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Micropropagation and tissue culture of *Eucalyptus*—a review

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Summary

Micropropagation has the potential to provide very high multiplication rates of selected tree genotypes, with resulting short-term silvicultural gains. Aseptic cultures have been established from seeds, seedlings, shoots, flowers and lignotubers. Callus cultures have been established from a wide range of tissue sources for at least 30 species of *Eucalyptus*. Plant regeneration from callus was successful for 12 of these species. Micropropagation through axillary proliferation, or adventitious shoot proliferation on nodal explants, or both, has been successful. An agar-based medium of Murashige and Skoog with a low auxin/cytokinin ratio is most commonly used for shoot multiplication. Vitrification and shoot senescence remain problems. Gibberellic acid was added in some media to stimulate shoot elongation. Various media are used for *in vitro* root initiation. Suspension and protoplast cultures have been achieved and plants have been regenerated from protoplasts. *In vitro* techniques are presently being applied to *Eucalyptus* to achieve genetic transformations.

Introduction

Today there is a demand for plants with superior growth potential. Clonal propagation through tissue culture has the potential to provide high multiplication rates of uniform genotypes, resulting in short-term production gains. The first *in vitro* callus cultures of *Eucalyptus* were obtained from seedling tissues. Jacquiot [77,78] derived callus from cambial tissues of *E. cladocalyx*, *E. gomphocephala*, *E. gunnii*, *E. tereticornis*, and *E. camaldulensis*. Sussex [130] was able to produce callus and suspension cultures of *E. camaldulensis*. The early work of de Fossard and co-workers was on callus cultures of *E. bancroftii*, *E. urnigera*, *E. melliodora* and *E. nicholii* [27,35,88]. Research on nodal cultures of seedlings and mature trees of the ornamental *E. ficifolia* followed [30–33].

As research progressed, media became more defined and new tissue culture techniques were applied to *Eucalyptus* species for a variety of purposes. These techniques have not yet been widely used for large-scale commercial operations, however.

Eucapyptus grandis has been micropropagated because of its importance in the pulp and mining industries. Other important timber species that have been micropropagated include *E. gunnii*, *E. dalrympleana*, *E. pauciflora*, *E. delagatensis*, *E. globulus*, *E. nitens*, *E. nova-anglica* and *E. viminalis* [89,98].

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Eucalyptus camaldulensis and *E. rudis* trees are being selected for salt-tolerance, because mass propagation of such clones would provide trees for plantations in areas normally hostile to afforestation [5,67,68,70]. Propagation of clones of *E. citriodora* and *E. polybractea*, selected for high oil content, provides the potential for rapidly establishing high oil yielding plantations. Researchers are also considering the potential of *in vitro* oil production [61,141].

A summary of reports on tissue culture of *Eucalyptus* species is given in Table 1. The table format is based on that used by George et al. [51] in their text on plant tissue culture media. The classification used is that of Pryor and Johnson [119].

Establishing aseptic cultures from explants

Seeds

Most papers report a sterilization technique involving three steps: pretreatment, treatment and posttreatment. Pretreatments usually involve rinses or a soak in water [69,84,99]. Hartney and Barker [69] and Bennett and McComb [11] included an alcohol rinse in their pretreatments. The sterilization step involves treatment of seeds with 0.1% (w/v) $HgCl_2$ for 5 to 20 min [82,84,101] or 10 to 20 min with either NaOCl or $Ca(OCl)_2$ in the range 1 to 7% [30,41]. A few drops of detergent are added to the treatment solutions. Posttreatments are usually three rinses in sterile distilled water.

McComb and Bennett [96] noted that tolerance of seeds to sterilization depends on size and thickness of the seed coat. *Eucalyptus citriodora* and *E. diversicolor* seeds are small and thin walled and can be sterilized in 2% NaOCl for 20 min, whereas *Eucalyptus marginata* seeds, which are larger and have thicker seed coats, can tolerate 5% NaOCl for 60 to 90 min [11,96]. MacRae (personal communication) obtained good results sterilizing *Eucalyptus* seeds with one part H_2O_2 (30% w/v) to one part absolute ethanol for 10 to 20 min, followed by three rinses in sterile distilled water. Sterilization success was improved by sorting seeds into groups of uniform size before treatment.

Shoots

The sterilization of mature, field-grown material has proved difficult because of endogenous microbial contamination [30]. Age of material and season are important factors determining success in establishing aseptic cultures [60]. It is virtually impossible to sterilize mature, field-grown shoots without severely damaging the tissues. Various preharvest techniques have been employed to provide shoots suitable as explant sources. The ideal material should have many of the qualities of young juvenile shoots.

Aseptic cultures have been established from stump coppice shoots [19,48], scion shoots [42,45,54], epicormic shoots [74] and from young, vigorously growing shoots on mature trees [30,95,124]. Ikemori [74] artificially induced epicormic shoots on severed branches of *E. grandis* (30 to 44 mm in diameter and 700 mm long) by

Table 1. Summary of *in vitro* media and techniques applied to *Eucalyptus* species. Stage (Column 2) indicates the successive steps in the experimental procedure described for a species in one paper. Explant (Column 2) describes the starting material for each stage. The morphogenetic response (Column 3) obtained for each experimental stage is listed in Table 1.1. The results (Column 4) describe the effects recorded at each stage. Column 5 lists a reference for the medium used and the superscript numbers refer to notes in Table 1.2 giving additional information about specific media. Column 6 indicates the pH of the medium and its content of sucrose and agar (the letters S, L, A and LA indicate solid medium, liquid medium, agar and liquid agar, respectively. Column 7 summarizes the combinations of growth substances that gave the most favorable results at each stage. The figures in brackets indicate the concentrations used in mg l^{-1}. Coconut milk and other undefined supplements are given in Table 1.3. References are listed (last column) by number as identified in the reference list.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
Eucalyptus sp.	Hypocotyl	Gen	Co-cultivation with *Agrobacterium tumefaciens* for plasmid transfer				[18]
Eucalyptus sp.	1 Shoots	ST-P	Shoot multiplication				[76]
	2 Shoots(1)	Pp-Co	Protoplasts co-cultured with protoplasts of *Hibiscus cannabinus*	[50] Med-B5 (modified)			
	3 Cell colonies(2)	S	Shoot cultures				
	4 Shoots(3)		Shoots rooted, plants				
E. alba	1 Hypocotyls	C	Callus culture	[137]	–/–/12	(1)2,4-D (15%)CM	[82]
	2 Callus(1)	C-ISR	Callus, shoots and roots	[137]	–/–/12	(1)IAA (1)2,4-D (15%)CM	
E. alba	1 Nodes	S-DR	Shoot development, roots	[105]		(1)IBA	[53]
E. alba	1 Coppice shoots or rejuvenated scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. bancroftii	1 Seeds	G	Germination	[27] Med-B	5.5/2.05/8	None	[21]
	2 Shoot tips(1)	DRS	Shoots rooted, plants	[27] Med-B	5.5/2.05/L	None	
E. bancroftii	1 Stem internodes (5 mm)	C	Callus culture	[27] Med-B	5.5/30.8/8	(0.88)IAA (0.43)Kin	[27]
E. bancroftii	1 Mature lignotuber	C	Callus culture	[88] Med-AB[1]	5.5/20.5/8	(4.42)2,4-D (0.45)BA	[88]
	2 Stem tissue from 5-week-old seedlings	C	Callus culture	[88] Med-AB[1]	5.5/20.5/8	(4.04)NOA (3.6)BA	[35]

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. botyroides	1 Hypocotyls, cotyledons or seedling leaves	DE	Embryo-like structures	[102] salts (mod.) RV vit. + amino acids		(1.1)2,4-D	[120]
	2 Embryo-like structures(1)	DE/DS	Embryo-like structures and adventitious shoots	[102] salts (mod.) RV vit. + amino acids		(1.1)BA	
E. bridgesiana	1 Nodes from 5-year-old tree	ST-P/C	Axillary shoots, semi-differentiated callus	[22]	5.8/20/8	(1)IBA	[43]
E. calophylla	1 *In vitro* seedling tissues		Shoot multiplication				[95]
	2 Shoots(1)	DR	Shoots rooted, plants				[11]
	3 *In vitro* seedling tissues	C	Callus culture				
	4 Stamens	C	Callus culture				
E. camaldulensis	1 Seedling root tips	O-R	Root culture	[14]	4.7/40/L	(14%)CM	[7]
E. camaldulensis	Cambial tissue	C	Callus culture				[77]
E. camaldulensis		C	Callus culture				[78]
E. camaldulensis	1 Hypocotyls	C	Callus culture	[137]	–/–/8	(1)2,4-D (15%)CM	[130]
	2 Friable callus(1)	Su	Suspension culture	[137]	–/–/LA	(1)2,4-D (15%)CM	
	3 Single cells(2)	Su	Nodular aggregates	[137]	–/–/LA	(1)2,4-D (15%)CM	
	4 Aggregates(3)	C	Friable callus	[137]	–/–/8	(1)2,4-D (15%)CM	
E. camaldulensis		C	Callus culture				[44], [116]
E. camaldulensis	1 Nodes	S-DR	Shoot development, roots	[102] Med-MX1, or [104]	6.0/20/10	(1)IBA	[53]
E. camaldulensis	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	
E. camaldulensis	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	

Table 1, Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. camaldulensis	1 Node (mature tree)	ST	Shoot development	[102][6]	–/20/8	(0.2)Kin (0.5)BA	[64]
	2 Shoots(1)	ST-P	Shoot proliferation	[102][2]	–/20/8	(0.2)Kin (0.5)BA	
	3 Shoots(2)	S	Shoot elongation	[102][2]	–/20/LA	(0.1)BA (0.05)Kin	
	4 Shoots (45mm)(3)	DR-Pre	72 h in dark	0.5 x [102]	–/20/L	(10)each IBA,IAA,NAA and 2iP	
	5 Shoots(4)	DR	Rooted plantlets	0.5 x [102][3]	–/20/L	None	
E. . camaldulensis	1 Seeds	G	Germination	Agar	–/–/8	None	[37]
	2 Hypocotyls or cotyledons(1)	C-IS	Callus initiation	[102] salts, vit. of [107]	5.5/20/8	(1)NAA (0.5–0.7)BA	
	3 Shoots(2)	DR	Shoots rooted	[102] salts, vit. of [107]	5.5/20/8	(0.1or 1)NAA	
E. camaldulensis	1 Leaves from *in vitro* shoots	C	Callus initiation	[130] salts (mod.), [102] vit., glyc.[4]	5.6/50/4	(3)NAA (0.1)BA (1000)CH [dark 10 days)	[101]
	2 Callus(1)	C-IS	Shoot regeneration	[130] salts (mod.), [102] vit., glyc.[4]	5.6/50/4	(0.5)BA (10%)CM [light]	
	3 Shoots(2)	DR-Pre	Pre-treatment	0.5 x [102]	5.6/20/L	(4)NAA [dark 42 h]	
	3 Shoots	DR	Shoots rooted	[102]	5.6/20/L	None [light]	
E. camaldulensis	1 Coppice shoots or rejuvenated scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. citriodora	1 Seedling root tips	C	Callus growth	[102] salts + (?) [88] Med-Alt[5]	–/20/4	(10)NOA (15%)CM	[4]
	2 Stem tissue	C	Callus initiation	[102] salts + (?) [88] Med Alt[5]	–/20/4	(10)NOA (15%)CM	
	3 Callus(2)	C	Callus growth	[102] salts + (?) [88] Med-Alt[5]	–/20/4	(1)NOA (15%)CM	
	4 Lignotuber tissue	C-ISR	Callus, shoots, roots	[102] salts + (?) [88] Med-Alt[5]	–/20/4	(1)NOA (15%)CM	
E. citriodora	1 *In vitro* seedling tissues	C	Callus culture	[105]	6/20/7	(2)IAA (1)Kin (1000)CH (1000)YE	[123]
E. citriodora	1 Shoot tips of 5-year-old trees	ST-P	Shoot multiplication	[102][6]	5.8/20/8	(0.5–2)BA	[87]
	2 Shoots(1)	DR	Shoots rooted	[102][6]	5.8/20/8	None	
E. citriodora	1 Hypocotyl or cotyledon	C	Callus initiation	[84]	6.0/20/10	(1.0–5)NAA or 2,4-D	[84]
	2 Cotyledon callus(1)	C-IS	Shoot regeneration	[84]	6.0/20/10	(0.2)IAA (1)Z	
	3 Shoots(2)	DR	Rooted plantlets	[84]	6.0/20/10	(1)IAA or IBA	
E.citriodora	1 Apical shoot tips	ST-P	Axillary shoot proliferation	[102][7]	–/–/A	(1.75)NAA (1.13)BA	[60]

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. citriodora	1 Seedling apex	ST-P	Shoot proliferation	[102][2]	5.8/20/A	(0.2)Kin (0.3)BA	[65]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	[102][2]	5.8/20/A	(0.2)Kin (0.3)BA	
	3 Shoots(1)(2)	DR	Shoots rooted, plants	[139][5]	5.8/20/L	(1)NAA [48 h., then none]	
	4 Vegetative buds from 20-y-old trees	ST-P	Axillary bud proliferation	[102][2]	5.8/20/L	(0.2)Kin (0.3)BA	[63]
	5 Shoots(4)	DR	Shoots rooted	[139][5]	5.8/20/L	(2)NAA [48h, then none]	
	6 Plantlets(5)	S	Root development	[102][2]	5.8/20/L	None	
E. citriodora	1 Terminal buds from 10- to 20-y-old trees	ST-Pre	15 °C for 72 h	[102][2]	5.8/20/A	(0.2)Kin (0.3)BA	[94]
	2 Explants(1)	ST-P	Axillary shoot proliferation	[102][2]	5.8/20/LA	(0.2)Kin (0.3)BA	
	3 Explants(2)	S	Shoot elongation	[102][2]	5.8/20/8	(0.2)Kin (0.3)BA	
	4 Shoots(3)	DR-Pre	48 h pre-treatment	[139][5]	5.6/20/L	(2)NAA	
	5 Shoots(4)	DR	Shoots rooted	0.5 × [102][3]	5.8/20/8	None	
	6 Shoots(5)	S	Root elongation	0.5 × [102]	5.8/20/L	None	
E. citriodora	1 Leaf and stem tissues from seedling and mature trees	C	Callus culture	[102][2]	5.8/20/–	(2)NAA (0.3)BA (0.2)Kin	[61],[62]
	2 Callus(1)	C-IS	Shoot regeneration	[102][2]	5.8/20/–	(0.3)BA (0.2)Kin	
	3 Shoots(2)	DR	Shoots rooted, plants	[139][5]	5.8/20/L	(2)NAA [48 h, then none]	
	4 Plants(3)	S	Root and shoot elongation	[102][2]	5.8/20/L	None	
E. citriodora	1 *In vitro* seedling tissues		Shoot multiplication				[95]
	2 Shoots(1)	DR	Shoots rooted, plants				
	3 Stamens	C	Callus culture				
E. citriodora	1 Embryos	DE	Initiation of somatic embryogenesis	[50] Med-B5	5.6/50/0.4	(3)NAA [dark]	[101]
	2 Embryoids(1)	DE	Somatic embryogenesis	[130] Salts (mod.), [102]vit., glyc.[4]	5.6/50/0.4	(0.5)BA (10%)CM [light]	
	3 Embryos(2)	E-Pl	Development of plants	0.5 × [102]	5.6/20/L	None	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH/Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. citriodora	1 Decoated seeds	G-DE	Germination, primary somatic embryogenesis on cotyledons	[50] Med-B5	–/50/1.5G	(3)NAA [dark]	[100]
	2 Embryos(1)	DE	Clusters of embryos	[50] Med-B5 [five subcultures]	–/50/1.5G	(3)NAA [dark]	
	3 Embryos(2)	DE	Embryogenic mass	[50] Med-B5[9]	–/30/1.5G	(5)NAA (500)CH [dark]	
	4 Embryos(3)	E-Pl	Plant development	[50] Med-B5	–/20/1.5G	None [light]	
E. citriodora	Cotyledons	Pp	Protoplast culture	K8P [liq.-on-agar or imbedded in agar]	–/–/A		[36]
E. citriodora	1 Coppice shoots or rejuvenated scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 × [102] or [63]	–/–/S	IAA or IBA	
E. cladocalyx		C	Callus culture				[78]
E. cladocalyx	1 Cambial tissue	C	Callus culture				[77],[53]
	2 Cotyledon callus(1)	C-IS	Shoot regeneration	[84]	6.0/20/10	(0.2)IAA (1)Z	
	3 Shoots(2)	DR	Rooted plantlets	[84]	6.0/20/10	(1)IAA or IBA	
E. coccifera	1 Juvenile leaf	C	Callus induction	[90] salts	–/30/15	(1)2,4-D (0.4)Kin (4000)ME (7days)	[75]
	2 Callus(1)	C	Optimal growth	[90] salts[10]	–/30/15	(0.22)2,4-D (2.15)Kin	
E. coccifera	1 Partially rejuvenated nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts, vit. of [29] med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	[15]
	2 Shoots(1)	DR	Root initiation	[83], vit. of [29] Med-M[11]	5.5/15/7	(1.5 or 3)IBA [dark 10 to 20 days]	
	3 Rooted shoots(2)	S	Root and shoot elongation	[83], vit. of [29] Med-M[12]	5.5/30/7	None	
E. curtisii	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH/Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. curtisii	1 Nodes from seedlings or coppice	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)IBA	
E. dalrympleana	1 Nodes from 5-year-old tree	DR	Rooted plantlet	[22]	5.8/20/8	(1)IBA	[43]
	2 Leaf from adult tree	DR	Root formation	[22]	5.8/20/8	(1)IBA	
E. dalrympleana	1 Seed	G	Germination	[22]	5.8/20/8	None	[41]
	2 Shoot tips, nodes (1) Shoot tips, nodes from 4-year-old trees	DRS	Shoot and root formation	[22]	5.8/10/8	(0.25)BA	
E. dalrympleana	1 Scion nodes (rejuvenated)	S	Shoot development	[102][13]	–/–/A	(0.01)NAA (1)IBA [dark 8 days]	[42, [45]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	[102] salts, vit. of [29] Med-M[11]	–/–/A	(0.01)NAA (0.1)BA	
	3 Shoots(2)	S	Shoot elongation	[102] salts, vit. of [29] Med-M[12]	–/–/A	(0.01)NAA (0.1)BA (1)GA_3	
	4 Shoots(3)	DR	Shoots rooted	[83]	–/–/A	(1)IBA [dark 7 days]	
E. dalrympleana	1 Scion nodes (rejuvenated)	SN-P	Axillary shoot proliferation	0.5 x [102] salts, vit of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	[15]
	2 Shoots(1)	DR	Root initiation	[83], vit. of [29] Med-M[11]	5.5/15/7	(1.5 or 3)IBA [dark 10 to 20 days]	
	3 Rooted shoots(2)	S	Root and shoot elongation	[83], vit of [29] Med-M[12]	5.5/30/7	None	
E. dalrympleana	1 Anthers	A-C	Callus culture	[136][14]	5.5/30/7	(2.21)2,4-D (1.13)BA	[17]
	2 Callus	C-IR	Root development	[71][15]	5.5/30/7	(0.11–1.1)2,4-D or (0.11–1.1)2,4-D (0.11–1.1)Kin, or (0.19)NAA (2.15)Kin or (0.04)NAA (1.1)BA	
E. deglupta	1 Seeds	G	Germination	[27]	5.5/2.05/8	None	[21]
	2 Shoot tips, nodes(1)	DRS	Shoots rooted, plants	[27]	5.5/2.05/8	None	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. delagatensis	1 *Partially rejuv. nodes*	SN-P	*Axillary shoot proliferation*	0.5 x [102] salts, vit. of [29] Med-M[13]	5.5/30/7	(0.03)NAA (0.2)BA	[15]
	2 Shoots(1)	DR	Root initiation	[83], vit. of [29] Med-M[11]	5.5/15/7	(1.5)or 3)IBA [dark 10 to 20 days]	
	3 Rooted shoots(2)	S	Root and shoot elongation	[83], vit. of [29] Med-M[12]	5.5/30/7	None	
E. diversicolor	1 *In vitro* seedling tissues	C	Callus initiation				[95]
	2 Callus(1)	C-IS	Shoot regeneration				
	3 Shoots(2)	ST-P	Shoot multiplication				
	4 Shoots(3)	DR	Shoots rooted, plants				
	5 Nodes from 10-year-old trees	SN-P	Shoot multiplication				
	6 Shoots(5)	DR	Shoots rooted				
	7 Stamens	C	Callus culture				
E. dunnii		C	Callus culture				[55]
E. dunnii	1 Hypocotyls, cotyledons or seedling leaves	DE	Embryo-like structures	[102] salts (mod.), RV vit. + amino acids		(1.1)2,4-D	[120]
	2 Embryo-like structures(1)	DE DS	Embryo Like structures and adventitious shoots	[102] salts (mod.), RV vit. + amino acids		(1.1)BA	
E. ficifolia	1 Seeds	G	Germination	[28] Med UNE-B	5.5/2.05/8	None	[32], [33]
	2 Nodes(1)	SN-P	Axillary shoot proliferation	[34] Med-MHMH	5.5/41.1/8	(1.75)IAA (2.03)IBA (1.86)NAA (2.03)NOA (2.21)2,4-D (1.86)p-CPA (0.22)Kin (0.23)BA	
	2 Nodes			[33]	5.5/20.5 or 41.1/8	(2.03)IBA (0.22)Kin (0.23)BA	
	3 Nodes from mature trees	SN-P	Axillary shoot proliferation	[34] Med-MHMH	5.5/41.1/8	(1.75)IAA (2.03)IBA (1.86)NAA (2.02)NOA (2.21)2,4-D (1.86)p-CPA (0.22)Kin (0.23)BA	
	3 Nodes from mature trees			[33]	5.5/20.5 or 41.1/8	(2.03)IBA (0.22)Kin (0.23)BA	
	4 Shoots(2)(3)	DR	Shoots rooted	[33]	5.5/20.5 or 41.1/8	(2.03)IBA	
E. ficifolia	1 Seeds	G	Germination	[28] Med-UNE-B	5.5/2.05/8	None	[30]
	2 *In vitro* seedling node	SN-P	Multiple buds & shoot growth	[34] Med-MHMH	5.5/41.1/8	(1.75)IAA (2.03)IBA (1.86)NAA (2.02)NOA (2.21)2,4-D (1.86)p-CPA (0.22)Kin (0.23)BA	
	3 Node(1)	SN-P/C-IS	Vigorous organogenic callus	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)IBA (0.22)Kin (0.23)BA	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. ficifolia cont'd.	4 Nodes(1) or (2)	DR	Shoot growth and roots	[34] Med-MHZH[17]	5.5/20.5 or 41.1/8	(2.03)BA	[30]
	4 Nodes			[22]	5.5/20.5/8	(1)IBA	
	5 Adult node	SN-P	Shoot growth, multiple buds	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)BA (0.22)Kin (0.23)BA	
	6 Nodes (4)	SN-P/C	No shoots from callus	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)BA (0.22)Kin (0.23)BA	
	7 Nodes (5)	DR	Shoots root poorly	[34] Med-MHZH[17]	5.5/41.1/8	(2.03)BA	
	7 Nodes	DR	Shoots root but degenerate	[22]	5.5/20.5/8	(1)IBA	
E. ficifolia	7 Nodes	DR	Shoots rooted, plants	1:9 combination[18]	5.5/20.5/8	(1)IBA	
E. ficifolia	1 Adult or seedling nodes	SN-P	Multiple buds	[34] Med-MHMH	5.5/41.1/8	(1.75)IAA (2.03)IBA (1.86)NAA (2.02)NOA (2.2)2,4-D (1.86)p-CPA (0.22)Kin (0.23)BA	[10]
	2 Buds(1)	ST-P	Axillary shoot proliferation	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)BA (0.22)Kin (0.23)BA	
	3 Nodes(2)	DR	Rooted plantlets	[10] Med-B	5.5/20.5/8	(1)IBA	
E. ficifolia	1 *In vitro* seedling shoots	DR	Shoots rooted, plants	[29] Med-R[19]	5.5/41.1/9	(1.02)IBA	[31]
	2 Shoots from adult nodal cultures	ST-P	Axillary shoot proliferation	[34] Med-MHMH[20]	5.5/20.5/9	(1.02)IBA (0.05)BA	
	3 Shoots(2)	DR	Shoots rooted	[29] Med-R[19]	5.5/41.1/9	(1.02)IBA (0.005)BA	
E. ficifolia	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	
E. ficifolia	1 Adult or seedling nodes	SN-P	Multiple buds	[34] Med-MHMH[20]	5.5/41.1/8	(1.02)IBA (0.45)BA	[29]
	2 Nodes ex subcultures(1)	DR	Only plants from seedlings survived	[27], [29] Med-R	5.5/20.5/8	(1.02) IBA	
E. ficifolia	1 Nodes from seedlings or coppice	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	
E. globulus	1 Seedling tissues	C	Callus culture	[102][21]	6.0/30/10	(1)2,4-D	[80]
	2 Callus	Su	Suspension culture (poor growth)	[102][21]	6.0/30/LA	(0.1)2,4-D	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. globulus	1 Terminal buds from 10- to 20-year-old trees	ST-Pre	15 C for 72 h	[102][2]	5.8/20/8	(0.2)Kin (0.5)BA	[62], [94]
	2 Explants(1)	ST-P	Axillary shoot proliferation	[102][2]	5.8/20/LA	(0.2)Kin (0.5)BA	
	3 Explants(2)	S	Shoot elongation	[102][2]	5.8/20/LA	(0.05)Kin (0.1)BA	
	4 Shoots(3)	DR-Pre	72 h pre-treatment	[139][9]	5.6/20/L	(2)IAA (2)NAA	
	5 Shoots(4)	DR	Root growth	0.5 x [102][3]	5.8/20/8	None	
	6 Shoots(5)	DR	Root elongation	0.5 x [102]	5.8/20/L	None	
E. globulus	1 Seeds	G	Germination	Water-agar	–/20/6	None	[108]
	2 Hypocotyls(1)	C	Nodular callus	[128] Mod-1	5.6/20/8	(3.72)NAA (11.26)BA	
	3 Nodular callus(2)	C-ISR	Shoots and roots	[128] Mod-1	5.6/20/8	None	
E. globulus	1 Nodes from 3-year-old trees	SN-P	Initiation of axillary shoot proliferation	[102] salts, [138] vit. + supplements[22]	5.7/20/7	(0.01)NAA (1)BA	[126]
	2 Shoots (1)	ST-P	Axillary shoot proliferation	[102] salts, [138] vit. + supplements[22]	5.7/20/7	(0.01)NAA (1)BA	
	3 Shoots(2)	DR-Pre	Pre-treatment	Aqueous	–/–/–	(1)IBA [basal dip 1 min]	
	3 Shoots	DR	Shoots rooted	[102] salts, [138] vit. + supplements[22]	5.7/20/7	None	
E. globulus	1 Explants from 14-y-old trees	ST/N-P	Shoot multiplication		–/–/–		[134]
	2 Shoots(1)	DR	Shoots rooted, plants		–/–/–	IBA	
E. globulus subsp. *bicostata*	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	
E. globulus subsp. *bicostata*	1 Nodes from seedlings or coppice	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	
E. globulus subsp. *globulus*	1 Embryos	DS	Adventitious shoots	[102]	–/–/–	2iP IAA Z	[142]
	2 Shoots(1)	S	Shoot development	[102]	–/–/–	None	
	3 Shoots(2)	DR	Shoots rooted		–/–/–	IBA	
	4 Seedlings, embryos or shoots	S-Gen	Plasmid transfer by *Agrobacterium tumefaciens*	[102]	–/–/–		

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. gomphocephala		C	Callus culture		–/–/–		[78]
E. gomphocephala	1 Cambial tissue	C	Callus culture		–/–/–		[77], [53]
E. grandis	1 Stem internode	C	Callus initiation and growth	[27] Med-A	5.5/30.8/8	(3.5)IAA (0.43)Kin	[27]
	2 Callus(1)	C-R	Roots formed, no shoots	[27] Med-A	5.5/30.8/8	(4.42)2,4-D (0.43)Kin	
E. grandis	1 Seeds	G	Germination	[27] Med-B	5.5/2.05/8	None	[21]
	2 Shoot tips, nodes(1)	DRS	Shoots rooted, plants	[27] Med-B	5.5/2.05/8	None	
E. grandis	1 *In vitro* seedling roots	O-R	Root cultures	[21] Med-F salts	–/20.5/L	None [dark]	[21]
E. grandis	1 Seeds	G	Germination	[27] Med-B	5.5/2.05/8	None	[35]
	2 Nodes(1)	DSR	Axillary shoots, roots	[27] Med-B	5.5/2.05/8	None	
	3 Nodes from 4-m-old trees	DR-Pre	Root initiation pre-treatment	[35] Med-LLP	5.5/20.5/10	(0.5)NAA [dark 15 days]	
	4 Rooted shoots	S	Root and shoot elongation	[35] Med-LLP	5.5/20.5/10	None	
	5 Nodes from 7-m-old trees	DR-Pre	Root initiation pre-treatment	[35] Med-LLP	5.5/20.5/10	(1)IBA [dark 28 days]	
	6 Rooted shoots	S	Root and shoot elongation	[35] Med-LLP	5.5/20.5/10	None	
E. grandis	1 Nodes	Pre	2 h soak in dist. water	Aqueous	–/–/–		[22]
	2 Nodes above node 14(1)	S/C-IR	Plant with roots via basal callus	[22]	5.8/20.5/8	(0.2)IBA	
E. grandis	1 Nodes	S-DR	Shoot development, roots	[103] Med-MX1, or [105]	6.0/20/10	(1)IBA	[53]
E. grandis	1 Leaves	C	Callus culture	[105]	–/20–40/–	Various combinations of 2,4-D and Kin	
	2 Callus(1)	C-R	Root development	[105]	–/–/–	(1)IBA	
E. grandis	1 Anthers	A-C	Callus culture	[139][23]	–/20/–	(1)2,4-D (10%)CM	[53]
E. grandis	1 Nodes of 1- to 2-y-old trees	DR	Shoot and root development, plants	[22]	5.8/20.5/8	(0.18)IAA [dark 7 days]	[22]
E. grandis	1 Stem, leaf & petiole tissues	C	Callus culture	[137]	–/–/12	(1)2,4-D (15%)CM	[82]

Table 1. *Continued.*

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. grandis	1 Seeds	G	Germination	[28] Med UNE-B	5.5/2.05/8	None	[10]
	2 Coppice or seedling nodes	SN-P	Good shoot growth	[34] Med-MHMH[16]	5.5/41.1/8	(2.68)IBA (0.22)Kin (0.23)BA	
	3 Nodes(2)	DR	Shoots root poorly	[34] Med-MHZH[17]	5.5/41.1/8	(2.68)IBA	
	3 Nodes	DR	Shoots root but degenerate	[22]	5.5/20.5/8	(1)IBA	
	3 Nodes	DR	Shoots rooted, plants	1/9 combination[18]	5.5/20.5/8	(1)IBA	
E. grandis	1 Nodes from 4-year-old tree	DR	Rooted plantlet	[22]	5.8/20/8	(1)IBA	[43]
E. grandis	1 Nodes from seedlings, coppice and young trees	DR	Rooted plantlet	[1] Med-B	5.5/20.5/8	(1.02)IBA	[10]
E. grandis	1 *In vitro* seedlings nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	
E. grandis	1 Nodes from young trees	SN-P	Shoot multiplication		–/–/–		[72]
	2 Shoots(1)	DR	Shoots rooted		–/–/–		
E. grandis	1 Nodes of seedlings or coppice	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.54)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	
E. grandis	1 Nodes of seedlings or coppice	SN-P	Shoot development	0.5 x [102] salts, vit. of [29] med-M[11]	5.5/30/7	(0.01)NAA (0.1)BA [dark 7 days]	[48]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	0.5 x [102] salts, vit. of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	
	3 Shoots(3)	S	Shoot elongation	0.5 x [102] salts, vit of [29] Med-M[12]	5.5/30/7	(0.01)NAA (0.1)BA (0.1)GA_3	
	4 Shoots(3)	DR	Shoots rooted	[83] vit. of [29] Med-M[11]	5.5/30/7	(1)IBA	
E. grandis	1 Nodes from 5-year-old trees	SN-P	Axillary shoot proliferation	[102][24]	–/20/8	(1)NAA (1)BA	[85]
	2 Shoots(1)	S	Shoot elongation	[102][24]	–/20/8	(1)GA_3	
	3 Shoots(2)	DR	Shoots rooted	0.5 x [102][24]	–/20/8	(1–5)IBA or (1–5)NAA	
	3 Shoots(2)	DR	Shoots rooted	[139]	–/–/–	(0.5)IAA	
E. grandis	1 Nodes from juvenile and mature trees	SN-P	Axillary shoot proliferation	[102][24]	–/20/A	(9.3)NAA (22.5–45)BA	[125]
	2 Shoots(1)	S	Shoot elongation	[102] salts	–/20/A	(10.39GA_3 [7 days]	
	3 Shoots(2)	DR	Root induction—Stage 1	[139]	–/20/A	(0.35)IAA (9.3)NAA (4.1)IBA [4 days]	
	4 Shoots(3)	DR	Root induction—Stage 2	0.5 x [102] salts[3]	–/20/A	None [14 days]	
	5 Shoots(4)	DR	Shoots rooted	0.5 x [102] salts	–/20/L	None [21 days]	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH/Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. grandis	1 Nodes from 4-year-old trees	SN-P	Axillary shoot proliferation	[102][25]	–/20/–	(0.1–1)NAA (0.5–1)BA	[86]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] or [139]	–/–/–	(5)IBA or (0.5)NAA (0.5)IAA	
	3 Shoots(1)	C	Callus initiation	[102][25]	–/–/–	(5)2,4-D	
	4 Callus(3)	C-IE	Embryogenesis	[102][25]	–/–/–	(0.1)NAA (5)Kin	
	5 Callus(3)	C-IE	Embryogenesis	[102]	–/–/L	(1)NAA (1)2,4-D (1)Kin (1)BA	
E. grandis	1 Induced epicormic shoots	ST/N-P	Shoot multiplication	[121](mod.)[26]	–/–/A		[74]
	2 Shoots(1)	DR	Shoots rooted	[83][27]	–/–/A	(1)IBA	
E. grandis	Seedling tissues	Su	Suspension culture		–/–/–		[38]
E. grandis	Epicormic shoots	ST/N-P	Shoot multiplication	[102]	–/–/–	(1.8)2iP (0.2)BA	[131]
E. grandis	1 Coppice shoots, rejuv. scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. grandis	1 Hypocotyls, cotyledons, seedling leaves, young leaves from *in vitro* shoots from adult trees	DE	Embryo-like structures	[102] salts (mod.), RV vit. + amino acids	–/–/–	(1.1)2,4-D	[120]
	2 Embryo-like structures(1)	DE/DS	Embryo-like structures and adventitious shoots	[102] salts (mod.), RV vit. + amino acids	–/–/–	(1.1)BA	
E. grandis hybrids	1 Hypocotyls or internodes from young adult shoots	C	Friable callus	[112]	–/–/–	(2.21)2,4-D (0.37)NAA [dark]	[112]
	2 Callus(1)	Su	Suspension culture		–/–/L	(0.44)2,4-D (0.37)NAA (0.0002)Kin	
	3 Callus(1), suspension cells(2) or young leaves from *in vitro* shoots	Pp	Protoplast culture		–/–/–		[113]
E. gunnii		C	Callus culture		–/–/–		[78]
E. gunnii	Cambial tissue	C	Callus culture		–/–/–		[77]
E. gunnii	1 Seeds	G	Germination	[22]	5.8/20/8	None	[41]
	2 Shoot tips, nodes(1) Shoot tips, nodes from 4-m-old trees	DRS	Shoot and root formation	[22]	5.8/10/8	None	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH/Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. gunnii	1 Scion nodes (rejuvenated)	SN-P	Shoot development	[102][3]	–/–/A	(0.01)NAA (1)BA [dark 8 days]	[42], [45]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	[102] salts, vit. of [29] Med-M[11]	–/–/A	(0.01)NAA (0.1)BA	
	3 Shoots(2)	S	Shoot elongation	[102] salts, vit of [29] Med-M[12]	–/–/A	(0.01)NAA (0.1)BA (1)GA_3	
	4 Shoots(3)	DR	Shoots rooted	[83]	–/–/A	(1)IBA [dark 7 days]	
E. gunnii	1 Partially rejuvenated nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts, vit. of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	[15]
	2 Shoots(1)	DR	Root initiation	[83], vit. of [29] Med-M[11]	5.5/15/7	(1.5–3)IBA [dark 10 to 20 days]	
	3 Rooted shoots(2)	S	Root and shoot elongation	[83], vit. of [29] Med-M[12]	5.5/30/7	None	
E. gunnii	1 Partially rejuvenated scion shoots	SN-P	Initiation of axillary shoot proliferation	[36] Med 1	–/30/7	(0.01)NAA (1)BA	[36]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	[36] Med 2	–/30/7	(0.01)NAA (0.1)BA	
	3 Shoots(2)	S	Shoot elongation	[36] Med 2[28]	–/30/7	(0.01)NAA (0.1)BA (1)GA_3	
	4 Shoots(3)	DR	Shoots rooted	[36] Med 3 (modified)	–/30/7	(1)IBA [dark 10 days]	
E. gunnii	1 Seeds	G	Germination	[102]	–/–/–	None	[1]
	2 Roots(1)	O-R	Root culture	[102]	–/–/A	None [dark 3 to 4 subcultures]	
	2 Roots			[102]	–/–/L	None [16 hour photoperiod]	
E. gunnii	1 Seeds	G	Germination	[102][29]	5.5/30/7	None	[17]
	2 Seedling leaves(1)	C-IR	Callus, root initiation & some undeveloped embryonic structures	[102][30]	5.5/30/7	Various combinations	
	3 Hypocotyls and internodes(1)	C-IE	Primary embryogenic callus	[102][30] or [107][31]	5.5/30/7 5.5/30/7	(1,9)NAA (0.11–1.1)BA (1,9)NAA (0.11–1.1)BA (+ other combinations)	
	4 Embryogenic callus(3)	C-IE	Secondary embryogenic callus	[102][30] or [107][31]	5.5/30/7 5.5/30/7	Various combinations Various combinations	
E. gunnii	1 *In vitro* seedling hypocotyls and leaves	DS	Adventitious buds	[102]	–/–/–	(1.1)BA	[46]
	2 *In vitro* seedlings tissues	C	Callus cultures	[102]	–/–/–		
	3 White hypocotyl callus(2)	C-IE	Somatic embryogenesis	[102][5]	–/–/–	(3,7)NAA (1.1)BA	
	3 White leaf callus	C-IE	Somatic embryogenesis	[102]	–/–/–	(9.3)NAA (1.1)BA	
	3 Callus(2)	C	Microcalli	[102]	–/–/L	(3.7)NAA (1.1)BA	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. gunnii, cont'd.	4 *In vitro* seedling internodes	C	Callus initiation	[107]	–/–/–	(0.93–9.3)NAA (1.08)Kin	
	5 Callus(4)	C-IE	Somatic embryogenesis	[107]	–/–/–	(0.11–0.44)2,4-D with or without (1.08)Kin	
				[107][3]	–/–/–	None	
E. gunnii	Callus or suspension cells	Pp-Gen	Protoplast culture: plasmid transfer		–/–/–		[133]
E. gunnii	1 Seedling nodes	SN-P	Shoot multiplication	[25] Med-A	5.8/41/8	(0.2)BA	[23], [24], [25]
	2 Shoots(1)	DR-Pre	Rooting pre-treatment	0.5 x [102] salts	5.8/20/8	(0.1)NAA (0.5)Kin or (0.5)Z	
	3 Shoots(2)	DR	Shoots rooted, pants	[25] Med-O	5.8/20/8	(0.5)IBA	
E. gunnii	1 Leaves	C	Callus initiation	[102]	–/–/7.5	(2)2,4-D [dark]	[132]
E. gunnii x *E. dalrympleana*	2 Friable callus(1)	Su	Suspension culture	[102], or [137]	–/–/LA	(3)2,4-D (0.1)BA [2 mths], then (1.5)2,4-D (0.1)BA	
					–/–/LA	(3)2,4-D (10%)CM [2 mths], then (1.5)2,4-D (0.1)BA	
	3 Cells(2)	Pp	Protoplast cultures	[132]	6.5/–/–		
	4 Cells(3)	Pp-C	Callus regeneration		–/–/7.5		
E. henryi	1 Coppice shoots or rejuvenated scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. lasvopinea	1 Stem internodes (5mm)	C	Callus culture	[27] Med A	5.5/30/8	(1.75)IAA (0.22)Kin	[27]
E. x leichow	1 *In vitro* seedlings	C	Callus		–/–/–		[109], [110]
	2 Callus	C-IE	Embryogenic callus		–/–/–		
	3 Embryoids	E-Pl	Plants		–/–/–		
E. macarthurii	1 Crown leaf from 5-y-old tree	DR	Root formation	[22]	5.8/20/8	(1)IBA	[43]
E. macrorhyncha	1 Shoots, nodes from 8- to 18-m-old trees	C	Callus culture	[102]	–/–/–	IAA IBA NAA 2,4-D BA (unspecified)	[2]
E. maculata		C	Callus culture		–/–/–		[55]

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. maculata	1 Coppice shoots	ST-P		[102][32]	–/20/8		[97]
	2 Shoots(1)	DR	Shoots rooted			(20)IBA	
E. marginata	1 Shoot tips from mature tree	ST-P	Shoot multiplication	[102][33]	–/20.5/10	(0.46)IAA (0.56)BA	[95]
	2 Shoots(1)	DR	Shoots	[95] Med-Rt	–/20.5/8	(2.0)IBA	
	3 Stamens from mature tree	C	Filament callus	[95]	–/31/8	(1.9)NAA (2.2)Kin [dark]	
	4 Callus	C-ISR	Shoots rooted	[95]	–/31/8	(0.09)IAA (2.2)Z	
	5 Shoots(4)	DR	Shoots rooted	[102] Salts (mod.), [50] Med-B5 vit. and inositol[34]	–/20.5/8	(2.03)IBA	
E. marginata	1 Cotyledon petiole, stamen filaments	C	Callus induction	[102] Salts + [50] Med-B5 vit. and inositol	5.7/30.8/10	(2.2)Kin (1.9)NAA [dark]	[11]
	2 Callus(1)	C-IS	Shoot induction	[11]	6.0/20.5/10	(0.09)IAA (2.2)Z	
	3 Shoots(2)	C-IS	Shoot proliferation	[87]	5.8/20.5/10	(0.46)NAA (0.56)BA	
	4 Nodes from mature tree	SN-P	Axillary shoot proliferation	[87]	5.8/20.5/10	(0.46)NAA (0.56)BA	
	5 Shoots(3)(4)	DR	Shoots rooted	[102] Salts	5.8/20.5/10	(2.03–3.04)IBA	
E. megacarpa	1 *In vitro* seedling tissues		Shoot multiplication		–/–/–		[96]
E. melliodora	1 Stem internodes	C	Callus cultures	[27] Med-A	5.5/30.8/8	(0.88)IAA (0.43)Kin	[27]
E. nicholii	1 Stem internodes	C	Callus cultures	[27] Med-A[35]	5.5/30.8/8	(3.5)IAA (0.1)Kin	[27]
E. nicholii	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
E. nitens	1 Nodes from 5-y-old tree	S/N-P/C	Axillary shoots, semi-differentiated callus	[22]	5.8/20/8	(1.0)IBA	[43]
E. nitens	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
E. nitens	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[68]
E. nitens	1 Nodes from 2-m-old trees	SN-P	Shoot development	0.5 x [102] salts, vit. of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.1)BA [dark 7 days]	[48]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	0.5 x [102] salts, vit. of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	
	3 Shoots(3)	S	Shoot elongation	0.5 x [102] salts, vit. of [29] Med-M[12]	5.5/30/7	(0.01)NAA (0.1)BA (0.1)GA_3	
	4 Shoots(3)	DR	Shoots rooted	[83] vit. of [29] Med-M	5.5/30/7	(1)IBA	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. nova-anglica	1 Seeds	G	Germination	0.5 x [99] Med-GD	5.6/20/7	None	[98]
	2 Shoot tips, nodes(1)	SN/T-P	Adventitious buds	[99] Med-GD	5.6/20/7	(0.09)IBA (1)Z	
	3 Buds(2)	ST-P	Adventitious buds	[99] Med-GD	5.6/20/7	(0.09)IBA (1)Z	
	4 Buds(2)(3)	S	Shoot growth	0.5 x [99] Med-GD[28]	5.6/2.5/7	None	
	5 Shoots(4)	DR	Shoots rooted, plants	0.5 x [99] Med-GD	5.6/2.5/7	(1)IBA	
E. obliqua	1 Lignotubers	C	Callus		–/–/–		[140]
	2 Callus(1)	C-ISR	Callus, shoots and roots		–/–/–		
E. obtusiflora	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	
E. obtusiflora	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.92)NAA	
E. oreades	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
E. oreades	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[69]
E. pauciflora	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/4.1/8	(1)IBA (0.34)BA	[69]
E. pauciflora	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 × [102] salts	–/20/8	(0.19NAA (0.23)BA	[67], [68]
E. pauciflora	1 Partially rejuvenated nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts, vit. of [29] Med-M[11]	5.5/30/7	(0.01)NAA (0.2)BA	[15]
	2 Shoots(1)	DR	Root initiation	[83], vit. of [29] Med-M[11]	5.5/15/7	(1.5 or 3)IBA [dark 10 to 20 days]	
	3 Rooted shoots(2)	S	Root and shoot elongation	[83], vit. of [29] Med-M[12]	5.5/30/7	None	
E. polybractea	1 Adult nodes	SN-P	Good shoot growth, no roots	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)IBA (0.22)Kin (0.23)BA	[10], [30]
E. polybractea	1 Adult nodes	SN-P	Multiple shoots, no roots	[34] Med-MHMH[16]	5.5/41.1/8	(1.02)IBA (0.22)Kin (0.23)BA	[10]
E. polybractea	1 Seedling tissues	C	Callus culture	[90]	–/30/–	(0.5)2,4-D (1)Kin (15%)CM [dark]	[141]
E. regnans	1 Adult nodes	SN-P	Good shoot growth, no roots	[34] Med-MHMH[16]	5.5/41.1/8	(2.03)IBA (0.22)Kin (0.23)BA	[10], [30]
E. regnans	1 Seedling or adult nodes	SN-P	Multiple shoots, no roots	[34] Med-MHMH[16]	5.5/41.1/8	(1.02)IBA (0.22)Kin (0.23)BA	[10]
E. regnans	1 *In vitro* seedling nodes	SN-P	Axillary shoot proliferation	[69] Med-1	5.5/41.1/8	(1)IBA (0.34)BA	[69]
	2 Shoots(1)	DR	Shoots rooted	[69] Med-2	5.5/20.5/8	(1)IBA	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. regnans	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	
E. resinifera	1 Seedling shoots	ST-P			–/20/8		[97]
E. robusta	1 Nodes	S-DR	Shoot development, roots	[103] Med-MX1, or [104]	6.0/20/10	(1)IBA	[53]
E. robusta	Stem, leaf, lignotuber tissues	C	Callus culture		–/–/–		[53]
E. robusta	1 Seedling tissues	C	Callus culture	[90]	–/30/–	(0.5)2,4-D (1)Kin [dark]	[141]
	2 Callus(1)	C	Callus culture	[90][36]	–/10/–	(0.4)2,4-D (1)Kin [light]	
E. robusta	1 Seedling tissues	C	Callus culture	[102]	–/–/–	(0.5)2,4-D (1)Kin	[127]
E. rudis	1 Seedling or coppice nodes	SN-P	Axillary shoot proliferation	0.5 x [102] salts	–/20/8	(0.19)NAA (0.23)BA	[67], [68]
	2 Shoots(1)	DR	Shoots rooted	0.5 x [102] salts	–/20/8	(0.93)NAA	
E. rudis	1 Nodes from adult trees	SN-P	Axillary shoot development	[8]	5.8/30/8	(1)IBA (0.2)Kin (0.3)BA	[8], [9]
	2 Shoots(1)	SN-P	Axillary shoot proliferation	[8]	5.8/30/8	(1)IBA (0.3)BA	
	3 Shoots(2)	DR	Shoots rooted	0.5 x [8]	5.8/15/8/	None	
E. rudis	1 Hypocotyls, cotyledons seedling leaves	DE	Embryo-like structures	[102] Salts (mod.), RV vit. + amino acids		(1.1)2,4-D	[120]
	2 Embryo-like structures(1)	DE DS	Embryo-like structures and adventitious shoots	[102] Salts (mod.) RV vit. +amino acids		(1.1)BA	
E. saligna	1	C	Callus culture		–/–/–		[53]
E. saligna	Seedling tissues	Su	Suspension culture		–/–/–		[38]
Eucalyptus sp. (*E. saligna*?)	1 Shoots	ST-P	Shoot multiplication		–/–/–		[76]
	2 Shoots(1)	Pp-Co	Protoplasts co-cultured with protoplasts of *Hibiscus cannabinus*	[50] Med-B5 (mod.)	–/–/–		
	3 Cell colonies(2)	S	Shoot cultures		–/–/–		
	4 Shoots(3)		Shoots rooted, plants				

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH/Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. saligna	1 Shoots	Pp-Gen	Protoplasts: electroporated with plasmid DNA		–/–/–		[79]
	2 Protoplasts(1)	Pp-Co	Protoplasts: co-cultured with protoplasts of *Hibiscus cannabinus*	[50] Med-B5 (mod.)	–/–/–		
	3 Transformed cells(2)	S-Gen	Regeneration of transformed plants		–/–/–		
E. sideroxylon	1 Buds (7–10mm)	C	Callus induction	[102]	–/–/A	(0.05–5)NAA or (0.05–5)2,4-D	[6]
	2 Callus(1)	C-IS	Callus, adventitious shoots	[102]	–/–/A	(0.05–5)NAA, IAA or IBA (4)Kin	
	3 Shoots(2)	C-IS/ST-P	Shoot proliferation	[102]	–/–/A	(0.05–4)NAA, IAA or IBA + (1.5)Kin (1.5)BA	
	4 Buds (7–10mm)	DS	Axillary shoot proliferation	[102]	–/–/A	(0.05–4)NAA, IAA or IBA + (1.5)Kin (1.5)BA	
	5 Shoots(3)(4)	DR	Shoots rooted	0.5 x [102]	–/–/5 or 6	(2.5)IBA	
	6 Rooted shoots(5)	S	Plantlet growth	0.25 x [102]	–/–/A	None	
E. sideroxylon	1 Nodes from coppice or mature trees	DS	Axillary shoot proliferation	0.5 x [102] salts, [102] vit.	5.8/–/6	(0.09–0.19)NAA (0.45–0.9)BA	[19]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] salts, [102] vit.	5.8/–/–	(2)IBA	
E. stuartiana (= *E. ovata*)	1 Seedling nodes	SN-P	Shoot multiplication	[33] (mod.)	5.8/4.1/8	(0.2)BA	[23], [24]
	2 Shoots(1)	DR-Pre	Rooting pre-treatment	0.5 x [102] salts	5.8/20/8	Kin or Z	
	3 Shoots(2)	DR	Shoots rooted, plants	[23]	5.8/20/8	IBA	
E. tereticornis		C	Callus culture		–/–/–		[78]
E. tereticornis	1 Cambial tissue	C	Callus culture		–/–/–		[77] [53]
E. tereticornis	1 Terminal buds from 10- to 20-year-old trees	ST-P	Shoot proliferation	[102][2]	5.8/20/LA	(0.2)Kin (1)BA	[94]
	2 Explants(1)	S	Shoot elongation	[102][2]	5.8/20/8	(0.2)Kin (1)BA	
	3 Shoots(2)	DR-Pre	72 h pre-treatment	[139][3]	5.6/20/L	(2)IAA (2)IBA (2)NAA (2)IPA	
	4 Shoots(3)	DR	Root initiation	0.5 x [102][2]	5.8/20/8	None	
	5 Shoots(4)	DR	Root elongation	0.5 x [102]	5.8/20/L	None	

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. tereticornis	1 Nodes from adult trees	SN-P	Initiation of shoot proliferation	[102][25]	–/20/A	(0.2)AA (1)NAA (1)BA	[124]
	2 Shoots(1)	ST-P	Axillary shoot proliferation	[102][25]	–/20/A	(0.02)NAA (0.1–0.2)BA	
	3 Shoots(2)	DR	Shoots rooted, plants	0.5 x [102]	–/–/L	(1)NAA (1)IBA (1)IAA [dark 4 days]	
	3 Shoots			0.5 x [102][3]	–/–/–	None [14 days]	
	3 Shoots			0.5 x [102]	–/–/L	None [21 days]	
	4 Shoots(2)	DR	Shoots rooted, plants	[139]	–/–/A	(1)IBA [dark 14 days]	
	4 Shoots			[139]	–/–/A	None	
E. torelliana	1 Nodes from mature trees	S	Shoot formation	[102][2]	–/20/L	(0.2)Kin (0.5)BA	[64]
	2 Explants(1)	S	Shoot development	[102][2]	–/20/8	(0.2)Kin (0.5)BA	
	3 Shoots(2)	ST-P	Shoot proliferation	[102][2]	–/20/8	(0.2)Kin (0.5)BA	
	4 Shoots(3)	S	Shoot elongation	[102][2]	–/20/8	(0.02)Kin (0.1)BA	
	5 Shoots(45mm)(4)	DR-Pre	48 h in dark	0.5 x [102]	–/20/L	(2)NAA	
	6 Shoot(5)	DR	Rooted plantlets	0.5 x [102]	–/20/L	None	
E. torelliana	1 Coppice shoots or rejuvenataed scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA, BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. x trabuti (*botryoides x camaldulensis*)	1 Stem tissue	C	Callus culture		–/–/–		[92]
E. urnigera		C	Callus culture		–/–/–		[35]
E. urophylla		C	Callus culture		–/–/–		[55]
E. urophylla	1 Scion nodes	SN-P	Shoot development	[54]	5.5/30/8	(2)BA	[54]
	2 Shoots(1)	ST-P	Axillary shoot development	[54]	5.5/30/8	(2)BA	
	3 Shoots(2)	DR	Shoots rooted, plants	[54]	5.5/30/8	None	
E. urophylla	Seedling tissues	Su	Suspension culture		–/–/–		[38]
E. urophylla	1 Coppice shoots or rejuvenated scions	SN-P	Shoot proliferation	[102]	–/–/S	IAA, BA	[59]
	2 Shoots(1)	DR	Shoots rooted, plants	0.5 x [102] or [83]	–/–/S	IAA or IBA	
E. viminalis	1 Lignotubers	C	Root callus		–/–/–		[140]
	2 Callus(1)	C-ISR	Callus, shoots and roots		–/–/–		

Table 1. Continued.

Species	Stage and explant	Response	Result	Medium	pH /Sucr./Agar (g/l) (g/l)	Plant growth regulators	Reference
E. viminalis	1 Juvenile leaf	C	Callus induction	[90]	–/30/15	(1)2,4-D (0.4)Kin	[75]
	2 Callus	C	Callus culture	[90]	–/30/15	(2.2)2,4-D (2.5)Kin	
E. viminalis	1 Seeds	G	Germination	0.5 x [99] Med-GD	5.6/20/7	None	[98]
	2 Shoot tips, nodes(1)	SN/T-P	Adventitious buds	[99] Med-GD	5.6/20/7	(0.05)IBA (1)Z	
	3 Buds(2)	ST-P	Adventitious buds	[99] Med-GD	5.6/20/7	(0.05)IBA (1)Z	
	4 Buds(2)(3)	S	Shoot growth	0.5 x [99] Med-GD[28]	5.6/2.5/7	None	
	5 Shoots(4)	DR	Shoots rooted, plants	0.5 x [99] Med-GD	5.6/2.5/7	(1)IBA	
E. viminalis x *E. dalrympleana*	1 Root tips	O-R	Root culture	[135]	5.5/20/L	(2)IAA	[135]
	2 Roots(1)	O-R S	Shoot regeneration	[135][37]	5.5/20/L	(1)IBA (0.2)Kin (0.3)BA [red light 660nm]	
E. viridis	1 Buds (7–10mm)	C	Callus induction	[102]	–/–/A	(0.05–5)NAA or (0.05–5)2,4-D	[6]
	2 Callus(1)	C-IS	Callus, adventitious shoots	[102]	–/–/A	(0.05–5)NAA, IAA or IBA (2)Kin	
	3 Shoots(2)	C-IS/ST-P	Shoot proliferation	[102]	–/–/A	(0.01–4)NAA, IAA or IBA + (1)Kin (1)BA	
	4 Buds (7–10mm)	ST-P	Axillary shoot proliferation	[102]	–/–/A	(0.01–4)NAA, IAA or IBA + (1.5)Kin (1.5)BA	
	5 Shoots(3)(4)	DR	Shoots rooted	0.5 x [102]	–/–/5–6	(2.5)IBA	
	6 *Rooted shoots*(5)	S	*Plantlet growth*	*0.25 x [102]*	–/–/A	None	
E. wandoo	1 *In vitro* seedling tissues		Shoot multiplication		–/–/–		[95] [96]
	2 Shoots(1)	DR	Shoots rooted		–/–/–		
E. youmanni	1 Shoots, nodes from 8- to 18-m-old trees	C	Callus cultures	[102]	–/–/–	IAA IBA NAA 2,4-D BA (unspecified)	[2]

Table 1.1. Description of morphogenetic responses in Table 1. Codes linked in Table 1 indicate sequential (-) or alternative (/) events.

Code	Description
A	Anther culture
A-C	Callus from anthers
C	Callus formed/callus culture
C-IE	Embryonic callus
C-IR	Indirect adventitious root formation from callus
C-IS	Indirect adventitious shoot formation from callus
C-ISR	Indirect adventitious shoot and root formation from callus
C-R	Roots formed on callus
DE	Direct embryogenesis
DR	Direct adventitious root formation
DR-Pre	Root initiation pretreatment
DRS	Direct root and shoot formation
DS	Direct adventitious shoot formation
E-Pl	Plants developed from embryos
G	*In vitro* germination
Gen	Genetic transformations
IE	Indirect embryogenesis
O-R	Isolated root culture
O-R-S	Root culture and shoot formation
Pp	Protoplast culture
Pp-C	Callus formation from protoplasts
Pp-Co	Protoplast cocultivation
Pp-Gen	Protoplast culture for genetic transformations
Ppi	Protoplast isolation
Pre	Preconditioning
S	Shoot growth (no propagation)
S-DR	Shoot development and direct adventitious root formation
S-Gen	Regeneration of transformed plants
SN	Nodal culture
SN-P	Multiple shoots from single node culture
ST	Shoot tip culture
ST-P	Multiple shoots from shoot tip culture
ST-Pre	Shoot preconditioning
Su	Suspension culture

Table 1.2. Notes to Table 1.

Note No.	Comment
1	Equivalent de Fossard [27] Med-A, no casein hydrolysate
2	Supplemented with 0.01 mg l^{-1} calcium pantothenate, 0.01 mg l^{-1} biotin, no casein hydrolysate
3	Supplemented with 2.5 g l^{-1} activated charcoal
4	Modified by exclusion of K_2SO_4
5	Equivalent to the medium of Aneja and Atal [4] deduced from Aneja and Atal [4] and from personal communication from Atal to Cremer (Forest Research Institute, Canberra) giving details of the medium used [88]. Note that the version of Cremer did not state that coconut

Table 1.2. Continued.

Note No.	Comment
	milk was added or whether NAA was to be substituted for 2,4-D or was in addition to it; the agar, sucrose and inositol concentrations also differed between the published version of the medium and the version sent to Cremer
6	Iron source replaced by 20 mg l^{-1} $FeC_6H_5O_7$ (ferric citrate)
7	Modified by KNO_3 at 950 mg l^{-1}, NH_4NO_3 at 825 mg l^{-1}, $CaCl_2$ at 880 mg l^{-1}, supplemented with 100 mg l^{-1} ascorbic acid
8	Supplemented with 0.25 mg l^{-1} Na_2MoO_4, 0.25 mg l^{-1} $CuCl_2$
9	Supplemented with 500 mg l^{-1} glutamine
10	Supplemented with 0.5 mg l^{-1} nicotinic acid, 0.5 mg l^{-1} biotin
11	Equivalent to vitamins of de Fossard et al. [34] without folic acid
12	Equivalent to vitamins of de Fossard et al. [88] without folic acid, supplemented with 15 g l^{-1} activated charcoal
13	Modified by $CaCl_2.2H_2O$ at 293 mg l^{-1}
14	Medium consisted of von Arnold and Eriksson [136] macro-salts, Murashige and Skoog [102] micronutrients with thiamine HCl at 10 mg l^{-1}, supplemented with 100 mg l^{-1} inositol
15	Medium consisted of Heller [71] macro-salts, Murashige and Skoog [102] micronutrients with thiamine HCl at 10 mg l^{-1}, supplemented with 100 mg l^{-1} inositol.
16	Modified by $FeSO_4.7H_2O$ at 27.8 mg l^{-1}, $Na_2EDTA.2H_2O$ at 37.2 mg l^{-1}, Na_2SO_4 at 92.3 mg l^{-1}, no folic acid, no KI
17	Modified by $FeSO_4.7H_2O$ at 13.9 mg l^{-1}, $Na_2EDTA.2H_2O$ at 18.6 mg l^{-1}, Na_2SO_4 at 63.9 mg l^{-1}, no folic acid, no KI
18	One part de Fossard et al. [88] Med-MHZH (see Note 17) to 9 parts Cresswell and Nitsch [22]
19	Supplemented with 92.3 mg l^{-1} Na_2SO_4
20	Modified by $FeSO_4.7H_2O$ at 27.8 mg l^{-1}, $Na_2EDTA.2H_2O$ at 37.2 mg l^{-1}, Na_2SO_4 at 92.3 mg l^{-1}, no folic acid
21	Vitamins as follows: 1.5×10^{-3} mg l^{-1} cyanocobalamine, 0.5 mg l^{-1} folic acid, 0.5 mg l^{-1} riboflavin, 1 mg l^{-1} biotin, 1 mg l^{-1} choline chloride, 1 mg l^{-1} calcium pantothenate, 1 mg l^{-1} pyridoxinephosphate, 1 mg l^{-1} thiamine HCl, 2 mg l^{-1} nicotinamide, supplemented with 55 g l^{-1} inositol, no glycine
22	Supplemented with 100 mg l^{-1} inositol
23	Modified by KNO_3 at 1011 mg l^{-1}
24	Modified by thiamine HCl at 4 mg l^{-1}, supplemented with 800 mg l^{-1} PVP
25	Supplemented with 800 mg l^{-1} PVP
26	No reference given. Reference taken from George et al. [51]
27	Supplemented with 1.2 g l^{-1} $Ca(NO_3)_2.4H_2O$
28	Supplemented with 10 g l^{-1} activated charcoal
29	Medium consisted of 0.5 × macro-salts, full micronutrients with thiamine HCl at 10 mg l^{-1}, supplemented with 100 mg l^{-1} inositol
30	Medium consisted of Murashige and Skoog [102] macro- and micro-salts, with vitamins of von Arnold and Eriksson [136]
31	medium consited of Norstog and Rhamstine [107] macro-salts, with vitamins of von Arnold and Eriksson [136]
32	Medium consisted of 0.25 × Murashige and Skoog [102] macro-salts, full micro-salts with NaFeEDTA at 36.7 mg l^{-1}
33	No glycine, no casein hydrolysate
34	Medium consited of 0.25 × macro-salts, but with $CaCl_2$ at 220 mg l^{-1}, full micro-salts
35	Modified by NH_4NO_3 at 2401.5 mg l^{-1}
36	Supplemented with 15 g l^{-1} sorbitol
37	Vitamins replaced by vitamins of Badia [8]
38	Supplemented with activated charcoal

Table 1.3 Abbreviations used in the text and in Table 1.

BA	benzyladenine
Benlate	benomyl; 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester
CH	casein hydrolysate
CM	coconut milk
p-CPA	*p*-chlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
GA_3	gibberellic acid
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
IPropA	indole-3-propionic acid
IPyrA	indole-3-pyruvic acid
Kaptan	captab; N-trichloro methyl mecapto-4-cyclohexene 1,2-dicarboximide
Kin	kinetin; 6-furfurylaminopurine
ME	malt extract
NAA	naphthaleneacetic acid
α-NAA	α-naphthaleneacetic acid
β-NAA	β-naphthaleneacetic acid
β-NOA	β-naphthyloxyacetic acid
2iP	N^6-(2-isopentenyl)-adenine
PVP	polyvinylpyrolidone
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
YE	yeast extract
Z	zeatin;4-hydroxy-3-methyl-trans-2-butenylaminopurine
RV	vitamins, reference not given

placing the branches in a greenhouse under intermittent mist irrigation. After 2 to 3 weeks, epicormic shoots approximately 30 mm in length were harvested from the branches and used as explants. The shoots were sprayed with Benlate (1 g l^{-1} active ingredient) plus streptomycin (0.1 g l^{-1}) 24 and 48 h before collection.

The protocols for sterilizing shoots are similar to those employed for seeds. Most procedures begin with a wash in running water [11,60] followed by treatment with 0.05 to 0.1% (w/v) $HgCl_2$ for 5 to 15 min, or 1 to 10% NaOCl or $Ca(OCl)_2$ for 10 to 30 min, followed by several rinses in sterile distilled water. McComb and Bennett [95] reported good survival of *E. marginata* nodal explants sterilized with 1% Zephiran (benzalkonium chloride) in 10% alcohol for 10 min. Holden and Paton [72] sterilized field-grown *E. grandis* shoots by 75 min immersion in saturated $Ca(OCl)_2$, followed by 4 h of UV-irradiation. They obtained little contamination and 50% of the explants grew.

Floral buds

Floral buds of almost full size of *E. marginata* were soaked in 95% ethanol for 5 min, flamed, treated with NaOCl for 20 min, and then rinsed three times in sterile distilled water. Buds were then sectioned to provide stamens for callus cultures. Effective sterilization (100%) was achieved by this technique [11].

Lignotubers

Lignotubers are swellings produced at the base of young trees of some *Eucalyptus* species. They contain many vegetative buds, vascular tissue, and substantial food reserves [118]. *Eucalyptus bancroftii* lignotubers were surface sterilized by swabbing with 0.1% (v/v) 7X detergent and covering with cotton wool saturated with $Ca(OCl)_2$ solution for 20 min before being cut from the tree. The cut surface was dipped in molten wax and the lignotuber transferred to the laboratory where slices were soaked in 7X detergent for 5 min before treatment with saturated $Ca(OCl)_2$ solution for 20 min. Slices were cut into smaller pieces to provide explants [35,88]. Aneja and Atal [4] used lignotubers of *E. citriodora* to establish shoot cultures, but no details of sterilization were given.

Some authors have recommended spraying source material with fungicides, insecticides or antibiotics before harvesting explants to increase the success rate of sterilization procedures [13,42,45,74]. Boulay [15,16] recommended spraying donor trees with systemic fungicides every two days during the week before initiating cultures. Debergh and Maene [26] and de Fossard [29] suggested that, before cultures are initiated, donor plant material should be transferred to a greenhouse where good sanitation can be maintained. Less rigorous sterilization protocols can then be employed and explant survival is improved.

Establishing cultures

Dormancy and senescence of explants are obstacles to the establishment of cultures. Sterilization procedures may elicit a wound response in addition to that caused by excision. In woody plants, phenolic compounds are produced in response to wounding and these can be deleterious to growth *in vitro* [52]. The oxidation of phenolic compounds in the tissue and explant exudates is evident by browning of the medium, or the explant or both [22,27,43,45,54].

Durand-Cresswell [40] cited by Durand-Cresswell et al. [42] reported two types of exudates in *E. grandis* organ cultures. One, which appeared within one hour of excision of the explants (wounding effect), was aggravated by constituents of the culture medium such as high concentrations of sucrose, serine, chlorogenic acid or cytokinins [40], high boron concentrations [55] and light [42]. Creasy [20] reported that the activity of enzymes catalyzing the oxidation of phenolics is increased by light. The second exudate appeared during incubation and seems to be a product of senescing cells.

The following precautions reduce phenolic production in *Eucalyptus* explants:

1. Selection of actively growing young explants [43];
2. Use of sterilization procedures that do not severely damage the explant tissues. This may require transfer of donor tree material into a controlled environment, establishment of higher standards of sanitation (for example fungicidal sprays) and partial rejuvenation of the material by grafting or rooting of cuttings [26,29,45];

3. Washing sections of source material in running water for at least 1 h before sterilization [11,30,32,54,60];
4. Soaking explants in sterile water after sterilization, before placing on medium [22,43];
5. Soaking explants in antioxidant solutions after sterilization, before placing on medium [6];
6. Including antioxidants such as polyvinylpyrolidone [85,86,124,125] and ascorbic acid [60] in the medium. Although activated charcoal has been used to adsorb toxic or inhibitory substances formed by callus explants [3,52], it was found to inhibit callus formation on various *Eucalyptus* explants when added to media at 8 g l^{-1} [55];
7. Placing cultures in the dark for an initial period of 7 to 14 days [10,15,22,45,48];
8. Frequent subculture during the initial months [54];
9. Using explants from established shoot cultures [101].

Callus cultures

In vitro callus formation and culture have been reported for at least 30 *Eucalyptus* species. Plant regeneration, however, has only been reported for 12 species (Table 1).

A variety of basal media and concentrations and combinations of plant growth regulators have been used for the callus culture of *Eucalyptus*. Earlier reports [4,123,130] included undefined components such as coconut milk, casein hydrolyzate and yeast extract in media. Although most recent studies listed well defined media additives, malt extract was used for callus cultures of *E. viminalis* and *E. coccifera* [75], and coconut milk for callus cultures of *E. polybractea* [141] and suspension cultures of *E. gunnii* [132]. Callus cultures have been grown in the dark [11,27,141] or in low light [53,82,84], usually at 25 °C.

The nature of calli produced depends on the explant source and the medium. Hard yellow calli developed from cotyledons, whereas initially grey, then yellow lumpy calli developed from stamen filaments of *E. marginata* [11]. Compact red-pigmented callus formed on cotyledons and hypocotyls of *E. citriodora* [84]. Compact red, yellow or pale-green callus formed on hypocotyls of *E. globulus* [108]. Sussex [130] produced friable callus from which he was able to initiate suspension cultures. A wide variety of callus types were produced in experiments with different media and auxin/cytokinin ratios [35,88].

Shoot and root regeneration from callus cultures

Shoot regeneration from *Eucalyptus* callus is difficult. Aneja and Atal [4] reported regeneration of plants from lignotuber callus. Lee and de Fossard [88] reported no organ regeneration from stem calli of *E. bancroftii* exposed to 175 auxin/cytokinin ratios, nor from lignotuber calli exposed to 100 auxin/cytokinin ratios. All cultures in these experiments were incubated in the dark at 25 °C. Light is essential for shoot regeneration from *Eucalyptus* calli [95].

Kitahara and Caldas [82] reported shoot and root regeneration from hypocotyl

callus grown on White's [137] medium with 1 mg l^{-1} IAA (see Table 1.3 for definitions of abbreviations), 60 days after subculture of the callus to fresh medium of the same composition in a 12-h photoperiod provided by diffuse fluorescent light. Lakshmi Sita [84] found that shoot regeneration from cotyledonary callus of *E. citriodora* required 0.2 mg l^{-1} IAA plus 1 mg l^{-1} zeatin and a 12-h photoperiod of diffuse fluorescent light. These shoots were able to form roots on a medium with auxin alone. Root initiation was achieved on calli of a number of *Eucalyptus* species in a 12-h photoperiod, whereas no shoot or root regeneration occurred in the dark [53,55].

Shoots regenerated from stamen callus of *E. marginata* showed low rooting ability (5%) compared to rooting of shoots regenerated from cotyledonary callus (35%) [11].

Organ cultures

Roots

Bachelard and Stowe [7] were able to maintain cultures of seedling roots in a liquid medium containing 14% coconut milk. Root tips could be subcultured on the same medium, but no shoot regeneration was achieved. Adam [1] was able to culture isolated roots of *E. gunnii* on solid or liquid Murashige and Skoog [102] medium without plant growth regulators.

Lignotubers

Callus cultures have been initiated from tissues of lignotubers of *E. bancroftii* [27,35], *E. citriodora* [4], *E. obliqua*, *E. viminalis* [140] and *E. robusta* [53]. Plant regeneration has only been reported for *E. citriodora* [4], which is surprising because lignotubers contain many dormant shoot meristems and these have been used for vegetative propagation [12,66].

Anthers

Callus cultures of anthers have been established for *E. callophyla*, *E. citriodora*, *E. diversicolor*, *E. marginata* [11,95], *E. dalrympleana* [17], and *E. grandis* [53]. Bennett and McComb [11] and McComb and Bennett [95] regenerated shoots from calli that could subsequently be rooted. Boulay [17] reported root initiation on calli with a range of plant growth regulator combinations, but no shoot regeneration was achieved.

Microcuttings

Early attempts at *in vitro* propagation of *Eucalyptus* aimed at rooting microcuttings from the crown branches of young trees, because these branch cuttings could no longer be rooted by conventional propagation techniques. Paton et al. [111] isolated a rooting inhibitor in older leaves that was responsible for the loss of rooting ability in cuttings taken from mature branches. Rooting of microcuttings was achieved with

nodes of *E. grandis* up to node number 80 (the cotyledonary node taken as the first node) [22], nodes from the crown of 5-year-old trees of *E. dalrympleana*, leaf discs of 5-year-old *E. macarthurii* trees [43] and nodes from the crowns of 22-year-old *E. ficifolia* trees [10,29].

Cresswell and Nitsch [22], Durand-Cresswell and Nitsch [43] and Durand-Cresswell and Boudet [41] used agar media supplemented with IBA in the range 0.25 to 1.0 mg l^{-1} to initiate rooting. Cresswell and de Fossard [21] used liquid media with filter paper bridges and no plant growth regulators to obtain roots on seedling shoot tips of *E. bancroftii*.

Shoot tips

Micropropagation of *Eucalyptus* species is best achieved by inducing axillary or adventitious shoot proliferation on nodal explants from seedlings [32,33,69], mature trees [32,33,64,94,125], coppice [9,67,68] or mature shoots grafted onto seedling rootstocks [15,42,45]. Rooting is attempted once shoot growth has occurred.

Nodal explants usually consist of stem segments 10 to 20 mm in length, 2 to 3 mm in diameter, with one or two preformed buds (depending on the arrangements of the leaves), the petiole, and a small portion of leaf.

Various terms are used in reports on shoot multiplication and some confusion exists concerning the nature of shoot proliferation. Two types of buds are present in young shoots. In the axil of the leaf there is a visible stalked bud, known as the naked bud [74,114,118], as well as one or more buds which are dormant and covered by petiolar tissue. The dormant buds are referred to as accessory buds by Penfold and Willis [114] and as concealed buds by Pryor [118]. The concealed buds [118] give rise to secondary branching when the naked buds are damaged by fire or insects. As the stem matures and thickens, the concealed bud tissues continue to grow as small shafts of vascular tissue extending radially toward the stem surface. In older stems, their presence can be detected as small depressions ("eyes") on the stem surface [74]. At this stage of development, they are called proventitious buds [74,114]. The proventitious buds develop into epicormic shoots along the stem branches of older trees, particularly after fire.

Multiple shoots produced *in vitro* could arise either from the development of preformed buds (naked or concealed), or from the development of buds formed *de novo* in culture (true adventitious buds). Both forms of bud development and growth could lead to extensive branching by further axillary bud development.

In nodal cultures, new buds and shoots continue to arise in the axils of leaves and new shoots, often without elongation, resulting in dense clumps of buds [10]. This process is usually associated with the development of callus at the base of the explant, where the explant is in contact with the medium [10]. In some cases, internodes elongate more and the result is more branches as the axillary buds grow out at each succeeding generation of nodes [60,63,67,68].

Various agar based media have been used to stimulate shoot multiplication, usually with a low auxin/cytokinin ratio. Hartney [68] successfully multiplied shoots of 12 *Eucalyptus* species on an agar based medium of half strength Murashige and Skoog

[102] salts with 2% sucrose, NAA and BA. Liquid media were used to stimulate axillary shoot proliferation from buds of 20-year-old *E. citriodora* trees [63] and from nodes of mature *E. tereticornis* trees [94]. Recently MacRae and Van Staden [91] reported excellent shoot proliferation from axillary buds of *E. grandis* using gelrite instead of agar.

Multiplication rates depend on species, clone, explant source, and juvenility. Hartney [68] achieved rapid multiplication rates for 12 *Eucalyptus* species with a potential production of 100 million shoots from a single shoot within a year. *E. marginata* seedling clones showed even higher multiplication rates [11].

Shoot cultures have potential for long-term storage of selected lines. Hartney [67] stored viable shoot cultures of *E. camaldulensis* and *E. grandis* on a simple medium in a domestic refrigerator for over 8 months. Franclet and Boulay [45] stored shoot cultures of *E. gunnii* and *E. dalrympleana* in the cold for six months without affecting their appearance or subsequent growth. Muralidharan et al. [100] stored embryonic cultures of *E. citriodora* at 10 °C on B-5 medium [50] for more than 9 months without subculture and without loss of embryogenic potential.

Vitrification

Vitrification, also known as tissue waterlogging or hyperhydric transformation, is the phenomenon whereby shoots become glassy and transparent, with swollen, brittle leaves and stems [52]. Boulay [15] found that when vitrification occurred in shoot multiplication cultures, usually only a few buds were affected. If the affected culture was transferred to multiplication medium containing 1.5 g l^{-1} activated charcoal, then the development of normal buds was promoted. Alternatively, vitrified buds can be excised from cultures at each passage and discarded. Boulay [16] noted that vitrified shoots were difficult to multiply and almost impossible to root, and recommended rapid subculturing as a possible solution to the problem. Durand-Cresswell et al. [42] recommended a reduction in cytokinin concentrations. This leads to a lower multiplication rate, but brings about greater physiological stability in cultures.

Callus development on shoots

Eucalyptus shoot cultures frequently produce a white callus, which is sugary in appearance, on leaf surfaces, stem nodes, and in the axils of leaves of *E. dalrympleana*, *E. delagatensis*, *E. ficifolia*, *E. grandis*, *E. regnans* and *E. gunnii* [10,15,32,42]. This nodal callus begins its development from the abscission layers on the petiole [42] and often develops into a mass that overtakes the axillary bud, causes leaf drop and senescence and abscission of the apical shoot and axillary branches. Boulay [15] recommended frequent subculturing (every 15 days) to prevent this problem. Multiple buds of *E. regnans* free of white sugary callus on leaves were obtained by excising affected leaves at each subculture and transferring the healthy shoots to fresh medium [10].

Shoot elongation

Before rooting is attempted it is essential that the shoots first elongate. Gupta et al.

[64] transferred *E. camaldulensis* shoots to liquid medium and reduced the concentrations of Kin and BA from 0.2 and 0.5 mg l^{-1} to 0.05 and 0.1 mg l^{-1} respectively. A reduction in cytokinins brought about shoot elongation of *E. torelliana* shoots [64], whereas a transfer from liquid to solid medium achieved a similar effect with *E. tereticornis* shoots [94].

Gibberellic acid has been added to media to obtain shoot elongation. Satisfactory results were obtained for *E. dalrympleana* (1 mg l^{-1}) [45], *E. grandis* (1 mg l^{-1}) [85], *E. grandis* and *E. nitens* (0.1 mg l^{-1}) [48]. Depommier [36], Franclet and Boulay [45] and Furze and Cresswell [48] combined 15 g l^{-1} activated charcoal with GA_3 to achieve shoot elongation.

Root initiation

Root initiation of *in vitro* shoots of *Eucalyptus* is variable. Shoots obtained from seedling explants generally root easily [66]. Hartney [68] obtained over 70% rooting of seedling shoots in six out of eight species and less than 30% rooting for two species. *Eucalyptus marginata* shoots originating from seedling shoots or from cotyledonary callus both showed an average of 35% rooting, whereas the mean rooting percentage for shoots from mature nodes was 5% [11]. The range of rooting percentages for seedling clones was 5 to 80%, whereas the range for adult clones was 2 to 20%. Burger [19] achieved 4% rooting with adult tissues of *E. sideroxylon* and 100% rooting from shoots derived from coppice explants.

Goncalves [54] found that the rootability of *E. urophylla* shoots obtained from grafted scions was increased with each subculture. Gupta et al. [64] reported good rooting ability of shoots from mature trees of *E. torelliana* and *E. camaldulensis* over six and 20 subcultures with rooting percentages of 50 and 70%, respectively. Shoots of *E. citriodora* from 20-year-old trees failed to root until the fourth subculture, when a rooting percentage of 35 to 40% was achieved. Rooting percentages of 45 to 50% were achieved during subsequent passages [63]. The rooting percentage of shoots from adult trees of *E. rudis* increased from around 20 to 100% from the third to the sixth subculture [8]. McComb and Bennett [95] found that an increase in the rooting percentage of *E. marginata* shoots from mature explants took place only after 12 to 15 months of culture.

Changes in season affect rooting of cuttings [106]. Durand-Cresswell and Nitsch [43] found that there was a decline in rooting ability of *E. grandis* shoots after stock plants had been maintained under constant conditions for long periods. Sudden changes in light intensity, temperature, or day length stimulated rooting. Cresswell and de Fossard [21] reported that faster rooting of cuttings occurred after parent plants of *E. grandis* had been given a series of short days, or had experienced a change in nutritional status.

Root initiation media are often simpler than those used for shoot multiplication. Examples are White's [139] medium [94,125], Knop's [83] medium [15,45,48], and half strength Murashige and Skoog [102] medium [6,64,68,85]. Solid and liquid media have been used, although de Fossard and Bourne [32] reported that agar-based media were superior to liquid media for both shoot development and root formation

in nodal cultures of *E. ficifolia*. Indole butyric acid is most frequently included as the root-inducing auxin, and is used most often at a concentration of 1 mg l^{-1}.

Excessive callus formation at the base of shoots, absence of rooting, browning and senescence of shoots can be minimized by placing cultures in the dark for an initial period of 2 to 20 days [15,39]. High auxin concentrations induce excessive callus formation and senescence of shoots [15]. Naphthyl acetic acid and IBA concentrations in the range of 0.5 to 2.0 mg l^{-1} were tested on shoots of *E. grandis*. The lower concentrations of NAA resulted in little or no callus formation but promoted rooting. Indole acetic acid at 0.5 mg l^{-1} gave a similar result [86].

De Fossard et al. [31] showed that shoots grown on media containing riboflavin and incubated in a light intensity of 10 µmol m^{-2} s^{-1} developed one to three long subsurface roots with either no laterals, or very short laterals (Type I roots). Shoots grown without riboflavin and incubated in 10 µmol m^{-2} s^{-1} or in darkness, developed numerous short roots with many laterals, i.e., a more fibrous root system (Type II roots). Shoots grown on media with riboflavin and incubated in the dark were also stimulated to form Type II roots. Riboflavin acted as a photoreceptor in the reaction leading to Type I root development. It did not act directly on the explant, but changed the medium, thereby causing Type I root development.

Indole-3-butyric acid alone resulted in Type II roots plus some callus on shoots of *E. ficifolia*. On media without IBA, a Type I root system developed with only small amounts of callus [56]. Riboflavin is able to photo-oxidize IAA [49] and IBA [57]; thus the effects of IBA on *in vitro* rooting of *E. ficifolia* shoots could be modified by the inclusion of riboflavin and incubation in light.

Badia [9] showed that vitamin E acted as an antioxidant against polyphenolic compounds produced by *E. rudis* shoot cultures, and had a synergistic effect with BA. Vitamin E increased the percentage of shoots forming roots, speed of root initiation, root elongation and shoot development. Boulay [15], however, reported that vitamin E had no effect on the elongation of axillary buds in shoot cultures of *Eucalyptus*.

In *E. ficifolia*, auxins with a phenolic oxygen between the aromatic ring and the side chain (2,4-D; 2,4,5-T; *p*-CPA; β-NOA) induced callus formation and poor root development, whereas auxins without the phenolic oxygen (α-NAA; β-NAA; IBA; IAA; IPropA; IPyrA) promoted good root development [57]. In cases where seedling shoots of *E. ficifolia* produced teratomas instead of roots, trimming the base with a scalpel and replanting on fresh rooting medium generally resulted in good root development [10].

Serial rooting has been adopted for some clones and species, a technique that usually involves a brief passage of shoots on a medium with auxins, followed by transfer to hormone-free medium which often contains activated charcoal. *E. citriodora* shoots derived from mature trees were pulsed in a liquid medium with 2 mg l^{-1} NAA for 48 h before transfer to fresh, hormone-free, liquid [63] or agar medium [94]. Gupta et al. [64] treated shoots of *E. camaldulensis* with 10 mg l^{-1} each of IBA, IAA, NAA and 2iP for 72 h before transfer to hormone-free liquid medium with activated charcoal (2.5 g l^{-1}). A similar technique was used for shoots

of *E. grandis* [125] and *E. tereticornis* [124].

An alternative strategy to the establishment of rooted plantlets *in vitro* is to transfer the shoots to non-sterile conditions as soon as root primordia become visible [42]. Rajbhandary [122] treated *in vitro* derived shoots of *E. camaldulensis* as conventional cuttings, and rooted these directly in sand in non-sterile conditions. Leaves touching the medium often initiate roots [15] which means that the lower leaves on microcuttings need to be trimmed before insertion into root initiation medium.

Suspension cultures

Sussex [130] established friable callus from *E. camaldulensis* hypocotyls on White's medium [137] using 1 mg l^{-1}, 2,4-D and 15% coconut milk. He established suspension cultures from this callus in an agitated liquid medium with the same growth regulators as before. Nodular aggregates formed from single cells in liquid culture, and these could be reestablished as friable callus cultures on agar medium. Khanna and Staba [80] tried to prepare suspension cultures from callus of seedling tissues of *E. globulus* on a modified Murashige and Skoog [102] medium, but reported poor growth. They used 1 mg l^{-1} and 0.1 mg l^{-1} 2,4-D for callus and suspension cultures respectively.

Friable callus of *E. gunnii* and an *E. gunnii* × *E. dalrympleana* hybrid were established from leaves on Murashige and Skoog [102] medium with 2 mg l^{-1} 2,4-D [132]. Suspension cultures were achieved in two steps on liquid Murashige and Skoog [102] medium or White's [137] medium. The first step involved subculturing the suspensions for two months with 0.1 mg l^{-1} BA plus 3 mg l^{-1} 2,4-D on Murashige and Skoog [102] medium or with 10% coconut milk and 3 mg l^{-1} 2,4-D on White's medium [137]. Following this step, suspension cultures could be maintained on media with 0.1 mg l^{-1} BA and 1.5 mg l^{-1} 2,4-D. Cell densities doubled within 3 days on Murashige and Skoog [102] medium. Callus regeneration was achieved after transfer of cells from the suspension to an agar-based medium.

Protoplast cultures

Suspension cultures have been initiated to provide protoplasts of *E. grandis*, *E. saligna* and *E. urophylla* [38,112,113,132]. Protoplasts, in contrast to complete cells, showed variable resistance to low temperature and their viability was correlated to the degree of frost-resistance of the donor tree. Cells plated onto solid medium proliferated into callus. Teulieres et al. [132] suggest that this technique could be used for the selection and propagation of frost resistant variants.

Protoplasts of *E. grandis* have been isolated and cultured from hypocotyls, cotyledons, hypocotyl-derived callus and cell suspensions, internodes from young shoots of adult trees, and from young leaves of *in vitro* grown shoots of adult trees [112,113]. Cotyledons of *E. citriodora* produced protoplasts, of which 80% were viable, which were cultured on a liquid-on-agarose medium or embedded in agarose, in the dark at 27 °C [38].

Plant regeneration from protoplasts of *E. saligna* derived from *in vitro* shoots, was achieved by facilitating initial cell divisions by co-culture with protoplasts of *Hibiscus cannabinus* L. (Kenaf) [76,79]. Protoplasts have been used to facilitate plasmid gene transfers in *E. saligna* [79], and *E. gunnii* [133].

Somatic embryogenesis

Muralidharan and Mascarenhas [101] reported somatic embryogenesis using embryos of *E. citriodora* on semisolid agar based B5 medium [50] with 3 mg l^{-1} NAA, and sucrose at 50 mg l^{-1}. Somatic embryos only developed on embryos that had been soaked in sterile distilled water at 29 °C for 2 days before placement on medium. When somatic embryos were transferred to the medium of Smith and McCown [129] to which was added 0.5 mg l^{-1} BA and 10% coconut milk, somatic embryogenesis was sustained. Growth and development of isolated embryos was possible on half-strength Murashige and Skoog [102] liquid medium without plant growth regulators. Similar results were reported by Muralidharan et al. [100]. Enhanced somatic embryogenesis was achieved by establishing an "embryogenic mass." The "embryogenic mass" cultures were maintained without loss or reduction of embryogenic competence for over 36 months on modified B5 medium [50] with 5 mg l^{-1} NAA. Casein hydrolysate (500 mg l^{-1}) and glutamine (500 mg l^{-1}) were added to the medium and cultures were maintained in the dark. Embryo growth and development occurred on fresh B5 medium without growth regulators and in the light.

Somatic embryogenesis was reported on callus derived from shoots of 4-year-old trees of *E. grandis* on Murashige and Skoog [102] medium with 0.1 mg l^{-1} NAA and 5 mg l^{-1} Kin [86]. Somatic embryos were also obtained by culturing friable callus in liquid medium containing 1 mg l^{-1} each of BA, Kin, NAA and 2,4-D. Cultures were grown in a 16-h photoperiod at 25 °C. Somatic embryogenesis and plant regeneration has been reported from callus of seedlings of "*E.* × *liechow* (which is thought to be a hybrid of *E. exserta* or *E. camaldulensis*) [109,110]. Boulay [17] achieved somatic embryogenesis with hypocotyl and internode calli derived from seedlings of *E. gunnii* on two different media and a variety of hormone concentrations. Secondary somatic embryogenesis was achieved by subculturing embryogenic calli.

Qin Chang-Le and Kirby [120] induced embryo-like structures in cultures of hypocotyls, cotyledons, and young seedling leaves of *E. botryoides*, *E. dunnii*, *E. grandis* and *E. rudis*, as well as from young leaves of cultured shoots of superior adult *E. grandis* clones. A sequential culture technique was used with Murashige and Skoog [102] salts and RV (reference not given) vitamins and amino acids as medium. Slow growing green protuberances developed from cut surfaces of explants after 2 weeks in culture on a medium containing 1.1 mg l^{-1} 2,4-D. These developed into adventitious shoots and embryo-like structures when transferred to medium with 1.1 mg l^{-1} BA. If red or white fast growing callus developed on the initial explants, no shoots or embryo-like structures could be induced.

Hardening and establishing regenerated plants

Various procedures for hardening plants have been described. All are based on the principle of gradually reducing the humidity around the plants and altering plant metabolism from partial dependence to full independence of an external carbohydrate source. The particular conditions reported in different papers probably reflect the climatic conditions of the region, season and the facilities available.

Durand-Cresswell and Boudet [41], Franclet and Boulay [45], and Boulay [15] recommended transfer of plantlets with or without agar to small unwoven bags (Melfert Container) containing a mixture of 70% pine bark, 25% sphagnum peat, 5% brown coal ash and 4 g dm^{-3} Osmocote (slow release) fertilizer. Roots develop freely in these containers without spiralling. The containers were placed together on an irrigation sheet in a greenhouse and a plastic sheet was placed over the containers to maintain a saturated atmosphere for the first week or longer depending on season. A survival rate of over 90% was obtained by this method and the *in vitro* derived plants were indistinguishable from normal seedlings [45].

Poissonier et al. [117] have shown that the acclimatization and survival of *in vitro* plants, after transfer to the greenhouse, depends on the quality of the plants. Boulay [16] states that maintenance of high relative humidity during the initial period is preferable to mist and that during this period the roots should be stimulated more than the shoots by heating the substrate. The young plantlets are often sensitive to attack by pathogens. Sprays with fungicides and insecticides are, therefore, recommended [16,96,117]. Durand-Cresswell et al. [42] recommended transferring plants to the greenhouse as soon as root primordia appeared, whereas others established rooted plants *in vitro* first [15,35,62,63]. McComb and Bennett [96] noted that *E. citriodora, E. diversicolor, E. calophylla* and *E. carnabyi* were easier to transfer than *E. marginata*.

Conclusions and future prospects

Vegetative propagation will play a significant role in tree improvement programs with eucalypts, the greatest demand being for increased biomass production by fast-growing trees. The most serious obstacle to this goal is the loss of rooting ability of cuttings with increasing age of donor trees. Therefore, many commercially important selections are difficult to propagate vegetatively at the stage at which the selection is made. In the past 10 years, the selection and cloning of superior trees from natural hybrids has had considerable impact on biomass production. However, the gene pool available for selection is restricted for commercial cloning by the requirement that at least 70% of the cuttings must root.

In many instances the extent of rejuvenation through horticultural techniques is insufficient to permit commercial propagation by cuttings. The phenomena of juvenility, maturity and rejuvenation are still poorly understood [46,115]. Grattapaglia et

al. [59] listed three key factors for successful large-scale propagation of *Eucalyptus* species:

1. The intrinsic propagation potential of the clone;
2. The level of juvenility of the initial explants; and
3. Strict observance of a maximum subculture period of four weeks.

The importance of selecting the appropriate explants of *Eucalyptus* species for the initiation of *in vitro* cultures should receive more attention [17,47,93]. Specific zones within the complex architecture of the tree, for example, basal epicormic shoots and the zones near floral and apical meristems [17], have juvenile characteristics or the potential for rejuvenation. The combination of horticultural rejuvenation techniques (for example, successive grafting of adult scions onto seedling rootstocks) and *in vitro* rejuvenation techniques merits further investigation [115]. Franclet et al. [47] reported the successful rejuvenation of over 200 adult clones of *Sequoia sempervirens* (some ortets over 500-year-old) and believe that their multiple-technique studies could serve as a model for the rejuvenation of other mature woody species.

Epicormic shoots on branches of mature trees have been used to initiate rejuvenated cultures of *E. grandis* [74,131]. Repeated *in vitro* grafting of adult scions onto seedling rootstocks has resulted in the rejuvenation of *Sequoia* and *Citrus* clones [73]. Grattapaglia et al. [59] successfully micropropagated seven *Eucalyptus* species using coppice shoots or graft-rejuvenated adult shoots as initial explants.

In vitro techniques have been employed to rejuvenate tissues of mature *Eucalyptus* over the past 25 years, and there exists the potential for mass production of selected material. However, the techniques are not yet sufficiently refined for commercial application. A number of large-scale field trials with *in vitro* derived clones are in progress [59,67,81] and it is expected that these will show whether commercial application is possible or not. Grattapaglia et al. [59] have planted over 250 000 micropropagated plants, consisting of 12 clones, into field trials. Some of the material will be used for commercial propagation by cuttings, and the remainder will be harvested for charcoal.

Field trials will establish the degree of somaclonal variation and other possible post-*in vitro* effects such as early maturation and changes in growth form, for example, the development of plagiotropism. Trials, when allowed to proceed to at least half-rotation age, will provide reliable cost and profit comparisons between horticultural and *in vitro* propagated plantations. The field trials will determine the current potential of micropropagation techniques for commercial afforestation.

Cell, tissue and organ culture techniques may be used for the rapid establishment of clonal and breeding seed orchards, particularly of trees which flower early, thus hastening and facilitating breeding programs. Clonal hedges can be established through micropropagation to provide shoot material for conventional propagation by cuttings. The techniques can also be used for the *in vitro* selection of cell lines possessing selected properties.

In vitro techniques are presently being applied to *Eucalyptus* to achieve genetic

transformations. These techniques are likely to play a vital role in future tree-improvement programs [18,79,133,142].

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