

Contents

1 Exclusive Rights in Life: Biotechnology, Genetic Manipulation, and Intellectual Property Rights

E.R. GOLD

1.1	Introduction	1
1.2	Biotechnological Innovation	2
1.2.1	Physical Innovations	3
1.2.1.1	DNA and Protein Molecules	3
1.2.1.2	Cells	3
1.2.1.3	Whole Organisms	4
1.2.2	Information and Other Intangibles	4
1.2.2.1	DNA Sequences and Cells	4
1.2.2.2	Processes Using Biological Matter	5
1.2.2.3	Bioinformatics	5
1.2.3	Summary	6
1.3	Introduction to Intellectual Property Rights	6
1.3.1	Exclusive Rights vs. Rights to Things	6
1.3.2	Property and Intellectual Property Rights	7
1.3.3	Trade Secrets	7
1.3.3.1	Subject Matter	8
1.3.3.2	Requirements	8
1.3.4	Patents	8
1.3.4.1	Subject Matter	9
1.3.4.1.1	Invention vs. Discovery	10
1.3.4.1.2	Exclusions	10
1.3.4.2	Requirements	11
1.3.4.2.1	Substantive Criteria	11
1.3.4.2.1.1	Novelty	12
1.3.4.2.1.2	Inventive Step (Nonobviousness)	12
1.3.4.2.1.3	Industrial Application (Utility)	12
1.3.4.2.2	Procedural Criterion: Disclosure	13
1.3.4.3	Remedies	14
1.3.5	Copyright and Database Protection	14
1.3.5.1	Subject Matter	15

1.3.5.2	Requirements	15
1.3.5.3	Remedies	16
1.3.6	Plant Variety Protection	16
1.3.6.1	Subject Matter	16
1.3.6.2	Requirements	17
1.4	Challenges	17
1.4.1	Incentive vs. Access	18
1.4.1.1	Justification for Property Rights	18
1.4.1.2	Economic Reality	19
1.4.2	Fairness to Providers of Biological Matter	20
1.4.2.1	Rights to Biological Matter	20
1.5	Conclusion	21
References		21

2 *Agrobacterium rhizogenes*-Mediated Transformation of Plants

W. VAN DE VELDE, M. KARIMI, G. DEN HERDER, M. VAN MONTAGU,
M. HOLSTERS, and S. GOORMACHTIG

2.1	Introduction	23
2.2	Aspects Influencing <i>A. rhizogenes</i> Transformation	
	Efficiency	26
2.2.1	Choice of <i>A. rhizogenes</i> Strain	26
2.2.2	Choice of Explant	28
2.2.3	Preparation of Bacterial Inoculum and Infection	
	of Explants	29
2.2.4	Co-cultivation	29
2.3	Establishing the Transformed Nature of Hairy Roots	31
2.4	Cotransformation of Binary T-DNA	31
2.5	Propagation of Hairy Root Lines in Liquid Cultures	33
2.5.1	The Clonal Status of Hairy Roots	33
2.5.2	Stability of Long-Term Hairy Root Cultures	34
2.6	Regeneration of Plants from Hairy Roots	34
2.7	The Multi-Auto-Transformation (MAT) Vector System	35
2.8	Conclusions	37
	Protocol 1: Production of Transformed Hairy Roots	37
	Protocol 2: Plant Regeneration from Hairy Roots	38
	Protocol 3: Hairy Root Liquid Culture	39
References		39

3 Transformation of *Petunia hybrida* by the *Agrobacterium* Suspension Drop Method

S.J. WYLIE, D. TJOKROKUSUMO, and J.A. McCOMB

3.1	Introduction	45
3.2	Transformation	47
3.3	Analysis of Transformants	47

3.3.1	Screening <i>Petunia</i> Seedlings for Herbicide Resistance	47
3.3.2	Transmission of Basta Resistance Phenotype to T ₂ Progeny	48
3.3.3	β-Glucuronidase Assay	48
3.3.4	DNA Analysis	49
3.4	Conclusion	49
	References	49

4 Onion, Leek and Garlic Transformation by Co-cultivation with *Agrobacterium*

C.C. EADY

4.1	Introduction	53
4.1.1	Current Applications of <i>Allium</i> Transformation Technology	53
4.1.1.1	Physiological Studies	53
4.1.1.2	Herbicide Resistance	54
4.1.1.3	Antimicrobial Resistance	54
4.1.1.4	Insect Resistance	55
4.2	Onion Transformation Protocols	55
4.2.1	Transformation Using Antibiotic and Visual Selection	56
4.2.1.1	Bacterial Strain and Plasmids	56
4.2.1.2	Transformation Procedure (Modified from Eady et al. 2000	56
4.2.2	Transformation Using Herbicide Selection	57
4.2.2.1	Bacterial Strain and Plasmids	58
4.2.2.2	Transformation Procedure	58
4.2.3	Ex-Flasking and Growth in Containment	58
4.2.4	Transgene Detection	59
4.2.5	Transgene Expression and Stability	60
4.2.5.1	Visual Reporter Genes	60
4.2.5.2	Expression of Herbicide Resistance	61
4.2.5.3	Antisense Alliinase Gene Expression	61
4.3	Leek Transformation	62
4.4	Garlic Transformation Protocol	63
4.4.1	Bacterial Strain and Plasmids	63
4.4.2	Transformation Procedure	63
4.5	Concluding Remarks	64
	References	65

5 Electroporation Transformation of Barley

F. GÜREL and N. GÖZÜKIRMIZI

5.1	Introduction	69
5.2	Background of Electroporation Procedures	71

5.2.1	Pre- and Post-Electroporation Period	71
5.2.2	Electrical Variables	73
5.3	Culture and Electroporation of Barley Explants	74
5.3.1	Protoplasts	74
5.3.2	Microspores	76
5.3.3	Intact Tissues	77
	5.3.3.1 Analysis and Inheritance of Transgenes in Electroporated Tissues	81
5.4	Conclusions and Future Perspectives	83
	References	84

6 Sorghum Transformation

Z. ZHAO and D. TOMES

6.1	Introduction	91
6.2	Sorghum Transformation Process and Optimization	92
6.2.1	Plant Materials and Transformation Systems	92
6.2.2	Transformation Via Microprojectile Bombardment	93
6.2.3	<i>Agrobacterium</i> -Mediated Transformation	94
6.3	Analysis of Transgenic Plants and the Progeny	96
6.3.1	Molecular Analysis of T ₀ Plants	97
6.3.2	Foreign Gene Expression in T ₀ Plants	98
6.3.3	Genetic and Molecular Analysis of the Progeny	99
6.4	Marker-Free Sorghum Transgenic Plants	99
6.4.1	Importance of Marker-Free Transgenics in Sorghum	100
6.4.2	Methods to Eliminate Markers from Transgenic Plants	100
6.4.3	<i>Agrobacterium</i> 2 T-DNA Co-Transformation System	101
	References	102

7 Transgenic Sunflower: PEG-Mediated Gene Transfer

P.C. BINSFELD

7.1	Introduction	109
7.2	Genetic Variability and Transgenic Breeding	109
7.3	Gene Transfer Systems	110
7.3.1	PEG-Mediated Gene Transfer	111
	7.3.1.1 Short DNA Molecule Uptake	111
	7.3.1.2 Large DNA Molecule Uptake	112
7.4	Plant Regeneration	114
7.5	General Analytical Considerations	115
7.5.1	Molecular Analysis	115
	7.5.1.1 DNA Extraction	116
	7.5.1.2 Southern Hybridization	116
	7.5.1.3 Polymerase Chain Reaction	116
	7.5.1.4 Random Amplified Polymorphic DNA	117

7.5.2	Biochemical Analysis	118
7.5.2.1	Multiple Molecular Forms of Enzymes	118
7.5.2.2	Enzymatic Assay	119
7.5.3	Cytogenetic Analysis	120
7.5.3.1	Flow Cytometric Analysis	120
7.5.3.2	Mitotic and Meiotic Cell Analysis	122
7.5.3.3	In Situ Hybridization	122
7.5.4	Morphological Analysis	123
7.6	Conclusions and Future Perspectives	124
	References	124

8 Transformation of Norway Spruce (*Picea abies*) by Particle Bombardment

D.H. CLAPHAM, H. HÄGGMAN, M. ELFSTRAND, T. ARONEN,
and S. VON ARNOLD

8.1	Introduction	127
8.2	Types of Particle Accelerator	127
8.3	Transformation of Embryogenic Cultures	128
8.3.1	Transient Expression in Embryogenic Cultures	128
8.3.2	Production of Stably Transformed Cell Cultures and Transgenic Plants	129
8.3.3	Stability of Transgene Expression	131
8.3.4	Trends in Transgenic Plant Production	131
8.4	Transformation of Pollen	133
8.4.1	The Reproductive Biology of Norway Spruce	133
8.4.2	Transient Expression in Pollen	134
8.4.3	Development of Controlled Pollination Techniques for Bombarded Pollen	135
8.5	Applications of Transgenic Norway Spruce in Research	136
8.5.1	Genes Regulating Embryogenesis	136
8.5.2	Genes with Similarity to Defense Genes	137
8.6	Prospects for Transgenic Norway Spruce in Practical Forestry	139
	References	143

9 WHISKERS-Mediated Transformation of Maize

J.F. PETOLINO, M. WELTER, and C. QIHUA CAI

9.1	Introduction	147
9.2	Preparation of Purified DNA Fragments	147
9.3	Establishment and Maintenance of Embryogenic Suspension Cultures	150
9.4	DNA Delivery via WHISKERS	152
9.5	Transgene Copy Number Estimation	153
9.6	Regeneration of Transgenic Plants and Progeny	157

9.7 Conclusions and Future Perspectives	157
References	158

10 Genetic Transformation of Soybean with Biolistics

D. SIMMONDS

10.1 Introduction	159
10.2 Tissue Culture and Plant Regeneration	160
10.2.1 Genotype Specificity	160
10.2.2 Initiation and Repetitive Proliferation of Somatic Embryogenic Cultures	161
10.2.3 Embryo Histodifferentiation and Maturation	163
10.2.4 Germination, Conversion and Plant Fertility	163
10.3 Transformation	164
10.3.1 Gene Delivery	164
10.3.2 Target Tissue Optimization	165
10.3.3 Selection	165
10.3.4 Transgenic Plant Recovery	166
10.4 Conclusions	167
10.5 Protocol	168
10.5.1 Induction and Maintenance of Proliferative Embryogenic Cultures	168
10.5.2 Transformation	168
10.5.3 Selection	169
10.5.4 Plant Regeneration	169
References	170

11 Genotoxic Effects of Tungsten Microparticles Under Conditions of Biolistic Transformation

J. BUCHOWICZ and C. KRYSIAK

11.1 Introduction	175
11.2 Biological Significance of Tungsten	175
11.2.1 Early Observations on Biological Effects of Tungsten	175
11.2.2 Catalytic Activity of Simple Tungsten Compounds	176
11.2.3 Tungstoenzymes	176
11.2.4 Tungsten-DNA Interaction	177
11.3 Tungsten Microparticles in Biotechnological Applications	178
11.3.1 Biolistic Transformation	178
11.3.1.1 An Overview	178
11.3.1.2 Technical Details	180
11.3.2 Biolistic Inoculation and Related Applications of Tungsten Particles	181
11.4 Assessment of Tungsten-Induced DNA Lesions	182
11.4.1 Electrophoretic Analysis of Tungsten-Damaged Plasmid DNA	182

11.4.2 A Modified TUNEL Method for Detection of Cellular DNA Fragmentation	184
11.5 Post-Bombardment Inhibition of Somatic Embryogenesis	186
11.6 Concluding Remarks	188
References	188
Subject Index	195

T. ANDRZEJCZAK

Forest Research Institute, Pankajczyk Research Station,
Poland; ul. 12, 34-430 Pankajczyk, Poland

P.C. BARRON

Center of Biotechnology, Federal University of Paraná - UFPR, Brazil Campus
Universitário, Caixa Postal 334, 81531-900, Paraná-RS, Brazil

J. BODZIMSKI

Institute of Biochemistry and Biophysics, Polish Academy of Sciences,
ul. Dąbrowskiego Street, 01-406 Warsaw, Poland

D.H. CLAPHAM

Department of Plant Biology and Forest Genetics, Swedish University of
Agricultural Sciences, Box 7000, 750 07 Uppsala, Sweden

G. DINE-HARPER

Department of Plant Systems Biology, Flinders Interuniversity Institute
for Microbiology, Flinders University, P.O. Box 211, Bedford Park, South
Australia

C.C. EMM

New Zealand Institute for Crop & Food Research Limited, Private Bag 479,
Christchurch, New Zealand

H. ERIKSSON

Department of Plant Biology and Forest Genetics, Swedish University of
Agricultural Sciences, Box 7000, 750 07 Uppsala, Sweden

H. GÖRGENBERG

TUM/TAF Research Institute for Genetic Engineering and Biotechnology,
74474 Göttingen, Germany