Contents

Preface				
1.	. Introduction			
	1.1	Totipotency	1	
	1.2	Organized Growth	3	
	1.3	Unorganized Growth	4	
	1.4	Callus Cultures	6	
	1.5	Cell Suspensions	6	
	1.6	Protoplast Cultures	6	
	1.7	Anther or Microspore Cultures	6	
	1.8	Gynogenesis	7	
	1.9	Plant Propagation, Meristem Culture, Somatic Embryogenesis	8	
	1.10	History of Plant Tissue Culture	8	
2.	Basic	Equipment and Facilities for Plant Tissue Culture	22	
	2.1	Environment Required During Culture	22	
	2.2	Laboratory Space for Tissue Culture	24	
	2.3	Media Preparation Room	26	
	2.4	Culture Room	27	
	2.5	Data Collection Area and Specialized Facilities	28	
	2.6	Equipment and Facilities	29	
	2.7	Sterilization	29	
	2.8	Media Composition	31	
	2.9	Solidified Media	38	
	2.10	Liquid Media	39	
	2.11	Major Types of Media	40	
	2.12	Subculturing	44	
3.	3. Media Composition		46	
	3.1	Complex Mixture of Salts	46	
	3.2	Plant Growth Regulators	47	
	3.3	Antimicrobial Compounds and Action Mechanism	48	
	3.4	Plant Tissue Culture Media	49	
4.	Tissu	e Culture Techniques	63	
	4.1	Cultures of Organized Structures	63	
	4.2	Cultures of Unorganized Tissues	64	

viii Contents

	4.3	Using Tissue Cultures for Plant Propagation	64
	4.4	Initiating Tissue Cultures	65
	4.5	Types of Tissue Culture	66
	4.6	Culture of Unorganized Cells	72
	4.7	Cultures of Single Cell Origin	76
	4.8	Cytodifferentiation	82
	4.9	Differentiated Cells in Callus and Cell Cultures	82
	4.10	Morphogenesis: Nature and Induction	84
	4.11	Haploid Plants (Anther and Pollen Culture)	85
	4.12	Gynogenesis	85
	4.13	Applications of Cell and Tissue Culture	91
5.	Plant	t Cell Culture	100
	5.1	Isolation of Single Cell	101
	5.2	Growth and Subculture of Suspension Cultures	102
	5.3	Synchronous Cell Cultures - Study of the Cell Cycle	109
	5.4	Morphogenesis in Cell Cultures	113
	5.5	Culture Medium for Cell Suspensions	119
	5.6	Synchronization of Suspension Cultures	121
	5.7	Measurement of Growth in Suspension Cultures	122
	5.8	Viability of Cultured Cells	123
	5.9	Culture of Isolated Single Cells	124
	5.10	Bioreactor for Large-Scale Culture	1 2 8
6.	Proto	pplast Isolation and Culture	130
	6.1	Protoplast Isolation	133
	6.2	Visualization of Cell Wall	136
	6.3	Viability	137
	6.4	Protoplast Yield and Culture Density	137
	6.5	Protoplast Culture Techniques	137
	6.6	Incubating Conditions	143
	6.7	Regeneration of Protoplast Derived Colonies/Calli	144
	6.8	Uptake Properties of Protoplasts	145
	6.9	Somatic Hybridization of Plants	146
7.	Somatic Hybridization 1		
	7.1	Protoplast Isolation	148
	7.2	Sketch of a Successful Somatic Hybridization Procedure	150
	73	Protoplast Fusion	150

			Contents ix
	7.4	Action of Polyethylene Glycol (PEG)	151.
	7.5	Electrofusion	153
	7.6	Somatic Hybrids	153
	7.7	Selection of Hybrid Cells	154
	7.8	Regeneration of Hybrid Plants	156
	7.9	Symmetric Hybrids	156
	7.10	Asymmetric Hybrids	157
	7.11	Fate of Plasma Genes	158
	7.12	Application of Somatic Hybridization	158
	7.13	Disadvantages	159
	7.14	Cybrids	160
	7.15	Somatic Hybridization in Lower Plants	160
	7.16	Somaclonal Variation	161
	7.17	Gametoclonal Variation	167
8.	Som	atic Embryogenesis	168
	8.1	Somatic Embryogenesis in Dicots Cultures	170
	8.2	Somatic Embryogenesis in Monocots Culture	170
	8.3	Steps in Somatic Embryogenesis	170
	8.4	Orchard Grass Embryogenesis: A Case Study	172
	8.5	Important Phases of Somatic Embryogenesis	174
	8.6	Applications of Somatic Embryogenesis	178
9.	Synt	hetic Seed	191
	9.1	General Procedure for the Production of Synthetic Seed	192
	9.2	Parts of a Typical Synthetic Seed	197
	9.3	The Genetic Stability of Synthetic Seeds	199
	9.4	Advantages of Synthetic Seeds	199
	9.5	Disadvantages	201
	9.6	Looking Ahead	201
10.	Micr	opropagation	202
	10.1	Stages of Micropropagation	202
	10.2	Methods of Micropropagation	204
	10.3	Propagule Multiplication	208
	10.4	Rooting and Hardening	209
	10.5	Hardening	211
	10.6	Plant Establishments	211
	10.7	Factors Influencing Acclimatization	211
	10.8	Advantages of Micropropagation	212
	10.9	Disadvantages of Micropropagation	213

x Contents

11.	Hapl	oid Production	214
	11.1	Culture of Excised Anthers/Microspore	214
	11.2	Microspore Culture	213
	11.3	Culture Environment	218
	11.4	Induction and Ontogeny of Androgenesis	219
	11.5	Problems Associated with Anther Culture	219
	11.6	Ovary or Ovule Culture	219
	11.7	Exclusion of Chromosomes by the Bulbosum Method	220
	11.8	Application of Haploid Production	223
	11.9	Problems Associated with Haploid Plants	222
12.	Tripl	oid Production	223
	12.1	Selection from Natural Population	224
	12.2	In Vitro Culture of Triploid Endosperm	224
	12.3	Polyploid Production Through Colchicine Treatment followed by	
		Hybridization between Tetraploid Female and Diploid Male	225
	12.4	Production of Tetraploid Parent through Somatic	
		Hybridization and Crossing with a Diploid Male Line	226
	12.5	Protoplast Fusion between Haploid and Diploid Genome	227
	12.6	Selection from Anther or Pollen Culture	228
	12.7	Importance of Triploid Production	228
13.	Emb	ryo Culture	230
	13.1	Embryogenesis	230
	13.2	Embryo Culture	231
	13.3	Embryo Rescue	232
	13.4	Requirements for Embryo Culture	232
	13.5	Culture Environment	236
	13.6	Applications of Embryo Culture	236
	13.7	Other Applications	238
	13.8	Modern Strategies of Embryo Culture	238
14.	Gern	nplasm Storage	240
	14.1	Importance of Germplasm	240
	14.2	Genetic Susceptibility and Slow Destruction	241
	14.3	In Situ Conservation	243
	14.4	Ex Situ Conservation	244
	14.5	Cryopreservation Techniques	248
	14.6	Complementary Conservation Strategy	252
	14.7	Documentation	253

		C	ontents	X	i
15.	Gene	tic Transformation		25	4
	15.1	Methods of Genetic Transformation		25	4
	15.2	Direct DNA Transfer Methods		26	6
	15.3	Use of Selectable Reporter or Marker Genes		27	3
	15.4	Applications in Plant Improvement		27	4
	15.5	Present Scenario		27	8
16.	Gene	tically Modified (GM) Plants: Risks and Benefits		27	9
	16.1	Goals of Technology		27	9
	16.2	Benefits and Risks of Genetically Modified Plants		28	1
	16.3	Possible Advantages of GM Food Plants		28	4
	16.4	Controlling Mechanisms in India		28	6
	16.5	Essential Questions and Answers Related to GM Plants		29	1
17.	Metal	bolic Engineering		29	7
	17.1	Engineering Strategies		29	8
	17.2	Metabolic Control Theory and Metabolic Engineering		30	5
	17.3	Limitations Due to Lack of Knowledge about Concentrations			_
		of Intracellular Proteins and Metabolites		30	
	17.4	Limitations in Metabolic Engineering Due to Network Rigidity		30	
	17.5	Creating Whole New Product and Reactants		30	
	17.6	Completion of Partial Pathways Giving New Products		30	
	17.7	Transfer of Promising Natural Motifs		30	
	17.8	Important Secondary Metabolites Obtained from Plants		30	
18.		Genetic Engineering – Genes of Interest		31	
		Genes and Traits		31	
	18.2	Increased Crop Yield		31	
	18.3			31	
	18.4			31	
	18.5	Herbicide Resistance and Weed Control		31	
	18.6	Pest and Disease Control		31	
	18.7	Resistance to Insect Pests		31	
	18.8	Virus Resistance		31	
	18.9	Resistance to Fungal Diseases		32	
	18.10	Control of Bacterial Diseases		32	
	18.11	Resistance to Plant Nematodes		32	
	18.12	Better Crop Characteristics		32	
	18.13	Delayed Ripening of Fruits		32	
	1X 1A	Higher Nutritive Value for Foods		-32	/4

xii Contents

1	18.15	Other Applications	328
	18.16	Genes for Other Novel Traits	331
-	18.17	Genes for the Future	333
-	18.18	Metabolic Engineering	333
	18.19	Biological Nitrogen Fixation in Non-Legumes	333
	18.20	Prevention of Physiological Deterioration in Cassava	334
	18.21	Plant Sex Determination	334
	18.22	Rubber from Sunflower	334
	18.23	Terminator Technology	335
	18.24	Gene Containment	345
19.	Mole	cular Markers and Their Applications in Plant Species	346
	19.1	Morphological Markers	346
	19.2	Biochemical Markers	347
	19.3	DNA-based Markers	347
	19.4	Hybridization-based Markers	348
	19.5	Specific Sequence-based Markers	356
	19.6	Single Nucleotide Polymorphism (SNP)	359
	19.7	Methodology for Screening Germplasm	361
	19.8	Applications of Molecular Markers	362
	19.9	Future of Molecular Markers	365
20.	Plant	Tissue Culture and Scope for Entrepreneurs	366
	20.1	Current Scope in Plant Tissue Culture	366
	20.2	Emerging Areas in Plant Tissue Culture	370
	20.3	Micropropagation Industry in India	371
	20.4	Current Status of (Plant) Tissue Culture from a Trade Viewpoint	375
	20.5	Role of Government in Plant Tissue Culture Industry	376
	20.6	Indian Set-up Against Rest of the World	377
	20.7	Future Growth of Plant Tissue Culture Industry	377
	20.8	Special Opportunities for Women in Plant Tissue	070
	20.0	Culture Research and Industry	378
	20.9	Constraints	378
		Conclusion	379 381
Gloss	Glossary		
Sugg	Suggested Readings		
Index	index		