

STRUCTURE OF THE COURSE

I TERM IN ACADEMIC INSTITUTE (6 MONTHS)			
NO.	NAME OF THE PAPER	HOURS/WEEK	CREDITS
PTC 1:	PLANT TISSUE CULTURE AND MICROPROPAGATION	4	4
PTC 2:	ADVANCED TECHNIQUES IN PLANT TISSUE CULTURE	4	4
PTC 3:	PLANT DEVELOPMENT, BREEDING AND TRENDS IN BIOTECHNOLOGY	4	4
PTC 4:	LAB TO LAND	4	4
LAB 1	PRACTICAL 1	4 hrs x 2session	4
LAB 2	PROJECT RELATED TO PRACTICAL TECHNIQUES	4 hrs x 2 session	4
			24
II TERM IN THE INDUSTRY (6 MONTHS)			
	TO BE DECIDED BY THE INDUSTRY		24
	TOTAL NUMBER OF CREDITS		48

PTC 1: PLANT TISSUE CULTURE AND MICROPROPAGATION

THEORY

TOTAL : 40 HRS

UNIT 1	<p>INTRODUCTION TO PLANT TISSUE CULTURE Definition, brief history, principle and significance of tissue culture; Cellular totipotency – Cytodifferentiation: factors affecting vascular tissue differentiation, cell cycle and TE differentiation; Organogenic Differentiation: induction, factors affecting shoot bud differentiation.</p>	4
UNIT 2	<p>LABORATORY ORGANIZATION: Design and layout for wash area, media preparation, sterilization and storage room, transfer area for aseptic manipulations, Culture rooms, and observation/data collection areas. Labwares <i>Good laboratory practices, Good safety practices.</i></p>	2
UNIT 3	<p>INSTRUMENTATION AND STERILIZATION TECHNIQUES Working principle, maintenance and management of following instruments: Laminar air flow, autoclave, distillation unit, pH meter, orbital shaker, microscope, deep freezer, growth chamber Sterilization: Importance, Methods of sterilization of equipments, culture media and explants.</p>	4
UNIT 4	<p>TISSUE CULTURE MEDIA Introduction, Types of Media and its importance; <i>Preparation of stocks, pH and Buffers and their significance in media.</i> Media Constituents (Vitamins, Unidentified supplements, carbohydrate for energy source, Nitrogen source and organic supplements, complex substances, hormones, Activate charcoal) An appraisal of different media, selection of media, Preparation and Designing.</p>	4
UNIT 5	<p>TYPES OF CULTURES, PRINCIPLES, METHODS, APPLICATIONS, ADVANTAGES AND DISADVANTAGES: Cell suspension Culture, Callus Culture, Embryo Culture, Haploid Culture : microspore and macrospore culture. Triploid culture: Endosperm Culture, Protoplast: isolation, Culture & Fusion, Somatic hybridization and cybridization. Somatic Embryogenesis and Synthetic Seed Production</p>	6
UNIT 6	<p>REGENERATION Current status of <i>in vitro</i> propagation in agriculture, horticulture crops and forestry Techniques, <i>Chemistry and Role of plant growth regulators</i>, Axillary bud</p>	2 6

	proliferation, adventitious shoot regeneration, Meristem and shoot tip culture, Bud organogenesis, embryogenesis, Virus indexing, Transfer of Plants to Green House; Advantages and Limitations of Micropropagation, Importance of Micropropagation in Crop Improvement. Methods to detect pathogens in propagation sources, Procedures to eliminate pathogens from plant parts	
UNIT 7	ROOTING <i>In vitro</i> rooting: use of PGRs, effect of media, use of alternative substrates (perlite, vermiculite), use of activated charcoal, culture environment <i>Ex-vitro</i> rooting: Advantages of microcutting rooting, use, composition of sowing mixtures and additives, culture environment.	2
UNIT 8	HARDENING Need for hardening micropropagated plantlets: Reasons: water stress, cuticle, stomata, hydrathodes. Techniques in acclimatization: Humidity, anti-transpirants, Soil and containers, Control of temperature and Light, Control of Diseases	2
UNIT 9	COMMERCIAL MICROPROPAGATION Designing laboratory for small, medium and large scale production of plants, Cost of micropropagation, methods to reduce cost of micropropagation, Preparation for planting and Dispatch, increasing rate of plant multiplication, Importance of timing, Mechanization and Automation, Alternative cultural techniques: Hydroponics, aeroponics.	4
UNIT 10	CHALLENGES IN PLANT TISSUE CULTURE Contamination, Vitrification, malformation, browning, phenolic and latex compounds, decline in vigour, Habituation, Shoot tip necrosis, Rooting and Hardening, Acclimatization of tissue cultured plants.	2
UNIT 11	IPR AND CERTIFICATION OF TISSUE CULTURED PLANTS IPR – Overview with case study; Certification of Plant Tissue Culture labs, Rules and regulation. Export potentials of Tissue Culture plants	2

References:

1. Plant Propagation by Tissue Culture. Edwin F. George and Paul Sherington, D. 1984. Exegetics Ltd., Edington, Westbury, England.
2. Biotechnology in Agriculture and forestry Bajaj YPS series. Springer Verlag pub., 1986
3. Biotechnology of higher plants-Russell G. Publ: Scholium Intl, 1988
4. Plant biotechnology-Mantell and Smith. Cambridge univ press, 1986
5. Introduction to plant biotechnology HS Chawla. Edition, 2nd ed. Publication, Enfield, NH : Science Publishers, 2002
6. Biochemistry and Molecular Biology of Plants Buchanan BB, Gruissem W, Jones RL, 2000,. American Society of Plant Physiologists, Rockville Pub: Springer Netherlands.
7. Plant Tissue Culture: Theory and Practice (revised edition) Edited by S S Bhojwani and M K Razdan Elsevier 1996
8. Plant Biochemistry and Molecular Biology Second Edition Edited by Peter J. Lea and Richard C. Leegood, John Wiley & Sons Ltd, Chichester, United Kingdom, 1999

PTC 2: ADVANCED TECHNIQUES IN PLANT TISSUE CULTURE

THEORY

TOTAL : 40 HRS

UNIT 1	SOMACLONAL AND GAMETOCLONAL VARIATIONS IN CROP IMPROVEMENT AND ORNAMENTAL SPECIES Definition, Importance, Screening; Epigenetic variation, genetic variation, identification of somaclonal variation. somaclonal variations for Biotic & Abiotic stress. Genetic basis of clones, Mutations, kinds of genetic variation within clones (chimeras)	4
UNIT 2	GERMPLASM STORAGE AND CRYOPRESERVATION Germplasm resources, Genebanks, Types and Methods of Conservation, Cryopreservation Techniques, revival of cryoprotected materials, Advantages and Limitations	4
UNIT 3	CELL SUSPENSION CULTURE AND SECONDARY METABOLITES Dynamics of callus growth, callus subculture and maintenance. Establishing cell cultures, Types of suspension culture, Growth Measurements, Plating techniques, Production of secondary Metabolites – Immobilized cell culture system, Hairy root cultures, Bioreactors and Biotransformation, Role of elicitors and precursor feeding on stimulation of chemical production.	6
UNIT 4	GENETIC ENGINEERING: Introduction to transgenic plants, methods of gene transfer – Agrobacterium tumefaciens mediated, Agrobacterium rhizogenes mediated. Recovery of transformed plants, gene expression and applications. Selectable markers, reporter gene and promoter in plant vectors. Chloroplast engineering	4
UNIT 5	METHODS OF GENE TRANSFER: Vectorless or direct DNA transfer- Physical gene transfer methods- electroporation, particle bombardment or biolistic method, macroinjection, microinjection, liposome mediated transformation, silicon carbide fiber mediated transformation, ultrasound mediated gene transformation; chemical methods of gene transformation- PEG mediated gene transfer, Calcium phosphate co-precipitation, DEAE dextran procedure	6
UNIT 6	DEVELOPMENT OF TRANSGENIC PLANTS: Selection, regeneration and reproduction of transgenic plants. Production of virus, bacteria, fungi, insect resistance plants. Transgenic crops for improved quality, herbicide tolerant, stress resistant plants, Transgenic plants for molecular farming: edible vaccines, plantibodies,	6

	<p>plantigens, bioplastics, biofuels, biofarming for carbohydrates.</p> <p>Anti-sense RNA technology-iRNA and its application in crop improvement. mechanisms and applications in post harvesting improvement.</p> <p>Golden rice technology and biotransformation of high value metabolites through cell culture.</p>	
UNIT 7	<p>ACCLIMATIZATION:</p> <p>Growth parameters (measurements and interpretation with respect to environment factors), Factors affecting acclimatization and field planting; Relative humidity, light and oxygen requirement for plant growth in nursery, requirement of water, fertilizers and other agronomic practices for continuous quality harvests, environment and other factors important in proper management of cultivations.</p>	4
UNIT 8	<p>GM CROPS</p> <p>The current state of transgenic crops, concerns about GM crops, Public acceptance of genetically modified crops; Plant breeders rights, Farmers rights, Plant variety protection, convention on biodiversity; case studies on plant patents- patenting of Basmati rice in USA, Revocation of Turmeric patent and Neem patent.</p> <p>Trangenic plants: field containment, Bt and terminator – technology protecting systems. (GURT). GM Foods: substantial equivalence, safety testing and labeling.</p>	6

References:

1. Plant Biotechnology: The genetic manipulation of plants. 1st edition. Slater A and others, Oxford University Press, New York, 2004
2. Plant propagation by tissue culture: vol 1. The background. George E.F. Springer, 2007
3. Cell Culture and Somatic Cell Genetics of Plants .Indra K. Vasil. 1980. Academic Press inc., New York.
4. In vitro culture in higher plants R.L.M. Pierik, 1987.. Martinus Nijhoff Publishers, Boston.
5. An Introduction to Plant Tissue Culture. Kalyanakumar De. 1997., New Central Book Agency, Calcutta
6. Environmental and ecological impacts from transgenic plants .: Unintended effects. Wolfenbarger, 2003. Information System for Biotechnology, Virginia Tech.USA.
7. Varshney RK and Tuberosa R. 2007. Genomics-Assisted Crop Improvement. Springer, Dordrecht, vol. 1 and 2.

PTC 3: PLANT DEVELOPMENT, BREEDING AND TRENDS IN BIOTECHNOLOGY

THEORY

TOTAL : 40 HRS

UNIT 1	PLANT GENOME: Plant genome structure, organization of chloroplast genome, mitochondrial genome and their interaction with nuclear genome, RNA editing in plant mitochondria. Mitochondrial DNA and Cytoplasmic male sterility.	6
UNIT 2	PLANT DEVELOPMENT Molecular basis of photosynthesis, gene expression in the developing seed, gene expression in ripening fruits, molecular basis of flower development, molecular basis for sexual incompatibility in plants, seed development and seed storage proteins.; Role of Plant hormones (auxins, cytokinins, abscissic acid, ethylene and Gibberellins) in plant development	8
UNIT 3	CONVENTIONAL PLANT BREEDING: Breeding methods in self-pollinated crops: Mass and pure-line selection, hybridization and selection in segregating populations; Breeding methods in cross-pollinated crops: Population improvement and hybrid development, male sterility and self-incompatibility in hybrid seed production, synthetics	4
UNIT 4	MOLECULAR PLANT BREEDING: Introduction - Molecular markers; Molecular marker techniques in plant improvement - Marker maps, marker assisted selection, PCR based and non PCR based molecular markers. Biochemical markers. NTSYS software for analysis of data	6
UNIT 5	BIOTECHNOLOGY AND MEDICINAL PLANTS Potential plants for medicine from India and their impact on human health. Natural products (secondary metabolites) : Phenolic, Terpenoids, Organic acids, lipids, Nitrogen compounds, sugar and their derivatives, Flavonoids, coumarins, stilbenes and fibres.	5
UNIT 6	METABOLIC ENGINEERING OF PLANTS Metabolic engineering of lipids, flavanoids, vitamin E biosynthesis, flavoring agents (monoterpenes and sesquiterpene), Carotenoid biosynthesis, secondary metabolites. Engineering plants for environmental remediation: phytoremediation	5

UNIT 7	METHODS AND EMERGING TRENDS: Tropical and subtropical fruit crops, Forestry, cereal crops eg:rice/corn/wheat. Ornamental flowering and foliage plants, Orchids, Bamboo Medicinal plants: Herbs, Shrubs and trees: eg. <i>Coleus forskohlii/ Taxus sps.</i> Spices and tuber crops. Biofuel plants,	5
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Reference:

- 1.. Gone to seed. Union of Concerned Scientists. Mellon and Rissler. 2004.. Cambridge, USA
2. Unintended consequences of plant transformation: A molecular insight. Filipecki and Malepszy. 2006. J. Appl. Genet. 47(4): 277-286.
3. Developmental Regulation of Plant Gene Expression (Grierson, D. ed.) 1991; Reviews and current journal articles.
4. Plant Physiology (Taiz, L and Zeiger, E.) 1991; Biotechnology in Agriculture (Bajaj series, (Vol 1-20) 1990-1999,
5. Journal of Plant Physiology; Agro-biotechnology related journals.
6. Introduction to Quantitative Genetics (Falconer, D.S.) 1989;
7. The Genetic Analysis of Quantitative Traits (Kearsey, M.J. and Pooni, H.S.) 1996;
8. Plant Breeding: Theory and Practice (Stoskopf, N.C., Tomes, D.T. and Christie, B.R.) 1993.

**PTC 4: LAB TO LAND
THEORY**

TOTAL : 40 HRS

UNIT 1	PLANT MICROBIAL INTERACTION: Bacterial, fungal, nematodes. Molecular basis for stress tolerance in plants: biotic and abiotic factors involved in stress pathways. Nitrogen Fixation: Root nodule bacteria, mechanism of root nodule development, nodulins, leghaemoglobin, nitrogenase enzyme complex. Mychorrhizae: types and associations	5
UNIT2	DETECTION OF PLANT PATHOGENS & DIAGNOSIS OF PLANT DISEASES - Serological / Immunochemical methods, nucleic acid methods; Biological Control Methods – Weed control by pathogens, control of plant pathogens: parasitism, antibiosis, competition, disease suppressive soils, control of insects & other pests.	5
UNIT 3	MAXIMIZING THE PRODUCTION THROUGH INTERCROPPING; Selection of suitable crops, proper harvesting, crop management; germination, dormancy, senescence and abscission: Physiology, biochemistry, mechanism and regulation of the processes, applications in cultivation;	4
UNIT4	POST HARVEST TECHNOLOGY: Overview of Post Harvest Handling – Extent of post harvest losses, importance & advantages of appropriate technology; Biological Aspects – Characteristics of perishable crops, morphological, anatomical & physiological basis of post harvest technology; Post Harvest Diseases – Types of diseases, sources of infection, factors effecting disease development;	6
UNIT 5	HARVESTING OF PRODUCE Concept of maturity, maturity indices, considerations during harvesting, pre-harvest modifiers of quality ; Preparation / Treatment of Produce – Trimming, cleaning & water elimination, curing, waxing & grading, Ripening, de-greening and colour adding, precooling	6
UNIT 6	PACKING HOUSE PREPARATION Purpose & function of packing houses , packinghouse features; Packaging & Transport of Produce – Functions of packaging, damage caused during packaging , types of packages, manual packing, improving package performance, prepackaging, bulk transport, transport conditions;	6
UNIT 7	STORAGE Refrigerated (low temperature) storage: Objectives, structural requirements,	8

	physiological disorders; Supplements to refrigeration: Irradiation, chemical treatments, controlled atmosphere, modified atmosphere, silicone membrane systems, biological treatments; Alternatives to refrigeration: Ambient storage, evaporative cooling, storage in water, ash storage, air-cooled storage, clamp storage, solar cooling, use of leaves.	
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References:

1. Biochemistry and Molecular Biology of Plants Buchanan BB, Gruissem W, Jones RL, 2000,. American Society of Plant Physiologists, Rockville Pub: Springer Netherlands.
2. Plant Physiology (Taiz, L and Zeiger, E.) 1991; Biotechnology in Agriculture (Bajaj series, (Vol 1-20) 1990-1999,
3. Journal of Plant Physiology; Agro-biotechnology related journals.
4. Plant Tissue Culture: Theory and Practice (revised edition) Edited by S S Bhojwani and M K Razdan Elsevier 1996
5. Plant Biochemistry and Molecular Biology Second Edition Edited by Peter J. Lea and Richard C. Leegood, John Wiley & Sons Ltd, Chichester, United Kingdom, 1999
6. Seeds Handbook: Biology, production processing and storage Babasaheb B.Desai Marcel Dekker, 2004
7. Principles of horticulture Adams CR, Bamford KM and Early MP 6th edition, ISBN-10: 0080969577 2011

PRACTICALS

@ 4 hrs / practical (40 practicals)

i) Laboratory organisation: (1)

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)

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)

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

c) Inoculation: (8)

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

d) Maintenance of cultures: (1)

Maintaining the cultures in growth room. Clean room operations.

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

Conditioning and Maintenance of Plants in Green house.

iii) Micropropagation technique: (3)

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)

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

v) Haploid production: (3)

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)

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)

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)

Biochemical methods, TLC / HPLC.

ix) Agrobacterium transformation: (4)

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

Reference:

- 1..Plant cell and tissue culture. A Lab manual-Reinert J. Narosa pub., 1982
2. 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.