



Contents

Preface

xxiii

1. Biotechnology

1-10

1.1	Definitions of Biotechnology	3
1.2	Scope of Biotechnology	4
1.2.1	Biotechnology in Crop Improvement	4
1.2.2	Biotechnology in Chemical Industry	4
1.2.3	Biotechnology in Medicine	4
1.2.4	Biotechnology in Protein Engineering	5
1.2.5	Microbial Biotechnology	5
1.2.6	Livestock Biotechnology	5
1.2.7	Biotechnology and Environment	5
1.2.8	Genomics for Biotechnology	6
1.3	Bioinformatics and Biotechnology	6
1.4	Biotechnology and Ethical Issues	7
1.5	Biotechnology and Safety Measures	7
1.6	Intellectual Property Rights and Biotechnology	7
	Summary	7
	Questions for Practice	9

2. Molecular Basis of Genetics

11-18

2.1	History of Molecular Genetics	11
2.2	DNA as Genetic Material	11
2.2.1	Hershey and Chase Experiment	13
2.3	RNA as Genetic Material	13
2.4	Direct Evidences that DNA is Genetic Material in Eukaryotes	14

2.5	Characteristics for DNA being Genetic Material	15
-----	--	----

	<i>Summary</i>	16
--	----------------	----

	<i>Questions for Practice</i>	17
--	-------------------------------	----

3. Structure of Nucleic Acids

19-33

3.1	DNA Components and Structure	19
-----	------------------------------	----

3.1.1	DNA is a Double-Helix	21
-------	-----------------------	----

3.1.2	DNA Structures	25
-------	----------------	----

3.2	Analysis of Nucleic Acids	28
-----	---------------------------	----

3.3	Sedimentation Behaviour of Nucleic Acid	29
-----	---	----

3.4	Denaturation and Renaturation of Nucleic Acids	29
-----	--	----

3.5	Reassociation Kinetics and Repetitive DNA	29
-----	---	----

	<i>Summary</i>	31
--	----------------	----

	<i>Questions for Practice</i>	32
--	-------------------------------	----

4. Synthesis and Replication of DNA

34-54

4.1	DNA Synthesis	34
-----	---------------	----

4.2	Fidelity of Synthesis	35
-----	-----------------------	----

4.3	Molecular Hybridization	37
-----	-------------------------	----

4.3.1	Solution Hybridization	37
-------	------------------------	----

4.3.2	Filter Hybridization	37
-------	----------------------	----

4.4	Replication and Synthesis of DNA	38
-----	----------------------------------	----

4.4.1	Experimental Proof for Semi-conservative Replication	39
-------	--	----

4.5	DNA Polymerase Enzymes	41
-----	------------------------	----

4.5.1	Proof-reading Activity	42
-------	------------------------	----

4.6	Replication of DNA	42
-----	--------------------	----

4.6.1	Relaxing DNA	43
-------	--------------	----

4.6.2	Evidence of Replication of Circular DNA Molecule	43
-------	--	----

4.6.3	Origin of Replication (<i>Ori</i>)	44
-------	--------------------------------------	----

4.6.4	<i>de novo</i> Initiation	44
-------	---------------------------	----

4.6.5	The Rolling Circle Model of Replication	45
-------	---	----

4.6.6	Bidirectional Replication	46
-------	---------------------------	----

4.6.7	Unwinding of DNA Helix	46
-------	------------------------	----

4.6.8	Nick Translation	47
-------	------------------	----

4.6.9	Initiation of Synthesis	48
-------	-------------------------	----

4.6.10	Continuous and Discontinuous DNA Synthesis	48
--------	--	----

4.7	Features of DNA synthesis	50
-----	---------------------------	----

4.7.1	Eukaryotic DNA Synthesis	50
-------	--------------------------	----

4.8	The Complete DNA Replication System	50
-----	-------------------------------------	----

	<i>Summary</i>	51
--	----------------	----

	<i>Questions for Practice</i>	52
--	-------------------------------	----

5. Mutation and DNA Repair	55-72
5.1 Characteristics of Mutation	55
5.2 Types of Mutations	55
5.2.1 Biochemical Basis of Mutation	58
5.3 Mutagenesis-Mechanism	58
5.4 Hot Spots of Mutation in DNA	60
5.5 Chemical Mutagens	61
5.6 Induced Mutations	63
5.7 Ultraviolet Irradiation	64
5.8 Mutagenesis by Transposable Elements	65
5.9 Mutator Genes	65
5.9.1 Mutagens and Antimutagens	65
5.9.2 Mutator and Antimutation DNA Polymerase	65
5.10 Mutation-Variation	65
5.10.1 Intragenic Reversion	66
5.10.2 Intergenic Suppression	66
5.11 Mutation Repair	67
5.11.1 Repair of Spontaneous Mutation	67
5.11.2 Repair of Mutation Caused Due to Depurination	67
5.11.3 Repair of Mutation Caused Due to Deamination	68
5.11.4 Repair of Direct Reversal	68
5.12 SOS Response System	69
Summary	70
Questions for Practice	71
6. Linkage, Chromosome Mapping and Crossing-over	73-86
6.1 Linkage and Independent Assortment	74
6.2 Study of Linkage	74
6.2.1 Linkage Ratio	76
6.3 Chromosome Mapping	77
6.4 Crossing-over	78
6.4.1 Crossing-over Molecular Basis	79
6.4.2 Experimental Evidence for Breaking and Rejoining Hypothesis	80
6.4.3 Crossing-over and DNA Duplication are Independent Processes	80
6.4.4 Single-stranded Tail Pairing Involved During Recombination Process	81
6.5 Removal of Errors Arising During Crossing-over	83
Summary	84
Questions for Practice	84

7. Recombinant DNA and Genetic Engineering 87-97

- 7.1 Types of Restriction Enzymes 88
 - 7.1.1 Type I 88
 - 7.1.2 Type II 89
 - 7.1.3 Type IIs 89
 - 7.1.4 Type III (EcoP15) 90
- 7.2 Naming of Restriction Endonucleases 90
- 7.3 Target Sites 92
 - 7.3.1 Recognition Sequences and Cleavage Pattern 92
 - 7.3.2 Cleavage Patterns 92
 - 7.3.3 Blunt End Cleavage 93
 - 7.3.4 Methylation 93
 - 7.3.5 Mechanical Shearing of DNA 94
- 7.4 Restriction Mapping 94
 - 7.4.1 Technique for Preparing a Restriction Enzyme Map 95
 - 7.4.2 Construction of a Restriction Map 95
- Summary 96
- Questions for Practice 96

8. Linking of Foreign DNA 98-110

- 8.1 Cleavage of DNA 98
- 8.2 DNA Ligase 99
- 8.3 Linker-aided Joining 101
- 8.4 Double Linker 102
- 8.5 Adaptors 103
- 8.6 Homopolymer Tailing 105
 - 8.6.1 Cloning of cDNA by Homopolymer Tailing 106
- 8.7 Reporter or Marker Genes 106
 - 8.7.1 Selectable Reporter Genes 107
- 8.8 Promoters 108
- Summary 109
- Questions for Practice 109

9. Vectors 111-138

- 9.1 *Escherichia coli* Vectors 112
 - 9.1.1 Plasmids 112
 - 9.1.2 Plasmid Vectors 116
- 9.2 Virus Vectors 119
 - 9.2.1 Bacteriophage Vectors 119
 - 9.2.2 Stages of the Lytic Life Cycle of a Typical Bacteriophage 119

9.2.3	Bacteriophage λ Vectors	121
9.2.4	Examples of λ Phage Vectors	122
9.2.5	λ gt Vectors	122
9.2.6	λ EMBL Vectors	123
9.2.7	λ DASH 11 and λ FIXII Vectors	123
9.2.8	Infection of <i>E. coli</i> Cells with λ Vectors	123
9.2.9	Phage M13 Vectors	123
9.2.10	Construction of M13 Vectors	124
9.2.11	M13 mp Series	125
9.2.12	Characteristics of M13 Vectors	125
9.3	Cosmids	125
9.4	Phagemids	127
9.4.1	Phagemid Vectors	129
9.5	Artificial Chromosome Vectors	130
9.5.1	Bacterial Artificial Chromosome (BAC) Vectors	131
9.6	Fosmid Vectors	131
9.7	Vectors for other Bacteria	132
9.8	Shuttle Vectors	132
9.9	Yeast Vectors	132
9.9.1	Yeast Plasmid Vectors	133
9.9.2	Yeast Artificial Chromosome (YAC) Vectors	134
9.10	Vectors for Plants	135
	Summary	135
	Questions for Practice	136

10. Insertion of a DNA Fragment into a Vector 139-147

10.1	Insertion of DNA	139
10.1.1	Insertion of DNA Fragment in a Vector with Both Ends Cohesive and Compatible	139
10.1.2	Both Ends are Compatible and Separately Matched	141
10.1.3	Both Ends Cohesive and Unmatched	142
10.1.4	Both Termini are Blunt/Flush	143
10.1.5	One End Cohesive and Compatible, the Other End Blunt	143
10.1.6	Insertion into a Vector of a DNA Molecule that is Complementary to an mRNA	143
10.2	Detection of Recombinant DNA Molecules	144
10.2.1	Direct Selection for the Desired Gene	144
10.2.2	Indirect Selection (Colony Hybridization)	145
	Summary	146
	Questions for Practice	146

11. DNA Sequencing	148-157
11.1 Maxam and Gilbert Procedure	148
11.2 Sanger Method	149
11.2.1 Synthesis of the Complementary DNA Strands	150
11.2.2 Comparing the Lengths of the Terminated DNA Strands by Gel Electrophoresis	151
11.2.3 Reading the DNA Sequence from the Autoradiograph	152
11.3 Polymerase Chain Reaction Method (PCR)	152
11.3.1 Reaction Mechanism of PCR	153
11.3.2 Fluorescence-based Procedure	154
11.4 Shotgun Sequencing	155
Summary	156
Questions for Practice	156
12. DNA (Gene) Libraries	158-177
12.1 Types of DNA Libraries	158
12.1.1 Genomic DNA Libraries	158
12.1.2 cDNA Libraries	158
12.2 Genomic Library	161
12.2.1 The Preparation of DNA Library or Genomic Library	161
12.2.2 Large Maps for Gene Libraries and Chromosome Walks	162
12.2.3 Chromosome Jumping	164
12.3 cDNA Library	164
12.3.1 Methods of Synthesis of cDNA Library	164
12.3.2 Cloning Vectors for Construction of cDNA Libraries	167
12.3.3 Methods to Detect a Specific Gene in a Library	167
12.3.4 Differential Screening and Subtracted Libraries	168
12.4 DNA Chips-Microarrays	168
12.4.1 Production of Oligonucleotide Microarrays	170
12.4.2 Production of DNA Microarrays	171
12.4.3 Hybridization and Detection Methods	173
12.4.4 Applications of DNA Microarray	173
12.4.5 Noise	175
Summary	175
Questions for Practice	176
13. Genomics, Proteomics and Metabolomics	178-192
13.1 Genomics	178
13.1.1 Gene Mapping	179
13.1.2 Sequencing of Genomes	179

13.1.3	Clone-by-Clone Sequencing	179
13.1.4	Shotgun Sequencing	179
13.1.5	Gene Identification	180
13.1.6	Comparative Genomics and its Applications	180
13.1.7	Functional Genomics	182
13.1.8	Cluster Analysis of ESTs	182
13.1.9	Transcriptome	182
13.2	Proteomics	182
13.2.1	Proteomic Analysis or Analytical Protein Chemistry	183
13.2.2	Expression Proteomics or Differential Display Proteomics	184
13.2.3	Functional Proteomics	184
13.3	Proteome Analysis	184
13.3.1	Importance of Proteomics in Genomic Studies	184
13.4	Cellular Complexity and Proteomic	185
13.5	Integrated or Total System Biology	185
13.6	Metabolomics	186
13.6.1	Metabolomics Analytical Techniques	186
13.6.2	Applications	187
13.7	Bio-nanotechnology	188
13.7.1	Applications of Nanotechnology in Biology	188
	Summary	189
	Questions for Practice	190

14. Bioinformatics 193-204

14.1	Development of Bioinformatics	194
14.2	Nomenclature	195
14.3	Database and Search Tools	197
14.4	Analysis Tools and Use of Database	198
14.4.1	The Gen Info Backbone (GIBB) Database	199
14.4.2	Bioinformatics and Analysis of Biological Data	201
	Summary	202
	Questions for Practice	203

15. Plant Cell, Tissue and Organ Culture—History, General Requirements and Techniques 205-227

15.1	History of Plant Tissue Culture	205
15.2	Setting of Tissue Culture Laboratory	211
15.2.1	Washing Facilities	212
15.2.2	Surface Sterilization	213
15.2.3	Glassware	213
15.2.4	Media Preparation Room	214

15.2.5	Chemicals Required	214
15.2.6	Other Facilities Required in Media Room	214
15.2.7	Sterilization Facilities	214
15.2.8	Inoculation Chamber	215
15.2.9	Incubation Chamber	216
15.2.10	Cold Storage	217
15.2.11	Greenhouse	217
15.3	Plants Nutrient Media Components	217
15.3.1	Mineral Nutrients	217
15.3.2	Macronutrients	217
15.3.3	Micronutrients	220
15.3.4	Carbon and Energy Source	220
15.3.5	Vitamins	220
15.3.6	Amino Acids	221
15.3.7	Undefined Organic Supplements	221
15.3.8	Solidifying Agents	221
15.3.9	pH of the Tissue Culture Media	221
15.3.10	Auxins	222
15.3.11	Cytokinins	222
15.3.12	Gibberellins	222
15.3.13	Absciscic Acid	222
15.4	Media Preparation	223
15.4.1	Stock Solution	223
15.4.2	Macronutrients	223
15.4.3	Micronutrients	223
15.4.4	Vitamins and Amino Acids	223
15.4.5	Growth Regulators	223
15.5	Preparation of Murashige and Skoog (MS) Medium	224
	<i>Summary</i>	225
	<i>Questions for Practice</i>	226
16.	Tissue Culture (Callus)	228-236
16.1	Techniques of Callus Production	228
16.1.1	Source of Plant Material	228
16.1.2	Preparation of Explants	229
16.1.3	Nutrient Media	229
16.1.4	Maintenance of Callus Cultures	230
16.2	Morphology of Callus	230
16.3	Changes During Callus Establishment	230
16.3.1	Induction of Callus	230
16.4	Measurements of Growth Parameters	231
16.5	Biochemical Methods	232

16.6	Applications of Callus Culture	232
16.7	Tissue Culture in Liquid Media	233
16.7.1	Stationary Liquid Media Culture	233
16.7.2	Agitated Liquid Media (Shake Cultures)	233
	<i>Summary</i>	234
	<i>Questions for Practice</i>	235

17. Cell Suspension Cultures

237-248

17.1	Techniques of Raising Single Cell Cultures	237
17.1.1	Isolation of Single Cells Directly from Plants	237
17.1.2	Isolation of Cells from Callus	238
17.1.3	Culture Stirring Systems	238
17.2	Types of Cultures	238
17.2.1	Batch Cultures	238
17.2.2	Continuous Cultures	239
17.2.3	Continuous Culture Vessels	239
17.3	Culture Media	240
17.4	Synchronization in Cell Divisions	240
17.4.1	Starvation Method	241
17.4.2	Inhibition Method	241
17.5	Estimation of Growth and Metabolism	241
17.5.1	Cell Counting	241
17.5.2	Cell Volume	241
17.5.3	Cells Fresh and Dry Weight	242
17.5.4	Biochemical Method	242
17.6	Methods of Single Cell Culture	243
17.6.1	The Filter Paper Raft-nurse Technique	243
17.6.2	Bergmann's Plating Technique	243
17.6.3	Microchamber Technique	244
17.7	Factors Effecting Single Cell Culture	245
17.8	Applications of Cell Culture	246
17.8.1	Mutant Selection	246
17.8.2	Industrial Uses	246
	<i>Summary</i>	246
	<i>Questions for Practice</i>	247

18. Morphogenesis and Differentiation in Culture

249-258

18.1	Cytodifferentiation	249
18.1.1	Factors Controlling Vascular Element Differentiation	250
18.1.2	Process of Cytodifferentiation	252

18.2	Organogenesis	252
18.2.1	Shoot Bud Differentiation	253
18.2.2	Explant and Organogenesis	254
18.2.3	Genotype Factor	254
18.2.4	Physical Factors	255
18.2.5	Mechanism of Differentiation	255
18.2.6	Ontogeny and Histology of Differentiation	256
18.2.7	Advantages of Cell and Tissue Differentiation Studies	256
	<i>Summary</i>	256
	<i>Question for Practice</i>	257

19. Somatic Embryogenesis

259-271

19.1	Control of Somatic Embryogenesis	260
19.1.1	Explants	260
19.1.2	Role of Growth Substances	260
19.1.3	Control of Nutrients	261
19.1.4	Oxygen Stress	262
19.1.5	Miscellaneous Factors	262
19.2	Ultra Structural Changes in Embryogenic Cells	262
19.3	Mechanism of Somatic Embryogenesis	263
19.3.1	Induction of Somatic Embryogenesis	263
19.3.2	Development of Embryo	264
19.4	Molecular Basis of Somatic Embryogenesis	264
19.4.1	Genes Involved in Cell Division	265
19.4.2	Genes Involved in Organ Formation	265
19.4.3	Embryo-specific Genes	265
19.5	Maturation of Somatic Embryos	265
19.6	Synchronization of Embryo Development	266
19.7	Large Scale Production of Somatic Embryos	266
19.8	Synthetic Seeds	267
19.8.1	Types of Synthetic Seeds	267
19.9	Commercial Production	269
	<i>Summary</i>	269
	<i>Question for Practice</i>	270

20. Protoplast Isolation and Culture

272-285

20.1	Isolation of Protoplasts	272
20.1.1	Source of Plant Material	273
20.1.2	Osmoticum	273

20.1.3	Use of Enzymes	274
20.1.4	Isolation of Protoplasts-methods	275
20.1.5	Isolation of Protoplast from Cultured Cells	277
20.1.6	Isolation of Protoplasts from Pollen	277
20.1.7	Purification of Protoplast	277
20.1.8	Viability of Protoplasts	278
20.2	Cell Wall Synthesis	278
20.2.1	Cell Division	278
20.2.2	Factors Controlling Protoplasts' Culture	279
20.2.3	Methods of Protoplast Culture	281
20.2.4	Protoplasts-raised Plants	282
	<i>Summary</i>	283
	<i>Questions for Practice</i>	283

21. Protoplast Fusion (Somatic Hybridization) 285-298

21.1	Fusogens	285
21.1.1	Sodium Nitrate	285
21.1.2	High pH and High Ca^{2+}	286
21.1.3	Polyethylene Glycol Treatment (PEG)	286
21.1.4	Electrofusion	286
21.2	Mechanism of Fusion	287
21.3	Selection of Somatic Hybrids	288
21.3.1	Genetic Complementation	288
21.3.2	Physiological Complementation	289
21.3.3	Mechanical Isolation	289
21.3.4	Regeneration of Growth-capacity in Hybrids upon Fusion of Inactivated Cells	290
21.3.5	Physical Enrichment	290
21.4	Analysis of Hybridity and Genetic Composition	291
21.4.1	Genetic Methods of Analysis	291
21.4.2	Biochemical Analysis	292
21.5	Protoplasts Fusion Products	293
21.5.1	Symmetric Hybrids	293
21.5.2	Asymmetric Hybrids	293
21.6	Cybrids	294
21.6.1	Cybridization	295
21.6.2	Cybridization for Crop Improvement	295
	<i>Summary</i>	296
	<i>Questions for Practice</i>	297

22. Genetic Transformation—Delivery System**299–317**

22.1	<i>Agrobacterium</i> Mediated Transformation	299
22.1.1	<i>Agrobacterium</i> Plasmid	300
22.1.2	Organization of T-DNA	301
22.1.3	Vir Region	302
22.1.4	Mechanism of <i>Agrobacterium</i> Infection	302
22.2	Ti-plasmid Based Vectors	303
22.2.1	Disarming of Ti-plasmids	303
22.2.2	Co-integrating Vectors	303
22.2.3	Binary Vector/Trans Vectors	305
22.3	Plant Virus Vectors	305
22.3.1	Brome Mosaic Virus (BMV)	305
22.3.2	Tobacco Mosaic Virus (TMV)	306
22.3.3	Gemini Viruses as Vectors	306
22.3.4	Caulimoviruses (CaMV)	307
22.3.5	CaMV Vector	307
22.4	Transposable Genetic Elements	307
22.5	Direct Gene Transfer	308
22.5.1	Chemical Method	308
22.5.2	Microparticle Bombardment Method	308
22.5.3	Electroporation	309
22.5.4	Microinjection	310
22.5.5	UV Laser Microbeam	310
22.5.6	Liposome System	311
22.5.7	Silicon Carbide Fibre-vertex	311
22.5.8	Sonication Method	312
22.5.9	Transformation through Pollen	312
22.5.10	Direct DNA Uptake by Mature Zygotic Embryos	312
22.6	Transformation Technique in Plants	312
22.6.1	<i>Agrobacterium</i> Mediated Transformation	312
22.6.2	Virus Genome Mediated Transformation	313
22.7	Genetic Transformation	314
22.7.1	Stable Transformants	314
22.7.2	Transient Transformants	314
22.7.3	Unstable Transient	314
22.7.4	Inter-Transformant Variability	315
	Summary	315
	Questions for Practice	316

23. Transgenic Plants**318-359**

23.1	Transgenic Plants for Biotic Stress Resistance	318
23.1.1	Insect Resistance	318
23.1.2	Herbicide Resistance	320
23.1.3	Virus Resistance	323
23.1.4	Transgenics for Fungal Resistance	326
23.1.5	Transgenic for Bacteria Resistance	328
23.1.6	Transgenic for Nematode Resistance	329
23.2	Transgenic Plants for Abiotic Stress Resistance	329
23.2.1	Tolerance against Oxidative, Salinity, Drought and Cold Stress	329
23.3	Transgenics for Quality Improvement of Crop Plants	330
23.3.1	Transgenics for the Modification of Starch Quality	330
23.3.2	Transgenic for the Modification of Oils	331
23.3.3	Transgenics for Modification of Proteins	332
23.4	Transgenic for Pharmaceuticals	333
23.4.1	Transient Expression	333
23.4.2	Stable Expression	334
23.4.3	Plants Molecular Farming (Plants as Bioreactors)	336
23.4.4	Alkaloids and Phenolics	337
23.4.5	Essential Oils	337
23.4.6	Production of Enzymes by Transgenics	338
23.5	Edible Vaccine	338
23.5.1	Recombinant Protein Production through Transgenic Plants	339
23.5.2	Crop Plants Used for Oral Vaccines	339
23.5.3	Production of Recombinant Proteins	340
23.6	Male Sterility (Plants without Pollen)	342
23.7	Transgenic for Vitamin (Golden Rice)	343
23.8	Transgenic for Improvement in Post-harvest Qualities	344
23.9	Transgenics of the Improved and Modified Ornamental Plants	345
23.9.1	Extension of Flower Life	345
23.9.2	Floral Scent	346
23.9.3	Pigmentation	346
23.10	Chloroplast Genome and Transplastomics	347
23.10.1	Organization of Chloroplast Genome	348
23.10.2	Genes Identified in cpDNA	348
23.10.3	Genetic Transformation of Chloroplast	348
23.10.4	Introduction of Novel Proteins in Plastids	349
23.10.5	Techniques of Transformation of cpDNA	349
23.10.6	Examples of Transplastomic Plants	350

23.11	Limitations in Producing Transplastomic Plants	351
23.12	Terminator Seed Technology	352
23.12.1	Terminator Technology	352
23.12.2	Verminator Technology or Traitor Technology	353
23.12.3	Gene-deletor Technology	354
23.12.4	Principle of Gene-deletor Technology	354
	Summary	355
	Questions for Practice	356
24.	Haploid Production	360-375
24.1	Androgenesis	361
24.1.1	Techniques of Androgenesis	362
24.1.2	Anther Culture	362
24.1.3	Pollen Culture	363
24.1.4	Factors Affecting Androgenesis	364
24.1.5	Induction of Androgenesis	368
24.1.6	Pathways of Pollen Embryo Development	369
24.1.7	Ploidy and Polysomy	371
24.2	Gynogenesis	371
24.3	Production of Haploids through Distant Hybridization	372
24.4	Diplodization of Haploids to Raise Homozygous Plants	372
24.5	Applications of Haploids	372
	Summary	373
	Questions for Practice	374
25.	Somaclonal Variation and Cell Line Selection	376-387
25.1	Genetic Flux and Plant Tissue	376
25.2	Source of Genetic Variability	377
25.2.1	Presence of Cellular Variability in Explants	377
25.2.2	<i>In vitro</i> Induced Variability	377
25.3	Mechanism Causing Genetic Variation	379
25.3.1	Chromosomal Aberration	379
25.3.2	DNA Amplification	379
25.3.3	Transposable Elements	380
25.4	Selection of Variants	380
25.5	Cell Cloning for Somaclone Variants (Cell Line Selection)	382
25.5.1	Single Step Selection	383
25.5.2	Multiple Step Selection	383
25.5.3	Disease Resistance	383
25.5.4	Herbicide Resistance	383
25.5.5	Salt Tolerance	384

Summary	385
Questions for Practice	385

26. *In Vitro* Pollination and Fertilization 388-396

26.1	<i>In vitro</i> Pollination and Fertilization	388
26.2	Techniques of <i>in vitro</i> Pollination and Fertilization	389
26.3	Overcoming Post-fertilization Barrier	390
26.3.1	Ovary Culture	390
26.3.2	Ovule Culture	391
26.3.3	Nucellus Culture	391
26.3.4	Endosperm Culture	392
26.3.5	Differentiation of Endosperm Callus	392
26.4	<i>In vitro</i> Fertilization	393
26.4.1	Isolation of Male Gamete	393
26.4.2	Isolation of Egg Cell	393
26.4.3	Fusion of Sperm and Egg Cell	393
26.4.4	Culture of Fertilized Egg	394
26.5	Applications	394
	Summary	395
	Questions for Practice	395

27. Embryo Culture 397-404

27.1	Techniques of Embryo Culture	398
27.1.1	Plant Material	398
27.1.2	Sterilization	398
27.1.3	Dissection of Embryo	398
27.1.4	Nutrition	399
27.2	Methods of Embryo Rescue	400
27.2.1	Culture of Excised Embryos using Nurse Endosperm	400
27.2.2	Embryo Transplantation Technique	401
27.3	Role of Suspensor in Embryo Development	401
27.4	Applications of Embryo Culture	401
27.5	Precocious Germination	402
	Summary	403
	Questions for Practice	403

28. Micropropagation 405-417

28.1	Objectives of Micropropagation	406
28.1.1	Strategies for Clonal Propagation	406

28.2	Techniques of Micropropagation	406
28.2.1	Preparation of Donor Plants (Stage 0)	406
28.2.2	Initiation of Cultures (Stage I)	406
28.2.3	Multiplication and Maintenance of Culture (Stage II)	407
28.2.4	Rooting of Shoots (Stage III)	409
28.2.5	Transplantation (Stage IV)	409
28.3	Factors Affecting Micropropagation	411
28.3.1	Factors Affecting Culture Initiation and Shoot Multiplication	411
28.3.2	Factors Affecting Rooting	412
28.4	Applications of Micropropagation	412
28.5	Problems Associated with Micropropagation	413
28.5.1	Hyperhydration	413
28.5.2	Off-types	413
28.5.3	Contamination	414
28.5.4	Oxidative Browning	414
28.5.5	Recalcitrance of Some Plants	415
28.5.6	High Cost	415
	Summary	415
	Questions for Practice	415

29. Germplasm Conservation of Plants

418-427

29.1	Strategy for Germplasm Conservation	418
29.1.1	<i>In situ</i> Conservation	418
29.1.2	<i>Ex situ</i> Conservation	419
29.2	Biotechnology in Germplasm Conservation	419
29.2.1	Short-term Storage or <i>in vitro</i> Active Gene Bank	419
29.3	Cryopreservation of Germplasm (Long-term Storage)	421
29.3.1	Selection of the Plant Material	421
29.3.2	Pre-freezing Treatments	422
29.3.3	Desiccation	422
29.3.4	Vitrification	422
29.3.5	Cryoprotectants	423
29.3.6	Freezing	423
29.3.7	Storage	424
29.3.8	Thawing	424
29.3.9	Regeneration	425
29.3.10	Viability Test	425
	Summary	425
	Questions for Practice	426

30. *In Vitro* Production of Secondary Metabolites of Plants

428–441

- 30.1 Plant Tissue-Culture and Secondary Metabolites 429
- 30.2 Plant Products of Interest of Industry 431
- 30.3 Protocol for the Production of Plant Metabolites using Cell Culture 433
 - 30.3.1 Culture Conditions 433
 - 30.3.2 Hairy Roots 434
 - 30.3.3 Elicitation 435
 - 30.3.4 Immobilization of Cells 436
 - 30.3.5 Photoautotrophy in Hairy Roots 437
 - 30.3.6 Genetic Engineering 437
 - 30.3.7 Biotransformation 437
- 30.4 Scale-up Process 438
- 30.5 Down-scaling Process 438
 - 30.5.1 Permeabilization of Cells 438
 - 30.5.2 Removal of Secreted Products 438
- Summary 439
- Questions for Practice 439

Index

445–456