Conservation Attempts of Woody Medicinal Plants of India by Biotechnological Tools

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1 Introduction

About 50% of plant species have been reported as endemic to the 34 global biodiversity hotspots; each hotspot contains 1500 endemic species [1]. The species with compact populations are not considered to be vulnerable or endangered at present but they are at risk and scattered within the restricted geographical regions and moderately more extensive range [2]. Plants act as the best natural purifiers of the environment and support an essential role in retaining the oxygen cycle, which is essential for the survival of all forms of life and reducing carbon dioxides in the air [3]. Ecologically, woody plants support windbreaks and shelterbelts and are used for the protection of soil erosion, floods, and deserts [4]. The woody tree plants reduced temperature in the environment through shade and by intercepting, absorbing, and reflecting solar radiation, especially in warmer places [5]. Tree species are houses of the majority of wild creatures including animals like insects, birds, small mammals, and reptiles. The woody shrubs and trees on roadsides may protect the travellers and curves, thus making a natural guide for safe driving. Woody trees provide timber for the construction of buildings, agricultural implements, boat and shipbuilding, matches and matchboxes, mathematical instruments, musical

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instruments, furniture and cabinetwork, pencil and pen holders, railway carriage railway sleepers, packing cases and boxes, picture framing, etc. [4]. Moreover, woody plants play an important role in the ecological balance and forest structure.

In India, people collect the barks, leaves, roots, and sometimes the whole plant body. About 30% of the entire plant body was used for one-time purposes. In developed countries such as the United States, they contribute 25% of total drug production whereas fast-developing countries such as China and India contribute 80%. The main reason for decreasing the population rate of woody medicinal plants is the introduction of alien species and anthropogenic further activities. Hamilton [6] documented that several hundreds of medicinal plants have been categorized under the threatened category with extinction risk. Conservation of wild medicinal plants is difficult through conventional methods such as layering budding, seed germination, cuttings, and grafting. However, the availability of plant material is not sufficient to propagate plantlets through conventional methods. To solve this problem, in vitro propagation can be used and grown successfully.

The plant tissue culture technique is the most efficient technology for large-scale plant multiplication through micropropagation. In recent years, it is imperative in the area of plant propagation, secondary metabolites production, pathogen-free plant production, production of high-yielding plants, and plant improvement. Endangered and rare plant species have successfully propagated and conserved by micropropagation. Tissue cultures were employed to preserve plant genetics, develop more energetic plants, and rapid production of many uniform plants.

2 Effect of Various Sterilant on Various Explants of Shoot, Node, Leaves, and Seeds

Explant surface sterilization was an essential and most perceptive step of plant tissue culture. Surface sterilization has concerned with explants immersed into a suitable concentration of chemical sterilant or decontaminators for a particular time of establishment in contamination-free culture. The axillary and apical bud of *Hildegardia populifolia,* an endangered tree, was sterilized by using various sterilant at different time duration, Teepol was treated for 5 min and ethanol (70%) for 3 min, by mercuric chloride (0.1%) for 5 min, and finally cleaned with sterilized distilled water for 4–5 times [7]. *Hildegardia populifolia* nodal explants were sterilized by 1% Bavistin for 30 min and washed with distilled water and 5% teepol for 15 min and mercuric chloride (0.1%) 3 min. The explants were washed using distilled water before culture [8]. *Syzygium densiflorum* explants from mother trees were kept under running tap water (30 min) and Tween-80 (15 min), the earlier to exterior sterilization. Further, explants were sterilized by mercuric chloride (0.1%) for 5–10 min, subsequently rinsed with distilled water afterwards by 2% NaOCl for 5–10 min and ethanol (70%) for 5–10 min. The explants were rinsed with sterile distilled water in a laminar airflow chamber [9]. Explants (leaf and stem) of

Nothapodytes foetida were washed in running tap water for 10 min and washed with 1–2 drops of Tween-10 followed sterile distilled water (3 times). The explant sterilization was attempted to submerge and shack explants in ethanol (70%) for 30 s and rinsed with sterilized water (2 times). Surface sterilization was carried out with mercuric chloride (0.1%) for 3 min in addition to sterile distilled water (3 times) [10]. The in vitro-derived leaves of *Zanthoxylum armatum* were excised from the 40-day-old shoots by a sterile blade and soaked in a WPM liquid medium containing different concentrations of TDZ and shaken for different periods of 12, 24, and 36 h followed by cultured on a medium [11]. Shoot tip and nodal explants of *Gaultheria fragrantissima* were washed by running tap water (10–15 min) then treated with Bavistin (2 g^{-1}) for 15–20 min and kept overnight at 25 °C with 70–80% RH. The explants were again washed with tap water for 20–25 min followed by 4 g^{-1} Tata Master (15 min). The explants were treated with antibiotics plantomycin (50 mg^{-1}) and rifampicin (50 mg^{-1}) for 20 min and washed with sterile water. Further, the explants were sterilized with 0.1% of $HgCl_2$ for 3 min and 100% of Tween-80 (one to two drops) for 3 min, eventually sterilized in distilled water for 10 min (4–5 times) [12]. The shoot apex and tip explants of *Elaeocarpus blascoi* were cleaned by running tap water (30 min) and then immersed in 10% of sodium hypochlorite (5–8 min) with two or three drops of Teepol over again with sterile distilled water (3 times). The explants were further treated with ethanol (70%) for 30 s, 0.05% $HgCl_2$ and washed with sterile distilled water or 2–3 times and immersed in the antimycotic solution for 5 min [13]. Leaf explants of *Leptadenia reticulata* were initially treated with 0.1% Bavistin for 10–15 min and 0.1% of mercuric chloride for 3–4 min and washed by sterile distilled water (6–8 times) in a laminar airflow chamber [14]. *Leptadenia reticulate* nodal and apical shoots were surface-sterilized by mercuric chloride (0.1%) for 4–5 min and immersed in ethyl alcohol (90%) for 30–40 s, followed by washing with sterile water (6–7 times). Then, explants were treated with additives such as adenine sulphate (25 mg^{-1}), arginine and citric acid, and ascorbic acid (50 mg^{-1}) for 10–15 min [15]. Mature and healthy seeds of *Pterocarpus marsupium* were sterilized in two stages. The first stage was washed with running water for 10 min and sterilized by Bavistin (1%) for 5 min followed by rinsed with running tap water (8 min) and imbibed 24 h in distilled water. In the second step, the seeds were cleaned with $HgCl_2$ (0.1%) for 4 min and washed with distilled water (4 times) and cultured on nutrient media [16].

Young shoots of *Rauvolfia serpentine* were washed by using tap water for 30 min and soaked in labolene (5%) for 5 min. Further, the explants were exteriorly sterilized by $HgCl_2$ (0.1%) for 3 min and eventually washed with sterile water 4–5 times [17]. Plant materials of *Atropa acuminata* were sterilized with Tween-20 (10 min) and washed with tap water for 30 min. The nodal and shoot tip explants excised from shoots were treated with 0.1% of Bavistin (10 min) and surface-sterilized with $HgCl_2$ (0.1%) for 4 min and rinsed with sterile distilled water 5 time [18]. Shoots of *Syzygium travancoricum* were washed with 10% of Nocidet B-300 (wetting agent) for 5 min and placed running tap water (30 min) and surface disinfected by 70% ethanol (3 min) followed by $HgCl_2$ (0.2%) for 5 min. Finally, explants were washed with sterile distilled water (5–6 times) for 10 min [19]. The young leaves and seeds

of *Berberis aristata* were washed with tap water and immersed in Tween-20 (0.1%) and agitated leaves (5–10 min) and seeds (15 min). The explants were treated with fungicide such as Bavistin, carbendazim for leaves (20 min) and seeds (20 min) and surface-sterilized by 0.1% of mercuric chloride for leaves (5 min) and seeds (7 min) and washed with sterilized double-distilled water for (4–6 times) [20]. The healthy seeds of *Sterculia urens* were washed with tap water and 5% of Tween-20 was additionally used with distilled water 3 times. Then, seeds were surface-sterilized with mercuric chloride (0.5%) for 5–7 min followed by autoclaved distilled water 2–3 times [21]. Explants of *Tylophora indica* shoots were washed with running tap water (30 min) and used 5% labolene to soak for 5 min. After that, 0.1% of $HgCl_2$ (3 min) were used for sterilization and finally rinsed with sterile distilled water (4–5 times) [22]. The nodal and shoot tips of *Garcinia travancorica* were used as explants and washed thoroughly with tap water for 15 min and washed with liquid detergent several times. Explants were also sterilized with 1% Clorox for 15 min. Further, explants were surface-sterilized by $HgCl_2$ (0.01%) for 20 min and subsequently washed with distilled water. Then, explants were surface-sterilized by ethanol (70%) for 10 min and also rinsed with sterile distilled water 3 times [23].

5% Tween (10–15 min), 80% ethanol (30 s), and 0.1% $HgCl_2$ (5 min) were reported to be suitable for sterilizing the axillary bud and node of *Ceropegia intermedia* [24]. The cotyledonary explant of *Terminalia bellirica* was washed with lanolin soap solution followed by 0.1% $HgCl_2$ for 15 min [25]. But at the same time, nodal explants of *Terminalia bellirica* were treated with 20% Bavistin for 5–7 min and followed by 0.1% $HgCl_2$ for 7 min for in vitro culture [26]. Patel et al. [14] reported to eradicate the microorganism from the leaf explants of *Leptadenia reticulata*; it has washed with 0.1% Bavistin (10–15 min) and 0.1% $HgCl_2$ (3–4 min). Likewise, 0.1% Bavistin (20 min) 0.1% mercuric chloride (4 min) and 70% ethanol were used for sterilizing the node of *Nilgirianthus ciliates* [27]. Surface sterilization of explants *Santalum album* was effectively done by using Tween-80 (5 min) and 0.075% mercuric chloride (5–6 min) [28].

The nodal explants of *Clerodendrum serratum* were treated with 1% lanolin and followed by 0.1% mercuric chloride was reported as effective to sterilize the explants *Couroupita guianensis* [29]. Singh et al. [30] studied and recorded that leaf of *Meizotropis pellita* was rinsed with two fungicides such as Bavistin 30 min and 0.1% Tween-20 for 5 min. Further, the explants were washed with 70% ethanol (1 min) followed $HgCl_2$ 0.1% for 4 min. The individual concentrations of sodium hypochlorite and calcium hypochlorite were effective for seed and node sterilization [31, 32], whereas mercuric chloride has alone used to sterilize *Decalepis hamiltonii* [33]. The application of Tween-20, 70% ethanol, and mercuric chloride was used to eliminate the microorganism from the leaf explant [34]. The collected seeds of *Entada pursaetha* were disinfected with Tween-20 (15 min), 70% ethanol, and finally immersed in 0.1% $HgCl_2$ for 10 min and also with distilled water [35].

Sharmila et al. [36] reported that various sterilization methods have been tested for eliminating fungal contamination. Among these, Teepol solution (5–10 min), 10% Bavistin, and antibiotics such as ampicillin and rifampicin (15–20 min) followed by 70% alcohol (30–60 s) and 0.1% mercuric chloride solution (3–7 min)

were considered as effective for leaf and stem explants. The leaf explant of *Mahonia leschenaultia* was washed with 1% Labolene detergent for 10–20 min and 0.1% $HgCl_2$ for 5–10 min [37]. Similarly, Sahoo and Chand [38] studied the nodal portion of *Vitex negundo* rinsed with 5% Laboline, 7% sodium hypochlorite (7–10 min), and subsequently washed with 0.1% mercuric chloride for 8 min. Kumar et al. [39] reported that *Caesalpinia bonduc* was soaked in sulphuric acid to break the seed dormancy during the sterilization procedure seeds after that the explants were washed with mercuric chloride solution.

The seeds of *P. santalinus* were treated with a combination of 50% HCl + 50 ethyl alcohol for 3 h and air-dried for 2 days before inoculation [40]. The axillary bud segments, leaf, and nodal explants of *Exacum wightianus* were washed under running tap water for 30 min. 0.1% Tween-20 (15 min) followed by 0.5% Bavistin were used for sterilizing the explants. After subsequent washes with distilled water, it was treated with 70% ethanol and finally sterilized with (0.1%) $HgCl_2$ [41]. The pods were soaked with distilled water for 24 h and washed with Tween-20 (15 min) and followed by savlon antiseptic solution (0.06%). Further sterilization procedures were carried out with 0.1% $HgCl_2$ (20 min) followed by 70% ethanol (1 min) [42]. De-pulped seeds of *Ilex khasiana* were washed under running tap water for 10 min soaked in 5% teepol for 5 min and treated with 70% ethanol (1 min) and finally surface-sterilized with 15% sodium hypochlorite [43]. Lal and Singh [44] experimented and recorded that the nodal explants of *Celastrus paniculatus* were washed with teepol running tap water. Surface sterilization of nodal explants was successfully done under aseptic conditions using 0.1% $HgCl_2$ for 3–5 min. Finally, the nodal region was exposed to absolute alcohol and washed with sterilized distilled water. The explant of *Tylophora indica* was soaked in 5% teepol solution for 5 min followed by freshly prepared $HgCl_2$ (for 3 min) [45, 46]. The nodal segments with the axillary region of *Gymnema sylvestre* were surface-sterilized with Tween-20 for 3 min and 0.1% mercuric chloride (8 min) [47]. The seeds of *G. sylvestre* were disinfected with 0.5% $HgCl_2$ containing laboline for 5 min [48]. The successfully germinated 15-day-old seedlings of *P. santalinus* were collected and soaked in soap solution to remove the contaminants and soil and the shoots were treated with 0.1% mercuric chloride solution [49]. The sterilize nodal explants of *Celastrus paniculatus* in $HgCl_2$ containing Tween-20 solutions (5 min) were washed with distilled water. Further, the nodal explants were disinfected with 0.1% $HgCl_2$ and washed with double sterile distilled water [50]. Padmalatha and Prasad [51] documented that the dried pods were soaked in boiling water (100 °C) overnight and treated with 5% sulphuric acid for 10 min. Seeds were further washed with 2% Bavistin for 30 min and nodes were exposed to 15 min by 70% ethanol and 0.1% $HgCl_2$ were used for disinfecting the pods and seeds.

Celastrus paniculatus nodal and internode explants were washed by running tap water (30 min) followed by 25% NaOCl (10 min) and consequently washed with autoclaved distilled water (4–5 times). The explants were further sterilized with 0.1% mercuric chloride (10 min) and washed 4–5 times by autoclaved double-distilled water and cultured on the medium [52]. The leaf and stem explants of *Rauwolfia tetraphylla* washed running tap water (2 min) with Tween-20 (1 min)

followed by 60% ethanol (2 min) 0.1% mercuric chloride ($HgCl_2$) (2 min) although the stem and root explants were sterilized with 0.5% Bavistin (10 min), followed by Tween-20 (2 min) and 0.1% mercuric chloride (4 min) [53]. The pods of *Pterocarpus marsupium* were washed by running tap water (15 min) followed by 2% of Teepol (10 min) and 5% of Tween-20 (4 min) and disinfected with 0.1% mercuric chloride ($HgCl_2$) for 6 min and cultured on medium [54]. The healthy seeds of *Pterocarpus santalinus* were washed with 70% alcohol (1 min) and 0.1% $HgCl_2$, followed by 0.1% sodium dodecyl sulphate (10 min) and washed by double sterile water for 5 times [55].

3 Effect on Various Media on In Vitro Shoot Multiplication

Plant tissue culture media provide essential components such as macro, micro, vitamins, hormones, and carbon source to the plant for their growth and development. Lavanya et al. [7] reported axillary and apical buds were cultured on MS medium fortified with different plant growth regulators (PGRs) to induce multiple shoots in *Hildegardia populifolia*. Nodal explants inoculated in MS and WPM containing PGRs were induced shoots [8]. The nodes of *Syzygium densiflorum* were cultured on WPM with combinations of PGRs to develop multiple shoots and microshoots were cultured on half-strength WPM to induce rooting [9]. The leaves and stem explants of *Nothapodytes foetida* were induced somatic embryos on MS medium in addition to PGRs and organic supplements [10]. In vitro raised aseptic leaf explants of *Zanthoxylum armatum* were induced calli on WPM with PGRs [11]. *Gaultheria fragrantissima* shoots tips and nodal explants cultured on WPM were induced multiple shoots. The nodal explants induced multiple shoots on MS medium supplemented with different concentrations of PGRs in *Leptadenia reticulata*, *Syzygium travancoricum*, *Sterculia urens*, *Tylophora indica*, and *Garcinia travancorica* [15, 19, 21–23]. *Syzygium travancoricum* developed multiple shoots on both MS and WPM [19], whereas nodal explants of *Elaeocarpus blascoi* and *Rauvolfia serpentina* were induced multiple shoots on WPM [13, 17].

The leaf of *Leptadenia reticulata* has induced calli on MS medium with various PGRs and the leaf calli of *Leptadenia reticulate* transfer to shooting MS medium to induce multiple shoots [14]. Seeds of *Pterocarpus marsupium* were induced multiple shoots on MS medium containing suitable PGRs and cotyledonary shoots were induced roots when transferred to MS medium containing PGRs [16]. The in vitro raised cotyledonary nodal explants of *Pterocarpus marsupium* were cultured on MS medium [56]. The explants of *Rhododendron wattii* were cultured on WPM-induced multiple shoots and microshoots were cultured on WPM with suitable rooting PGRs which induced roots [57]. While microshoots of *Rauvolfia serpentina* induced root on WPM [17], explants of shoot tips and nodal of *Atropa acuminata* were cultured on MS medium fortified with PGRs and induced shoot proliferation and RT (Revised tobacco) medium were used for shoot elongation rooting [18]. Leaf-derived callus of *Berberis aristata* have induced multiple shoot on WPM [20]. Nodal explants of

Garcinia travancorica were initiated shoots on MS medium containing PGRs [23]. *Ceropegia intermedia* axillary bud and node to induce multiple shoots [24] and petiole and leaf explants developed calli on MS medium with PGRs [62]. *Terminalia bellirica* cotyledonary nodal explants induced shoot proliferation and multiple shoots on MS medium [25]. *Leptadenia reticulata* leaf explants induced calli and leaf-derived calli developed multiple shoots on MS with various concentrations of PGRs. The microshoots were induced root on one-fourth strength of MS medium [14]. The cotyledonary nodes of *Sterculia urens* cultured on MS medium to induced multiple shoots [58]. The nodal explants of *Nilgirianthus ciliates* induced multiple shoots on MS medium and the addition of PGRs and microshoots were developed roots on half-strength MS medium [27]. The nodal explants of *Santalum album* induced multiple shoots on MS medium and developed root from microshoots which have cultured on quarter-strength MS basal medium [28]. *Clerodendrum serratum* nodal explants were induced the greatest shoot bud induction and multiple shoots and microshoots induced root on MS medium [59]. Seeds of *Couroupita guianensis* were germinated on MS medium containing PGRs and nodal explants were cultured on MS medium with additional combinations of PGRs to induce multiple shoots. The roots were developed from microshoots cultured on half-strength MS medium addition of PGRs [29]. *Meizotropis pellita* leaf explants have induced calli and leaf-derived calli induced shoots were cultured on MS medium containing different concentrations and combinations of PGRs. The roots were developed from microshoots on MS medium [30]. The nodal explants of *Commiphora wightii* showed shooting response on MS medium containing PGRs [60]. The nodal explants of *Pterocarpus marsupium* have induced shoot on basal MS medium and multiple shoots on MS medium containing PGRs [31] (Table 1).

The nodal explants of *Decalepis arayalpathra* and *Vitex negundo* were exhibited growth response on MS medium [16, 38] whereas embryos cultured on MS Medium influenced the growth of shoot formation. The bud and leaf explants of *Decalepis hamiltonii* inoculated on MS medium showed a good culture response, and for further growth, the medium was supplemented with PGRs. Most of the seed explants were successfully germinated on MS medium and supplemented with plant growth regulators [32, 39]. But in the case of *Entada pursaetha*, rooting has been initiated on half-strength MS medium [35]. Likewise, some of the researchers studied and reported that the leave explant was successfully grown on MS medium [34, 36, 37].

Anuradha and Pullaiah [40] documented that mesocotyl explants of *Pterocarpus santalinus* were induced multiple shoots on B5 medium. In B5 medium shoot tip, necrosis and leaf fall were observed whereas, on MS medium, these abnormalities were recorded. Sita et al. [49] reported that shoot tips from seedlings were cultured on B5 medium showed better shoot response. The different explants of *E. wightianus* have shown callus induction on MS medium supplemented with various plant growth regulators [41]. The cotyledonary explants of *P. marsupium* induced single shoot on MS medium, and for better rooting, the plantlets were transferred to half- and quarter-strength MS medium [42]. The seeds of *Ilex khasiana* cultured on MS medium were failed to induced microshoots without hormones [43]. Some of the researchers inoculated the nodal part in the MS medium that has failed to induce

Table 1 Conservation efforts of selected endemic and endangered woody medicinal plants of India

S. No	Name of the plant	Explant	Types of sterilant used	Medium	Conc. hormone	Response	Hardening (%)	Restoration	Reference
1.	*Hildegardia populifolia* (Malvaceae)	Axillary and apical bud	Teepol (5 min), 70% ethanol (3 min), 0.1% mercuric chloride (5 min)	MS	BA and KIN (0.5–3 mg^{-1}) with GA_3 (0.1–3 mg^{-1})	Shoot multiplication	Soil, sand, and vermiculate (2:1:1) with MS basal (¼ strength)	100%	Lavanya et al. [7]
		Nodal	1% Bavistin (30 min), 5% teepol (15 min), 0.1% mercuric chloride (3 min)	MS and WPM	BA, KIN and TDZ (0.5–10 µM)	Shoot induction	Garden soil+manure (3:1) and soil rite, vermicompost, irrigated with MS basal (¼ strength)	98.3%	Upadhyay et al. [8]
2.	*Syzygium densiflorum* (Myrtaceae)	Nodal	Tween-80 (15 min), 0.1% mercuric chloride (5 min), 2% NaOCl (5–10 min), 70% ethanol (5–10 min)	WPM	BAP (1.5 mg^{-1}) with IBA (1.5 mg^{-1})	Shoot multiplication	Sand and sterile soil (1:1) sprayed distilled water with micronutrients and macronutrients	58.5%	Ramasubbu and Divya [9]
		Shoots	Sterile distilled water	Half-strength WPM	IBA (0.5 mg^{-1})	Rooting			
3.	*Nothapodytes foetida* (Icacinaceae)	Leaf and stem	Washing in liquid detergent (1–2 drops of Tween-10 in distilled water), 70% (v/v) ethanol (30 s), 0.1% mercuric chloride (3 min)	MS	TDZ (0.5–3.0 mg^{-1}) coconut water (20%)	Somatic embryos induction	–	–	Khadke and Kuvalekar [10]
4.	*Zanthoxylum armatum* (Rutaceae)	Aseptic leaf explants	Soaked in distilled water (24 h)	WPM	TDZ (15 µM) and combination of TDZ (6.0 µM) and NAA (0.5 µM)	Calli induction	Farmyard manure and soil (3:1)	–	Purohit et al. [11]
5.	*Gaultheria fragrantissima* (Ericaceae)	Shoot tips and nodal	2.0 g^{-1} Bavistin (15–20 min), 0.1% mercuric chloride (3 min) with containing 1–2 drops of 100% (v/v) Tween-80	WPM	TDZ (0.22 mg^{-1})	Shoot multiplication	1:1:9 farmyard, sand, and virgin soil	–	Bantawa et al. [12]

6.	*Elaeocarpus blascoi* (Elaeocarpaceae)	Nodal	10% Sodium hypochlorite (5–8 min), 70% ethanol (30 s), 0.05% mercuric chloride (5 min)	WPM	TDZ (0.22 μM)	Shoot multiplication	Garden soil, farmyard, and river sand mixture (1:2:1)	–	Siva et al. [13]
		Regenerated microshoots	Sterile distilled water	WPM (half-strength)	IBA (2 mg^{-1})	Rooting			
7.	*Leptadenia reticulata* (Asclepiadaceae)	Leaf	0.1% (w/v) Bavistin (10–15 min) 0.1% $HgCl_2$ (3–4 min)	MS	2,4-D (0.5 mg^{-1}) and BAP (0.5 mg^{-1})	Callus proliferation	Sand: FYM: Clay (3:1:1)	95%	Patel et al. [14]
		Leaf-derived calli	Sterile distilled water	MS	BAP (0.5 mg^{-1}) and NAA (0.1 mg^{-1})	Shoot multiplication			
		Nodal	0.1% $HgCl_2$ (3–4-5 min), dipped in 90% ethyl alcohol (30–40 s)	MS	IAA (0.6 μM) and BA (9 μM)	Shoot multiplication	Soil rite and sandy soil: farmyard manure: soil rite (2:1:1)	95%	Arya et al. [15]
8.	*Pterocarpus marsupium* (Fabaceae)	Seeds	1% Bavistin (5 min), seeds were kept in sterilized distilled water for imbibition (24 h), 0.1% $HgCl_2$ (4 min)	MS	GA_3 (0.50 μM) with TDZ (0.50 μM)	Shoot multiplication	Soil rite	86.7%	Ahmad et al. [16]
		Microshoots	Sterile distilled water	MS	IBA (100 μM)	Rooting			
		In vitro raised cotyledonary nodal	Sterile distilled water	MS	TDZ (0.4 μM)	Shoot multiplication	Soil and soil rite (1:1)	–	Husain et al. [56]
9.	*Rhododendron wattii* (Ericaceae)	In vitro raised nodal	Sterile distilled water	WPM	2iP (39.36 μM)	Shoot multiplication	–	–	Mao et al. [57]
		Microshoots	Sterile distilled water	WPM	IBA (2.45 μM)	Rooting			

(continued)

Table 1 (continued)

S. No	Name of the plant	Explant	Types of sterilant used	Medium	Conc. hormone	Response	Hardening (%)		Restoration	Reference
10.	*Rauvolfia serpentine* (Apocynaceae)	Nodal	5% (v/v) detergent (5 min), 0.1% $HgCl_2$ (3 min)	WPM	BA (5.0 µM) with NAA (1.0 µM)	Multiple shoot induction	Garden soil	50	–	Alatar et al. [17]
		Microshoots	Sterile distilled water	WPM	NAA (1.0 µM)	Rooting	Soil rite	90		
		Microshoots	Sterile distilled water	RT medium	IBA (1 mg^{-1})	Rooting	Vermiculite			
11	*Syzygium travancoricum* (myrtaceae)	Nodal	70% ethanol (3 min) 0.2% $HgCl_2$ (5 min)	MS and WPM	BA (17.7 µM) and NAA (1.3 µM)	Multiple shoot induction	Sterilized sand and soil mixture (1:1)		40%	Anand et al. [19]
12	*Berberis aristata* (Berberidaceae)	Young leaves (leaf-derived callus)	1% Tween-20 (5–10 min), 0.5% Bavistin (20 min), 0.1% $HgCl_2$ (5 min)	WPM	TDZ (0.5 µM) and BA (8.88 µM) with NAA (2.68 µM)	Shoot multiplication	Sterile soil and farmyard manure (3:1)		–	Brijwal et al. [20]
13.	*Sterculia urens* (Sterculiaceae)	Nodal	5% Tween-20 (10–15 min), 0.1% $HgCl_2$ (5 min)	MS	TDZ (0.33 mg^{-1})	Shoot multiplication	Garden soil, organic manure, coco peat, and vermiculite (3:3:2:1)		80%	Dhiman et al. [21]
14.	*Tylophora indica* (Asclepiadaceae)	Nodal	5% detergent, labolene (5 min), 0.1% $HgCl_2$ (3 min)	MS	BA (2.5 µM), NAA (0.5 µM) and lAA (100 mg^{-1})	Shoot multiplication	Sterile garden soil, soil rite, or vermiculite		100%	Faisal et al. [22]
15.	*Garcinia travancorica* (Clusiaceae)	Nodal	Shaking with 1% Clorox (15 min), 0.01% HgCl (20 min), 70% ethanol (10 min)	MS	BAP (4.0 mg^{-1})	Shoot initiation	Sterile soil and sand (1:1)		–	Ramasubbu et al. [23]
				MS	BAP (4.0 mg^{-1}) and NAA (1.0 mg^{-1})	Multiple shoots and shoot elongation				

16.	*Ceropegia intermedia* (Asclepiadaceae)	Axillary bud and node	5% Tween (10–15 min), 80% ethanol (30 s), 0.1% $HgCl_2$ (5 min)	MS	BA (6.66 µM)	Multiple shoots	Peat moss and garden soil(1:1)	–	Karuppusamy et al. [24]
		Petiole and leaf explants	–	MS	Vermicompost extract and coelomic fluid in 3:1 ratio	Calli induction	–	–	Kashyap et al. [62]
17.	*Terminalia bellirica* (Combretaceae)	Cotyledonary nodal	Lanolin soap solution 0.1% $HgCl_2$ (15 min)	MS	BAP (2.0 mg^{-1}) BAP (3.5 mg^{-1}) and KN (0.5 mg^{-1})	Shoot proliferation multiple shoots	Soil rite and sprayed with 1/4 MS salt solution	–	Mehta et al. [25]
		Node	20% (v/v) Extran® (Merck, India) (5–7 min) 0.1% $HgCl_2$ (Merck, India) (7 min)	MS	½ MS + BA (8.8 µM) + NAA (2.6 µM)	Shoot multiplication	Organic manure and garden soil (1:1)	–	Dangi et al. [26]
					IBA (2.5 µM)	Rooting			
18.	*Leptadenia reticulate* (Asclepiadaceae)	Leaf	0.1% (w/v) Bavistin (10–15 min) and 0.1% (w/v) $HgCl_2$ (3–4 min)	MS	2,4-D (0.5 mg^{-1}) and BAP (0.5 mg^{-1})	Calli proliferation	Sand, farm yard manure, and clay (3:1:1)	–	Patel et al. [14]
		Leaf-derived calli		MS	BAP (0.5 mg^{-1}) and NAA (0.1 mg^{-1})	Multiple shoots			
		Microshoots		One-fourth strength MS	IBA (1.5 mg^{-1}) and 100 mg^{-1} Ascorbic acid	Rooting			
19.	*Sterculia urens* Roxb. (Sterculiaceae)	Cotyledonary node	–	MS	BAP (2.0 mg^{-1})	Multiple shoots	Farmyard manure and soil (1:3)	–	Purohit and Dave [58]
20.	*Nilgirianthus ciliatus* (Acanthaceae)	Nodal	0.1% (w/v) Bavistin (20 min) 0.1% mercuric chloride (HgCl2) (4 min) and 70% ethanol	MS	BA (3 mg^{-1}) and IAA (0.1 mg^{-1})	Multiple shoots	Vermicompost and sand, horticulture soil (1:1:1)	100% survival rate	Ramakrishnan et al. [27]
		Microshoots		Half-strength MS	IBA (1.0 mg^{-1})	Rooting			

(continued)

Table 1 (continued)

S. No	Name of the plant	Explant	Types of sterilant used	Medium	Conc. hormone	Response	Hardening (%)	Restoration	Reference
21.	*Santalum album* (Santalaceae)	Nodal	Dipped in a CPT 0.1% (5 min) Tween-80, [5] 0.075% mercuric chloride [5, 6]	MS	NAA (0.53 µM), BA (4.44 µM)	Multiple shoots	40%, garden 50%, sieved sand, and soil 10%)	100%	Sanjaya et al. [28]
		Microshoots	–	Quarter-strength MS basal	IBA (98.4 µM)	Rooting			
22.	*Clerodendrum serratum* (Lamiaceae)	Nodal	Laboline detergent 1% (10 min) 0.1% mercuric chloride (10 min)	MS	BAP (0.5 mg^{-1})	Best shoot bud induction and multiplication	Sterilized vermiculite	–	Upadhyay and Koche [59]
				MS	NAA (0.5 mg^{-1})	Rooting			
23.	*Couroupita guianensis* (Lecythidaceae)	Seeds	Bavistin (7–8 min) 0.1% mercuric chloride (5–6 min)	MS	IBA (2.0 mg^{-1})	Germination	–	86%	Shekhawat and Manokari [29]
		Nodal		MS	BAP (4.0 mg^{-1}) alone and combinations of BAP and KIN (1.0 mg^{-1}) + NAA (0.5 mg^{-1}) with additives	Multiple shoots			
		Microshoots	Distilled water	½ MS	IBA (2.5 mg^{-1})	Rooting			

24.	*Meizotropis pellita* (Fabaceae)	Leaf	Fungicide (Bavistin) 30 min 0.1% Tween-20 (5 min). 70% ethanol (1 min) and 0.1% $HgCl_2$ (4 min)	MS	2–4, D (9.06 µM) and 2–4, D (9.06 µM) + 2-iP (7.38 µM)	Callus induction and proliferation	Sand and farmyard manure (3:1:1 ratio)	55–65%	Singh et al. [30]
		Leaf calli		MS	BA (17.6 µM) + GA3 (1.0 µM)	Shoot initiation			
		Cotyledonary node		MS	KN+ GA3 (4.6 µM + 1.0 µM) or BA (13.2, 17.6 µM) + GA3 (1.0 µM)	Multiple shoots			
		Microshoots		MS	IBA (4.9 µM)	Rooting			
25.	*Commiphora wightii* (Burseraceae)	Node (with two nodes and two internodes)	–	MS	BAP (2 mg^{-1}) + GA3 (0.5 mg^{-1})	Shoot response	–	–	Tejovathi et al. [60]
26.	*Pterocarpus marsupium* (Fabaceae)	Nodal	10% sodium hypochlorite (10 min)	MS	Without hormones	Shoot induction	Sand soil and fame yard (1:1:1)	68%	Tiwari et al. [31]
				MS	IBA (0.2 mg^{-1})	Multiple shoots			
		Seeds	2% Teepol (10 min), 5% Tween-20 (4 min) and 0.1% mercuric chloride (6 min)	MS	BA (3.0 mg^{-1}) and IAA (0.5 mg^{-1})	Shoot multiplication	Sterilized soil and vermiculite (1:1)	74%	Tippani et al. [54]
		Microshoots	Distilled water	½ MS	IBA (3.0 mg^{-1})	Rooting			
27.	*Decalepis arayalpathra* (Apocynaceae)	Nodal	–		BA (2.0 mg^{-1})	Shoot proliferation	Not mentioned	85%	Seeni and Decruse [61]

(continued)

Table 1 (continued)

S. No	Name of the plant	Explant	Types of sterilant used	Medium	Conc. hormone	Response	Hardening (%)	Restoration	Reference
28.	*Calamus nagabettai* (Arecaceae)	Embryo	–	MS	TDZ (0.1 mg^{-1})	Shoot multiplication	–	90%	Seeni and Decruse [61]
29.	*Decalepis hamiltonii* (Apocynaceae)	Buds and leaves	0.1% $HgCl_2$ (5 min)	MS	BA (1.5 mg^{1}) + IAA (0.5 mg^{-1})	Shoot multiplication	Farmyard manure, red soil and sand (1:1:1)	–	Giridhar et al. [33]
					IBA (2.0 0.2 mg^{-1})	Rooting			
30.	*Citrus indica* (Rutaceae)	Seeds	5% Calcium hypochlorite	MS	TDZ (0.01 mg^{-1}) and NAA (90.1 mg^{-1})	Calli multiplication	Mixture of garden soil, leaf mould compost, and river sand (1:2:3)	–	Laskar et al. [32]
					BAP (0.5 mg^{-1}), TDZ (0.25 mg^{-1}) and NAA (0.25 mg^{-1})	Shoot multiplication			
					NAA (10 mg^{-1})	Rooting			
31.	*Vitex negundo* (Lamiaceae)	Leaf	5% Tween-20 (10 min), 70% ethanol (10–15 s and 0.1% mercuric chloride (2–3 min)	MS	IAA (0.3 mg^{-1}) and BAP (0.3 mg^{-1})	Shoot induction	Manure, soil, and sand (1:1:1)		Jawahar et al. [34]
					IBA (0.5 mg^{-1})	Rooting			
		Nodes	5% Laboline, 7% sodium hypochlorite (7–10 min), and 0.1% mercuric chloride (8 min)	MS	BA (2.0 mg^{-1}) + GA_3 (0.4 mg^{-1})	Shooting	Vermicompost	93%	Sahoo and Chand [38]
					IBA (1.0 mg^{-1})	Rooting			
32.	*Entada pursaetha* (Mimosae)	Seeds	Tween-20 (15 min), 70% ethanol and 0.1% $HgCl_2$ (10 min)	MS	5.0 mg^{-1}BAP	Shoot organogenesis	Garden soil	70%	Vidya et al. [35]
					5.0 mg^{-1} NAA	Rooting			

33.	*Cayratia pedata* (Vitaceae)	Leave and stem	Teepol (5–10 min) 10% Bavistin (15–20 min), 70% alcohol (30–60 s) 0.1% mercuric chloride solution (3–7 min)	MS	BAP (0.5 mg^{-1}) + NAA (0.2 mg^{-1})	Calli formation	–	–	Sharmila et al. [36]
34.	*Mahonia leschenaultii* (Berberidaceae)	Leave	1% Labolene detergent (10–20 min), and 0.1% $HgCl_2$ (5–10 min)	MS	BA (1.0 mg^{-1}) and IAA (0.02 mg^{-1})	Shoot formation	Top soil and river sand (1:1)	78.8 and 90.6	Radha et al. [37]
					IBA (1.0 mg^{-1})	Rooting			
35.	*Caesalpinia bonduc* (Ceasalpiniacae)	Seeds	Tween-20 (15 min), 70% ethanol (5 min), H_2SO_4 (8 min) and 0.1% $HgCl_2$	MS	BAP (17.57 µm) and IBA (2.85 µm)	Shooting	–	–	Kumar et al. [39]
					IBA (2.95 µm)	Rooting			
336.	*Pterocarpus santalinus* (Fabaceae)	Mesocotyl explant	50% HCL + 50 ethyl alcohol (3 h)	B5	BAP (3 mg^{-1}) + NAA (1 mg^{-1})	Shooting	–	–	Anuradha and Pullaiah [40]
					IAA (0.1 mg^{-1}), NAA and IBA	Rooting			
		Seeds	70% alcohol (1 min), 0.1% $HgCl_2$, and 0.1% sodium dodecyl sulphate (10 min)	MS	NAA + BAP + Kin (0.1 + 1.0+ 1.0 mg^{-1})	Shoot multiplication	Garden soil and sand (1:1)	90%	Arockiasamy et al. [55]
		Microshoots	Distilled water	¼ MS	IAA 1.0 mg^{-1}	Rooting			
		Nodal	2% Bavastin (15 min), 70% ethanol (2 min), and 0.1% mercuric chloride (15 min)	MS	TDZ (1.0, 2.0 and 3.0 mg^{-1})	Shoot multiplication	Soil rite	20%	Padmalatha and Prasad [51]
		Seed	2% Bavistin (30 min), 70% ethanol (2 min) and 0.1% mercuric chloride (12 min)	MS	KIN (2.0 mg^{-1}) and BA (1.0 mg^{-1})	Shoot multiplication			
		Microshoots	Distilled water	MS basal	–	Rooting			

(continued)

Table 1 (continued)

S. No	Name of the plant	Explant	Types of sterilant used	Medium	Conc. hormone	Response	Hardening (%)	Restoration	Reference
37.	*Celastrus paniculatus* (Celastraceae)	Internode	25% NaOCl (10 min), 0.1% mercuric chloride (10 min)	MS	BA (4.44 µM)	Shoot buds regeneration	Sand and farmyard manure (1:1)	80%	Rao and Purohit [52]
		Microshoots	Distilled water	¼ MS salts	IBA (2.45 mM)	Rooting			
		Nodal	0.1% mercuric chloride and Tween-20 (5 min)	MS	NAA (0.1 mg^{-1}) and BA (1.5 mg^{-1})	Shoot multiplication	–	–	Martin et al. [50]
		Nodal	0.1% mercuric chloride (3–5 min) and absolute alcohol	MS	BAP (1.0 mg^{-1})	Shoot multiplication	Soil and sand mixture (3:1)	70%	Lal and Singh [44]
		Microshoots	Distilled water	½ MS	NAA (0.5 mg^{-1})	Rooting			
38.	*Rauwolfia tetraphylla* (Apocynaceae)	Leaf	Tween-20 (1 min), 60% ethanol (2 min), 0.1% mercuric chloride (2 min)	MS	NAA (5.0 mg^{-1})	Calli induction	2:1 ratio of sterile garden soil and sand	86%	Rohela et al. [53]
		Stem and root	0.5% Bavistin (10 min), Tween-20 (2 min), 0.1% mercuric chloride (4 min)	MS	NAA (5.0 mg^{-1})	Calli induction			
		Leaf and stem calluses	Distilled water	MS	TDZ (0.25 mg^{-1}) and BAP (2 mg^{-1})	Shoot multiplication			
		Microshoots	Distilled water	MS	IAA (1.0 mg^{-1}) and IBA (1.0 mg^{-1})	Rooting			

39.	*Ilex khasiana* (Aquifoliaceae)	Seeds	5% Teepol (10 min), 70% ethanol (1 min), and 15% sodium hypochlorite (15 min)	MS	BA (8.88 μM) and Kn (4.64 μM)	Shoot response	Black soil: vermiculite: sand (2:1:1)	70%	Dang et al. [43]
		Cotyledonary nodal	Distilled water	MS					
		Leaf	Distilled water	MS	2,4-D (9.04 μM) and BA (2.32 μM)	Callus initiation			
		Leaf calli	Distilled water	MS	BA (6.63 μM)	Shoot bud proliferation			
		Microshoots	Distilled water	½ MS	IBA (9.84 μM)	Rooting			
40.	*Tylophora indica* (Asclepiadaceae)	Petiole	5% Teepol (5 min), 0.1% $HgCl_2$ (3 min)	MS	2,4-D (10 μM) and TDZ (2.5 μM)	Calli induction	Sterile vermiculite	100%	Faisal et al. [45]
		Friable callus	Distilled water	MS	TDZ (2.5 μM)	Shoot multiplication			
		Microshoots	Distilled water	½ MS	IBA (0.5 μM)	Rooting			
		Leaf	5% Teepol (5 min), 0.1% HgCl (3 min)	MS	2,4,5-T (10 μM)	Calli induction	Sterile vermiculite	–	Faisal and Anis [46]
		Leaf calli	Distilled water	MS	Kin (5 μM)	Adventitious shoots			
		Microshoots	Distilled water	½ MS	IBA (0.5 μM)	Rooting			
41.	*Gymnema sylvestre* R.Br (Apocynaceae)	Nodal	Tween-20 (3 min), 0.1% mercuric chloride (8 min)	MS	2,4-D (0.5 mg^{-1})	Calli	–	–	Gopi and Vatsala [47]
		In vitro axillary node explants	Distilled water	MS	BA, KIN and NAA (91.0, 0.5 and 0.1 mg^{-1})	Shoot multiplication	–	–	Komalavalli and Rao [48]
		Microshoots	Distilled water	½ MS	IBA (3 mg^{-1})	Rooting			

microshoots, which devoid of hormone [44, 48, 51]. Similarly, the petiole explant of *Tylophora indica* showed callus induction and shoot multiplication on MS medium [22]. The callogenesis of leaf explant of *Tylophora indica* has shown better response on MS medium with various PGRs [46]. The cell suspension culture of *Gymnema sylvestre* performed better response on MS medium [47]. Martin et al. [50] reported that nodal explant of *C. paniculatus* achieved bud breakage and elongation in MS medium without growth regulator.

Celastrus paniculatus internode explants were cultured on MS medium addition of PGRs to initiate shoot buds. Microshoots were inoculated on one-fourth MS medium supplemented with PGRs to induced roots [52]. The leaf, stem, and root of *Rauwolfia tetraphylla* were cultured on MS medium addition of PGRs to induced calli and in vitro-derived leaf and stem calluses were cultured on MS medium with PGRs to induce multiple shoots although microshoots were cultured on MS medium helped to develop roots [53]. The healthy seeds of *Pterocarpus marsupium* and *P. santalinus* were inoculated on MS medium to develop multiple shoots and microshoots on one-half and one-fourth MS strength medium to induced shoots and roots, respectively [54, 55].

4 Effect on Various Hormones or PGRs

Plant growth regulators (PGRs) are natural organic compounds that have stimulated or inhibited the specific enzymes or enzyme systems inside plant cells and facilitated to regulate plant metabolism. The nodal explants of *Syzygium densiflorum* were cultured on WPM supplemented with IBA (1.5 mg^{-1}) with BAP (1.5 mg^{-1}) and obtained 7.7 number of shoots per explants [9]. *Nothapodytes foetida* leaf and stem explants induced maximum culture response (90%) of somatic embryo germination and regeneration on MS medium addition of TDZ (0.5–3.0 mg^{-1}) along with coconut water (20%) [10]. In vitro regenerated leaves of *Zanthoxylum armatum* were soaked in distilled water (24 h) and cultured on WPM containing TDZ (15 μM) and NAA (0.5 μM) in which TDZ (6.0 μM) combination induced 90% of calli from explants [11]. The shoots tips and nodal explants of *Gaultheria fragrantissima* induced 35 shoots per explants from medium fortified with TDZ (0.22 mg^{-1}) [12]. Similarly, TDZ induced multiple shoots in *Elaeocarpus blascoi*, *Pterocarpus marsupium*, and *Sterculia urens* [13, 21, 56]. The leaf explants of *Leptadenia reticulata* induced calli on medium addition of 2,4-D (0.5 mg^{-1}) and combinations of NAA (1.0 mg^{-1}) + BAP (0.5 mg^{-1}) induced 48% more compact calli. The leaf-derived calli developed a greater number of shoots [30] per explants with 8.62 cm of average length from medium containing BAP (0.5 mg^{-1}) and NAA (0.1 mg^{-1}) [14]. *Leptadenia reticulata* nodal explants induced [3, 4] multiple shoots on medium containing IAA (0.6 μM) and BA (9 μM) [15]. Seeds of *Pterocarpus marsupium* were developed maximum (23.0) number of shoots with 5.14 cm shoot length on medium fortified with GA_3 (0.50 μM) and TDZ (0.50 μM) [16]. The in vitro raised cotyledonary nodal explants of *Pterocarpus marsupium* induced highest [15] number of

shoots per explants were obtained from medium containing TDZ (0.4 μM) [56]. In vitro raised *Rhododendron wattii* nodal explants were developed maximum (7.72) no. of shoots along with highest shoot length (2.30 cm) from WPM addition of 2iP (39.36 μM) [57]. *Atropa acuminata* shoot tips and nodal explants were induced shoot proliferation 80% with average shoot number (5.8) on medium with IBA (1 mg^{-1}) and BAP (1 mg^{-1}) [18]. The nodal explants of *Syzygium travancoricum* were cultured on both MS and WPM containing combinations of PGRs BA (17.7 μM) and NAA (1.3 μM) induced 25 shoots per explant [19]. The young leaf-derived callus of *Berberis aristata* induced multiple shoots (17.6–26.5) per callus on medium containing combinations of PGRs TDZ (0.5 μM) and NAA (2.68 μM) with BA (8.88 μM) [20]. *Tylophora indica* nodal explants showed a maximum (8.6) number of shoots with 5.2 cm of average shoot length on medium containing NAA (0.5 μM), BA (2.5 μM) [22]. The nodal explants of *Garcinia travancorica* were inoculated on MS medium fortified with BAP (4.0 mg^{-1}) have obtained 86% of shoot initiation and combinations of NAA (1.0 mg^{-1}) and BAP (4.0 mg^{-1}) induced 2.8 shoots per node with an almost equal number of shoots (1.6) and shoot elongation [23]. The half-strength WPM containing IBA induced root on *Syzygium densiflorum* and *Elaeocarpus blascoi* [9, 13] although WPM containing IAA and NAA induced root on *Rhododendron wattii* and *Rauvolfia serpentina* [17, 57]. Microshoots of *Pterocarpus marsupium* were induced root on full-strength MS medium with IBA [16]. *Ceropegia intermedia* axillary bud and nodal explants have showed maximum (5.5) shootlets on MS medium containing BA (6.66 μM) [24] although petiole and leaf explants of *Ceropegia intermedia* have induced calli on MS medium fortified with PGRs [62]. The cotyledonary node of *Terminalia bellirica* showed best shoot proliferation (80%) on MS medium containing BAP (2.0 mg^{-1}), and a higher number of shootlets [5] were obtained on MS medium containing combinations of BAP (3.5 mg^{-1}) and KN (0.5 mg^{-1}) [25]. *Leptadenia reticulata* leaf explants induced maximum calli on MS medium with BAP (1.0 mg^{-1}) and 2,4-D (0.5 mg^{-1}), respectively. The leaf-derived calli have induced the highest number (30 per explants) of shoots on BAP (0.5 mg^{-1}) and NAA (0.1 mg^{-1}) and microshoots cultured on one-fourth strength MS containing IBA (1.5 mg^{-1}) with ascorbic acid (100 mg^{-1}) exhibited (81%) rooting [14]. The cotyledonary nodes of *Sterculia urens* induced 4.3 shoots on MS medium containing BAP (2.0 mg^{-1}) [58]. *Nilgirianthus ciliata* nodal explants induced higher shoots [24] on MS medium containing BA (3 mg^{-1}) and IAA (0.1 mg^{-1}). The microshoots developed were exhibited 82% of rooting (14 number roots) on half-strength MS medium addition of IBA (1.0 mg^{-1}) [27]. The nodal explants of *Santalum album* showed maximum no. of shoots and shoot length on MS medium with NAA (0.53 μM) and BA (4.44 μM) responded for a maximum of 50% of roots from microshoots on quarter-strength MS with IBA (1230 μM) [28]. The nodal explants of *Clerodendrum serratum* have developed higher shoot induction and number of shoots with higher shoot length (5.2 cm) on MS medium containing BAP (0.5 mg^{-1}) and maximum [7] number of root were induced from the microshoot on MS medium with NAA (0.5 mg^{-1}) [59]. *Couroupita guianensis* seeds were germinated (100%) on a medium containing IBA (2.0 mg^{-1}) and nodal explants induced multiple shoots (4.1 per explants) on a medium containing BAP (4.0 mg^{-1}).

A maximum of 8.2 shoots were induced on combinations of BAP and KIN (1.0 mg^{-1}) + NAA (0.5 mg^{-1}) with additives. The microshoots were induced root (97%) on half-strength MS medium addition of IBA (2.5 mg^{-1}) [29]. The leaf explants of *Meizotropis pellita* induced calli on medium containing 2–4, D (9.06 μM) and 2–4, D (9.06 μM) + 2-iP (7.38 μM). The cotyledonary node of *Meizotropis pellita* induced multiple shoots (2.5 per explants) on medium containing KN+ GA3 (4.6 μM + 1.0 μM) or BA (13.2, 17.6 μM) + GA3 (1.0 μM) and microshoots induced the highest 8.2 number of roots on medium with IBA (4.9 μM) [30]. The nodal explants of *Commiphora wightii* exhibited best shootlet response (27%) on MS medium combined with BAP (2 mg^{-1}) + GA3 (0.5 mg^{-1}) [60]. The nodal explants of *Pterocarpus marsupium* induced 2.26 multiple shoots from MS medium with IBA (0.2 mg^{-1}) [31].

The nodal explant of *Decalepis arayalpathra* showed shoot proliferation on 2.0 BA mg^{-1} and produced mean no. of shootlets (1.0). The embryos of *Calamus nagabettai* were effectively grown on 0.1 mg^{-1} TDZ induced medium and formed 5 no. of shoots [61]. In *Decalepis hamiltonii*, shoot multiplication was observed on BA (1.5 mg^{-1}) + IAA (0.5 mg^{-1}) with 18 ± 1.2 shoots and 11.2 ± 0.4 cm shoot length. For rooting, the medium was supplemented with IBA (2.0 mg^{-1}) and observed profuse rooting [33]. Laskar et al. [32] reported that seeds of *Citrus indica* have induced calli formation on 0.01 mg^{-1} TDZ and 0.1 mg^{-1} NAA and the calli were treated for shoot multiplication in supplemented with 0.5 mg^{-1} BAP, 0.25 mg^{-1} TDZ, and 0.25 mg^{-1} NAA. For rooting, the microshoots were treated with 1.0 mg^{-1} NAA. Similarly, the seeds of *Entada pursaetha* showed 95% of culture response on 5.0 mg^{-1} BAP and the highest shooting frequency was observed on the proximal transverse division of cotyledon. The synergistic effect of BA (0.5 mg^{-1}) + NAA (0.5 mg^{-1}) promoted shootlets 9.8 ± 1.23 with shoot length 12.8 ± 0.78 [35]. The calli formation of *Cayratia pedata* was observed on BAP + NAA (0.2 mg^{-1}) [36].

The leaf explant of *Mahonia leschenaultii* showed shoot multiplication on 1.0 mg^{-1} BA and 0.02 mg^{-1} IAA containing medium [37]. In *Vitex negundo*, maximum no. of shoots (17.39 ± 0.71) was observed in 0.3 mg/l IAA + 0.3 mg/l BAP. Similarly, the highest no. of rooting frequency was observed on 0.5 mg/l IBA [34]. Similar studies have been done in *Vitex negundo* [38]. The nodal explants showed shoot multiplication on 2.0 mg^{-1} BA + 0.4 mg^{-1} GA3 and produced 2.0 + 0.4 mean no. of shoots. The optimal rooting was observed on 1.0 mg^{-1} IBA and about 94% of microshoots induced rooting. The maximum no. of shoots was observed on *Caesalpinia bonduc* at 17.57 μmol BAP and 2.85 μmol IBA and 2.95 μmol IBA has induced rooting calli [39].

The high frequency of shooting in (10–15 shoots) *P. santalinus* was observed on B5 medium supplemented with 3 mg^{-1} BAP+ 1 mg^{-1} NAA induced. For better shooting, the medium was fructified with 15% coconut milk exhibited 85% response. Half-strength MS medium was supplemented with 0.1 mg^{-1} IAA, NAA, and IBA in each concentration which has shown 50% rooting [40]. The in vitro-grown callus was induced shoots on (25 shoots) BA (2.0 mg^{-1}) + NAA (0.5 mg^{-1}) within the 2 weeks of observation [41]. The nodal segments of *I. khasiana* induced maximum shoots (10.2 ± 0.22) on 8.8 μm BA and 4.6 μm Kn. Similarly, the leaf disc initiate callus on

9.04 μm 2, 4-D, and 2.32 μm BA in combination. For rooting, the half-strength MS medium was supplemented with 9.84 μm IBA showed 93.3% of response with 5.56 ± 0.11 roots [43]. Lal and Singh [44] documented a maximum no of shoots (8.9 ± 0.5) and 100% bud breakage 1.0 mg^{-1} BAP. The half-strength MS medium induced 100% rooting on 0.5 mg^{-1} NAA. About 90% shoot multiplication of *Tylophora indica* was achieved on MS medium supplemented with 2.5 mM TDZ and 0.5 mM IBA [45]. Similar studies were carried out in *Tylophora indica*, in which the leaf callogenesis was observed on 5 μM Kinetin and at 0.5 μM IBA induced rooting on half-strength MS medium [46]. Gopi and Vatsala [47] recorded maximum callus formation on the MS medium supplemented with 0.5 mgl^{-1} 2, 4-D and followed by IAA, NAA, IBA at 2.5 mg^{-1}. Similarly, cytokinin such as 2.5 mg^{-1} BA and 5.0 mg^{-1} Kn have also shown better callusing. The multiple shoot induction of *Gymnema sylvestre* was noted on the combination of 1.0 mg^{-1} BA and 0.1 mg^{-1} Kn with average shoot no. 6.2 ± 0.07 and shooting frequency 76 ± 2.00. The medium was supplemented with different extracts, among these malt extract influences the shoot multiplication and prevents yellowing of leaves and also effectively prevents the callus formation at the cut end of the explant. For rooting the shootlets formation, the half-strength MS medium was supplemented with various auxins among which 3 mg^{-1} IBA showed a high frequency of rooting [48]. On single cytokinin treatment, 2–3 multiple shoots were induced in *P. santalinus* to increase the shooting frequency with the combination of Kn and BA. The combination of 1 mg^{-1} Kn and BA produced 8 shoots within 4–6 weeks and at 5 mg^{-1} IBA showed optimum rooting [49]. For the shoot elongation and multiplication, MS medium was supplemented with various hormones either alone or combination established with 1.5 mg l^{-1} BA and 0.1 mg l^{-1} NAA with mean no. of shoots 5.0 ± 1.2. At the same time, ex vitro rooting with hardening was performed [50]. The combination of BA and Kn (2.0 mg^{-1} + 1.0 mg^{-1}) have induced 19–20 shoots and rooting in *P. santalinus* [51].

Celastrus paniculatus internode explants showed a maximum number of (3.89) shoot bud regeneration on medium supplemented with BA (4.44 μM) and one-fourth MS medium addition of IBA (2.45 mM) induced maximum (80%) rooting [52]. The stem explants of *Rauwolfia tetraphylla* obtained a maximum of 412.8 mg of fresh calli on MS medium supplemented with NAA (5.0 mg^{-1}). In vitro-derived stem calli has showed maximum of 20 shoot buds on MS medium addition of TDZ (0.25 mg^{-1}) and BAP (2 mg^{-1}) and microshoots were cultured on MS medium with IAA (1.0 mg^{-1}) and IBA (1.0 mg^{-1}) to induce rooting [53]. The seeds of *Pterocarpus marsupium* were cultured on MS medium supplemented with BA (3.0 mg^{-1}) and IAA (0.5 mg^{-1}) inducing a maximum of 17.3 [54]. The seeds of *Pterocarpus santalinus* showed the highest number of in vitro shoots (10.4) on MS medium supplemented with NAA + BAP + KIN (0.1 + 1.0 + 1.0 mg^{-1}) and microshoots induced a maximum of 76% of rooting on one-fourth MS medium with IAA 1.0 mg^{-1} [55].

5 Hardening

Hardening is one of the most important steps in plant tissue culture in which the micropropagated plants were allowed to grow under controlled environmental conditions. If the plants were immediately transferred to field conditions, they will get shock from the outside environment. Due to the inconsistency of weather and humidity, in vitro plantlets get so much stress from the outside environment. There are so many factors that make it difficult for plants to acclimatize outside environment.

Seeni and Decruse [61] have standardized the micropropagation protocol of some of the rare, endangered, and threatened plants (*Decalepis arayalpathra* and *Calamus nagabettai*). After rooting, *invitro* propagated plantlets were transferred to pots containing vermiculite and kept in the greenhouse. About 73% of the rooted plants were reported as survived and transferred to vermiculite-containing planting medium [59]. The agar adhered from the root of *Decalepis hamiltonii* were removed and planted into the soil of the pots, and the humidity was maintained at 60–70% and allowed to grow for 2 months. The hardening mixture contained 1:1:1 ratio of farmyard manure, red soil, and sand [33]. The in vitro-developed plantlets of *Citrus indica* were hardened with the mixture of garden soil, leaf mould compost and river sand (1:2:3) [32]. In vitro the rooted *Rauvolfia serpentina* plantlets were introduced into different planting substratum with garden soil, soil rite, and vermiculite. After the 4 weeks of observation, 30–54 plants were reported to survive in soil rite whereas in vermiculite 51 plants and in garden soil 30 plants were recorded as survived [17].

The in vitro-developed shootlets of *Terminalia bellirica* were transferred to the vermiculite-containing polybags [25]. The leaf of in vitro-grown plantlets was expanded after 3 weeks of observation and transferred into the mist chamber. Patiel et al. [63] recorded that at 15 °C temperature, the plantlets of *Picrorhiza kurroa* were shown 100 ± 0.00^{a} % survival rate on the sand. Where sprayed with Hoagland solution in a regular 12-day interval, it exhibited better growth. The rooted plantlets of *Commiphora wightii* were planted in vermiculite medium wetted with Hoagland's solution. On primary hardening, the rooted microshoots were planted into vermiculite and maintained humidity [64].

Before the introduction of in vitro-grown *P. marsupium* to the soil, it was transferred to the culture tubes without sucrose. After that, plants were transferred to clay pots and 70% of the plants were survived with a high degree of uniformity [56]. Similar studies have been done by Ahmad et al. [16] in *P. marsupium*, in which the rooted microshoots were transferred to a potted medium containing soil rite. To maintain the humidity, the microshoots were covered with transparent polybags and supplemented 16/8 h of photoperiodism. The half-strength MS liquid was watered for 20 days followed by tape water for alternative days. The acclimatized plants were slowly transferred to a mixture of soil rite and garden soil containing medium. The study conducted on the micropropagation of *Oroxylum indicum* using different additives was exhibited better shootlets. The rooted plantlets were immersed in distilled water for 4 days and transplanted to the sand and soil mixtures [65].

Bantawa et al. [12] have treated the microshoots of *Gaultheria fragrantissima* with 500 mg^{-1} IBA for 30 min before transplanting. The potting mixture containing 1:1:9 farmyard, sand, and virgin soil and supplemented with one-quarter strength of WPM liquid medium for 7 days of interval. Different potting mixtures were tried for the hardening procedure of shootlets hardened which vermiculite, garden soil and cocopeat (1:2:3). After biotization with endophytes, plantlets showed better growth response [21]. Similarly, this potting mixture was effectively used for the growth of *Garcinia xanthochymus* [66].

The in vitro-grown plantlets of *Decalepis arayalpathra* were reintroduced to the natural habit after rooting and observed the survival rate [67]. Husain et al. [56] reported that prior to the introduction of in vitro-developed *P. marsupium* into the soil, the shoots were allowed to culture without sucrose and transferred to clay pots and 70% of the plants were survived. On in vitro and ex vitro rooting methods on *Leptadenia reticulata*, it has shown that 95% of survival rates were observed under greenhouse conditions. The ex vitro rooted plantlets were slowly adapted to low humidity (50–55%) and high temperature (34–36 °C), high humidity (80–85%), and low temperature (26–28 °C) [14].

The root of *Zanthoxylum armatum* was washed with Bavistin to eradicate fungal contamination. The washed plantlets were introduced into the farmyard manure and soil (3:1) and covered with polythene bags. After 1 week of observation, the plantlets were transferred to the garden soil and kept in the mist chamber [11]. The similar procedure has been done for the development of in vitro-developed *Rhododendron wattii* with different potting mixture [57]. Purohit and Dave [58] have reported that to avoid desiccation, it has directly transferred to the rooting medium. The plantlets were easily adapted to the *invitro* hardening condition, mixture of sand and soil rite (1:1).

Previous studies documented that ex vitro rooted plants were easily adapted to external environments compared to micropropagated plants. In ex vitro conditions low humidity high temperature and vice versa were maintained [29, 68]. Some of the researchers have documented that vermicompost and soil rite as an effective substratum to harden in vitro-grown plantlets [38, 69] whereas soil rite alone showed a good growth response on the plantlets [70]. Similarly, in garden soil, a significant plant growth response was observed [22, 35, 39]. The microshoots were hardened and 80–82% of the plantlets were successfully hardened and placed under the mist chamber [37]. Farmyard manure, sand, and soil (1:1:3) were also used for hardening with humidity by 60% [30]. Siva et al. [13] studied the micropropagation protocol for *E. blascoi,* after the completion of the rooting procedure, the plantlets were slowly transferred to garden soil, farmyard, and river sand mixture (1:2:1). The hardening medium of *P. marsupium* has reported to filled with farmyard manure, sand, and soil in the ratio of 1:1:1. The plantlets were maintained in the greenhouse with relative humidity 60 ± 5% and 30 ± 2 °C temperature [31]. The rooted explants were planted in the mixture of sand: soil: peat moss and irrigated with one-fourth MS salt solution [25]. The garden soil, sand, and vermiculite mixture has induced the growth of *Celastrus paniculatus* during hardening [71].

The in vitro-grown plantlets were directly transferred to greenhouse conditions and irrigated quarter-strength MS medium for 2 weeks. Then, plantlets were uncovered and exposed to natural light and transferred to earthen pots containing garden soil. About 70% of the plantlets were shown rooting on sand, black soil, and vermiculite mixture [43]. The rooted plantlets were maintained in high humidity conditions and planted in soil and sand mixture for 15 days [40]. The regenerated plantlets of *C. paniculatus* were successfully transferred to autoclaved sand and soil mixture (3:1) and sprayed with MS medium [44]. The rooted plantlets showed 100% survival rate on garden soil [44]. In the first step, the regenerated plantlets were successfully transferred to vermiculite-containing pots and maintained the humidity of potted plantlets with polythene bags covered. The transferred plantlets were irrigated at every 3 days with half-strength MS medium without sucrose. After 2 weeks of observation, plantlets were shifted to garden soil containing pots [46]. About 100 rooted plantlets with 6–5 fully expanded leaves and well-rooted plantlets transferred to soil were observed with normal growth [48]. The hardened plantlets of *P. santalinus* were successfully transferred to a combination of soil and sand (1:1) and about 50% of the plants were survived. The rooted plantlets were transferred to test tubes containing water and covered with parafilm. Similarly, the plantlets transferred to the pots were covered with plastic covers to maintain the high humidity, and 60% of the plants were survived [49]. Martin et al. [50] reported that the simultaneous ex vitro rooting and hardening steps were done for the development of *C. paniculatus*. Among the treatments at 100 mg l^{-1}, each of IBA and NOA and 10 mg l^{-1} chlorogenic acid-treated microshoots was planted into the sand- soil containing pots. After 5 weeks, root establishment was observed and the plantlets were transferred to a coir-containing liquid medium. The plantlets were kept in a greenhouse and 98–99% rooting was observed. Ramasubbu and Divya [9] reported that plantlets hardened in sand soil (1:1) were sprayed with distilled water showed a better response. Rao and Purohit [52] reported the in vitro regeneration of shootlets of *Celastrus paniculatus* was transferred to polybags with sand and farmyard manure (1:1) and kept nursery shade conditions to grow. The in vitro-developed plantlets of *Rauwolfia tetraphylla* were transplanted into a plastic pot containing a 2:1 ratio of sterile garden soil and sand [53]. In vitro rooted plantlets of *Pterocarpus marsupium* were transferred into polybag containing a mixture of sterilized soil and vermiculite (1:1) [55].

6 Restoration to the Field

To enhance the plant growth and establishment, a successful acclimatization process has been undertaken which decreases the percentage of dead and damage of the plant [72]. According to Ahmed et al. [16] plantlets, that complete their acclimatization process, showed an 86.7% survival rate without morphological changes. After 3 months of hardening, the plantlets of C. *paniculatus* were successfully introduced into the field conditions and obtained 70% of survival rate [71]. Karuppusamy et al. [24] described that the gradual exposure of plantlets to the field slowly balances the

humidity. Seventy-four healthy plantlets were planted into the field and regenerated plantlets have not shown any morphological variance. The micropropagated plantlets of *Nilgirianthus ciliates* were planted into the campus of Alagappa University and 100% of the survival rate was obtained [27]. Mao et al. [57] reported successfully acclimatized plantlets were reintroduced into the natural habitats and observed growth of the plant. Sahoo and Chand [38] have also observed after 6 weeks of observation of plantlets produces flowers and from this experiment reported that, all the in vitro propagated plantlets of *Santalum album* showed 100% of survival rate during field transfer [28]. The nursery-grown plantlets of *Mahonia leschenaultia* were planted into Vattakanal shola forests at Palani hills showed 90.6% of survival rate [37]. Some of the endogenous fungi treated with the root of *Sterculia urens* showed high potency of survival rate in field conditions [21].

The fully developed plantlets of *Celastrus paniculatus* were successfully transferred to field condition and about 77% of the plantlets were survived [44]. Komalavalli and Rao [48] reported that about 80–85% of the hardened plantlets of *G. sylvestre* were survived in field. The ex vitro rooted plantlets were directly introduced in the field condition and showed normal growth [50]. The successfully hardened plantlets of *S. densiflorum* were planted in the natural habitat of Megamalai Wildlife Sanctuary and Kodaikanal Wildlife Sanctuary for the better survival rate. After 2 months of observation, leaf senescence and slow vegetative growth were observed [9]. At monsoon season, about 100 plantlets of *Decalepis arayalpathra* were planted in Kallar and Aryankavu and about 280 plantlets of *Calamus nagabettai* were reintroduced [61]. *Celastrus paniculatus* were successfully planted in field conditions and obtained 80% of survival rate [52]. The in vitro-developed plantlets of *Rauwolfia tetraphylla* were transplanted on the field and obtained 86% of survival rate [53]. The in vitro plantlets of *Pterocarpus marsupium* were successfully transferred to the field with 74% survival rate [54]. The in vitro rooted plantlets were successfully transformed on the field and obtained 90% of survival rate [55].

7 Conclusion

The present review investigates the importance of woody medicinal plants and their mass propagation. Medicinal plants are prospective sources of therapeutic medicines and have a considerable role in health systems for humans and animals and maintaining proper health. They are disappearing under exploitation for their medicinal, ornamental, perfumery uses. However, woody trees timbers are overexploited for construction of the building, agricultural implements, boat and ship building, musical instruments, and railway sleepers. Therefore, the rare and endangered woody medicinal plants urgent need their conservation. In recent years, in vitro culture techniques have been envisaged for germplasm conservation to ensure the survival of endangered plant species and mass multiplication for commercial and conservation purposes.

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