

K. L. E. SOCIETY'SS.NIJALINGAPPA COLLEGE



DEPARTMENT OF BIOTECHNOLOGY

Certificate course ON PLANT TISSUE CULTURE TECHNIQUES

Submitted By H.O.D Department of Biotechnology

Submitted to Principal K.L.E Society's S Nijalingappa College, Rajajinagar, Bangalore-10

i) Laboratory organisation: (1hr)

Designing - low cost, commercial, export-oriented laboratory and R&D laboratory. Comparison and cost evaluation. Fumigation and sterilization of culture room.

ii) Basic techniques

a) Media preparation: (2 hrs)

Preparation of stocks, storage, documentation of media preparation, precautions and common trouble shooting while media preparation. Large-scale media preparations and automation in media preparation.

b) Explant preparation: (1 hr)

Surface sterilization protocols for leaf, buds, rhizomes, flower buds, tubers and bulbs. Documentation to identify or optimize sterilization protocols.

c) Inoculation: (8 hrs)

Aseptic techniques, dissections, inoculation of axillary buds, terminal buds, leaves, roots, anthers, rhizomes, meristem, seeds and embryos.

d) Maintenance of cultures: (1 hr)

Maintaining the cultures in growth room. Clean room operations.

e) Transfer of Plants to Green house: (2 hr)

Conditioning and Maintenance of Plants in Green house.

iii) Micropropagation technique: (3 hrs)

Optimization of various protocols of all stages. Development of technical know-how. Documentation and assessment of multiplication rate. Micropropagation of Commercially important Crop (Banana / Vanilla)

iv) Somatic embryogenesis: (2 hrs)

Optimization of initiation, synchronization, maturation and germination techniques. Artificial seed technology.

v) Haploid production: (3 hrs)

Techniques involved in anther culture, pollen culture, ovary culture, ovule culture. Optimization of procedures for haploid plant production. Diploidization of haploids.

vi) Protoplast culture: (3 hrs)

Techniques of isolation of protoplasts from leaves and callus. Culture of protoplasts by hanging drop technique and plating technique. Screening and studying the viability of protoplasts.

vii) Secondary metabolite production: (4 hrs)

Initiation and establishment of cell and suspension cultures. Growth studies, packed cell volume and other growth measurement techniques. Single cell clones, Bergman's cell plating technique, selection and screening of single cell colonies. Selection of high yielding lines, yield enhancement techniques, biotic and abiotic elicitation, precursor feeding, immobilization and biotransformation.

viii) Analysis of Secondary Metabolites: (6 hrs)

Biochemical methods, TLC / HPLC.

ix) Agrobacterium transformation: (4 hrs)

Vector based - Agrobacterium mediated transformation. Induction of hairy roots for secondary metabolite production. Growth and yield enhancement studies.

COURSE DETAILS

CERTIFICATE COURSE ON PLANT TISSUE CULTURE TECHNIQUES :

- ▶ Year : From the Academic year 2018-2023
- Eligibility : Candidates who have completed two year pre-University course of Karnataka or equivalent & Students studying at B.Sc combination with at least one life science subject
- > **Duration :** The course study will extend over a period of 1 year
- Teaching hours :100 Hrs (includes both theory,practicals & Field/industrial visits)
- Maximum Marks:100
- Certificate course admission fees :Nil
- Maximum intake :100 Students
- Medium of Instruction: The medium of instruction shall be English and

the candidate must answer the examination in English.

- ATTENDENCE: A student must satisfy the requirement of attendance for the course ,if he/ she has attend less then 75% of attendance in the course then student shall not be permitted to take the examination.
- PEDAGOGY: It will be a blend of lectures, seminars, guest lectures, Field visits designed by theH.O.D. There will be 40 theory hours and 60 practical hours.

> Examination:

Paper I: Theory examination of 3 hours duration (100 marks reduced to 40 marks)Paper II: Practical examination of 4 hours duration (40 marks)Viva voce on Field visit and lab report (20 marks)

Introduction :

- The plant tissue culture technique is a skill based new technology helps the student community to develop the Research career in Biotechnolog, Botany, Horticulture, Forestry, Horticulture, Floriculture and Agricultural Sciences.
- The course initiates and self empower the student and to start small scale or large scale agro based industries .It increases the self employability in students.
- It gives the job opportunity in Agriculture and agro based industries, Agricultural Research institutes, Wood sciences, Horticulture department, Botanical research institutes, National Plant tissue culture laboratories, Phaarmacognostical & Pharmaceutical industries.
- Students are strengthened to get the employability in Biotechnology industries and Research Institutes of Life Sciences

OBJECTIVES:

- To develop professionals in the area of plant tissue culture.
- To develop entrepreneurs in the field of plant tissue culture.
- To make the students competent and capable for self employment.
- This area can be taken up as micropropagation/nursery business with smaller

investment by entrepreneurs.

• The students will be technically trained with good practical exposure to perform

the plant tissue culture, which is the at most required in this field of science, skilled

candidates are absorbed in well established and commercial tissue culture units.

Outcome / Scope:

- Self employability
- Entrepreneurship
- Jobs in MNC/Agricultural companies/Plant tissue culture laboratory
- Research Associate Fellowship in R&D/Research projects/Major & minor projects

COURSE MATRIX: <u>outline of syllabus – theory units</u>

SI. No.	TOPICS	No. Of Allotted Hours
1	Introduction: Introduction to Plant Tissue Culture, History of Plant Tissue Culture	1 Hr
	Laboratory Organization and Techniques in Plant Tissue	
2	Culture: Tissue Culture Laboratory, Glass, Goods and Instruments, Culture Media and Preparation of Stock Solutions. Techniques in Plant Tissue Culture-Sterilization techniques-, filter, heat, wet and chemical Inoculation techniques, Incineration	6Hrs
3	Basic plant regeneration and plant propagation Procedures	
	-Organogenesis: Brief History, Principle, Protocol, Factors Affecting Organogenesis, Applications. Callogenesis, Organogenesis from callus, Adventious roots/shoot proliferation	7 Hrs
4	Somatic Embryogenesis and Artificial Seeds Applications. : History, Different Stages of Somatic Embryogenesis, Protocol,	4Hrs
	Artificial Seeds Techniques of Organ Culture & applications Definition	
5	Different Types-Principle, Protocol, Applications - Root Culture, Shoot Tip / Meristem Culture, Ovary Culture, Ovule Culture, Anther Culture, Embryo Culture.	5Hrs
	Micropropagation: Techniques Used in Clonal Propagation of	
6	Elite Species in Agriculture, Horticulture and Forestry, Steps Involved in Micropropagation, Applications.	4Hrs
7	Protoplast Culture and Fusion: Protocol for Isolation and Culture, Important Properties of Isolated Protoplasts. Applications of Protoplast Isolation. Protoplast Fusion Methods, Mechanism, Post Fusion Events, Somatic Hybridization, Cybrids.	5 Hrs
8	Somaclonal and Gametoclonal Variations	2 Hrs
9	Micro techniques and Analytical Procedures	3 Hrs
10	Applications of Plant Tissue Culture: Brief Applications of Plant Tissue Cultures in Agriculture, Forestry, Horticulture, Floriculture and Propagation of Transgenic Plants.	3 Hrs.

Total hours allotted: 40 Hrs

PRACTICALS

Sl. No.	TOPICS	No. Of Allotted Hours
1	Laboratory Management –Facility Management and Operation Management ,Designing - low cost, commercial, export-oriented laboratory and R&D laboratory. Comparison and cost evaluation. Fumigation and sterilization of culture room.	4Hrs
2	Study of Instruments used in Tissue Culture	3 Hrs
3	Media Preparation – Washing and cleaning glass wares methodolo ,preparation of Basal media,MS, Nitsch, White's Media,Preparation Stock solutions(storage, documentation of media preparati precautions and common trouble shooting while media preparati Large-scale media preparations and automation in media preparation	6Hrs
4	Preparation of Explants, Culture techniques, Media preparation and inoculation – Nodal, Shoot Tip, Leaf Disc, Anther, Meristem, Ovary, Ovule, Axillary and Adventitious Buds, Endosperm, Seed culture	12 Hrs
5	Clonal Propagation of Elite Species – Fruit – Banana Floriculture – Geranium Medicinal – Oscimum	3 Hrs
6	Protoplast Isolation	3 Hrs
7	Callus, callus subculture and Suspension Cultures	6 Hrs
8	Somatic Embryogenesis and Synthetic Seeds	3 Hrs
9	Field visit and practical report ,Visit to Wood science,IIHR,Premier Research centers, plant tissue culture Research lab,Poly houses,Project Discussion *Field visit (20 Hrs) to acquaint the propagation of skill and management *	20Hrs

Total hours allotted: 60 Hrs

Total Teaching Hours Of Theory= 40 Hrs.Total Hours of Practical= 60 Hrs

Grand Total = 100 Hrs.

Laboratory works will be conducted at Department of Biotechnology Laboratory Faculty:

1. H.O.D & 3 Faculty from department of Biotechnology

2. Dr.Arunkumar.B.Sonappanavar, Ph.D in plant tissue culture ,Principal, KLESNC

3. Resource person from Universities /Different Tissue culture laboratory /Premier Research institute

REFERENCES:

- Bhojwani. S. S (ed.) 1990. Plant Tissue Culture: Applications and Limitations. Elsiever, Amsterdam.
- Bhojwani. S. S and Razdaan M. K 1983. Plant Tissue culture: Theory and Practice. Elsiever, Amsterdam.
- Gamborg. O. L and Wetter. L. R. (eds.) 1975. Plant Tissue Culture Methods. National Res Council, Canada, PRL, Saskatoon.
- Street. H. E. 1977. Plant Cell and Tissue Culture. Blackwell, London.
- Vasil. I. K and Thrope. T. A. (eds.) 1994, Plant Cell and Tissue Culture. Kluwer Academic Publishers, London.
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- Clark, M.S. 1997. Plant molecular biology: a laboratory Manual. Springer-Verlag, Berlin, Heidelberg.
- 3.Potrykus, I and Spangenberg, G. 1997. Gene Transfer to Plants (Springer Lab Manual), Springer Verlag. AN INTRODUCTION TO PLANT TISSUE CULTURE.
- Kumar De. K, 1992, New Central Agency, Calcutta
- > PLANT BIOTECHNOLOGY.
- Mantell S h and Smith H, 1983.Cambridge University Press. Cambridge.
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- Oxford and IBH Publishing Company
- APPLIED AND FUNDAMENTAL ASPECTS OF PLANT CELL, TISSUE AND ORGAN CULTURE.Reinart. J and Bajaj Y P S, 1977.
- Springer Verlag, Berlin

I) SCHEME OF EXAMINATION:

There shall be examination at the end of the course.

- 1. Evaluation of each paper shall be for 100 marks comprising of 40 marks in theory and 40 marks in practical and 20 marks for Viva voce on Field visit and lab report
- 2. The composition of the theory paper including written
- 3. The duration of examination for the theory paper of 40 marks shall be 3 hours.
- 4. The duration of examination per practical paper of 40 marks shall be 4 hours.
- 5. In case of practical examinations, the students will be assessed on the basis of knowledge of processes, skills, operations involved, results and observations and reporting. Practical records will be evaluated during the examination.
- 6. The minimum for pass in the examination is 40 % per paper inclusive of theory and practicals.

II) EXAMINATION VALUATION:

The question paper for the examination shall be set by the college. The candidate shall take the examination as per the syllabus and the scheme of examination in force.

III) PATTERN OF QUESTION PAPER:

Each theory paper shall ordinarily consist of three sections to test conceptional skills, understanding skills, comprehensive skills and application skills.

SECTION A – Answer All Questions (Multiple Choice)

(10 x 1 MARK = 10)

SECTION B-Answer all the questions (One sentence answer)

(1 x 5 MARKS = 5)

SECTION C- Answer Any Five Questions (Out Of 7) (Short Answer Questions) (5 x 3 MARKS = 15)

SECTION D–Answer Any Two Questions (Out Of four) (Medium Length Answers) (2 x 5 MARKS = 10)

IV) CLASSIFICATION OF SUCCESSFUL CANDIDATES: SUCCESSFUL CANDIDATES SHALL BE CLASSIFIED AS FOLLOWS:

- ▶ FIRST CLASS: Those who obtain 60% and above of the aggregate marks.
- SECOND CLASS: Rest of the successful candidates who obtain 50% and above of the aggregate marks.

SCHEME OF PRACTICAL EXAMINATION

Max. Marks: 40 Time: 3 Hours 1. Isolate the protoplast from the given plant material **A**. Write the principle and procedure followed. 10 Marks. 2. Prepare the explant for tissue culture using the given material **B**. Discuss its significance. 10 Marks. 3. Prepare synthetic seeds using the given material **C**. 4. Identify and comment on **D**, **E**, **F**, **G**, **H**. 5. Records. 10 Marks.

SCHEME OF VALUATION

1.	Procedure +	Performance – 4	4 Marks	+4 Marks

Result + Comment -1 Mark + 1 Mark

- 2. Performance 8 Marks
- Comment 2Mark
- 3. Performance 5 Marks
- 4. Spotters -5×3 Marks = 15 Marks