

B.Sc. III Year

Semester - V

BBO-E503

DSE-1 Analytical Techniques in Plant Sciences

MM : 100
Time : 3 hrs

Sessional : 30
ESE : 70
Pass Marks : 40

Learning objective:

- To understand the basic knowledge of analytical techniques in plant sciences.
- To acquire the basic information about the imaging and related techniques.
- To become familiar with Principles of microscopy, radioisotopes and spectrophotometry, and Chromatography.
- To become familiar with characterization of proteins and nucleic acids, applications of Biostatistics.

Learning outcomes:

At the end of course student will be able

- The student will be able to familiar with various tools used in analytical techniques in plant sciences, applications of fluorescence microscopy such as Chromosome banding, FISH, chromosome painting and sample preparation for electron microscopy.
- The student will be able to understand the differential and density gradient centrifugation and ultracentrifugation, marker enzymes, Principle of paper chromatography; column chromatography, TLC, GLC, HPLC and Mass spectrometry like X-ray diffraction; X-ray crystallography; characterization of proteins and nucleic acids; electrophoresis: AGE, PAGE, SDS-PAGE etc.
- The student will be to learned and understand the statistics, data, population, samples, parameters; representation of data: tabular, graphical; measures of central tendency: arithmetic mean, mode, median; measures of dispersion.
- The student will be able take the decisions for carrier point of views in research, industries and academia entrepreneurs etc.

Unit 1: Imaging and Related Techniques:

(15 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 and spectrophotometry:

(6 Lectures)

Use in biological research, auto-radiography, pulse chase experiment. Principle (Beer & Lambert's law). Application in biological research.

Unit 4: Chromatography:

8 Lectures)

Principle; paper chromatography; column chromatography, TLC, GLC, HPLC, ion-exchange chromatography; molecular sieve chromatography; affinity chromatography.

Unit 5: Characterization of Proteins and Nucleic Acids, Biostatistics:

(20 Lectures)

Mass spectrometry; X-ray diffraction; X-ray crystallography; characterization of proteins and nucleic acids; electrophoresis: AGE, PAGE, SDS-PAGE. 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.

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Practical's

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 chromatographic method.
7. To estimate protein concentration through Lowry's methods.
8. To separate proteins using PAGE.
9. To separate 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).
12. Calculation (numerical) of central tendencies (mean, mode & median), standard deviation, standard error.

Suggested readings:

1. Dubey, R.C. A Text Book of Biotechnology. S. Chand & Company Pvt. Ltd. Ram Nagar, New Delhi-110 055.
2. Campbell A. M., Heyer L. J. (2006) Discovering Genomics, Proteomics and Bioinformatics. II Edition. Benjamin Cummings.
3. Smith, R. 2000 Plant Tissue Culture: Techniques and Experiments, 2nd edition, Academic.

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