Propagation of Ornamental Plants
Vol. 12, № 1, 2012: 25-51

Invited paper

IN VITRO PROPAGATION OF TROPICAL HARDWOOD TREE SPECIES – A REVIEW (2001-2011)

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Abstract

Tropical hardwood tree species are important economically and ecologically, and play a significant role in the biodiversity of plant and animal species within an ecosystem. There are over 600 species of tropical timbers in the world, many of which are commercially valuable in the international trade of plywood, roundwood, sawnwood, and veneer. Many of these tree species are being threatened and are endangered because of logging practices, conversion to agricultural lands, non-optimal management strategies, and overall deforestation rates that cannot keep up with natural regeneration of native forests. Tropical tree species provide timber for commercial uses because of the beauty of the wood-grain, -color, or -pattern, strength, durability, and versatility of finishing applications for a vast array of markets. Because of the high value of tropical tree species, *in vitro* (adventitious shoot regeneration, cryopreservation, genetic transformation, micrografting, protoplast culture, shoot tip and nodal culture, and somatic embryogenesis) propagation technologies are an integral component in tree improvement and conservation programs, in order to complement seed banking and *ex situ* measures for long-term conservation and clonal propagation of germplasm. Tropical tree species are also important because wildlife populations may be affected, soils can be stabilized and organic matter and nutrients in the forest floor altered, degraded areas can be restored, and tropical trees also provide socio-economic development for local communities. In addition, tropical tree species are valuable as ornamentals for landscaping (shade, flower, fall foliage, and fruit production), and as resources for a multitude of known and yet unknown commercial properties, such as medicinal drugs, natural insecticides, industrial uses, and non-timber products. This paper is a literature review (2001-2011) on *in vitro* propagation of tropical tree species grown or harvested for timber.

Key words: adventitious shoot regeneration, cryopreservation, micrografting, nodal culture, organogenesis, regeneration, shoot tip culture, somatic embryogenesis, transformation

INTRODUCTION

Tropical hardwood tree species are important economically and ecologically, and play a significant role in the biodiversity of plant and animal species within an ecosystem, similar to temperate hardwood tree species (see Pijut et al. 2011). There are over 600 species of tropical timbers in the world (Chudnoff 1984, USDA FPL 2011), many of which are commercially valuable in the international trade of plywood, roundwood, sawnwood, and veneer (GTWPTN 2011, ITTO 2011, USDA FAS 2011). Many of these tree species are being threatened and are endangered (IUCN 2011) because of logging practices, conversion to agricultural lands, non-optimal management strategies, and overall deforestation rates that cannot keep up with natural regeneration of native forests. Climate change models also project that tropical forests may have significant and unprecedented heat regimes in the near future, which will impact productivity (Diffenbaugh and Scherer 2011).

Tropical forests can be found 23 ½º North latitude and 23 ½º South latitude at elevations below 1000 m, and divided into four major regions (Neotropical, Afrotropical, Indomalayan Tropical, and Australian Tropical) (Duery and Vlosky 2006). Hardwood plantations in the tropics would be a valuable complement to natural tropical forest regeneration, in order to assure a future supply of these commercially valuable timbers (FAO 2001). Tropical tree species provide timber for commercial uses because of the beauty of the wood-grain,

Received: December 12, 2011 *Accepted: January 30, 2012*

-color, or -pattern, strength, durability, and versatility of finishing applications for a vast array of markets (boat construction, cabinetry, decking, doors, flooring, furniture, heavy and light building construction, interior finishing, marine docks or pilings, millwork, musical instruments, paneling, turnery, and various specialty products). Because of the high value of tropical tree species, *in vitro* propagation technologies are an integral component in a tree improvement or conservation program, in order to complement seed banking and *ex situ* measures for long-term conservation and clonal propagation of germplasm (Pence 2010, Sarasan 2010). Tropical tree species are also important because of the impacts on wildlife populations (Chapman et al. 2010), soil stabilization, and organic matter and nutrients in the forest floor (Binkley and Menyailo 2005). These trees are also important as "living fences" for restoration of degraded areas (Zahawi 2005), and they provide socio-economic opportunities for local communities. In addition, tropical tree species are valuable as ornamentals for landscaping (shade, flower, fall foliage, and fruit production), and as sources for medicinal drugs and cytotoxic compounds, natural insecticides (Isman 2005), industrial (oils, dyes, and rubber), and non-timber products (nuts and fruits).

Some of the more well-known tropical tree species grown or harvested for wood include: teak (*Tectona grandis*), mahogany (*Swietenia macrophylla*), eucalypt (*Eucalyptus grandis*), rosewood (*Dalbergia* spp.), primavera (*Tabebuia donnell-smithii*), acacia (*Acacia* spp.), Spanish or Brazilian cedar (*Cedrela* spp.), purpleheart (*Peltogyne* spp.), neem (*Azadirachta* spp.), meranti (*Shorea* spp.), bocote (*Cordia* spp.), and African ebony (*Diospyros* spp.), but there are many other lesser-known species that potentially could command a high market value for the beautiful wood.

This review focuses on the literature (2001-2011) with *in vitro* (adventitious shoot regeneration, cryopreservation, genetic transformation, micrografting, protoplast culture, nodal culture, shoot tip culture, and somatic embryogenesis) propagation of tropical tree species grown or harvested for timber. There are several common names for many of these species, but we have tried to focus on names (trade) used in the commercial wood markets (The Wood Explorer 2011). There is also considerable interest in molecular markers to assess genetic variation in tropical tree species (Muchugi et al. 2008), but that subject matter would require a separate review. Some research on tropical tree technologies has been undertaken by private companies, and therefore the data is confidential, patented, or often only partially shared as scientific publications. Although a comprehensive review of the literature is never really possible, we have tried to briefly summarize the most recent information on *in vitro* propagation of tropical hardwood tree species.

IN VITRO TECHNOLOGIES

Adventitious shoot regeneration

Adventitious shoot regeneration (shoot organogenesis) arises from unusual points of origin either directly or indirectly through a callus stage (e.g. from anthers, cotyledons, hypocotyls, leaves, petioles, stem internodes, or root segments) and may not be desirable for clonal propagation of a species because of the possibility of somaclonal variation. However, these protocols are extremely valuable in the genetic improvement and conservation of a species. Adventitious shoot regeneration is a key step in regenerating plants that have been genetically modified via *Agrobacterium*-mediated transformation.

Albizia

The addition of 3 mg l^{-1} hymexazol (HMI) to a modified Murashige and Skoog (1962) medium (MS) containing 2.22 µM N^6-benzyladenine (BA) and 0.05 µM α-naphthaleneacetic acid (NAA) increased adventitious shoot development on cotyledons and hypocotyls of *Albizia julibrissin* (Albizzia). Roots developed on shoots cultured on 0.3 mg l^{-1} HMI on a half-strength MS medium (Yin et al. 2001).

Azadirachta

Direct organogenesis and plant regeneration using leaf explants of *Azadirachta excelsa* (Limpaga) was achieved, by culturing shoot tips from a 6-month-old nursery grown plant on MS medium with 6.66 µM BA. After 4 months, the *in vitro* leaves were excised and cultured on medium with combinations of BA, kinetin (KIN), and adenine sulfate. An average of five adventitious shoots per leaf explant were produced on 8.88 µM BA, 5.58 µM KIN, and 6 mg l^{-1} adenine sulfate. The best shoot development and elongation was observed on MS medium with 4.44 µM BA and 12.5 mg l^{-1} magnesium sulfate. Rooting (100%) occurred on MS medium with 53.7 µM NAA, and all plantlets survived 30 days after acclimatization (Foan and Othman 2006).

Salvi et al. (2001) investigated the regeneration potential of 7-day-old hypocotyl, epicotyl, cotyledonary node, root-shoot zone, cotyledon, leaves, and roots from *A. indica* (Neem or Chinaberry). Explants were cultured on MS medium with 8.88 µM BA and 0.57 µM indole-3-acetic acid (IAA). All explants exhibited shoot development, with leaves and roots giving the highest and lowest number of shoots per explant, respectively. Shoots were cultured on MS medium with 4.92 µM indole-3-butyric acid (IBA), where 40% of shoots produced roots.

Callus formation on *A. indica* anthers was achieved through the application of 21.5 µM NAA and 11.9 µM KIN in MS basal medium. Plant regeneration was best on MS medium with 4.4 µM BA with the addition of

0.25 mg l^{-1} silver nitrate enhancing shoot elongation. Rooting (85%) was achieved on half-strength MS medium with 5.7 µM IAA alone, or in combination with 0.046 µM KIN (Dhillon et al. 2005).

Organogenesis from unfertilized ovaries from various sized flower buds of a 54-year-old neem tree was developed by Srivastava et al. (2009). To induce callus, ovaries were cultured on MS medium with 1 µM 2, 4-dichlorophenoxyacetic acid (2,4-D), 5 µM BA, and 90 g l^{-1} sucrose. Callus was then transferred to MS medium with 0.5 µM 2,4-D alone, or in combination with 4.5 µM KIN. Best shoot regeneration (78%) occurred using 4 mm flower buds cultured on MS medium with 0.05 µM 2,4-D and then cultured on MS medium with 5 µM BA. Multiplication was achieved through forced axillary branching on MS medium with 1.0 µM BA and the addition of casein hydrolysate. Rooting (79%) occurred on quarter-strength MS medium with 0.5 µM IBA, with 81.8% plantlet survival after transplantation.

Cariniana

Indirect organogenesis of *Cariniana pyriformis* (Abarco) was achieved using woody plant medium (WPM; Lloyd and McCown 1980) supplemented with 1.21 µM IAA and 7.6 µM BA. This combination produced 75% callus formation and bud initiation after 4 weeks using hypocotyls collected from 2-week-old *in vitro* explants (Yaya et al. 2005).

Cedrela

Adventitious shoot regeneration was achieved using 12-day-old hypocotyl segments from *Cedrela odorata* (Central American cedar). The addition of 20% coconut water to TY17 medium (full-strength WPM plus half-strength MS vitamins) increased the number of adventitious shoots per explant. No cytokinin studied provided similar results. The use of juvenile material in BioMINT bioreactors for root and shoot elongation resulted in a 3.5- and 4-fold increase in shoot and root elongation, respectively, and resulted in 98% acclimatization to soil of *in vitro* grown plants. Using juvenile material was more efficient, as it was possible to propagate 16,000 plants per mother plant in 6 months in comparison to 125 with mature material (Peña-Ramirez et al. 2010).

Dalbergia

Dalbergia latifolia (Indian Rosewood) regeneration was initiated using excised root cultures from field-grown plants cultured on MS medium with 0.2 µM thiamine-HCl, 0.5 µM pyridoxine-HCl, 0.8 µM nicotinic acid, 0.2 µM folic acid, 0.4 µM d-biotin, 0.2 µM riboflavin, and 0.05 µM NAA for root culture establishment. However, after 8 years of culture, the addition of 0.9 µM potassium nitrate and 111 µM myo-inositol were necessary to maintain viability. Shoots were induced from root cultures using a three-step process: Schenk and Hildebrandt (1972) medium (SH) plus 1.1 µM BA, 0.14 µM IAA, 37 µM adenine sulfate, 94.9 µM chlormequat chloride, followed by 0.44 µM BA, 0.14 µM IAA, 37 µM adenine sulfate, 31.6 µM chlormequat chloride, and finally 0.44 µM BA, 0.14 µM IAA, 37 µM adenine sulfate, and 1.89 µM ancymidol. A two-step rooting procedure was reported, where growth in agar medium with 1.43 µM IAA was followed by a filter-paper-bridged liquid medium culture without IAA to minimize callus formation (Chaturvedi et al. 2004b).

Cerdas and Guzmán (2004) studied adventitious shoot regeneration from hypocotyls of *D. retusa* (Cocobolo) and reported optimal shoot formation was obtained on half-strength MS medium plus 8.8 µM BA and 20 g l^{-1} sucrose. Half-strength MS medium with 19.7 µM IBA resulted in the greatest ratio of shoot to root formation (91%), however half-strength MS medium with 24.6 µM IBA produced the most roots, with thicker roots produced at higher IBA concentrations.

Adventitious shoot organogenesis on *D. sissoo* (Sissoo) semi-mature cotyledons required MS medium with 4.44 µM BA and 0.26 µM NAA. Cotyledon explants required MS medium with 22.2 µM BA, however pre-culture in liquid MS medium with 8.88 µM BA for 48 h greatly improved regeneration. Shoots derived from cotyledons were rooted on half-strength MS medium with 1.23 µM and 4.92 µM IBA, respectively (Singh et al. 2002).

Chand and Singh (2005) regenerated plants of *D. sissoo* from callus derived from semi-mature zygotic embryos. Callus was maintained on MS medium with 9.04 µM 2,4-D and 1.16 µM KIN for 40 days, and then transferred to adventitious shoot regeneration medium where 45% of the explants cultured on MS medium with 1.34 µM NAA and 8.88 µM BA produced shoots. Shoots were rooted on half-strength MS medium with 1.23 µM IBA, 0.27 mM myo-inositol, and 30 g l^{-1} sucrose for 15 days, then acclimatized in half-strength or quarter-strength MS medium for 10 days before being transferred to pots.

Diospyros

Adventitious shoot regeneration of *Diospyros kaki* 'Rojo Brillante' (Chinese Persimmon) was achieved from young leaf explants using a MS medium with 41 µM zeatin (ZT) and 0.5 µM NAA, as well as a 20-day dark pulse. Regenerated shoots were micrografted onto seedling rootstocks of *D. virginiana* (Naval et al. 2009).

Garcinia

Shoot regeneration (63.9%) of *Garcinia indica* (Kokum) was achieved with leaves from *in vitro* raised seedlings using a MS medium containing 1 µM thidiazuron (TDZ; 1-Phenyl-3-(1,2,3,-thiadiazol-5-yl)urea. Thidiazuron was superior for regeneration of adventitious buds compared to BA and KIN, or in combination

with NAA. Maximum rooting (83.3%) was achieved when shoots were cultured on half-strength MS medium with 10 µM IBA (Malik et al. 2010).

Lagerstroemia

Adventitious shoots were induced from leaf-derived callus of *Lagerstroemia speciosa* (Banaba or Queens Crapemyrtle) on a MS medium with 5 µM BA, 3 µM NAA, 10% coconut water, and 568 µM ascorbic acid. Rooting of shoots was achieved on a MS medium with 1 µM IBA (Rahman et al. 2010).

Melia

Neo-formation of flower buds, vegetative shoots, and shoot regeneration from callus of *Melia azedarach* (Persian Lilac) was reported by Handro and Floh (2001). Hypocotyl segments cultured on MS medium without plant growth regulators (PGRs) regenerated (80%) vegetative buds and normal shoots.

The effect of genotype and explant source on indirect organogenesis of *M. azedarach* was investigated using different leaf portions at various developmental stages from seven genotypes. Explants were cultured on MS medium with 4.44 µM BA, 0.465 µM KIN, and 3 mg l^{-1} adenine sulfate, with the adaxial side of the explant in contact with the medium. Regeneration was influenced by developmental stage with regeneration increasing with increasing leaf age. All portions of the leaf regenerated shoots, however the greatest number of shoots per explant and percent shoot regeneration occurred from the rachis (10-30%) except for two genotypes. Regenerated shoots were rooted on MS medium with 12.3 µM IBA for 4 days and then transferred to plant growth regulator (PGR)-free MS medium for 26 days with 85% survival (Vila et al. 2004).

A plant regeneration system via organogenesis using cotyledons of *M. azedarach* was developed by Sharry and Silva (2006). Callus was induced on cotyledons cultured on MS medium with 2.69 µM NAA, 4.44 µM BA, 1 g l^{-1} casein hydrolysate, and 50 g l^{-1} sucrose. Shoot proliferation occurred on MS medium with 4.44 µM BA, 200 mg l^{-1} casein hydrolysate, 40 mg l^{-1} adenine, 80.5 mg l^{-1} putrescine, and 20 g l^{-1} sucrose, with shoot elongation achieved on MS medium with 0.89 µM BA, 0.029 µM gibberellic acid (GA_3), 161.1 mg l^{-1} putrescine, and 30 g l^{-1} sucrose. Shoots were rooted on MS medium with 0.05 µM NAA and 10 g l^{-1} sucrose.

Pterocarpus

Adventitious shoots and roots formed on callus proliferating from nodal sections of *in vitro* germinated *Pterocarpus marsupium* (Bijasal) embryos on MS medium without PGRs. Plantlets were acclimatized in the greenhouse and survived under field conditions (Tiwari et al. 2004).

Ricinodendron

Adventitious buds were induced from callus obtained on *Ricinodendron heudelotii* (African nut tree) microcuttings cultured on half-strength MS medium with 16.3 µM KIN. Eighty-five-percent of microcuttings developed roots when exposed to 8.1 µM NAA (Fotso et al. 2007a).

Santalum

Induction of adventitious shoots of *Santalum album* (Sandalwood) was achieved using leaf explants (0.5-1.5 cm in length) with liquid MS medium or WPM containing 0.44 or 2.22 µM BA. Shoot buds were induced mainly on the leaf lamina without a callus stage (Mujib 2005).

Sapium

Adventitious shoots were successfully regenerated from *in vitro* hypocotyls, leaves, and stem segments of *Sapium sebiferum* (Chinese tallow). The optimum medium for shoot regeneration for hypocotyls was a MS medium with 0.27 µM NAA and 1.33 µM BA, resulting in up to six buds. The best medium for leaves was MS medium with 2.69 µM NAA and 2.22 µM BA, and for stem segments was MS medium with 0.27 µM NAA and 0.44-1.33 µM BA. Shoots produced roots (100%) when placed on half-strength MS medium with 2.46 µM IBA and were successfully acclimatized (Chen et al. 2010).

Swietenia

Leaf and root fragments from *Swietenia macrophylla in vitro* cultures were used for organogenesis induction. Leaf explants formed callus (90%) on a modified MS medium with three-quarter-strength nutrients and vitamins supplemented with 4.4 µM BA and 0.54 µM NAA, or 8.9 µM BA and 0.11 µM or 0.54 µM NAA. Root explants formed callus (55%) on MS medium with 2.2 µM BA and 0.54 µM NAA. Adventitious roots were regenerated from leaf callus on medium with 1.2 µM KIN and 2.7 µM or 5.4 µM NAA (Rocha and Quoirin 2004).

Tabebuia

Adventitious shoot regeneration of *Tabebuia donnell-smithii* (Primavera) was achieved from twigs of elite trees and initiating axillary shoots on WPM containing half-strength MS vitamins and 30 µM ZT. The addition of coconut water increased the number of shoots per explant. Shoots were successfully rooted on WPM with 20 µM IBA and plantlets were acclimatized (González-Rodríguez et al. 2010).

Tectona

Widiyanto et al. (2005) noted that the best medium for callus formation on internodal segments of *Tectona grandis* (Teak) was MS medium with 0.01 µM IBA and

1 μM TDZ. The best medium for shoot formation was MS medium with 10 μM BA and 1 μM GA_3. Elongated shoots were rooted in a soil-sand mixture.

Cryopreservation

Cryopreservation, the ultralow temperature (-196°C) storage of cells, tissue, embryos, or seeds, is a viable secondary storage system that can be considered as a secure backup to living collections (Benson 2008). Cryopreservation may involve multiple steps to be successful with tree species, and there are mainly three standard protocols in plant cryopreservation; controlled-rate cooling, vitrification, and encapsulation-dehydration (Reed 2008). Cryopreservation techniques aid in the long-term storage, conservation, and biodiversity of tropical hardwood tree species throughout the world.

Azadirachta

Varghese and Naithani (2008) cryopreserved seeds of *Azadirachta indica* for 1 year with a 45% survival rate after drying to 0.16 g H_2O g^{-1} dry mass. The amount of lipid peroxidation, superoxide dismutase, catalase, and ascorbate peroxidase were monitored during the storage period to investigate the possible causes of cryo-injury.

Cedrela

An *in vitro* conservation approach for *Cedrela fissilis* (Brazilian cedar) was developed. Alginate encapsulated propagules (shoot tips and cotyledonary nodal segments) from 30-day-old aseptic seedlings could be stored at 25°C for 2 months with 96-100% viability. Short-term storage of 6 months greatly decreased viability, with shoot tips having the highest survival (44%) and cotyledonary nodes the lowest (6-8%), with viabilities of 6-8% after 9 months for all explant types. The authors were also able to conserve seeds by direct immersion in liquid nitrogen. After 1 h of liquid nitrogen immersion seeds were thawed at 45ºC for 3 min and planted in a river-sand : soil (1 : 1) mixture with a 90-92% germination rate (Nunes et al. 2003).

Cinnamomum

Cryopreservation studies involving *Cinnamomum cassia* (Cassia) explored the effect of moisture content and dehydrogenase activity on freezing, thawing, and survival of excised embryos. The authors concluded that the optimal moisture range was 30-40% and that a rapid freeze-thaw cycle was preferable (Chen et al. 2005).

Diospyros

Several *Diospyros* species were cryopreserved by vitrifying dormant shoot tips before encapsulation-dehydration and freezing. A pretreatment with a sucrose-glycerol solution was essential to osmoprotect the tropical germplasm against the vitrification process, but was unnecessary for the temperate species. Shoot growth resumed successfully after warming (Matsumoto et al. 2001).

A protocol for cryopreservation of dormant *D. kaki* vegetative buds was developed using a step-wise freezing method. The genetic stability of regenerated plantlets was confirmed by molecular analysis (Ai and Luo 2005).

A one-step procedure using plant vitrification solution 2 (PVS2) was described for shoot tips from dormant *D. kaki* buds. With a 90 min PVS2 treatment, survival (52-80%) and genetic fidelity of regrown shoots was confirmed to be stable (Benelli et al. 2009).

Garcinia

Cryopreservation of *Garcinia indica* beginning with seed explants was not successful. Seeds desiccated below 30% moisture content had significantly reduced viability, and were unable to tolerate the extreme low temperatures of cryopreservation (Normah et al. 2002). Cryopreservation through shoot tip culture was a possible viable alternative, but any successful reports remain unconfirmed for this species (as reviewed by Noor et al. 2011).

Garcinia cowa (Kandis) had an increased tolerance of a PVS2 vitrification protocol with a 3 day preculture on a 0.3 M sucrose medium, however successful cryopreservation remains elusive (Yap et al. 2011).

Mangifera

Cryopreservation using vitrification was described for *Mangifera indica* embryogenic cultures variety 'Zihua' derived from nucellar and cotyledon explants. Direct somatic embryogenesis alleviated the problem of tissue browning during cryopreservation and plant regeneration. Regeneration potential of the cultures was not affected by the process (Wu et al. 2007).

Melia

Cryopreservation of *Melia azedarach* was achieved using embryonic axes. Tissues were either encapsulated with the addition of salicylic acid (0-200 μM) and 0.75 M sucrose, or unencapsulated. The tissues were then exposed to 4°C or cryopreserved for 4 months. Encapsulated tissues with 200 μM salicylic acid had higher viability (83%) in comparison with other experimental conditions (Bernard et al. 2002).

Cryopreservation of *M. azedarach* apical meristems was established by Scocchi et al. (2004) using an encapsulation-dehydration technique. Apical meristem tips from *in vitro* grown *M. azedarach* were cultured on MS medium with 2 μM BA and 0.5 μM IBA, and then encapsulated in 3% sodium alginate. Encapsulated apical meristems were then pretreated for 3 days in liquid medium with increasing sucrose concentrations every

24 h. The encapsulated beads were then dehydrated for 5 h in silica gel until 25% water content was reached. The beads were then exposed to liquid nitrogen for 1 h or cooled 1°C per min until the temperature reached -30°C, and then immersed in liquid nitrogen for 1 h. Meristems were warmed to 30°C for 2 min before being plated onto MS medium with 2 µM BA and 0.5 µM IBA for recovery. Survival for rapid and slow-cooling resulted in 63% and 80%, respectively.

Mroginski and Rey (2007) reported that cryopreservation of apical meristems and somatic embryos of *M. azedarach* resulted in 60% and 36% regeneration, respectively.

Kaviani (2007) attempted to enhance the resistance of embryonic axes of *M. azedarach* to cryopreservation. Embryonic axes were suspended in MS medium with 0.75 M sucrose, 3% sodium alginate, and 200 µM salicylic acid. The axes were then suspended in MS medium with 200 µM salicylic acid, 100 mM $CaCl_2$, and 0.75 M sucrose for 30 min with slow agitation. The alginated beads were then dehydrated for 1 h in the laminar flow hood. The viability of axes encapsulated with 200 µM salicylic acid was enhanced (71.9%).

Long-term storage of cryopreserved *M. azedarach* was investigated by Kaviani (2010). Embryonic axes were preserved by the encapsulation-dehydration technique. Embryonic axes pretreated with 0.75 M sucrose and encapsulated had a 42% germination rate after exposure to liquid nitrogen. Pretreatment with sucrose increased the tolerance of the explants to low temperatures (-196°C).

Sterculia

Cryopreservation of *Sterculia cordata* (Kalumpang) was achieved using zygotic embryos. Seeds in a later stage of development had a significantly higher tolerance of the freezing process (Nadarajan et al. 2006). Cryopreservation of *S. cordata* zygotic embryos was optimized by treating the plant material with PVS2 before storage in liquid nitrogen. Culture on MS medium with sucrose, loading solution, and PVS2 increased the success of germination after cryopreservation (Nadarajan et al. 2007).

Swietenia

Cryopreservation of *Swietenia macrophylla* (Honduras mahogany) seeds was investigated. For seeds with testa, the highest survival (63%) was obtained using direct immersion in liquid nitrogen for 6 h whereas, for both seeds without testa and excised embryos, 6 h desiccation using a slow-cooling technique resulted in the highest survival of 77% and 87%, respectively (Marzalina 2002).

Tabebuia

Seeds of *Tabebuia impetiginosa* (Lapacho) with a moisture content of 4.2% were successfully stored in liquid nitrogen for 360 days, and these seeds maintained a 67% germination rate (Martins et al. 2009). Similar results have been achieved for *T. chrysotrica* (Golden trumpet tree) where seeds were dried to 4% moisture and successfully preserved at -196°C (Tresena et al. 2010).

Genetic transformation

Genetic transformation allows for the incorporation of foreign genes into plant species. For tropical tree species, much like temperate hardwood tree species, stable and cost-effective methods must be developed for many species, in addition to gathering long-term field trial data for commercialization. Development of efficient protocols for genetic transformation of tropical tree species will be crucial in order to develop elite germplasm with increased tolerance to biotic or abiotic factors, and for the overall genetic improvement and utilization of certain traits for wood quality, form, and transgene containment.

Cinnamomum

A successful *Agrobacterium*-mediated transformation protocol for *Cinnamomum camphora* (Camphor tree) was established. Embryogenic callus was used as targets and 50 mg l^{-1} hygromycin was optimum for screening transgenic plants. High transformation rates were achieved with an *Agrobacterium* OD_{600} of 0.6, an inoculation period of 40 min, and a 3 day co-culture period (Du et al. 2008).

Eucalyptus

Girjashankar (2011) has discussed recent advances in transgenic *Eucalyptus* research.

Mangifera

An efficient transformation protocol was developed for *Mangifera indica* somatic embryos using *A. rhizogenes rolB* and Gamborg B5 (Gamborg et al. 1968) medium containing cefotaxime and carbenicillin. Complete plants were regenerated, but without hairy root formation (Chavarri et al. 2010).

Santalum

Agrobacterium-mediated genetic transformation of *Santalum album* was achieved with embryogenic suspension cultures derived from stem internode callus, using *A. tumefaciens* strain EHA105 harboring pCAMBIA 1301 binary vector. Somatic embryos were recovered from transformed tissues on MS medium with 4.4 µM BA, 5.7 µM IAA, and 5 mg l^{-1} hygromycin, and shoots developed on half-strength MS medium with 0.44 µM BA and 0.29 µM GA_3. Complete plantlet formation was achieved using White's (1940) medium containing 0.1% activated charcoal. Analysis of β-glucuronidase

expression and specific activity in the transformed cultures were conducted, and stable insertion of T-DNA was confirmed by Southern blot analysis (Shekhawat et al. 2008).

Tectona

Agrobacterium-mediated transformation of *Tectona grandis* was achieved, and a high frequency of β-glucuronidase expression was observed in leaf and internodal tissue, with less in young leaves and shoot tips (Widiyanto et al. 2009).

Terminalia

Genetic transformation of *Terminalia chebula* (Alale) was confirmed in callus initiated from cotyledonary explants exposed to *Agrobacterium tumefaciens* strain C-58 cultured with 100 μM acetosyringone. The production of tannins was analyzed in the transformed callus (Shyamkumar et al. 2007).

Protoplast culture

Protoplasts are plant cells, but whose cell wall has been removed enzymatically. The cell wall regenerates during subcultures, thereby enabling cell division. Protoplast culture can be utilized as an alternative technique for genetic manipulation that includes protoplast fusion, electroporation, microinjection, and transformation.

Cinnamomum

Cinnamomum camphora protoplasts derived from embryogenic suspension cultures yielded $13.1 \pm 2.1 \times 10^6$ g^{-1} fresh weight with 91.8% viability with a mixture of 3% cellulase Onozuka R10 and 3% macerozyme Onozuka R10 in a 12.7% mannitol solution with 0.12% 2-(N-morpholino) Ethanesulphonic acid, 0.36% $CaCl_2 \bullet 2H_2O$, and 0.011% $NaH_2PO_4 \bullet 2H_2O$. The greatest plating efficiency (6.88%) was achieved in a liquid medium supplemented with 30 g l^{-1} sucrose, 0.7 M glucose, 0.54 μM NAA, 4.44 μM BA, and 2.88 μM GA_3. Somatic embryos were recovered from protoplasts. The somatic embryos germinated on an agar-based MS medium with 4.92 μM IBA (Du and Bao 2005).

Shoot tip culture, nodal culture, and micrografting

Clonal propagation of plants from shoot tips, meristem-tips, or nodal explants, usually has an accelerated proliferation of (axillary) shoots during subcultures. There are four stages to this type of micropropagation; establishment, multiplication, rooting, and acclimatization. *In vitro* micrografting is comparable to conventional grafting and budding, and is often used to overcome recalcitrance to rooting. Development of these types of protocols for tropical tree species is important for the mass production of clonal germplasm, that otherwise may be lost because of the inability to propagate this material or the incompatibility of plant material using conventional propagation methods (e.g. rooted cuttings or grafting).

Albizia

In vitro propagation has been accomplished for *Albizia lebbeck* (Lebbek) using three different explants. This work was aimed at developing a successful tissue culture protocol for *A. lebbeck* using cotyledons, *in vitro* seedling derived shoots, or nodal segments from juvenile trees. All explants were cultured on MS medium containing 30 g l^{-1} sucrose, 0.6% agar, and supplemented with 0.44-17.76 μM BA, or 4.44-13.32 μM BA in combination with 0.54-1.07 μM NAA, or 4.65-13.95 μM KIN in combination with 0.54-1.07 μM NAA, and finally 0.47-9.3 μM KIN in combination with 0.06-11.42 μM IAA. Cotyledonary segments responded preferentially to 11.1 μM BA, producing nine shoots per explant at a rate of 83%. *In vitro*-derived nodal segments regenerated 7.3 shoots per explant when supplemented with 11.1 μM BA and 1.07 μM NAA, however the highest shoot regeneration for nodal segments was reported when BA was reduced to 8.88 μM. Nodal segments from juvenile trees failed to respond to many of these conditions producing only 1.5 shoots per explant at a 25% rate on 11.1 μM BA and 2.69 μM NAA. Adventitious roots were formed on 90% of shoots cultured with 4.92 μM IBA (Mamun et al. 2004).

Xanthan gum and gum katira were investigated for seed germination, caulogenesis, rhizogenesis, or somatic embryogenesis in *A. lebbeck*, and were suitable replacements for agar (Jain and Babbar 2002, 2006).

Gamborg B5 medium with 20 g l^{-1} sucrose and solidified with either 1% xanthan gum or 0.9% agar provided similar results for all parameters studied, with the exception of excised embryos sinking into the medium containing xanthan gum when the vessels were disturbed (Jain and Babbar 2006).

Albizia odoratissima (Bansa) micropropagation was developed beginning with *in vitro* germinated seeds and cotyledonary or leaf nodal explants. All explants were cultured on MS medium with 30 g l^{-1} sucrose and 0.8% Bacto agar. The optimal conditions for cotyledonary nodes were reached when supplemented with 10 μM BA and 10 μM N^6-(2-isopentyl) adenine (2iP), while 5 μM 2iP was optimal for leaf nodal sections. Microshoots were successfully rooted on a medium with 25 μM IBA for 24 h, prior to transfer to a PGR-free medium. During acclimatization, plantlets were exposed to half-strength MS medium biweekly (Rajeswari and Paliwal 2006).

Micropropagation of *A. odoratissima* was achieved with apical buds from 7-day-old *in vitro* seedlings using MS medium with 3.3 μM BA. The best rooting of shoots was achieved using PGR-free half-strength MS medium. The highest percentage (40%) of acclimatization and pot establishment of the rooted plantlets was obtained in soilrite (Borthakur et al. 2011).

Albizia guachapele (Albizzia) was successfully cultured on MS medium with BA, KIN, or TDZ. Although there were high rates of *in vitro* germinated seeds (100%) all microshoots of *A. gauchapele* failed to substantially elongate, and abnormal leaf physiology was reported for all cytokinins investigated (Valverde-Cerdas et al. 2008).

Aspidosperma

Disinfestation and aseptic culture of *Aspidiosperma polyneuron* (Peroba Rosa) was achieved by Ribas et al. (2002). The best method using 2-year-old apical shoots was a 10 min soak in 0.05% mercuric chloride, and 84.1% of shoots survived after 3 weeks in culture on WPM with 30 g l^{-1} sucrose, 0.01% myo-inositol, and 0.65% agar. Plant material collected during the spring and summer yielded better results. Culture induction of *A. polyneuron* was achieved from juvenile material on WPM with 4.48-8.8 µM ZT or BA. Shoot elongation was achieved with WPM with 2.25 µM ZT or BA and 1.25 µM IBA. Treatment with 10 mM IBA for 15 min produced 80% rooting. Plantlet survival rates of 90% were observed (Ribas et al. 2005).

Aspidiosperma ramiflorum (Yellow guatambu) juvenile material was propagated on WPM with varying levels of BA and NAA. Explants responded positively with multiple shoots initiated when BA levels were higher than NAA. WPM produced the best multiple shoot induction with 6.66, 13.32, or 22.2 µM BA with 5.37 µM NAA (Hubner et al. 2007).

Azadirachta

Propagation of *Azadirachta indica* via axillary buds and nodal segments was investigated by Rodríguez and Ortiz (2001). Explants were cultured on MS medium with 8.88 µM and 13.32 µM BA, and 9.30 µM and 13.95 µM KIN, alone or in combination with NAA. The best medium for shoot regeneration was 13.32 µM BA. Rooting was accomplished on MS medium without PGRs and, after acclimatization, rooted plantlets showed the same characteristics as the donor plant.

In vitro propagation of a 50-year-old *A. indica* tree was achieved using nodal explants. Cultures were initially established on half-strength MS medium, and multiple shoot formation occurred on half-strength MS medium with 1 µM BA and 0.5 µM GA_3. The number of shoots increased when cultures were transferred to half-strength MS medium with 1 µM BA and 500 mg l^{-1} casein hydrolysate, but this did not support shoot elongation. To elongate the shoots, explants were transferred to MS medium with 1 µM BA and 250 mg l^{-1} casein hydrolysate, and cultures were able to be maintained for 5 years. Eighty-two-percent of the shoots rooted on quarter-strength MS medium with 0.5 µM IBA with an 87.5% survival rate when transplanted to soil (Chaturvedi et al. 2004a).

Clonal propagation of *A. indica* was also achieved using buds and basal sprouts from a 15-year-old tree and a 1-year-old juvenile plant. Explants were cultured on MS medium with 0.44 µM BA and 12.5 µM polyvinyl pyrrolidone-40 to control phenolics. Once elongated, the explants were transferred to either MS medium or Driver and Kuniyuki (1984) walnut (DKW) medium with 0.22 µM BA for shoot proliferation and multiplication. The DKW medium with 0.22 µM BA was significantly better for producing shoots, and juvenile and basal sprouts had a uniform number of shoots, however, shoot length and number of nodes were greater in juvenile explants than basal sprouts. Rooting occurred on half-strength DKW medium with 4.9 µM IBA in juvenile (100%) and basal sprouts (90%), and survival after acclimatization was 90% for both explant types (Quraishi et al. 2004).

Reddy et al. (2006) established a micropropagation protocol for *A. indica* using cotyledonary nodes from 15-20-day-old *in vitro* seedlings. Best shoot proliferation was on MS medium with 1.5 µM TDZ, 0.5 µM 2, 4-D, 40 mg l^{-1} adenine, 100 mg l^{-1} glutamine, and 10 mg l^{-1} thiamine HCL. For shoot multiplication, explants were subcultured on MS medium with 2.5 µM TDZ and 1.0 µM BA. For shoot elongation, explants were subcultured on 1.5 µM TDZ, 0.5 µM BA, and activated charcoal to reduce browning of the medium. Rooting occurred on half-strength MS medium with 2 µM IBA, where 86.6% of plantlets rooted and 80% survived after acclimatization.

Clonal propagation of *A. indica* from root and shoot tips was reported by Shahin-uz-zaman et al. (2008). Both root and shoot tips formed shoot buds although shoot tips had a higher percentage of regeneration than root tips, however, root tips gave the best average number of shoots per explant. Shoot initiation was best on MS medium with 1.32 µM BA and 0.25 µM NAA for both root and shoot explants. Rooting occurred on MS medium with 0.25 µM IBA and 0.3 µM IAA, with 94.3% and 93.5% rooting in shoot and root explants, respectively.

In vitro propagated microshoots of *A. indica* were acclimatized via three semi-sterile methods. One-hundred-percent acclimatization was achieved using sand : soil (2 : 1) with cocopeat : biofertilizer and addition of *Trichoderma viride* (Lavanya et al. 2009).

Arora et al. (2010) clonally propagated a 40-year-old *A. indica* tree using stem nodal segments cultured on MS medium with 1.11 µM BA, 1.43 µM IAA, and 81.43 µM adenine hemisulphate. Explants collected in March-April responded the best *in vitro* with mid-April being the best for maximum bud break (80%). Shoots were rooted on medium with 2.46 µM IBA. The authors also observed that thicker nodal explants obtained from the middle order nodes performed better.

Calophyllum

Pale green shoots collected from mature *Calophyllum apetalum* (Poonspar of Travancore) trees during the flush season resulted in the best shoot proliferation on MS medium with 8.8 μM BA. Rooting was achieved on quarter-strength MS medium with a 9.8 μM IBA pulse. Plantlets were successfully acclimatized and outplanted (Nair and Seeni 2003).

Shoot multiplication was initiated from *in vitro* germinated seedlings of *C. inophyllum* (Beach calophyllum) on WPM with 0.91 μM TDZ. Elongation of shoots was achieved by supplementing WPM with 2.22 μM BA. Rooting was observed on WPM amended with 2.46 μM IBA, and plantlets were acclimatized successfully (Thengane et al. 2006a).

Cedrela

Juvenile nodal segments were used to develop a micropropagation protocol for *Cedrela fissilis*. MS medium with 1.25-5 μM BA produced the highest multiplication rates. Shoot proliferation and growth was inhibited by NAA, but NAA promoted callus formation. Rooting (87-100%) was achieved after 10-12 days on half-strength MS medium without PGRs or with 2.5 μM IBA. Survival of acclimatized plantlets was 100% after 3 months (Nunes et al. 2002).

Nunes et al. (2007) reviewed protocols for shoot tip culture and *in vitro* conservation of *C. fissilis*. Valverde-Cerdas et al. (2008) cultured *C. odorata* on MS medium with BA, KIN, or TDZ. Normal development and optimal conditions for elongation were obtained with 1.33 μM BA, and rooting (70%) was achieved when shoots were exposed to 4.92 μM IBA.

Anatomy of rooted microcuttings of *C. odorata* was investigated with 0 and 4.44 μM IBA cultures. The development of primordial roots was similar in the control and the 4.44 μM IBA treatment, however there were more roots developed using 4.44 μM IBA (Millán-Orozco et al. 2011).

Ceiba

Ceiba pentandra (Kapok tree) was established *in vitro* using shoot tips from 5-month-old seedlings. Shoots were successfully cultured *in vitro* on MS medium with 1 μM BA or 10 μM KIN (Silva et al. 2010).

Cinnamomum

Mature nodal and apical sections of *Cinnamomum camphora* when cultured on WPM with 13.32 μM BA and 4.65 μM KIN was successful. The combination of BA with KIN was superior to KIN and ZT for shoot proliferation. Adventitious rooting occurred with all elongated shoots with WPM supplemented with activated charcoal. However, the highest rooting (100%) occurred when 4.9 μM IBA was added to the medium (Nirmal Babu et al. 2003).

Azad et al. (2005) reported a protocol for *C. camphora* using cotyledonary nodes from *in vitro*-derived seedlings using MS medium containing 4 μM BA. Rooting was achieved on MS medium with 1 μM IBA and 90% of plantlets were acclimatized.

Cordia

Schuler et al. (2005) produced 134 plantlets of *Cordia alliodora* (Light American Cordia) using apical buds containing at least two leaves initiated on half-strength MS medium with 100 ml l^{-1} ascorbic acid. Thirty days after initiation, explants were transferred to a medium with 0.47 μM GA_3 and 6.04 μM KIN, and shoots were successfully elongated and multiplied. Rhizogenesis was achieved on MS medium containing 2 g l^{-1} activated charcoal and 15 g l^{-1} sucrose. Rooted plantlets were then slowly acclimatized.

Dalbergia

Culture of nodal explants from a 60-year-old tree of *Dalbergia sissoo* was achieved on MS medium with 4.44 μM BA and 1.34 μM NAA. Efficient rooting (76.9%) of shoots was attained on half-strength MS medium with 4.92 μM IBA with 70% survival after acclimatization (Joshi et al. 2003).

Chand and Singh (2004) using encapsulated nodal segments of *D. sissoo* produced plantlets using half-strength MS medium without PGRs, or when material was directly sown into pots containing peat moss. Thirunavoukkarasu et al. (2010) micropropagated *D. sissoo* from axillary buds from epicormic and coppice shoots. Coppice shoots performed better than epicormic shoots on MS medium with 6.6 μM BA and 1.14 μM IAA. Rooting of shoots was best on half-strength MS medium with 7.5 μM IBA.

Enterolobium

Nodal explants from *in vitro* seedlings of *Enterolobium cyclocarpum* (Guanacaste) produced shoots on MS medium with 2.2 μM BA and 10.7 μM NAA. Rooting was obtained on half-strength MS medium without PGRs, and acclimatized plantlets had a survival rate of 90% (Rodríguez-Sahagún et al. 2007).

Eucalyptus

Nakhooda et al. (2011) investigated the variation between *in vitro* rooting of *Eucalyptus grandis* (Rose gum) explants derived from shoot cultures versus those from cuttings. With the use of an auxin transport inhibitor (2, 3, 5-triiodobenzoic acid) and IBA, IAA, or NAA, it was noted that IAA was required for development of root graviresponse. Rooting with IBA (100%), IAA (71%), and NAA (21%) was reported with some abnormal graviperception.

Garcinia

Beginning with immature seed segments, a high frequency multiplication system was developed for *Garcinia indica* using a MS medium with 12.5-50 µM BA. Adventitious rooting (92%) was obtained when microshoots were placed on half-strength MS medium with 10 µM IBA (Malik et al. 2005).

Adventitious rooting of *in vitro*-derived shoots from immature seeds of *G. indica* reached 100% when shoots were dipped for 30 s in 9800 µM IBA and then transferred to half-strength MS medium (Chabukswar and Deodhar 2005). Optimum substrate for acclimatization was cocopeat with a 76% survival rate. Adventitious rooting (55%) of *in vivo* shoots occurred when dipped in Rootex-3. Chabuskwar and Deodhar (2006) reported successful *in vitro* rooting of *G. indica* through the use of successive *in vitro* grafts to rejuvenate mature explants. Initiating mature material was best accomplished in the spring to ensure survival and reduce contamination on WPM with 8.9 µM BA and 0.5 µM TDZ. All mature explants failed to root until after the fifth successive graft, at which point rooting was 75% after a 60 s dip in 9800 µM IBA followed by culture on half-strength WPM.

Mature clones of *G. indica* were successfully micropropagated using nodal segments from root suckers. Explants were initiated on WPM containing 13.2 µM BA, multiplied on WPM with 4.4 µM BA and 0.45 µM TDZ, and elongated for rooting on half-strength WPM with 0.44 µM BA and 0.5 g l^{-1} activated charcoal. Rooting (65%) was achieved when shoots were dipped for 30 s in 19.6 mM IBA before transfer to half-strength WPM (Tembe and Deodhar 2011).

Micropropagation of *G. mangostana* (Manggis) was successful when seed segments or shoot tips were the original explants. Seeds and shoot tips proliferated on MS medium with 40 µM BA and 2.5 µM NAA, and WPM with 5 µM BA, 1 µM NAA, and 10 nM TDZ, respectively. Rooting was achieved when shoots were placed on WPM containing 147.6 µM IBA (Normah 2000).

A study aimed at developing a photoautotrophic micropropagation system for *G. mangostana* produced no significant difference in dry or fresh weight between plants grown on agar containing medium and those grown on a vermiculite-based medium regardless of conditions (Kozai and Kubota 2001). The authors did note an increase in adventitious root formation in those plants grown exclusively on vermiculite with or without PGRs compared to the control.

Breeding, molecular genetics, micropropagation, genetic manipulation, and protoplast culture of *G. mangostana* was reviewed by Te-chato and Lim (2004). The authors described work that focused on assessing genetic diversity, organogenesis, and inducing variation of *G. mangostana* through mutagenesis.

Micropropagation of *G. mangostana* was developed with shoot tips, stems, and seed explants. MS medium with 4.44 µM BA and 0.23 µM KIN was optimal for shoot regeneration with shoot tips and stem segments, whereas the best medium for shoot regeneration with seed explants was MS medium with 17.76 BA and 1.07 µM NAA. The highest incidence of adventitious root formation (90.4%) was obtained on quarter-strength MS medium with 0.54 µM NAA (Sirchi et al. 2008).

Genipa

Genipa americana (Jagua) *in vitro* rooting and acclimatization protocols were developed using two genotypes and various concentrations of BA, IBA, and commercial additives. Both genotypes were rooted (43.3% and 70%) in MS medium with 9.8 µM IBA. Addition of the substrate Ecoterra® improved the health and quality of the root system and aerial plant parts during acclimatization (Rocha et al. 2008).

Gmelina

Naik et al. (2003) studied the effect of origin and subculture on the *in vitro* propagation of *Gmelina arborea* (Batinj) by comparing axillary shoot elongation, formation of multiple shoots, and rooting of seedlings from six provenances over several subcultures. The protocol involved nodal segments using MS medium with 1 µM KIN and 1 µM BA. Rooting was achieved using half-strength MS medium containing 0.1% activated charcoal for root induction, followed by immersion in 100 µM IBA for 24 h and then culture on a modified MS medium for root growth. Significant provenance-dependent variation of the parameters was observed, and a subculture-dependent decrease in multiple shoot formation and rooting was also observed. A transient decrease in chlorophyll fluorescence and a gradual increase in ribulose-1,5-bisphosphate carboxylase levels in leaves of the *in vitro* plants undergoing acclimatization was observed.

Valverde-Cerdas et al. (2004) developed a complete micropropagation and acclimatization protocol with nodal segments of *in vitro* seedlings of 10 families of *G. arborea* obtained from controlled crosses. MS medium with BA (0.4-3.9 µM) was used for shoot multiplication, and half-strength MS medium containing 14.8 µM IBA for root induction. After exposure to IBA, shoots were transferred to Jiffy® pellets under the same conditions of *in vitro* culture. Longer exposure of shoots to auxin increased the number of roots per shoot with increased rooting (65-87%). The effect of BA on bud induction, the effect of light intensities and periods of exposure to IBA in liquid or semi-solid medium on the rooting of several genotypes, and significant differences among genotypes and families were also reported.

Irvingia

Embryos excised from kernel or ripe *Irvingia gabonensis* (African mango) fruits were grown to full plantlets on quarter-strength MS medium supplemented with 0.93 µM KIN (Fajimi et al. 2007).

Khaya

In vitro propagation of *Khaya senegalensis* (African mahogany) was achieved with mono-nodal cuttings from *in vitro* seedlings using MS medium with 0.26 µM IBA and 2.2 µM BA. The highest rooting (90%) of shoots was achieved with a 7-day induction on half-strength MS medium with 260 µM IBA followed by transfer to a PGR-free medium. The rooted shoots were successfully acclimatized. Micrografting of *K. senegalensis* was also developed by grafting apices or axillary buds from young shoots onto the epicotyl of *in vitro* seedlings (Danthu et al. 2003). Hung and Trueman (2011a) micropropagated *K. senegalensis* using *in vitro* seedlings cultured on MS medium containing 4.4 µM BA. High frequency (76-90%) of root formation was obtained when shoots were cultured in darkness on half-strength MS medium with 19.6 µM IBA for 1 week, followed by transfer to PGR-free-half-strength MS medium with almost all plantlets successfully acclimatized.

Hung and Trueman (2011b) developed a protocol for short-term preservation and distribution of *K. senegalensis* using alginate-encapsulated shoot tips from four different clones. Optimal shoot regrowth from capsules was obtained on MS medium with 4.4 µM BA. The rooting and acclimatization of encapsulated shoot tips was achieved by preculture on half-strength MS medium with 245 µM IBA before encapsulation, followed by culturing on PGR-free half-strength MS medium for 4 weeks. High frequency plantlet recovery under non-aseptic conditions was also achieved after the IBA pretreated shoot tips were maintained under light in moistened Petri dishes for 4 weeks before transfer to organic compost.

Okere and Adegeye (2011) investigated different concentrations and combinations of NAA, BA, and KIN for propagation of *K. grandifoliola* (African mahogany) using embryos. MS medium with 4.4 µM BA, 0.05 µM NAA, and 27.1 µM adenine sulphate was the optimum medium for shoot and root length, and number of nodes and roots.

Melaleuca

Shoot proliferation from nodal segments was optimized for *Melaleuca alternifolia* (Tea tree) when cultured either in liquid or on agar-based MS medium containing 1.11 µM BA or 0.55 µM BA, respectively. MS medium was found to be more effective for shoot initiation than WPM. All shoots produced roots without exogenous auxin on MS medium (Oliveira et al. 2010).

Melia

Apical meristem-tips were used for *in vitro* conservation of *Melia azedarach*. Cultures were established from axillary buds on MS medium with 2 µM BA, 0.5 µM IBA, and 0.3 µM GA_3. Explants proliferated on MS medium with 2 µM BA and 0.3 µM GA_3, and meristems could be maintained for 1 year on quarter-strength MS medium with 0.5 µM BA at 4°C. After 1 year, 67% of the explants proliferated when recultured on MS medium with 2 µM BA and 1 µM IBA at 27°C. Rooting was induced (60%) on MS medium with 17.5 µM IBA for 4 days followed by transfer to MS medium without PGRs for 30 days (Scocchi and Mroginski 2004).

A rapid micropropagation protocol for *M. azedarach* was achieved using axillary buds from juvenile plants. After 65 days of culture, the best regeneration was achieved on MS medium with 30 g l^{-1} sorbitol, 8.88 µM BA, and 1 mg l^{-1} 2,3,5-triiodobenzoic acid. Roots developed on shoots using a horticultural soilrite mix with half-strength MS medium and 4.92 µM IBA. The plantlets were transferred to the field with a 65% survival rate (Chennaveeraiah et al. 2006).

A micropropagation protocol was established for *M. azedarach* using nodal segments from a 20-year-old tree. Shoot regeneration was best on MS medium with 5 µM BA, 0.5 µM IAA, and 30 µM adenine sulfate. Ammonium sulfate (250 mg l^{-1}) and 100 mg l^{-1} potassium sulfate were added to the culture medium to alleviate tip burning and defoliation. For shoot multiplication, BA levels were reduced to 2.5 µM, with IAA and adenine sulfate levels remaining the same. Rooting was induced (90%) by pulse treating shoots for 15 min with 250 µM IBA, followed by transfer to soil (Husain and Anis 2009).

Nothofagus

In vitro propagation of mature phenotypes of *Nothofagus alpine* (Coique) from field and grafted plants was achieved on broad-leaved tree medium (BTM; Chalupa 1981) with MS vitamins supplemented with 0.55 µM BA and 13.7 µM glutamine. Rhizogenic potential showed no change after 4 years in culture, and could be induced on half-strength BTM with MS vitamins and 5.9 µM IBA using a 7 day dark pulse. Plantlets were successfully acclimatized (Sabja et al. 2008).

Rooting of *N. nervosa* (Coique) microshoots was induced on BTM by initially supplementing the medium with 0.61 µM IBA, incubating cultures in darkness for 7 days, and then transferring shoots to the light on medium ± 20 µM naringenin (Martínez Pastur et al. 2003). The incorporation of flavonoids during the root expression phase improved the speed of appearance, quantity, and quality of the roots formed. A histological study was conducted to understand tissue differentiation during adventitious rooting which showed early stages of differentiation in the pith and cambial cells, allowing

rooting phases to be visualized. The individual stages were also quantified by measuring the changes in cell diameter, density, and cellular content (Martínez Pastur et al. 2005).

Rhizogenesis of microshoots was improved by the addition of 10 µM spermine to the medium during the root expression phase, and a polyamine inhibitor during the induction phase (Martínez Pastur et al. 2007). Polyamines and peroxidases were determined to be potentially useful as biochemical indicators to define rooting phases. Vigorous *in vitro* seedling growth and bud and shoot induction were achieved from mature *N. procera* (Anis) embryos on MS medium with 2.2 µM BA and 3.4 µM IBA. Induced organs grew significantly better when transferred to PGR-free MS medium where roots formed spontaneously (Sanchez-Olate et al. 2004).

Pterocarpus

Axillary shoot proliferation of *Pterocarpus angolensis* (Muninga) was stimulated on MS medium with 4.4 or 22.2 µM BA. Rooting of shoots was induced on MS medium without PGRs, but was more uniform and efficient in the presence of 5-20 µM IBA, although roots that formed were brittle (Chisha-Kasumu et al. 2006).

Multiple shoots of *P. marsupium* were regenerated from cotyledonary nodes excised from 20-day-old seedlings. Shoot cultures proliferated on MS medium with 4.44 µM BA and 0.26 µM NAA for 15 weeks before being rooted on half-strength MS medium with 9.84 µM IBA, or as microcuttings. Plants were successfully acclimatized (Chand and Singh 2004).

Proliferation of axillary buds from cotyledonary nodes of 18-day-old seedlings were used to regenerate multiple shoots of *P. marsupium*. Shoots proliferated on MS medium with 5 µM BA and 0.25 µM IAA. Rooting of shoots was induced on half-strength MS medium with a 5 day pulse of 200 µM IBA before the auxin was reduced to 0.5 µM, and plantlets were successfully acclimatized (Anis et al. 2005).

Axillary buds from cotyledonary nodes from 18-day-old axenic seedlings were used to stimulate multiple shoots of *P. marsupium*. High shoot regeneration was achieved on MS medium with 0.4 µM TDZ. Shoots elongated on MS medium containing 5 µM BA. Rooting was induced on half-strength MS medium with 0.2 µM IBA and 3.96 µM phloroglucinol, and plants were successfully acclimatized (Husain et al. 2007).

Multiple shoots were obtained from nodal explants from 18-day-old *in vitro* seedlings on MS medium with 4 µM BA, 0.5 µM IAA, and 20 µM adenine sulphate. Microshoots rooted on PGR-free MS medium after a 7 day pulse of half-strength MS liquid medium with 100 µM IBA and 15.84 µM phloroglucinol. Plantlets were successfully acclimatized (Husain et al. 2008).

Shoot proliferation of *P. santalinus* (Red Sandalwood) was achieved with mature nodal explants using liquid MS medium with 4.4 µM BA and 2.2 µM TDZ. Rooting of shoots was induced using a semi-solid half-strength MS medium containing 4.9 µM IBA, and plantlets were successfully acclimatized (Prakash et al. 2006).

In vitro seed establishment and micropropagation of *P. santalinus* was achieved using Anderson medium without charcoal. Germination was significantly better with pods greater than 4 cm in diameter, cultured soon after harvest, and exposed at least 15 min to 0.1% mercuric chloride. Seedling appearance and rooting improved with the addition of 1 g l^{-1} activated charcoal. Leaf and root formation was highest on WPM without PGRs (Chaturani et al. 2006).

Shoot proliferation of *P. santalinus* was achieved from cotyledonary nodes on MS medium containing 2.5 µM BA and 2 µM 2iP. Rooting (83%), superior to previous literature, was induced by dipping microshoots into a 5 µM IAA plus 1 µM IBA solution. Acclimatization was improved by hardening plantlets in a mixture of coarse sand : clay : farmyard manure (1 : 1 : 1) (Rajeswari and Paliwal 2008).

Seedling shoot apices were proliferated on MS medium with 4.44 µM BA and 0.45 µM TDZ. Microshoots produced roots (60%) on MS medium with 0.49 µM IBA, and survived acclimatization (73.3%) in a substrate of organic manure and sand (Balaraju et al. 2011).

Ricinodendron

A propagation protocol for *Ricinodendron heudelotii* was described where single-node cuttings were initially cultured on half-strength MS medium with 20 µM BA to proliferate buds, then transferred to medium with 11.6 µM KIN to stimulate bud burst. Culture medium containing 10.7 µM NAA induced rooting, and plantlets were acclimatized (Fotso et al. 2004).

Santalum

Santalum album nodal bud segments were established on MS medium with 0.53 µM NAA and 11.09 µM BA, and shoot proliferation occurred on MS medium with 0.53 µM NAA, 4.44 µM BA, 283.93 µM ascorbic acid, 118.1 µM citric acid, 104.04 µM cystine, 342.24 µM glutamine, and 10% coconut milk (Sanjaya et al. 2006a). Rooting (41.7%) was induced by pulsing the shoots with 98.4 µM IBA for 48 h followed by transfer to PGR-free quarter-strength MS medium with vitamin B_5 and 20 g l^{-1} sucrose. In comparison, 50% rooting was achieved using shoots pulsed with 1230 µM IBA for 30 min in soilrite rooting medium, and 100% survival was observed after acclimatization. Micrografts of *S. album* were reported using 1- to 2-cm-long nodal shoot segments as scions and the decapitated hypocotyl of 45-day-old *in vitro* seedlings producing 60% graft success (Sanjaya et al. 2006b).

Sapium

MS medium with 4.44 μM BA, 2.33 μM KIN, and 2.69 μM NAA was optimal for shoot multiplication of nodal segments of *Sapium sebifera* (Chinese tallow tree). Relatively high rooting (93%) was also induced for *S. sebifera* by using a half-strength MS medium containing 2.45 μM IBA (Qie et al. 2009).

Schinopsis

Schinopsis balansae (Barauna) was propagated using nodal segments cultured on quarter-strength MS medium with 100 mg l^{-1} ascorbic acid, 30 g l^{-1} sucrose, and 5-15 μM BA. Medium without cytokinin promoted shoot development in 73% of the explants, while medium supplemented with 1-15 μM TDZ was ineffective for shoot production. Optimal rooting (>40%) was obtained with quarter-strength MS medium with 7.5 μM IBA in vermiculite (Sansberro et al. 2003).

Spondias

Spondias mombin (Jobo) proliferating shoot cultures were recovered from nodal segments from 4-6-month-old seedlings on WPM with 20 g l^{-1} sucrose and 0.7% agar, and rooted plantlets were obtained. However, shoots did not fully elongate, reaching a maximum of 8.1 mm on medium containing 0.22 μM BA. The NAA concentration negatively affected shoot length, but did induce root formation at 4.44 μM in the absence of BA (Carvalho et al. 2002).

Swietenia

Swietenia macrophylla proliferating shoot cultures were established with nodal segments on MS medium (half-strength nitrates) and 0.89 μM BA (Collado et al. 2004).

Schottz et al. (2007) studied the effect of BA (2.5, 5, 10, 20, or 50 μM) in combination with 2iP (0. 1.1, 2.2, 4.4, or 8.8 μM) in MS medium or Quoirin and Lepoivre (Quoirin and Lepoivre 1977) medium (QL). The highest multiplication rate was obtained with juvenile nodal explants using MS or QL medium with 18.51 μM BA and 2.2 μM 2iP.

Tabebuia

Tabebuia rosea (Roble) was successfully propagated using apical leaf buds derived from seedlings and placed on three successive media formulations. Apical buds containing at least two leaves were established on half-strength MS medium with 100 ml l^{-1} ascorbic acid and kept at 27°C with a 16 h photoperiod. Thirty days after initiation, explants were transferred to medium with 0.47 μM GA_3 and 6.04 μM KIN, and shoots were successfully elongated and multiplied. Rhizogenesis was induced on MS medium with 2 g l^{-1} activated charcoal and 15 g l^{-1} sucrose. Rooted plantlets were slowly acclimatized (Schuler et al. 2005).

Nery et al. (2008) and Abbade et al. (2010) sought to develop more efficient germination of recalcitrant *Tabebuia* seeds. Seeds of *T. serratifolia* (Lapocho) had the highest *in vitro* germination frequency when cultured on MS medium or WPM 40-50 days after anthesis (Nery et al. 2008).

Disinfestation using sodium hypochlorite resulted in phytotoxicity, and a 2 min wash in 2% carbendazin was preferable. Germination of *T. roseo-alba* was optimal when seeds were placed on half-strength MS medium with 2.89 μM or 8.66 μM GA_3 (Abbade et al. 2010).

Tectona

Tiwari et al. (2002) improved *Tectona grandis in vitro* shoot numbers from an average of 1.46 to an average of 5.76 using MS medium with 22.2 μM BA and 0.57 μM IAA. Shoots were rooted *in vivo*.

Gangopadhyay et al. (2002) showed that root induction, total root number, and percent survival of *T. grandis* after transplanting were all significantly improved when shoots were grown in liquid coir medium rather than on agar-based plates. Survival rates increased from 54% to 77% for shoots maintained in MS-coir medium with 0.44 μM BA, 0.46 μM KIN, and 100 mg l^{-1} adenine and transferred to White's basal medium with 9.8 μM IBA and 1.14 μM IAA. Plants grown in liquid coir medium suffered fewer structural defects (mostly epicuticular) than those grown on agar in the later developmental stages.

Shoot multiplication rates of *T. grandis* increased from 1.66 to 6.33 in MS medium with 10 μM BA and 1.0 μM NAA. Shoots did not root efficiently on medium with various concentrations of IBA or indole-3-propionic acid (IPA). However, 66.6% rooting was achieved in MS medium with 15 μM NAA (Shirin et al. 2005).

Yasodha et al. (2005) demonstrated good shoot production in *T. grandis* from *in vitro* germinated seedlings using alternate PGR media, with the final subculture on MS medium containing 4.4 μM BA and 2.32 μM KIN.

Gyves et al. (2007) reported the most efficient shoot proliferation and elongation medium for *T. grandis* was MS medium with 0.29 mM GA_3 and 3 g l^{-1} activated charcoal, with 100% rooting achieved using MS medium with 2.46 μM IBA and 160 mg l^{-1} putrescine.

Nor Aini et al. (2009) reported varied rooting results when shoots of *T. grandis* were exposed to light or dark culture conditions in conjunction with different IBA concentrations. Dark-grown shoots on MS medium with 0.98 μM IBA had longer roots and more roots than any other dark culture treatment. Light-exposed shoots grown on MS medium with 24.6 μM or 2.46 μM IBA had the highest root number and length, respectively, for any of the shoot cultures.

Terminalia

Terminalia arjuna (Arjun) was propagated from

nodal explants of a mature tree on half-strength MS medium with 4.44 µM BA and 0.53 µM NAA. Seasonal variations affected proliferation rates, with the best rate obtained from material collected during April or May. Rooting was obtained on medium with 4.92 µM IBA, and plants were acclimatized (Pandey et al. 2006).

Terminalia bellirica (Hean) shoots were induced from nodal explants cultured on MS medium with 13.3 µM BA, followed by subculture on medium containing 4.4 µM BA. Modified Gamborg B5 medium and WPM, both supplemented with 4.9 µM IBA were used for root induction, and rooted plants were acclimatized (Ramesh et al. 2005).

Nodal segments from *in vitro* germinated *T. bellerica* seedlings were cultured on MS medium with 6.66 µM BA for establishment and shoot proliferation (Rathore et al. 2008).

Quarter-strength MS medium containing 0.49 µM IBA induced rooting, and plantlets were acclimatized to a mist house. The endosymbiotic root fungus *Piriformospora indica* was used to bioprime *in vitro* rooted plantlets of *T. bellerica*, increase plantlet survival and chlorophyll content, as well as promoted root and shoot growth (Chittora et al. 2010).

Wrightia

In vitro propagation of *Wrightia tomentosa* (Atkuri) under high carbon dioxide levels resulted in increased shoot production and root growth. Acclimatization and *in vivo* growth of plants was also improved when plants were grown in a carbon dioxide-enriched environment (Purohit and Habibi 2010).

Somatic embryogenesis

Somatic embryogenesis from vegetative or non-gametic cells can provide a means for mass propagation of important tropical timber species. Somatic embryogenesis produces a bipolar embryo with similar developmental stages that occur in the normal zygotic embryo. Somatic embryogenesis can occur directly from the original explant tissue or indirectly from callus, cell suspension, or via repetitive somatic embryogenesis. There are four stages to successful somatic embryogenesis; induction, maintenance, development or maturation, and germination or plantlet recovery.

Anthocephalus

Direct somatic embryogenesis from internodes of *Anthocephalus cadamba* (Kadam) occurred on MS medium with 9.3 µM KIN and 5.4 µM NAA. Coconut water increased the frequency and number of roots formed. The optimum medium for root development was MS medium with 4.6 µM KIN, 5.4 µM NAA, and 15% coconut water (Apurva and Thakur 2009).

Azadirachta

Somatic embryos were induced directly from root and nodal explants and indirectly from callus when leaf explants of *Azadirachta indica* were used. Somatic embryos were induced on PGR-free MS1 medium (half-strength MS macronutrients with full-strength micronutrients) with adenine sulfate, L-glutamine, myo-inositol, and casein hydrolysate for root and nodal explants. However, with leaf explants, 2.3-4.5 µM TDZ and 0.5 µM 2,4-D were necessary for somatic embryo induction. The addition of 0.3 µM GA_3 and 2.2 µM BA to MS1 medium were necessary for somatic embryo germination with > 60% conversion (Akula et al. 2003).

Rout (2005) induced embryogenic cultures of *A. indica* on MS medium with 1.11 µM BA and 4.52-6.78 µM 2,4-D. To initiate globular somatic embryo development, embryogenic cultures were transferred to MS medium with 1.11 µM BA and 0.45 µM 2,4-D. To germinate (64.2%) the somatic embryos, cultures were transferred to half-strength MS medium with 0.94 µM abscisic acid (ABA). Sixty-five-percent survived acclimatization and exhibited normal growth.

Gairi and Rashid (2005) induced somatic embryos directly from the abaxial side of immature *A. indica* cotyledons on MS medium with 0.5 µM TDZ. Mature cotyledons were less responsive, however, when TDZ levels were increased to 1 µM, 50% of the cotyledons formed somatic embryos. Washing of the cotyledons resulted in less regeneration.

Singh and Chaturvedi (2009) developed a protocol for cyclic somatic embryogenesis of *A. indica*. Somatic embryos were directly induced from immature zygotic embryos on MS medium with 0.1 µM TDZ and 4 µM ABA with a 76% success rate. Primary embryos were transferred to MS medium with 5 µM IAA and 5 µM GA_3 for secondary somatic embryogenesis with a 12.5% success rate. Embryos were maintained for an extended period with a 10% conversion rate.

Shekhawat et al. (2009) developed a protocol to induce embryogenic cultures from leaflets of *A. indica* on MS medium with 30 g l^{-1} sucrose, 6.98 µM KIN, 8.57 µM IAA, 50 mg l^{-1} $(NH_4)_2SO_4$, and 100 mg l^{-1} KNO_3, and produced all stages of somatic embryos. The somatic embryos matured and germinated (82%) on half-strength MS medium without PGRs. Eighty to 83.5% of the plantlets survived acclimatization and transfer to field conditions.

Somatic embryogenesis of *A. indica* was reported by Das (2011) using immature zygotic embryos. Explants cultured on MS medium with 2.22 µM BA and 6.79-9.05 µM 2,4-D developed somatic embryos. After 20 days of culture, 62% of somatic embryos germinated with the addition of 0.95 µM ABA. The embryos that germinated were rooted on half-strength MS medium with 3.78 µM ABA and 20 g l^{-1} sucrose. Plantlets were acclimatized and transferred to field conditions with a 50% survival rate.

Baillonella

Embryogenic cultures were induced on leaf fragments of *Baillonella toxisperma* (Moabi) on half-strength MS medium with 2.2 µM BA and 13.6 µM 2,4-D. An average of 38 embryos was observed per embryogenic culture clump, and bipolar stage embryos were obtained (Fotso et al. 2008b).

Caesalpinia

Induction of embryogenic cultures from *Caesalpinia echinata* (Pau-Brasil) leaf discs at different developmental stages was studied. Juvenile leaves on 22.62 µM and 90.5 µM 2,4-D and young leaves on 226.2 µM and 452.5 µM 2,4-D responded with no significant differences, whether cultured in the dark or light. Proembryonic masses (PEMs) developed on MS medium with 22.62 µM, 45.25 µM, and 90.5 µM 2,4-D. The number of embryogenic cultures and PEMs increased on medium without PGRs. Globular- and heart-shaped somatic embryos developed on medium with 2.26 µM 2,4-D (Werner et al. 2009).

Werner et al. (2010) investigated different basal media (MS, Gamborg B5, White, and WPM), nitrogen sources (KNO_3, NH_4NO_3, and glutamine), and PGRs (2,4-D, IBA, IAA, KIN, and BA) for induction on leaf tissue of *C. echinata*. Proliferation was increased with 4.52 µM 2,4-D, 22.2 µM BA with either 2.4 g l^{-1} NH_4NO_3 or 4.11 g l^{-1} KNO_3 and 1.35 g l^{-1} glutamine, and was best with MS medium supplemented with 2.26 µM 2,4-D, 24.6 µM IBA, 22.2 µM BA, and 2.4 g l^{-1} NH_4NO_3.

Cedrela

Cedrela fissilis somatic embryogenesis was induced from zygotic embryos on MS medium with 2,4-D or picloram. Embryogenic cultures were transferred to 45 µM or 22.5 µM 2,4-D, or 22.5 µM 2,4-D with 0.4 µM BA. These combinations resulted in 56.7, 30, and 36.7% callus formation, respectively, after 6 months of culture. The embryogenic cultures were transferred to MS medium with 4.5 µM 2,4-D for somatic embryo production. To stimulate somatic embryo development, they were transferred to MS medium without PGRs and there was a 12.5% conversion frequency. Plantlets were successfully acclimatized with 85% survival (Vila et al. 2009).

Embryogenic *C. odorata* cultures were induced from young inflorescences from 40-year-old trees on MS medium with 0.45 µM TDZ (Daquinta et al. 2004).

Induction of PEMs was investigated from roots (main and secondary), leaves, and apical meristems of *C. odorata*. WPM with MS vitamins, 2.26-13.57 µM dicamba, 5% coconut water, 1% activated charcoal, 100 mg l^{-1} citric acid, and 100 mg l^{-1} ascorbic acid was optimal for roots. Medium with 14.54 µM TDZ and 1.92 µM ZT enabled somatic embryo development from PEMs (González-Rodríguez and Peña-Ramírez 2007).

Somatic embryogenesis in *C. odorata* was reported by Cameron (2010). DKW medium with 5 µM BA and 50 µM 2,4-D produced the best results with 20-25% nodule occurrence. Nodules were cultured on PGR-free DKW medium for secondary somatic embryo development, and rooted plants were transplanted to pots. Nodule formation was further improved by orienting cotyledons with the abaxial surface in contact with the medium, pre-treatment with a heat shock of 47°C for 8-12 h, or when used together.

Cedrela odorata somatic embryogenesis was induced by culturing immature zygotic embryos on MS medium containing 13.57 µM dicamba. Conversion frequency of 12% was observed on MS medium with 3.8 µM ABA. A transfer efficiency of 75% was achieved after planting in the field (Peña-Ramírez et al. 2011).

Cinnamomum

Embryogenic cultures were induced from immature embryos of *Cinnamomum camphora* on MS medium with 4.44 µM BA, 0.45 µM 2,4-D, and 700 mg l^{-1} casein hydrolysate. Somatic embryos developed on all PGR combinations. Somatic embryos developed normally and were successfully transferred to greenhouse conditions (Du et al. 2007).

Shi et al. (2009) sought to further optimize direct somatic embryogenesis for *C. camphora*. This study explored the effects of osmotic stress on zygotic embryo explants, as well as other factors such as the addition of activated charcoal and light periodicity. Osmotic stress from elevated sucrose concentration greatly increased the frequency of somatic embryogenesis in *C. camphora* on a PGR-free medium. Shi et al. (2010) reported a procedure in which secondary somatic embryos of *C. camphora* were regenerated from somatic embryos maintained through subculturing for over 4 years. Proliferation of somatic embryos was achieved when cultured in darkness on MS medium with 5.37 µM NAA and 1 g l^{-1} malt extract. Secondary somatic embryos developed and matured on MS medium with 0.91 µM TDZ, and the optimum medium contained 1.89 µM ABA, producing normal shoots with a 31.7% and 30.4% frequency, respectively. Somatic embryos, germinated successfully on MS medium with 0.45 µM TDZ, 1 µM IBA, and 2.22 µM BA, were then transferred to half-strength MS medium with 2.46 µM IBA for rooting, and plantlets were eventually transferred to the greenhouse.

Embryogenic cultures of *C kanehirae* were achieved from leaves on WPM containing 0.58 µM GA_3 and 150 ml l^{-1} coconut water at 5°C for 14 days prior to transfer to a PGR-free medium. The optimal medium was half-strength MS medium with 4.44 µM BA and 2.69 µM NAA. Somatic embryos germinated and were successfully transplanted to ambient growing conditions (Chen

and Chang 2009).

Somatic embryogenesis was achieved from immature embryos of *C. pauciflorum* on MS medium with 2.5 µM TDZ. Direct somatic embryos developed along the hypocotyl and radicle when 0.8 g l^{-1} casein, 0.4 g l^{-1} glutamine, and 10 g l^{-1} sucrose were added to the medium. Thidiazuron was more effective then 2,4-D, BA, or picloram. Eighty-two-percent of somatic embryos germinated on PGR-free medium supplemented with 30 g l^{-1} sucrose and 5 g l^{-1} Phytagel (Kong et al. 2009).

Dalbergia

Embryogenic cultures derived from semi-mature cotyledon segments of *Dalbergia sissoo* were reported and conversion of somatic embryos was achieved. Cultures were induced on 89% of the segments after 12-15 days on MS medium with 9.04 µM 2,4-D and 0.46 µM KIN. Fifty-five-percent of embryogenic culture clumps formed somatic embryos after transfer to half-strength MS medium without PGRs, however 0.68 mM l^{-1} L-glutamine increased the number of somatic embryos to 66.6%. Transfer of somatic embryos to half-strength MS medium with 10 g l^{-1} sucrose for 15 days prior to transfer to half-strength MS medium with 20 g l^{-1} sucrose for 20 days increased plantlet formation from 50% to 75% (Singh and Chand 2003).

Singh and Chand (2010) transferred embryogenic cultures to half-strength MS medium plus 10 g l^{-1} sucrose for 14 days prior to alginate encapsulation. Forty-three-percent of encapsulated somatic embryos converted after 20 days on half-strength MS medium with 20 g l^{-1} sucrose, while 72% of non-encapsulated somatic embryos developed into plantlets. Fourteen-percent of encapsulated somatic embryos stored at 4°C for 30 days before being transferred *in vivo* were converted, while only 22.2% of non-stored, non-encapsulated somatic embryos underwent conversion.

Garcinia

Somatic embryos were induced on immature seeds of *Garcinia indica* with an 80% frequency on WPM with 22.2 µM BA. The addition of NAA reduced somatic embryo frequency. The combination of WPM with 16 µM BA, 5.7 µM IAA, and 4.6 µM KIN resulted in somatic embryo maturation and germination. Somatic embryos on half-strength WPM with 4.9 µM IBA formed roots and plantlets were acclimatized (Thengane et al 2006b).

Somatic embryogenesis was successful in *G. mangostana* using MS medium with 4.92 µM IBA, 0.54 µM NAA, and 10% coconut water. Shoots elongated on MS medium with 0.44 µM BA, 0.54 µM NAA, 2 g l^{-1} yeast extract, and 10% coconut water. Rooting (85%) was accomplished when shoots were placed on WPM with 0.44 µM BA, 24.6 µM IBA, 2 g l^{-1} yeast extract, and 10% coconut water (Minh 2005).

Irvingia

Fragments of young *Irvingia gabonensis* leaves were used for induction of embryogenic cultures on medium with 13.6 µM 2,4-D. Somatic embryos developed from the cultures on half-strength MS medium with 4.4 µM BA, and later the BA concentration was increased to 8.9 µM. Somatic embryos cultured on medium with 17.8 µM BA and 10.7 µM NAA germinated (Fotso et al. 2008a).

Mangifera

Nucellar tissues from immature fruits of *Mangifera indica* cultivars produced somatic embryos when exposed to various previously published culture media formulations. Some cultivars germinated and produced roots (Flórez-Ramos et al. 2007).

Direct somatic embryogenesis was achieved for *M. indica* var. *Zihua* by culturing immature cotyledons and nucelli on a modified Gamborg B5-MS medium supplemented with 500 mg l^{-1} glutamine, 23.3 µM KIN, 22.6 µM 2,4-D, 10% coconut water, 40 g l^{-1} sucrose, and 2 g l^{-1} activated charcoal. Cultures occasionally produced mature somatic embryos, but most remained at the pro-embryo stage (Wu et al. 2007).

Krishna and Singh (2007) reviewed some of the problems encountered in somatic embryogenesis of *M. indica*.

Pateña and Barba (2011) developed techniques for successful transfer of *in vivo* grafted somatic plants to the field.

Melia

Embryogenic cultures were induced from immature zygotic embryos of *Melia azedarach* on MS medium with 0.45, 4.54, or 13.62 µM TDZ. A 25% conversion rate was obtained on quarter-strength MS medium with 30 g l^{-1} sucrose. Well-developed plantlets were successfully transferred to soil with a 95% acclimatization rate (Vila et al. 2003).

Embryonic cultures were induced on MS medium with 16.11 µM NAA, 4.44 µM BA, 14.4 µM GA_3, and 30 g l^{-1} sucrose. Somatic embryos developed on MS medium with 44.4 BA, 40 mg l^{-1} adenine, 161.1 mg l^{-1} putrescine, and 60 g l^{-1} glucose. Following germination rooted plants were successfully transferred to the greenhouse (Sharry and Silva 2006, Sharry et al. 2006).

The factors affecting somatic embryogenesis induction in *M. azedarach* were evaluated by Vila et al. (2007). Somatic embryogenesis was affected by PGRs, light, gelling agent, carbohydrate source, explant type, and induction time. Induction of embryogenic cultures was best on MS medium with 4.54 µM TDZ solidified with Sigma A-1296 agar. Torpedo- and cotyledonary-stage somatic embryos responded best to conversion. Medium supplemented with 30 g l^{-1} raffinose produced the best results for somatic embryo induction and

conversion. Explants incubated at 160 μmol m^{-2} s^{-1} or pretreated for 1 week in darkness before maintenance under 160 μmol m^{-2} s^{-1} had a higher embryogenic index.

The effect of morphological heterogeneity on somatic embryo conversion in *M. azedarach* was investigated by Vila et al. (2010). Embryogenic cultures were induced from immature zygotic embryos on MS medium with 4.45 μM TDZ or 0.45 μM 2,4-D. After 6 weeks, somatic embryos were categorized into morphological classes and histological analyses were conducted. Explants were then transferred to quarter-strength MS medium without PGRs to determine conversion rates. Bipolar somatic embryos with well-defined root and shoot apical meristems had a conversion rate of 28% when cultured individually, and when cultured in groups the rate declined to 6.8%. Fused embryos, when subcultured in groups, had a conversion rate of 2.1% and produced shoots with fused stems. Plants that successfully converted were successfully acclimatized with 95% survival.

Pterocarpus

Embryogenic cultures were induced from hypocotyl segments of 12-day-old *in vitro* seedlings of *Pterocarpus marsupium* on MS medium with 5 μM 2,4-D and 1 μM BA. Somatic embryos developed from cultures on MS medium with 5 μM 2,4-D and 2 μM BA. Maturation of the somatic embryos was enhanced using MS medium with 0.5 μM BA, 10 μM ABA, and 0.1 μM NAA. Somatic embryos were successfully converted into plantlets on half-strength MS medium with 2 μM BA and 0.1 μM NAA, rooted on half-strength MS medium with 0.2 μM 1,2,3-trihydroxy benzene and 0.2 μM IBA, and acclimatized (Husain et al. 2010).

Ricinodendron

Embryogenic cultures of *Ricinodendron heudelotii* were induced from leaf fragments on medium containing 13.6 μM 2,4-D or 4.5 μM 2,4-D and 4.7 μM KIN, or 4.5 μM 2,4-D and 8.9 μM BA (Fotso et al. 2007b).

Santalum

Das et al. (2001) determined the optimal levels of medium constituents for induction of embryogenic cultures of *Santalum album* in suspension cultures. The optimal medium contained 37.56 g l^{-1} sucrose, 11.6 mM nitrate, 7.89 mM ammonium, and 5.0 μM ABA with an embryogenesis efficiency of 57.35%.

Ravishankar Rai and McComb (2002) developed a direct somatic embryo and plantlet regeneration system for *S. album* using mature zygotic embryos as explants. Using a MS induction medium with 4.5 μM TDZ or 6.4 μM BA, MS medium with PGRs for secondary embryogenesis, and half-strength MS medium with 2.8 μM GA_3 for conversion of somatic embryos, and 70% of plantlets were successfully acclimatized.

Swietenia

Germination of somatic embryos of *Swietenia macrophylla* was achieved at the cotyledonary stage on half-strength MS medium without PGRs (Collado et al. 2005).

Embryogenic cultures of *S. macrophylla* were induced from epicotyl explants on MS medium with BA and NAA (Brunetta et al. 2006).

González-Rodríguez and Peña-Ramírez (2007) established a protocol of somatic embryogenesis for *S. macrophylla*. WPM with MS vitamins, 13.6 μM dicamba, 5% coconut water, 1% activated charcoal, 520.5 μM citric acid, and 567.8 μM ascorbic acid at pH 7.5 was used to induce embryogenic cultures from leaf explants.

Maruyama (2009) investigated the effect of polyethylene glycol (PEG) on somatic embryo maturation and subsequent germination of *S. macrophylla*. The highest number of cotyledonary somatic embryos and percentage of germination and regeneration was obtained using WPM with 7.5% PEG and 1 μM BA as the maturation medium. Somatic embryogenesis of *S. macrophylla* was also induced from cotyledons of mature zygotic embryos on MS medium containing 18.1 μM 2,4-D, and half-strength MS medium with 4.4 μM BA was used for the development and maturation of somatic embryos.

The maturation, germination, and root formation of somatic embryos was also achieved using half-strength MS medium without PGRs with 60 g l^{-1} sucrose (Collado et al. 2010).

Terminalia

Embryogenic cultures were induced from mature zygotic embryos of *Terminalia chebula* on MS medium with 4.5 μM 2,4-D, 0.05 μM KIN, and 50 g l^{-1} sucrose. Germination of somatic embryos was maximized with 2.2 μM BA and 30 g l^{-1} sucrose (Anjaneyulu et al. 2004).

Table 1. Summary of *in vitro* technologies for propagation of tropical hardwood tree species (2001-2011).

Genus	Research studies	References
Albizia	CM, MP, R	Yin et al. 2001, Jain and Babbar 2002, 2006, Mamun et al. 2004, Rajeswari and Paliwal 2006, Valverde-Cerdas et al. 2008, Borthakur et al. 2011
Anthocephalus	SE	Apruva and Thakur 2009
Aspidosperma	MP	Ribas et al. 2002, 2005, Hubner et al. 2007

Table 1. Continues

Azadirachta	ASR, CC, CP, MP, SE	Salvi et al. 2001, Akula et al. 2003, Chaturvedi et al. 2004a, Quraishi et al. 2004, Rodríguez and Ortiz 2001, Dhillon et al. 2005, Gairi and Rashid 2005, Rout 2005, Foan and Othman 2006, Reddy et al. 2006, Shahin-uz-zaman et al. 2008, Varghese and Naithani 2008, Lavanya et al. 2009, Shekhawat et al. 2009, Srivastava et al. 2009, Arora et al. 2010, Das 2011
Baillonella	SE	Fotso et al. 2008b
Caesalpinia	CC	Werner et al. 2009, 2010
Calophyllum	MP, R	Nair and Seeni 2003, Thengane et al. 2006a
Cariniana	ASR, CC	Yaya et al. 2005
Cedrela	ASR, MP, SE	Nunes et al. 2002, 2003, Daquinta et al. 2004, Gonzalez-Rodriguez et al. 2007, Nunes et al. 2007, Vila et al. 2009, Cameron 2010, Peña-Ramírez et al. 2010, 2011
Ceiba	MP	Silva et al. 2010
Cinnamomum	CP, GT, MP, PC, SE	Nirmal Babu et al. 2003, Azad et al. 2005, Chen et al. 2005, Du and Bao 2005, Du et al. 2007, 2008, Chen and Chang 2009, Kong et al. 2009, Shi et al. 2009, 2010
Cordia	MP	Schuler et al. 2005
Dalbergia	ASR, CC, CM, EC, MP, R, SE	Singh et al. 2002, Joshi et al. 2003, Singh and Chand 2003, 2010, Cerdas et al. 2004, Chand and Singh 2004, 2005, Chaturvedi et al. 2004b, Thirunavoukkarasu et al. 2010
Diospyros	ASR, CP, MG	Matsumoto et al. 2001, Ai and Luo 2005, Benelli et al. 2009, Naval et al. 2009
Enterolobium	MP, SG	Rodríguez-Sahagún et al. 2007
Eucalyptus	GT, MP, R	Fett-Neto et al. 2001, Girjashankar 2011, Nakhooda et al. 2011
Garcinia	ASR, CP, MP, R, SE	Normah 2000, Kozai and Kubota 2001, Normah et al. 2002, Te-chato and Lim 2004, Chabukswar and Deodhar 2005, 2006, Malik et al. 2005, 2010, Tran 2005, Thengane et al. 2006b, Sirchi et al. 2008, Noor et al. 2011, Tembe and Deodhar 2011, Yap et al. 2011
Genipa	MP, R	Rocha et al. 2008
Gmelina	MP, R	Naik et al. 2003, Valverde-Cerdas et al. 2004
Irvingia	ASR, CC, MP, R, SE	Fajimi et al. 2007, Fotso et al. 2008a
Khaya	EC, MG, MP	Danthu et al. 2003, Hung and Trueman 2011a,b, Okere and Adegeye 2011
Lagerstroemia	ASR	Rahman et al. 2010
Mangifera	CP, GT, R, SE	Flórez-Ramos et al. 2007, Wu et al. 2007, Chavarri et al. 2010, Pateña and Barba 2011
Melaleuca	MP	Oliveira et al. 2010
Melia	ASR, CP, MP, SE	Handro and Floh 2001, Bernard et al. 2002, Vila et al. 2003, 2004, 2007, 2010, Scocchi and Mroginiski 2004, Scocchi et al. 2004, Chennaveeraiah et al. 2006, Sharry and Silva 2006, Sharry et al. 2006, Kaviani 2007, 2010, Mroginski and Rey 2007, Hussain and Anis 2009
Nothofagus	MP, R	Martínez Pastur et al. 2003, 2005, 2007, Sanchez-Olate et al. 2004, Sabja et al. 2008
Pterocarpus	ASR, MP, R, SE, SG	Chand and Singh 2004, Tiwari et al. 2004, Anis et al. 2005, Chaturani et al. 2006, Chisha-Kasumu et al. 2006, Prakash et al. 2006, Husain et al. 2007, 2008, 2010, Rajeswari and Paliwal 2008, Balaraju et al. 2011
Ricinodendron	ASR, MP, R, SE	Fotso et al. 2004, 2007a,b
Santalum	ASR, GT, MP, SE	Das et al. 2001, Ravishankar Rai and McComb 2002, Mujib 2005, Sanjaya et al. 2006a,b, Shekhawat et al. 2008
Sapium	MP	Qie et al. 2009, Chen et al. 2010
Schinopsis	MP	Sansberro et al. 2003
Spondias	MP	Carvalho et al. 2002

Table 1. Continues

Sterculia	CP	Nadarajan et al. 2006, 2007
Swietenia	CP, MP, R, SE	Marzalina 2002, Collado et al. 2004, 2005, 2010, Rocha and Quoirin 2004, Brunetta et al. 2006, Schottz et al. 2007, González-Rodríguez and Peña-Ramírez 2007, Maruyama 2009
Tabebuia	CP, MP, SG	Schuler et al. 2005, Nery et al. 2008, Martins et al. 2009, Abbade et al. 2010, Tresena et al. 2010, González-Rodríguez et al. 2010
Tectona	ASR, GT, MP, R	Gangopadhyay et al. 2002, Tiwari et al. 2002, Shirin et al. 2005, Widiyanto et al. 2005, 2009, Yashoda et al. 2005, Gyves et al. 2007, Nor Aini et al. 2009
Terminalia	MP, GT, R, SE	Anjaneyulu et al. 2004, Ramesh et al. 2005, Pandey et al. 2006, Shyamkumar et al. 2007, Rathore et al. 2008, Chittora et al. 2010, Purohit and Habibi 2010
Wrightia	MP	Purohit and Habibi 2010

ASR - Adventitious shoot regeneration, CC - Callus culture, CM - Culture medium, CP - Cryopreservation, EC - Encapsulation, GT - Genetic transformation, MG - Micrografting, MP - Micropropagation, PC - Protoplast culture, R - Rooting, SG - Seed germination, SE-Somatic embryogenesis.

CONCLUDING REMARKS

The use of *in vitro* approaches for the propagation, conservation, and genetic improvement of tropical tree germplasm has improved slightly in the last 10 years (2001-2011). Tropical timber trees are long-lived and require many years to reach reproductive maturity. Once harvested, many more years are required for natural regeneration to occur (if at all), or for pure stands cultivated in plantations to reach harvestable market-size. In addition to providing timber for a vast array of wood products, tropical tree species are invaluable biological resources and provide unique environmental protection, such as reducing soil erosion and compaction, increasing soil fertility, absorbing pollutants, releasing oxygen, sustaining watersheds, and affecting climate change on a global scale. Tropical tree species also provide shade, shelter, biological and migration corridors, and food for countless species of wildlife; provide habitats for a rich diversity of plant species; and enable the sustainability of organisms such as fungi and microbes. Apart from meeting the demands for high-quality wood, tropical trees provide ornamental value and valuable medicinal, insecticidal, industrial, and many yet unknown commercial properties. Trees are also very aesthetically pleasing. For these and many more reasons, we must make a concerted effort to conserve habitats and ecological processes within native tropical tree forests before more tropical trees species become threatened or endangered.

Appropriate biotechnological and sustainable conservation strategies for many tropical hardwood tree species needs further research and development. *In vitro* clonal propagation presents many challenges in production costs, labor required to produce the plant material, and potential losses suffered during acclimatization of plants to the field. Rejuvenation of elite, mature phase tissue for *in vitro* biotechnology is critical for the establishment of plantations with elite characteristics or traits (e.g. growth rate, form, wood quality). Many *in vitro* studies have not been optimized based on the classical literature for a genus or species, but involve a single genotype. Tropical fruit, nut, and ornamental species may be useful as model systems to help overcome problems associated with *in vitro* propagation technologies of tropical hardwood (timber) species. Conservation through *in vitro* propagation technologies will have a global economic and ecological impact on sustaining tropical forest tree biodiversity.

Acknowledgements: The authors gratefully acknowledge Drs. Richard E. Litz, Stephen J. Trueman, and Wagner A. Vendrame for their review and suggestions for the improvement of this manuscript.

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