



FOURTH EDITION

Biotechnology

Fundamentals and Applications

S. S. Purohit

FOURTH
EDITION

Biotechnology

Fundamentals and Applications

S. S. Purohit

Ex-Head, P.G. Department of Botany
Dungar College, Bikaner



AGROBIOS (INDIA)



FOURTH EDITION

Published by:

AGROBIOS (INDIA)

Agro House, Behind Nasrani Cinema, Chopasani Road, Jodhpur 342 002

Phone: 91-0291-2642319, Fax: 2643993, E. mail: agrobios@sify.com

Web Site: agrobiosindia.com



Agrobios (India)

© All Rights Reserved (2005)

Reprinted 2005

All rights reserved. No part of the book or part thereof, including the title of the book, be reprinted in any form or language without the written permission of the author and the publishers. The copyists shall be prosecuted.

Rs. 1200.00 / US\$ 80.00

ISBN: 81-7754-139-0

Published by: Updesh Purohit for Agrobios (India), Jodhpur

Laser typeset at: Yashee Computers

Cover Design by: Reena

Printed at: Shyam Printing Press, Jodhpur

FOURTH
EDITION

Biotechnology

Fundamentals and Applications

PREFACE

Biotechnology is the manipulation of biological organisms to make products that benefit human beings. Biotechnology contributes to such diverse areas as food production, waste disposal, mining, and medicine. Although biotechnology has existed since ancient times, some of its most dramatic advances have come in more recent years. Modern achievements include the transferal of a specific gene from one organism to another (by means of a set of genetic engineering techniques known as transgenics); the maintenance and growth of genetically uniform plant- and animal-cell cultures, called clones; and the fusing of different types of cells to produce beneficial medical products such as monoclonal antibodies, which are designed to attack a specific type of foreign substance.

Biotechnology contributes to such diverse areas as food production, waste disposal, mining, and medicine. Although biotechnology has existed since ancient times, some of its most dramatic advances have come in more recent years. Modern achievements include the transferal of a specific gene from one organism to another (by means of a set of genetic engineering techniques known as transgenics); the maintenance and growth of genetically uniform plant- and animal-cell cultures, called clones; and the fusing of different types of cells to produce beneficial medical products such as monoclonal antibodies, which are designed to attack a specific type of foreign substance.

When these technologies are applied at industrial level, they constitute bio-industry which include, on the one hand, industrial activities where biotechnologies can replace technologies normally or currently in use and, on the other hand, industrial activities where biotechnologies play an important driving role. There are several areas in which these technologies are being used. The important are chemical industry, food industry (mass production of yeast, algae and bacteria with a view of providing proteins, amino acids, vitamins, and use of enzymes), agricultural productivity, pharmaceutical industry, environmental protection and abatement of pollution, etc. Biotechnology is also helpful in feeding the World.

Biotechnology has multiple faces. The expansion of biotechnology in the medical arena, agricultural techniques (like, breeding and growth control, including genetic engineering for yield and quality traits, metabolite production, rapid clonal propagation, and germplasm conservation, etc.), in microbial agro-biotechnology (including new approaches to plant pest control, biofertilization, bioremediation, and fermentation of agricultural products), livestock biotechnology (including genetic manipulations for improving fertility and reproduction, growth, milk quality, egg production and disease control), marine

biotechnology (including the use and improvement of fish, macro- and micro-algae for food and biochemical production and environmental uses) made the studies of biotechnology essential for students. All these domains are concerned with food production and the conservation of our environment.

Biotechnology is an applied science and has made advances in two major areas, viz., molecular biology and production of industrially important biochemicals (including enzymes). The science has multiple applications in agriculture: (1) Automated bio-screening, (2) Bio-processing alkenes to valuable oxides and glycols, (3) Developing immobilized cell and enzyme systems for chemical process industries, (4) Engineering of a series of organisms for specific industrial use, (5) Genetical improvement of microorganisms for production of pharmaceutical products, (6) Human gene therapy, (7) Improved production of Vitamin B₁₂, (8) Large scale production of fructose from inexpensive forms of glucose, (9) Manufacturing ethanol by continuous fermentation, (10) Microbiological based production of human insulin and interferons, (10) Microbiologically upgradation of hydrocarbons, (11) Production and development of vaccine to prevent calicivirus (a disease develops in newborn calves and piglets), (12) Production of biopesticide and biofertilizers, (13) Production of diagnostic kits for toxoplasmosis identification, (14) Production of monoclonal antibodies for organ transplant tissue typing, (15) Production of photosynthetically efficient plants, (16) Production of transgenic plants and animals, (17) Production of xanthan gum in oil fields for recovery of crude mineral oils, etc.

The book includes two important chapters viz., **Bioinformatics** and **Nanotechnology**. These are new emerging fields of biotechnology.

The text has been prepared with the help of several authentic literature. I do not claim for my original writing, however the way of presentation of the text is new. I thankfully acknowledge all the sources. I shall welcome suggestions for improvement of next edition.

I express my gratitude to Dr. Updesh Purohit, Managing Director, Agrobios (India) for cooperation and attractive presentation of the text.

Author

CONTENTS

Chapter 1 1

BIOTECHNOLOGY: AN OVERVIEW

Origin and Definitions	1
Definitions	2
Feeding the World	3
Multiple Faces of Biotechnology	5
The Power of Biotechnology.....	7
<i>Biotechnology: Strategies for Agricultural Development</i>	7
Genetic Engineering Techniques	8
<i>Cloning Technology</i>	8
<i>Genetic Modification Technology.....</i>	9
<i>Hybrid Technologies</i>	10
<i>Gene Transfer</i>	11
<i>Isolation of Important Genes</i>	11
<i>Gene Transfer Technology.....</i>	12
<i>Molecular Markers.....</i>	12
<i>Marker-Assisted Selection: Fast Track to New Crop Varieties.....</i>	13
<i>Molecular Shortcut.....</i>	13
<i>Marked Benefits</i>	14
<i>Marker-Assisted Selection.....</i>	14
<i>DNA Fingerprinting</i>	15
<i>Genetic Engineering of Microorganisms Associated with Plants</i>	15
<i>Genetically Modified (GM) Crops.....</i>	15
Protein (or Enzyme) Engineering.....	16
Cell Culture Technology.....	16
Tissue Culture Techniques	17

<i>Tissue Culture: Production of True to Type Plant.....</i>	20
<i>Tissue Culture Techniques to Increase Genetic Variability</i>	21
<i>Protoplast Culture and Micropropagation</i>	21
<i>Somatic Hybrids and Cybrids</i>	21
<i>Production of Virus-Free Plants.....</i>	22
<i>Shoot Tip Micrografting</i>	22
<i>Micropropagation Techniques.....</i>	22
<i>Propagation by Axillary Shooting</i>	22
<i>Propagation by Direct or Indirect Organogenesis.....</i>	23
<i>Improvement of Axillary Branching</i>	23
<i>Somatic Embryogenesis Propagation.....</i>	23
<i>Synseeds.....</i>	23
<i>Somaclonal Variations</i>	24
<i>Haplodiploidisation</i>	24
<i>Germplasm Conservation (Gene Bank)</i>	24
<i>Tissue Engineering Technology</i>	25
<i>DNA Chip Technology.....</i>	25
<i>Bioinformatics Technology</i>	26
<i>Microbial Biopesticides, Biofungicid, Herbicides and Agricultural Antibiotics</i>	26
<i>Use of Biotechnology in Biocide Production</i>	27
<i>Natural Protections for Plants</i>	28
<i>Biosensor Technology</i>	28
<i>Biochips Technology.....</i>	29
<i>Biofilm Technology.....</i>	29
<i>Biosurfactants Technology</i>	29
<i>Monoclonal Antibody Technologies.....</i>	29
<i>New Approaches to Crop Production</i>	30
<i>The Genetic Engineering of Plants.....</i>	31

<i>The Genetic Engineering of Microorganisms Associated with Plants</i>	32
<i>Genetic Engineering for Crop Protection</i>	33
New Approaches to Animal Agriculture	33
<i>Animal Breeding</i>	33
<i>Pregnancy Tests</i>	34
<i>Growth Hormones</i>	34
<i>Booroola Gene</i>	35
<i>Muscle vs Fat</i>	35
<i>Fish Farming</i>	35
Microorganisms Associated with Animals	36
<i>Vaccines Against Animal Diseases</i>	36
<i>Vaccines from Vaccinia Virus</i>	37
<i>Altering Intestinal Organisms</i>	37
Bioprocessing Opportunities	38
<i>Alternative Fuels</i>	38
<i>Alternative Feed and Food Sources</i>	39
<i>Other Products</i>	39
Chapter 2	52
DNA, GENE, GENOME, PROTEIN SYNTHESIS AND DNA CHIP TECHNOLOGY	
The Physical Structure of DNA:	
<i>the Double Helix</i>	53
<i>Native DNA</i>	53
<i>Sugar</i>	54
<i>Bases</i>	54
<i>The Phosphates</i>	55
Types of DNA	56
<i>Features of A-DNA and B-DNA</i>	57
<i>Features of Z-DNA</i>	58
<i>DNA in the Cell</i>	58
<i>Bends in DNA</i>	59
<i>Size, Organization, and Topology of DNA</i>	59
<i>Supercoiled DNA</i>	60
<i>Genetic Polymorphism</i>	60
DNA Replication.....	61
<i>How Replication is Achieved?</i>	62
<i>Replication of Bacterial DNA</i>	66
Ribonucleic Acid (RNA)	67
<i>RNA can also Function as a Genetic Molecule</i>	68
Gene	69
<i>Prokaryotic Genes</i>	72
<i>Structure of Cyanobacterial Genes</i>	73
<i>Gene Arrangement in the Cauliflower Mosaic Virus</i>	73
<i>Eukaryotic Genes</i>	73
<i>Organelle Genes</i>	73
<i>Gene that Code for Proteins</i>	74
<i>Gene that Code for tRNA and tRNA</i>	75
<i>Eukaryotic Gene Organization and Expression</i>	75
<i>Prokaryotic Gene Organization and Expression</i>	76
<i>Gene Isolation</i>	78
<i>Important Terms Related to Gene</i>	78
Genome	80
<i>Genome of Bacteriophages</i>	80
<i>Genomes of Viruses of Eukaryotic Hosts</i>	82
<i>Retroviruses</i>	82
<i>Genomes of Microorganisms</i>	83
<i>Bacterial Genomes</i>	84
<i>Plasmids</i>	84
<i>Yeast (Saccharomyces cerevisiae)</i>	89
<i>Transposons</i>	90
<i>Genomes of Eukaryotes</i>	91
<i>DNA Outside the Nucleus</i>	93
Genetic Code	94
<i>Concepts of Degeneracy</i>	97
<i>DNA Encodes Genetic Information</i>	100
Molecular Cloning of DNA	101
<i>Restriction Enzymes</i>	101
<i>DNA Fingerprinting</i>	102
Mapping Genomes by Genetic Techniques	102
<i>Genetic and Physical Maps</i>	103

<i>Markers for Genetic Maps</i>	104
<i>Simple Sequence Length Polymorphisms (SSLP)</i>	105
<i>Southern Hybridization</i>	105
DNA Chip and its Technology.....	106
<i>Structure</i>	106
Chapter 3	109
APPLICATIONS OF GENETIC ENGINEERING	
Biological Molecular Markers Assisted Breeding.....	109
Transgenic Plants	110
Engineered Resistance Against Herbicide.....	111
<i>Modification of the Target</i>	112
<i>Resistance Against Glyphosate</i>	112
<i>Resistance Against Sulfonylurea and Imidazolinone Herbicides</i>	113
<i>Resistance Against Phosphinothricin</i>	113
<i>Resistance Against Atrazine</i>	113
<i>Detoxification or Degradation of Herbicide</i>	113
Engineered Resistance Against Pest	114
<i>Bacillus Thuringiensis Endotoxins</i>	114
<i>Pseudomonas Flourescens that Secretes Bt Toxin</i>	115
<i>Protease Inhibitors</i>	115
<i>Gene for Other Insecticidal Secondary Metabolites</i>	115
Engineered Resistance Against Viral Infection.....	116
<i>Cross Protection</i>	116
<i>Coat Protein Mediated Protection</i>	117
<i>Antisense RNA Mediated Protection</i>	117
<i>Satellite RNA Mediated Protection</i>	117
<i>Defective Interfering Mediated Protection</i>	117
<i>Replicase Mediated Protection</i>	117
Engineered Resistance Against Fungal Pathogen	118
Genetic Engineering and Plant Lipids	118
Genetic Engineering and Storage Proteins	119
Genetic Engineering for Nitrogen Fixation	120
<i>Transfer of Nif-Genes from Klebsiella Pneumoniae to E. Coli</i>	120
<i>Transfer of Nif-Genes of Eukaryotic Organisms</i>	120
<i>Transfer of Nod-Genes to Increase Host Range</i>	120
<i>Plants that Produce Plastic</i>	120
Miscellaneous Applications.....	120
Chapter 4	122
ENZYMES IN GENETIC ENGINEERING (NUCLEIC ACID ENZYMOLOGY)	
Storage of DNA and Handling of Enzymes.....	123
Restriction Endonucleases	123
<i>Types of Restriction Enzymes</i>	123
<i>Naming of Restsriction Endonucleases</i>	124
<i>Target Sites of Restriction Endonucleases</i>	125
<i>Restriction Endonucleases in Genetic Engineering</i>	129
Ligases	130
<i>Activity of Ligases</i>	130
<i>Blunt-Ended Ligation</i>	131
<i>Source of DNA Ligases</i>	131
<i>Applications of DNA Ligases</i>	133
Alkaline Phosphatase	133
<i>Applications of Alkaline Phosphatase</i>	133
Polynucleotide Kinase.....	133
<i>Applications of Polynucleotide Kinase</i>	134
Terminal Deoxynucleotidyl Transferase.....	134
S1 Nuclease	135
DNA Polymerase I, Holoenzyme.....	135
DNA Polymerase I, Klenow Fragment.....	136
T4 DNA Polymerase	136
<i>Taq DNA Polymerase</i>	137
<i>Ribonuclease (RNase H)</i>	137

Reverse Transcriptase	137	<i>Camv as a Gene Vector</i>	156
<i>Poly (A) Polymerase</i>	138	Shuttle Vectors	157
<i>Dexoxyribonuclease I</i>	138	<i>Yeast Episomal Plasmids (YEPss)</i>	157
Chapter 5	139	Yac Vectors	159
GENE CLONING VECTORS			
Plasmids.....	140	<i>Expression Vectors</i>	159
<i>Replication of Plasmids</i>	140	Gene Cartridges	162
<i>Size of Plasmids</i>	140	<i>Synthetic Regulator Sequences</i>	163
<i>Copy Number</i>	141	<i>Universal Translation Terminator</i>	163
<i>Amplification of the Plasmid</i>	141	<i>Portable Translation Initiation Sites (PTIS)</i>	163
<i>Types of Plasmids</i>	141	<i>Trp a Transcription Terminator</i>	163
<i>Isolation of Plasmid DNA</i>	141		
<i>Criteria for Plasmid Cloning</i>	142		
Cloning Vectors Based on Bacterial		Chapter 6	165
Plasmids.....	143	RECOMBINANT DNA TECHNOLOGY	
<i>Plasmid Pbr322</i>	144	Isolation of Total Cellular DNA.....	165
<i>Col E1 Plasmid DNA</i>	146	Nucleic Acid Hybridization	166
<i>Col E 1 Amp Plasmid DNA</i>	146	Methods for Labelling Nucleic Acids	167
<i>Pbr 325 Plasmid DNA</i>	146	Methods of Labelling Nucleic Acid and Probes	168
<i>Pmb9 Plasmid DNA</i>	147	<i>Nick Translation</i>	168
<i>Ptz Plasmids</i>	147	<i>Primer Extension Method</i>	169
Bacteriophage Vectors for <i>E. Coli</i>	147	<i>Methods Based on RNA Polymerases</i>	169
<i>Phage λ as a Vector</i>	148	End-Labelling of Nucleic Acids.....	171
M13 Bacteriophage.....	149	Choice of Label	172
<i>Genetic Organisation of Wild Type</i>		<i>Radioactive Labels</i>	172
<i>Bacteriophage M13</i>	150	<i>Non Radioactive Labels</i>	172
<i>Construction of M13 Based Vectors</i>	151	Mapping Genes on Specific	
<i>M13 P1 and M13 Mp2</i>	151	<i>Chromosomes</i>	173
<i>M13 Mp7</i>	151	<i>Somatic Cell Hybrids</i>	173
<i>M13-Plasmid Hybrid Vectors</i>	151	<i>In Situ Hybridization</i>	173
Puc 118 and Puc 119.....	152	<i>Transposon Tagging</i>	176
The Pembl8 Cloning Vehicle	153	<i>Genetic Linkage Mapping</i>	176
Cosmids	153	Genomic Library	176
<i>Cosmid Phc79</i>	153	<i>Colony Hybridization</i>	178
<i>Cosmid Pjb8</i>	154	<i>Plaque Hybridization</i>	178
<i>Cloning Using a Cosmid Vector</i>	154	<i>Chromosome Walking</i>	178
Vectors for Plant Cells	155	<i>Chromosome Jumping</i>	179
<i>The Chromosome of Camv</i>	156	Cleaving DNA with Restriction Enzymes	179
<i>Multiplication Cycle of Camv</i>	156	<i>Gel Eletrophoresis</i>	180

Blotting Techniques	181
<i>Analysis of DNA by Southern Blotting</i>	181
<i>Analysis of RNAs by Northern Blot Hybridizations</i>	182
<i>Analysis of Proteins by Western Blot Techniques</i>	183
Detection of Rflps	184
DNA Sequencing	185
<i>Methods for Analyzing DNA Sequence</i>	185
<i>Sequencing by Chemical Degradation</i>	185
<i>Sequencing by Chain Termination</i>	186
<i>Automated DNA Sequence Analysis</i>	189
Mechanical Shearing of DNA	190
Transcript Mapping Techniques	190
<i>Primer Extension</i>	191
<i>S1 Mapping</i>	191
Synthesis of Cdna from Mrna	193
<i>Preparation of Double-Stranded Cdna</i>	194
<i>Cdna Library</i>	194
Searching for Gene Using Computers	195

Chapter 7 196

GENETIC ENGINEERING TECHNIQUES

The Goal for Improved Crop Varieties	196
Transgenic Techniques	197
Steps for Developing New Crop Varieties	197
Bacteria: Nature's Genetic Engineers	200
<i>Bacteria and Gene Transfers in Plants</i>	200
<i>How Bacteria are Used to Modify Plants</i>	200
<i>Agrobacterium Mediated Gene Transfer</i>	204
<i>Plant-Transformation Vectors</i>	205
<i>Gene Cloning</i>	205
<i>Creating Recombinant DNA</i>	206
<i>DNA Ligase</i>	206
<i>T4 DNA Ligase</i>	206
<i>Linkers</i>	207
<i>Stop Linker</i>	208
<i>Adaptors</i>	208
<i>Double-Linkers</i>	208
<i>Terminal Transferase</i>	209
Cloning in Bacteria and Eukaryotes	209
<i>Cloning in Bacteria</i>	209
<i>Cloning in Eukaryotes</i>	209
Amplification of DNAs by Polymerase Chain Reaction (PCR)	210
<i>Applications of PCR</i>	211
Gene Transfer Technology	215
<i>Sexual Method</i>	216
<i>Asexual Methods</i>	216
Biological Delivery System	218
<i>Agrobacterium Tunefaciens: An Important Vector for Genetic Engineering</i>	218
<i>Disarmed Ti Vectors</i>	222
<i>Vectors</i>	222
Gene Transfer Using Particle Bombardment Technique	222
<i>History</i>	223
<i>Applications of Biolistic</i>	223
<i>Particle Gun Design</i>	224
<i>Preparation of DNA Coated Microparticles</i>	224
<i>Gene Gun</i>	224
<i>Modified Bombardment Devices</i>	225
<i>Factors Affecting Particle Bombardment-Mediated Transformation</i>	226
<i>Components of the Introduced Plasmid DNA</i>	228
<i>Target Tissue</i>	229
<i>Tissue Treatment</i>	231
<i>The Fate of the Introduced DNA(s)</i>	231
Gene Transfer by Microinjection Technique	233
<i>Applications of Microinjection Technique</i>	234
<i>The Technique</i>	235
Silicon Carbide Fiber-Vortex (Silicon Whiskers)	236
Sonication	236
Electroporation	237

Poly-Cation Mediated DNA Uptake.....	238	<i>Double Transformations and Gene Silencing.....</i>	248
Ca-DNA Co-Precipitation Method	238	<i>Post-Transcriptional Gene Suppression.....</i>	248
Ultrasonication	239		
Uv Laser Microbeam	239		
Genetic Transformation in Monocotyledonous Plants	240	Chapter 9	249
<i>Factors Involved in Agrobacterium- Mediated Transfer of Genes to Monocots</i>	240		
<i>Direct Gene Transfer Monocotyledonous Plants</i>	240		
Problems Involved in Gene Transfer	241	GENE SILENCING AND ANTISENSE TECHNOLOGY	
<i>Expression System</i>	241		
<i>Cellular Localization.....</i>	241		
<i>Proteolysis, Protein Folding and Prosthetic Group Acquistion</i>	242	Reasons for Gene Silencing	250
<i>Precursor Availability</i>	242	Types of Gene Silencing	251
<i>Inhibitory Environments.....</i>	242	<i>Homology-Dependent Gene Silencing</i>	251
<i>Side Reaction of New Compounds.....</i>	242	<i>Position Effects.....</i>	251
Chapter 8	243	<i>Environmental Stress</i>	251
EXPRESSION OF INDUCED GENES		<i>Antisense Gene Silencing</i>	252
Factors Affecting Gene Expression	243	<i>Endogenous Genes Silencing by Co- Suppression.....</i>	252
<i>Regulation of Gene Expression.....</i>	243	Post-Transcriptional Gene Silencing (PTGS)	253
Reporter Gene for Transient Expression	245	<i>Characteristics of PTGS.....</i>	253
<i>β-Glucuronidase Gene</i>	245	<i>Possible Mechanisms of Post- Transcriptional Gene Silencing.....</i>	254
<i>Luciferase Gene</i>	245	<i>Elimination of Homologous Transcripts</i>	255
<i>Chloramphenicol Acetyl Transferase Gene</i>	245	<i>Ptgs of Endogenous Genes and the Presence of Specific Transgene Loci.....</i>	255
<i>Nopaline (Nos) Synthase Gene</i>	246	Gene Silencing in Crop Plants	256
<i>Neomycin Phosphotransferase Gene (Npt II).....</i>	246	<i>Tomato.....</i>	256
<i>Anthocyanins.....</i>	246	<i>Brassica.....</i>	256
<i>Dihydrofolate Reductase Gene</i>	246	<i>Rice</i>	256
Promoter Elements	246	<i>Development of Viral Resistance in Plants.....</i>	256
Marker Gene.....	247	<i>Effect of Transgene Dose on Silencing</i>	257
Inactivation of Foreign Gene Expression	247	<i>Silencing Depends on Similarity of Transcribed Sequences</i>	257
<i>Transgene Inactivation and Methylation.....</i>	248	<i>Class II Chitinase Host Genes are Not Silenced by a Class I Transgenes</i>	258
		<i>Silencing Occurs During Seedling Development</i>	258
		<i>Stability of the Silent State During Vegetative Growth.....</i>	258
		<i>Genetic Factors Influencing Silencing</i>	258

<i>Silencing of Chitinase Gene Expression is Likely to Be a Post Transcriptional Event</i>	259
<i>Occurrence of Silencing and Resetting</i>	259
<i>Antisense Technology</i>	260
<i>Formation of Antisense mRNA</i>	261
<i>Inhibition of Gene Expression by Antisense RNA</i>	261
<i>Plants from Antisense Technology</i>	261
Chapter 10	263
SCOPE, ACHIEVEMENTS AND HAZARDS OF GENETICALLY MODIFIED CROPS	
<i>Our Need</i>	263
<i>Fungal Resistance</i>	265
<i>Long Lived Fruits and Vegetable</i>	266
<i>Race-Specific Resistance Genes</i>	267
<i>Two-Component Systems for Non- Specific Resistance</i>	267
<i>Compounds Toxic to Fungi</i>	268
<i>Anti-Fungal Protein</i>	268
<i>Ribosome-Inactivating Proteins (RIPS)</i>	269
<i>Engineered Resistance Against Virus Diseases</i>	269
<i>Coat Protein-Mediated Protection (CPMP)</i>	270
<i>Defective Interfering RNA/ DNA Protection</i>	271
<i>Satellite RNA-Mediated Disease Attenuation</i>	271
<i>Anti-Sense RNA/ Ribozyme-Mediated Protection</i>	271
<i>Replicase and Protease-Mediated Protection</i>	271
<i>Movement Protein-Mediated Protection</i>	272
<i>Resistance Through Transgenic Expression of Antiviral Proteins</i>	272
<i>Herbicide Tolerance Genes</i>	273
<i>Herbicide Target Modification</i>	273
<i>Over Production of Target Enzyme</i>	274
<i>Detoxifying Enzymes</i>	274
<i>Insect Tolerance</i>	274
<i>Coat Protein Genes for Virus Protection</i>	275
<i>Light Regulated Genes</i>	276
<i>Antisense RNA</i>	276
<i>Transposable Elements</i>	276
<i>Seed Storage Proteins</i>	276
<i>Bioactive Peptides</i>	276
<i>Human Proteins</i>	277
<i>Enzymes</i>	277
<i>Antibodies</i>	278
<i>Vaccines</i>	278
<i>Industrial Products</i>	278
<i>Metal Resistance in Plants</i>	279
<i>Transgenic Plants with Higher Stress Tolerance</i>	279
<i>Water Stress</i>	279
<i>Temperature Stress</i>	279
<i>Salt Stress</i>	280
<i>Strategy for Stress Tolerant Transgenic Plants</i>	280
<i>Properties/Characters Required for Improved Stress Tolerance</i>	280
<i>Alteration of Oil Composition and Content with Reference to Transgenic Rapeseed</i>	281
<i>Recommendations and Priorities for Future Research</i>	282
<i>Priorities for Future Research</i>	283
<i>When Transgenes Wander, Should We Worry?</i>	284
<i>Gene Flow in Crops</i>	285
<i>Plant Improvement are Not Absolutely Safe</i>	286
<i>Why Bt Cotton Should Be Banned</i>	286
<i>Bt Cotton Creating Resistance to Bt?</i>	287

Chapter 11	288
-------------------	------------

ECO-SOCIAL IMPACT OF GENETICALLY MODIFIED CROPS / FOODS

Potential Hazards	288
Potential Ecological Impact of Transgenic	
Virus- Resistance in Plants	289
<i>Areas for the Assessment of the Ecological Impact</i>	290
<i>Agricultural and Natural Habitats</i>	290
<i>Virus Location and Virus-Host Co-Evolution.....</i>	290
<i>Virus Epidemiology</i>	291
<i>Fitness Impact.....</i>	291
<i>Plant Population Genetics</i>	291
Assessment	292
<i>Risk Assessment of Gene Flow</i>	
<i>Associated with the Release of Virus Resistant Transgenic Crop Plants.....</i>	292
Adoption of Transgenic Seeds: Profit or Loss	292
<i>Who Has Benefited?</i>	293
Legal Rights in the New Biotechnology	294
<i>What is a Patent?</i>	294
<i>Impact on Farmers and Consumers</i>	295
<i>Ethical and Practical Problems.....</i>	295
Transgenic are Not Terminator Crops	296
<i>The Hypothetical Risks.....</i>	297
Government Alerted: Did Gm Foods Reach India?	298
Transgenic Plants: Bioethical and Moral Concerns	299
<i>Bioethics</i>	300
<i>Ethical Concerns</i>	300
Transgenic Plants: Risks, Benefits and Impacts on Society and the Environment	301
Transgenics and the Environment.....	302
<i>Objections to Development and Deployment of Transgenic Crops.....</i>	303
<i>Transgenic Trees</i>	303

Transgenics and Human Health	304
<i>Socio-Economic Impact and Benefits</i>	305
Ownership of Genes and the Need for Patents	305
Biotechnological Development and Risks.....	306
<i>Situation in the Developing Countries</i>	306
<i>Regulation of Agricultural Products of Biotechnology in Canada.....</i>	308

Chapter 12	310
-------------------	------------

PLANT TISSUE CULTURE: PRINCIPLES AND METHODOLOGY

Importance of Tissue Culture Technique.....	310
<i>Global Picture of Plant Tissue Culture</i>	311
History of Plant Tissue Culture	
<i>Researches</i>	311
Tissue Culture Researches in India	314
Plant Tissue Culture: Principles.....	316
<i>Callus Culture</i>	316
<i>Meristem Culture</i>	317
<i>Organ Culture</i>	317
The Concept of Totipotency of Cells	318
<i>Contrasts Between Plant and Animal Cells.....</i>	318
<i>The Autonomous Organelles: Their Behaviour During Growth Induction and Morphogenesis</i>	320
<i>Sites and Modes of Action of Growth Regulating Substances</i>	320
<i>The Behaviour of Carrot Cells in Vitro</i>	320
<i>Clonal Development from Animal and Plant Cells</i>	320
Plant Tissue Culture: Methods	321
<i>Media Preparation: Area/Room</i>	322
<i>Culture Media, Washing Powder/ Liquid Detergent, Disinfectants</i>	322
<i>Aseptic Transfer Chamber Area</i>	322
<i>Environmentally Controlled Culture Room</i>	322
<i>Analytical Room.....</i>	323

<i>Acclimatization Room</i>	323	<i>Maturation of Embryos</i>	351
<i>Miscellaneous Items</i>	324	<i>Important Parameters for Consideration</i>	351
<i>Specifications of Laboratory Equipments</i>	324	<i>Simplification of the Technique</i>	351
<i>Aseptic Technique</i>	325	<i>Different Causes of Failures (and Remedies)</i>	352
<i>Sterilization of Plant Tissues</i>	325		
<i>Cleaning (Preparation of Glasswares/Plastic Wares (Autoclavable))</i>	326		
<i>Sterilization</i>	326		
<i>Surfactants</i>	327		
<i>Control of Bacterial and Fungal Contaminants by Antibiotics in Plant Tissue Culture</i>	328		
<i>in Vitro Environment</i>	329		
<i>Pretreatment to Explant Tissues Prior to Culture</i>	330	<i>Histological Techniques</i>	353
<i>Procedure</i>	330	<i>General Considerations for Paraffin Studies</i>	354
<i>Micropropagation Through Organogenesis</i>	332	<i>Immobilization of Specimens for Paraffin Sectioning</i>	358
<i>Culture Media and Preparation</i>	334	<i>Dissection and Mounting of Specimens</i>	360
<i>Media Components</i>	335	<i>Preparation of Specimens for Scanning Electron Microscopy</i>	360
<i>Inorganic Salts</i>	335	<i>Photographic Methods for Plant Cell and Tissue Culture</i>	361
<i>Plant Growth Regulators</i>	336	<i>Type of Photography for Plant Cell and Tissue Culture Research</i>	361
<i>Carbon Source</i>	336	<i>Macrophotography</i>	361
<i>Gelling Agent</i>	337	<i>Microphotography with Stereomicroscopes</i>	362
<i>Amino Acids and Amides</i>	337	<i>Focusing and Cropping the Image</i>	363
<i>Antibiotics</i>	337	<i>Types of the Films</i>	363
<i>Natural Complexes</i>	337	<i>Composition</i>	364
<i>Additional Requirements</i>	337		
<i>Sterilization of Media</i>	342		
<i>Use and Storage of Coconut Water</i>	343		
<i>Related Procedures</i>	343		
<i>Ultraviolet Light</i>	343		
<i>Preparation of Phenol</i>	343		
<i>Working with ³²p Labelled Compounds</i>	343		
<i>Silanization of Plastic and Glassware</i>	344		
<i>Preparation of Dialysis Tubing</i>	344		
<i>Initiation of Embryogenesis in Suspension Culture</i>	347		
<i>Method</i>	348		
<i>Establishment of Embryogenic Suspension Culture</i>	348		
		CHAPTER 13	353
		HISTOLOGICAL AND PHOTOGRAPHIC TECHNIQUES FOR PLANT TISSUE CULTURE	
		<i>Histological Techniques</i>	353
		<i>General Considerations for Paraffin Studies</i>	354
		<i>Immobilization of Specimens for Paraffin Sectioning</i>	358
		<i>Dissection and Mounting of Specimens</i>	360
		<i>Preparation of Specimens for Scanning Electron Microscopy</i>	360
		<i>Photographic Methods for Plant Cell and Tissue Culture</i>	361
		<i>Type of Photography for Plant Cell and Tissue Culture Research</i>	361
		<i>Macrophotography</i>	361
		<i>Microphotography with Stereomicroscopes</i>	362
		<i>Focusing and Cropping the Image</i>	363
		<i>Types of the Films</i>	363
		<i>Composition</i>	364
		Chapter 14	365
		PROTOPLAST TECHNOLOGY	
		<i>Importance of Protoplast Isolation and Culture</i>	365
		<i>Isolation of Protoplast from Various Plant Parts</i>	366
		<i>Enzymatic Method</i>	367
		<i>Mechanical Method</i>	367
		<i>Enzymic Isolation of Protoplasts: Method</i>	368

Methods of Protoplasts Culture	372	Incorporation of Non-Biological Materials	390
<i>Laboratory Facilities</i>	372	Selection of Fusion Hybrids	390
<i>Enzyme Mixture and Osmotic Stabilizer (Osmotica)</i>	372	<i>Visual Selection</i>	390
Purification of Isolated Protoplasts	374	<i>Fluorescent Labels</i>	390
<i>Sedimentation and Washing</i>	374	<i>Fluorescence Activated Cells Sorting</i>	390
<i>Flotation</i>	374	<i>Nutritional Selection</i>	391
<i>Other Purification Methods</i>	374	<i>Drug Sensitivity and Resistance</i>	391
Protoplast Viability Testing	374	Somatic Cell Hybridization or Cybrid or	
<i>General Steps of Protoplast Culture</i>	375	<i>Cytoplast</i>	393
Culture Media for Protoplast Culture	375	Protoplasts for Isolation of Cell	
<i>Protoplast Culture Media for Pc I Group</i>	375	<i>Components</i>	393
<i>Protoplast Culture Media Pc II Group</i>	377	<i>Plasmalemma</i>	393
<i>Proroplast Culture Media Pc III Group</i>	377	<i>Chloroplasts</i>	394
<i>Agar Embedded Culture</i>	378	<i>Mitochondria</i>	394
<i>Microchambers</i>	378	<i>Vacuoles</i>	394
<i>Hanging Drop Cultures (HDC) Techniques</i>	378	<hr/>	
<i>Multidrop Array (MDA) Techniques</i>	378	Chapter 15	395
Growth and Division of Protoplast	380	MICROPROPAGATION IN PLANTS	
<i>Protoplast Culture: Regeneration of Cell Wall</i>	380	Advantages of Micropropagation	395
<i>Development of Callus/Whole Plant</i>	380	<i>Other Advantages of Micropropagation Methods</i>	396
Determination of Protoplast Plating Efficiency	381	<i>Advantages of Micropropagation Over Traditional Methods</i>	398
Technique for Isolation of Sub-Protoplasts	382	<i>Uses of Micropropagation in Horticultural Industry</i>	398
Handling of Regenerated Plantlets	382	<i>Disadvantages of Micropropagation</i>	399
Protoplast Fusion	382	Commercial Uses of Micropropagation	400
<i>General Steps of Protoplast Fusion</i>	383	Materials for Micropropagation	400
Protoplast Culture: Regeneration of Plants	386	<i>Plant Health</i>	403
<i>Organogenesis</i>	387	<i>Germplasm Storage</i>	403
<i>Embryogenesis</i>	387	Types of Micropropagation	404
<i>Handling of Regenerated Plantlets</i>	387	<i>Micropropagation Through Shoot Bud Proliferation</i>	404
Protoplast Culture: Uptake of Foreign Materials	388	<i>Micropropagation Through Adventitious Buds</i>	406
<i>Incorporation of Foreign DNA</i>	388	<i>Meristem Tip Culture</i>	408
<i>Incorporation of Nuclei</i>	388	<i>in Vitro Tuberization</i>	408
<i>Incorporation of Chloroplasts</i>	388	<i>Somatic Embryogenesis</i>	408
<i>Incorporation of Cyanobacteria</i>	389	Methods of Micropropagation (General)	411
<i>Incorporation of Bacteria</i>	389	<i>Which Material Should Be Used?</i>	412
<i>Incorporation of Virus</i>	389		

<i>Stages of Micropropagation</i>	412	<i>ABA Levels</i>	430
<i>Materials</i>	412	<i>Water Saturation</i>	431
Important Considerations and Precautions	416	Genetic Control of Embryo Cloning	431
<i>Problems and Contamination in Culture</i>	416	<i>Genetic Variability</i>	431
Micropropagation in Forest Trees	418	<i>Cytoplasmic Effects</i>	431
<i>Conifers</i>	419	Applications of Somatic Embryogenesis	432
Commercial Aspects of Micropropagation.....	419	<i>Mass Propagation</i>	432
<i>Large Potential Market</i>	420	<i>Scale-Up Potential</i>	433
<i>Futuristic Outlook of Micropropagation</i>	421	<i>Use of Bioreactors</i>	433
<hr/>		<i>Protoplast Culture</i>	433
Chapter 16	422	Embryo Cloning and Gene Transfer	436
SOMATIC EMBRYOGENESIS		Somatic Embryogenesis in Trees	437
<i>Carrot as a Embryogenic System</i>	423	<hr/>	
Somatic and Zygotic Embryos	423	Chapter 17	440
Totipotency and Role of Auxin	423	SOMACLONAL AND GAMETOCLONAL VARIANT SELECTION	
<i>Proembryogenic Masses and Role of Auxin</i>	423	<i>Genetic Variations and Crop Improvement</i>	441
<i>Acquisition of Totipotency</i>	424	<i>Why Variations Occur?</i>	442
<i>Expression of the Programme in the Absence of Auxin</i>	425	The Mechanism of Somaclonal Variation	442
<i>Methylated Cytosine Level and Development</i>	425	Source Material and Culture Conditions.....	442
<i>The Genetic Approach of Somatic Embryogenesis</i>	426	<i>Determination of Cell Number</i>	442
<i>The Need</i>	426	Forms of Somaclonal Variation.....	443
<i>The Process of Somatic Embryogenesis</i>	426	<i>Detection and Isolation of Somaclonal Variants</i>	443
<i>Induction of the Embryogenic State</i>	426	Mutagenesis and Somaclonal Variation.....	444
<i>Recurrent Embryogenesis</i>	427	Somatic Genetics of Nitrogen Metabolism.....	445
<i>Problems</i>	429	<i>Method for Isolation of Desired Variant Cells for NaCl -Tolerant from Callus/ Suspension Cultures</i>	445
<i>Maintenance</i>	429	<i>Applications in Plant Breeding</i>	446
<i>Auxin vs Cytokinin for Induction of Embryogenesis</i>	429	<hr/>	
How to Obtain Embryos from Embryogenic Cultures?	429	Chapter 18	449
Embryo Maturation and the Development of Germinability	430	GYNOGENESIS AND CROP IMPROVEMENT	
<i>Auxin Levels</i>	430	<i>Gynogenesis Induced by Irregular Pollination in Situ</i>	450
<i>Sucrose Level</i>	430		
<i>Heat Shock Treatment</i>	430		