

CONTENTS

Preface

xvii

Foreword to Plant Biotechnology and Genetics

xix

Contributors

xxiii

1. Plant Agriculture: The Impact of Biotechnology

1

Graham Brookes

1.0 Chapter Summary and Objectives

1

1.0.1 Summary

1

1.0.2 Discussion Questions

1

1.1 Introduction

1

1.2 Biotechnology Crops Plantings

2

1.3 Why Farmers Use Biotech Crops

4

1.3.1 Herbicide-Tolerant Crops

7

1.3.2 Insect-Resistant Crops

7

1.3.3 Conclusion

8

1.4 How the Adoption of Plant Biotechnology Has Impacted the Environment

8

1.4.1 Environmental Impacts from Changes in Insecticide and Herbicide Use

9

1.4.2 Impact on Greenhouse Gas (GHG) Emissions

12

1.5 Conclusions

14

References

19

2. Mendelian Genetics and Plant Reproduction

21

Matthew D. Halfhill and Suzanne I. Warwick

2.0 Chapter Summary and Objectives

21

2.0.1 Summary

21

2.0.2 Discussion Questions

21

2.1 Genetics Overview

22

2.2 Mendelian Genetics

25

2.2.1 Law of Segregation

28

2.2.2 Law of Independent Assortment

28

2.3 Mitosis and Meiosis

30

2.3.1 Mitosis

31

2.3.2 Meiosis

32

2.3.3 Recombination

32

2.3.4 Cytogenetic Analysis

33

2.4	Plant Reproductive Biology	34
2.4.1	History of Research	34
2.4.2	Mating Systems	35
2.4.2.1	Sexual Reproduction	35
2.4.2.2	Asexual Reproduction	38
2.4.2.3	Mating Systems Summary	38
2.4.3	Hybridization and Polyploidy	39
2.5	Conclusion	41
	References	45
3.	Plant Breeding	47
	<i>Nicholas A. Tinker</i>	
3.0	Chapter Summary and Objectives	47
3.0.1	Summary	47
3.0.2	Discussion Questions	47
3.1	Introduction	48
3.2	Central Concepts in Plant Breeding	49
3.2.1	Simple versus Complex Inheritance	49
3.2.2	Phenotype versus Genotype	51
3.2.3	Mating Systems, Varieties, Landraces, and Pure Lines	52
3.2.4	Other Topics in Population and Quantitative Genetics	55
3.2.5	The Value of a Plant Variety Depends on Many Traits	56
3.2.6	Varieties Must Be Adapted to Environments	56
3.2.7	Plant Breeding Is a Numbers Game	57
3.2.8	Plant Breeding Is an Iterative and Collaborative Process	57
3.2.9	Diversity, Adaptation, and Ideotypes	58
3.2.10	Other Considerations	61
3.3	Objectives for Plant Breeding	62
3.4	Methods of Plant Breeding	63
3.4.1	Methods of Hybridization	63
3.4.1.1	Self-Pollinated Species	64
3.4.1.2	Outcrossing Species	69
3.4.1.3	Synthetic Varieties	72
3.4.1.4	Hybrid Varieties	72
3.4.2	Clonally Propagated Species	74
3.5	Breeding Enhancements	74
3.5.1	Doubled Haploidy	74
3.5.2	Marker-Assisted Selection	75
3.5.3	Mutation Breeding	76
3.5.4	Apomixis	77
3.6	Conclusions	77
	References	82
4.	Plant Development and Physiology	83
	<i>Glenda E. Gillaspay</i>	
4.0	Chapter Summary and Objectives	83
4.0.1	Summary	83
4.0.2	Discussion Questions	83
4.1	Plant Anatomy and Morphology	84

4.2	Embryogenesis and Seed Germination	85
4.2.1	Gametogenesis	85
4.2.2	Fertilization	88
4.2.3	Fruit Development	88
4.2.4	Embryogenesis	89
4.2.5	Seed Germination	91
4.2.6	Photomorphogenesis	91
4.3	Meristems	92
4.3.1	Shoot Apical Meristem	92
4.3.2	Root Apical Meristem and Root Development	94
4.4	Leaf Development	96
4.4.1	Leaf Structure	96
4.4.2	Leaf Development Patterns	97
4.5	Flower Development	98
4.5.1	Floral Evocation	98
4.5.2	Floral Organ Identity and the ABC Model	99
4.6	Hormone Physiology and Signal Transduction	101
4.6.1	Seven Plant Hormones and Their Actions	101
4.6.2	Plant Hormone Signal Transduction	103
4.6.2.1	Auxin and GA Signaling	104
4.6.2.2	Cytokinin and Ethylene Signaling	105
4.6.2.3	Brassinosteroid Signal Transduction	105
4.7	Conclusions	106
	References	110
5.	Tissue Culture: The Manipulation of Plant Development	113
	<i>Vinitha Cardozo</i>	
5.0	Chapter Summary and Objectives	113
5.0.1	Summary	113
5.0.2	Discussion Questions	113
5.1	Introduction	113
5.2	History	114
5.3	Media and Culture Conditions	115
5.3.1	Basal Media	115
5.3.2	Growth Regulators	116
5.4	Sterile Technique	118
5.4.1	Clean Equipment	118
5.4.2	Surface Sterilization of Explants	118
5.5	Culture Conditions and Vessels	119
5.6	Culture Types and Their Uses	120
5.6.1	Callus Culture	120
5.6.1.1	Somaclonal Variation	122
5.6.2	Cell Suspension Culture	122
5.6.2.1	Production of Secondary Metabolites and Recombinant Proteins Using Cell Culture	122
5.6.3	Anther/Microspore Culture	123
5.6.4	Protoplast Culture	123
5.6.4.1	Somatic Hybridization	124
5.6.5	Embryo Culture	124
5.6.6	Meristem Culture	124

5.7	Regeneration Methods of Plants in Culture	125
5.7.1	Organogenesis	125
5.7.1.1	Indirect Organogenesis	125
5.7.1.2	Direct Organogenesis	125
5.7.2	Somatic Embryogenesis	126
5.7.2.1	Synthetic Seeds	127
5.8	Rooting of Shoots	127
5.9	Acclimation	128
5.10	Conclusions	128
	Acknowledgments	128
	References	132
6.	Molecular Genetics of Gene Expression	135
	<i>Maria Gallo and Alison K. Flynn</i>	
6.0	Chapter Summary and Objectives	135
6.0.1	Summary	135
6.0.2	Discussion Questions	135
6.1	The gene	135
6.1.1	DNA Coding for a Protein via the Gene	135
6.1.2	DNA as a Polynucleotide	136
6.2	DNA Packaging into Eukaryotic Chromosomes	136
6.3	Transcription	140
6.3.1	Transcription of DNA to Produce Messenger RNA (mRNA)	140
6.3.2	Transcription Factors	143
6.3.3	Coordinated Regulation of Gene Expression	144
6.3.4	Chromatin as an Important Regulator of Transcription	144
6.3.5	Regulation of Gene Expression by DNA Methylation	146
6.3.6	Processing to Produce Mature mRNA	146
6.4	Translation	148
6.4.1	Initiation of Translation	150
6.4.2	Translation Elongation	152
6.4.3	Translation Termination	152
6.5	Protein Postranslational Modification	152
	References	156
7.	Recombinant DNA, Vector Design, and Construction	159
	<i>Mark D. Curtis</i>	
7.0	Chapter Summary and Objectives	159
7.0.1	Summary	159
7.0.2	Discussion Questions	159
7.1	DNA Modification	160
7.2	DNA Vectors	163
7.2.1	DNA Vectors for Plant Transformation	166
7.2.2	Components for Efficient Gene Expression in Plants	167
7.3	Greater Demands Lead to Innovation	170
7.3.1	Site-Specific DNA Recombination	171

7.3.1.1 Gateway Cloning	172
7.3.1.2 Creator™ Cloning	175
7.3.1.3 Univector (Echo™) Cloning	175
7.4 Vector Design	177
7.4.1 Vectors for High-Throughput Functional Analysis	177
7.4.2 Vectors for RNA Interference (RNAi)	179
7.4.3 Expression Vectors	179
7.4.4 Vectors for Promoter Analysis	180
7.4.5 Vectors Derived from Plant Sequences	181
7.4.6 Vectors for Multigenic Traits	183
7.5 Targeted Transgene Insertions	184
7.6 Safety Features in Vector Design	186
7.7 Prospects	188
References	190
8. Genes and Traits of Interest for Transgenic Plants	193
<i>Kenneth L. Korth</i>	
8.0 Chapter Summary and Objectives	193
8.0.1 Summary	193
8.0.2 Discussion Questions	193
8.1 Introduction	194
8.2 Identifying Genes of Interest via Genomic Studies	194
8.3 Traits for Improved Crop Production	197
8.3.1 Herbicide Resistance	197
8.3.2 Insect Resistance	200
8.3.3 Pathogen Resistance	202
8.4 Traits for Improved Products and Food Quality	205
8.4.1 Nutritional Improvements	205
8.4.2 Modified Plant Oils	207
8.4.3 Pharmaceutical Products	208
8.4.4 Biofuels	209
8.5 Conclusions	210
References	216
9. Marker Genes and Promoters	217
<i>Brian Miki</i>	
9.0 Chapter Summary and Objectives	217
9.0.1 Summary	217
9.0.2 Discussion Questions	217
9.1 Introduction	218
9.2 Definition of Marker Genes	218
9.2.1 Selectable Marker Genes: An Introduction	218
9.2.2 Reporter Genes: An Introduction	222
9.3 Promoters	224
9.4 Selectable Marker Genes	227
9.4.1 Conditional Positive Selectable Marker Gene Systems	227

9.4.1.1	Selection on Antibiotics	228
9.4.1.2	Selection on Herbicides	229
9.4.1.3	Selection Using Nontoxic Metabolic Substrates	229
9.4.2	Nonconditional Positive Selection Systems	230
9.4.3	Conditional Negative Selection Systems	230
9.4.4	Nonconditional Negative Selection Systems	230
9.5	Nonselectable Marker Genes or Reporter Genes	231
9.5.1	β -Glucuronidase	231
9.5.2	Luciferase	232
9.5.3	Green Fluorescent Protein	232
9.6	Marker-Free Strategies	233
9.7	Conclusions	237
	References	242
10.	Transgenic Plant Production	245
	<i>John Finer and Taniya Dhillon</i>	
10.0	Chapter Summary and Objectives	245
10.0.1	Summary	245
10.0.2	Discussion Questions	245
10.1	Overview	246
10.2	Basic Components for Successful Gene Transfer to Plant Cells	246
10.2.1	Visualizing the General Transformation Process	246
10.2.2	DNA Delivery	247
10.2.3	Target Tissue Status	248
10.2.4	Selection and Regeneration	248
10.3	Agrobacterium	249
10.3.1	History of Our Knowledge of <i>Agrobacterium</i>	249
10.3.2	Use of the T-DNA Transfer Process for Transformation	251
10.3.3	Optimizing Delivery and Broadening the Range of Targets	253
10.3.4	Agroinfiltration	254
10.3.5	Arabidopsis Floral Dip	254
10.4	Particle Bombardment	255
10.4.1	History of Particle Bombardment	255
10.4.2	The Fate of Introduced DNA	257
10.4.3	The Power and Problems of Direct DNA Introduction	259
10.4.4	Improvements in Transgene Expression	259
10.5	Other Methods	260
10.5.1	The Need for Additional Technologies	260
10.5.2	Protoplasts	261
10.5.3	Whole-Tissue Electroporation	262
10.5.4	Silicon Carbide Whiskers	262
10.5.5	Viral Vectors	263
10.5.6	Laser Micropuncture	263
10.5.7	Nanofiber Arrays	263
10.6	The Rush to Publish	265
10.6.1	Controversial Reports of Plant Transformation	265
10.6.1.1	DNA Uptake in Pollen	265
10.6.1.2	<i>Agrobacterium</i> -Mediated Transformation of Maize Seedlings	265

10.6.1.3	Pollen Tube Pathway	266
10.6.1.4	Rye Floral Tiller Injection	266
10.6.1.5	Electrotransformation of Germinating Pollen Grain	267
10.6.1.6	<i>Medicago</i> Transformation via Seedling Infiltration	267
10.6.2	Criteria to Consider: Whether My Plant Is Transgenic	268
10.6.2.1	Resistance Genes	268
10.6.2.2	Marker Genes	268
10.6.2.3	Transgene DNA	269
10.7	A Look to the Future	269
References		272
11. Transgenic Plant Analysis		275
<i>Janice Zale</i>		
11.0	Chapter Summary and Objectives	275
11.0.1	Summary	275
11.0.2	Discussion Questions	275
11.1	Introduction	276
11.2	Directionally Named Analyses: As the Compass Turns	276
11.3	Initial Screens: Putative Transgenic Plants	277
11.3.1	Screens on Selection Media	277
11.3.2	Polymerase Chain Reaction	278
11.3.3	Enzyme-Linked Immunosorbent Assays (ELISAs)	279
11.4	Definitive Molecular Characterization	280
11.4.1	Intact Transgene Integration	280
11.4.2	Determining the Presence of Intact Transgenes or Constructs	284
11.4.3	Transgene Expression: Transcription	284
11.4.3.1	Northern Blot Analysis	284
11.4.3.2	Quantitative Real-Time Reverse Transcriptase (RT)-PCR	286
11.4.4	Transgene Expression: Translation: Western Blot Analyses	286
11.5	Digital Imaging	287
11.6	Phenotypic Analysis	287
11.7	Conclusions	288
References		288
12. Regulations and Biosafety		291
<i>Alan McHughen</i>		
12.0	Chapter Summary and Objectives	291
12.0.1	Summary	291
12.0.2	Discussion Questions	291
12.1	Introduction	291
12.2	History of Genetic Engineering and its Regulation	293
12.3	Regulation of GE	296
12.3.1	United States	296
12.3.1.1	USDA	297
12.3.1.2	FDA	297
12.3.1.3	EPA	298
12.3.2	EU	299
12.3.3	Canada	300

12.3.4	International Perspectives	301
12.4	Conclusions	302
	References	309
13.	Field Testing of Transgenic Plants	311
	<i>Detlef Bartsch, Achim Gathmann, Christiane Saeglitz, and Arti Sinha</i>	
13.0	Chapter Summary and Objectives	311
13.0.1	Summary	311
13.0.2	Discussion Questions	311
13.1	Introduction	312
13.2	Environmental Risk Assessment (ERA) Process	312
13.2.1	Initial Evaluation (ERA Step 1)	313
13.2.2	Problem Formulation (ERA Step 2)	313
13.2.3	Controlled Experiments and Gathering of Information (ERA Step 3)	313
13.2.4	Risk Evaluation (ERA Step 4)	313
13.2.5	Progression through a Tiered Risk Assessment	313
13.3	An Example Risk Assessment: The Case of Bt Maize	314
13.3.1	Effect of Bt Maize Pollen on Nontarget Caterpillars	315
13.3.2	Statistical Analysis and Relevance for Predicting Potential Adverse Effects on Butterflies	317
13.4	Proof of Safety versus Proof of Hazard	319
13.5	Proof of Benefits: Agronomic Performance	319
13.6	Conclusions	320
	References	323
14.	Intellectual Property in Agricultural Biotechnology: Strategies for Open Access	325
	<i>Alan B. Bennett, Cecilia Chi-Ham, Gregory Graff, and Sara Boettiger</i>	
14.0	Chapter Summary and Objectives	325
14.0.1	Summary	325
14.0.2	Discussion Questions	325
14.1	Introduction	326
14.2	Intellectual Property Defined	327
14.3	Intellectual Property in Relation to Agricultural Research	329
14.4	Development of an "Anticommons" in Agricultural Biotechnology	331
14.4.1	Transformation Methods	331
14.4.2	Selectable Markers	332
14.4.3	Constitutive Promoters	332
14.4.4	Tissue- or Development-Specific Promoters	332
14.4.5	Subcellular Localization	333
14.5	Freedom to Operate (FTO)	333
14.6	Strategies for Open Access	336
14.7	Conclusions	338
	References	339

15. Why Transgenic Plants Are So Controversial 343*Douglas Powell*

15.0 Chapter Summary and Objectives	343
15.0.1 Summary	343
15.0.2 Discussion Questions	343
15.1 Introduction	343
15.1.1 The Frankenstein Backdrop	344
15.1.2 Agricultural Innovations and Questions	344
15.2 Perceptions of Risk	345
15.3 Responses to Fear	347
15.4 Feeding Fear: Case Studies	348
15.4.1 Pusztai's Potatoes	349
15.4.2 Monarch Butterfly Flap	349
15.5 How Many Benefits are Enough	350
15.6 Continuing Debates	351
15.6.1 Process versus Product	351
15.6.2 Health Concerns	352
15.6.3 Environmental Concerns	353
15.6.4 Consumer Choice	353
15.7 Business and Control	353
15.8 Conclusions	354
References	354

16. The Future of Plant Biotechnology 357*C. Neal Stewart, Jr. and David W. Ow*

16.0 Chapter Summary and Objectives	357
16.0.1 Summary	357
16.0.2 Discussion Questions	357
16.1 Introduction	357
16.2 Site-Specific Recombination Systems to Provide Increased Precision	359
16.2.1 Removal of DNA from Transgenic Plants or Plant Parts	361
16.2.2 More Precise Integration of DNA	362
16.3 Zinc-Finger Nucleases	363
16.4 The Future of Food (and Fuel and Pharmaceuticals)	364
16.5 Conclusions	365
References	368

Index 371