

Contents

1. Preface	1
2. Introduction	3
2.1. Outline	3
2.2. Abbreviations and glossary	7
3. History	21
3.1. Introduction	21
3.2. Developments in the Netherlands and other countries	25
4. Types of cultures	29
5. Laboratory equipment	31
5.1. Supplies	31
5.2. The laminar air-flow cabinet	34
5.3. Sterilization of nutrient media	36
5.3.1. Introduction	36
5.3.2. Autoclaving	36
5.3.3. Sterilization by irradiation	39
5.3.4. Sterilization by filtration	40
5.3.5. Sterilization at school and in the home	41
5.4. Preparation room	42
5.5. Cleaning glassware	42
5.6. Robots in the plant tissue culture laboratory	42
6. Preparation and composition of nutrient media	45
6.1. Introduction	45
6.2. Glassware and plastics	48
6.3. Preparation	50
6.4. Composition	54
6.4.1. Water	54
6.4.2. Agar	54

6.4.3. Sugar	60
6.4.4. Mineral nutrition	63
6.4.5. pH	65
6.4.6. Osmotic potential	66
6.4.7. Regulators	67
6.4.7.1. Introduction	67
6.4.7.2. Auxins	69
6.4.7.3. Cytokinins	70
6.4.7.4. Gibberellins	71
6.4.7.5. Other regulators	71
6.4.8. Vitamins	76
6.4.9. Miscellaneous	76
6.5. Commercially prepared media	82
6.6. Storage of nutrient media	82
7. Closure of test tubes and flasks	83
8. Care of plant material	87
9. Sterilization of plant material	89
9.1. Introduction	89
9.2. Chemical sterilization	90
9.3. Apparently sterile cultures	92
9.4. Internal infections	93
9.5. Symbiotic cultures	94
10. Isolation, inoculation and subculturing	95
10.1. Introduction	95
10.2. Isolation	96
10.3. Inoculation	97
10.4. Subculturing	99
11. Mechanization	101
12. The influence of plant material on growth and development	107
13. The influence of physical factors on growth and development	115
13.1. The culture room	115
13.2. Discussion of special physical factors	118
14. The transfer from nutrient medium to soil	127
15. Aids to study	133
15.1. Literature study	133
15.2. Societies and associations	136
15.3. Laboratory notebook, photographs and slides	137

16. Embryo culture	139
16.1. Introduction	139
16.2. Techniques	141
16.3. Factors affecting the success of embryo culture	143
16.4. Practical applications	146
17. Germination of orchid seeds	149
17.1. Introduction	149
17.2. Factors affecting germination and growth	154
18. Vegetative propagation of orchids	159
18.1. Introduction	159
18.2. Meristem culture	161
18.2.1. Production of virus-free plants	161
18.2.2. Propagation through meristem culture	162
18.3. Other methods of propagation	165
18.4. Variation arising during culture	166
18.5. Practical applications	167
19. Production of disease-free plants	169
19.1. Introduction	169
19.2. Production of virus-free plants	171
19.2.1. Heat treatment	171
19.2.2. Meristem culture	172
19.2.2.1. History	172
19.2.2.2. Accomplishment	174
19.2.3. Heat treatment and meristem culture	177
19.2.4. Adventitious shoot formation, eventually followed by meristem culture	177
19.2.5. Virus-free plants produced from callus and protoplasts	178
19.2.6. Grafting of meristems on virus-free (seedling) rootstocks (micrografting)	179
19.2.7. Virus identification	179
19.3. Production of bacteria- and fungal-free plants by meristem culture	180
20. Vegetative propagation	183
20.1. General introduction	183
20.2. Single-node culture	190
20.3. The axillary bud method	194
20.4. Regeneration of explants	200
20.4.1. Introduction	200
20.4.2. Adventitious root formation	203
20.4.3. Adventitious shoot formation	209

20.5. Callus induction, callus culture and regeneration of organs and embryos	213
20.5.1. Introduction	213
20.5.2. Callus induction	214
20.5.3. Callus culture	217
20.5.3.1. Introduction	217
20.5.3.2. On solid media	218
20.5.3.3. In liquid media	218
20.5.4. Regeneration of organs and embryos	219
20.5.4.1. Introduction	219
20.5.4.2. Regeneration of organs	220
20.5.4.3. Regeneration of embryos	222
20.5.4.4. Nucellar polyembryony	226
20.6. Regeneration of plants from single cells	227
20.7. Synthetic seeds	229
21. Somaclonal variation	231
22. Test tube fertilization	239
23. Production of haploids	243
23.1. General introduction	243
23.2. Obtaining haploids in vitro	247
23.3. Problems associated with haploid induction	256
24. Genetic manipulation	259
24.1. General introduction	259
24.2. Description	261
24.3. Prerequisites for the use of genetic manipulation	263
24.4. Somatic hybridization	263
24.4.1. Outline	263
24.4.2. Discussion of the different phases involved	264
24.5. The relevance of somatic hybridization	270
24.6. Disadvantages and problems of somatic hybridization	272
24.7. Selection procedures after somatic hybridization	274
24.8. Selection of mutants	275
24.9. Transformation by <i>Agrobacterium tumefaciens</i>	276
25. Miscellaneous applications	281
25.1. In phytopathology	281
25.1.1. Outline	281
25.1.2. Transport of disease-free plant material	282
25.2. In plant breeding	284
25.2.1. The development of chimeras in vitro	284
25.2.2. Separation of chimeras and isolation of mutants	286

25.2.3. Obtaining tetraploids and triploids; induction of chromosome loss	287
25.2.4. Mutation induction in vitro	291
25.2.5. Storage of plant material in vitro	296
25.3. The biosynthesis of substances in vitro	301
26. In vitro cloning of plants in the Netherlands	305
27. Index	309
28. Literature cited	321