CHAPTER 6

APPENDICES

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Appendix I: LSA and LSB callus induction medium (Modified from Linsmaier and Skoog (1965) and N6M callus maintenance medium (Modified from Chu *et al.*, 1975).

Component	Concentration (mg l^{-1})		
	LSA medium	LSB medium	N6M medium[2]
Macronutrients			
KNO_3	1900.0	1900.0	2830.0
NH_4NO_3	1650.0	1650.0	
$CaCl_2$	440.0	440.0	166.0
KH_2PO_4	170.0	170.0	400.0
$MgSO_4$	370.0	370.0	185.0
$(NH_4)_2SO_4$			463.0
Micronutrients			
KI	0.83	0.83	0.8
H_3BO_3	6.20	6.20	1.6
$MnSO_4$	22.30	22.3	4.4
$Na_2MoO_4.2H_2O$	0.25	0.25	
$ZnSO_4.7H_2O$	8.60	8.60	1.5
$CuSO_4.5H_2O$	0.025	0.025	
$CoCl_2.6H_2O$	0.025	0.025	
$FeSO_4.7H_2O$	27.85	27.85	27.85
Na_2EDTA	37.25	37.25	37.25
NaFeEDTA			
Vitamins			
Myo-inositol	100.0	100.0	
Nicotinic acid		0.5	0.5
Pyridoxine HCl		0.5	0.5
Kinetin	1.0		
Thiamine HCl	1.0	0.1	1.0
Glycine	2.0		
amino acids			
Glutamine		500.0	2.0
Other Supplements			
Casein hydrolysate		100.0	
Tryptophan	50.0		
2,4-dichlorophenoxyacetic acid	4.0	4.0	4.0
Sucrose	30000	30000	20000
Mannitol			30000
pH 5.8			

LSA, LSB and N6M media were prepared as single strength in distilled water and made solidified with 0.8% (w/v) agar; autoclaved at 121°C for 20 minutes.

Appendix II : MS0 seed germination medium an and MSB plant regeneration medium (Modified from Murashige and Skoog, 1962)

Component	Concentration (mg l^{-1}) MS0 medium	MSB
Micronutrients		
NH_4NO_3	1650	1650
KNO_3	1900	1900
$CaCl_2.2H_2O$	440	440
$MgSO_4.7H_2O$	370	
KH_2PO_4	170	
Macronutrients		
KI	0.83	0.83
$CoCl_2.6H_2O$	0.025	0.025
H_3BO_3	6.2	6.2
$Na_2MoO_4.2H_2O$	0.25	0.25
$MnSO_4.4H_2O$	22.3	22.3
$CuSO_4.5H_2O$	0.025	0.025
$ZnSO_4.7H_2O$	8.6	8.6
$FeSO_4.7H_2O$	27.85	27.85
Na_2EDTA		
	37.25	37.25
Vitamins		
Inositol	100	100
Nicotinic acid	0.5	0.5
Pyridoxine HCl	0.5	0.5
Thiamine HCl	0.1	0.1
Glycine		
	2.0	2.0
Carbon Source		
Sucrose		
	30000	30000
Growth Regulators		
Kinetin		3.0
NAA		0.5
pH 5.8		

MS0 medium was made up as a single strength solution in distilled water and autoclaved at 121°C for 20 min made solidified with 0.8% (w/v) agar. MSB medium was made up as a single strength solution in distilled water and autoclaved at 121°C for 20 minutes made solidified with 0.8% (w/v) agarose (Sigma Type 1)

Appendix III: AA4 suspension culture medium (Modified from Muller and Grafe 1978)

Component	Concentration (mg l^{-1}) AA4 medium	AAP medium
Macronutrients		
$CaCl_2$	440.0	440.0
KH_2PO_4	170.0	170.0
$MgSO_4$	370.0	370.0
KCl	2940.0	2940.0
KNO_3		
Micronutrients		
KI	0.83	0.83
H_3BO_3	6.20	6.20
$MnSO_4$	22.3	22.3
$Na_2MoO_4.2H_2O$	0.25	0.25
$ZnSO_4.7H_2O$	8.60	8.60
$CuSO_4.5H_2O$	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025
$FeSO_4.7H_2O$	27.85	27.85
Na_2EDTA	37.25	37.25
Vitamins		
Myo-inositol	100.0	100.0
Nicotinic acid	0.5	0.5
Pyridoxine HCl	0.1	0.1
Thiamine HCl	0.5	0.5
Glycine	75.0	75.0
L-Glutamine	877.0	877.0
L-Aspartic acid	266.0	266.0
L-Arginine	228.0	228.0
L-Proline		1.0
Other Supplements		
2,4-dichlorophe-noxyacetic acid	4.0	4.0
Gibberellic acid	0.1	0.1
Kinetin	0.2	0.2
Sucrose	30000	30000
pH 5.8		

AA4 and AAP medium was made up as a single-strength solution in reverse osmosis water and filter sterilized through a 0.2 μm pore size membrane.

Appendix IV : N6PCMZ protoplast culture medium (Modified from Chu *et al.*, 1975)

Component	Concentration (mg l^{-1}) N6PCMZ medium
Macro nutrients	
KNO_3	2860
$(NH_4)_2SO_4$	463
KH_2PO_4	400
$MgSO_4.7H_2O$	185
$CaCl_2.2H_2O$	166
Micro nutrients	
H_3BO_3	1.6
$MnSO_4.4H_2O$	4.4
$ZnSO_4.7H_2O$	1.5
KI	0.8
$FeSO_4.7H_2O$	27.85
Na_2EDTA	37.25
Amino acid	
Glycine	2.0
Vitamins	
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	1.0
Carbon Source	
Sucrose	20000
Glucose	90000.08
Growth Regulator	
2,4-D	1.5
Zeatin	0.4
Casein hydrolysate	500
pH 5.8	

N6PCMZ medium was made up as single strength solutions in distilled water and filter sterilized by passage through a 0.2 μm membrane.

Appendix V: Composition of Enzyme Mixture (Bhattacharjee and Gupta, 1995)

Component	Concentration (mg l^{-1})
Cellulase RS	10000
Pectolyase Y23	1000
MES	11000
pH 5.8	

Enzyme Mixture was made up in CPW13M solution and filter sterilised by passing through a 0.2 μm pore size membrane.

Appendix VI: CPW 13M Solution (Modified from Frearson *et al.*, 1973)

Component	Concentration (mg l^{-1})
KH_2PO_4	27.2
KNO_3	101.0
$CaCl_2.2H_2O$	1480.0
$MgSO_4.7H_2O$	246.0
KI	0.16
$CuSO_4.5H_2O$	0.025
Mannitol	130000
pH 5.8	

CPW 13M solution was made up as a single strength solution in reverse osmosis water and autoclaved at 121^{0}C for 20 min.

Appendix VII : W5 solution for protoplast fusion (After Medgyesy *et al.*, 1980)

Component	Concentration (mM)
$CaCl_2$	125 mM
NaOH	155 mM
KC	15 mM
Glucose	5 mm
pH 5.6	

W5 solution was made up as a single strength solution in reverse osmosis water and autoclaved at 121^{0}C for 20 min.

Appendix VIII : Iodoacetamide solution for inactivation of protoplasts (Bhattacharjee *et al.*, 1999)

Component	Concentration (mg ml^{-10})
Iodoacetic Acid	18.6
pH 5.6	

18.6 gm of iodoacetic acid was dissolved in 10 ml of 10mM W5 solution and filter sterilized by passing through a 0.2 μm pore size membrane.