

(Semester V)

Paper 14: Analytical Techniques in Plant Sciences

THEORY

Unit 1: Imaging and related techniques

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. **(12 lectures)**

Unit 2: Cell fractionation

Centrifugation: Differential and density gradient centrifugation, sucrose density gradient, CsCl₂ gradient, analytical centrifugation, ultracentrifugation, marker enzymes. **(6 lectures)**

Unit 3: Radioisotopes

Use in biological research, auto-radiography, pulse chase experiment. **(3 lectures)**

Unit 4: Spectrophotometry

Principle and its application in biological research. **(3 lectures)**

Unit 5: Chromatography

Principle; Paper chromatography; Column chromatography, TLC, GLC, HPLC, Ion-exchange chromatography; Molecular sieve chromatography; Affinity chromatography. **(6 lectures)**

Unit 6: Characterization of proteins and nucleic acids

Mass spectrometry; X-ray diffraction; X-ray crystallography; Characterization of proteins and nucleic acids; Electrophoresis: AGE, PAGE, SDS-PAGE **(5 lectures)**

Unit 7: Biostatistics

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. **(13 lectures)**

PRACTICALS

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).

ESSENTIAL 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.