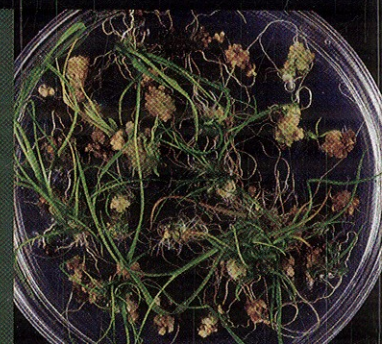


Plant Cell Culture

Essential Methods

Editors | Michael R. Davey and Paul Anthony

 WILEY-BLACKWELL



Contents

Preface	xi
Contributors	xiii
1 Plant Micropropagation	1
<i>Ivan Iliev, Alena Gajdošová, Gabriela Libiaková, Shri Mohan Jain</i>	
1.1 Introduction	1
1.2 Methods and approaches	2
1.2.1 Explants and their surface disinfection	2
1.2.2 Culture media and their preparation	4
1.2.3 Stages of micropropagation	6
1.2.4 Techniques of micropropagation	7
1.3 Troubleshooting	19
References	20
2 Thin Cell Layers: The Technique	25
<i>Jaime A. Teixeira da Silva and Michio Tanaka</i>	
2.1 Introduction	25
2.2 Methods and approaches	26
2.2.1 TCL	26
2.2.2 Choice of material: <i>Cymbidium</i> hybrid	26
2.3 Troubleshooting	35
2.3.1 General comments	35
References	36
3 Plant Regeneration – Somatic Embryogenesis	39
<i>Kim E. Nolan, Ray J. Rose</i>	
3.1 Introduction	39
3.2 Methods and approaches	40
3.2.1 Selection of the cultivar and type of explant	40
3.2.2 Culture media	41
3.2.3 Preparation of culture media	44
3.2.4 Sterilization of tissues and sterile technique	48
3.2.5 Culture and growth of tissue	51

3.2.6	Culture and induction of somatic embryos	52
3.2.7	Embryo development	52
3.2.8	Transfer to soil – the final stage of regeneration	56
3.3	Troubleshooting	57
	References	57
4	Haploid Plants	61
	<i>Sant S. Bhojwani and Prem K. Dantu</i>	
4.1	Introduction	61
4.2	Methods and approaches	62
4.2.1	Androgenesis	62
4.2.2	Diploidization	67
4.3	Troubleshooting	74
	References	75
5	Embryo Rescue	79
	<i>Traud Winkelmann, Antje Doil, Sandra Reinhardt and Aloma Ewald</i>	
5.1	Introduction	79
5.2	Methods and approaches	80
5.2.1	Identification of the time and type of barrier in hybridization	80
5.2.2	Isolation of plant material after fertilization	81
5.2.3	Culture conditions and media	82
5.2.4	Confirmation of hybridity and ploidy	83
5.2.5	Conditions for regeneration of embryos to plants	86
5.3	Troubleshooting	93
	References	93
6	<i>In vitro</i> Flowering and Seed Set: Acceleration of Generation Cycles	97
	<i>Sergio J. Ochatt and Rajbir S. Sangwan</i>	
6.1	Introduction	97
6.2	Methods and approaches	98
6.2.1	Protein legumes [7]	98
6.2.2	Arabidopsis thaliana [13]	105
6.3	Troubleshooting	108
	References	109
7	Induced Mutagenesis in Plants Using Physical and Chemical Agents	111
	<i>Chikelu Mba, Rownak Afza, Souleymane Bado and Shri Mohan Jain</i>	
7.1	Introduction	111
7.2	Methods and approaches	112
7.2.1	Determination of the optimal doses of mutagens for inducing mutations	112
7.3	Troubleshooting	126
7.3.1	Factors influencing the outcome of mutagenesis using chemical mutagens	126

7.3.2	Factors influencing the outcome of mutagenesis using physical mutagens	128
7.3.3	Facts about induced mutations	129
	References	129
8	Cryopreservation of Plant Germplasm	131
	<i>E.R. Joachim Keller and Angelika Senula</i>	
8.1	Introduction	131
8.2	Methods and approaches	132
8.2.1	Main principles	132
8.2.2	Slow (two-step) freezing	134
8.2.3	Vitrification	134
8.2.4	Encapsulation–dehydration	135
8.2.5	DMSO droplet freezing	135
8.2.6	Combined methods	136
8.2.7	Freezing of cold-hardened buds	136
8.2.8	Freezing of orthodox seeds	136
8.2.9	Freezing of pollen and spores	137
8.3	Troubleshooting	149
	References	150
9	Plant Protoplasts: Isolation, Culture and Plant Regeneration	153
	<i>Michael R. Davey, Paul Anthony, Deval Patel and J. Brian Power</i>	
9.1	Introduction	153
9.2	Methods and approaches	154
9.2.1	Protoplast isolation	154
9.2.2	Protoplast culture	156
9.3	Troubleshooting	170
	References	171
10	Protoplast Fusion Technology – Somatic Hybridization and Cybridization	175
	<i>Jude W. Grosser, Milica Čalović and Eliezer S. Louzada</i>	
10.1	Introduction	175
10.2	General applications of somatic hybridization	176
10.3	Methods and approaches	179
10.4	Troubleshooting	195
	References	196
11	Genetic Transformation – <i>Agrobacterium</i>	199
	<i>Ian S. Curtis</i>	
11.1	Introduction	199
11.2	Methods and approaches	200
11.2.1	<i>Agrobacterium</i> as a natural genetic engineer	200
11.2.2	Vector systems for transformation	201
11.2.3	Inoculation procedures	202

11.3	Troubleshooting	213
	References	214
12	Genetic Transformation – Biolistics	217
	<i>Fredy Altpeter and Sukhpreet Sandhu</i>	
12.1	Introduction	217
12.2	Methods and approaches	218
12.2.1	Biolistic technology	218
12.2.2	Optimization of gene delivery parameters	219
12.2.3	Target tissues	220
12.2.4	Reporter gene assays	230
12.2.5	Selection and plant regeneration	231
12.3	Troubleshooting	237
	References	237
13	Plastid Transformation	241
	<i>Bridget V. Hogg, Cilia L.C. Lelivelt, Aisling Dunne, Kim-Hong Nguyen and Jacqueline M. Nugent</i>	
13.1	Introduction	241
13.2	Methods and approaches	243
13.2.1	Principles of plastid transformation	243
13.2.2	Biolistic-mediated plastid transformation	244
13.2.3	PEG-mediated plastid transformation	250
13.2.4	Identification and characterization of transplastomic plants	254
13.3	Troubleshooting	257
13.3.1	Biolistic-mediated transformation	257
13.3.2	PEG-mediated transformation	258
	References	258
14	Molecular Characterization of Genetically Manipulated Plants	261
	<i>Cristiano Lacorte, Giovanni Vianna, Francisco J.L. Aragão and Elíbio L. Rech</i>	
14.1	Introduction	261
14.2	Methods and approaches	262
14.2.1	Plant DNA extraction	263
14.2.2	Polymerase chain reaction	266
14.2.3	Southern blot technique	268
14.2.4	Analysis of the integration site: inverse PCR (iPCR) and thermal asymmetric interlaced PCR (Tail-PCR)	272
14.3	Troubleshooting	278
	References	279
15	Bioreactors	281
	<i>Spiridon Kintzios</i>	
15.1	Introduction	281
15.2	Methods and approaches	283

15.2.1	Medium scale disposable or semidisposable airlift reactors	283
15.2.2	The RITA temporary immersion reactor	284
15.2.3	The LifeReactor	286
15.2.4	Immobilized cell bioreactors	289
15.2.5	Mini-bioreactors	289
15.3	Troubleshooting	292
	References	294
16	Secondary Products	297
	<i>Kexuan Tang, Lei Zhang, Junfeng Chen, Ying Xiao, Wansheng Chen and Xiaofen Sun</i>	
16.1	Introduction	297
16.2	Methods and approaches	298
16.2.1	Plant cell cultures	298
16.2.2	Scale-up and regulation of secondary metabolite production	303
16.2.3	Detection of secondary products	310
16.3	Troubleshooting	313
	References	314
17	Plant Cell Culture – Present and Future	317
	<i>Jim M. Dunwell</i>	
17.1	Introduction	317
17.2	Micropropagation	317
17.3	Embryogenesis	318
17.3.1	Background	318
17.3.2	Commercial exploitation of somatic embryos	318
17.3.3	Molecular aspects of somatic embryogenesis	318
17.3.4	Microspore derived embryos	319
17.4	<i>Haploid methodology</i>	319
17.4.1	Haploids and their exploitation	319
17.4.2	Induction of haploid plants	320
17.4.3	Molecular aspects of haploid induction from microspores	320
17.4.4	<i>Ab initio</i> zygotic-like embryogenesis from microspores	321
17.5	Somaclonal variation	321
17.6	Transgenic methods	322
17.6.1	Background	322
17.6.2	Regeneration and transformation techniques	322
17.6.3	Chloroplast transformation	322
17.6.4	Biopharming	323
17.7	Protoplasts and somatic hybridization	323
17.8	Bioreactors	323
17.8.1	Production of plant products	323
17.8.2	Production of pharmaceuticals	323
17.8.3	Production of food ingredients	324
17.8.4	Production of cosmetics	324
17.8.5	Analytical methodology	324

17.9	Cryopreservation	324
17.10	Intellectual property and commercialization	324
17.10.1	Background	324
17.10.2	Sources of patent and other relevant information	325
17.11	Conclusion	325
	References	325

Index

333