

Course Code: BD501T

DSE Course – I: Analytical Techniques in Plant Sciences

The objective of this course is to expose the students to different techniques which can be used to study different Biological processes

(Credits: Theory-4, Practical-2)

THEORY

Lectures: 60

**Unit 1: Imaging and related techniques
(lectures)**

Principles of microscopy; Light microscopy; Fluorescence microscopy; Confocal microscopy; Use of fluorochromes: (a) Flow cytometry (FACS); (b) Applications of fluorescence microscopy: Chromosome banding, FISH, chromosome painting; Transmission and Scanning electron microscopy – sample preparation for electron microscopy, cryofixation, negative staining, shadow casting, freeze fracture, freeze etching.

Unit 2: Cell fractionation (8 lectures)

Centrifugation: Differential and density gradient centrifugation, sucrose density gradient, CsCl₂ gradient, analytical centrifugation, ultracentrifugation, marker enzymes.

Unit 3: Radioisotopes (4 lectures)

Use in biological research, auto-radiography, pulse chase experiment.

Unit 4: Spectrophotometry (4 lectures)

Principle and its application in biological research.

Unit 5: Chromatography (8 lectures)

Principle; Paper chromatography; Column chromatography, TLC, GLC, HPLC, Ion-exchange chromatography; Molecular sieve chromatography; Affinity chromatography.

Unit 6: Characterization of proteins and nucleic acids (6 lectures)

Mass spectrometry; X-ray diffraction; X-ray crystallography; Characterization of proteins and nucleic acids; Electrophoresis: AGE, PAGE, SDS-PAGE

Unit 7: Biostatistics (15 lectures)

Statistics, data, population, samples, parameters; Representation of Data: Tabular, Graphical; Measures of central tendency: Arithmetic mean, mode, median; Measures of dispersion:

Range, mean deviation, variation, standard deviation; Chi-square test for goodness of fit.

Course Code: BD501P
DSE Course – I - Practical: Analytical Techniques in Plant Sciences

Practical

1. Study of Blotting techniques: Southern, Northern and Western, DNA fingerprinting, DNA sequencing, PCR through photographs.
2. Demonstration of ELISA.
3. To separate nitrogenous bases by paper chromatography.
4. To separate sugars by thin layer chromatography.
5. Isolation of chloroplasts by differential centrifugation.
6. To separate chloroplast pigments by column chromatography.
7. To estimate protein concentration through Lowry's methods.
8. To separate proteins using PAGE.
9. To separation DNA (marker) using AGE.
10. Study of different microscopic techniques using photographs/micrographs (freeze fracture, freeze etching, negative staining, positive staining, fluorescence and FISH).
11. Preparation of permanent slides (double staining).

Suggested Readings

1. Plummer, D.T. (1996). An Introduction to Practical Biochemistry. Tata McGraw-Hill Publishing Co. Ltd. New Delhi. 3rd edition.
2. Ruzin, S.E. (1999). Plant Microtechnique and Microscopy, Oxford University Press, New York. U.S.A.
3. Ausubel, F., Brent, R., Kingston, R. E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. (1995). Short Protocols in Molecular Biology. John Wiley & Sons. 3rd edition.
4. Zar, J.H. (2012). Biostatistical Analysis. Pearson Publication. U.S.A. 4th edition.