

# Handbook on Plants and Cell Tissue Culture

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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 variants, 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.

## 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 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 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 limbbuds

Preparation of trypsinised embryonic carcass

Trypsinisation of monkey kidney tissue

Preparation of primary human amnion cells

Trypsinisation procedure

Trypsinisation 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 Mutagen and Expression of Mutations  
Site Specific Mutagenesis  
Applications of Mutation to Antibiotic-producing  
Microorganisms  
RECOMBINATION  
Protoplast Fusion  
Conjugation and Natural Plasmids  
Transformation  
Transduction  
Sexuality and Parasexuality in Fungi  
Recombinant DNA Technology  
Transposable Elements  
Applications of Recombination to Antibiotic-producing 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 vector 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  $\lambda$   
Phage vectors  
Cosmids vectors  
Special Purpose Cloning Vectors  
Expression vectors  
Single-stranded 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 Recombinant 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 Environmen

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. ColflourWhiteTest
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

### DISSECTION OF THE CHICK EMBRYO

#### Chick embryonic limb bones 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 COLD BLOODED 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 cortisone-treated 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  
1. 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

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