Handbook on Plants and Cell Tissue Culture

Author:- NIIR Board of Consultants & Engineers
Format: paperback
Code: NI157
Pages: 640
Price: Rs.1275**US$** 125
Publisher: NIIR PROJECT CONSULTANCY SERVICES
Usually ships within **5** days

Plants cell tissue culture is a rapidly developing technology which holds promise of restructuring agricultural and forestry practices. During the last two decades cell culture have made considerable advanced in the field of agriculture, horticulture, plant breeding, forestry, somatic cell genetics, phytopathology etc. Plant cells can be grown in isolation from intact plants in tissue culture systems. The cells have the characteristics of callus cells, rather than other plant cell types. These are the cells that appear on cut surfaces when a plant is wounded and which gradually cover and seal the damaged area. Plant cells and tissue culture are often used for the production of primary and secondary metabolites. Plant tissue cultures can be initiated from almost any part of a plant. The physiological state of the plant does have an influence on its response to attempts to initiate tissue culture. The parent plant must be healthy and free from obvious signs of disease or decay. The source, termed explant, may be dictated by the reason for carrying out the tissue culture. Younger tissue contains a higher proportion of actively dividing cells and is more responsive to a callus initiation programme. The plants themselves must be actively growing, and not about to enter a period of dormancy. Plant tissue culture is used widely in plant science; it also has a number of commercial applications. Tissue culture is employed in; micropropagation, elimination of pathogens from plant materials, germoplasm storage, production of somaclonal varients, embryo rescue, production of haploids, production of artificial seeds, production of secondary metabolities, production of transgenic plants etc. Some of the fundamentals of the book are plant tissue culture, basic requirements for tissue culture laboratory, surface sterilization of explant materials, development of tissue culture techniques, principles of cell culture cell, special factors influencing growth and metabolism, media for culturing cells and tissues, sterilisation procedures, design and equipment of a tissue culture laboratory, isolation method for microorganisms for culture, culture preservation and stability, genetic modification of industrial microorganisms mutation etc.
The present book discuss about the methods, culture preservation and stability procedures, storage and transportation of plant cell tissue culture. This book is an invaluable resource for research workers, students, technocrats, entrepreneurs, institutional libraries etc.

4. A Constant Temperature Room
5. A Shaker System
Formulation of Tissue Culture Medium
1. Composition of M.S. Medium
2. Preparation of M.S. Medium
Collection of Explant Materials
Surface Sterilization of Explant Materials
Preparation of Explants and inculcation
Incubation of Culture Flasks
2. SUBCULTURE OF CALLUS
Regeneration of Plants from Callus
Organogentic Method
Embryogenesis Method
3. NUCELLUS CULTURE
4. EMBRYO CULTURE
Uses of Embryo Culture
5. MERISTEM CULTURE
Uses of Meristem Culture
6. ANTHER CULTURE
Procedure For Anther Culture
Uses of Anther Culture
7. SUSPENSION CULTURE
Methods For Growth Measurement
Experiments to Assess the Cell Viability
Uses of Suspension Culture
8. DEVELOPMENT OF TISSUE CULTURE TECHNIQUES
9. PRINCIPLES OF CELL CULTURE
CELL
Fine Cell Structure
Nuclearcytoplasmic Relationships
Cellular Activity
CELL DIVISION
CELLTYPES AND TISSUES
BEHAVIOUR OF CELLS IN CULTURE GROWTH,
DIFFERENTIATION AND METABOLISM
Primary And Established Cell Lines
The Nature Of Cell Alteration Or Transformation

Do Cultured Cells Differentiate?
KINETICS OF CELL GROWTH
(a) Established cell lines
(b) Primary cell lines
The cell cycle
Interaction among cells
Genetics of cultured cells
METABOLISM
Carbohydrate metabolism
Synthetic mechanisms
Protein Metabolism
Lipid metabolism
Nucleic acids
Structural elements
Relation of metabolism to growth

SPECIAL FACTORS INFLUENCING GROWTH AND METABOLISM
THE CELL AND ITS ENVIRONMENT PRESUMABLY
Temperature
Osmotic pressure
Hydrogen ion concentration
Other inorganic ions
Carbohydrates
Gases
Amino acids
Vitamins
Proteins and peptides
Supplementary metabolites
Hormones
Other specific factors
The matrix
Balance among factors
MEDIA FOR CULTURING CELLS AND TISSUES
I. NATURAL MEDIA
PLASMA
BLEEDING FROM THE WING
BLEEDING FROM THE HEART
BLEEDING FROM THE CAROTID ARTERY
COLLAGEN
BIOLOGICAL FLUIDS
Preparation of serum
Placental cord serum
Aminiotic fluid
Ascitic and pleural fluid
Aqueous humour
Serum ultrafiltrates
Dialysed serum
Insect haemolymph
Coconut water (coconut milk)
TISSUE EXTRACTS
The preparation of embryo extract
Preparation of chick embryo extract
Preparation of embryo extract from young embryos
The preparation of bovine embryo extract
Ultrafiltrates of embryo extract
Other tissue extracts
Other media of biological origin
MEDIA FOR CULTURING CELLS AND TISSUES
II. DEFINED MEDIA
MEDIA FOR TISSUES FROM WARMBLOODED VERTEBRATES
Solubility of materials.
Compatibility of components
Purity of materials.
Chemical instability
Stock solutions.
BALANCED SALT SOLUTIONS
Materials
Preparing a balanced salt solution
PARTIALLY COMPLETE SYNTHETIC AND COMPLETE MEDIA

Preparation of Eagles Medium
MEDIA FOR CULTURE OF TISSUES FROM COLD
BLOODED VERTEBRATES
MEDIA FOR INVERTEBRATE TISSUES
MEDIA FOR PLANT TISSUES
10. PREPARATION OF MATERIALS
PREPARATION OF APPARATUS
Glassware
Plastic vessels
Stoppers for culture vessels
Rubber tubing
Instruments, etc
CLEANING PROCEDURES GLASSWARE
Detergents
Alkalies
Oxidising acids
Ultrasonics
Special problems
Automatic washing machines
PREVENTION OF CONTAMINATION
I. STERILISATION PROCEDURES
Sterilisation by dry heat
Sterilisation by moist beat
Radiations
Antiseptics
Antibiotics
Filtration
Storage of sterile materials
Chronic contamination (especially PPLO and L forms)
Sterility testing
Elimination of contamination
Outbreaks of contamination
PREVENTION OF CONTAMINATION
II. ASEPTIC TECHNIQUE
Contamination from tissue
Contamination from the air
Contamination from the operator
DESIGN AND EQUIPMENT OF A TISSUE CULTURE LABORATORY
Sterilisation and cleaning facilities
Sterile working area
Storage for media
Incubator facilities
Special glassware and apparatus
General equipment
Special apparatus
Coverslip techniques
Rollertube techniques
Organ culture
Handling of strains
Sources of materials
LABORATORY DESIGN
A singleroom unit
Laboratory suite for tissue culture

Sterilisation room
The preparation room
The aseptic room
Aseptic cubicle
Hot room
General facilities
11. PRIMARY EXPLANATION TECHNIQUES
I. TISSUE CULTURES
SLIDE CULTURES
THE PREPRATION OF SLIDE CULTURE
Single coverslip with plasma clot
Maximow double coverslip method with plasma clot
Single coverslip with liquid medium. Laying and hanging drop cultures
AFTERCARE OF SLIDE CULTURES
Washing and feeding double coverslip cultures
Patching
Transferring coverslips cultures
CARREL FLASK TECHNIQUE
PREPARATION OF CULTURES
Renewal of medium
The transfer of tissue
TESTTUBE CULTURES
Plasma clot technique
Feeding testtube cultures.
Patching testtube cultures
Transfer of cultures from testtube
Culture of primary explants in roller tubes without
plasma.
Flying coverslips in test tubes
THREEDIMENSIONAL SUBSTRATES
PRIMARY EXPLANTATION TECHNIQUES
II. ORGAN AND EMBRYO CULTURE
Organ cultures on plasma clots
Cultures on agar
Fluid media
PREPARING AN ORGAN CULTURE ON A CELLULOSE ACETATE RAFT
SETTING UP AN ORGAN CULTURE OF EMBRYONIC LIMB BONES ON A GRID
Set up apparatus
Prepare dishes
Prepare explants
Set up explants (e.g. chick limb bones)
Subculture (The medium should be changed every
48 hours.)
CHOPPED TISSUE TECHNIQUE
Cultivation of poliomyelitis virus in minced tissue
suspensions
CUTTING CHICK EMBRYONIC HEART EXPLANTS BY MEANS OF THE McILWAIN TISSUE CHOPPER
WHOLE EMBRYO CULTURE
Culture of preimplantation mammalian embryos
Culture of postimplantation mammalian embryos
PRIMARY EXPLANTATION TECHNIQUES
III. DISAGGREGATION METHODS

Object of Preservation
Good Record Keeping of Previous Treatment and Lineage
Notation of Reported Characteristics of a Culture
Culture Preservation and Stability
DETERMINANTS FOR CULTURE IDENTITY, CHARACTERISTICS AND PURITY
Authenticated Cultures Confirmation of Stated Traits
Morphological
Biochemical
Physiological
Research and Development Strains
Elimination of leaky mutants
Assurance of auxotrophic traits (elimination of mixed genetic bag)
Selective pressure for maintaining specific culture traits
Longterm Storage
Cost efficiency
Minimal maintenance
Endurance of label
Precise inventory system
Shortterm Storage
Ease of sample preparation
Label reliability
Economic aspects
Reliability
Ease of retrieval
Rapid retrieval
SELECTION OF MAINTENANCE CONDITIONS AND PROCEDURES FOR IMPLEMENTATION, BASED ON CULTURE USE
Longterm Storage
Analytical organisms
Comparison strains
Manufacturing plant cultures
Shortterm Storage
.New metabolite producers for investigative studies
Clones from populations for improved metabolite producers
Working stocks of analytical organisms
CULTURE RESTORATION AND GROWTH CONSIDERATIONS
Restoration
Concentration of inocula
Nutrition
Osmotic (rehydration)
Temperature (rehydration and/or rate of melting)
Growth
Requirements
Temperature
Aeration (including dissolved gases)
Duration
Verification of Purity
15. GENETIC MODIFICATION OF INDUSTRIAL MICROORGANISMS
MUTATION

DNA Repair Mechanisms
Mutagen Specificity
Survival Curves and Optimum Conditions for the Use of a Motagen and Expression of Mutations
Site Specific Mutagenesis
Applications of Mutation to Antibioticproducing
Microorganisms
RECOMBINATION
Protoplast Fusion
Conjugation and Natural Plasmids
Transformation
Transduction
Sexuality and Parasexuality in Fungi
Recombinant DNA Technology
Transposable Elements
Applications of Recombination to Antibioticproducing Microorganisms
GENETICS AND SCREENING
16. IN VITRO RECOMBINANT DNA TECHNOLOGY
GENERATION AND CLONING OF DNA FRAGMENTS
Fragmentation of DNA
Class II restriction enzymes
Random DNA fragments and the generation of genomic libraries
Enrichment for specific D.N.A. sequences
Synthesis of cDNA
Chemical synthesis of DNA
Covalent Linkage of DNA Fragments to Vector Molecules
Ligation to sector molecules
Methods favouring formation of hybrid DNAmolecules
Modification of DNA Extremities
Isolation of Recombinant Molecules and Interspecies DNA Transfer
Transformation and transfection
In vitro packaging
CLONING VECTORS
Plasmid Vectors
Vectors Derived from Bacteriophage l
Phage vectors
Cosmids vectors
Special Purpose Cloning Vectors
Expression lectors
Singlestranded phage vectors
Plasmid vectors for subcloning and sequencing
Vectors for the detection of transcription and translation signals
Vector Systems for Organisms other than E. coli
DETECTION AND ANALYSIS OF CLONES
Screening Recominant Clones
Nucleic acid homology
Translation in vitro
Immunological screening
Characterization of Cloned DNA
Isolation of cloned DNA
Physical characterization of cloned fragments
Characterization of products expressed by cloned
fragments
MANIPULATION OF CLONED GENES

GROWTH OF UNICELLULAR ORGANISMS
Cocci
Grampositive Rods
Gramnegative Rods
Budding Yeasts (Saccharomyces)
THE CELL CYCLE
GROWTH OF FILAMENTOUS ORGANISMS
Germination of Fungal Spores
Hyphal Morphology
Growth of Individual Hyphae
The extension zone
Cytology of the nonextending part of fungal hyphae
The peripheral growth zone
Growth of Mycelia
YEASTMYCELIAL DIMORPHISM
COLONY GROWTH
Growth of Colonies on Solid Media
Growth of Colonies in Liquid Media
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Analysis of Multispecies Communities
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Genetic Interactions
Mixed Culture Processes
Spontaneous mixed culture processes
Defined mixed cultures
Contamination and Degradation
Contamination
Industrial fermentations with unstable strains
Environmental Biotechnology
MIXED SUBSTRATES
Patterns of Mixed Substrate Utilization
Control of Mixed Substrate Utilization in Batch Culture
Control by regulation of substrate transport
Control by regulation of enzyme synthesis
Control by regulation of enzyme activity
Mixed Substrate Utilization in Continuous Culture
Double substrate limited growth
Efficiency of growth on mixed substrates
COMETABOLISM
Cometabolism in the Environmen
Technological Potential
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ISOLATION OF PROTOPLASTS
1. Mechanical Method
2. Enzymatic Method

MAINTENANCE OF PROTOPLASTS
Viability Tests for Protoplasts
1. FAD Test
2. Phenol Safranin Test
3. ColflourWhiteTest
4. Microscopic Observation of Cytfoplasmic Streaming
Plant Regeneration from Protoplasts
Applications of Protoplast Culture
PROTOPLAST FUSION
Methods of Protoplast Fusion
Selection of Hybrid protoplasts
Regeneration of Plantlets
Uses of Protoplast Fusion
INVITRO MUTATION BREEDING
Induction of invitro Mutagenesis
Uses of Invitro Mutation Breeding

23. GERMPLASM STORAGE
GERMPLASM STORAGE BY CRYOPRESERVATION
1. Collection of Plant Materials
2. Addition of Cryoprotective Agents
3. Freezing Treatment
4. Longterm Cold Storage
REUSE OF PRESERVED TISSUE
1. Thawing
2.Removal of Cryogen
3. Callus Induction
4. Plant Regeneration
Achievements
Advantages of Cryopreservation
STORAGE OF GERM PLASM OF POTATO
24. GENETIC ENGINEERING THROUGH THE TRANSFER OF CELL ORGANELLES
1. Isolation of Cell Organelles
2. Isolation of Protoplasts
3. Induction of protoplast to uptake cell Organelles
4. Selection of Transformed Protoplast
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Production of Cybrids
Applications of Cybrids
25. SPECIAL CONSIDERATIONS FOR DIFFERENT TISSUES
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Embryonic tissues
DISSECTION OF THE CHICK EMBRYO
Chick embryonic limbbones for organ culture
MAMMALIAN EMBRYONIC TISSUES
ADULT TISSUES
PREPARATION OF EXPLANTS OF THE BUFFY COAT
Culture of peripheral blood leucocytes
Human skin fibroblasts
PROLONGED CULTURE OF DIFFERENTIATED CELLS
CULTIVATION OF TISSUES FROM COLDBLOODED VERTEBRATES

Determining the mitotic coefficient
Planimetry
Examination of living cells
Photography
PERFUSION OR CIRCUMFUSION CHAMBERS
Timelapse cinemicrography
QUANTITATIVE OPTICAL METHODS
Auto radiography
Preparation of cultures for electron microscopy
30. APPLICATIONS OF TISSUE CULTURE
I. Micropropagation
2. Elimination of Pathogens
3. Germplasm Storage
4. Somaclonal Variation
5. Embryo Rescue
6.The Production of Haploids
7. Artificial Seeds
Types of Artificial Seeds.
8. Production of Secondary Metabolites
9. Production of Somatic Hybrids
10. Transgenic Plants
Secondary Metabolites
Culture of Plant Cells for the Extraction of Secondary Metabolites
1. Designing of Bioreactor
2. Selection of Explant Source
3. Surface Steriflization
4. Preparation of Explant
5. Callus Culture
6. Suspension Culture
7. Cell Plating
8. Testing for Biosynthetic Activity
9. Culture of more Productive Clones
10. Extraction of Secondary Metabolites
Biotransformation In Plant Cells
Elicitor dependent Biosynthesis
Immobilization of Plant Cells
Hairy Root Clones
31. LIST OF CULTURE
NCTC 109 AND NCTC 135
32. SOURCES OF MATERIALS FOR TISSUE CULTURE
General suppliers of laboratory apparatus
General glassware (in addition to above firms)
General biological products and biochemicals
General chemicals
Special tissue culture media
Suppliers of cell cultures

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Fri, 09 May 2025 06:29:36 +0000