

# Plant Biotechnology Handbook

**Author:-** NIIR Board

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Plant biotechnology is a precise process in which scientific techniques are used to develop molecular and cellular based technologies to improve plant productivity, quality and health; to improve the quality of plant products; or to prevent, reduce or eliminate constraints to plant productivity caused by diseases, pest organisms and environmental stresses. It can be defined as human intervention on plant material by means of technological instruments in order to produce permanent effects, and includes genetic engineering and gene manipulation to obtain transgenic plants. Plant genetic engineering is used to produce new inheritable combinations by introducing external DNA to plant material in an unnatural way. The results are genetically modified plants (GMPs) or transgenic plants. The key instrument used in plant biotechnology is the plant tissue culture (PTC) technique which refers to the in vitro culture of protoplasts, cells, tissues and organs. Plant biotechnology in use today relies on advanced technology, which allows plant breeders to make precise genetic changes to impart beneficial traits to plants. The application of biotechnology in agriculture has resulted in benefits to farmers, producers and consumers. Plant biotechnology has helped make both insect pest control and weed management safer and easier while safeguarding plants against disease. The worldwide demand for food, feed and modern textile fibers can only be met in the future with the help of plant biotechnology. It has the potential to open up whole new business areas that will totally redefine the current market scope and perception.

This book majorly deals with the organisms of biotechnology, herbicide resistant plants, transgenic plants with improved storage proteins, engineering for preservation of fruits, enhancing the photosynthetic efficiency, basic requirements for nitrogen fixation, animal and plant cell cultures, insecticides, cellular characteristics which influence the choice of cell, the growth of animal and plant cells immobilized within a confining matrix, virus free clones through plant tissue culture, microbial metabolism of carbon dioxide, organisms involved in the conversion of hydrogen, hydrogen utilization by aerobic hydrogen oxidizing bacteria, overproduction of microbial metabolites, regulation of metabolite synthesis etc.

The book contains measurement of plant cell growth, plant tissue culture, initiation of embryo genesis in suspension culture, micro propagation in plants, isolation of plant DNA and many more. This is very helpful book for entrepreneurs, consultants, students, institutions, researchers etc.

## 1. The organisms of biotechnology

Cells - The Basic Units

Types of Microorganism

Viruses

Prokaryotes

Eukaryotes

Algae

Protozoa

Fungi

Tissue Cultures

Animal Cells

Plant Cells

2. Transgenic plants

Herbicide Resistant Plants

1. Glyphosate Tolerant Plants

2. Sulphonylurea Tolerant Plants

3. Atrazine Tolerant Plants

4. Phosphinothricin Tolerant Plants

5. Bromoxynil Tolerant Plants

Insect Resistant Plants

1. Transgenic Plants with Bt Toxin

2. Transgenic Plants with Bt Toxin and Serine Protease

Inhibitor Gene

3. Transgenic Plants with Cowpea Trypsin Inhibitor

4. Transgenic Plants with Nicotiana glauca Proteinase Inhibitor

Virus Resistant Plants

1. Transgenic Plants with Viral Coat Protein

2. Transgenic Plants with Viral Nucleoprotein

3. Transgenic Plants with Viral SAT RNA

4. Transgenic Plants with Antisense RNA

Transgenic Plants Resistant to Fungi and Bacteria

Transgenic Plants with Improved Storage Proteins

Sweet Proteins

Enriching the Carbohydrate Contents

Improving the Quality of Oils and Fats

Male Sterility and Fertility Restoration

Changing the Flower Colours

Stress Tolerant Plants

Cold Tolerant Plants

Drought Tolerant Plants

Plant Tolerant to High Light Intensity

Engineering for Preservation of Fruits

Enhancing the Photosynthetic Efficiency

Transgenic Plants as Bioreactors

Vaccines

Interferons

Pharmaceutical Compounds

Biodegradable Plastics

3. Biological Nitrogen fixations

Non-symbiotic Nitrogen Fixation

Features Favourable for Non-symbiotic Nitrogen Fixation

1. Special separation of Nitrogen Fixing Cells

2. Protein-Nitrogenase Association

3. High Rate of Respiration

4. Time specific Nitrogenase Activity

5. Association with Rapid Oxygen Consumers

6. Presence of hydrogenase

## 7. Colonization

### Nitrogenase

#### Basic requirements for Nitrogen Fixation

#### Mechanism of Nitrogen Reduction

#### Assimilation of Ammonia

#### Route I

#### Route II

#### Symbiotic Nitrogen Fixation

#### Host Specificity

#### Root Nodulation

#### Mechanism of Nitrogen Fixation

##### (a) Oxygen Transport by Leghaemoglobin

##### (b) Utilization of Oxygen by Hydrogenase

#### Nitrogenase

#### Requirement for Nitrogen reduction

#### Assimilation of Ammonia

## 4. Genetics of Nitrogen Fixation

### Nif-genes of Klebsiella Pneumoniae

#### Regulation of Nif Genes

#### Nif-genes of Azotobacter

#### Nif-genes of Anabaena

### Genetics of Legume-Rhizobium Nitrogen Fixation

#### 1. Rhizobial Genes

##### a) Nod Genes

##### b) Nif Genes

##### c) Hup Genes

#### 2. Legume Nodulin Genes

#### Leghaemoglobin Gene

#### Overall Regulation of Genes

#### Gene Transfer for Nitrogen Fixation

##### 1. Transfer of Nif Genes to Non-Nitrogen Fixing Bacteria

##### 2. Transfer of Nif Genes to yeasts

##### 3. Transfer of Nif-Genes to plants

##### 4. Transfer of Nod Genes

##### 5. Transfer of Hup Genes

##### 5. Mycorrhizae for Agriculture and Forestry

### Mycorrhizal types and their structural and nutritional features

#### Ectomycorrhizae

#### Mechanism of ECM formation

#### Morphology and structure

#### Synthesis of mycorrhiza

#### Cultural study

### Vesicular arbuscular Mycorrhiza

#### Introduction

#### Evolution

#### Taxonomy

#### Classification

#### Distribution

#### Lifecycle

#### Reproduction

#### Sexual reproduction

#### A sexual production

Method of Inoculum production of VAM  
Some important steps in production of VAM  
Host plant/growth medium  
Fertilizations/micronutrients  
Chemical application  
Control of fungal pathogens  
Plant vesicular arbuscular mycorrhizal fungal interactions  
VAM and soil biota  
Control of root diseases  
Endomycorrhiza fungi and tree diseases  
Mechanism of disease control  
6. Animal and plant cell cultures  
Historical perspectives  
Products and potentials  
Animal cells  
Immuno biologicals  
1. Virus vaccines  
2. Monoclonal antibodies  
3. Immunoregulator materials  
Insecticides  
Enzymes  
Hormones  
Whole cells  
Plant cells  
Pharmaceuticals  
Food additives  
Agrochemicals  
Perfumes  
Enzymes  
Speciality Chemicals  
Biomass applications of plant cell cultures  
Cell culture and product synthesis  
The nature of animal and plant cells in culture  
Cell culture initiation  
Culture development  
Secondary cultures  
Culture replication  
Industrially useful cell cultures  
Substrate independent cultures  
Individuality of cell lines in relation to the productivity  
Culture media  
Growth media  
Water  
Inorganic salts  
Trace elements  
Vitamins  
Buffers  
Sources of energy and carbon  
Nitrogen sources  
1. Defined nitrogen sources  
2. Undefined nitrogen sources  
Growth factors  
Other ingredients

Maintenance media

Cell culture technologies

Cellular characteristics which influence the choice of cell culture technology

Mixing

Aeration

Doubling times

1. Sterilization of media

2. Sterilization of equipment

Cell stickiness

Immobilized cell systems

The growth and exploitation of cell grown on the surface of a supporting solid substratum

1. Multiple process

2. Unit process

The growth of animal and plant cells immobilized within a confining matrix

1. Gel entrapment systems

2. Applications of entrapped cells

Dynamic cell systems

Air driven systems

Impeller and air driven systems

Impeller mixed systems

7. Somaclonal variation, cell selection and genotype improvement

Somaclonal variation

Historical perspective

The manifold incidence of somaclonal variation

Range of species

Characters displaying variation

Genetic nature of somaclonal variants

Pre-existing or culture induced variation

Genetic and explant sources effects

The origin of somaclonal variation

Chromosomal abnormalities

Molecular possibilities

Gene amplification and diminution

Transposable elements

Cell selection

Disease resistance

Herbicide tolerance

Nutritional quality

Other cell selection systems

8. Virus-free clones through plant tissue culture

Distribution of viruses in plants

Techniques for eradication

Heat treatment

Chemotherapy

Meristem culture

Culture media

Factors affecting developments and rooting

Virus eradication

Major use of virus-free clones

Study effect of virus infection

Source for clonal propagation

Source for in vitro mass propagation

Concluding remarks

9. Microbial metabolism of carbon dioxide

Autotrophic carbon dioxide fixation

The calvin cycle

Molecular structure and properties of RuBP case

Phosphoribulokinase

Carboxysomes

Regulation of ribulose 1,5-biphosphate carboxydase and phosphoribulokinase synthesis

The reductive carboxylic acid cycle

The anaerobic non-phototrophic autotrophs

Heterotrophic carbon dioxide fixation

10. Microbial metabolism of Hydrogen

Introduction

The role of Hydrogen in the biosphere

Enzyme catalysing the evolution and oxidation of Hydrogen

H<sub>2</sub> :+ Ferredoxin Oxidoreductase

H<sub>2</sub> : Ferricytochrome C3 oxidoreductase

H<sub>2</sub> : NAD- Oxidoreductase

H<sub>2</sub> : Coenzyme F420 oxidoreductase

Membrane-bound hydrogenases

Formate hydrogenlyase

Nitrogenase

Organisms involved in the conversion of hydrogen

Hydrogen-producing micro-organisms

Anaerobic conditions

1. Fermentation and fermentative bacteria
2. Anoxygenic photosynthesis and phototrophic bacteria
3. Oxygenic Phototrophic bacteria (Cyanobacteria)
4. Oxygenic green algae

Aerobic conditions : Nitrogen fixing bacteria

Hydrogen consisting organisms

Hydrogen utilization by anaerobes

1. Nitrate-reducing dentifying bacteria
2. Sulfate reducing bacteria
3. Methanogenic bacteria
4. Acetogenic bacteria
5. Furmarate-reducing bacteria

Hydrogen utilization by phototrophs

1. Anoxygenic phototrophs
2. Cyan bacteria
3. Green algae

Hydrogen utilization by aerobic hydrogen-oxidizing bacteria

The potential use of Hydrogenases and hydrogen in biotechnology

11. Microbial grwoth dynamics

Microbial growth in unlimited environments

Basic growth equation from cell number increase

Basic growth equation from increment increase in the population over a small growth time.

Basic growth equations.

Microbial growth in limited environments

Growth limitation by substrate exhaustion

Variation in the observed growth yield  
Influence of the growth-limiting substrate on growth rate  
Deviation of the Monod equation at High substrate concentrations  
Basic growth limiting substrate equation  
Modelling microbial growth in limited environments  
The logistic equation  
The saturation model  
Microbial growth in open environments  
Chemostat growth kinetics  
The dilution rate  
The dilution rate and biomass concentration  
The dilution rate and growth limiting substrate concentration  
Biomass and growth-limiting substrate concentrations in the steady state  
Determination of  $\mu_{\max}$  from washout kinetics  
Establishing and maintaining the steady state  
Deviations from theoretical chemostat kinetics  
Influence of variation in the observed growth yield  
Microbial competition  
Competition in closed environments  
Competition in open environments  
12. Stoichiometry of microbial growth  
Growth yields and material balances  
Relation between ATP production and growth yields, YATP  
Influence of growth rate and maintenance energy on YATP :  
anaerobic chemostat cultures  
Aerobic yield studies and the influence of the efficiency of  
oxidative phosphorylation on growth yields  
Theoretical calculations on the ATP requirements for the formation  
of microbial biomass  
Influence of Cell Composition  
Influence of the carbon source and complexity of the medium  
Theoretical calculations on the ATP requirement for the  
formation of  
microbial biomass  
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Energy-dissipating mechanisms during growth with excess  
carbon and source.  
Influence of the degree of reduction of the growth substrate  
Heat production  
The stoichiometry of product formation  
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Basic principles  
Death of microbes  
Ageing of microbes  
Viability among microbes  
Survival of populations : Cryptic growth  
Injury among microbes  
Stress and survival  
The physiological status of the population  
Overt and actual stress  
Starvation  
Substrate accelerated death (SAD)

Metabolic and structural injury  
Thymine less death  
Survival of slowly growing bacteria  
Differentiation and survival  
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Mechanisms of micro-organisms response to the environment  
Primary response due to direct chemical or physicochemical effects  
Enzyme inhibition and stimulation  
Induction and repression of protein synthesis  
Changes in cell morphology  
Change in genotype  
Dissolved oxygen  
Cell Interactions with oxygen  
Respiration  
Oxygen incorporation  
Oxygen as an inhibitor  
Oxygen as an enzyme regulator  
Measurement of dissolved oxygen  
Generalized response to DOT  
Diffusion limitation  
Response of growing micro-organisms  
Respiration rate  
Change in cell constituents  
Changes in metabolic products  
Transient responses to changes in DOT  
Control of DOT  
Redox potential  
Responses to carbon dioxide  
Requirement for carbon dioxide  
Inhibition by carbon dioxide  
Water activity  
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Halotolerance and halophily  
Effects of pH  
Introduction  
Cellular level responses  
Intracellular pH  
Effects of pH membrane function  
Effects of pH on uptake of substrate  
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Effects of pH on cell morphology and structure  
Effects of pH on the chemical environment  
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Causes of pH changes in cultures  
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By means of a buffer  
By balancing metabolism  
By feedback control



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Temperature ranges for growth  
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2. DNA  
3. RNA  
4. Proteins  
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Response to temperature shifts  
Effects on substrate utilization  
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Patterns of lipid accumulation  
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Growth rate  
Substrate  
Temperature  
Growth substrate  
Oxygen  
pH and salinity  
Other factors  
Lipid biosynthesis  
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Fatty acid synthetase  
Origin of acetyl - CoA  
Bacteria  
Eukaryotic micro-organism  
Biosynthesis of unsaturated fatty acids  
Biosynthesis of other fatty acids  
Biosynthesis of lipids from fatty acids  
Triacylglycerols  
Phospholipids  
Waxes  
Poly  $\beta$ -hydroxybutyrate  
Microbial metabolism of alkanes and fatty acids

Alkane-utilizing organisms

Uptake of alkanes

Mechanisms of alkane oxidation

Oxidation of primary alcohols to fatty acids

Metabolism of fatty acids derived from alkanes

α-oxidation

ω-oxidation

Microbial products derived from alkanes

Fatty alcohols and aldehydes

Fatty acids

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16. Microbial metabolism of aromatic compounds

Fission of the Benzene nucleus

Preparation of nucleus for aerobic fission

Reactions which follow ring fission

Pathways of degradation

Meta fission pathways

Degradation of 4-hydroxyphenylacetic, homopropionic, homoglutamic

Homogentisinic and gentisic acids

Proocatechuate 4,5 dioxygenase

Degradation of 3-O-Methylglucic acid: Biological formation of  
methanol

Ortho fission pathway

Separation of pathways used for aromatic catabolism by bacteria

Catabolism of aromatic compounds in *Trichosporon cutaneum*

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Catabolic plasmids

Release of halogen substrates from benzene nucleus

Incomplete degradation of aromatics

17. Bacterial respiration

The generation of the proton motive force

Bacterial respiratory chains

Respiration linked proton translocation

The proton motive force

The utilization of the proton motive force

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18. Mechanisms of enzyme catalysis

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Enzyme mechanisms

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Binding of the substrate to the enzyme

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Induction

Nutritional repression

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- Limiting accumulation of end products
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- Additional types of regulations
- Permeability consideration
- Recent approaches to strain construction
- Amino-acid production by genetically engineered strains of E-Coli and related organisms
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- Aspects of enzyme secretion in fungi
- Regulation of Extracellular enzyme synthesis
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- Catabolite repression
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- RNA polymerase modification
- Catabolite repression
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22. Overproduction of microbial metabolites  
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Modular pathways  
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Properties of the wild-type proteins  
Evolution of lactose utilization  
Evolution of new activities for ebg enzymes  
Evolution of the ebg repressor  
Decryptifying Existing Genes

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**NIIR PROJECT CONSULTANCY SERVICES**, 106-E, Kamla Nagar, New Delhi-110007, India.  
Email: [npcs.india@gmail.com](mailto:npcs.india@gmail.com) Website: [NIIR.org](http://NIIR.org)

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