Punyashlok Ahilyadevi Holkar Solapur University, Solapur

SKILL DEVELOPMENT CENTRE

Certificate Course in Plant Tissue Culture

Syllabus

Theory: Paper-I: Introduction and Techniques of Plant Tissue Culture

Objectives of the Paper:

1. To get the knowledge about introductory history of plant tissue culture
2. To get the knowledge about Laboratory organization in plant tissue culture
3. To get the knowledge about Media preparation in plant tissue culture
4. To get the knowledge about aseptic manipulation in plant tissue culture
5. To get the knowledge about the Cell Culture & Cellular Totipotency

Expected Outcome of the Paper:

1. Student will come to know introductory history of plant tissue culture
2. Student can understand Laboratory organization in plant tissue culture
3. Student can understand Media preparation in plant tissue culture
4. Student will come to know about aseptic manipulation in plant tissue culture
5. Student will come to know about Cell Culture & Cellular Totipotency

Unit 1: Introductory History

1.1 Concept of Cell culture
1.2 Development of tissue culture
1.2.1 Root tip culture
1.2.2 Embryo culture
1.2.3 Stem-tip culture
1.3 Role of Auxin
1.4 Discovery of Cytokinin
1.5 Hormonal control of organ formation
1.6 Improvement of Media
1.7 Preparation and Cloning of Single Cell Cultures
1.8 Regeneration of single cell to whole plant
1.9 Practical applications and recent advances
1.9.1 Morphological aspects
1.9.2 Production of secondary metabolites
1.9.3 Production of pathogen-free plants
1.9.4 Germplasm conservation
1.9.5 Genetic manipulation

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Unit 2: Laboratory Organisation

2.1 Washing and storage facilities
2.1.1 Cleaning glassware
2.1.2 Using Plastic Labware
2.2 Media preparation room
2.3 Transfer area
2.4 Culture room
2.5 Data collection area and specialised facilities
2.6 Transplantation area

Unit 3: Media

3.1 Media Composition
3.1.1 Inorganic nutrition
3.1.2 Carbon and energy source
3.1.3 Organic supplements
3.1.4 Growth regulators
3.1.5 Solidifying Agents
3.1.6 pH
3.2 Media Preparation
3.3 Selection of New Medium

Unit 4: Aseptic Manipulation

4.1 Sterilising the culture vessels and instruments
4.2 Sterilisation of Nutrient Media
4.3 Sterilising Culture rooms and transfer area
4.4 Aseptic Culture technique
4.4.1 Sterilising plant material
4.4.2 Transfer of the explant

Unit 5: Cell Culture & Cellular Totipotency

5.1 Isolation of Single Cell
5.2 Culture medium for cell suspensions
5.3 Viability of Cultured Cell
5.4 Vascular Differentiation
5.5 Phloem Differentiation
5.6 Organogenic Differentiation
5.7 Totipotency of Epidermal Cells
5.8 Totipotency of Crown-gall cells

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Theory: Paper-II: Applications to Plant Breeding

Objectives of the Paper:

1. To get the knowledge about Haploid Production
2. To get the knowledge about Triploid Production
3. To get the knowledge about *In Vitro* Pollination & Fertilization
4. To get the knowledge about Zygotic Embryo Culture
5. To get the knowledge about the Somatic Hybridization, Cybridisation and Genetic transformation

Expected Outcome of the Paper:

1. Student will come to know Haploid Production
2. Student can understand Triploid Production
3. Student can understand *In Vitro* Pollination & Fertilization
4. Student will come to know about Zygotic Embryo Culture
5. Student will come to know about the Somatic Hybridization, Cybridisation and Genetic transformation

Unit 1: Haploid Production

1.1 Techniques of androgenesis
1.2 Factors influencing anther culture
1.3 Differentiation of pollen into gametophytic or Sporophytic cell
1.4 Development of Androgenic Haploids
1.5 Haploids from Isolated microspore or pollen culture
1.6 Induction of haploids from unpollinated overies/ovules
1.7 Diplodisation of haploid plants
1.8 Applications of haploids in plant breeding
1.9 Problems associated with haploid production

Unit 2: Triploid Production

2.1 The technique
2.1.1 Explant
2.1.2 Nutrient Media
2.1.3 Development of Shoot Buds and Plantlets
2.1.4 Maintenance of cultures
2.1.5 Biochemical markers in Endosperm culture
2.2 Histology and Cytology of Callus
2.3 Application of triploids in plant improvement

Unit 3: *In Vitro* Pollination & Fertilization

3.1 Methodology
3.1.1 Materials
3.1.2 Disinfection of material

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3.1.3 Culture of Ovules and Ovary
3.2 Factors affecting seed set after *in vitro* pollination
3.3 Applications of *in vitro* pollination
3.4 *In vitro* fertilization using isolated single gametes

Unit 4: Zygotic Embryo Culture

4.1 Types of embryo culture
4.2 Technique
4.3 Nutritional requirement
4.4 Role of suspensor in embryo culture
4.5 Precocious germination
4.6 Morphogenesis in the cultures of seeds with partially differentiated embryos
4.7 Organogenic Potential of Embryo Callus
4.8 Practical Applications

Unit 5: Somatic Hybridization, Cybridisation and Genetic transformation

5.1 Isolation of protoplasts
5.2 Culture of protoplasts
5.3 Protoplasts regeneration
5.4 Protoplast fusion
5.5 Selection of somatic hybrids and cybrids
5.6 Assessments of somatic hybrid and cybrid nature of plants
5.7 Practical applications of somatic hybridisation and cybridisation
5.8 Uptake of DNA by pollen; transformation of protoplasts; integration and expression of foreign DNA in plant cells
5.9 Use of selectable markers and reporter genes

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Practical: Paper-III: General Applications of Plant Tissue Culture

Objectives of the Paper:

1. To get the knowledge about General Applications of Plant tissue culture through various practicals

Expected Outcome of the Paper:

1. Student will come to know General Applications of Plant tissue culture through various practicals in the laboratory

1. Sterilization and Preparation of media
2. Isolation of explants, establishments, and maintenance of culture
3. Subculture of Callus, organogenesis, and transfer of plants to soil
4. Micropropagation by proliferation of axillary bud
5. Micropropagation by adventitious shoot proliferation
6. Initiation and establishment of cell suspension cultures
7. Microspore/anther culture for haploid production
8. Protoplast isolation and culture
9. Embryogenesis and embryo culture, synthetic seeds
10. Density gradient centrifugation for isolation of chloroplast and mitochondria
11. Isolation of total RNA from plant
12. Isolation of total DNA from chloroplast
13. Demonstration of southern hybridization to check the plant transformation
14. Isolation of total DNA from plant

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Practical: Paper-IV: Project on Plant Tissue Culture

Objectives of the Paper:

1. To get the knowledge about Plant tissue culture through laboratory/industry

Expected Outcome of the Paper:

1. Student will get hands on training through field work-based project in laboratory/industry.

Field Work and Project based on field work	
Student should complete the field work of 45 hrs from Plant Tissue Culture based laboratory/industry and submit the project based on same. The Project should clearly mention Title of the Project, Material Methodology implemented in the project, Review of Literature, Result and Discussion.	**3 Credits (100 Marks)**

Reference Books:

1. Plant Tissue Culture by **K.K. Dey**
2. Methods in Plant tissue culture by **Razdan**
3. Plant tissue culture by **Jha & Ghosh**
4. Trends in plant tissue culture by **Pareek**
5. Plant Cell, Organ & tissue culture by **Norasa**
6. Plant Cell & Tissue Culture by **S. Narayaswami**

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Scheme of Examination:

Scheme of the examination: The examination will be carried out as guidelines given by the P.A.H. Solapur University, Solapur

The Proposed scheme is as follows:

Sr. No.	Heads	Total Marks
1.	Theory Paper-I: Introduction and Techniques of Plant Tissue Culture (100 Marks) Paper-II: Applications to Plant Breeding (100 Marks)	200
2	Practical Paper-III: General Applications of Plant Tissue Culture (100 Marks) Paper-IV: Project on Plant Tissue Culture (100 Marks)	200
	Total	400

Qualification for the Course: 10th Pass

Medium of Instruction for the Course: Marathi & English

Course Fee: 5000/- Rs.

Nature of Theory Question Paper

University Assessment:		**Total Marks 80**
Q.1.	Objectives	20
Q.2.	Short Notes (Any four)	20
Q.3.	Short Answer (Any two)	20
Q.4.	Long Answer (Any one)	10
Q.5.	Long Answer (Any one)	10

College Assessment:

Collect Home Assignment/Open Book Examination **Total 20 Marks**

Nature of Practical Question Paper		**Total Marks 100**
Short Experiments	2 (15 marks each)	30 marks
Long Experiments	3 (20 marks each)	60 marks
Practical Record	10 marks	10 marks
Total Marks		**100 marks**

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Nature of Project

Submission of Dissertation **100 marks**

Passing Criteria: As per the guidelines of P.A.H. Solapur University, Solapur

The Proposed passing criteria is as follows:

The candidate obtaining minimum of 40 marks each in theory examination & practical examination will be declared as pass. Certificates will be issued for such candidates.

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