

Plant Tissue Culture

(Nucleic Acids, Amino Acids, Callus Culture, Transgenic Plants, Embryo Rescue, Embryonic Tissues, Cometaabolism, Fungi and Actinomycetes, Grampositive Rods, Cloning Vectors, Biodegradation, Batch Cultures, Organ Culture)



Introduction

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 a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micro propagation. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.

To quickly produce mature plants.

The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.

The regeneration of whole plants from plant cells that have been genetically modified.

The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.

The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e.: orchids and Nepenthes.

To clear particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

Table of 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. Glass wares 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

Nuclear cytoplasmic 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 PPL0 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

- **PREPARATION OF CELL SUSPENSIONS FROM FRESH TISSUES**

Disaggregation of embryonic limb buds

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 nitrate-reducing 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 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 vector 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 λ

Phage vectors

Cosmids vectors

Special Purpose Cloning Vectors

Expression vectors

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 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. Col flour White Test

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 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

- 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

Plant Tissue Culture in India, Commercialization of Plant Tissue Culture in India, Role of Plant Tissue Culture in Agriculture, Plant Tissue Culture Industry in India, Industrial Plant Tissue Culture, Tissue Culture in Agriculture, Plant Tissue Culture, Tissue Culture, Cell Culture and Tissue Culture, Tissue Culture and Cell Culture, Tissue Culture in Plants, Plant Cell and Tissue Culture, Commercial Plant Tissue Culture in India, Plant Tissue Culture Business Plan, Plant Tissue Culture and Biotechnology, Tissue Culture Plants, Plant Tissue Culture Business Plan, Business Opportunities in Plant Tissue Culture, Tissue Culture Methods, Cybrid Production, Process of Cybrids Production, Production of Cybrids, Production of Cybrid Plants, Production of Haploid Plants, Haploid Production, Plant Secondary Metabolism, Production of Secondary Metabolites, Production of Secondary Metabolites Using Plant Cell Cultures, Plant Tissue Cultures in Production of Secondary Metabolites, Secondary Metabolites Production, Production of Somatic Hybrid Plants, Somatic Hybridization of Plants, Somatic Hybrid, Somatic Hybrid Production, Production of Enriched Biomass, Enrichment on Biomass Production, Formulation of Tissue Culture Medium, Collection of Explant Materials, Subculture of Callus, Regeneration of Plants from Callus, Preparation of Chick Embryo Extract, Preparation of Embryo Extract from Young Embryos, Preparation of Bovine Embryo Extract, Preparation of Eagles Medium, Media for Plant Tissues, Organ Culture,

Preparation of Trypsinised Embryonic Carcass, Enrichment Culture Methods, Genetic Modification of Industrial Microorganisms Mutation, Methods Favouring Formation of Hybrid DNA Molecules, Modes of Growth of Bacteria and Fungi, Mixed Culture and Mixed Substrate Systems, Spontaneous Mixed Culture Process, Maintenance of Protoplasts, Collection of Plant Materials, Storage of Germ Plasm of Potato, Mammalian Embryonic Tissues, Preparation of Tissues from Plants, Largescale Culture Methods, Preparation and Sterilisation of Apparatus, Preparation and Sterilisation of Media, Reservation, Storage and Transportation of Living Tissues and Cells, Culture of Plant Cells for Extraction of Secondary Metabolites, Preparation of Explant, Suspension Culture, Extraction of Secondary Metabolites, Biotransformation in Plant Cells, Immobilization of Plant Cells, Special Tissue Culture Media, Manufacturing Plant Cultures, Products from Plant Tissue Culture, Cultivation of Plant Tissue, Cultures of Tomato Roots, Tissue Culture of Tomato Roots, Preparation of Carrot Callus Culture, Tissue Culture of Carrot Callus, Carrot Callus Tissue for Culture, Cultivation of Cells in Vivo Transplantation, Cultures on Agar, Npcs, Niir, Process Technology Books, Business Consultancy, Business Consultant, Project Identification and Selection, Preparation of Project Profiles, Startup, Business Guidance, Business Guidance to Clients, Startup Project, Startup Ideas, Project for Startups, Startup Project Plan, Business Start-Up, Business Plan for Startup Business, Great Opportunity for Startup, Small Start-Up Business Project, Best Small and Cottage Scale Industries, Startup India,

Stand Up India, Small Scale Industries, New Small Scale Ideas for Haploid Production Industry, Cybrid Production Business Ideas You Can Start on Your Own, Indian Secondary Metabolites Production Industry, Small Scale Somatic Hybrid Production, Guide to Starting and Operating Small Business, Business Ideas for Enriched Biomass Production, How to Start Secondary Metabolites Production Business, Starting Enriched Biomass Production, Start Your Own Somatic Hybrid Production Business, Secondary Metabolites Production Business Plan, Business Plan for Cybrid Production, Small Scale Industries in India, Haploid Production Based Small Business Ideas in India, Small Scale Industry You Can Start on Your Own, Business Plan for Small Scale Industries, Set Up Cybrid Production, Profitable Small Scale Manufacturing, How to Start Small Business in India, Free Manufacturing Business Plans, Small and Medium Scale Manufacturing, Profitable Small Business Industries Ideas, Business Ideas for Startup

Niir Project Consultancy Services (NPCS) can provide Process Technology Book on **Plant Tissue Culture**

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- *We adopt a systematic approach to provide the strong fundamental support needed for the effective delivery of services to our Clients' in India & abroad*

We at NPCS want to grow with you by providing solutions scale to suit your new operations and help you reduce risk and give a high return on application investments. We have successfully achieved top-notch quality standards with a high level of customer appreciation resulting in long lasting relation and large amount of referral work through technological breakthrough and innovative concepts. A large number of our Indian, Overseas and NRI Clients have appreciated our expertise for excellence which speaks volumes about our commitment and dedication to every client's success.



We bring deep, functional expertise, but are known for our holistic perspective: we capture value across boundaries and between the silos of any organization. We have proven a multiplier effect from optimizing the sum of the parts, not just the individual pieces. We actively encourage a culture of innovation, which facilitates the development of new technologies and ensures a high quality product.



What do we offer?

- *Project Identification*
- *Detailed Project Reports/Pre-feasibility Reports*
- *Business Plan*
- *Industry Trends*
- *Market Research Reports*
- *Technology Books and Directory*
- *Databases on CD-ROM*
- *Laboratory Testing Services*
- *Turnkey Project Consultancy/Solutions*
- *Entrepreneur India (An Industrial Monthly Journal)*

How are we different ?

- *We have two decades long experience in project consultancy and market research field*
- *We empower our customers with the prerequisite know-how to take sound business decisions*
- *We help catalyze business growth by providing distinctive and profound market analysis*
- *We serve a wide array of customers , from individual entrepreneurs to Corporations and Foreign Investors*
- *We use authentic & reliable sources to ensure business precision*

Our Approach

Requirement collection

Thorough analysis of the project

Economic feasibility study of the Project

Market potential survey/research

Report Compilation

Who do we serve?

- *Public-sector Companies*
- *Corporates*
- *Government Undertakings*
- *Individual Entrepreneurs*
- *NRI's*
- *Foreign Investors*
- *Non-profit Organizations, NBFC's*
- *Educational Institutions*
- *Embassies & Consulates*
- *Consultancies*
- *Industry / trade associations*

Sectors We Cover

- *Ayurvedic And Herbal Medicines, Herbal Cosmetics*
- *Alcoholic And Non Alcoholic Beverages, Drinks*
- *Adhesives, Industrial Adhesive, Sealants, Glues, Gum & Resin*
- *Activated Carbon & Activated Charcoal*
- *Aluminium And Aluminium Extrusion Profiles & Sections,*
- *Bio-fertilizers And Biotechnology*
- *Breakfast Snacks And Cereal Food*
- *Bicycle Tyres & Tubes, Bicycle Parts, Bicycle Assembling*

Sectors We Cover Cont...

- *Bamboo And Cane Based Projects*
- *Building Materials And Construction Projects*
- *Biodegradable & Bioplastic Based Projects*
- *Chemicals (Organic And Inorganic)*
- *Confectionery, Bakery/Baking And Other Food*
- *Cereal Processing*
- *Coconut And Coconut Based Products*
- *Cold Storage For Fruits & Vegetables*
- *Coal & Coal Byproduct*

Sectors We Cover Cont...

- *Copper & Copper Based Projects*
- *Dairy/Milk Processing*
- *Disinfectants, Pesticides, Insecticides, Mosquito Repellents,*
- *Electrical, Electronic And Computer based Projects*
- *Essential Oils, Oils & Fats And Allied*
- *Engineering Goods*
- *Fibre Glass & Float Glass*
- *Fast Moving Consumer Goods*
- *Food, Bakery, Agro Processing*

Sectors We Cover Cont...

- *Fruits & Vegetables Processing*
- *Ferro Alloys Based Projects*
- *Fertilizers & Biofertilizers*
- *Ginger & Ginger Based Projects*
- *Herbs And Medicinal Cultivation And Jatropha (Biofuel)*
- *Hotel & Hospitability Projects*
- *Hospital Based Projects*
- *Herbal Based Projects*
- *Inks, Stationery And Export Industries*

Sectors We Cover Cont...

- *Infrastructure Projects*
- *Jute & Jute Based Products*
- *Leather And Leather Based Projects*
- *Leisure & Entertainment Based Projects*
- *Livestock Farming Of Birds & Animals*
- *Minerals And Minerals*
- *Maize Processing(Wet Milling) & Maize Based Projects*
- *Medical Plastics, Disposables Plastic Syringe, Blood Bags*
- *Organic Farming, Neem Products Etc.*

Sectors We Cover Cont...

- *Paints, Pigments, Varnish & Lacquer*
- *Paper And Paper Board, Paper Recycling Projects*
- *Printing Inks*
- *Packaging Based Projects*
- *Perfumes, Cosmetics And Flavours*
- *Power Generation Based Projects & Renewable Energy Based Projects*
- *Pharmaceuticals And Drugs*
- *Plantations, Farming And Cultivations*
- *Plastic Film, Plastic Waste And Plastic Compounds*
- *Plastic, PVC, PET, HDPE, LDPE Etc.*

Sectors We Cover Cont...

- *Potato And Potato Based Projects*
- *Printing And Packaging*
- *Real Estate, Leisure And Hospitality*
- *Rubber And Rubber Products*
- *Soaps And Detergents*
- *Stationary Products*
- *Spices And Snacks Food*
- *Steel & Steel Products*
- *Textile Auxiliary And Chemicals*

Sectors We Cover Cont...

- *Township & Residential Complex*
- *Textiles And Readymade Garments*
- *Waste Management & Recycling*
- *Wood & Wood Products*
- *Water Industry(Packaged Drinking Water & Mineral Water)*
- *Wire & Cable*

Contact us

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THANK YOU!!!

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