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APPENDIX

ศูนย์วิทยทรัพยากร
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Preparation Culture Media

The culture media used in this study included B5 (Gamborg *et al.*), MS (Murashige and Skoog), RM (Root Medium) and WPM (Woody Plant Medium). The chemical composition of each medium are shown in Table 2.

Each culture media were prepared by mixing its stock solutions (Table 3 and 4) according to the volume describe in Table 5. The final pH of each medium was adjusted to its desired value with 0.1 N. NaOH or 0.1 N. HCl. The semisolid media were prepared by adding with 0.6-0.8 % w/v agar (Difco, Detroit Michigan, USA) and sterilized by autoclaving at 121°C, 15 lb/in² for 15 min. Liquid media were also prepared similiary without adding the agar.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 2 Composition of plant tissue culture media B5, MS, RM and WPM

Constituent	concentration(mg/l)			
	B5	MS	RM	WPM
Macronutrients:				
CaCl ₂ ·2H ₂ O	150.0	440.0	440.0	96.0
Ca(NO ₃) ₂ ·4H ₂ O	-	-	-	556.0
KH ₂ PO ₄ ·H ₂ O	-	170.0	170.0	170.0
KNO ₃	2500.0	1900.0	1900.0	-
MgSO ₄ ·7H ₂ O	250.0	370.0	370.0	370.0
NaH ₂ PO ₄ ·H ₂ O	150.0	-	-	-
NH ₄ NO ₃	-	1650.0	1650.0	400.0
(NH ₄) ₂ SO ₄	134.0	-	-	-
Micronutrients:				
CoCl ₂ ·6H ₂ O	0.025	0.025	0.025	-
CuSO ₄ ·5H ₂ O	0.025	0.025	0.025	0.25
FeSO ₄ ·7H ₂ O	-	27.8	27.8	27.8
H ₃ BO ₃	3.0	6.2	6.2	6.2
KI	0.75	0.83	0.83	-
MnSO ₄ ·H ₂ O	10.0	15.6	22.30	37.3
Na ₂ EDTA	-	37.3	37.3	37.3
Na ₂ MoO ₄ ·2H ₂ O	0.25	0.25	0.25	0.25
ZnSO ₄ ·2H ₂ O	2.0	8.6	8.6	8.6
Vitamins:				
Nicotinic acid	1.0	-	0.5	0.5
Pyridoxine HCl	1.0	-	0.5	0.5
Thiamine HCl	10.0	0.5	0.1	1.0
Amino acid:				
Glycine	-	-	-	2.0
K ₂ SO ₄	-	-	-	990.0

Abbreviations: B5 = Gamborg et al.

MS = Murashige and Skoong

RM = Root Medium

WPM = Woody Plant Medium

Table 3 Stock solutions of B5 and MS

B5		MS	
Stock 1	g/l	Stock 1	g/l
KNO ₃	50.00	KH ₂ PO ₄	3.4
MgSO ₄ .7H ₂ O	5.00	KNO ₃	38.0
NaH ₂ PO ₄ .H ₂ O	3.00	MgSO ₄ .7H ₂ O	7.4
(NH ₄) ₂ SO ₄	2.68	NH ₄ NO ₃	33.0
Stock 2		Stock 2	
CoCl ₂ .6H ₂ O	0.025	CoCl ₂ .6H ₂ O	0.025
CuSO ₄ .5H ₂ O	0.025	CuSO ₄ .5H ₂ O	0.025
H ₃ BO ₃	3.000	H ₃ BO ₃	6.200
MnSO ₄ .H ₂ O	10.000	MnSO ₄ .H ₂ O	16.900
Na ₂ MoO ₄ .2H ₂ O	0.250	Na ₂ MoO ₄ .2H ₂ O	0.250
ZnSO ₄ .7H ₂ O	2.000	ZnSO ₄ .7H ₂ O	8.600
Stock 3		Stock 3	
CaCl ₂ .2H ₂ O	30.0	CaCl ₂ .2H ₂ O	87.0
Stock 4		Stock 4	
KI	0.75	KI	0.75
Stock 5		Stock 5	
myo-Inositol	100.00	myo-Inositol	100.00
Nicotinic acid	1.00	Thiamine HCl	0.08
Pyridoxine HCl	1.00	Stock 6	
Thiamine HCl	10.00	FeSO ₄ .7H ₂ O	5.56
Stock 6		Na ₂ EDTA	7.46
FeSO ₄ .7H ₂ O	5.56		
Na ₂ EDTA	7.46		

Table 4 Stock solutions of RM and WPM

RM		WPM	
Stock 1	g/l	Stock 1	g/l
CaCl ₂ .2H ₂ O	8.8	Ca(NO ₃) ₂ .4H ₂ O	27.8
KH ₂ PO ₄	3.4	NH ₄ NO ₃	20.0
KNO ₃	38.0	Stock 2	
MgSO ₄ .7H ₂ O	7.4	K ₂ SO ₄	49.5
NH ₄ NO ₃	33.0	Stock 3	
Stock 2		CaCl ₂ .2H ₂ O	19.2
CoCl ₂ .6H ₂ O	0.025	Stock 4	
CuSO ₄ .5H ₂ O	0.025	H ₃ BO ₃	34.00
H ₃ BO ₃	6.200	KH ₂ PO ₄	1.24
KI	0.830	Na ₂ MoO ₄ .2H ₂ O	0.05
MnSO ₄ .4H ₂ O	22.300	Stock 5	
Na ₂ MoO ₄ .2H ₂ O	0.250	CuSO ₄ .5H ₂ O	0.05
ZnSO ₄ .7H ₂ O	8.600	MgSO ₄ .7H ₂ O	74.00
Stock 3		MnSO ₄ . H ₂ O	7.46
Nicotinic acid	0.5	ZnSO ₄ .7H ₂ O	1.72
Pyridoxine HCl	0.5	Stock 6	
Thiamine HCl	0.1	FeSO ₄ .7H ₂ O	5.56
Stock 4		Na ₂ EDTA	7.46
FeSO ₄ .7H ₂ O	5.56	Stock 7	
Na ₂ EDTA	7.46	Glycine	0.4
Stock 5		Nicotinic acid	0.1
myo-Inositol	10.00	Pyridoxine HCl	0.1
		Thiamine HCl	0.2
		Stock 8	
		myo-Inositol	20.0

Table 5 Preparation of various plant tissue culture media from their stock solutions described in Table 3 and 4.

	B5	MS	RM	WPM
Distilled water to	1000 ml	1000 ml	1000 ml	1000 ml
Stock 1	50 ml	50 ml	50 ml	20 ml
Stock 2	1 ml	1 ml	1 ml	20 ml
Stock 3	5 ml	5 ml	1 ml	5 ml
Stock 4	1 ml	1 ml	5 ml	5 ml
Stock 5	10 ml	10 ml	10 ml	5 ml
Stock 6	5 ml	5 ml	-	5 ml
Stock 7	-	-	-	5 ml
Stock 8	-	-	-	5 ml
Sucrose	30 g	30 g	30 g	30 g
Final pH adjust	5.5	5.8	6.0	5.8
Plant growth regulators	(---as needed---)			

ศูนย์วิทยทรัพยากร
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Vita

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