forests

Review

Somatic Embryogenesis of Representative Medicinal Trees in South America—Current Status

Raluca A. Mihai [1,†], Erly J. Melo Heras [1], Eliza A. Pinto Valdiviezo [1], Iván A. Espinoza Caiza [1], Nelson S. Cubi Insuaste [1], Jean Pierre Mejía [1], Rodica D. Catana [2,*,†], Mirela M. Moldoveanu [2,†] and Larisa I. Florescu [2,†]

[1] CICTE, Department of Life Science and Agriculture, Universidad de Las Fuerzas Armadas—ESPE, Av. General Rumiñahui s/n y, Sangolqui 171103, Ecuador; rmihai@espe.edu.ec (R.A.M.); ejmelo@espe.edu.ec (E.J.M.H.); eapinto@espe.edu.ec (E.A.P.V.); iaespinoza@espe.edu.ec (I.A.E.C.); nscubi@espe.edu.ec (N.S.C.I.); jpmejag@espe.edu.ec (J.P.M.)

[2] Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, Romania; mirela.moldoveanu@ibiol.ro (M.M.M.); larisa.florescu@ibiol.ro (L.I.F.)

* Correspondence: rodica.catana@ibiol.ro

† These authors contributed equally to this work.

Abstract: Human society is currently facing a growing demand for forest resources, which causes overexploitation and endangers biodiversity. In this regard, it is important to be aware that 10% of trees across the world are used in traditional and modern medicine. South America has the greatest diversity, with 40% of tree species in the world. The aim of our review consists of the assessment of the state of the art of micropropagation through somatic embryogenesis of representative medicinal trees in South America and of targeting the tree species that should be prioritized for conservation plans. From a total of 23,631 tree species from Central and South America, 31 are extinct, 7047 are threatened, and 1434 are possibly threatened. In this sense, in order to conserve them, various strategies are applied both in situ and ex situ. The application of in vitro multiplication protocols represents effective ways both in conservation and in the sustainable use of resources in order to obtain secondary metabolites of interest. Somatic embryogenesis is a well-known method in woody multiplication. According to the VOSviewer analyses, very few studies were available concerning aspects of somatic embryogenesis in medicinal trees. From the 10 representative species selected in our study, somatic embryogenesis protocols were established for 3 species, only for conservation purpose, not for secondary metabolites production. The development of protocols focused on obtaining secondary metabolites of medicinal trees will allow for the obtainment of valuable plant material as a non-invasive alternative.

Keywords: conservation; endangered; forest; *Myrcianthes pungens*; propagation; regeneration; secondary metabolites

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1. Introduction

Forests are among the most complex ecosystems, characterized by a high biodiversity of trees and also habitat for other species. Trees, as an integral part of forest ecosystems, have important functions and offer multiple ecological services to human society. They are important factors in preventing global warming but also provide us food, medicinal products, wood, fuel, timber, and fibers, as well as aesthetic and cultural values. Medicinal trees are considered the living pharmacy due to the sources (wood, bark, roots, leaves, flowers, fruits, seeds) with active principles that are fundamental to the well-being of millions of people. In 2021, 30% of tree species were threatened with extinction, while ~142 tree species were considered extinct. At the global level, almost 10% of trees with medicinal or aromatic properties are used in traditional and modern medicine [1]. The loss of the tree biodiversity leads to a decrease in the opportunities for the discovery of new potential medicines and biotechnologies, but also to the limitation of raw materials for the production of widely used ones [2].

For this reason, the sustainable exploitation of resources, as well as the conservation of trees, are important steps to support their environmental and economic values. At this time, all over the world, tree conservation measures are represented by in situ (protected areas ~64% of all tree species are conserved) and ex situ (botanic gardens and seed banks, ~30% of tree species) measures [1]. In vitro multiplication represents an important part of ex situ conservation; somatic embryogenesis plays an important role in the vegetative propagation of trees [3].

Somatic embryogenesis (SE) is a complex process of clonal propagation, which involves the ability of plant cells to differentiate and dedifferentiate into new cellular types [4]. In this process, somatic cells are transformed into embryos through numerous changes (morphological and biochemical), similar to zygotic embryos without the fusion of gametes [5]. The SE process's main goal is to produce synchronous populations of embryogenic cells or differentiating embryos. Just as with any reproduction method, the induction of SE has advantages (high multiplication, faster rate, haploid production, somatic hybridization, artificial seeds) [6] and disadvantages (lack of apical and radical meristems, loss of bipolarity, fusion of embryos, translucent embryos, multiple cotyledons) [7]. Finding a middle way is recommended, depending on the desired goal.

South America is one of the continents with major biodiversity (40% of tree species, 37% of rare species) in the world, being dominated mainly by tropical and subtropical humid lands [8]. The Living Planet Report 2014, published by WWF, mentions that biodiversity is declining in temperate and tropical regions (to a greater extent), with Latin America having the most dramatic decline (83%). Habitat loss, degradation, exploitation, and climate change are some of the major common threats and are likely to exert greater pressure on populations in the future [9]. The multiple pressures on the forests, through unsustainable exploitation, climate change, and pollution with effects including the galloping loss of flora species, led us to establish the objective of raising awareness of the importance of biodiversity conservation. Research concerning the micropropagation of native forests is scarce in this region because the research focuses on species of horticultural importance. The interest in the multiplication of threatened species has been growing lately.

The aim of the study was to assess the state of the art of micropropagation through somatic embryogenesis of representative medicinal trees in South America and to target the tree species that should be prioritized for conservation plans. The objectives of our study were (1) to evaluate the representative medicinal tree species of South America and their conservation status; (2) to identify the stage of the studies regarding SE of endangered medicinal trees. Since the regeneration of resources of this type requires a longer period, vegetative multiplication measures represent a suitable solution for the conservation of the representative species of medicinal trees in South America; (3) to identify if SE is a viable method to potentiate the synthesis of secondary metabolites, for their valorization in in vitro culture, as a non-invasive alternative to sustainable use of natural resources; and (4) to select suitable protocols for the propagation of medicinal trees of interest.

2. The Biodiversity of South America Trees and Conservation Status

A total of 60% of the planet's terrestrial life is found in the Latin American and Caribbean region [10], with forests having a great diversity of mostly endemic native flora [11]. Along the Pacific coast of South America, the orographic effect is exhibited in a marked biogeographic boundary around 30° S, with subtropical and temperate rainforests, which extend to the extreme south of the Chilean territory, and semi-deserts and hyper-arid deserts, which are distributed northward, near the Equator (Figure 1) [12].

Species diversity has been a consequence of the accumulation of a series of ecological, biogeographical, and evolutionary processes throughout history. Thanks to this, the continents have been able to be characterized by their own plant identity and distribution [13]. South America harbors a total of 73,300 tree species; these endemic species mainly inhabit the tropical and subtropical forests of Colombia, Peru, Ecuador, Bolivia, Venezuela, and Brazil [8]. The richness of species in this region is mainly associated with changes in the terrain, and drastic changes in climate, from humid tropical to dry and cold, which causes irregular rainfall [14]. Its tree biodiversity, mainly concentrated in its tropical humid zones, is subject to its water availability and reduced climatic seasonality. Rapid flooding during rainy seasons provides nutrients that are transported by water, which benefits the emergence of new plant specimens and the development of communities, unlike temperate and cold environments [15].

Figure 1. The State of Biodiversity in Latin America and the Caribbean: A mid-term review of progress towards the Aichi Biodiversity Targets. UNEP-WCMC, Cambridge, UK. Adapted from: UNEP-WCMC (2016).

The South American continent has some of the most important ecosystems such as the Cerrado, Chiquitania, and Amazonian forests [16]. Throughout history, human activities have modified the spatial structure of plant communities [17]. Industry, intensive agriculture, forest fires, and the domestication of different species are the causes that affect the organization, abundance, and richness of tree communities [8].

Currently, the world population is about 7,8 billion people, and their consumption of natural productivity reaches 175% of available resources [18]. The growth of the world population and the increase in levels of development have resulted in a greater demand for goods such as food, wood, minerals, and fuels, which require the transformation and appropriation of natural ecosystems for their extraction [16]. Human health has been linked to the use of medicinal plants for millennia, and the increased demand in the markets has led to the overexploitation of specimens and the advance of their extinction [16].

From a total of 23,631 tree species from Central and South America, 31 are extinct, 7047 are threatened, 1434 are possibly threatened, 11,002 are not threatened, 2451 are data deficient, and 1666 are not evaluated [1]. The forests of Latin America and the Caribbean (LAC) are being devastated by increasingly frequent and intense extreme events caused by a variety of factors such as climate change, forest fires, invasive species (insects, pathogens, weeds, and mammals), and outbreaks of native insects that threaten ecosystems, as well as more frequent and intense hurricanes and floods caused by the El Niño phenomenon [19].

According to ECLAC Statistical Briefings 2021 [20], deforestation between 1990 to 2020 led to a 7% loss of forests, the Amazon region being one of the most affected regions. The forested areas in South America are native forests (97.7%) and forest plantations (2.3%). In 1998, Oldfield et al. [21] showed that all over the world, 10% of all trees (>8000 species) were threatened with extinction. Today, according to WWF, only around 13% of the world's forests are protected but still threatened by encroachment and degradation [22].

The development of new propagation technologies has improved tree production and they have become an alternative to solve this problem. These have been aimed at using biotechnology methodologies such as in vitro propagation, genetic transformation, and assisted improvement [23]. Somatic embryogenesis is a well-known method to obtain in vitro woody and some conifer species. This technique is highlighted because it focuses on the mass propagation of clones and genetic improvement, especially in woody species, which present a long life cycle. Moreover, it becomes a tool for the cryogenic storage of useful germplasms.

3. Overview of Somatic Embryogenesis in Trees

3.1. VOS Viewer for Screening and Bibliometric Analysis

To perform the VOS viewer concerning our subject, we used elementary methods of bibliometrics and closely related informetrics or scientometrics, which are based on quantitative analysis and mapping of research in the scientific literature. For data collection and analysis, the study surveyed papers in Science Direct and WoS Core Collection (retrieval data last updated: June 2023). The keywords used were represented by somatic embryogenesis, forest, and somatic embryogenesis, medicinal trees. The titles and abstracts of publications were used in the bibliometric analysis. Full record and cited references of the included papers were extracted from other reference software file formats and imported into VOSviewer (version 1.6.19.0) Leiden University, Leiden, The Netherlands) for further citation analysis. The VOSviewer program was conducted on different literature databases, to determine co-occurrence and clusters of connected publications, as well as clusters of interrelated research topics (text data). VOSviewer was used to indicate the research trends through all keywords [24].

For somatic embryogenesis and forest keywords, in ScienceDirect, VOSviewer results highlighted 16,108 terms, from which 459 meet the threshold, at 10 minimum number of occurrences of a term. For each of the 459 terms, a default 60% relevance score was established, and based on this, 275 of the most relevant terms were selected in the analysis. The results were organized in 7 clusters, concerning subjects such as culture, medium, somatic embryo, mechanism, and protein, as follows: cluster 1—92 items, in red; cluster 2—76 items, in green; cluster 3—67 items, in blue; cluster 4—23 items, in light green; cluster 5—13 items, in purple; cluster 6—2 items in turquoise and cluster 7 in orange (Figure 2).

VOSviewer shows that most subjects concerning the Somatic embryogenesis and medicinal trees keywords are concentrated in the conservation aspect (Figure 3). In the results of these searches, a higher number of terms (6249) were found, from which only 153 meet the threshold, at 10 minimum number of occurrences of a term. For each of the 153 terms, at the default, 60% relevance score, 92 of the most relevant terms were selected. The results were organized into 5 clusters: cluster 1—49 items, in red; cluster 2—28 items, in green; cluster 3—10 items, in blue; cluster 4—3 items, in light green; and cluster 5—2 items, in purple (Figure 3).

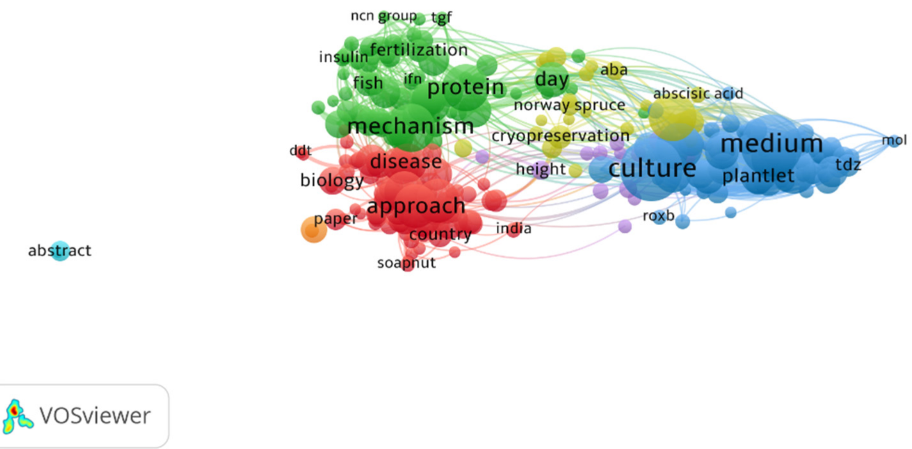

Figure 2. VOSviewer co-occurrence network visualization mapping of resulting terms of somatic embryogenesis and forest keywords in Science Direct databases.

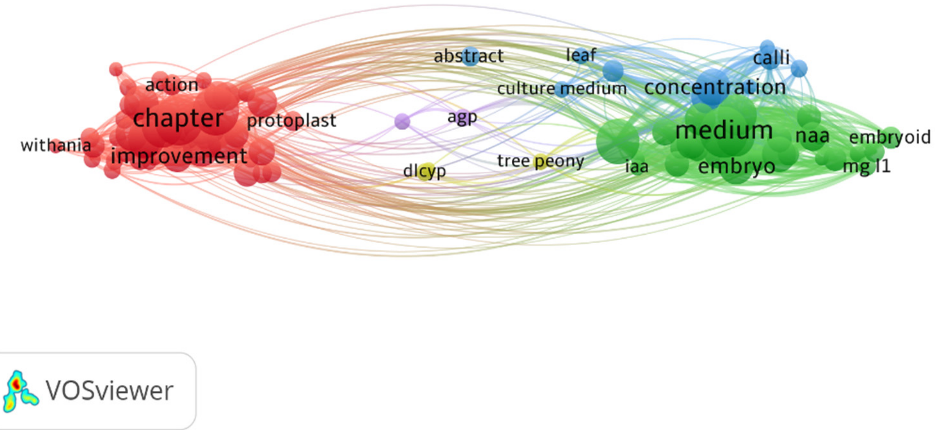

Figure 3. VOSviewer co-occurrence network visualization mapping of keywords such as somatic embryogenesis, and medicinal trees in Science Direct databases.

From the Web of Science database, most papers related to keywords such as somatic embryogenesis and forests were about genomic selection (3.38), epigenetic memory (2.81), somatic plant (2.49), and genetic gain (2.35) (Figure 4). From WOS, of the resulting 9474 terms in the literature, 331 met the threshold, from which 199 terms were the most relevant terms, grouped in 4 clusters.

Concerning the somatic embryogenesis and medicinal trees keywords, the relevance scores were shown on the subjects such as dry weight (3.65) and embryonic callus (3.58; highest significant value (3.35) (Figure 5). A total of 3062 terms were discovered, from which 62 met the threshold, from which only 37 terms were selected for generating 4 clusters.

Items are represented by a label and a circle. The size of the circles reflects the weight of an item. Some items are not displayed to avoid overlap. The colors in network visualization (text maps) represent clusters of similar items as calculated by the program. The strength of the relationships between the elements is indicated by the distance.

In woody plants, the first report in SE was on palm oil in 1970 [25]. After that, pioneering research on somatic embryogenesis was only observed to form embryo-like structures in sandalwood and several conifers, however, these embryo-like tissues did not develop into full plants. The first reports of regeneration through somatic embryogenesis in sandalwood were achieved using hypocotyl and nodal segments and in conifers was first reported in Norway spruce (*Picea abies*), for which immature and mature zygotic embryos were used as explants to establish a culture system [23].

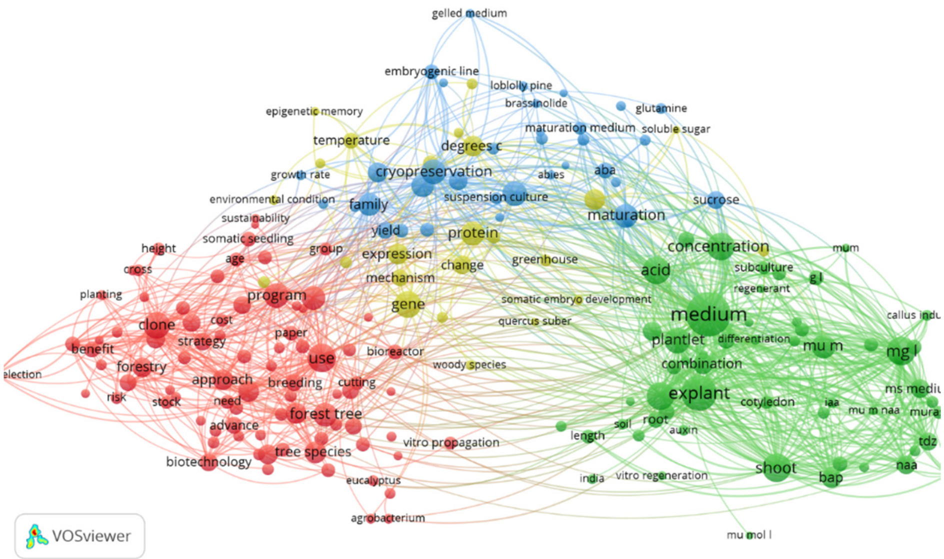

Figure 4. VOSviewer co-occurrence network visualization mapping of keywords such as somatic embryogenesis and forests in Web of Science databases.

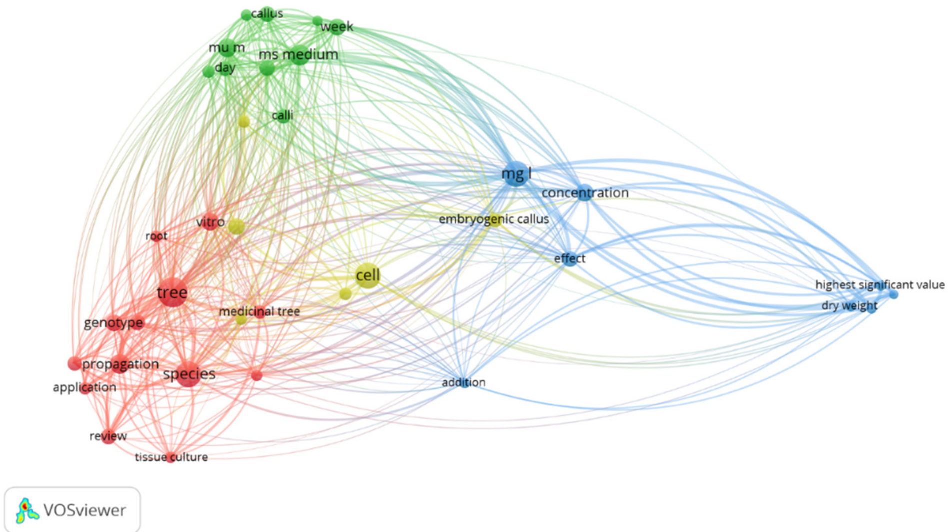

Figure 5. VOSviewer co-occurrence network visualization mapping of keywords such as somatic embryogenesis and medicinal trees in Web of Science databases.

The pattern of somatic embryo development is very similar among most of the woody species, starting from immature or mature embryos grown on a nutrient medium containing a high concentration of plant growth regulators (2,4-D, 6-BA, and picloram). The induction of somatic embryos using mature tree leaves as explants has already been achieved in a small number of species, but this difficulty remains unresolved in the initiation of embryogenic cultures from adult woody plants [23].

The somatic embryogenesis of some medicinal tree species from South America generally starts from explants such as nodal segments (*Ilex paraguariensis* [26]; *Ilex guayusa* [27]), semi-woody cutting, seeds (*Annona muricata* [28]), caulinar apices from in vitro germinated seedlings (*Cinchona pubescens/officinalis* [29]), flower buds (*Theobroma cacao* [30]), embryos extracted from mature and immature seeds (*Juglans neotropica* [31,32]), or cuttings (*Bixa orellana* [33]). Media variants such as MS [34], woody plant medium (WPM), and DKW [35] are the most common media variants used for somatic embryogenesis induction added with different plant growth factors.

3.2. Somatic Embryogenesis and Secondary Metabolites

In vitro plant culture has been a preferred method to obtain secondary metabolites, due to numerous drawbacks such as overexploitation, low productivity, phytogeographical, seasonal, and tissue/organ-specific variation, and economic cost. In vitro plants have been criticized because there is information that says that this technique cannot obtain a good concentration of secondary metabolites [36]. It could be due to plants naturally being espoused to different ecological factors that avoid their growth and development, and at the same time, stimulate the production of these natural products. Environmental stress can change the physiological and biochemical responses in medicinal species, and the most common factors that have influenced the production of secondary metabolites are temperature, carbon dioxide and ozone concentration, light, water availability, salinity, and soil fertility [37].

Somatic embryogenesis in woody plants has some advantages such as maintaining embryogenic competence for many years [23]. The development of this process in woody plants is similar in most of the species tested to date. The explants used could be nodal segments, leaves, ovules, anthers, cotyledons, petals, immature seeds, and roots from juvenile plants, but the most usual are immature or mature embryos, cultured in medium with regulators such as 2,4-D, 6-BA, and picloram to induce a callus [23,38].

In order to improve secondary metabolite production, elicitors (agents used to stimulate the defense responses of plants) have been applied as an alternative to stimulate the accumulation of these metabolites and avoid the variation in their quantity and quality in in vitro production. The elicitors with different origins biotic (fungus, bacteria, yeast, polysaccharides from plant membrane cells) and abiotic (hormones, physical, and chemicals) may be used to stimulate the secondary metabolites production. Methyl jasmonic (MJ) and salicylic acid (SA) are abiotic elicitors that are more advantageous for adventitious root growth in major plants. Both increase the alkaloids, flavonoids, terpenes, and polyphenol synthesis through signal transduction, which accelerates the enzymatic catalysis and the accumulation in biosynthetic [39]. Heavy metals such as $CuSO_4$ and $AgNO_4$ also affect the metabolism in plants, acting as abiotic inductors. For the biotic elicitors, yeast extract induces the defense response, increasing the natural secondary metabolites production. However, the biosynthetic capacity of vegetal cells in some medicinal species frequently results in the loss of or reduction in undifferentiated culture with specialization and accumulation of metabolites absent [40].

All types of elicitors act as a signal, which is perceived by an elicitor-specific receptor from the cell membrane. After perception is initiated, a transduction cascade occurs, which conducts expression level change of various regulatory transcription factors/genes, resulting in increased synthesis and accumulation of secondary metabolites [41,42]. Studies concerning the comparisons of metabolite compositions of both embryo types are scarce. Different studies concerning the comparison between metabolite profiles of somatic and zygotic embryos show that there are no rules.

However, some studies report the production of a high number of embryos depending on the age of the mother plant too; thus, when the mother plant or the tissue is younger, a better number of embryos has been reported [38] and this in combination with an appropriate concentration of growth regulators has shown good secondary metabolite production in a big quantity and the same secondary metabolites produced in wild leaves [23].

4. Tree Species with Medicinal Properties in South America

Traditionally in South America, many indigenous communities have used medicinal plants as palliative treatment for certain illnesses and all this knowledge has been passed through generations (Table 1) [42]. According to IUCN, after a screening of the literature concerning endemic medicinal trees, 3 of them are endangered, 8 are vulnerable, 10 are not threatened, and 27 are least concerned.

Table 1. A summary of the representative medicinal tree species with medicinal properties.

Species/Common Name	Origin	Medicinal Properties	Secondary Metabolites
		Endangered Species	
Myrcianthes pungens (O. Berg) D. Legrand) synonym: Eugenia punges/ Guaviyú, Guabijú, Yguaviyú, Ibabiyú, Yva viyú, Yva poreí	BO, BR, AR, UY, PY	Diuretic, digestive, and antidiarrheal properties, for sore throats and other throat conditions [43]	α and β-amyrins, quercetin, sesquiterpenes β-caryophyllene, 1,8-cineole, bicyclogermacrene, spathulenol, 5-epi-neointermedeol (leaves), gallic acid, catechin, vanillic acid, ellagic acid (fruit) [44–46]
Juglans neotropica Diels./ Nogal, cedro negro, Tocte	VE, CO, EC, PE	Antidiabetic, antioxidant, antifungal, antimicrobial, hypoglycemic, anti-hepatic, vaginal, gastric, and respiratory infections, open skin wounds, and treat sores. [47]	Gallic acid, ellagic acid, caffeic acid, tannins (seeds), hydroxycinnamic acid derivatives, flavonoid heterosides, sterol, beta-sitosterol, isoquinolin berberine, catechin, 3-0-glucosyl-galactosidequercetin, 5-hydroxy-tryptamine (leaves), juglone quinones, 1,4-naphthoquinone, currumidicin (fruit), juglone qinone, regiolone, betulin triterpenes, betulinic acid (stem) [48]
Ocotea quixos (Lam.) Kosterm./ Ishpingo, Espíngo, Canelillo	CO, EC, PE	Disinfectant, local anesthetic, against arthritis, antimicrobial, and antifungal activity; antiplatelet, antithrombotic, vaso-relaxing [49]	Transcinnamaldehyde (27.9%), methylcinnamate (21.6%), 1,8-cineole (8.0%), benzaldehyde (3.6%), β-selinene (2.1%) [50]
		Vulnerable species	
Cinchona pubescens Vahl, 1970/ Cascarilla, cinchona, Quinina, Quino, Kina	S. América (Andean Region)	Malaria treatment [51,52]	Cinchonine, cinchonidine, quinine, quinidine [53], triterpene heterosides, resins, catechin, and tannins [54].
Myrceugenia glaucescens (Cambess.) D. Legrand & Kausel/ Murta	UY, BR, PY, N. of AR	Astringent, antidiarrheal, and digestive [55]	It shares several characteristics with its native relative, the Anacahuita, since some members of the Myrtaceae family produce essential oils (camphor, monoterpenes, sesquiterpenes, triterpenes, phenylpropanoids) [56]
Polylepis spp. Ruiz & Pavón/ Quinual, Queñoa	BO, PE, EC	Antihypertensive properties, for respiratory diseases, hypoglycemic [57]	Flavonoids, anthocyanins, carotenoids, triterpenes oxygenated, and flavonoids glycosidated [58,59]
Sebastiania commersoniana (Baill.) L. B. Sm. & Downs/Guindillo, Palo de leche	AR, UY	Antiseptic, external use [55]	Quercetin, isoquercitrin, cumarin, kaempferol, isorhamnetin, gallicin, scopoletin, syringic, and caffeic acids [60,61]
Handroanthus chrysanthus (Jacq.) S.O.Grose/ Guayacán amarillo, Araguaney	VE, CO, EC	Antimicrobial, antioxidant, hepatitis, alleviate osteoporosis [62]	Flavonoids, cardiotonic glycosides, sesquiterpene lactones [63]
Jacaranda mimosifolia D. Don, 1822/ Jacaranda, tarco	BR, BO, PY, PE, CO, EC, N. AR	Treatment of amoebic dysentery and other acute gastrointestinal conditions, antiseptic, antitumor, spasmolytic [64]	Jacaranone, jacoumaric acid, methyl jacaranone [65]
Capparidaceae Juss./Sapote, Zapote	VE, CO, EC, N. PE, Antilles	Gastrointestinal, inflammation, anemia, liver dysfunction, rheumatism, analgesic, antispasmodic, vermifuge, anti-hemorrhoidal, laxative, cleansing, diuretic, expectorant, body tonic [66]	Catechin, epicatechin, gallocatechin, catechin 3-O-gallate, gallic acid, syringic acid, p-hydroxybenzoic acid, p-coumaric acid, proanthocyanidins [67]
Swietenia macrophylia King/ Mahogany	PE, BR	Antipyretic, astringent, tonic [68]	Scopoletin, stearic acid methyl ester, beta-siterol, swietenine, swietenolide, swietemahonin E, F, G (seeds), catechin, epicatechin, swietemacrophyllanin (bark) swietephragmin (H, I, J), germacrene (A, D), swietenine (J) (leaves), 3-hydroxycaruilignan C (stem) [69]

Legend: BO—Bolivia, BR—Brazil, AR—Argentina, UY—Uruguay, PY—Paraguay, VE—Venezuela, CO—Colombia, EC—Ecuador, PE—Peru, AN—Antilles.

5. Propagation of Medicinal Trees in South America

According to the VOSviewer analyses, very few studies were available concerning the aspects of *somatic embryogenesis* in *medicinal trees.* In the literature, we found that even though there are some preoccupations considering medicinal tree propagation, few of them are based on somatic embryogenesis. That underlines the difficulty of regeneration techniques, with the analyzed species having different problems concerning the propagation.

In the case of *Myrcianthes pungens*, in vitro culture, seedlings, and growing in greenhouses are some methods used to propagate this species. Different explants (apical or nodal), substrates (paper filters, vermiculite, sand in combination with gibberellic acid (GA_3), and media variants were used for in vitro regeneration [70].

Juglans neotropica possesses a great adaptive capacity. It is usually propagated through seeds. However, due to its irregular germinative capacity and latency, in vitro multiplication methods using embryos are employed [71]. Based on studies conducted on various forest species, DKW medium supplemented with BAP and TDZ has demonstrated an increase in the number of shoots [26].

The *Ocotea quixos* species is in demand in the domestic market because it is used to flavor traditional foods and beverages such as colada morada. In order to use this species and its derivatives, it takes 12 years to obtain a mature tree, so growing it from seed is a problem and it is recommended to use young plants [72]. Currently, new technologies have been developed for the preservation and propagation of endangered forest species. The micropropagation protocol mentioned by Narciso 2021 [73] shows effectiveness in shoots, as high multiplication rates were seen with MS medium with BAP.

Cinchona pubescens is an iconic species from the Andes since its species was used as the only effective treatment against malaria for more than three centuries. Most of the *Cinchona* species are threatened by various anthropogenic activities [74]. Some studies show that the germination and regeneration capacity under natural conditions is reduced or deficient, occurring only under certain conditions such as association or growth with other types of species [75]. An in vitro protocol [76] was developed starting from caulinar apices and nodal segments from in-vitro-germinated seedlings on MS media, supplemented with vitamins and plant growth factors such as NAA (0.2 mg/L), BAP (0.2 and 2.0 mg/L), and kinetin (0.2 and 2.0 mg/L). A high rate of germination and formation of shoots, nodes, and leaves were obtained in the in vitro multiplication phase of explants, which demonstrates that the in vitro culture technique can be used for the recovery and conservation of the species, as well as a model for other forest species of the Rubiaceae family [29,77].

Polylepis spp. trees are important in the Andean region due to their adaptability to heights, where some communities use them as timber resources because there are not many other trees that they would use as fuel. The seeds have low viability (30%–60%), and the germination percentage is low too [78]. The main method to propagate *Polylepis* is by cuttings, which allows for obtaining genetically identical plants and cuts down the time until obtaining an individual [79]. To help the rooting, the combination of natural rooting has been used together with some kind of substrate. The study of Huarhua Chipani et al., 2020 [80], indicates that the combination of coconut water (natural rooting) and a substrate composed of peat, humus, and sand shows a good development of cuttings, obtaining 94.67% of the establishment at greenhouse conditions. In vitro culture is another method to propagate *Polylepis*, but one of the major challenges is to avoid the explant's contamination, phenolization, and oxidation, which are characteristic of woody plants. However, some techniques such as establishing a disinfection treatment of the mother plant and cultivating explants in darkness have been shown to decrease these effects, allowing for obtaining more shoots per explant. Growth factors such as NAA (0.25 μM) and BAP (2 μM) worked well to obtain a big number of shoots per explant (17–19 shoots) of *Polylepis rugulosa* Bitter after three subcultures [81]. These studies indicate that natural and synthetic growth factors can help the *Polylepis* spp. propagation efforts, however, we need more studies to know which of them is better for each *Polylepis* species.

Concerning the *Sebastiania commersoniana*, there is not much information about the propagation methods of this species. Some studies indicate that propagation through cuttings has a low rate of rotting. Substrates with renewable components, such as carbonized rice husk, coconut fiber, and commercial substrate based on pine bark, were found to be viable for the production of *S. commersoniana* seedlings. Also, the time until the cuttings could have a good number of roots could take more than 90 days; however, rotting is not sufficient for transplantations. Another factor that influences rotting is the diameter and age of the plant matrix, where juvenile and thin cutting showed better development of seedlings [82,83].

A protocol for the *Handroanthus chrysanthus* micropropagation under in vitro conditions was developed by the Universidad Estatal del Sur de Manabí (UNESUM), starting from nodal segments of 3 months old. The culture media used were MS supplemented with agar, sucrose, gentamicin, Gamborg vitamin, and cysteine. In the multiplication phase, the established explants were transferred to the MS culture medium added with BAP and IBA, following the method described by Carranza-Patiño et al. (2012) [84]. Three sowings were made at 30 days intervals and in the rooting phase, 3-cm-long shoots were taken and transferred to a rooting medium with AIB in different concentrations. In the incubation and photoperiod phase, vitroplants were incubated with a high light intensity of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a photoperiod of 16 h and temperature of 25 ± 1 °C. In the multiplication phase, 82% of healthy shoots were generated, and in the rooting of shoots in combined doses with BAP/AIB and BAP/ANA. Therefore, the in vitro production of *T. crhysantha* plant material asserts the usefulness of micropropagation compared to the use of conventional techniques [85].

For *Jacaranda mimosifolia*, good results have been obtained by eliminating contamination in seeds. The highest survival rate in seeds was obtained in sterile seeds that were planted in 3/4 concentration of agarized MS medium with sucrose; in the multiplication stage, it is advisable to use MS medium at 1/2 of its concentration because a greater number of sprouts are obtained. On the other hand, MS medium at 1/4 of concentration is the most suitable for rooting because it gave better results in root and shoot length. In terms of length and fresh weight, the best results were obtained with 6-BAP (2.0 mg/L), 6-BAP (4.0 mg/L) gave the highest number of shoots, and 6-BAP (1.0 mg/L) and NAA (0.0 mg/L) gave the highest amount of flavonoids. It is mentioned that the highest number of roots and dry weight was obtained with AC (2.0 g/L) + NAA (0.5 mg/L), while the longest shoots were obtained with AC (0.0 g/L) + NAA (0.1 mg/L). At the acclimatization stage, the best plant height was obtained with peat moss and perlite at a ratio of 2:1 (v/v) [86].

Selective exploitation of *Swietenia macrophylla* for its timber has deteriorated its abundance and genetic resources. This situation has made it necessary to conserve its germplasm and use in vitro techniques for reforestation. Apical shoots, which are the product of aseptically germinated seedlings, are grown on WPM without growth factors, with 0.5% (w/v) activated charcoal. After a few months, excised shoot tips are grown in WPM supplemented with BAP (10 pM) or ZEA. For rooting, media without factors and WPM media supplemented with IBA together with NAA have been used. For elongation, media with low concentrations of IBA or without growth factors, but with activated charcoal, have been used. For acclimatization, pots filled with vermiculite are used at temperatures of 25–30 °C and high relative humidity (90%–95%) [87].

A brief representation of the process of inducing and recovery of somatic embryos in the case of medicinal tree species is presented in Table 2.

Table 2. Somatic embryogenesis process in the representative medicinal tree species.

Names of Trees	Type of Explant	Media Variant for Somatic Embryogenesis Induction and PGF	Media Variant for Somatic Embryo Recovery and PGF	References
Myrcianthes pungens	Nodal	WPM medium and BAP	WPM medium without PGF	[70]
Juglans neotropica	Disinfected embryos from mechanical rupture of the seed coat [71]	WPM medium and IBA (0.832 µM) and BAP (8.32 µM)	MS medium/DKW medium + BAP + TDZ	[88]
Ocotea quixos	-	-	-	-
Cinchona pubescens	-	-	-	-
Polylepis spp.	-	-	-	-
Sebastiania commersoniana	-	-	-	-
Handroanthus chrysanthus	-	-	-	-
Jacaranda mimosifolia	-	-	-	-
Swietenia macrophylla	Shoot tips of aseptically germinated seedlings Vegetative material from clones	WPM (solid or liquid) and ZEA (1–10 µM) and AC WPM liquid and L-cysteines, CH, malt extract, citric acid, IBA, 2,4-S, iP, +sucrose [87]	WPM without PGF/WPM and low ZEA (0.1 µM)/WPM and glutamine, arginine, asparagine, lysine, proline WPM liquid medium and 6-BAP [88]	[89]

In a review, Minocha and Minocha, 1995 [90], underlined that SE in some woody plants (especially conifers) has become routine; even if the process is similar, the requirements for differentiation and maturation of somatic embryos may vary among species. However, some recommendations for initiating a somatic embryogenesis induction protocol in medicinal trees can be drawn:

- Somatic embryogenesis induction step:
    - Plant material—should be carefully collected, transported, and prepared for introduction in in vitro culture;
    - Sterilization of plant material—is one of the most important steps assuring an aseptic but viable material for in vitro cultures;
    - Media variant—should be added with proper plant growth hormones that are able to induce differentiation and dedifferentiation of the somatic cells;
    - Control of the environmental conditions.
- Somatic embryo recovery step—for the development of the somatic embryos, these should be transferred on a media variant with a lower concentration of plant growth factors than in the first step.

6. Conclusions

In the case of the medicinal tree species taken in this study, the available literature shows that somatic embryogenesis induction protocols exist only in the case of three species (1—endangered and 2—vulnerable). Even though SE in trees has been well studied, still there are many aspects that require detailed analyses. Future studies on the selection, culture, physiology, and molecular biology of forest trees are requested for better sustainable use of them. The accurate assessment of the knowledge concerning multiplication through somatic embryogenesis in South American representative medicinal trees constitute an important basis for future research. To encounter the growing demand for trees as raw materials for different human needs, efforts should be made to develop standardized somatic embryogenesis protocols with the aim of conservation and sustainable use. These protocols will allow for obtaining valuable plant material for the discovery of new secondary metabolites, without destroying the species from natural habitats.

This assessment provides a guide to identify the aspects that are still missing to achieve a sustainable use of medicinal trees in South America as well as for their conservation. Even though SE has been well studied in some medicinal and fruit forest species, as the VOSviewer analysis suggests, more studies are needed to provide a major understanding in order to establish strategies that help maintain their population and at the same time satisfy the growing demand of trees as raw materials for the different human needs.

Furthermore, the development and standardization of protocols focused on the secondary metabolites production of medicinal trees will allow us to obtain valuable plant material that could be an alternative to replace the necessity of taking the resources of the tree from their natural habitats, allowing us to continue making use of the benefits that nature has without destroying it. No data concerning the secondary metabolites in somatic embryos for the representative medicinal tree are available until now.

On the other hand, to identify the tree species that would be the most threatened in the future, researchers could find support in the traditional knowledge of native communities of the South American region, because the people from these communities know how to use the medicinal properties from natural resources and how many applications they will have, providing an idea of the potential tree species that will be more exploited due to their medicinal properties and, in consequence, should be prioritized in conservation plans [91].

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Abbreviation

2,4-D—2,4-dichloro phenoxy acetic acid; 6-BA—BAP—6-benzyl-aminopurine; AC—activated charcoal; AIB—2-Aminoisobutyric acid; CH—hydrolyzed casein; DKW—Paradox walnut rootstock (*Juglans hindsii* x *J. regia*) (Driver and Kuniyuki, 1984); GA_3—gibberellic acid; IBA—indole-3-butyric acid; iP—N6 (D2-isopentenyl) adenine; IUCN—International Union for Conservation of Nature; LAC—Latin America and the Caribbean; MJ—methyl jasmonic; MS—Murashige and Skoog medium variant, 1962; NAA—naphthaleneacetic acid; PGF—plant growth factors; SA—salicylic acid; SE—somatic embryogenesis; TDZ—thidiazuron; WPM medium—modified woody plant medium.

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