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 metabolites, 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.

Contents

1. PLANT TISSUE CULTURE
   Historical Events in Plant Tissue Culture
   Basic Requirements for Tissue Culture Laboratory
   1. Area for Medium Preparation
   2. A Sterile Room
   3. Glasswares and Other Instruments
   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 inoculation
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
Amniotic 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 heat
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 PREPARATION 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
PREPARATION OF CELL SUSPENSIONS FROM FRESH TISSUES
Disaggregation of embryonic limb buds
Preparation of trypsinised embryonic carcass
Trypsination of monkey kidney tissue
Preparation of primary human amnion cells
Trypsination procedure
Trypsination in the cold
Cloning of primarily disaggregated cells
12. CELL LINES
STATIC CULTURE METHODS
SUSPENDING CELLS FROM A MONOLAYER
CULTURE
INOCULATION OF NEW VESSELS
FEEDING AND MAINTENANCE
Agar slope cultures
SUSPENSION CULTURES
Media for suspension cultures
Gas phase
General methods
General management of suspension cultures
Batch cultures
Continuous medium replacement
GROWTH OF PLANT CELLS IN SUSPENSION
CLONING CELLS
Cloning of HeLa cells by the dilution technique
Agar suspension technique
Cloning in fibrin gels
Cloning cells by the isolation technique
Technique
Characterisation of cell lines
SPECIAL ASPECTS OF HANDLING PRIMARY CELL LINES
General maintenance
Seed stocks
13. ISOLATION METHOD FOR MICROORGANISMS FOR CULTURE
SOURCES OF ORGANISMS AND SOME SAMPLING
STRATEGIES
DIRECT ISOLATION METHODS
Pretreatment of Samples
DILUTION AND INCUBATION OF SAMPLES
Media Considerations
ENRICHMENT CULTURE METHODS
Baiting Methods
General Chemical Enrichment
Specialized Enrichment Systems and their Applications
Enrichments from sea water
Enrichments for biomass production
Enrichments for nitratereducing bacteria
Enrichments in complex media
Biodegradation
Heterogeneous continuous flow systems
14. CULTURE PRESERVATION AND STABILITY
PROCEDURES PRIOR TO SELECTING A
PRESERVATION METHOD
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 DNA molecules

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 I

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

IN VITRO

Mutagenesis
Generation of deletions and insertions
Point mutations
Efficient Expression of Cloned Genes
Constructions that maximize expression
Secretion of cloned products

17. NUTRITIONAL REQUIREMENTS OF MICROORGANISMS
BACTERIA AND FUNGI
Macronutrients
Carbon
Nitrogen
Hydrogen
Oxygen
Phosphorus
Sulfur
Potassium
Magnesium
Micronutrients
Growth requirements
Effects of trace elements
Addition of trace elements
Chelation
Growth Factors
Vitamins
Amino acids
Miscellaneous growth factors
ALGAE
Macronutrients
Carbon, oxygen and hydrogen
Nitrogen
Phosphorus and sulfur
Potassium and magnesium
Micronutrients
Growth Factors
PROTOZOA

18. DESIGN, PREPARATION AND STERILIZATION OF FERMENTATION MEDIA
MEDIUM DESIGN
MEDIUM PREPARATION
STERILIZATION

19. NUTRIENT UPTAKE AND ASSIMILATION
NUTRIENT UPTAKE
Simple Diffusion
Transport Systems
Facilitated diffusion
Active transport
Redundancy of Transport Systems
ASSIMILATION
Assimilation of Carbon
Assimilation of Nitrogen
Control of nitrogen assimilation
Assimilation of Other Elements

20. MODES OF GROWTH OF BACTERIA AND FUNGI
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
EFFECT OF GROWTH RATE AND OTHER VARIABLES ON CELL COMPOSITION AND MORPHOLOGY
Unicellular Organisms
Fungi and Actinomycetes
21. MIXED CULTURE AND MIXED SUBSTRATE SYSTEMS
MIXED CULTURES
Methods of Study
Enrichment of Mixed Cultures
Analysis of Twospecies Systems
Analysis of Multispecies Communities
Kinetics of Mixed Cultures
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 Environment
Technological Potential
22. PROTOPLAST TECHNOLOGY
ISOLATION OF PROTOPLASTS
1. Mechanical Method
2. Enzymatic Method
MAINTENANCE OF PROTOPLASTS
Viability Tests for Protoplasts
1. FAD Test
2. Phenol Safranin Test
3. ColfleurWhiteTest
4. Microscopic Observation of Cytoplasmic 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
5. Regeneration of Plantlets

Advantages of Organelle Uptake Method

SUBPROTOPLASTS
Production of Cybrids
Applications of Cybrids

25. SPECIAL CONSIDERATIONS FOR DIFFERENT TISSUES

VERTEBRATE TISSUES

Embryonic tissues

DISSECTON 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

CULTURE OF INVERTEBRATE TISSUES

Arthropods
Other invertebrates
STORAGE OF TISSUE BEFORE CULTURING
CULTURE OF PLANT TISSUES
Preparation of tissues from plants
Cultivation of plant tissues
Culture of tomato roots
Culture of carrot callus

26. CULTIVATION OF CELLS IN VIVO TRANSPLANTATION
Transplantation into embryos

PROCEDURE
Transplantation into tolerant chimeras
Transplantation into genetically similar hosts
Transplantation into nonvascular areas
Procedure for anterior eye chamber implantation
Procedure for brain implantation
Diffusion chambers
Transplantation to irradiated and cortisonetreated animals
scites tumours

Maintenance of sterility

27. LARGESCALE CULTURE METHODS
Preparation and sterilisation of apparatus
Preparation and sterilisation of media
Cells and media

APPARATUS FOR MASSIVE CULTURE OF CELLS ON GLASS SURFACES
Largescale Roux flask cultures
Roller bottle methods
Solid matrix perfusion systems.
The multiple surface tissue culture propagator

MASSIVE SCALE SUSPENSION CULTURE
Culture vessels

CONTROL OF CULTURE CONDITIONS
Temperature
pH
Oxygen

28. PRESERVATION, STORAGE AND TRANSPORTATION OF LIVING TISSUES AND CELLS
Maintenance at slightly reduced temperatures
Maintenance at refrigerator temperature
Preservation by freezing

Equipment
General Procedure
Transportation of cells

29. MORPHOLOGICAL STUDIES
Morphological Studies

COMMON FIXATION AND STAINING TECHNIQUES
FOR TISSUE CULTURE MATERIAL
I. Commonly used fixatives
II. Routine stains
III. Special histochemical stains
Chromosome spreading technique
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 Sterilization
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

About NIIR

NIIR PROJECT CONSULTANCY SERVICES (NPCS) is a reliable name in the industrial world for offering integrated technical consultancy services. NPCS is manned by engineers, planners, specialists, financial experts, economic analysts and design specialists with extensive experience in the
related industries.


NPCS also publishes varies process technology, technical, reference, self employment and startup books, directory, business and industry database, bankable detailed project report, market research report on various industries, small scale industry and profit making business. Besides being used by manufacturers, industrialists and entrepreneurs, our publications are also used by professionals including project engineers, information services bureau, consultants and project consultancy firms as one of the input in their research.

Our Detailed Project report aims at providing all the critical data required by any entrepreneur vying to venture into Project. While expanding a current business or while venturing into new business, entrepreneurs are often faced with the dilemma of zeroing in on a suitable product/line.