

15

2725-672 7

R. Endress

Plant Cell Biotechnology

With 53 Figures and 234 Tables

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo
Hong Kong Barcelona
Budapest

Contents

Chapter 1 Introduction	1
Chapter 2 Basic Techniques	15
1 Definitions	15
1.1 Callus, Cell Suspensions and Protoplasts	15
1.2 Totipotency	15
2 Initiation	15
2.1 Callus	15
2.1.1 Explant	15
2.1.2 Phytohormones	17
2.1.3 Culture	18
2.1.4 Primary Calli	18
2.2 Cell Suspension Culture	19
2.3 Protoplast Culture	19
2.3.1 Enzyme Influence	19
2.3.2 Yield and Vitality of the Protoplasts.	20
3 Characterization.	20
3.1 Callus	20
3.2 Suspension Cultures	21
3.3 Protoplasts.	21
3.3.1 Stability.	21
3.3.2 Survival.	22
3.3.3 Source Material	22
3.4 Cell and Vitality Tests	22
3.4.1 Calcofluor White	22
3.4.2 Semipermeability	23
4 General Culture Techniques.	24
4.1 Sterilization	24
4.1.1 Sterilization of Plant Tissue.	24
4.1.1.1 Sterilization Solutions	24
4.1.2 Sterilization of Nutrient Solutions	25
4.1.2.1 Autoclaving: Sterilization in Water Vapor	25
4.1.2.2 Tyndallization: Sterilization in Flowing Vapor.	26
4.1.2.3 Temperature-Sensitive Substances	26

4.1.3	Sterilization of Glassware, Tools and Air; Transfer of Cultures	27
4.1.3.1	Sterilization of Glassware and Tools.	27
4.1.3.2	Air Sterilization	27
4.1.3.3	Transfer of Cultures	28
5	Composition of Media	28
5.1	Inorganic Components	29
5.1.1	Macroelements	30
5.1.1.1	Sulphur	30
5.1.1.2	Phosphorus	30
5.1.1.3	Nitrogen	30
5.1.1.4	Mg, K, Ca	31
5.1.2	Microelements	33
5.2	Organic Components	33
5.2.1	Amino Acids	33
5.2.2	Vitamins	34
5.3	Growth Regulators	34
5.3.1	Auxins	35
5.3.2	Cytokinins	36
5.4	Carbon Source	36
6	Choice of Medium	38
6.1	Testing Process	38
7	Solidification of the Medium	41
7.1	An Example: Agar-Agar	41
Chapter 3	Culturing of Plant Cells	46
1	Cultivation Methods	46
1.1	Protoplast Cultures	46
1.2	Cultures of Single Cells	48
1.2.1	Nurse Culture	49
1.2.2	Plating and Feeder-Layer Technique; Culture in a Microchamber	49
1.2.2.1	Conditioned Medium.	49
1.2.2.2	Plating and Feeder-Layer Techniques	49
1.2.2.3	Culture in a Microchamber	51
1.3	Mass Cultivation Methods	51
1.3.1	Determining Factors	51
1.3.1.1	Physical Factors.	53
1.3.1.2	Chemical Factors	53
1.3.1.3	Biochemical Factors	54
1.3.1.4	Biological Factors.	56
1.3.2	Fermenter Technology	57
1.3.2.1	Fermenter Design	57
1.3.2.2	Nutrient and Gas Supply	59

1.3.3	Examples	60
2	Growth of Cell and Tissue Cultures	62
2.1	Growth Process	62
2.1.1	Measurement Methods.	62
2.1.2	Culture Systems	63
2.1.2.1	Batch Culture	64
2.1.2.2	Closed and Open Systems	64
2.1.3	Synchronization	65
2.1.4	Growth of Static Cultures	65
2.2	Effects of Culture Conditions on Growth	67
2.2.1	Internal Culture Conditions	67
2.2.1.1	Components of the Basic Medium	67
2.2.1.2	Organic Components.	71
2.2.1.3	Carbon Source	72
2.2.1.4	Phytohormones	75
2.2.1.5	pH of the Medium, Exudates, O ₂ Supply, and Osmotic Effects	77
2.2.2	Influence of Medium Components on the Composition of Cell Suspension Cultures.	80
2.2.3	External Culture Conditions	81
2.2.3.1	Stirring Frequency	81
2.2.3.2	Light	82
2.2.3.3	Temperature	83
Chapter 4	Obtaining and Culturing Haploid Cells.	89
1	Definitions	89
2	Androgenesis.	89
2.1	Culture Techniques	91
2.2	Induction Factors.	92
3	Gynogenesis	93
3.1	Culture Technique	94
3.2	Induction Conditions.	94
4	Biotechnological Utilization.	95
Chapter 5	Plant Regeneration: Morphogenesis.	99
1	Definitions	99
2	Preconditions	102
3	Role of the Plant Material	103
3.1	Competence for Regeneration.	103
3.2	Loss of Morphogenetic Potential.	105
4	Effects of Culture Media and Culture Conditions	106
4.1	The Concept of Hormonal Regulation.	106
4.1.1	The Auxin/Cytokinin Ratio	107
4.1.2	Effects of Other Hormones	110

4.2	Effects of Other Components	112
5	Characterization of Somatic Embryogenesis	113
6	Vitrification	114
6.1	Description of the Phenomenon	114
6.2	Acclimatization	115
7	Biotechnological Utilization of Regeneration Potential	115
7.1	Clonal Propagation.	115
7.1.1	Reproduction from Cuttings.	115
7.1.2	Adventitious Buds	116
7.1.3	Shoot Tip Culture	116
7.1.3.1	Protocorm Formation	116
7.1.4	Axial Bud Reproduction.	117
7.1.5	The Method Via Callus	117
7.1.6	Somatic Embryogenesis	117

Chapter 6 Plant Cells as Producers of Secondary

	Compounds.	121
1	What Is Secondary Metabolism and What Are Secondary Compounds?	121
1.1	Definition.	121
1.2	The Function of Secondary Compounds	122
1.3	Accumulation	123
1.3.1	Spatial Compartmentalization	125
1.3.1.1	Synthesis	125
1.3.1.2	Storage	136
1.3.1.3	Transport Mechanisms.	141
1.3.2	Temporal Compartmentalization.	143
1.3.2.1	Rhythmic Phenomena	143
1.3.2.2	The Role of Individual Developmental Stages . . .	143
2	Significance of the Degree of Cell Differentiation .	149
2.1	The Advantages of Using Dedifferentiated Tissue .	150
2.1.1	Differences from Accumulative Processes in Fully Differentiated Tissues	153
2.1.2	Differences in Enzyme Patterns.	156
2.2	Linkage of Morphological and Biochemical Differentiation Processes.	156
2.2.1	Callus Heterogeneity	156
2.2.2	Shoot and Root Induction.	157
2.2.3	Plastid Differentiation	159
2.2.4	Differentiation of Glandular and/or Storage Cells. .	161
2.2.5	Artificial Induction Processes	161
2.2.6	Subcellular Differentiation.	162
3	Selection	163
3.1	Basic Selection.	163

3.2	Working with Cell Cultures	164
3.2.1	Callus Cultures	164
3.2.2	Cell Suspension Cultures.	164
3.2.3	Protoplast Cultures.	166
3.2.4	Selection Process	167
4	Downstream Processing	167
4.1	Identification.	167
4.1.1	Classic Methods.	167
4.1.2	Immunoassays.	168
4.2	Product Isolation	171
4.2.1	Extraction	171
4.2.1.1	Mechanical and Chemical Breakage	172
4.2.1.2	Use of Enzymes, Microorganisms and CO ₂	172
4.2.2	Secretion	176
4.2.2.1	Natural Secretion Mechanisms	177
4.2.2.2	Artificially Induced Excretion.	178
4.2.2.3	Use of Inert Secondary Phases	184
5	Influence of Culture Conditions on Secondary Metabolite Accumulation	187
5.1	The Role of Physiological Age	187
5.2	Influences of Production Conditions.	188
5.2.1	Internal Culture Conditions.	188
5.2.1.1	Inoculum and Preculture	188
5.2.1.2	Components of the Basal Medium.	189
5.2.1.3	Source of Carbon	195
5.2.1.4	Phytohormones	198
5.2.1.5	Antimetabolites	203
5.2.1.6	O ₂ and pH.	204
5.2.1.7	Exudates	205
5.2.2	Production Media	207
5.2.3	Two-Step Systems.	213
5.2.4	Change in Medium/Adsorbents.	215
5.2.5	Elicitors.	216
5.2.5.1	Definitions	216
5.2.5.2	Action Mechanism of Elicitors	222
5.2.5.3	Intracellular Reaction Messengers	225
5.2.6	External Culture Conditions	230
5.2.6.1	Temperature Effects.	230
5.2.6.2	Stirring Frequencies	230
5.2.6.3	Influence of Culture Containers.	230
5.2.6.4	Light Effects	232
5.2.7	Mechanisms of Light Effects	235
5.2.7.1	Coordinated and Uncoordinated Synthesis of Enzymes in Secondary Metabolism	238
5.2.7.2	Gene Activation.	239

5.3	Regulation Mechanisms	240
5.3.1	Key Enzymes/Initiation Enzymes.	240
5.3.2	Other Regulation Mechanisms	242

Chapter 7 Immobilization of Plant Cells 256

1	Background	256
2	Definitions	257
3	Characterization of Immobilization Processes	258
3.1	Criteria for Choice	258
3.2	Characterization.	259
3.2.1	Cross-Linking	259
3.2.2	Surface Immobilization.	260
3.2.3	Immobilization by Embedding or Micro-Encapsulation	260
3.2.3.1	Composition of the Matrix	262
3.3	Biological Aspects.	264
3.3.1	Effects of Mass Transfer	265
3.3.2	Culture Conditions	265
3.3.2.1	Vitality	265
3.3.2.2	Oxygen Supply	266
3.3.2.3	Phosphate Concentration	266
3.4	Effects on Secondary Metabolism	266
4	Advantages and Disadvantages of Immobilization.	267
4.1	Advantages.	267
4.2	Disadvantages	268

Chapter 8 Use of Altered or Previously Unused Genetic Information. 270

1	The Role of Biological Variability	270
1.1	Differences Between Families, Genera and Species	270
1.2	Differences Within a Species, Plant or Culture.	270
1.3	Causes of Variation.	271
1.3.1	Somaclonal Variation	272
1.3.1.1	Possible Causes	272
1.3.1.2	Jumping Genes	273
1.3.1.3	Examples	274
2	Gene Manipulation.	275
2.1	Mutants and Mutagens	275
2.1.1	Effect of Various Mutagens	275
2.1.1.1	Chemical Mutagens.	275
2.1.1.2	Physical Mutagens	275
2.1.2	Selection of Mutants	276
2.1.2.1	The System of Negative Selection	276
2.1.2.2	Positive Selection	276

2.1.3	Examples	278
2.2	Somatic Hybridization	281
2.2.1	Definitions	281
2.2.2	Fusion Methods	283
2.2.2.1	Chemical Methods	284
2.2.2.2	Physical Method	287
2.2.3	Examples of Biotechnological Applications	288
2.3	Genetic Engineering	290
2.3.1	Vectors	290
2.3.2	Examples of DNA Transfer Systems	291
2.3.2.1	The System of Plant Viruses	291
2.3.2.2	The Ti-Plasmid System	291
2.3.2.3	Artificially Constructed Vectors	292
2.3.2.4	Gene Transfer	297
2.3.3	Targeted Integration of Foreign DNA: Gene Targeting	299
2.3.4	Basic Prerequisites of Stable Transformation	299
2.3.5	Applications of Genetic Engineering	300
2.3.5.1	Case in Point: Secondary Metabolism	300
2.3.5.2	Case in Point: Agriculture	303
2.3.5.3	Case in Point: Food Processing	307
2.3.6	"Natural" Genetic Engineering	307
3	Biotransformation: Utilization of Quiescent Potentials	308
3.1	Definition	308
3.2	Examples	308
3.2.1	Dependence on the Degree of Differentiation and Age	308
3.2.2	Specific Reactions	314
3.2.3	In Vitro Biotransformation	315
Chapter 9	Storage of Cell Cultures	321
1	Retarded Growth	321
2	Cryopreservation	322
2.1	Freezing Process and Freezing Damage	323
2.1.1	Theoretical Foundations	323
2.1.1.1	Extracellular Ice Formation	323
2.1.1.2	Intracellular Ice Formation	324
2.1.1.3	Cooling-Off Process	325
2.1.1.4	Thawing Process	325
2.1.1.5	Shock Freezing	325
2.1.2	Summary	325
2.2	Process Techniques	325
2.2.1	Cooling	326

XIV Contents

2.2.2	Antifreezes	326
2.2.2.1	General Effects	326
2.2.2.2	Special Effects	326
2.3	Biological Effects	327
2.3.1	Role of Cell Stage.	327
2.3.2	Effects of Culture Conditions	328
3	Routinely Used Cryoprotection Methods.	329
Subject Index.		331