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Review

Tissue Culture of *Corymbia* and *Eucalyptus*

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Abstract: Eucalypts are among the world's most widely planted trees, but the productivity of eucalypt plantations is limited by their often-low amenability to true-to-type propagation from cuttings. An alternative approach to cutting propagation is tissue culture, which can be used to micropropagate valuable genotypes rapidly while simultaneously preserving germplasm in vitro. This review describes the use of tissue culture methods such as shoot culture, organogenesis, and somatic embryogenesis for micropropagating eucalypts. This review also discusses the use of cool storage, encapsulation, and cryopreservation methods for preserving eucalypt germplasm and delaying tissue maturation under minimal-growth conditions.

Keywords: adventitious roots; alginate encapsulation; clonal propagation; juvenility; plantlet conversion; plant propagation; plant preservation; rooting; synthetic seeds; temporary immersion

1. Introduction

Eucalypts are the world's most widely planted hardwood trees, with more than 20 million hectares established in plantations because of their wide diversity of species, suitability of individual species for different climates and soils, fast growth rates, and multiple products such as timber, pulp, fodder, biofuel, essential oil, and bioactive chemicals [1–11]. Eucalypt plantations are dominated by ten species, *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson, *Eucalyptus camaldulensis* Dehnh., *E. dunnii* Maiden, *E. globulus* Labill., *E. grandis* W.Hill, *E. nitens* (H.Deane & Maiden) Maiden, *E. pellita* F.Muell., *E. saligna* Sm., *E. tereticornis* Sm., and *E. urophylla* S.T.Blake, although well over 100 species have been planted globally for a range of forestry and horticultural purposes [4].

The productivity of eucalypt plantations has been limited by low amenability to clonal propagation from cuttings. Some species from high rainfall or riparian habitats, such as flooded gum (*E. grandis*), river red gum (*E. camaldulensis*), and rainbow gum (*E. deglupta* Blume), have long been considered amenable to cutting propagation [11–16]. Nonetheless, efficient commercial-scale propagation of these species has relied on the development of intensively managed 'mini-cuttings' or 'micro-cuttings' systems for maintaining stock plants and producing cuttings in the nursery (Figure 1a). The difference between these two systems is that nursery stock plants in the mini-cuttings system are raised from small and often serially-propagated rooted cuttings, whereas nursery stock plants in the micro-cuttings system are raised initially in tissue culture [16–26]. The species and hybrids that are propagated in these systems (e.g., many millions of *E. grandis* and *E. grandis* × *E. urophylla* plants per annum) are generally suited to high rainfall sites in the tropics or subtropics. Hardwood plantations are increasingly being established on drier and colder sites where land is more readily available and less expensive. These sites require other eucalypt species such as spotted gum (*C. citriodora*), Gympie messmate (*E. cloeziana* F.Muell.), southern blue gum (*E. globulus*), or shining leaf gum (*E. nitens*) that are more drought- or cold-tolerant, but which are also much more difficult to propagate from

cuttings [14,27–34]. One of the great challenges in hardwood forestry is to develop efficient methods for the clonal propagation of eucalypts, particularly for those species that are difficult to propagate from cuttings. One of the most promising approaches is tissue culture, which can be used to micropropagate valuable genotypes rapidly whilst simultaneously preserving germplasm in vitro (Figure 1b,c) [35–42].

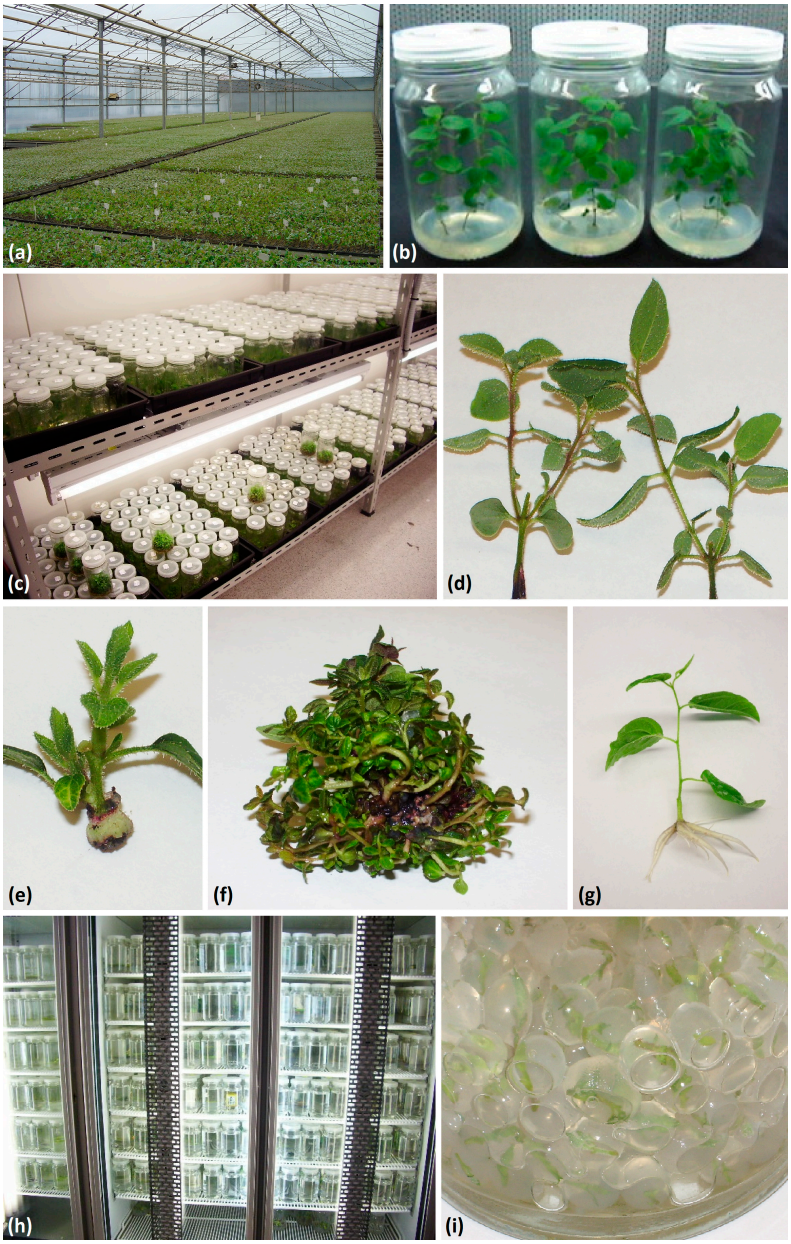

Figure 1. Eucalypt propagation and germplasm preservation: (**a**) *Eucalyptus grandis* × *E. urophylla* cuttings in a commercial nursery; (**b**–**i**) *Corymbia torelliana* × *C. citriodora*: (**b**,**c**) shoots in tissue culture; (**d**) axillary bud outgrowth in shoot culture; (**e**) callogenesis at the base of a shoot; (**f**) multiple shoot production in organogenic culture; (**g**) a plantlet; i.e., a shoot with adventitious roots; (**h**) shoots in cool storage; and (**i**) alginate-encapsulated shoot tips and nodes; i.e., synthetic seeds. Photographs: (**a**) I. Wendling, (**b**,**h**) S.J. Trueman, and (**c**–**g**,**i**) C.D. Hung.

This paper provides an overview of conventional techniques of shoot culture, node culture, organogenesis, and somatic embryogenesis that have been used for micropropagation of eucalypts. These methods allow rapid and true-to-type propagation of selected clones in a clonal plantation

program or of seedlings from selected families in a vegetative family plantation program [42–44]. Shoot culture and node culture use primary explants with an intact shoot meristem or node, respectively, to proliferate shoots by stimulating the outgrowth of axillary shoots (Figure 1d) [35,45]. These two methods of shoot proliferation are, in practice, very similar, often differing only in the type of primary explant used for culture initiation. Many authors have used the term 'shoot culture' to describe both shoot culture and node culture and so, henceforth, we use the term 'shoot culture' to describe both of these methods for shoot proliferation. Organogenesis involves the induction de novo of adventitious shoots, often via an intervening callus phase (Figure 1e,f), from tissues such as internodes or cotyledons that would not otherwise have formed shoots [35,46,47]. The shoots produced by shoot culture and organogenesis often lack roots (i.e., they are unipolar) but, once a sufficient number of shoots has been multiplied, roots are induced on the shoots to produce plantlets; i.e., shoots with roots (Figure 1g). Somatic embryogenesis, in contrast, induces bipolar embryo-shaped structures, possessing both a shoot and a root meristem, often via an intervening phase of embryogenic callus [35,36,47–49].

This paper also discusses the use of cool storage (Figure 1h), encapsulation (Figure 1i), and cryopreservation techniques to preserve eucalypt clones under minimal-growth conditions. These in vitro techniques can be highly effective for archiving plant germplasm without the space requirements, fertiliser costs, and pest and disease risks associated with nursery or broad-acre clone banks [42,50–54]. These techniques also have the potential to maintain juvenile clones in vitro for many years with little or no maturation, ensuring that the propagules retain their juvenile characteristics of high propagation potential and maximal stem elongation for the duration of clonal archiving [39,40,42,48,54–57].

This review focusses primarily on techniques that have been developed since the last comprehensive review of eucalypt tissue culture over 25 years ago [58]. The review describes, wherever possible, the optimal treatment among all the treatments attempted in each previous study, although many studies described only one method for some phases of their tissue culture process. The review, firstly, describes methods for the establishment of eucalypt cultures in vitro before outlining the shoot culture, organogenesis, and somatic embryogenesis methods that have been used for eucalypt micropropagation. The review then describes methods for converting eucalypt shoots to plantlets and for improving their ex-flasking capacity. The review, finally, describes the cool storage, encapsulation, and cryopreservation methods that have been used to store eucalypt shoots and callus.

2. Establishment of Aseptic Cultures

Culture initiation is the first and often the limiting phase during in vitro propagation of trees because primary explants are typically non-aseptic and are, therefore, the main source of inoculum for microbial contamination in vitro. The initial explants for eucalypt tissue culture are typically seeds, shoot tips, nodes, or axillary buds (Table A1). Seeds do not provide true-to-type clonal propagation from a selected mother tree, but they can be used as the starting point for producing multiple clones from selected families in a vegetative family forestry program. Seeds are also an appropriate explant source for producing a genetically diverse collection of plants for conservation, revegetation, fodder production, or horticulture. Seeds are often the simplest explants to initiate into tissue culture because they are easy to decontaminate and because the juvenility of young seedlings makes them conducive to callogenesis or rapid shoot proliferation [35,45,54,55]. The use of shoot tips, nodes, or axillary buds as explants allows true-to-type propagation of selected trees, but these explants can be difficult to decontaminate, especially for eucalypts that are covered in hairs. The proliferation capacity of shoot tips, nodes, or axillary buds and the subsequent growth of their plantlets may also be influenced strongly by the position of the tree from which the explant was harvested. Maturation effects such as reduced rooting capacity, shorter internode length, and decreased stem growth [54,55,59–62] can become evident from very early stages (i.e., from relatively low explant positions) during the development of eucalypt trees [44,63–67].

Surface sterilisation of the initial explant is required for contaminant-free initiation into a tissue culture medium. However, non-aseptic shoot cultures of *E. benthamii* Maiden & Cambage have been maintained successfully by incorporating an active chlorine source into all stages of the tissue culture to suppress microbial growth [68]. Surface sterilisation of eucalypt explants typically involves rinsing in non-sterilised water or detergent solution, soaking in 70% ethanol for 30–60 s, immersing in a sterilant such as mercuric chloride ($HgCl_2$), sodium hypochlorite (NaOCl), or calcium hypochlorite ($Ca(OCl)_2$) with constant agitation (Table A1), and then rinsing in sterile distilled water. Other surface sterilants such as hydrogen peroxide (H_2O_2), combinations of H_2O_2 and ethanol, and alkyldimethylbenzalkonium chloride have been used occasionally for decontaminating eucalypt explants [47,69–76]. A drop of detergent or wetting agent such as Tween 20® is often added to the solution to improve contact between the sterilant and the explant surface, which is often hairy in the case of eucalypt shoots or leaves. The use of chlorine-based sterilants such as NaOCl or $Ca(OCl)_2$ is strongly recommended over the use of $HgCl_2$ because of the high mammalian toxicity and long-term environmental persistence of $HgCl_2$ [35,77,78]. Eucalypt explants are generally treated with NaOCl at concentrations of 67–1340 mM for 1–30 min (Table A1). However, there can be a fine balance between applying sufficient sterilant to prevent microbial contamination and applying so much sterilant that it reduces seed germination or shoot growth. For example, raising the NaOCl concentration progressively from 134 to 402 to 670 mM reduced seed germination of *C. torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson × *C. citriodora* from 88% to 74% to 64%, respectively, and it reduced the percentage of plated seeds with shoots of sufficient length (>5 mm) for subculture from 78% to 65% to 52%, respectively [45].

An optimal balance of medium components including mineral salts, vitamins, organic supplements, and hormones contributes to the success of tissue culture for eucalypts and other plants. However, eucalypt explants are often placed initially onto simple culture media with minimal additives. Half- or full-strength Murashige and Skoog (MS) basal salts or media [79] are commonly used during culture establishment, typically with the addition of no organic additives other than 58.4 mM (2%) or 87.6 mM (3%) sucrose (Table A1). Establishment media sometimes include additives such as myo-inositol, thiamine, biotin, or calcium pantothenate, with or without hormones such as benzyladenine (BA), naphthalene acetic acid (NAA), or kinetin (Table A1). Reduced levels of mineral nutrients have also been used in some establishment media. For example, reduced levels of NH_4NO_3 and KNO_3 have been used in MS-based establishment media for *C. citriodora* nodes [80] and greatly reduced $CaCl_2$ levels have been used during in vitro germination of *E. dunnii*, *E. globulus*, and *E. saligna* seeds [81–84].

Eucalypt initiation and shoot proliferation are usually performed on semi-solid media that incorporate gelling agents such as 6–8 g L^{-1} agar, 1.5–4.0 g L^{-1} Gelrite, or 1.5–2.5 g L^{-1} Phytagel, which are adjusted to pH between 5.6 and 6.0 (Table A1). However, liquid media have been used to establish *E.* × *phylacis* L.A.S.Johnson & K.D.Hill nodes and shoot tips into culture [71]. Paper supports over liquid MS salts have been used during establishment of axillary shoot tips of *C. citriodora* trees into culture [69], while seeds of *C. maculata* (Hook.) K.D.Hill & L.A.S.Johnson, *E. sideroxylon* A.Cunn. ex Woolls, and *E. urophylla* have been germinated on sterile moistened filter paper prior to transfer to semi-solid media for shoot culture or callogenesis [85–87]. Anti-browning agents such as polyvinylpyrrolidone (PVP), ascorbate, and activated charcoal are sometimes added to the establishment medium to improve eucalypt explant survival [16,88–95]. Antibiotics can also be used during establishment to reduce bacterial or fungal contamination. Caution is warranted in the use of antibiotics because they may have only a bacteriostatic or fungistatic effect, with contaminants emerging at later and more-costly stages of the tissue culture process [96]. Establishment of eucalypt explants, including the germination of seeds, is usually performed in the light, although cultures are sometimes established in darkness if the primary explant is being used to induce callus.

3. Shoot Culture

Shoot culture relies on the capacity to promote the outgrowth of existing axillary and accessory buds that occur at the base of each leaf axil. Eucalypt shoots have mostly been proliferated on full-strength, or sometimes half-strength, MS media or MS salts (Table A1). Media that use MS salts are usually supplemented with organic additives, including some that are constituents of MS medium (e.g., 500–555 μM myo-inositol, 1–6.25 μM thiamine-HCl, 4.06 μM nicotinic acid, 2.43 μM pyridoxine-HCl, and/or 26.64 μM glycine) and some that are not (e.g., 0.4 μM biotin and/or 0.2 μM calcium pantothenate). Other proliferation media for eucalypts have included woody plant medium (WPM) [94,97], JADS media [98–100], DKW medium [101], mixtures of MS and de Fossard nutrients [102], and MS basal salts with either White vitamins or B5 vitamins [85,87,90,103]. Shoot proliferation is performed in the light, usually on semi-solid medium containing 58.4 mM or 87.6 mM sucrose (Table A1). Sucrose at 87.6 mM has been used in proliferation media for *Corymbia* species and hybrids even when 58.4 mM sucrose was used in the establishment medium [45,46,64,69,87]. A lowered sucrose level of 43.8 mM has been used during elongation [92] or both proliferation and elongation [93] of *E. benthamii* × *E. dunnii* shoots. The sucrose level has been dropped from 58.4 mM in the proliferation phase to 29.2 mM to promote elongation of *E. grandis* × *E. urophylla* shoots prior to transfer to root induction media [104].

Mineral nutrient levels have sometimes been adjusted in MS-based media for eucalypt shoot proliferation. The concentrations of KNO_3 and NH_4NO_3 have been reduced by half in full-strength MS media for shoot culture of *C. citriodora* [80]. Shoots of *C. torelliana* × *C. torelliana* exhibit micronutrient deficiencies in half-strength MS medium, but shoot proliferation and shoot length are not increased by using full-strength MS medium or by doubling the micronutrient levels in half-strength MS medium [46]. A greatly decreased KNO_3 concentration of 1.88 mM, but increased $MgSO_4 \cdot 7H_2O$ concentration of 3.76 mM, has been used during the establishment and proliferation of *E. dunnii*, *E. grandis* × *E. camaldulensis*, *E. grandis* × *E. urophylla*, and *E. urophylla* × *E. grandis* shoots [105]. Calcium chloride concentrations are often reduced to one-sixth of their full-strength MS levels, including for proliferation of *E. globulus* and *E. saligna* shoots [82,83].

Eucalypts have multiple buds within each leaf axil [106]. Outgrowth of these buds is promoted by cytokinins such as 0.44–6.66 μM BA and, occasionally, 0.23–9.29 μM kinetin that are added to the proliferation medium (Table A1). Cytokinins are sometimes supplemented with an auxin, usually 0.05–5.4 μM NAA. Auxins can promote eucalypt rooting and shoot elongation in cytokinin-free proliferation media [45,46]. However, cytokinins prevent adventitious rooting and so it is unclear why auxin rooting hormones are added to shoot proliferation media that contain cytokinins. Cytokinin levels are often reduced during long-term maintenance of cultures or for a single passage prior to root induction [80,92,93,107,108]. The gibberellins, GA_3 at 0.29–0.58 μM or GA_4 at 0.5 or 1 μM, have been added for a single passage to promote elongation of *E. benthamii* × *E. dunnii* and *E. impensa* Brooker & Hopper shoots, respectively, prior to root induction [70]. Shoot culture often provides lower plant production rates than alternative methods that include a callus phase, but the repeated use of intact organs is thought to minimize the risk of releasing or inducing somaclonal variation [35,45,46]. Genetic variation has been reported after shoot culture of *E. camaldulensis* and *E. tereticornis* clones, although much of this variation was attributed to mislabelling during the tissue culture process [16,109].

4. Organogenesis

Organogenesis involves the formation of adventitious buds in tissues that would not have otherwise formed buds. The production of adventitious eucalypt shoots usually seems to occur through an intervening callus phase (i.e., by indirect organogenesis) [35], although the anatomical origin and development of the new shoots is often not investigated thoroughly. Callus can be induced from eucalypt hypocotyls, cotyledons, nodes, internodes, shoot apices, leaves, immature flowers, and stamens (Table A1). Explants from the base of the seedling such as the hypocotyls and cotyledons are typically the most responsive because these organs contain juvenile cells that have undergone minimal

ageing [44,54,55,67]. However, eucalypt shoots regenerating from the hypocotyls are often easier to proliferate subsequently than shoots regenerating from the cotyledons.

Eucalypt callogenesis and shoot regeneration are usually performed on full- or half-strength MS salts or MS medium (Table A1). However, SP medium [108,110–112], WPM minerals [113], WPM [114], B5 medium [115–117], N7 medium [86,118], JADS medium [98], EDM [119], and MS medium with White vitamins [89] have also been used. Callus is often induced in darkness, although basal organogenesis on shoots can occur in eucalypt shoot cultures that are maintained under light [45,46,64,98,120]. Callogenesis, shoot regeneration, and shoot elongation are performed on semi-solid media, typically containing 87.6 mM or, occasionally, 58.4 mM sucrose (Table A1). However, liquid MS media have been used to induce axillary bud-break on *C. citriodora* nodal explants, and to establish shoot tips and nodes of *E.* × *phylacis*, prior to callogenesis on semi-solid media [71,106].

Mineral nutrient levels have only occasionally been adjusted in MS-based media for eucalypt callogenesis and shoot regeneration. Half-strength KNO_3 and NH_4NO_3 have been used in full-strength MS medium for *E. microtheca* F.Muell. organogenesis [121]. Standard and double MS concentrations of boron and calcium have both been used for organogenic culture of *E. grandis* [122], whereas $CaCl_2$ has been eliminated from N7 medium during callogenesis, but incorporated during shoot regeneration, of *E. urophylla* [86]. An MS medium with 4.90 mM NH_4NO_3, 5.68 mM K_2SO_4, 1 µM $CuSO_4{\cdot}5H_2O$, and no KNO_3 has been employed for organogenesis and subsequent shoot development of *E. grandis* × *E. urophylla* [123].

Organogenesis from eucalypt explants is induced using plant growth regulators, particularly 0.05–5 µM BA or a combination of 0.22–5 µM BA with 0.05–16 µM NAA (Table A1). Low cytokinin concentrations tend to be used when the callogenesis medium is supplemented with additives such as 100 mL L^{-1} coconut water. Other cytokinin–auxin combinations have been used for organogenesis, including 1.1 µM BA with 28.5 µM IAA for *E. grandis* and *E. grandis* × *E. urophylla* [124], 5 µM BA with 1 µM 2,4-D for *E. tereticornis* [125], 4.65 µM kinetin with 5.4 µM NAA for *E. microtheca* [121], and 5 µM kinetin with 10 µM NAA for *E. stricklandii* Maiden [126]. Thidiazuron (TDZ) and 2-Cl-PBU (i.e., 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea and *N*-phenyl-*N*′-[6-(2-chlorobenzothiazol)-yl] urea, respectively) are potent promotors of callus formation in eucalypts. Organogenesis can be induced using 0.89 µM BA with 0.91 µM TDZ, or 0.5 µM TDZ with 0.2 µM 2,4-D for *E. globulus* [127,128], 2 µM TDZ, or 0.23 µM TDZ with 0.05 µM NAA, or 3 µM TDZ with 0.1 µM NAA for *E. grandis* × *E. urophylla* [89,123,128], and 2.27 µM TDZ with 0.54 µM NAA, or 1.14 µM 2-Cl-PBU with 0.57 µM IAA for *E. urophylla* [89,111,112]. Picloram (i.e., 4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid) has been used alone at 20.7 µM for *E. urophylla* organogenesis [86] or at 0.04 µM in combination with 2.25 µM BA for *E. gunnii* Hook.f. organogenesis [129].

Adventitious eucalypt shoots usually appear to form via an intervening callus phase, but some media formulations and explant types may favour direct organogenesis. For example, nodular regenerating structures form on the hypocotyls of *E. globulus* zygotic embryos plated onto MS medium containing 16.2 µM NAA [47]. These resemble somatic embryos, but microscopic examination demonstrates that they are formed via an organogenic, rather than an embryogenic, developmental pathway. These authors [47] suggested that the numerous reports of eucalypt somatic embryogenesis using similar protocols may, in fact, have described organogenesis, and that histological examination of numerous serial sections is required to confirm an embryogenic pathway. Similarly, some reports of eucalypt organogenesis from nodes must be treated with caution because eucalypt leaf axils contain multiple accessory buds, which may be released from dormancy by the same growth regulators, especially cytokinins, that induce callus formation. Callus overgrowth may conceal these growing buds, and so shoot formation could be occurring by either (or both) axillary shoot proliferation or adventitious shoot regeneration. The use of callus to regenerate shoots can provide very rapid plant production, but it also has the potential to release or induce somaclonal variation [35,45]. There are only two reports of possible somaclonal variation arising during organogenesis of eucalypts. Haploid and triploid variants have been identified following callogenesis and organogenesis of *E. urophylla* [130]

and amplified fragment length polymorphism analysis has identified genetic variation within clones, without apparent phenotypic variation, following callogenesis and organogenesis of *E. globulus* [131].

5. Somatic Embryogenesis

Somatic embryogenesis involves the formation of bipolar structures (i.e., with both a shoot meristem and a root meristem), typically along a morphological and physiological pathway that resembles the development of zygotic embryos. Histological examination is required to ultimately confirm the developmental pathway of embryo-like structures, although this has not been attempted in many reports of eucalypt embryogenesis [47]. In practice, the morphology of the proliferative tissue might not be important provided that the tissue can be converted easily into either somatic emblings or plantlets.

Eucalypt somatic embryos appear to arise from zygotic embryos, hypocotyls, cotyledons, internodes, leaves, and shoot apices, although most studies have used zygotic embryos, hypocotyls, or cotyledons (Table A1), which are derived from the most-juvenile region of the plant [54,55]. Much research on eucalypt somatic embryogenesis has focused on species, particularly *E. globulus* and its related species, *E. dunnii* and *E. nitens*, that are difficult to propagate from cuttings [14,27,29,32–34].

Eucalypt somatic embryogenesis is initiated on semi-solid MS-based media typically with 87.6 mM or, sometimes, 58.4 mM sucrose (Table A1). However, B5 medium with 146 mM sucrose has been used for initiating somatic embryos on *C. citriodora* cotyledons [132], and N7 medium [118] has been used for callogenesis and somatic embryo formation from *E. urophylla* hypocotyls [86]. MS nutrient levels are generally not adjusted for somatic embryogenesis although half-strength MS medium with one-sixth $CaCl_2$ has been used for *E. dunnii* [81] and MS medium with half-strength KNO_3 and NH_4NO_3 has been used for *E. microtheca* [121]. Embryogenic callus is often, though not always, induced in darkness.

The induction of embryogenic tissue in eucalypts has been achieved using a diverse array of plant growth regulators (Table A1). NAA at concentrations between 10.8 and 81.0 μM has been used for somatic embryogenesis from *C. citriodora*, *E. camaldulensis*, *E. dunnii*, and *E. globulus* zygotic embryos, cotyledons, or germinating seedlings, sometimes with the addition of 100 mL L^{-1} coconut water or 0.5–1 g L^{-1} casein hydrolysate [73–76,81,132–134]. NAA at 5.4 μM has been used in combination with 2.22 μM BA or 4.52 μM 2,4-D for inducing embryogenic callus from zygotic embryos, hypocotyls, or cotyledons of *E. globulus* and *E. nitens* [134,135], with coconut water at 100 mL L^{-1} incorporated into the callogenesis medium for *E. nitens* [135]. Callogenesis has been induced using 100 μM indole-3-butyric acid (IBA) for *E. globulus* hypocotyls or cotyledons [136], while 2.22 μM BA has been used for callogenesis from *E. camaldulensis* hypocotyls [133]. Picloram at 20.7–50.0 μM induces somatic embryogenesis from *E. grandis* cotyledons, *E. globulus* hypocotyls and cotyledons, and *E. globulus* and *E. saligna* × *E. maidenii* F.Muell. shoot apices and leaf explants [136–138]. There are surprisingly few reports of TDZ-induced somatic embryogenesis in eucalypts, although a medium containing 2.37 μM kinetin, 21.6 μM NAA, and 0.45 μM TDZ induces embryogenic callus on *E. microtheca* internodes [121].

Embryogenic calli are typically transferred to standard shoot proliferation media for embryo development, including hormone-free media [81,86] and media containing 4.44–5 μM BA and 0.54–2.70 μM NAA [132,133,135,136]. Somatic embryos can then be germinated on hormone-free MS medium [73–76,132,134], MS medium with 0.89 μM BA, and 1.08 μM NAA [74], MS medium with 1.24 μM BA, 2.46 μM kinetin, and 2.48 μM NAA [74], or half-strength MS medium with 2.22 μM BA and 0.54 μM NAA [133]. Embryo germination has also been performed on filter paper suspended over liquid MS medium containing 0.44 μM BA [138].

6. Adventitious Root Formation

Germination of the bipolar structures formed during somatic embryogenesis requires media that stimulate growth from the existing root and shoot meristems. In contrast, the unipolar structures formed during shoot culture or organogenesis usually must be converted into plantlets by inducing adventitious roots at the base of the shoot. Root induction on eucalypt shoots is typically performed

on semi-solid media similar to those used during the shoot establishment and proliferation phases (Table A1). However, the sucrose concentration is sometimes reduced from 87.6 mM to 58.4 mM or from 58.4 mM to 43.8 mM, and root induction is often performed in darkness. Glucose at 88 or 176 mM has been used, instead of sucrose, during root induction on *E. globulus* and *E. saligna* shoots [139]. Activated charcoal at 83.3–833 mM is often incorporated into the root induction media, including for *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. grandis* × *E. urophylla*, *E. regnans* F.Muell., and *E. saligna* [14,82–84,88,139–144]. Activated charcoal may act by adsorbing inhibitory compounds, decreasing phenolic oxidation, altering medium pH, or reducing irradiance at the base of the shoot [145,146].

Levels of mineral nutrients are often reduced during the root induction phase for eucalypt shoots. Mineral adjustments have included the use of MS salts with 2.74 mM NaH_2PO_4, or a reduction in MS-medium strength to 1/10, for *C. citriodora* shoots [69,147]. They have also included the use of MS medium with half-strength macronutrients, or half-strength nitrates, for *E. camaldulensis* shoots [116,143], or the use of MS macronutrients with half-strength micronutrients for *E. grandis* × *E. urophylla* shoots [148]. More often, MS medium is simply reduced to half-strength during root induction, including for shoots of *C. citriodora* × *C. torelliana*, *C. ptychocarpa* (F.Muell.) K.D.Hill & L.A.S.Johnson,*C. torelliana* × *C. citriodora*, *E. camaldulensis*, *E. camaldulensis* × *E. tereticornis*, and *E. grandis* × *E. urophylla* [88,103,107,120,149,150]. Half-strength MS medium with 1/10 KNO_3 and 2.5× $MgSO_4$ has been used for *E. grandis* shoots [104], and half-strength MS medium with full-strength vitamins, 2.66 µM riboflavin, and 0.93 µM β-carotene has been used recently for *E. grandis* × *E. urophylla* shoots [114]. MS medium has also been reduced to quarter-strength during root induction, including for *E. grandis*, *E. grandis* × *E. nitens*, and *E. grandis* × *E. urophylla* shoots [124,151–153]. MS nutrients at quarter-strength, but with half- or three-quarters-strength $CaCl_2$ and $MgSO_4$, have been used for root induction on *E. grandis* × *E. nitens* and *E. grandis* × *E. urophylla* shoots [154,155]. MS macronutrients at quarter-strength but with one-eighth-strength nitrogen sources and full-strength micronutrients have been used for *E. marginata* Donn ex Sm. root induction [156].

MS-salt strength has been reduced from half to 3/10 for root induction on *E. globulus*, *E. grandis*, and *E. saligna* shoots [14,82–84,124,139,140]. This includes the use of 3/10-strength MS salts with no Fe, 3 mM Ca, 18 mM NO_3, and 60 µM Zn for *E. globulus* shoots [84]. MS macro-salts at quarter-strength with full-strength micro-salts and 1/20-strength Fe.Na.EDTA have also been used for *E. globulus*, as have half-strength MS salts without NH_4NO_3 but with quarter-strength Fe.Na.EDTA [157,158]. Other media used for root induction have included WPM minerals or Knop's medium [159] for *E. camaldulensis* [16,113], B5 medium for *E. globulus* [117], SP medium with MS micronutrients for *E. grandis* × *E. urophylla* [110], Knop macronutrients, MS micronutrients, and de Fossard organics without KI and riboflavin for *E. nitens* [101], half-strength DKW medium for *E. pellita* F.Muell. [100], Hoagland's salts [160] for *E. regnans* [141], and SP medium for *E. urophylla* [111].

Eucalypt shoots sometimes produce roots spontaneously in hormone-free medium or potting mix [45,46,64,88,92,96,104,113,119,143,144,150,161]. However, adventitious rooting on eucalypt shoots is usually induced with an auxin rooting hormone, typically IBA at a concentration between 0.49 and 49 µM (Table A1). IBA has also been used in combination with NAA to induce adventitious eucalypt roots, including 5 µM IBA with 0.5 or 1.0 µM NAA for *E. dolorosa* Brooker & Hopper [162], 0.25 µM IBA with 0.25 µM NAA, or 0.5 µM IBA with 0.5 µM NAA, for *E. drummondii* Benth. × *E. rudis* Endl. [162], 0.25, 2.5, or 5 µM IBA with 0.25 or 0.5 µM NAA for *E. impensa* [70], and 2.5 µM IBA with 2.5 µM NAA for *E. sideroxylon* [85]. NAA has been used alone to induce roots at 10.8 µM for *C. citriodora* [69], 2.7 µM for *E. grandis* and *E. grandis* × *E. urophylla* [124], 1.1 or 2.7 µM for *E. pellita* [100], and 12.5 µM for *E. urophylla* × *E. grandis* [163]. IAA at 57 µM induces roots on *E. globulus* and *E. grandis* shoots [14]. Cytokinins are almost always excluded from media during the root induction phase because they are potent inhibitors of adventitious rooting in eucalypt shoots [45,46,64]. However, low levels of cytokinin have occasionally been incorporated with auxins in root induction media (e.g., 0.04 µM BA with 5.4 µM NAA for *E. urophylla* [130] and 0.4 µM BA with 5 µM IBA for *E. urophylla* × *E. grandis* [164]).

Eucalypt shoots are frequently transferred to auxin-free media after a short period on root induction medium (Table A1). This allows root and shoot elongation, which can be inhibited by long periods of exposure to exogenous auxin [165,166]. Alternatively, shoots can be transferred to potting medium immediately after auxin treatment, bypassing one of the culture passages typically associated with rooting and ex-flasking. For example, *C. torelliana* × *C. citriodora* shoots can be transferred after 3–7 days on IBA-containing medium to tubes containing sterile potting mix, with the tubes placed in sterile 1-L plastic containers that are covered initially with another container to create a humid sealed volume of 2 L [41,45,46,64,120]. Shoots of *E. benthamii* × *E. dunnii*, *E. grandis* × *E. camaldulensis*, *E. grandis* × *E. tereticornis*, and *E. grandis* × *E. urophylla* have been transferred directly ex vitro after auxin treatment [92,167]. Shoots of *E. benthamii* × *E. dunnii* and *E. grandis* × *E. camaldulensis* have also been transferred directly ex vitro without an auxin treatment, as have *E. cloeziana* and *E. dunnii* shoots [92,96,99,104,119].

The process of ex-flasking shoots and acclimatising them to nursery conditions is one of the limiting steps in the micropropagation of many plants. One of the innovations in tissue culture that has been expected to improve ex-flasking capacity of eucalypt shoots has been the use of temporary immersion systems that provide repeated cycles of shoot wetting and drying [95,161,168–171]. These systems have the potential to increase nutrient and hormone uptake by repeatedly refreshing the medium in contact with the shoot surface during the wetting cycles while also conditioning the shoot for ex vitro conditions during the drying cycles (e.g., by promoting cuticle formation [170–172]). Temporary immersion has increased proliferation more than 2-fold and increased nursery survival from 5% to 67% for shoots of *E. grandis* × *E. nitens* [168], and increased proliferation more than 5-fold for shoots of *E. grandis* × *E. urophylla* [171]. Temporary immersion and continuous immersion systems have both provided high rooting (100%) and nursery survival (76%) with *E. camaldulensis* shoots [161]. Another technique that supports the acclimatisation capacity of eucalypt shoots is photoautotrophic culture, in which shoots are maintained under conditions of high CO_2 concentration, but low sugar concentration, to promote photosynthetic carbon fixation and transpiration [173–177]. Photoautotrophic culture has provided excellent nursery survival with shoots of *E. camaldulensis* (86–96%) and *E. urophylla* × *E. grandis* (100%) [175–177].

7. In Vitro Preservation

One of the advantages of tissue culture is the capacity to preserve germplasm in vitro for long periods without the large investments in land, labour, water, fertiliser, and pesticide that would be required for plantation- or nursery-based germplasm archives [42,51–53,178,179]. In vitro storage can also delay the maturation of valuable clones, especially if their shoots or callus are stored under minimal-growth or nil-growth conditions [39,40,42,180–182]. Plantation trees generally display higher adventitious rooting capacity, stem growth, internode length, and developmental commitment to vegetative growth when they are propagated from juvenile, rather than mature, explants or cuttings [54,55,61,62,183,184]. However, many eucalypt species progress through some of these juvenile-to-mature phase transitions at a very young age and low canopy height [11,39,40,44,63,64,67,185]. This may be the one of the reasons why seeds (or in vitro seedlings) have been the initial explant source in 54% of the eucalypt tissue-culture techniques in which an explant source has been stated (Table A1). Propagation of selected adult trees often relies on the ability to obtain juvenile tissue at the base of the tree by inducing coppice shoots or epicormic shoots [54,55]. Shoot tips, nodes, or axillary buds from nursery stock plants or adult trees have been the initial explant source in 46% of the eucalypt tissue-culture techniques in which an explant source has been stated (Table A1). This includes 24% of the techniques that used explants from nursery stock plants, 18% that used explants from the canopy of adult trees, and 4% that used explants from coppice shoots or epicormic shoots at the base of adult trees (Table A1). Coppice and basal epicormic shoots may be more juvenile than upper-canopy shoots but they are not as juvenile as seedling explants. Some plantation growers subculture difficult-to-root clones in vitro (e.g., for 10–12 passages) to rejuvenate their stock plant

material prior to use in the nursery. Other plantation growers have moved away from employing clonal forestry programs that clonally propagate selected adult individuals to employing vegetative family forestry programs that propagate multiple clones from selected seedling families. Tissue culture techniques such as cool storage, synthetic seed preservation, and cryopreservation can preserve juvenile tissue in vitro with little or no growth. These techniques, therefore, have great potential to improve nursery efficiency and tree productivity in forestry plantation programs. Nonetheless, there are few reports of eucalypt germplasm storage under growth-limiting conditions.

Cool storage of shoots has been attempted for *E. grandis* and *C. torelliana* × *C. citriodora*. Storage at 10 °C and reduced irradiance (4 μmol m^{-2} s^{-1}) allowed the preservation of *E. grandis* shoots on full-strength MS medium for 6 months, although shoots did not survive to 8 months [178]. However, *E. grandis* shoots could be stored for 10 months at 24–28 °C on half-strength MS medium, or on full-strength MS medium with 37.8 μM abscisic acid (ABA) [178]. Shoots of *C. torelliana* × *C. citriodora* have been stored on half-strength MS medium for 12 months at 14 °C and reduced irradiance (10 μmol m^{-2} s^{-1}) [40]. These shoots were subsequently ex-flasked and their performance as nursery stock plants compared with plants of the same clones that had been stored for the same 12-month period either ex vitro in the nursery or in vitro at 25 °C. Cool storage at 14 °C delayed clonal maturation, with adventitious rooting and total root mass of many clones being higher after cool storage than after ex vitro nursery storage [40]. Adventitious rooting was sometimes also higher after cool storage at 14 °C than after storage at 25 °C [40], providing empirical evidence that minimal-growth storage can delay germplasm maturation and improve subsequent plant growth.

Synthetic seed preservation has also been attempted for *E. grandis* and *C. torelliana* × *C. citriodora*. Plant germplasm can generally be preserved, as synthetic seeds, by encapsulating small explant such as shoot tips, nodes, or axillary buds in calcium alginate [53,186–191]. Encapsulation can limit the size of the shoots, especially when the synthetic seeds are preserved under minimal-growth conditions of low temperature, reduced irradiance, or decreased nutrient supply [56,178,192–196]. Almost 50% of encapsulated axillary buds of *E. grandis* have been preserved successfully for 6 months at 10 °C and 4 μmol m^{-2} s^{-1} irradiance when the synthetic seeds, containing full-strength MS medium, were stored in jars containing a small volume of sterile distilled water [178]. Between 76% and 100% of encapsulated shoot tips or nodes of *C. torelliana* × *C. citriodora* have been preserved successfully for 12 months at 14 °C in darkness when the synthetic seeds, containing highly-diluted MS medium, were stored in Petri dishes containing either agar alone, agar with 29.2 mM sucrose, or MS medium with 29.2 nM sucrose [56]. The most effective storage substrate, MS medium with 29.2 mM sucrose, provided 92–100% regrowth capacity [56]. This high regrowth capacity after 12 months of storage means that synthetic seed techniques can provide major commercial advantages in managing the workflow requirements for propagule production in commercial laboratories. Synthetic seeds can be constructed and stored throughout the year and then retrieved in one large batch, without requiring a peak labour commitment in the weeks prior to despatch. Storage of synthetic seeds beyond 6 months or 12 months has not been tested for either *E. grandis* or *C. torelliana* × *C. citriodora*, respectively. Further research is warranted to determine whether synthetic seeds could be stored for much longer than 1 year. If this were the case, alginate encapsulation would provide an extremely convenient, low-cost, and space-effective means to preserve germplasm.

Cryopreservation has been attempted for *E. globulus*, *E. grandis*, *E. grandis* × *E. camaldulensis*, *E. grandis* × *E. urophylla*, *E. gunnii*, and *E. gunnii* × *E. dalrympleana* Maiden. Cryopreservation has proven challenging because of the desiccation sensitivity of eucalypt buds [52,197]. However, axillary buds of *E. grandis* × *E. camaldulensis* have been cryopreserved successfully, with 49% regrowth, by placing encapsulated explants on semi-solid MS media with progressively increasing sucrose and glycerol concentrations (each 0.4, 0.7 then 1.0 M), drying them in empty Petri dishes to a moisture content ≤25% before freezing, and re-growing them on media with progressively decreasing sucrose and glycerol concentrations [198]. *E. gunnii* has also been cryopreserved successfully, with 62–73% regrowth, by transferring encapsulated shoot tips into liquid media with progressively increasing

sucrose concentrations (0.3, 0.5, 0.75 then 1.0 M), drying them over silica gel before freezing, and re-growing them on MS medium with BA, NAA, and 87.6 μM sucrose [199]. The same technique provided 43% and 13% regrowth from alginate-encapsulated shoot tips of *E. gunnii* × *E. dalrympleana* and *E. globulus*, respectively [199]. Shoot-tip and axillary-bud cryopreservation has proven challenging for some eucalypts, but it has a major advantage over cool storage and synthetic seed preservation in potentially being able to store plant germplasm for many years without the need for periodic subcultures for recovery and re-storage. Cryopreservation has been used very successfully to store embryogenic callus of other tree species [36,48], but there are no reports of embryogenic-callus cryopreservation for eucalypt species.

8. Conclusions

Tissue culture provides a means to rapidly propagate selected eucalypt trees, or their progeny, in a clonal forestry or vegetative family forestry program. Eucalypt tissue cultures are usually initiated from shoot tips, nodes, axillary buds, or seeds, typically after surface sterilisation using detergent, aqueous ethanol solution, and sterilants such as NaOCl or $Ca(OCl)_2$. Eucalypt plants can be multiplied through: (1) shoot culture, by proliferating shoots from existing axillary and accessory buds in the leaf axils; (2) organogenesis, by inducing adventitious buds, often through an intervening callus phase; or (3) somatic embryogenesis, by forming bipolar structures with both a shoot and root meristem, often following formation of an embryogenic callus. Eucalypt tissue culture is often performed on semi-solid MS-based media, although a wide range of media formulations and support systems have been employed. Shoots arising from shoot culture or organogenic culture are converted into plantlets using an auxin such as IBA to induce adventitious roots, although some eucalypts form adventitious roots spontaneously in the absence of exogenous auxin. Ex-flasking capacity can be improved by techniques such as temporary immersion and photoautotrophic culture that pre-acclimatise shoots for transfer to nursery conditions. There are few reports of eucalypt germplasm conservation in vitro despite the multitude of techniques for eucalypt plantlet or embling production. Nonetheless, cool storage, synthetic seed storage, and cryopreservation have all been successful, albeit following attempts with only a few eucalypt species. These preservation techniques for eucalypt germplasm have been under-utilised, given that in vitro preservation can delay or prevent the maturation of juvenile clones prior to their mass-production for hardwood plantations. The development of efficient clonal-propagation methods for eucalypts has been one of the great challenges in hardwood forestry. Micropropagation and in vitro preservation are now contributing to provide the best-possible hardwood trees for the global plantation estate.

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Appendix A

Table A1. Culture techniques, explants, and media used in the micropropagation of eucalypts. Media are aseptic and semi-solid unless stated otherwise.

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Corymbia citriodora					
Shoot culture [69]	Seeds 3.53 M H_2O_2 for 15 min	Murashige and Skoog (MS) salts with 58.4 mM sucrose	MS salts with 555 µM myo-inositol, 2.96 µM thiamine, 26.7 µM benzyl adenine (BA), 5.4 µM 1-naphthalene acetic acid (NAA), and 87.6 mM sucrose	MS salts with 2.74 mM NaH_2PO_4 or Schenk and Hildebrandt (SH) salts [200], each with 555 µM myo-inositol, 2.96 µM thiamine, 10.8 µM NAA, and 58.4 mM sucrose	8 g L^{-1} agar pH 5.5–5.7
Shoot culture [69]	Axillary shoot tips from adult trees 1.84 mM $HgCl_2$ for 10 min	Paper support over liquid MS salts with 555 µM myo-inositol, 2.96 µM thiamine, 26.7 µM BA, 2 µM NAA, and 87.6 mM sucrose	MS salts with 555 µM myo-inositol, 2.96 µM thiamine, 26.7 µM BA, 5.4 µM NAA, and 87.6 mM sucrose	MS salts with 2.74 mM NaH_2PO_4 or SH salts, each with 555 µM myo-inositol, 2.96 µM thiamine, 10.8 µM NAA, and 58.4 mM sucrose	8 g L^{-1} agar pH 5.5–5.7
Sugar-free shoot culture [173]	Shoots from established in vitro cultures from 8-year-old trees	–	–	Modified liquid MS medium with 0.1 µM indole-3-butyric acid (IBA) and no sucrose, in a rockwool system with 3000 µmol mol^{-1} CO_2	–
Shoot culture [80]	Nodal segments from 7-year-old trees 67 mM NaOCl for 20 min	Modified MS medium with $\frac{1}{2}$ NH_4NO_3, $\frac{1}{2}$ KNO_3, 0.9 µM BA, and 58.4 mM sucrose	Proliferation on modified MS medium with $\frac{1}{2}$ NH_4NO_3, $\frac{1}{2}$ KNO_3, 10% coconut water, 4.4 µM BA, and 58.4 mM sucrose, followed by same medium but with 0.9 µM BA	Modified MS medium with $\frac{1}{2}$ NH_4NO_3, $\frac{1}{2}$ KNO_3, 0.98 µM IBA, and 58.4 mM sucrose in a phenol resin foam	2.5→2 g L^{-1} gellan gum pH 5.8
Sugar-free shoot culture [174]	Shoots from established in vitro cultures	–	–	Liquid MS medium with 0.1 µM IBA and no sucrose, in a rockwool system under 80% red + 20% blue light emitting diodes (LEDs) and 3000 µmol mol^{-1} CO_2	–
Shoot culture [147]	Nodal segments from in vitro seedlings	–	MS medium with 9.3 µM kinetin	1/10 MS medium with 9.84 or 14.76 µM IBA	–
Organogenesis from internodes [106]	Axillary buds from mature trees 3.68 mM $HgCl_2$ for 3 min, with pre-treatment using 0.8% polyvinylpyrrolidone (PVP) at 3–6 °C for 36–48 h	MS medium with 120 µM thiamine-HCl	Bud break in liquid MS medium with 120 µM thiamine-HCl, 4.44 µM BA, and 5.37 µM NAA, then organogenesis on semi-solid $\frac{1}{2}$ MS medium with 1.11 µM BA and 5.37 µM NAA	–	8 g L^{-1} agar pH 5.8
Somatic embryogenesis [132]	Seeds 3.68 mM $HgCl_2$ for 5 min	Embryo plated onto B5 medium with 16.2 µM NAA and 146 mM sucrose, and cotyledon-derived somatic embryos subcultured onto the same medium	B5 medium with 27 µM NAA, 500 mg L^{-1} casein hydrolysate, 3.42 mM glutamine, and 87.6 mM sucrose	Germination in B5 medium with 58.4 mM sucrose	1.5 g L^{-1} Gelrite

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
C. citriodora* × *C. torelliana					
Shoot culture [107]	Nodal segments from a 17-year-old tree 3.68 mM $HgCl_2$ for 20 min	MS medium	MS medium with 6.67 µM BA, 5.4 µM NAA, and 87.6 mM sucrose, then long-term subculturing on MS medium with 4.44 µM BA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 24.6 or 49.2 mM IBA and 73 mM sucrose	7 g L^{-1} agar pH 5.8
C. maculata					
Shoot culture [87]	Seeds 134 mM NaOCl for 20 min	Moistened filter paper, then germinants transferred to MS basal medium with 60 mM sucrose	MS basal salts, Gamborg B5 vitamins [201], 1 µM BA, 0.05 µM NAA, 1.17 mM 2-(N-morpholino)ethanesulfonic acid (MES), and 90 mM sucrose	MS basal salts, Gamborg B5 vitamins, 0.4 µM calcium pantothenate, 0.4 µM biotin, 30 µM IBA, 6–25 µM silver thiosulphate (STS), 1.17 µM MES, and 90 mM sucrose, followed by $\frac{1}{2}$ MS basal salts, Gamborg B5 vitamins, 0.4 µM calcium pantothenate, 0.4 µM biotin, 1.17 µM MES, and 60 mM sucrose	7 g L^{-1} agar
C. ptychocarpa					
Shoot culture [103]	Nodal segments from a 3-year-old tree 3.68 mM $HgCl_2$ for 10 min	MS medium with 2.22 µM BA, 0.54 µM NAA, and 82.7 µM VB_2	MS medium with 6.67 µM BA, 1.1 µM NAA, and 82.7 µM VB_2	$\frac{1}{2}$ MS medium with 7.38 µM IBA	–
C. torelliana* × *C. citriodora					
Shoot culture [45]	Seeds 134 mM NaOCl for 10 min	$\frac{1}{2}$ MS basal salts with 58.4 mM sucrose	$\frac{1}{2}$ MS medium with 0.05 or 0.27 µM NAA and 87.6 mM sucrose, then $\frac{1}{2}$ MS medium with 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 4.9 µM IBA and 87.6 mM sucrose for 3 days, then sterile perlite and pine bark	8 g L^{-1} agar pH 5.8
Shoot culture [149]	Nodes from 30–32-year-old trees 5.52 mM $HgCl_2$ for 10 min	MS medium with 6.67 µM BA, 2.7 µM NAA, and 87.6 mM sucrose	MS medium with 4.44 µM BA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 2.46 µM IBA and 87.6 mM sucrose	6 g L^{-1} agar
Shoot culture [46,64]	Seeds 134 mM NaOCl for 10 min	$\frac{1}{2}$ MS basal salts with 58.4 mM sucrose	$\frac{1}{2}$ MS medium with 0 or 0.05 µM NAA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 19.6 µM IBA and 58.4 mM sucrose for 7 days, then sterile vermiculite and perlite	8 g L^{-1} agar pH 5.8
Organogenesis [45]	Seeds 134 mM NaOCl for 10 min	$\frac{1}{2}$ MS basal salts with 58.4 mM sucrose	$\frac{1}{2}$ MS medium with 0 or 0.05 µM NAA and 87.6 mM sucrose, then $\frac{1}{2}$ MS medium with 2.2 µM BA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 4.9 µM IBA and 87.6 mM sucrose, then sterile perlite and pine bark	8 g L^{-1} agar pH 5.8
Organogenesis [46,64]	Seeds 134 mM NaOCl for 10 min	$\frac{1}{2}$ MS basal salts with 58.4 mM sucrose	$\frac{1}{2}$ MS medium with 0 or 0.05 µM NAA and 87.6 mM sucrose, then MS medium with 2.2 µM BA, 0 or 0.05 µM NAA, and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 19.6 µM IBA and 58.4 mM sucrose for 7 days, then sterile vermiculite and perlite	8 g L^{-1} agar pH 5.8
Organogenesis [120]	Seeds 134 mM NaOCl for 10 min	$\frac{1}{2}$ MS basal salts with 58.4 mM sucrose	MS medium with 87.6 mM sucrose, then proliferation on MS medium with 4.4 µM BA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 19.6 µM IBA and 58.4 mM sucrose for 7 days, then sterile vermiculite and perlite	8 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Eucalyptus benthamii					
Non-aseptic shoot culture [68]	Nodal segments from nursery stock plants 201 mM NaOCl for 10 min	–	MS medium with 87.6 mM sucrose, incorporating 0.001 or 0.003% active chlorine	–	7 g L^{-1} agar pH 5.8
E. benthamii* × *E. dunnii					
Shoot culture [91]	Nodal segments from nursery stock plants 67–268 mM NaOCl for 10 min	MS medium with 250 mg L^{-1} PVP and 87.6 mM sucrose	Proliferation on $\frac{1}{2}$ MS medium with 1.11 µM BA, 0.054 µM NAA, 250 mg L^{-1} PVP, and 87.6 mM sucrose, then elongation in $\frac{1}{2}$ MS medium with 0.22 µM BA, 1.35 µM NAA, 250 mg L^{-1} PVP, and 43.8 mM sucrose	–	6 g L^{-1} agar pH 5.6
Shoot culture [92]	Nodal segments from nursery stock plants 67–268 mM NaOCl for 10 min	MS medium with 250 mg L^{-1} PVP and 87.6 mM sucrose	Proliferation on $\frac{1}{2}$ MS medium with 2.2 µM BA, 0.27 µM NAA, 250 mg L^{-1} PVP, and 43.8 mM sucrose, then elongation in $\frac{1}{2}$ MS medium with 0.44 µM BA, 0.29–0.58 µM GA, 250 mg L^{-1} PVP, and 43.8 mM sucrose	$\frac{1}{2}$ MS medium with 0 or 9.84 µM IBA, 210 µM calcium pantothenate, 409 µM biotin, 555 µM myo-inositol, and 43.8 mM sucrose, or ex vitro in carbonised rice bark/vermiculite ($1/v$, v/v) after treatment with 0 or 4.92 µM IBA	6 g L^{-1} agar pH 5.8
E.* × *brachyphylla					
Shoot culture [93]	Nodal segments from nursery stock plants 201 mM NaOCl for 10 min	–	Woody Plant Medium (WPM) with 284 µM ascorbate, 400 µM cysteine, 400 mg L^{-1} PVP, 4.44 µM BA, 5.4 µM NAA, and 87.6 mM sucrose	–	8 g L^{-1} agar pH 5.8
E. camaldulensis					
Shoot culture [202]	Nodes from 2-year-old nursery plants 630 mM $Ca(OCl)_2$ for 20 min	–	MS medium with 5 µM IBA, 87.6 mM sucrose, and 0.2% charcoal	–	5 g L^{-1} agar pH 5.6
Shoot culture [203]	Shoots from long-term in vitro cultures	–	MS medium with 2.5 µM BA, 0.1 µM NAA, and 58.4 mM sucrose	–	2.5 g L^{-1} agar + 2.5 g L^{-1} Gelrite pH 5.8
Shoot culture [16]	Nodal segments from coppice shoots of 10-year-old field ramets 80 mM NaOCl for 10 min	Initiation on MS medium with 400 mg L^{-1} PVP, 2.22 µM BA, 1.16 µM kinetin, 0.029 µM gibberellic acid (GA_3), and 87.6 mM sucrose	Proliferation on MS medium with 400 mg L^{-1} PVP, 0.44 µM BA, 0.23 µM kinetin, 0.029 µM GA_3, and 87.6 mM sucrose	Knop's medium [159] with 4.9 µM IBA and 43.8 mM sucrose	6 g L^{-1} agar pH 5.7–5.9

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Organogenesis [113]	Excised leaves from established cultures	Callogenesis on WPM minerals [204] with 1 g L^{-1} casein, 0.44 µM BA, 16.2 µM NAA, and 146 mM sucrose	Shoot regeneration on WPM minerals with 1.33 µM BA and 146 mM sucrose	WPM minerals with 146 mM sucrose	5 g L^{-1} Phytagar pH 5.9
Organogenesis from hypocotyl segments [116]	Seeds 134 mM NaOCl for 15 min	MS medium	Organogenesis on B5 medium [201] with 100 mL L^{-1} coconut milk, 1.37 mM glutamine, 100 mg L^{-1} casein hydrolysate, 4.44 µM BA, 16.2 µM NAA, and 87.6 mM sucrose	Modified MS medium with half-strength macronutrients, 4.92 µM IBA, and 87.6 mM sucrose	7.5 g L^{-1} agar pH 5.7
Callogenesis [205]	Immature flowers and stamens 10% commercial bleach for 4 min	–	MS medium with 0.01 or 0.1 µM BA, 0.01 or 0.05 µM 2,4-dichlorophenoxyacetic acid (2,4-D), and 58.4 mM sucrose	–	8 g L^{-1} agar pH 5.8
Organogenesis from cotyledons [143]	Seeds 804 mM NaOCl for 20 min	$\frac{1}{2}$ MS medium with 58.4 mM sucrose	MS medium with 4.44 µM BA, 5.4 µM NAA, and 87.6 mM sucrose	MS medium with $\frac{1}{2}$-strength nitrates and 0.2% charcoal	7 g L^{-1} agar pH 5.8
Organogenesis from cotyledons [150]	Seeds 804 mM NaOCl for 20 min	$\frac{1}{2}$ MS medium with 58.4 mM sucrose	Organogenesis on MS medium or WPM with 4.44 µM BA, 2.7 µM NAA, and 58.4 mM sucrose, then multiplication on MS medium with 2.96 µM thiamine, 2.64 µM BA, 0.5 µM NAA, and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 87.6 mM sucrose	7→6→7 g L^{-1} agar pH 5.8
Somatic embryogenesis from zygotic embryos [133]	Seeds 134 mM NaOCl for 20 min	Zygotic embryos plated on MS medium with 10.8 µM NAA and 87.6 mM sucrose	Callus transferred to MS medium with 4.44 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Germination on $\frac{1}{2}$ MS medium with 2.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	8 g L^{-1} agar pH 5.8
Somatic embryogenesis from cotyledons [133]	Seeds 134 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Cotyledons plated on MS medium with 10.8 µM NAA and 87.6 mM sucrose, then calli transferred to MS medium with 4.44 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Germination on $\frac{1}{2}$ MS medium with 2.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	8 g L^{-1} agar pH 5.8
Somatic embryogenesis from hypocotyls [133]	Seeds 134 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Hypocotyls plated on MS medium with 2.22 µM BA and 87.6 mM sucrose	Germination on $\frac{1}{2}$ MS medium with 2.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	8 g L^{-1} agar pH 5.8
E. camaldulensis* × *E. tereticornis					
Shoot culture [149]	Nodes from 30–32-year-old trees 5.52 mM $HgCl_2$ for 12 min	MS medium with 4.44 µM BA and 58.4 mM sucrose	MS medium with 4.44 µM BA, 0.49 µM IBA, and 58.4 mM sucrose	$\frac{1}{2}$ MS medium with 4.92 µM IBA and 58.4 mM sucrose	6 g L^{-1} agar

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. cloeziana					
Shoot culture [96]	Nodes from epicormic shoots on harvested branches of 26-year-old trees 134 mM NaOCl for 5 min	MS medium with 2.22 µM BA and 87.6 mM sucrose	Proliferation on WPM [204] with 2.22 µM BA, then on WPM with 3.33 µM BA, 0 or 0.27 µM NAA, and 87.6 mM sucrose, then elongation on WPM with 0.44 µM BA and 87.6 mM sucrose	Vermiculite and composted pine bark (2:1, *v*/*v*) ex vitro	4 g L^{-1} agar pH 5.75–5.85
E. dolorosa					
Shoot culture [162]	Shoots from wild adult trees	–	$\frac{1}{2}$ MS medium with 0.25 µM BA and sucrose, with or without 0.25 µM NAA	$\frac{1}{2}$ MS medium with sucrose, 5 µM IBA, and 0.5 or 1 µM NAA	Agar
E. drummondii* × *E. rudis					
Shoot culture [162]	Shoots from wild adult trees	–	$\frac{1}{2}$ MS medium with sucrose, and either 0.25 µM BA and 2.5 µM kinetin or 0.5 µM BA	$\frac{1}{2}$ MS medium with sucrose and either 0.25 µM IBA and 0.25 µM NAA or 0.5 µM IBA and 0.5 µM NAA	Agar
E. dunnii					
Shoot culture [104]	Shoots from established long-term cultures	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.18 µM BA, 87.6 mM sucrose, and 2 g L^{-1} Gelrite	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.04 µM BA, 87.6 mM sucrose, and either Gelrite (semi-solid) or EM2 or M-Gel (liquid)	–	2→(2 or 0) g L^{-1} Gelrite pH 5.6
Shoot culture [94]	Nodes from coppice of 3-year-old trees 201 mM NaOCl for 10 min	MS medium with 555 µM myo-inositol, 250 mg L^{-1} PVP, and 87.6 mM sucrose	Proliferation on $\frac{1}{2}$ MS medium with 278 µM myo-inositol, 1.11–2.22 µM BA, and 87.6 mM sucrose	–	6 g L^{-1} agar pH 5.8
Shoot culture [99]	1-m length epicormic shoots 201 mM NaOCl for 20 min	*Eucalyptus dunnii* medium (EDM) [119] with 58.4 mM sucrose	Modified EDM with 0.89 µM BA, 0.54 µM NAA, and 58.4 mM sucrose	Ex vitro in composted pine bark/perlite/vermiculite (2/1/1, *v*/*v*) with the mineral components of modified EDM	6→5 g L^{-1} agar pH 5.8
Organogenesis from hypocotyls [99]	Seeds 268 mM NaOCl for 30 min	EDM without sucrose	Callogenesis on EDM with 286 mg L^{-1} Basafer® as Fe source instead of $Fe(SO_4)_2$ and Na_2.ethylenediamine tetraacetic acid (Na_2EDTA), 0.44 or 2.22 µM BA, 0.54–5.40 µM NAA or indole acetic acid (IAA), and 87.6 mM sucrose, then multiplication on EDM with 286 mg L^{-1} Basafer ® instead of $Fe(SO_4)_2$ and Na_2EDTA, 0.89 µM BA, 0.054 µM NAA, and 58.4 mM sucrose	Excised shoots placed, without auxin treatment, in sterile mixture of composted pine bark, and perlite and vermiculite (2:1:1) ex vitro	6→5 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Somatic embryogenesis [81]	Seeds 16 mM NaOCl for 15 min	$\frac{1}{2}$ MS medium with 1/6 $CaCl_2$ and 58.4 mM sucrose	3-day-old seedlings to $\frac{1}{2}$ MS medium with 1/6 $CaCl_2$ and 5.5 or 16.5 µM NAA, 1 g L^{-1} casein hydrolysate or 10% coconut milk, and 58.4 mM sucrose, then to hormone-free medium	–	8 g L^{-1} agar
E. erythronema					
Organogenesis from hypocotyls [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 5 µM BA, or 5 µM kinetin, or 1 µM BA and 5 µM NAA, or 1 µM and 10 µM NAA, each with 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from cotyledons and youngest leaves [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 1 or 5 µM BA and 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from shoot apices [206]	Seeds 402 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Organogenesis on MS medium with 0.1 or 0.25 µM BA and 87.6 mM sucrose, then proliferation on Quoirin and Lepoivre (QL) medium [207] with 2.2 µM BA, 0.5 µM NAA, and 58.4 mM sucrose	–	7 g L^{-1} Phytagel pH 5.7
E. erythronema* × *E. stricklandii					
Organogenesis from hypocotyls and cotyledons [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 5 µM BA, 5 µM NAA, and 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from youngest expanding leaves [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 1 µM BA and 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from shoot apices [206]	Seeds 402 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Organogenesis on MS medium with 0.5 µM BA and 87.6 mM sucrose, then proliferation on QL medium with 2.2 µM BA, 0.5 µM NAA, and 58.4 mM sucrose	–	7 g L^{-1} Phytagel pH 5.7
Organogenesis from shoot apices [206]	In vitro shoots established from an 18-month-old stock plant	QL medium with 0.5 µM GA and 58.4 mM sucrose	Organogenesis on MS medium with 1 µM BA and 87.6 mM sucrose, then proliferation on QL medium with 2.2 µM BA, 0.5 µM NAA, and 58.4 mM sucrose	–	7 g L^{-1} Phytagel pH 5.7
Organogenesis from leaves [206]	Seeds 402 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Organogenesis on MS medium with 0.25, 0.5, or 1 µM BA and 87.6 mM sucrose, then proliferation on QL medium with 2.2 µM BA, 0.5 µM NAA, and 58.4 mM sucrose	–	7 g L^{-1} Phytagel pH 5.7

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. globulus					
Shoot culture [157]	Nodal segments from 4–5-year-old trees 134 mM NaOCl for 20 min	Establishment and maintenance on MS medium with 2.5 µM BA, 1.25 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium that alternated 1.25 µM BA with 1.9, 2.2, or 2.5 µM kinetin from passage to passage, and 58.4 mM sucrose	Shoots from the medium with kinetin transferred to $\frac{1}{4}$-strength MS macro-salts, full-strength micro-salts, 50 µM Fe.Na.EDTA, 10 µM IBA, and 20 nM sucrose	2.5 g L^{-1} agar + 2.5 g L^{-1} Gelrite pH 5.8
Shoot culture [82,83]	Seeds 201 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 658 µM $CaCl_2$ and 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine HCl, 555 µM myo-inositol, 10 or 492 µM IBA, and 87.6 mM sucrose, then the same medium without IBA but with 83.3 mM activated charcoal	7.5 g L^{-1} agar pH 5.8
Shoot culture [158]	Nodal segments from 4–5-year old trees 134 mM NaOCl for 20 min	Establishment and maintenance on MS medium with 2.5 µM BA, 1.25 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium that alternated 2.5 µM BA with 2.5 µM kinetin from passage to passage, and 58.4 mM sucrose	Shoots from the medium with kinetin transferred to $\frac{1}{2}$ MS salts without NH_4NO_3, with $\frac{1}{4}$-strength MS Fe.Na.EDTA, 1, 2.5, or 5 µM IBA, and 58.4 mM sucrose	2.5 g L^{-1} agar + 2.5 g L^{-1} Phytagel pH 5.8→5.5
Shoot culture [140]	Seeds 335 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine, 2775 µM myo-inositol, 49.2 µM IBA, and 87.6 mM sucrose, then same medium without IBA but with 83.3 mM activated charcoal	6 g L^{-1} agar pH 5.8
Shoot culture [139]	Seeds 335 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine, 2775 µM myo-inositol, 49.2 µM IBA, and 88 or 176 mM glucose, then same medium without IBA but with 83.3 mM activated charcoal and 87.6 mM sucrose instead of glucose	6 g L^{-1} agar pH 5.8
Shoot culture [84]	Seeds 201 mM NaOCl for 15 min	$\frac{1}{2}$ MS salts with 0.5 mM $CaCl_2$ and 58.4 mM sucrose	–	0.3× MS salts with no Fe, 3 mM Ca, 18 mM NO_3, 60 µM Zn, 1.18 µM thiamine HCl, 555 µM inositol, 49.2 µM IBA, and 87.6 mM sucrose, then same medium with 30 µM Fe, 0.9 mM Ca, 18 mM NO_3, 60 µM Zn, 83.3 mM activated charcoal, and no IBA	6 g L^{-1} agar pH 5.8
Root induction on hypocotyls [208]	Seeds 1.88 M NaOCl for 30 min	MS salts with 58.4 mM sucrose	–	MS medium with 100 µM IBA	2 g L^{-1} Phytagel
Root induction on seedling apical shoots [14]	Seeds 201 mM NaOCl	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	–	0.3× MS salts with 1.18 µM thiamine, 555 µM inositol, 57 µM IAA, and 87.6 mM sucrose for 4 days then same medium without IAA but with 83.3 mM activated charcoal	6 g L^{-1} agar pH 5.8
Organogenesis from hypocotyls and cotyledons [127]	Seeds 490 mM $Ca(OCl)_2$ for 20 min	MS basal medium with 87.6 mM sucrose	MS medium with 0.89 µM BA and 0.91 µM thidiazuron (TDZ)	$\frac{1}{2}$ MS basal salts with White's vitamins [209], 14.76 µM IBA, and 58.4 mM sucrose for 3–7 days, then the same medium but lacking IBA	7 g L^{-1} agar pH 5.6

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Organogenesis and occasional somatic embryogenesis from hypocotyl segments and cotyledons [135]	Seeds 40% commercial bleach for 30 min	MS salts and 87.6 mM sucrose	Callogenesis on MS nutrients with 10% coconut water, 2.22 µM BA, 5.4 µM NAA, and 87.6 mM sucrose, then shoot regeneration on the same medium but with 4.44 µM BA and 2.7 µM NAA	MS medium with 14.76 µM IBA and 58.4 mM sucrose, then the same medium without IBA	8 g L^{-1} agar pH 5.8
Organogenesis from hypocotyls and cotyledons [128]	Seeds 503 mM NaOCl for 30 min	MS medium	Bud induction on MS medium with 0.05 µM TDZ and 0.2 µM 2,4-D, then shoot regeneration on MS medium with 5 µM BA, both with 87.6 mM sucrose	–	2 g L^{-1} Phytagel pH 5.8
Organogenesis from seeds [117]	Seeds	87.6 mM sucrose	Organogenesis on B5 medium with 10% coconut water, 0.22 µM BA, 2.7 µM NAA, and 87.6 or 146.0 mM sucrose, then B5 medium with 87.6 mM sucrose, then shoot regeneration on B5 medium with 0.22 µM BA and 87.6 mM maltose	B5 medium with 2.46 µM IBA and 87.6 mM sucrose	6.5 g L^{-1} agar pH 5.8
Organogenesis from hypocotyls [117]	In vitro seedlings	–	Organogenesis on B5 medium with 10% coconut water, 0.22–2.22 µM BA, 0–5.4 µM NAA, and 146 mM sucrose, then B5 medium with 87.6 mM sucrose, then shoot regeneration on B5 medium with 0.22 µM BA and 87.6 mM maltose	B5 medium with 2.46 µM IBA and 87.6 mM sucrose	6.5 g L^{-1} agar pH 5.8
Organogenesis from cotyledons [117]	In vitro seedlings	–	Organogenesis on B5 medium with 10% coconut water, 0.22 or 1.11 µM BA, 2.7 µM NAA, and 146 mM sucrose, then B5 medium with 87.6 mM sucrose, then shoot regeneration on B5 medium with 0.22 µM BA and 87.6 mM maltose	B5 medium with 2.46 µM IBA and 87.6 mM sucrose	6.5 g L^{-1} agar pH 5.8
Organogenesis from leaves [117]	Established in vitro cultures of selected clones	–	Organogenesis on B5 medium with 10% coconut water, 0.22 µM BA, 2.7 µM NAA, and 146 mM sucrose, then B5 medium with 87.6 mM sucrose, then shoot regeneration on B5 medium with 0.22 µM BA and 87.6 mM maltose	B5 medium with 2.46 µM IBA and 87.6 mM sucrose	6.5 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Organogenesis [47]	Seeds Ethanol/8.83 M H_2O_2 (1/1, *v*/*v*)	Zygotic embryo plated on MS medium with 16.2 µM NAA and 87.6 mM sucrose	MS medium with 87.6 mM sucrose to induce nodular structures from hypocotyls, which were transferred to fresh MS medium with 87.6 mM sucrose	–	2.5 g L^{-1} Gelrite
Somatic embryogenesis [136]	Seeds 503 mM NaOCl for 30 min	Cotyledon segments or hypocotyls plated onto MS medium with 50 µM picloram or 100 µM IBA, and 87.6 mM sucrose	Callus transferred to MS medium with 5 µM BA and 1 µM NAA (cotyledons) or 10 µM IBA (cotyledons and hypocotyls), with 87.6 mM sucrose	–	2 g L^{-1} Phytagel
Somatic embryogenesis from cotyledons [134]	Seeds 0.1% Benlate®	MS medium with 58.4 mM sucrose	Embryogenesis on MS medium with either 5.4 µM NAA + 4.52 µM 2,4-D or 16.2–27.0 µM NAA or 27.0 µM NAA + 500 mg L^{-1} casein hydrolysate + 3.40 mM glutamate, each with 87.6 mM sucrose	MS medium with 87.6 mM sucrose	3 g L^{-1} Gelrite pH 5.8
Somatic embryogenesis from zygotic embryos [134]	Seeds 0.1% Benlate®	–	Embryogenesis on MS medium with either 5.4 µM NAA + 4.52 µM 2,4-D or 16.2–81.0 µM NAA or 27.0 µM NAA + 500 mg L^{-1} casein hydrolysate + 3.40 mM glutamate, each with 87.6 mM sucrose	MS medium with 87.6 mM sucrose	3 g L^{-1} Gelrite pH 5.8
Somatic embryogenesis from zygotic embryos [73,75,76]	Seeds Ethanol/8.83 M H_2O_2 (1/1, *v*/*v*) for 15 min	–	Embryogenesis on MS medium with 16.1 µM NAA and 87.6 mM sucrose	MS medium with 87.6 mM sucrose	2.5 g L^{-1} Gelrite pH 5.8
Somatic embryogenesis [74]	Seeds Ethanol/8.83 M H_2O_2 (1/1, *v*/*v*) for 15 min then 0.1% Benlate® for 15 min	–	Embryogenesis on MS medium with 16.1 µM NAA and 87.6 mM sucrose, then MS medium with 87.6 mM sucrose	Secondary embryogenesis and germination on MS medium with or without 0.89 µM BA + 1.08 µM NAA or 1.24 µM BA + 2.46 µM kinetin + 2.48 µM NAA, each with 87.6 mM sucrose	2.5 g L^{-1} Gelrite pH 5.8
Somatic embryogenesis [138]	2-year established cultures from 12-year-old elite trees	Shoot apex and leaf explants plated on MS medium with 500 mg L^{-1} casein hydrolysate, 40 µM picloram, 40 mg L^{-1} gum Arabic, and 87.6 mM sucrose	Secondary embryogenesis on MS medium with 16.11 µM NAA, 20 µM STS, and 87.6 mM sucrose	Germination on filter paper over liquid MS medium with 0.44 µM BA and 87.6 mM sucrose	6 g L^{-1} agar pH 5.6–5.7

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. grandis					
Shoot culture [210]	Nodal segments from nursery and field stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 2 min	MS nutrients and 58.4 mM sucrose	MS nutrients, 0.89 µM BA, 0.054 µM NAA, and 58.4 mM sucrose	–	4 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture [197]	Nodal segments from nursery rooted cuttings 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 1.56 µM BA, 0.054 µM NAA, and 73 mM sucrose	MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 1.56 µM BA, 0.054 µM NAA, and 73 mM sucrose	–	3 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture [152]	Nodal segments from nursery stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	Initiation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.44 µM BA, 0.23 µM kinetin, 0.21 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.21 µM NAA, and 58.4 mM sucrose, then elongation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.93 µM kinetin, 2.1 µM IAA, 0.25 µM IBA, 1.6 µM NAA, and 58.4 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, and 43.8 mM sucrose	4 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture [93]	Nodal segments from nursery stock plants 134 mM NaOCl for 5 min	MS medium	WPM with 284 µM ascorbate, 400 µM cysteine, 400 mg L^{-1} PVP, and 87.6 mM sucrose	–	8 g L^{-1} agar pH 5.7
Shoot culture [153]	Nodal segments from nursery stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	Initiation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.44 µM BA, 0.23 µM kinetin, 0.21 µM NAA, and 58.4 mM sucrose	Proliferation on the same medium used for initiation, then elongation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.93 µM kinetin, 0.49 µM IBA, 1.62 µM NAA, and 58.4 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, and 43.8 mM sucrose	4 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture with basal organogenesis [98]	Subapical nodes of in vitro seedlings	Immersion in 889 µM BA solution for 1–2 h	JADS medium [97]	–	–
Root induction on seedling apical shoots [14]	Seeds 201 mM NaOCl	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	–	0.3× MS salts with 1.18 µM thiamine, 555 µM inositol, 0 or 57 µM IAA, and 87.6 mM sucrose for 96 h, then the same medium without IAA but with 83.3 mM activated charcoal	6 g L^{-1} agar pH 5.8
Organogenesis [113]	Excised leaves from established cultures	Callogenesis on WPM minerals with 1 g L^{-1} casein, 0.44 µM BA, 16.2 µM NAA, and 146 mM sucrose	Shoot regeneration on WPM minerals with 1.33 µM BA and 146 mM sucrose	–	5 g L^{-1} Phytagar pH 5.9

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Organogenesis [124]	2–3 mm shoot segments from long-term in vitro cultures, with the apical and axillary buds removed	–	Callogenesis on MS medium with 1.11 µM BA, 28.5 µM IAA, and 87.6 mM sucrose, then shoot regeneration on the same medium, then shoots conditioned for root induction in MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.89 µM BA, 0.054 µM NAA, and 87.6 mM sucrose followed by MS medium with 43.8 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 2.7 µM NAA, and 43.8 mM sucrose	4 g L^{-1} Gelrite pH 5.6–5.8
Organogenesis [122]	Seeds 335 mM NaOCl for 20 min then 1% Benomyl® for 20 min	MS medium with 87.6 mM sucrose	Nodal segments transferred to MS medium with 2.22 µM BA, 0.27 µM NAA, with or without 2× boron or calcium concentrations, and 87.6 mM sucrose	–	9 g L^{-1} agar pH 5.8
Somatic embryogenesis from cotyledons [137]	Seeds 670 mM NaOCl for 15 min	$\frac{1}{2}$ MS medium with 278 µM myo-inositol and 43.8 mM sucrose	Embryogenesis on MS medium with 555 µM myo-inositol, 2.26 µM dicamba or 20.7 or 41.4 µM picloram, and 87.6 mM sucrose	–	2.8 g L^{-1} Phytagel pH 5.6–5.8
E. grandis* × *E. camaldulensis					
Shoot culture [167]	Nodal segments from 1-year-old trees 134 mM NaOCl for 20 min	MS medium with 0.41 µM biotin, 2.1 µM calcium pantothenate, 0.49 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 87.6 mM sucrose	MS medium with 0.89 µM BA and 0.054 µM NAA, then elongation on MS medium with 0.47 µM kinetin, 1.89 µM NAA, and 0.25 µM IBA	Pasteurised bark ex vitro	3.5 g L^{-1} Gelrite pH 5.6
Shoot culture [104]	Shoots from established long-term cultures	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.18 µM BA, 87.6 mM sucrose, and 2 g L^{-1} Gelrite	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.04 µM BA, 87.6 mM sucrose, and 5 g L^{-1} EM2 (liquid medium)	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 87.6 mM sucrose and 2 g L^{-1} Gelrite, or ex vitro in vermiculite	2→(2 or 0)→2 g L^{-1} Gelrite pH 5.6
Shoot culture [210]	Nodes from nursery and field stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 2 min	MS nutrients and 58.4 mM sucrose	MS nutrients, 0.89 µM BA, 0.054 µM NAA, and 58.4 mM sucrose	–	4 g L^{-1} Gelrite pH 5.6–5.8
E. grandis* × *E. globulus					
Shoot culture [90]	Nodes from nursery stock plants 134 mM NaOCl for 15 min	MS medium with 555 µM myo-inositol, 800 mg L^{-1} PVP, 2.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Proliferation on MS medium with 555 µM myo-inositol, 800 mg L^{-1} PVP, 2.22 µM BA, 0.054 µM NAA, and 87.6 mM sucrose	–	7 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. grandis* × *E. nitens					
Shoot culture [154]	Nodal segments from nursery stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	–	MS nutrients with 0.4 µM biotin, 0.2 µM calcium pantothenate, 0.5 µM BA, 0.05 µM NAA, and 0.09 M sucrose, then elongation on MS nutrients with 0.4 µM biotin, 0.2 µM calcium pantothenate, 0.9 µM kinetin, 0.05 µM IBA, 0.05 µM NAA, and 0.07 M sucrose	$\frac{1}{4}$ MS nutrients but with $\frac{3}{4}$-strength $CaCl_2$ and $MgSO_4$, and with 0.4 µM biotin, 0.2 µM calcium pantothenate, 0.5 µM IBA, and 0.04 M sucrose	4 g L^{-1} Gelrite pH 5.8
Shoot culture [151]	Nodal segments from nursery stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	Initiation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.44 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 1.37 µM *trans*-zeatin, 0.23 µM IAA, and 58.4 mM sucrose, then elongation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.93 µM kinetin, 2.1 µM IAA, and 58.4 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.49 µM IBA, and 43.8 mM sucrose	4 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture [155]	Nodal segments from 1-year-old stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	Initiation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.49 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.89 µM BA, 0.054 µM NAA, and 73 mM sucrose, then elongation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.47 µM kinetin, 0.49 µM IBA, 1.89 µM NAA, and 58.4 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.22 g L^{-1} $CaCl_2.2H_20$, 0.75 mM $MgSO_4$, 0.49 µM IBA, and 43.8 mM sucrose	3.5 g L^{-1} Gelrite pH 6.2→5.8
E. grandis* × *E. tereticornis					
Shoot culture [167]	Nodal segments from 1-year-old trees 134 mM NaOCl for 20 min	MS medium with 0.41 µM biotin, 2.1 µM calcium pantothenate, 0.49 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 87.6 mM sucrose	MS medium with 0.89 µM BA and 0.054 µM NAA, then elongation on MS medium with 0.47 µM kinetin, 1.89 µM NAA, and 0.25 µM IBA	Pasteurised bark ex vitro	3.5 g L^{-1} Gelrite pH 5.6
E. grandis* × *E. urophylla					
Shoot culture [88]	Nodal segments from nursery stock plants 7.36 mM $HgCl_2$ for 15 min	$\frac{1}{2}$ MS medium with 1 g L^{-1} PVP, 0.44 µM BA, and 58.4 mM sucrose	Proliferation on $\frac{1}{2}$ MS medium with 1 g L^{-1} PVP, 555 µM myo-inositol, 210 µM calcium pantothenate, 409 µM biotin, 0.89 µM BA, 0.047 µM kinetin, and 58.4 mM sucrose, then elongation on the same medium but with 0.93 µM kinetin instead of BA and NAA	$\frac{1}{2}$ MS medium with 1 g L^{-1} PVP, 210 µM calcium pantothenate, 409 µM biotin, 833 mM activated charcoal, and 58.4 mM sucrose	2→2.5 g L^{-1} Gelrite pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Shoot culture [167]	Nodal segments from 1-year-old trees 134 mM NaOCl for 20 min	MS medium with 0.41 µM biotin, 2.1 µM calcium pantothenate, 0.49 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 87.6 mM sucrose	MS medium with 0.89 µM BA and 0.054 µM NAA, then elongation on MS medium with 0.47 µM kinetin, 1.89 µM NAA, and 0.25 µM IBA	Pasteurised bark ex vitro	3.5 g L^{-1} Gelrite pH 5.6
Shoot culture [148]	Apical shoots from nursery stock plants 84 mM $Ca(OCl)_2$ for 10 min	MS medium	MS medium with 0.44 µM BA, 0.01 or 0.54 µM NAA, and 58.4 mM sucrose, followed by the same medium with $\frac{1}{2}$-strength KNO_3 and 29.2 mM sucrose to promote elongation	Full-strength MS macro-salts and vitamins, $\frac{1}{2}$-strength MS micro-salts, 1.48 µM IBA, and 29.2 mM sucrose	–
Shoot culture [142]	Shoots from field stock plants 67 mM NaOCl	–	Custom multiplication medium with 1 mM BA, 1 mM NAA, 55 µM myo-inositol, 68 µM L-glutamic acid, 300 µM thiamine-HCl, 8 µM nicotinic acid, 50 µM pyridoxine-HCl, and 58.4 mM sucrose	MS medium with 3 g L^{-1} charcoal	7.25 g L^{-1} agar pH 5.8
Shoot culture [104]	Shoots from established long-term cultures –	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.18 µM BA, 87.6 mM sucrose, and 2 g L^{-1} Gelrite	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.04 µM BA, 87.6 mM sucrose, and either Gelrite (semi-solid) or EM2 or M-Gel (liquid)	–	2→(2 or 0) g L^{-1} Gelrite pH 5.6
Shoot culture [210]	Nodes from nursery and field stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 2 min	MS nutrients and 58.4 mM sucrose	MS nutrients, 0.89 µM BA, 0.054 µM NAA, and 58.4 mM sucrose	–	4 g L^{-1} Gelrite pH 5.6–5.8
Shoot culture [89]	–	–	MS basal salts with White vitamins [209], 555 µM myo-inositol, 800 mg L^{-1} PVP, 1.33 µM BA, 0.054 µM NAA, and 87.6 mM sucrose	–	5 g L^{-1} agar pH 5.8
Shoot culture [211]	Seeds 67 mM NaOCl for 5 min	MS basal medium with 555 µM myo-inositol, 4.06 µM nicotinic acid, 2.43 µM pyridoxine-HCl, 26.64 µM glycine, 6.25 µM thiamine-HCl, and 87.6 mM sucrose	MS basal medium with 555 µM myo-inositol, 4.06 µM nicotinic acid, 2.43 µM pyridoxine-HCl, 26.64 µM glycine, 6.25 µM thiamine-HCl, 1 µM BA, 6 µM NAA, and 87.6 mM sucrose, with one dip in 20.9 µM 28-homocastasterone	–	6 g L^{-1} agar pH 5.7

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Shoot culture [155]	Nodal segments from 1-year-old stock plants 0.74 mM $HgCl_2$ for 10 min then 70 mM $Ca(OCl)_2$ for 10 min	Initiation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.49 µM BA, 0.23 µM kinetin, 0.22 µM NAA, and 58.4 mM sucrose	Proliferation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.89 µM BA, 0.054 µM NAA, and 73 mM sucrose, then elongation on MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.47 µM kinetin, 0.49 µM IBA, 1.89 µM NAA, and 58.4 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.22 g L^{-1} $CaCl_2.2H_20$, 0.75 mM $MgSO_4$, 0.49 µM IBA, and 43.8 mM sucrose	3.5 g L^{-1} Gelrite pH 6.2→5.8
Organogenesis from hypocotyls, cotyledons, cotyledonary nodes, and true leaves [110]	Seeds 1.2 M NaOCl for 10 min	SP medium [108]	Callogenesis on SP medium with 2 µM TDZ; regeneration on SP medium with 5 µM BA and 0.5 µM NAA; elongation on SP medium with 1 µM BA, 0.5 µM NAA, and 2 µM GA_3, then SP medium with MS micro-nutrients, 833 mM activated charcoal, and 58.4 mM sucrose	SP medium with full-strength MS micronutrients, 2.5 µM IBA, and 58.4 mM sucrose, then the same medium without IBA	2 g L^{-1} Gelrite pH 6.0
Organogenesis from nodal segments [89]	Shoots from long-term in vitro cultures	Shoots elongated on MS basal salts with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 0.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Callogenesis on MS medium with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 0.23 µM TDZ, 0.054 µM NAA, and 58.4 mM sucrose; regeneration on same medium but with 4.44 µM BA; elongation on same medium but with 0.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	MS basal salts with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 4.92 µM IBA, and 87.6 mM sucrose	5 g L^{-1} agar pH 5.8→5.6→5.8
Organogenesis [124]	2–3 mm shoot segments from long-term in vitro cultures, with the apical and axillary buds removed	–	Callogenesis on MS medium with 1.11 µM BA, 28.5 µM IAA, and 87.6 mM sucrose, then shoot regeneration on the same medium, then shoots conditioned for root induction in MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 0.89 µM BA, 0.054 µM NAA, and 87.6 mM sucrose followed by MS medium with 43.8 mM sucrose	$\frac{1}{4}$ MS medium with 0.41 µM biotin, 0.21 µM calcium pantothenate, 2.7 µM NAA, and 43.8 mM sucrose	4 g L^{-1} Gelrite pH 5.6–5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Organogenesis [123]	Leaf segments from established in vitro cultures	MS medium with 5.68 mM K_2SO_4, 18.79 mM KCl, no KNO_3, 29.28 mM NH_4NO_3, 1 µM BA, 0.5 µM 2,4-D, and 87.6 mM sucrose, then the same medium but with 2 µM NAA and no 2,4-D	Organogenesis on MS medium with no $COCl_2$, 1 µM $CuSO_4$, 5.68 mM K_2SO_4, no KI, no KNO_3, 4.9 mM NH_4NO_3, 2.44 mM $(NH_4)_2SO_4$, 339 µM arginine, 10% coconut water, 0.1 µM NAA, 3 µM TDZ, 500 µM putrescine, 100 µM spermidine, and 87.6 mM sucrose; shoot development on same medium with no arginine, coconut water or TDZ but with 2 µM BA, 0.5 µM NAA, 100 µM putrescine, and 10 µM spermidine	–	–
Organogenesis [114]	Leaves from established in vitro cultures	Callogenesis on WPM with 0.25–0.5 µM TDZ and 0.1 µM NAA	Shoot induction on WPM with 5 µM BA and 0.5 µM NAA	$\frac{1}{2}$ MS medium with full-strength vitamins, 2.66 µM riboflavin, 0.93 µM β-carotene, 2.46 µM IBA, and 43.8 mM sucrose	6 g L^{-1} agar pH 5.8
E. gunnii					
Organogenesis [129]	Leaves, nodes, and internodes from long-term shoot cultures	MS salts with 555 µM myo-inositol, 26.6 µM glycine, 8.1 µM nicotinic acid, 4.9 µM pyridoxine-HCl, 2.96 µM thiamine-HCl, 1 µM BA, 0.04 µM picloram, and 87.6 mM sucrose for 1 week	MS salts with 555 µM myo-inositol, 26.6 µM glycine, 8.1 µM nicotinic acid, 4.9 µM pyridoxine-HCl, 2.96 µM thiamine-HCl, 2.25 µM BA, and 0.04 µM picloram, then elongation on MS salts with 555 µM myo-inositol, 0.81 µM nicotinic acid, 0.49 µM pyridoxine-HCl, 1.48 µM thiamine-HCl, and 0.45 µM BA, both with 87.6 mM sucrose	–	2.5 g L^{-1} Gelrite pH 5.5
E. impensa					
Shoot culture [70]	20–40-mm long stem segments from wild plants 63 mM alkyl-dimethyl-benzalkonium chloride for 20 min and then again for 5–20 s	$\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine, 2.5 µM pyridoxine, 4 µM nicotinic acid, 500 µM myo-inositol, 0.5 µM BA, and 60 mM sucrose	Proliferation in $\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine, 2.5 µM pyridoxine, 4 µM nicotinic acid, 500 µM myo-inositol, 0.25 µM BA, 2.5 µM kinetin, and 60 mM sucrose, with 0.5 µM zeatin and 0.5 or 1 µM GA_4 used for a single passage to promote elongation	$\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine, 2.5 µM pyridoxine, 4 µM nicotinic acid, 500 µM myo-inositol, 0.25, 2.5 or 5 µM IBA, 0.25 or 0.5 µM NAA, and 60 mM sucrose	9 g L^{-1} agar pH 5.9

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. marginata					
Shoot culture [156]	Shoots from established cultures	–	MS medium with 2.5 µM BA, 1.25 µM NAA, 10 mM MES, and 58.4 mM sucrose	$\frac{1}{4}$ MS macronutrients with 1/8 nitrogen sources, full-strength MS micronutrients, 10 µM IBA, 10 mM MES, and 58.4 mM sucrose	2.5 g L^{-1} agar + 2.5 g L^{-1} Gelrite pH 5.8 → 5.5
Organogenesis [113]	Excised leaves from established cultures	Callogenesis on WPM minerals with 1 g L^{-1} casein, 0.44 µM BA, 16.2 µM NAA, and 146 mM sucrose	Shoot regeneration on WPM minerals with 1.33 µM BA and 146 mM sucrose	–	5 g L^{-1} Phytagar pH 5.9
E. microcorys					
Shoot culture [212]	Apical shoots from nursery seedlings and in vitro seedlings 34 mM NaOCl for 1 min	–	MS or $\frac{1}{2}$ MS medium with 0.5–1.0 µM BA and 58.4 mM sucrose	MS medium with 5–10 µM IBA and 58.4 mM sucrose	7 g L^{-1} agar pH 5.8
Organogenesis [212]	Apical shoots from nursery seedlings and in vitro seedlings 34 mM NaOCl for 1 min	–	MS medium with 0.25 µM BA and 58.4 mM sucrose	MS medium with 5–10 µM IBA and 58.4 mM sucrose	7 g L^{-1} agar pH 5.8
E. microtheca					
Shoot culture [121]	Internodes from 1-year-old nursery seedlings 0.37 mM $HgCl_2$ for 30 s	MS medium with 4.65 µM kinetin, 5.4 µM NAA, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	MS medium with 4.65 µM kinetin, 5.4 µM NAA, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	–	–
Organogenesis [113]	Excised leaves from established cultures	Callogenesis on WPM minerals with 1 g L^{-1} casein, 0.44 µM BA, 16.2 µM NAA, and 146 mM sucrose	Shoot regeneration on WPM minerals with 1.33 µM BA and 146 mM sucrose	–	5 g L^{-1} Phytagar pH 5.9
Organogenesis [121]	Internodes from 1-year-old nursery seedlings 0.37 mM $HgCl_2$ for 30 s	MS medium with 4.65 µM kinetin, 5.4 µM NAA, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	MS medium with 4.65 µM kinetin, 5.4 µM NAA, 0.05 or 2.27 µM TDZ, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	–	–
Somatic embryogenesis [121]	Internodes from 1-year-old nursery seedlings 0.37 mM $HgCl_2$ for 30 s	MS medium with 2.32 µM kinetin, 20.8 µM NAA, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	MS medium with 2.32 µM kinetin, 20.8 µM NAA, 0.45 µM TDZ, and $\frac{1}{2}$ strength KNO_3 and NH_4NO_3	–	–
E. nitens					
Shoot culture [101]	Seeds $Ca(OCl)_2$ for 20 min	Modified MS medium with half-strength macronutrients and 58.4 mM sucrose	Proliferation on MS medium with half-strength macronutrients, de Fossard organics [213], 0.9 µM BA, 0.05 µM NAA, and 87.6 mM sucrose, then elongation on the same medium but with 0.45 µM BA and 0.05 µM NAA	Knop macronutrients, MS micronutrients without KI, de Fossard organics without riboflavin, and 4.9, 9.8 or 14.8 µM IBA, then the same medium without hormones	7.5 g L^{-1} agar pH 5.7

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
Shoot culture [101]	Shoot tips from 1-year-old stock plants $Ca(OCl)_2$ for 20 min	–	De Fossard macronutrients, MS micronutrients, de Fossard organics, 0.9 µM BA, 0.05 µM NAA, and 87.6 mM sucrose, then elongation on the same medium but with 0.45 µM BA and 0.05 µM NAA	–	7.5 g L^{-1} agar pH 5.7
Organogenesis and occasional somatic embryogenesis from hypocotyl segments and cotyledons [135]	Seeds 40% commercial bleach for 30 min	MS salts and 87.6 mM sucrose	Callogenesis on MS nutrients with 10% coconut water, 2.22 µM BA, 5.4 µM NAA, and 87.6 mM sucrose, then shoot regeneration on the same medium but with 4.44 µM BA and 2.7 µM NAA	MS medium with 14.76 µM IBA and 58.4 mM sucrose, then the same medium without IBA	8 g L^{-1} agar pH 5.8
E. ochrophloia					
Organogenesis [113]	Excised leaves from established cultures	Callogenesis on WPM minerals with 1 g L^{-1} casein, 0.44 µM BA, 16.2 µM NAA, and 146 mM sucrose	Shoot regeneration on WPM minerals with 1.33 µM BA and 146 mM sucrose	–	5 g L^{-1} Phytagar pH 5.9
E. pellita					
Shoot culture [100]	Nodes from stock plants 268 mM NaOCl for 5 min	MS medium	Proliferation on Driver and Kuniyuki woody plant (DKW) medium [214] with 0.44 µM BA, 16.66 mM activated charcoal, and 87.6 mM sucrose	$\frac{1}{2}$ DKW medium with 1.08 or 2.70 µM NAA and 58.4 mM sucrose	3 g L^{-1} Gelrite pH 5.6
Non-aseptic shoot culture [102]	Nodes from in vitro seedling shoots	–	MS salts, White vitamins, 555 µM inositol, 9.87 µM BA, 11.4 µM IAA, 8.86 µM IBA, and 87.6 mM sucrose, incorporating 0.67 or 0.94 mM NaOCl	–	1.5 g L^{-1} Phytagel pH 6.0
E.* × *phylacis					
Organogenesis [71]	Single nodes and shoot tips from the only wild tree 63 mM alkyl-dimethyl-benzalkonium chloride for 10 min and 9.2 mM $HgCl_2$ for 30 s	Liquid $\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine HCl, 2.5 µM pyridoxine HCl, 4 µM nicotinic acid, 500 µM myo-inositol, 100 mg L^{-1} MES buffer, 0.01% potassium citrate:citrate (10:1), 416.5 mM activated charcoal, and 1 µM zeatin, followed by semi-solid medium with the same organic compounds and 0.5 µM zeatin	Nodular callogenesis on $\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine HCl, 2.5 µM pyridoxine HCl, 4 µM nicotinic acid, 500 µM myo-inositol, 100 mg L^{-1} MES buffer, and 5 µM TDZ, then shoot regeneration and development on the same medium but with 1 µM GA_4, then with 0.5 µM zeatin and 1 µM GA_4, then with 0.1 µM BA and 1 µM zeatin, then with 0.5 µM zeatin and 2 µM IAA, then with either 0.5 µM zeatin and 0.5 µM GA_4 or 1 µM zeatin and 0.5 µM IAA	$\frac{1}{2}$ MS medium with 100 µM Na.EDTA, 1 µM thiamine HCl, 2.5 µM pyridoxine HCl, 4 µM nicotinic acid, 500 µM myo-inositol, 100 mg L^{-1} MES buffer, and 5 µM IBA	pH 5.8 7 g L^{-1} agar

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. polybractea					
Shoot culture [72]	Nodal segments from nursery seedlings, field saplings, and field coppice shoots 3.1 mM alkyl-dimethyl-benzyl-ammonium chloride for 30 min	Initiation on $\frac{1}{2}$ MS nutrients, 2.5 µM zeatin, 5 µM NAA, and 87.6 mM sucrose	Proliferation on 2.3 g L^{-1} WPM basal salts, 1 mL L^{-1} MS modified vitamins, 4.5 µM BA, 16 µM NAA, and 87.6 mM sucrose, then elongation on the same medium but with 3 µM 2iP instead of BA and NAA	2.3 g L^{-1} WPM basal salts, 1 mL L^{-1} MS modified vitamins, 100 µM IBA, and 87.6 mM sucrose	7 g L^{-1} agar pH 5.6
E. regnans					
Shoot culture [141]	Seeds 4.02 M then 1.34 M NaOCl, each for 30 min	$\frac{1}{2}$ MS nutrients and 87.6 mM sucrose, then to filter paper over $\frac{1}{2}$ MS nutrients without sucrose	Proliferation on MS medium with 2.28 µM zeatin, 0.27 µM NAA, and 58.4 mM sucrose	Hoagland's salts [160] with 98 µM IBA and 58.4 mM sucrose, then to the same medium without IBA but with 416.5 mM activated charcoal	4→2.2 g L^{-1} Gelrite pH 5.7
E. saligna					
Shoot culture [82,83]	Seeds 201 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 658 µM $CaCl_2$ and 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine HCl, 555 µM myo-inositol, 4.92, 49.2 or 492 µM IBA, and 87.6 mM sucrose, then the same medium without IBA but with 83.3 mM activated charcoal	7.5 g L^{-1} agar pH 5.8
Shoot culture [140]	Seeds 335 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine, 2775 µM myo-inositol, 49.2 µM IBA, and 87.6 mM sucrose, then the same medium without IBA but with 83.3 mM activated charcoal	6 g L^{-1} agar pH 5.8
Shoot culture [139]	Seeds 335 mM NaOCl for 15 min	–	$\frac{1}{2}$ MS salts with 58.4 mM sucrose	0.3× MS salts, 1.18 µM thiamine, 2775 µM myo-inositol, 49.2 µM IBA, and 88 mM glucose, then the same medium without IBA, with 83.3 mM activated charcoal and with 87.6 mM sucrose instead of glucose	6 g L^{-1} agar pH 5.8
Shoot culture and organogenesis from cotyledonary nodes [144]	Seeds 804 mM NaOCl for 30 min	$\frac{1}{2}$ MS medium with 58.4 mM sucrose	Cotyledonary nodes transferred to MS medium with 10% coconut water, 4.4 µM BA, 3.6 µM NAA, and 87.6 mM sucrose in dark, then to MS medium with 1.1 µM BA, 2.7 µM NAA, and 87.6 mM sucrose in light, then shoot proliferation on MS medium with 1.1 µM BA and 87.6 mM sucrose, then elongation on MS medium with 208.3 mM activated charcoal and 87.6 mM sucrose	MS medium with 208.3 mM activated charcoal and 87.6 mM sucrose	7 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. saligna* × *E. maidenii					
Somatic embryogenesis [138]	2-year established cultures from 12-year-old elite trees	Shoot apex and leaf explants plated on MS medium with 500 mg L^{-1} casein hydrolysate, 40 µM picloram, 40 mg L^{-1} gum Arabic, and 87.6 mM sucrose	Secondary embryogenesis on MS medium with 16.11 µM NAA, 20 µM STS, and 87.6 mM sucrose	Emblings used to establish shoot cultures and then plantlets	6 g L^{-1} agar pH 5.6–5.7
E. sideroxylon					
Shoot culture [85]	Seeds 335 mM NaOCl for 20 min	Moist filter paper	$\frac{1}{2}$ MS salts, B5 vitamins, and 58.4 mM sucrose	$\frac{1}{2}$ MS salts, B5 vitamins, 2.5 µM IBA, 2.5 µM NAA, and 58.4 mM sucrose	6 g L^{-1} Phytagar pH 5.8
E. stricklandii					
Organogenesis from hypocotyls [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 1 or 5 µM BA with or without 5 µM NAA, or with 5 µM kinetin and 10 µM NAA, each with 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from cotyledons [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 1 or 5 µM BA each with 5 µM NAA, or with 5 µM BA and 10 µM NAA, each with 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from youngest expanding leaves [126]	Seeds 402 mM NaOCl for 20 min	–	MS medium with 1 µM BA, or with 5 µM BA and 5 µM NAA, each with 87.6 mM sucrose	–	7 g L^{-1} Phytagel
Organogenesis from shoot apices [206]	Seeds 402 mM NaOCl for 20 min	MS medium with 87.6 mM sucrose	Organogenesis on MS medium with 0.5 or 1 µM BA and 87.6 mM sucrose; proliferation on QL medium with 2.2 µM BA, 0.5 µM NAA, and 58.4 mM sucrose	–	7 g L^{-1} Phytagel pH 5.7
E. tereticornis					
Shoot culture [215]	Nodes from mature trees 3.68 mM $HgCl_2$ for 10 min	Das and Mitra nutrient medium [216] with 4.44 µM BA, 0.54 or 5.4 µM NAA, and 87.6 mM sucrose	Shoot multiplied and elongated on Das and Mitra nutrient medium with 0.44 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Modified MS medium (e.g., no NH_4NO_3) with 4.9 µM IBA	8 g L^{-1} agar
Organogenesis from hypocotyls [115]	Seeds 7.36 mM $HgCl_2$ for 2 min	B5 medium [201]	Hypocotyl segments transferred to B5 medium with 2.22 µM BA	WPM with 2.46 µM IBA	10 g L^{-1} agar pH 5.5
Organogenesis from leaves [125]	Nodal segments from coppice shoots of 12-year-old trees 114 mM NaOCl for 15 min	MS medium with 2.5 µM BA, 0.5 µM NAA, and 58 mM sucrose, then MS medium with 0.1 µM BA and 0.5 µM NAA	Organogenesis on MS medium with 5 µM BA and 1 µM 2,4-D	–	7 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. urophylla					
Organogenesis from hypocotyls [162]	Seeds 375 mM NaOCl for 30 min	MS medium with 58.4 mM sucrose	Organogenesis on MS medium with 0.89 µM BA, 1.08 µM NAA, and 58.4 mM sucrose, then shoot regeneration on MS medium with 0.46 µM zeatin and 58.4 mM sucrose	MS medium with 0.044 µM BA, 5.4 µM NAA, and 58.4 mM sucrose	8 g L^{-1} agar → 2 g L^{-1} Phytagel pH 5.6
Organogenesis from hypocotyls [86]	Seeds 1.34 M NaOCl for 20 min	Moistened filter paper	Callogenesis on N7 medium [118] without $CaCl_2$ but with 21.7 µM picloram, then organogenesis on N6 medium with 6.12 mM $CaCl_2$	–	–
Organogenesis from hypocotyls [111]	Seeds 2.68 M NaOCl for 20 min	$\frac{1}{2}$ MS medium	Callogenesis on SP medium [110] with 6.12 mM $CaCl_2$, 4.9, 6.6, or 8.2 µM *N*-phenyl-*N*′-[6-(2-chlorobenzothiazol)-yl] urea (2-Cl-PBU) and 0.57 µM IAA; regeneration on SP medium with 6.12 mM $CaCl_2$, 3.52 µM BA, and 0.28 µM NAA; proliferation and elongation on SP medium with 6.12 mM $CaCl_2$, 0.44 µM BA, 0.54 µM NAA, and 0.3 µM GA_3, then SP medium with 58.4 mM sucrose	SP medium with 2.5 µM IBA	–
Organogenesis from hypocotyls [112]	Seeds	$\frac{1}{2}$ MS medium	Callogenesis on SP medium with 6.12 mM $CaCl_2$, 1.14 µM 2-Cl-PBU, and 0.57 µM IAA; shoot regeneration on SP medium with 6.12 mM $CaCl_2$, 3.52 µM BA, and 0.28 µM NAA	–	–
Somatic embryogenesis from hypocotyls [86]	Seeds 1.34 M NaOCl for 20 min	Moistened filter paper	Callogenesis on N7 medium with 21.7 µM picloram but no $CaCl_2$; somatic embryogenesis on N7 medium with 6.62 mM $CaCl_2$	–	–
E. urophylla* × *E. globulus					
Shoot culture [90]	Nodes from nursery stock plants 134 mM NaOCl for 15 min	MS medium with 555 µM myo-inositol, 800 mg L^{-1} PVP, 2.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Proliferation on MS medium with 555 µM myo-inositol, 800 mg L^{-1} PVP, 2.22 µM BA, 0.054 µM NAA, and 87.6 mM sucrose	–	7 g L^{-1} agar pH 5.8

Table A1. *Cont.*

Taxon, Technique	Explant, Sterilisation	Establishment Medium	Proliferation Medium	Rooting Medium	Gelling Agent, pH
E. urophylla* × *E. grandis					
Shoot culture [217]	Shoots from long-term in vitro cultures	SEM [217] with 58.4 mM sucrose	SEM or modified SEM [217] with 58.4 mM sucrose	–	6.5 g L^{-1} agar pH 5.8
Shoot culture [104]	Shoots from established long-term cultures	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3 and 3.76 mM $MgSO_4$, and with 0.18 µM BA and 87.6 mM sucrose	$\frac{1}{2}$ MS medium but with 1.88 mM KNO_3, 3.76 mM $MgSO_4$, 0.04 µM BA, 87.6 mM sucrose, and Gelrite (semi-solid) or EM2 or M-Gel (liquid)	–	2→(2 or 0) g L^{-1} Gelrite pH 5.6
Shoot culture [89]	–	–	MS basal salts with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 1.33 µM BA, 0.054 µM NAA, and 87.6 mM sucrose	–	5 g L^{-1} agar pH 5.8
Shoot culture [163]	Shoots from long-term in vitro cultures from mature trees	–	$\frac{1}{2}$ MS medium with 278 µM myo-inositol, 26.6 µM glycine, 2.96 µM thiamine, 4.9 µM pyridoxine-HCl, 8.1 µM nicotinic acid, 0.4 µM BA, 0.05 µM NAA, and 87.6 mM sucrose	$\frac{1}{2}$ MS medium with 278 µM myo-inositol, 26.6 µM glycine, 2.96 µM thiamine, 4.9 µM pyridoxine-HCl, 8.1 µM nicotinic acid, 5 µM IBA or 12.5 µM NAA, and 87.6 mM sucrose	2.5 g L^{-1} Phytagel pH 5.6–5.8
Shoot culture [164]	Nodal segments from nursery stock plants and an in vitro-germinated seed	–	$\frac{1}{2}$ MS basal salts with 278 µM myo-inositol, 26.6 µM glycine, 2.96 µM thiamine, 4.9 µM pyridoxine-HCl, 8.1 µM nicotinic acid, 0.4 µM BA, 0.05 µM NAA, and 87.6 mM sucrose	$\frac{1}{2}$ MS basal salts with 278 µM myo-inositol, 26.6 µM glycine, 2.96 µM thiamine, 4.9 µM pyridoxine-HCl, 8.1 µM nicotinic acid, 5 µM IBA, 0 or 0.4 µM BA, and 87.6 mM sucrose	2.5 g L^{-1} Phytagel pH 5.6–5.8
Organogenesis [89]	Shoots from long-term in vitro cultures	Shoots elongated on MS basal salts with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 0.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	Callogenesis on MS medium with White vitamins, 555 µM, 800 mg L^{-1} PVP, 2.77 µM TDZ, 0.54 µM NAA, and 58.4 mM sucrose, then shoot regeneration on the same medium but with 0.89 µM BA and 1.08 µM NAA, then elongation on the same medium but with 0.22 µM BA, 0.54 µM NAA, and 87.6 mM sucrose	MS basal salts with White vitamins, 555 µM myo-inositol, 800 mg L^{-1} PVP, 4.9 µM IBA, and 87.6 mM sucrose	5 g L^{-1} agar pH 5.8→5.6→5.8

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