The Nucleus (2022) 65:379–389
https://doi.org/10.1007/s13237-022-00394-3

ORIGINAL ARTICLE

Rapid multiplication of mature *Eucalyptus* hybrids through macro-and-micropropagation

Maneesh S. Bhandari[1] · Sandeep Maikhuri[1] · Ajay Thakur[1] · Giriraj S. Panwar[2] · Arzoo Shamoon[1] · Shailesh Pandey[3]

Received: 11 October 2021 / Accepted: 5 July 2022 / Published online: 10 August 2022

Abstract

Eucalyptus species are globally recognized and extensively planted hardwood trees whose flexibility, growth and diversity make them popular renewable sources of fibre and energy. Across the globe, the interest to create ways to develop significant hardwoods, like eucalypts, is rapidly growing. Although micropropagation offers a means to clonally propagate the desirable cultivars, the technique is often variety-specific for eucalypts. The present study aimed to expand our understanding of root initiation through macro-and-micropropagation in difficult to propagate superior full-sib matured hybrid (7-years-old trees) of eucalypts. Analysis of Variance (ANOVA) revealed that hormonal treatment of IBA (2,000–4,000 ppm) supplemented with *Enterobacter* sp. (10^6 cfu ml^{-1}) significantly increased ($p < 0.05$) hybrid rooting through macropropagation. Notably, root initiation with maximum root development of 10.00% ± 0.91 and 3.75% ± 0.48 was observed at 3,000 ppm of IBA for hybrid genotypes FRI-PH3 (*Corymbia torelliana* × *C. citriodora*; 2A) and FRI-PH4 (*E. pellita* × *E. urophylla*; 1D), respectively. In the case of micropropagation studies, genotype FRI-4s (coppice shoots of *E. tereticornis* × *E. camaldulensis*, 10O) showed significant differences ($p < 0.01$) with various hormonal combinations (BAP, Kn and TDZ); and maximum number of shoot (4.0 ± 0.82) proliferation with average shoot length of 2.5 ± 0.15 cm was recorded in 0.5 mg L^{-1} BAP, ½ MS medium and 0.5 mg L^{-1} Kn in accordance to Duncan Multiple Range Test (DMRT). Afterward, for *in vitro* rooting, ¼ MS medium fortified with 1.0 mg L^{-1} IBA was proved to be the optimal medium for root induction, the maximum number of roots per explant (5.0 ± 0.91) with an average root length of 3.10 ± 0.15 cm was observed. Overall, the study signifies successful macro-and-micropropagation of mature eucalyptus hybrids through branch cuttings and nodal segments.

Keywords *Eucalyptus* · Full-sib · Hybrids · *In vitro* propagation · Branch cutting · Explants

Corresponding Editor : Maumita Bandyopadhyay; Reviewer : Arpita Roy.

✉ Maneesh S. Bhandari
maneesh31803@gmail.com; bhandarims@icfre.org

Sandeep Maikhuri
sandeepma94@gmail.com

Ajay Thakur
thakura@icfre.org

Giriraj S. Panwar
panwar_giriraj@rediffmail.com

Arzoo Shamoon
arzoo707@gmail.com

Shailesh Pandey
shailesh31712@gmail.com

[1] Division of Genetics & Tree Improvement, Forest Research Institute, 248 195 Dehradun, Uttarakhand, India

[2] Tissue culture Discipline, Northern Regional Centre, Botanical Survey of India, 248 195 Dehradun, Uttarakhand, India

[3] Forest Pathology Discipline, Division of Forest Protection, Forest Research Institute, 248 006 Dehradun, Uttarakhand, India

Significance Statement

Mature trees are difficult to propagate by vegetative methods. However, a successful asexual method has been developed for eucalypt hybrids.

Introduction

Eucalyptus (family: Myrtaceae), an outcross group of approximately 700 species native to continent Australia, can self-fertilize, where conceivable outcomes of pollen contamination are high. This group has tall and evergreen trees distributed worldwide [6]. Economically, *Eucalyptus* sp. provides fuelwood, biomass and reduces carbon dioxide levels from the atmosphere directly, including a variety of services to the industry in supplying raw material for plywood, pulp and paper, medicines, and essential oil used as insect/pest repellent [5, 32]. Importantly, tree breeding work over the decade supplies quality genetic stocks and germplasm in the forms of hybrids to fulfil stakeholders' needs. But genetic gain could not be harnessed from the stocks by progeny performance taking a long time to show genetic worth of parents or sometimes due to the lagging standardization of the propagation methods. Some specific examples belong to the conifers that are easily multiplied with immature embryos compared to matured ones [28, 33, 40]. Similarly, as an experimental model in family Fagaceae, reviewed works on several Oaks revealed the limitations of mature and selected hardwood tree species hinder the application of the process for mass clonal propagation [3].

Given these case studies, the advancement of eucalypts cloning, while its recognition as a functional strategy for imitating superior trees, has gone through a continuous process of improvement. Substantially, in the last two decades, cloning of *Eucalyptus* sp. through macro-and-micropropagation has yielded productive clonal and vegetative material by the forest-based companies and private plywood industries, thus, solving problems associated with diseases, e.g., canker caused by *Cryphonectria cubensis* [2, 16]. Further, focus has shifted in the direction of wood-based industries towards resistant germplasm and volumetric growth expansion of the eucalypts. In this connection, benefits of macro-and-micropropagation are enormous as abundant planting stocks could be generated from the limited source material. Many cuttings and germplasm stocks can be kept in the nursery, which consistently tends to be utilized without going for a repeated nursery foundation.

Baseline research revealed that eucalypts regeneration using tissue culture approaches was first reported way back in the 1960s [46], revealing the successful culture of juvenile tissue and eventually with mature tree-derived explants in the last few decades [25]. Considerable developments were subsequently reported, with successful plantlet regeneration from almost all plant organs [30], such as somatic embryogenesis [45, 50], axillary buds [23], cotyledons [4], shoot tips and nodes [17], and lignotubers [36], albeit with limited success. Similarly, macropropagation through rooted cuttings (macro-cuttings) has enabled foresters to develop and operate highly successful clonal programmes [20, 49].

Importantly, 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 *E. grandis* (Flooded gum), *E. camaldulensis* (River red gum), and *E. deglupta* (Rainbow gum), have long been considered amenable to cutting propagation [47]. Further, attempts to standardize regeneration protocols were also made in many *Eucalyptus* species and their hybrid variants [43]. For instance, a generic protocol for *in vitro* establishment of novel eucalypt hybrid, namely *E. grandis* × *E. nitens* was recently developed [26]. Similarly, the effects of light intensity as a limiting factor on *in vitro* introduction and multiplication phases were assessed for a commercial hybrid clone of *E. grandis* × *E. urophylla* [44].

However, research exploration for difficult to propagate mature trees is required whose rooting response significantly varies among and between the genotypes [8]. Since macropropagation and *in vitro* culture of the phenotypically selected trees can accelerate the genetic improvement programmes of fast-growing species on a sustainable basis. Thus, these techniques could be integrated directly with the conventional tree breeding and selection programs to enhance the agroforestry yield. Given these considerations, there is an urgent need to standardize propagation protocols from matured trees, particularly hybrids, to achieve 100% genetic gain. Therefore, the present study aims to standardize the macro-and-micropropagation methods for the 7-years-old mature *Eucalyptus* hybrids.

Materials and methods

Description of the study area

The macro-and-micropropagation studies were carried out in the Genetics and Tree Improvement Division, Forest Research Institute (FRI), Dehradun. Agro-climatic condition of Dehradun (geographical attributes: E 78°00'42.78", N 30°20'38.00", 679 m) is characterized by an average annual rainfall of 2073 mm as area falls under humid subtropical climatic condition. A seven-years-old matured hybrid eucalypts trial (founded: 2012) with four superior genotypes representing FRI-PH3 (*Corymbia torelliana* × *C. citriodora*, 2A), FRI-PH4 (*E. pellita* × *E. urophylla*, 1D

and 7O) and FRI-4s (coppice shoots of *E. tereticornis* × *E. camaldulensis*, 10O) were selected for further experimentation during 2019–20, whose turnover calculation was given in supplementary MS Excel sheet.

Sample collection, procedure and experimental setup for macropropagation

The shoot and root initiation performance experiments were conducted for four superior genotypes. Plant growth regulators, *viz*. Indole 3-Butyric Acid (IBA, 10–50 mg) or Indole 3-Acetic Acid (IAA, 10–50 mg) as well as Talcum powder (10 g) and methanol (10 ml) were used in various combinations for media preparation (1000–5000 ppm). Potential plant growth promoters (PGP), i.e., bacterial isolates, recovered from soil of reserve forest areas of FRI, Dehradun. Further, genomic DNA was isolated and 16S rDNA has been amplified using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTTGTTACGACTT-3') [29]. Polymerase Chain Reaction (PCR) cycle was programmed with an initial denaturation at 95 °C for 5 min, 30 cycles at 95 °C for 1 min; annealing at 55 °C for 1 min, 72 °C for 2 min, final extension at 72 °C for 5 min, and hold at 4 °C. The PCR reaction was conducted in a final volume of 50 µl containing 1.5 µl of template DNA, 1 µl deoxynucleotide triphosphates, 5 µl of *Taq* buffer, 0.8 µl of *Taq* DNA polymerase, 2 µl of each primer and 37.7 µl of nuclease-free water. The DNA fragments were visualized under UV light and image captured using gel documentation system (UVP, USA; Suppl. Figure 1). Afterwards, PCR products were sent to a commercial biotech company (M/s Biokart India Pvt. Ltd.) for sequencing. The amplified products were refined by polyethylene glycol (PEG)-NaCl precipitation and immediately sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA). Importantly, sequencing was paired-end and the sequence similarity search was done against a publicly available repository at National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST). For mass multiplication, the nutrient broth was prepared in a 250 ml flask, inoculated with the bacterial isolates' pure culture, and incubated at 28 ± 2 °C for 24 h. The concentration was adjusted to 10^6 cfu ml^{-1} using a spectrophotometer. Lastly, silver nanoparticles (0.2%) were used in the vermiculite to remove any fungal and bacterial contamination and kept overnight.

Initially, four hybrid genotypes (2A, 1D, 7O and 10O) of eucalypts were selected (based on the Index method) and lopped during winter (January–February-2019). The revitalized shoots (soft branch) produced during subsequent spring (March–April-2019) were excised with the help of secateurs. Cuttings were made up of 8–10 cm in length with 2–3 leaves cut on-side by scissors. Initially, cuttings were subjected to surface sterilization by placing them in fungicide solution [0.2% w v^{-1} Bavistin (half carbendazim, WP)] for 1 h. These processed cuttings were immediately immersed in varied hormonal concentrations (1,000–5,000 ppm) of either IBA and IAA for 4 h. The cuttings were further dipped in bacterial suspension and implanted in hyco-trays containing vermiculite. The experiments were conducted in Completely Randomized Design (CRD) with three treatments, and each treatment consisted of 100 cuttings, which were kept in the mist chamber for growth analysis. The branch cuttings dipped in distilled water served as a control. Observation and data recording were done on a day-to-day and weekly basis. The data were subjected to Analysis of Variance (One-Way ANOVA) with treatment means has been compared through Duncan Multiple Range Test (DMRT) using software SPSS ver. 16.0 (SPSS Inc. 233 S. Wacker Drive, 11th Floor, Chicago, Il 60,606 – 6307).

Explant collection, sterilization and standardization of the culture media for micropropagation

Twigs of the hybrids were used to derive nodal segments of length ranging between 2 and 3 cm, washed gently to evacuate the residue particles, such as dust, particulates, pollen, exo-and-epiphytes, invading microbes, etc., clinging to the surface. The explants were then washed with a fluid detergent (Teepol, 5–10 drops per 100 ml) and Tween-20 (2 drops per 100 ml arrangement) in delicate agitating conditions for 5 min, and splashed again followed by fungicide treatment [0.1% (w/v) Bavistin (half carbendazim, WP)] for 10 min. Further, explants were surface disinfected with sodium hypochlorite (NaClO; 4% accessible chlorine) for 5–10 min under aseptic conditions. Finally, explants were washed with cetrimide and disinfected refined water (4–5 times) to expel the traces of sterilant.

A different set of micropropagation experiments were conducted throughout the study period. Murashige and Skoog (MS) medium with 3% sucrose as a carbohydrate source and 0.7% agar (HI Media) were used for solidification. A 5.8 pH was used in the medium *via.* 1 N NaOH or 1 N HCl, autoclaved at 120 °C with a pressure of 1.0×10^5 Pa for 15 min, where growth hormone with varying concentrations was used. All the cultures were incubated in the culture room with 8/16 hours of photoperiod, which was illuminated by 40-Watt cool white fluorescent tubes of 1200 lx for 8 h in the dark under culture room maintained at 23 ± 1 °C.

For shoot induction– ½ MS medium, 6-Benzylaminopurine (BAP; 0.5–1.0 mg L^{-1}), Kinetin (Kn; 0.5–1.5 mg L^{-1}), and Thidiazuron (TDZ; 0.5–1.0 mg L^{-1}) were used. For proper growth and multiplication of shoots, cultures were then subcultured at a strength of ½ MS medium + ½ BAP and Kn (0.5–1.5 mg L^{-1}) in a regular interval up to eight weeks. Further, rooting was induced in MS and modified MS media (¼ and ½ strength) enriched with different concentrations of IBA (0.25, 0.50 and 1.00 mg L^{-1}). Root cultures were sub-cultured at a regular interval of four weeks.

The clonal explants showing root initiation were taken out from the MS media, mildly washed with tap and then distilled water to remove every possible trace of media from the roots. With successful establishment of roots, hardening and acclimatization were carried out after a month. The plantlets were placed in a sterilized soil (transfer to polybag) and kept under observation in the nursery.

Results

Macropropagation

Out of four hybrids initially selected for the study, promising results were shown by only two genotypes, i.e., 2A (*C. torelliana* × *C. citriodora*) and 1D (*E. pellita* × *E. urophylla*). A successful procedure for hybrid cuttings rooting has been summarized in Fig. 1(a–i). A BLASTn homology search of the 16 S rDNA sequence of bacterial isolate against accessions in NCBI GenBank resulted in 97.20% homology with unknown *Enterobacter* spp. (KX881780, KU362707, KU362706 and KU362633), *E. hormaechei* (MT507084, MG738315, MF138109), and *E. cloacae* (MT052568). The 16 S rDNA sequence of the isolate has been deposited in GenBank with accession number OM956154. ANOVA revealed a significant difference ($p < 0.05$) between auxin concentration and its combination with *Enterobacter* sp. for induction of rooting in eucalypts hybrids (Table 1). Shoot proliferation was observed in the cuttings derived from the genotypes 2A and 1D. In the DMRT analysis, auxins (IBA and IAA), and their concentrations (2000, 3000 and 4000 ppm) showed significant differences in proliferation of rooting for both the genotypes (Table 2). Importantly, root initiation was observed in concentrations ranging from 2,000 to 4,000 ppm and maximum proliferation of 10.00 ± 0.91% and 3.75 ± 0.48% at 3,000 ppm with the hormonal combination of *Enterobacter* sp. isolates. The cuttings in other auxin concentrations could not survive. Both genotypes rooted cuttings were transplanted to the field after 45 days of root initiation, but only 1% survived to abide the transplantation shock.

Micropropagation

Standardization of micropropagation protocol for development of shoots, roots and establishment of plantlets was conducted for hybrid genotypes of eucalypts. However, establishment of shoot proliferation was shown by hybrids 7O (*E. pellita* × *E. urophylla*) and 10O (*E. tereticornis* × *E. camaldulensis*), where micropropagated shoots of only the later genotype were successfully extended up to rooting and elaborated in detail under the following subsections.

Axillary bud growth, culture establishment, multiplication of shoots and roots, and hardening

A significant difference ($p < 0.05$) through ANOVA was observed among various hormonal combinations (BAP, Kn and TDZ) on standardization of culture media and axillary bud growth (Table 3). DMRT also revealed significant differences between treatments, where ½ MS medium supplemented with BAP (0.5 mg L^{-1}) showed maximum number (2.0 ± 0.41) of shoots per explant with an average shoot length of 1.70 ± 0.04 cm (Table 4).

After getting a successful media establishment, *in vitro* grown shoots were further extended for the shoot proliferation experiments (Table 5). The cultures were regularly transferred to a fresh medium at a periodic interval of 3–4 weeks. Herein, ½ MS (solid) medium and 0.5 mg L^{-1} BAP supplemented with Kn (0.5, 1.0 and 1.5 mg L^{-1}) showed significant variation ($p < 0.05$). DMRT showed significant differences between the treatments, where the maximum number (4.0 ± 0.82) of shoots with an average length of 2.5 ± 0.15 cm was observed for 0.5 mg L^{-1} Kn (Table 6). However, lesser success has been achieved in other combinations of Kn with MS media and BAP.

In the case of *in vitro* rooting, ANOVA revealed significant differences ($p < 0.05$) between IBA concentrations for the rooting response in a hybrid genotype– 10O (*E. tereticornis* × *E. camaldulensis*) (Table 7). DMRT also showed significant differences between the treatments, i.e., MS (1, ½ and ¼) and IBA (0.25, 0.50 and 1.00 mg L^{-1}) combinations, where the maximum number (5.00 ± 0.91) of roots per explant with an average root length of 3.10 ± 0.15 cm was observed in ¼ MS media and 1.00 mg L^{-1} IBA (Table 8). Notably, root initiation was observed in 20 explants after 3 weeks of incubation and hardening was done accordingly [Fig. 2(a–l)]. Plantlets were solidified and accustomed to field relocation, as they have developed inside closed vials under high humidity and have undeveloped cuticles.

Fig. 1 Macropropagation of hybrid eucalypts: (a) Potting mixture preparation, (b) Sample preparation, (c) Cuttings placed in Bavistin solution, (d-e) Cuttings in hycotrays (top view & side view), (f-g) Growth observed in the cuttings, (h) Profuse rooting after 2 months in stem cuttings, and (i) Transplantation of the cuttings to the polybag.

Discussion

Macropropagation

During March–May-2019, 1,500 soft cuttings of the four selected hybrid genotypes were collected from trials laid out in FRI. Hormonal treatment of IBA and IAA (1,000–5,000

Table 1 One-way ANOVA showed significance level of various concentrations and combinations of auxins on rooting of hybrid eucalypts.

Sources of Variation	Parameter: Rooting percent							
	Within auxin concentration (Pooled over the years, 2019–2020)							
	Genotype 2A: *C. torelliana* × *C. citriodora*				Genotype 1D: *E. pellita* × *E. urophylla*			
	IBA	IAA	IBA + *Enterobacter* sp.	IAA + *Enterobacter* sp.	IBA	IAA	IBA + *Enterobacter* sp.	IAA + *Enterobacter* sp.
MSS (df)	0.800* (4)	0.450 (4)	68.00* (4)	6.80* (4)	0.200 (4)	0.028 (4)	11.25* (4)	0.500* (4)
F value	6.00	2.46	51.00	8.50	3.00	2.46	61.36	4.29
P value (0.05)	0.004	0.091	0.000	0.001	0.053	0.091	0.000	0.016

*Superscript indicated significant differences for the parameters studied.

ppm) in various combinations along with *Enterobacter* sp. was given to the processed cuttings, which were placed in the mist chamber. The genus *Enterobacter* (Class: Gammaproteobacteria; Family: Enterobacteriaceae), are widely present in water, soil, and plants, and have a varied range of PGP features and act as plant growth enhancers [22, 27]. The *Enterobecter* sp. has the potential role in plant growth-promoting activity due to genes for nitrogen fixation, phosphorus mobilization, and phytohormone production [53]. For instance, *E. cloacae* strains designated MSR1 isolated from roots of non-nodulating *Medicago sativa* exhibited traits for plant growth promotion in *Pisum sativum* [27]. Further, in a study conducted at salt-affected soil of Kolhapur (Maharashtra, India); *E. cloacae* strain KBPD showed the potential for maintaining ACC deaminase activity, phosphate solubilization, IAA, siderophore, ammonia, hydrogen cyanide and exopolysaccharide production under salt stress [7]. Likewise, *E. buriae* strain RS83, isolated from the rhizosphere of cassava in Phitsanulok province of Thailand, promoted plant growth and yield of various vegetable crops [21]. Similarly, the same bacteria with strain H3 isolated from the root nodules promotes capability and compatibility to support the germination of *Vigna radiata* [51]. Recently, selected strain MG00145 (OS03) has been isolated from *Ocimum sanctum* reported to significantly enhance the growth of *Oryza sativa*, *Arachis hypogaea*, *Vigna mungo* and *Brassica rapa* var. Toria [39]. Moreover, *E. hormaechei* has been reported to solubilize K-feldspar into potassium, tri-calcium phosphate into phosphate, also produced IAA, enhanced the biomass and shoot length of *Lycopersicum esculentum*, thus, leading to improved crop productivity [42]. However, no single study has been reported on the application of *Enterobacter* sp. along with IBA as a rooting agent in tree species. Though treatments with quercetin (root initiating chemical), IBA and their combination improved biochemical parameters in developing roots, suggesting potential use in hard-to-root clones of hybrid eucalypts [14].

In current study, ANOVA results revealed a significant difference between hormonal combinations (IBA and IAA) and *Enterobacter* sp. for the cuttings length between 8 and 10 cm, where root initiation was observed in twenty (10%) and seven cuttings (~3.75%) at a concentration of 3,000 ppm for genotypes 2A (*Corymbia torelliana* × *C. citriodora*) and 1D (*E. pellita* × *E. urophylla*), respectively. Further, in one of the reviews of *Eucalyptus* species on propagation and conservation, there are so many standard tables have been given for plant growth hormonal concentrations but mostly restricted to juvenile explants only, except few studies conducted on macro-and-micropropagation belongs to mature tree-derived explants [35]. For instance, in the case of *E. cloeziana*, macro-cuttings were derived from five-years-and 15-years-old trees with IBA concentration varies from 0.0 to 6.0 mg L^{-1} IBA, and the success rate lies between 10 and 82.5% [12].

Notably, a decline in the rooting ability of *E. globulus* macro-cuttings was observed with longer length and

Table 2 Mean performance of various concentrations and combinations of auxins on rooting of stem cuttings of hybrid eucalypts.

Auxin concentration (ppm)	Rooting percent (Mean ± S. Em.)							
	Pooled over the years (2019 and 2020)							
	Genotype 2A: *C. torelliana* × *C. citriodora*				Genotype 1D: *E. pellita* × *E. urophylla*			
	IBA	IAA	IBA + *Enterobacter* sp.	IAA + *Enterobacter* sp.	IBA	IAA	IBA + *Enterobacter* sp.	IAA + *Enterobacter* sp.
1000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.41^{b}	1.00 ± 0.58^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.50 ± 0.29^{ab}
3000	1.00 ± 0.41^{b}	0.75 ± 0.48^{b}	10.00 ± 0.91^{c}	3.00 ± 0.82^{b}	0.50 ± 0.29^{b}	0.19 ± 0.12^{b}	3.75 ± 0.48^{b}	0.75 ± 0.25^{b}
4000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.00 ± 0.82^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5000	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Range	0.00 ± 0.00–1.00 ± 0.41	0.00 ± 0.00-0.75 ± 0.48	0.00 ± 0.00–10.00 ± 0.91	0.00 ± 0.00–3.00 ± 0.82	0.00 ± 0.00-0.50 ± 0.29	0.00 ± 0.00-0.19 ± 0.12	0.00 ± 0.00-3.75 ± 0.48	0.00 ± 0.00-0.75 ± 0.25

*Superscript indicated by the same letter (a, b and c) showed non-significant differences according to DMRT.

Table 3 One-way ANOVA showed the effect of various combinations of hormones and additive on shoot multiplication of nodal explants in hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sources of Variation	Parameters: number and length of shoots	
	MSS (df)	MSS (df)
Between auxin concentrations in various combination	1.778* (8)	1.284* (8)
F value	24.00	1734.00
P value (0.05)	0.000	0.000

*Superscript indicated significant differences for the parameters studied.

Table 4 Mean performance of the ½ MS medium and effect of various hormones on the shoot development of nodal explants of hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sl. No.	½ MS medium + hormone (mg L^{-1})	Number of shoots per explant (Mean ± S.Em.)	Length of shoots (cm) (Mean ± S.Em.)
		After 4 weeks	After 4 weeks
BAP (mg L^{-1})			
1.	0.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
2.	0.5	2.0 ± 0.41^{b}	1.70 ± 0.04^{b}
3.	1.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
Kn (mg L^{-1})			
4.	0.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
5.	0.5	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
6.	1.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
TDZ (mg L^{-1})			
7.	0.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
8.	0.5	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}
9.	1.0	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}

*Superscript indicated by the same letter (a and b) showed non-significant differences according to DMRT.

Table 5 One-way ANOVA showed the effect of various combinations of Kn along with the ½ MS medium + ½ BAP on shoot proliferation and multiplication of hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sources of Variation	Parameters: number and length of shoots	
	MSS (df)	MSS (df)
Between Kn in various combination	12.00* (4)	6.90* (4)
F value	6.75	110.79
P value (0.05)	0.016	0.000

*Superscript indicated significant differences for the parameters studied.

woodiness of the stem [52]. Likewise, the extent of hybrid *E. grandis* × *E. urophylla* cuttings sourced from conventional hedgebanks has been stated to expressively affect rooting percentage and growth [34]. Further, mature trees of *Ziziphus spina-christi* showed > 16 mm length were found to be more effective for rooting [1]. According to these authors, it was recommended that an optimal cutting length lies between 8 and 10 cm for hybrids. However, too many eucalypt clones remain hard to root through macro-cuttings,

Table 6 Mean performance of the effect of ½ MS medium, ½ BAP and Kn in various combination on shoot multiplication of nodal explants of hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sl. No.	Kn (mg L^{-1}) in various combination	Number of shoots per explant (Mean ± S.Em.)	Length of shoots (cm) (Mean ± S.Em.)
		After 4 weeks	After 4 weeks
1.	0.5	4.0 ± 0.82^{b}	2.50 ± 0.15^{b}
2.	1.0	1.0 ± 0.41^{a}	0.20 ± 0.02^{a}
3.	1.5	1.0 ± 0.71^{a}	0.20 ± 0.16^{a}

*Superscript indicated by the same letter (a and b) showed non-significant differences according to DMRT.

Table 7 One-way ANOVA showed the effect of various combinations of MS media and hormones on root multiplication of nodal explants of hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sources of Variation	Parameters: number and length of roots	
	MSS (df)	MSS (df)
Between auxin concentrations in various combination	10.78* (8)	4.14* (8)
F value	14.55	262.25
P value (0.05)	0.000	0.000

*Superscript in the table indicated significant differences for the parameters studied.

leading to the implementation of a mini-cutting system ranging from 2 to 3 cm. Recently, weak genetic control with very low heritability (0.3%) was specified in the cuttings of *Eucalyptus* sp., where a highly diverse rooting fraction might be due to the physiological positioning of the cuttings [31]. The affirmative discussion concludes that cuttings from *Eucalyptus* hybrids, specifically matured ones, are difficult to root due to genotypic, environmental and interaction effects. Still, proper length and combinations of hormones and genotypes adaptive traits (e.g., bark texture and pattern, GBH, wood anatomical properties, etc.) determine the ability to survive under natural conditions.

Micropropagation

Eucalyptus sp. *in vitro* propagation was first described through axillary bud proliferation and somatic embryogenesis using seedling tissues and micropropagation during 1964 [41], which showed the importance of the technique to obtain clones with mass-scale production [54]. Numerous micropropagation procedures have already been established for diverse *Eucalyptus* sp. Still, it is tough to reiterate due to each passing step, such as establishment, multiplication, elongation and rooting, every genotype showed varying behaviour. It means novelty is required in micropropagation methods at different stages to enhance each clone for viable production [8].

Table 8 Mean performance of the effect of different strengths of MS media and IBA on root development in *in vitro* developed shoots in hybrid (*E. tereticornis* × *E. camaldulensis*; 10O).

Sl. No.	MS medium in various combinations	IBA hormone in various combinations (mg L^{-1})	Number of roots per explant (Mean ± S.Em.)	Length of roots (cm) (Mean ± S. Em.)
			After 4 weeks	After 4 weeks
1.	1/4	0.25	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2.	1/4	0.50	0.00 ± 0.41^{a}	0.00 ± 0.04^{a}
3.	1/4	1.00	5.00 ± 0.91^{b}	3.10 ± 0.15^{d}
4.	1/2	0.25	1.00 ± 0.58^{a}	0.50 ± 0.11^{c}
5.	1/2	0.50	1.00 ± 0.71^{a}	0.25 ± 0.04^{b}
6.	1/2	1.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
7.	MS	0.25	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
8.	MS	0.50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
9.	MS	1.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

*Superscript indicated by the same letter (a, b, c and d) showed non-significant differences according to DMRT.

Current study revealed that 10O (*E. tereticornis* × *E. camaldulensis*) genotypes showed a positive response in culture establishment with repeated sub-culturing of explants for 5th, 6th and 7th times. This is important as mature plant juvenile cuttings take more time for culture establishment. For instance, it seems that morphogenetic potential of the explants of *Taxus mairei* showed immense response to shoot multiplication and elongation only after 7 weeks in culture [11]. Similarly, the differences were observed in the rooting success among the micro-cuttings derived from adult trees of *E. cloeziana*, which was related to the genotypic effects, as the rooting capacity of propagules showed specificity in hormonal combination whose range varied from ½–1 MS (sometimes with modified 1 mL L^{-1} vitamin) with 30 g L^{-1} sucrose and the PGRs 5–16 mM NAA, 2.5 mM zeatin, 4.5 mM BAP, 100 mM IBA, and 2.3 g L^{-1} McGowan's woody plant basal salt mixture [18].

In vitro cultures and regular sub-culturing were successfully established, which leads to maximum shoot proliferation and shoot length in ½ MS media fortified with 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} Kn. This study utilized the first pathway of direct organogenesis, where ¼ MS and 1.0 mg L^{-1} IBA leads to established rooting in two explants of a hybrid 10O. Otherwise, organogenesis via. callus has been the other pathway utilized for difficult to root species. It is noticed that adult tree-derived cultures produced fewer shoots than explants derived from coppice material due to physiological maturity as depicted in *Eucalyptus radiata* spp. *radiata* [10]. Further, a mature hybrid of *E. tereticornis* × *E. grandis* revealed shoot multiplication from 1.0 mg L^{-1} each of BAP and NAA, and 1.0 mg L^{-1} IBA for rooting [24]. Similarly, nodal segments from matured hybrid of *E. urophylla* × *E. grandis* revealed shoot multiplication and elongation from 0.1 mg L^{-1} BAP and 0.01 mg L^{-1} NAA, and 1.0 mg L^{-1} IBA/NAA for rooting [37]. Notably, in *E. benthamii*, Woody Plant Medium (WPM) supplemented with 0.5 mg L^{-1} BAP and 0.05 mg L^{-1} NAA showed significant results in *in vitro* bud multiplication and nutrient medium free of GA3 and BAP for the elongation of shoots. The study further revealed that the culture medium, concentration of plant growth regulators, and the viability of cuttings has been enhanced through mini-incubators mostly depended on the genotype of a clone [8]. In *E. cloeziana* epicormic shoots were induced in branches of adult trees shoots were multiplied *in vitro* using WPM culture supplemented with 0.60 mg L^{-1} BA and 0.10 mg L^{-1} NAA, elongation at 0.1 mg L^{-1} BA, and *ex vitro* rooting and acclimatisation was successful in mini-incubators [13]. These findings on different *Eucalyptus* hybrids illustrated the congruence to our findings on hybrid *E. tereticornis* × *E. camaldulensis*, i.e., 10O genotype.

In case of other matured explant-derived tree species, *T. mairei* showed shoot production at 2.5 mg L^{-1} BA and rooting at 2.5 mg L^{-1} IBA with 35% success [11]. Multiple shoots and roots were obtained from the mature trees of *Pistacia vera* on MS supplemented with 8.8 mM benzyl amino purine (BA) and 9.8 µM IBA [38]. Further, a mature tree (20–25-years-old) of *Dalbergia sissoo* was used for shoot bud induction on MS medium complemented with 8.88 µM of BAP, multiplication of shoots at 2.22 µM BAP and 0.002 µM TDZ, and finally, *ex vitro* rooted shoots were treated with 984 µM of IBA for 5 min. showing the success of *in vitro* methods [48]. In contradiction to the findings of a current study, the culture medium supplemented with 0.05 or 0.10 mg L^{-1} BAP was failed to induce elongated shoots, which postulates that sometimes shoot proliferation and establishment is more stressful than root initiation and development [8]. These results signify the key level of hormonal concentration and incubation period determine the shoot and root proliferation in matured trees; nevertheless, multiplication ability varies across the genotypes and species in eucalypts.

In case of hardening and acclimatization, after two weeks of culture, the uppermost new leaves shrivelled and appeared yellowish-green, which may be due to the iron deficiency in rooting media. With a successful root growth, hardening

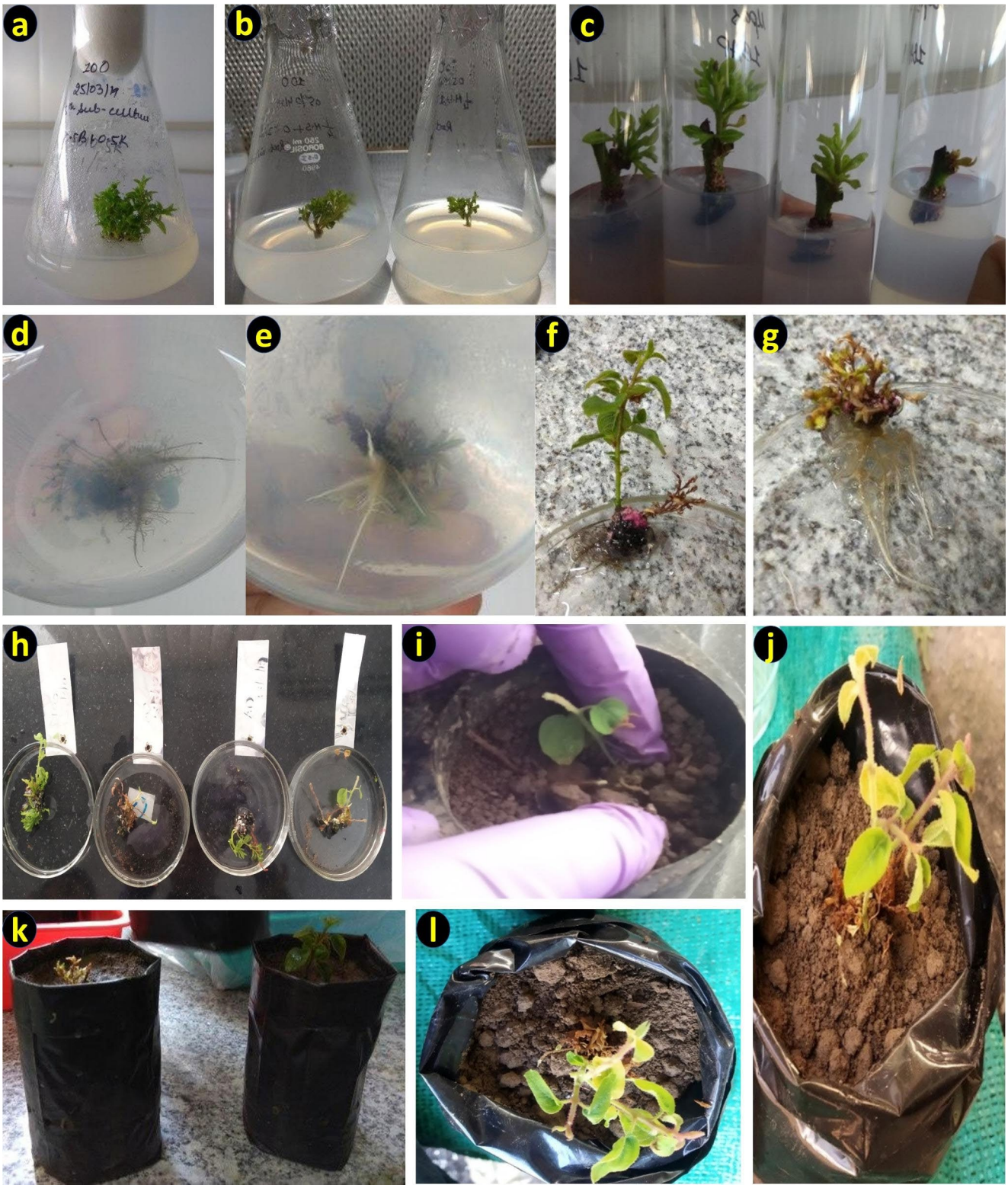

Fig. 2 *In vitro* propagation of hybrid *E. tereticornis* × *E. camaldulensis* (10O) through axillary bud: (a-b) Shoot initiation and multiple-shoot formation after 46 days, (c) Optimal shoot growth for transferring to the rooting media after 76 days, (d-e) Root initiation and expansion was observed after 91 days, (f-g) Optimum root growth for transferring *in vitro* plantlet to *ex vitro* conditions, (h) Washing and fungicide treatment (0.2% carbendazim) was given to the plantlet, (i) Transplanting the treated plantlet into the soil, and i*n vitro* hardening (j) side view and (k-l) top view.

(transfer to polybag) was carried out after a month of root development in hybrid genotype 10O (*E. tereticornis* × *E.*

camaldulensis) with 1% success. A report of less than 10% success in rooting of stem cuttings was observed in *T. mairei* derived from adult trees after 6 months of greenhouse cultivation [19]. A success rate of 45–66% plantlets establishment was observed for *E. benthamii* × *E. dunnii* [9], which was considered appropriately great for profitable *Eucalyptus* micropropagation industries [15].

Conclusions

In the present study, effective and commercially viable macro-and-micropropagation techniques were successfully developed through branch cuttings of mature trees, which could be further utilized for mass multiplication of superior eucalypts and their hybrids.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13237-022-00394-3.

Acknowledgements The financial support by the Indian Council of Forestry Research & Education (ICFRE), Dehradun, under Grant No. 5-1/2017-RCS; dated 23rd June, 2017 is gratefully acknowledged. The authors are thankful to the Director, FRI for providing the research facilities and Ms. Upasana Chaubey for conducting experimentation on micropropagation. Authors wish to thank the Handling Editor and the anonymous reviewer for their constructive and positive comments in the manuscript.

Author contribution MSB conceived the research ideas, conducted the analysis, data interpretation, wrote the first draft of the manuscript and led the writing. SM and AS performed the macropropagation-based field experimentation. GSP and AT performed the micropropagation experimentation. SP performed the experimentation on microbes and provided the bacterial culture isolates. All authors edited the draft and critically revised the manuscript.

Funding The financial support by the Indian Council of Forestry Research & Education (ICFRE), Dehradun, under Grant No. 5-1/2017-RCS; dated 23rd June, 2017 is gratefully acknowledged. The authors are thankful to the Director, FRI for providing the research facilities.

Availability of data and material (data transparency) All data files have been uploaded and clearly written in the manuscript.

Code Availability Not applicable.

Declaration None declared.

References

1. Assareh MH, Sardabi H. Macropropagation and micropropagation of *Ziziphus spina-christi*. Pesquisa Agropecuária Brasileira. 2005;40(5):459–65.
2. Assis TF, Fett-Neto AG, Alfenas AC. Current techniques and prospects for the clonal propagation of hardwood with emphasis on *Eucalyptus*, in Plantation Forest Biotechnology for the 2004. Walter and M. Carson (Kerala: Research Signpost).
3. Ballester A, Corredoira E, Viéitez Martín AM. Limitations of somatic embryogenesis in hardwoods trees. 2016.
4. Bandyopadhyay S, Cane K, Rasmussen G, Hamill JD. Efficient plant regeneration from seedling explants of two commercially important temperate eucalypt species–*Eucalyptus nitens* and *E. globulus*. Plant Science. 1999;140(2):189 – 98.
5. Barton A. The oil mallee project: a multifaceted industrial ecology case study. J Ind Ecol. 1999;3(2-3):161–76.
6. Batish DR, Singh HP, Kohli RK, Kaur S. *Eucalyptus* essential oil as a natural pesticide. For Ecol Manag. 2008;256(12):2166–74.
7. Bhise KK, Bhagwat PK, Dandge PB. Plant growth-promoting characteristics of salt tolerant *Enterobacter cloacae* strain KBPD and its efficacy in amelioration of salt stress in *Vigna radiata* L. J Plant Growth Regul. 2017;36(1):215–26.
8. Brondani GE, de Wit Ondas HW, Baccarin FJ, Gonçalves AN, de Almeida M. Micropropagation of *Eucalyptus benthamii* to form a clonal micro-garden. Vitro Cell Dev Biology-Plant. 2012;48(5):478–87.
9. Brondani GE, Dutra LF, Grossi F, Wendling I, Hornig J. Establishment, multiplication and elongation in vitro of *Eucalyptus benthamii* Maiden & Cambage × *Eucalyptus dunnii* Maiden. Revista Árvore. 2009;33(1):11–9.
10. Chang SH, Donald DG, Jacobs G. Micropropagation of *Eucalyptus radiata ssp. radiata* using explants from mature and coppice material. S Afr For J. 1992;1(1):43–7.
11. Chang SH, Ho CK, Chen ZZ, Tsay JY. Micropropagation of *Taxus mairei* from mature trees. Plant Cell Rep. 2001;20(6):496–502.
12. de Almeida F, Xavier A, Dias JM. Vegetative propagation of selected *Eucalyptus cloeziana F*. Muell. trees through cutting technique. Revista Árvore. 2007;31:445–53.
13. de Oliveira LS, Brondani GE, Batagin-Piotto KD, Calsavara R, Gonçalves AN, de Almeida M. Micropropagation of *Eucalyptus cloeziana* mature trees. Australian Forestry. 2015;78(4):219–31. DOI:https://doi.org/10.1080/00049158.2015.1073211.
14. do Prado DZ, Dionizio RC, Vianello F, Baratella D, Costa SM, Lima GP. Quercetin and indole 3-butyric acid (IBA) as rooting inducers in '*Eucalyptus grandis* × *E. urophylla*'. Aust J Crop Sci. 2015;1(11):1057–63.
15. Dutra LF, Wendling I, Brondani GE. A micropropagação de *eucalipto*. Embrapa Florestas-Artigo em periódico indexado (ALICE). 2009.
16. Ferreira M, Santos PET. Proceedings IUFRO Conference on Silviculture and Improvement of *Eucalypts*, v.1, Salvador, Colombo (CNPF-EMBRAPA, 14.) 1997.
17. Gomes F, Canhoto JM. Micropropagation of *Eucalyptus nitens* Maiden (shining gum). *In Vitro* Cellular & Developmental Biology-Plant. 2003;39(3):316 – 21.
18. Goodger JQD, Heskes AM, King DJ, Gleadow RM, Woodrow IE. Research note: micropropagation of *Eucalyptus polybractea* selected for key essential oil traits. Funct Plant Biol. 2008;35:247–51. doi:https://doi.org/10.1071/FP07241.
19. Ho CK, Chang SH, Tsai JY. Selection breeding, propagation and cultivation of *Taxus mairei* in Taiwan. Taiwan For Res Inst. 1998;88:65–82.
20. Ikemori YK, Penchel RM, Bertolucci FLG. Integrating biotechnology into eucalypt breeding. International Symposium of Wood Biotechnology. Tokyo University of Agriculture and Technology, Tokyo. 1994.
21. Jetiyanon K. Multiple mechanisms of *Enterobacter asburiae* strain RS83 for plant growth enhancement. Songklanakarin Journal of Science & Technology. 2015;1:37(1).
22. Jha CK, Aeron A, Patel BV, Maheshwari DK, Saraf M. *Enterobacter*: role in plant growth promotion. In: Bacteria in agrobiology: Plant growth responses. Berlin: Springer; 2011. pp. 159–82.

23. Jones NB, Van Staden J. Micropropagation and establishment of *Eucalyptus grandis* hybrids. South Afr J Bot. 1994;1(2):122–6.
24. Joshi I, Bisht P, Sharma VK, Uniyal DP. In vitro Clonal Propagation of Mature *Eucalyptus F*. Silvae Genetica. 2003;52:3–4.
25. Kendurkar SV, Rangaswamy M. *In Vitro* Approaches for the Improvement of Eucalyptus. In: Biotechnologies of Crop Improvement. Cham: Springer; 2018. pp. 159–214.
26. Keret R, Nakhooda M, Jones NB, Hills PN. Optimisation of micropropagation protocols for temperate eucalypt hybrids in South Africa, with a focus on auxin transport proteins. South Forests: J For Sci. 2022;20:1–0.
27. Khalifa AY, Alsyeeh AM, Almalki MA, Saleh FA. Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi Journal of Biological Sciences. 2016;23(1):79–86.
28. Kvaalen H, Daehlen OG, Rognstad AT, Grønstad B, Egertsdotter U. Somatic embryogenesis for plant production of *Abies lasiocarpa*. Can J For Res. 2005;35(5):1053–60.
29. Lane DJ. 16S/23S rRNA Sequencing. Chichester: John Wiley and Sons; 1991.
30. Le Roux JJ, Van Staden J. Micropropagation and tissue culture of *Eucalyptus*—a review. Tree Physiol. 1991;9(4):435–77.
31. Makouanzi G, Bouvet JM, Denis M, Saya A, Mankessi F, Vigneron P. Assessing the additive and dominance genetic effects of vegetative propagation ability in *Eucalyptus*—influence of modeling on genetic gain. Tree Genet Genomes. 2014;10(5):1243–56.
32. Martin B. *Eucalyptus*: A strategic forest tree. In: Wei RP, Xu D, editors. *Eucalyptus* Plantations: Research, Management and Development. Proceedings of the International Symposium, Guangzhou, China. 2002. World Scientific Publishing Co. Pte. Ltd., Singapore; pp. 3–18.
33. Miguel C, Gonçalves S, Tereso S, Marum L, Maroco J, Margarida Oliveira M. Somatic embryogenesis from 20 open-pollinated families of Portuguese plus trees of maritime pine. Planr Cell Tissue Organ Cult. 2004;76(2):121–30.
34. Naidu RD, Jones NB. The effect of cutting length on the rooting and growth of subtropical *Eucalyptus* hybrid clones in South Africa. South Forests. 2009;71(4):297–301.
35. Nakhooda M, Jain SM. A review of *Eucalyptus* propagation and conservation. Propag Ornam Plants. 2016;16(4):101–19.
36. Nakhooda M, Mandiri E. Using synergistic exogenous phytohormones to enhance somatic embryogenesis from leaf explants of a *Eucalyptus grandis* clone. South Forests: J For Sci. 2016;78(1):73–80.
37. Nourissier S, Monteuuis O. In vitro rooting of two *Eucalyptus urophylla* × *Eucalyptus grandis* mature clones. *In Vitro* Cellular & Developmental Biology-Plant. 2008;44(4):263–72.
38. Onay A. Micropropagation of pistachio from mature trees. Planr Cell Tissue Organ Cult. 2000;60(2):159–63.
39. Panigrahi S, Mohanty S, Rath CC. Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South Afr J Bot. 2020;134:17–26.
40. Park YS, Pond SE, Bonga JM. Initiation of somatic embryogenesis in white spruce (*Picea glauca*): genetic control, culture treatment effects, and implications for tree breeding. Theor Appl Genet. 1993;86(4):427–36.
41. Pinto G, Araújo C, Santos C, Neves L. Plant regeneration by somatic embryogenesis in *Eucalyptus* spp.: current status and future perspectives. South Forests: J For Sci. 2013;1(2):59–69.
42. Ranawat B, Bachani P, Singh A, Mishra S. *Enterobacter hormaechei* as plant growth-promoting bacteria for improvement in *Lycopersicum esculentum*. Curr Microbiol. 2021;78:1208–17. https://doi.org/10.1007/s00284-021-02368-1.
43. Saafi H, Borthakur D. In vitro plantlet regeneration from cotyledons of the tree-legume *Leucaena leucocephala*. Plant Growth Regul. 2002;38(3):279–85.
44. Santana Costa Souza DM, Fernandes SB, Oliveira Silva E, Politi Duarte V, Santos Gonçalves D, de Carvalho D, Leal Teixeira G, Ebling Brondani G. Effect of light intensity on in vitro introduction and multiplication of *Eucalyptus grandis* × *Eucalyptus urophylla*. *In Vitro* Cellular & Developmental Biology-Plant. 2021;23:1–5.
45. Termignoni RR, Wang PJ, Hu CY. Somatic embryo induction in *Eucalyptus dunnii*. Plant Cell, Tissue and Organ Culture. 1996;45(2):129–32.
46. Timmis R, El-Nil MM, Stonecypher RW. Potential genetic gain through tissue culture. In: Cell and Tissue Culture in Forestry. Dordrecht: Springer; 1987. pp. 198–215.
47. Trueman SJ, Hung CD, Wendling I. Tissue culture of *Corymbia* and *Eucalyptus*. Forests. 2018;9(2):84.
48. Vibha JB, Shekhawat NS, Mehandru P, Dinesh R. Rapid multiplication of *Dalbergia sissoo* Roxb.: a timber yielding tree legume through axillary shoot proliferation and *ex vitro* rooting. Physiol Mol Biology Plants. 2014;20(1):81–7.
49. Wallis JT. Recirculating hydroponic systems: evaluating cuttings yield and rooting ability of cold tolerant eucalyptus hybrids (Doctoral dissertation). 2004.
50. Watt MP, Blakeway F, Cresswell CF, Herman B. Somatic embryogenesis in *Eucalyptus grandis*. S Afr For J. 1991;157:59–65.
51. Widowati T, Sukiman H. Production of indole acetic acid by *Enterobacter cloacea* H3 isolated from Mungbean (*Vigna radiata*) and its potential supporting the growth of soybean seedling. In IOP Conference Series: Earth and Environmental Science. IOP Publishing; 2019;308. pp. 012040.
52. Wilson PJ. Propagation characteristics of *Eucalyptus globulus* Labill. ssp. *globulus* stem cuttings in relation to their original position in the parent shoot. J Hortic Sci. 1993;68(5):715–24.
53. Witzel K, Gwinn-Giglio M, Nadendla S, Shefchek K, Ruppel S. Genome sequence of *Enterobacter radicincitan*. DSM16656T, a plant growth-promoting endophyte. 2012.
54. Xiao Y, Niu G, Kozai T. Development and application of photoautotrophic micropropagation plant system. Plant Cell Tissue and Organ Culture (PCTOC). 2011;105(2):149–58.