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.

Note: Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.