze-fracture, freeze-etching, negative staining, FISH, chromosome banding).
2. Isolation of chloroplasts by differential centrifugation.
3. Separation of nitrogenous bases by paper chromatography.
4. Separation of sugars by thin layer chromatography
5. Separation of chloroplast pigments by column chromatography (demonstration)
6. Amplification of DNA by PCR and visualization of PCR products.
7. Detection of DNA polymorphism (SSR based DNA fingerprinting).
8. Gel based and capillary based DNA sequence data analysis.
9. Estimation of protein concentration by Bradford method.
10. PAGE to study overexpression of proteins/ Separation of proteins by PAGE.
11. Blotting techniques: Southern, Northern and Western using digital resources.

Suggested Reading:

18. Hofmann, A., &Clokie, S. (2018) Wilson and Walker's Principles and Techniques of Biochemistry and Molecular Biology (8th ed.). Cambridge University Press.
19. Gerald Karp, Janet Iwasa, Wallace Marshall (2019).Karp's Cell and Molecular Biology, 9th Edition: Wiley
20. O' Brien, T.P. and Cully M.E (1981). The Study of Plant Structure. Principles and selected Methods, Termarcarphi Pty. Ltd., Melbourne.

Additional Resources:

1. Cooper, G.M., Hausman, R .E. (2009). The Cell: A Molecular Approach, 5th edition. Washington, D.C.: ASM Press & Sunderland, Sinauer Associates, MA.

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POOL OF DISCIPLINE SPECIFIC ELECTIVES

DISCIPLINE SPECIFIC ELECTIVE COURSE (DSE -07): Recombinant DNA Technology and Proteomics

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Recombinant DNA Technology and Proteomics BOT-DSE-07	**4**	**2**	**0**	**2**	Class XII pass with Biology/ Biotechnology	**Nil**

Learning Objectives: This course structure is designed to:

- familiarize the students with the essential knowledge and technical skills/ methodology involved in creating recombinant DNA molecules.
- provide knowledge on generating modified organisms, synthesize a product or modify a biological process by tailoring and/or incorporating DNA from one organism into another.

Learning outcomes:

After completion of the course students will:

7. be able to identify, locate, isolate and functionally characterize DNA sequences/genes.
8. Get familiarized with technologies used to create recombinant DNA.
9. be able to design strategies adopted to generate genetically modified organisms for various applications.
10. be aware of the application of recombinant DNA in pharmaceuticals, agriculture, environment management, etc.

Unit 1: **Enzymes inrecombinant DNA technology** **04 hours**

Nucleases: DNAses, RNAses, Restriction endonucleases (discovery, classification, isoschizomers and cleavage action), exonucleases, polymerases (DNA, RNA, Reverse transcriptase, *Taq* polymerase), ligases, kinases, alkaline phosphatase.

Unit 2: Cloning vectors **04 hours**

Plasmids (basic features and types - pBR322, pUC18, pUC19, TA vectors), lambda vectors (insertion and replacement vectors), M13, cosmids and phagemids, pBluescript II; Artificial chromosomes as vectors (BACs, YACs). Expression vectors and shuttle vectors, YeP; strategies for over-expression of proteins

Unit 3: Isolation and cloning of target DNA **03 hours**

PCR, Strategies: isolation/generation of target sequence (restriction-based and PCR-based), generation of compatible cohesive ends, linkers and adaptors.

Unit 4: **Creating and screening DNA libraries** **03 hours**

Construction of genomic and cDNA libraries, screening and identification of target sequence by DNA hybridization and immunological methods.

Unit 5: **Introduction of DNA into host cell** **06 hours**

Preparation and transformation of competent bacterial cells (heat shock and electroporation). DNA delivery into plant cells and protoplasts: *Agrobacterium* mediated (disarmed Ti plasmid), electroporation, microinjection, liposomes and biolistic methods). Selection and identification of transformants (alpha-complementation, antibiotic resistance and reporter genes (GUS and GFP).

Unit 6: Protein purification and Identification **03 hours**

Chromatography-based methods (ion exchange chromatography and affinity chromatography), antibody-based methods (ELISA and Western blotting).

Unit 7: **Proteomics** **04 hours**

Introduction to proteomics: gel-based methods (Native and SDS PAGE, 2D gel electrophoresis, differential gel electrophoresis), mass spectrometry.

Unit 8: Applications **03 hours**

Application of recombinant DNA technology and Proteomics in medicines (insulin, vaccines), agriculture (insecticide delta endotoxin, golden rice, antisense strategy in tomatoes).

Practicals **60 hours**

15. Plasmid DNA isolation using Bacterial cultures.
16. Agarose Gel electrophoresis of plasmid DNA
17. Quantification of DNA by spectrophotometry
18. Extraction of protein and its Quantification by Lowry's method

19. Constructing Restriction map of linear and circular DNA using the data provided
20. Study of recombinant DNA techniques through photographs (Biolistic technique, electroporation, microinjection, PCR, western blotting, artificial chromosomes YAC, BAC, Cosmid, Phagemid, Ti plasmid)
21. Demonstration of SDS-PAGE and affinity Chromatography

Suggested reading:

- Brown, T. A. (2016) Gene Cloning an Introduction: Chapman & Hall.
- Zlatanova, J. and Van Holde, K.E. (2016) Molecular Biology Structure and Dynamics of Genomes and Proteomes: Taylor and Francis; .
- Glick, Bernard R, Jack J. Pasternak, Patten Cheryl L. 2018. Molecular Biotechnology; principles and applications of recombinant DNA, ASM Press, Washington.
- Lovric, J., 2011. Introducing Proteomics. Wiley-Blackwell
- S.B. Primrose, R. M. Twyman, R.W.Old. 2001. Principles of Gene manipulation: Blackwell Science; 2001

Additional reading:

- Banks, K (2022) Introduction to Proteomics. Larsen & Keller Education
- Kreuzer, H. Massey, A (1996) Recombinant DNA and Biotechnology; A guide for teachers; ASM Press.

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