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REVIEW

Tissue culture mediated biotechnological interventions in medicinal trees: recent progress

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Abstract

Many tree species are an excellent source of a wide range of bioactive compounds of pharmaceutical importance. However, overexploitation of medicinal trees to fulfil the demand for plant-based herbal drugs puts pressure on its natural population. Therefore, many tree species are declining continuously, particularly those used in pharmaceutical industries. In addition, limited production of secondary metabolites from a specific part and only at a particular developmental stage and sometimes very-low yield are some major bottlenecks when secondary metabolites are isolated directly from a tree species. Tissue culture-based biotechnological interventions for propagation, in vitro conservation and secondary metabolite production in medicinally important tree species have been practiced during the last two–three decades. Many medicinally important trees successfully propagated in vitro through different modes of regeneration i.e., axillary shoot proliferation, adventitious organogenesis and somatic embryogenesis. Success in in vitro propagation of most of the medicinal trees has been achieved through axillary shoot proliferation. Recent studies on medicinal trees showed that ex vitro rooting is an ideal method of rooting of microshoots. Gene targeted molecular markers have now been preferred for genetic fidelity of tissue culture-raised plants of medicinal trees. In recent years, newly developed droplet-vitrification and cryo-plate methods increased the applicability of cryopreservation for the long-term conservation of many medicinal trees. Several bioactive compounds of pharmaceutical importance are produced from trees via in vitro culture technique. There are a few success stories of producing secondary metabolites at a commercial scale from medicinal trees i.e., taxol, camptothecin and azadirachtin. This review paper presents the recent progress on plant tissue culture-mediated biotechnological advances in medicinal trees, emphasizing different aspects of in vitro propagation, conservation, and production of bioactive compounds of pharmaceutical importance.

Keywords Cryopreservation · Ex vitro rooting · Genetic fidelity · In vitro conservation · Micropropagation · Secondary metabolites

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Key message

- This review provides a comprehensive overview on the recent biotechnological progress in medicinal trees, emphasizing in vitro propagation, conservation, and secondary metabolites production.

Introduction

According to World Health Organization, more than three-fourth population of the world, mostly in poor and developing countries, depends mainly on plant-based traditional ethnomedicine for primary health care (Ekor 2014). The ingredients and phytoconstituents found in such medicinal

plants are the base of many traditional systems of medicine, like Ayurveda, Unani, Siddha, and Homoeopathy. Variations in the qualitative and quantitative contents of active principles highly depend on the genetic make-up of the plants, climate, soil quality and developmental stage of the plant itself (Isah 2019). The medicinal plants constitute many plant groups, including herbs, shrubs, trees, climbers, woody climbers, etc., providing raw material for drug formulation. Many prescribed phytomedicines used today are usually derived from herbs. However, trees are also a powerful source of many phytoconstituents and beneficial because they are perennial and available throughout the year. Despite the worldwide importance, many tree species are under threat in the wild. The habitat loss due to anthropogenic activities, overexploitation for the timber or medicinal values, deforestation, urbanization and industrialization, exotic species, pest and diseases and global climate changes are significant threats to the tree species (Shekhawat et al. 2014). These factors posed a danger and have led to the extinction of many economically important tree species. Recent data of the IUCN Red List indicated that out of 58,343 plant species described and evaluated by IUCN Red List version 2021-2, 23,335 species are listed as threatened (IUCN 2021). The increasing demand for raw drugs from medicinal trees puts pressure on its natural population. The wild populations of threatened tree species are declining at an alarming rate, particularly those used in pharmaceutical industries. Therefore, these species need more attention for conservation and management.

Conservation and management of many threatened tree species are sometimes difficult in their natural habitat due to several factors, like, inefficient natural propagation system, pollination regime, seed dormancy, shift in the seed set, biotic and abiotic stresses etc. (Oldfield 2009; Rai et al. 2021a). The conventional methods of vegetative propagation of trees are slow and inefficient in many cases because they lose the ability to root at maturity and make their regeneration under in vivo conditions difficult (Pena and Seguin 2001; Giri et al. 2004). Conventionally, ex-situ conservation of many tree species in seed bank or field gene bank is inhibited mainly due to seed dormancy and poor germination of seeds after storage, high risk of disease transfer and loss of genetic resources (Potter et al. 2017; Wyse et al. 2018). Recent advances in biotechnological technique, i.e., plant tissue culture, have facilitated a new way to propagate and conserve several commercially important plant species, including medicinal trees, and have been extensively studied in recent three-four decades. The main application of plant tissue culture technique in medicinal trees includes micropropagation and production of genetically pure plants, production of bioactive compounds and in vitro conservation for short- to medium-term or long-term. This paper discusses a brief insight into the status of plant tissue culture-mediated biotechnological advances in medicinal tree species, emphasizing recent progress.

In vitro propagation of medicinal trees

Plant tissue culture technique provides a novel approach for the large-scale propagation and germplasm preservation of commercially and economically important plants, including medicinal trees. The most applicable aspect of plant tissue culture technique is micropropagation, which is highly acclaimed for its practicability and commercial use. It offers an excellent system for the maintenance and multiplication of pathogen-free plants and for the safe exchange of their germplasm across the world (Bhojwani and Dantu 2013). Micropropagation of trees has great relevance to overcoming the overexploitation of trees. However, very little progress has been made with medicinal trees compared to herbaceous medicinal plant species. The slow-growing nature, the problem of juvenility versus maturity and long and complex life cycles of trees are some main hurdles associated with the micropropagation of trees (Pena and Seguin 2001; Giri et al. 2004; Rai et al. 2021b).

In tree species, mainly fruit and medicinal trees with great commercial values, explants derived from mature tissue facilitate direct tree improvement. Usually, plants propagated in vitro directly from mature tissue can capture the genetic make-up of an elite genotype resulting in the development of plants with desired characteristics (Rai et al. 2010). The response of explants obtained from mature trees is significantly influenced by the source and types of explants, physiological state of tissue and collection season. The performance of different types of explants of trees in different seasons is highly associated with endogenous hormone status, which is impacted by environmental factors. Earlier, nodal segments or other explants obtained from mature trees have been used for the micropropagation of many medicinal trees. However, the success rate of micropropagation of trees is comparatively low when using mature explants rather than seedling explants (Bonga 1987). Axillary bud proliferation through nodal explants obtained from mature plants is one of the best methods of micropropagation of medicinal trees. In most plant species, this method assures the production of true-to-type plants and maintains the genetic fidelity in tissue culture-raised plants (Rani and Raina 2000). However, leaching of phenols and browning of explants after wounding, exogenous and endogenous microbial contamination, in vitro recalcitrance of explants and low shoot proliferation are major obstacles in establishing culture when using explants from mature trees (Giri et al. 2004; Rai et al. 2010).

Most of the tree species produce phenolic compounds after wounding during culture establishment. Leaching of

phenolics from the explants hampers the in vitro regeneration and has proven to be lethal to explants (Ahmad et al. 2013). The major cause of the browning of explants is the oxidation of phenols within the tissue. This problem is more evident in tissue culture of tree species, which makes tissue culture of woody plants difficult. Many workers combat this problem using different compounds as antioxidants and adsorbents (Singh 2018). To overcome the problem of phenolic exudation, the pre-treatment of explants with antioxidant solutions, like, ascorbic acid and citric acid was found effective in the tissue culture of many tree species (Harry and Thrope 1994; Giri et al. 2004). In some cases, explants were pre-treated with polyvinylpolypyrrolidone (PVPP), a polyamide, to control browning. Activated charcoal also plays an important role in reducing browning and preventing the leaching of phenol during the establishment of culture in many tree species (Thomas 2008).

Culture contamination caused by exogenous and endogenous fungi and bacteria is one of the most serious problems for culture establishment of many tree species when using explants from mature tissues (Harry and Thrope 1994; Giri et al. 2004; Singh 2018). The major consequence of microbial contamination is growth retardation, necrosis and finally the demise/loss of cultures. In addition, it can cause a substantial economic loss, particularly in commercial tissue culture laboratories and the loss of time and efforts spent in developing cultures. The major microbial contamination in culture is associated with plants, but sometimes it may be due to error in the surface sterilization processes or by transfer of microbes from human body hair and clothing into the sterile area. The contamination of some latent and slow-growing bacteria, which appears at a very late stage, can cause reduced shoot and root growth, tissue necrosis, and finally, loss of culture (Bhojwani and Dantu 2013). To overcome the problem of culture contamination, explants can be surface sterilized using various sterilizing agents like mercuric chloride, sodium hypochlorite, hydrogen peroxide, ethanol, etc., and treated with antibacterial and antifungal agents. However, types of sterilizing agents, their concentration and the duration of treatment may vary with tree species, the juvenility and maturity of plant tissue and the infestation rate.

The success of plant tissue culture technique can largely depend upon the ability to regenerate complete plants in vitro from explants either derived from mature trees or juvenile tissue. However, in vitro recalcitrance is one of the main bottlenecks of tissue culture of trees. In many tree species, explants obtained from mature trees are not amenable to in vitro culture procedures (Benson 2000). In vitro recalcitrance highly depends on the physiology of donor plants and the requirement of specific nutrients and plant growth regulators (PGRs) for in vitro manipulations. Therefore, selection of donor plants, explants at a specific responsive stage, nutritional and PGRs requirements are of great importance to overcome recalcitrance (Arya and Shekhawat 1986; Bonga 2017). In most of the tree species, successful plant regeneration was observed from explants obtained from seedlings. Other than seedlings, the procurement of juvenile explants mostly shoot tips and nodal segments that arise from the base of the main stem, i.e., offshoots, are an alternative and efficient way to establish cultures in many recalcitrant woody plants (Benson 2000; Rai et al. 2010). In general, three modes of in vitro propagation system, i.e., axillary bud proliferation, adventitious organogenesis, and somatic embryogenesis, have been practiced through tissue culture techniques.

Axillary bud proliferation

Propagation of plants in vitro through shoot tips, shoot segments with single or multiple nodes, or axillary buds from mature plants has proved to be the most common and reliable clonal propagation method. In this method, newly formed apical shoots, lateral buds, or a piece of shoots having one or multiple nodes bearing shoot meristems serve as explants for shoot proliferation and multiplication by the repeated formation of axillary branches (George et al. 2008). Although, establishing cultures from shoots or nodal segments acquired from a mature tree is difficult in some tree species. In such cases, nodes or cotyledonary nodes excised from in vitro grown seedlings may be used as suitable alternatives to shoot tips and nodal segments of a mature plant. Most of the in vitro propagation studies in medicinal trees were carried out using nodal segments explants obtained from mature plants or seedlings (Table 1). In plant tissue culture of any plant species, media compositions play a vital role in the morphogenesis. Different basal media have been tested for the micropropagation of medicinal trees. However, MS (Murashige and Skoog 1962) and WPM (woody plant medium, i.e., Lloyd and McCown 1980) media were found most suitable for the tissue culture of tree species.

Usually, the incorporation of cytokinins in the growth medium promotes the proliferation of axillary shoots by eliminating the dominance of apical meristems. However, a permissible balance of cytokinin with endogenous hormones is required. So, the desirable cytokinin concentration may vary from species to species, and it has become necessary to standardize for a specific species. The types and concentrations of cytokinins are the two most critical factors for shoot multiplication in tissue culture studies. N^6-benzylaminopurine (BAP) was the most common cytokinin used for many medicinal tree species. Other cytokinins, like, kinetin (Kin), zeatin, thidiazuron (TDZ) and meta topolin (mT) were also reported to be effective for axillary shoot proliferation in some medicinal tree species. There are also several reports in which single cytokinin was

Table 1 Tissue culture studies in some selected medicinal tree species

Plant name	Explant	Mode of regeneration and morphogenic responses	References
Acacia auriculiformis	Shoot segments	Micropropagation and assessment of genetic fidelity analysis	Yadav et al. (2016)
Acacia catechu	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Sahini and Gupta (2002)
Acacia nilotica	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration	Rathore et al. (2014, 2015)
Acacia senegal	Immature cotyledon	Induction of somatic embryogenesis	Rathore et al. (2012)
Aegle marmelos	Cotyledonary node	Shoot multiplication, in vitro rooting and plant regeneration	Nayak et al. (2007)
	Nodal segment	Direct shoot multiplication, plant regeneration and genetic fidelity analysis using molecular marker	Pati et al. (2008)
	Nodal segment and root suckers	Axillary bud proliferation and plantlet regeneration	Parveen et al. (2015)
Alangium salviifolium	Seedling nodal segments and shoot apices	Shoot multiplication, in vitro rooting and plant regeneration	Pandey et al. (2022)
Azadirachta indica	Nodal, root and leaf	Direct somatic embryogenesis from nodal and root explant and indirect from leaf explant, plant regeneration and in vitro azadirachtin production	Akula et al. (2003)
	Anther culture	Production of haploid plants	Chaturvedi et al. (2003a)
	Immature endosperm	Triploid plant regeneration from callus	Chaturvedi et al. (2003b)
	Nodal explants from 50 years old mature tree	Axillary shoot proliferation, multiplication and in vitro rooting	Chaturvedi et al. (2004)
	Buds from basal sprouts of mature and juvenile plant	Shoot multiplication, in vitro rooting and plant regeneration	Quraishi et al. (2004)
	Leaflet segments	Adventitious shoot induction, proliferation and plantlet production	Arora et al. (2009)
	Unpollinated ovary	Organogenesis and plant regeneration	Srivastava et al. (2009)
	Nodal segment	Axillary bud proliferation and plantlet production	Arora et al. (2010)
	Root explants	Shoot induction, plant regeneration and genetic fidelity analysis using RAPD	Arora et al. (2011)
	Anther culture	Haploid plant production from callus and increased production of azadirachtin	Srivastava and Chaturvedi (2011)
	Nodal segment from 8-month-old grown in green house conditions	Shoot multiplication in flasks with caps containing PTFE membranes	Rodrigues et al. (2012)
Balanites aegyptiaca	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Siddique and Anis (2009)
Bauhinia racemosa	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration and genetic fidelity analysis using CBDP and SCoT marker	Sharma et al. (2017, 2019a)
Calophyllum apetalum	Young shoots from mature trees	Shoot multiplication, in vitro rooting and plant regeneration	Nair and Seeni (2003)
Campomanesia xanthocarpa	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Machado et al. (2020)
Camptotheca acuminata	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Nacheva et al. (2020)
Cassia angustifolia	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Siddique and Anis (2007)

Table 1 (continued)

Plant name	Explant	Mode of regeneration and morphogenic responses	References
Cinnamomum camphora	Immature zygotic embryos	Somatic embryogenesis from callus and plant regeneration	Shi et al. (2009)
Couroupita guianensis	Nodal segment	Shoot multiplication, ex vitro rooting and plant regeneration	Shekhawat and Manokari (2016)
Crataeva adansonii	Nodal segments	Induction of axillary buds, shoot multiplication, in vitro rooting and plant regeneration	Sharma et al. (2003)
Crataeva magna	Nodal segment	Shoot multiplication, plant regeneration and genetic fidelity analysis using ISSR marker	Bopana and Saxena (2009)
Elaeocarpus serratus	Nodal segments	Shoot multiplication, plant regeneration and genetic fidelity analysis using RAPD and ISSR markers	Raji and Siril (2021)
Elaeocarpus sphaericus	Nodal segments	Shoot multiplication, in vitro rooting and plant regeneration	Saklani et al. (2015)
Ficus religiosa	Nodal segments	Shoot multiplication, in vitro rooting and plant regeneration	Siwach and Gill (2011)
Garcinia indica	Seed segments	Induction of multiple adventitious shoot buds, in vitro rooting and plant regeneration	Malik et al. (2005)
Gymnocladus assamicus	Cotyledonary node and node from seedlings	Direct induction of adventitious shoots, in vitro rooting and plant regeneration	Gupta et al. (2020)
Hildegardia populifolia	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration and genetic uniformity analysis using ISSR and RAPD marker	Upadhyay et al. (2020)
Hovenia dulcis	Leaf from seedlings	Callus induction and shoot regeneration and in vitro rooting	Jeong et al. (2009)
Kalopanax pictus	Immature zygotic embryos	Embryogenic callus, somatic embryogenesis and plant regeneration	Moon et al. (2005)
Maytenus emarginata	Nodal segments	Shoot multiplication, in vitro rooting and plant regeneration	Shekhawat et al. (2021)
Melaleuca alternifolia	Apical shoots from one-year-old greenhouse stem cutting-derived plants	Shoot proliferation, in vitro rooting and plant regeneration	Iiyama and Cardoso (2021)
Melia azedarach	Immature zygotic embryos	Direct somatic embryogenesis and plant regeneration	Vila et al. (2003)
	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration	Husain and Anis (2009)
	Immature endosperm	Triploid plant regeneration from callus	Thang et al. (2018)
Millettia pinnata	Hypocotyls	Direct adventitious shoot bud formation, in vitro rooting and plant regeneration	Nagar et al. (2015)
Mitragyna parvifolia	Nodal segments	Shoot multiplication and concurrent ex vitro rooting and acclimatization	Patel et al. (2020)
Morinda coreia	Nodal segments	Shoot multiplication, in vitro and ex vitro rooting and plant regeneration	Shekhawat et al. (2015a)
Morinda citrifolia	Nodal segments	Shoot multiplication, in vitro and ex vitro rooting and plant regeneration	Shekhawat et al. (2015b)
Moringa oleifera	Nodal segments and shoot-apices	Somatic embryogenesis and organogenesis	Chand et al. (2019)
	Seedling nodal explants	Shoot proliferation, in vitro rooting and plant regeneration	Gupta et al. (2020)

Table 1 (continued)

Plant name	Explant	Mode of regeneration and morphogenic responses	References
Murraya koenigii	Cotyledon and zygotic embryonic axis	Direct and indirect somatic embryogenesis and plant regeneration	Paul et al. (2011)
Neolamarkia cadamba	Cotyledon	Direct adventitious shoot organogenesis and plant regeneration	Huang et al. (2014)
	Cotyledon and hypocotyl	Callus induction and shoot regeneration and in vitro rooting	Huang et al. (2020)
Nothapodytes foetida	Hypocotyl segments	Shoot multiplication, in vitro rooting and plant regeneration	Rai (2002)
Oplopanax elatus	Immature zygotic embryos	Embryogenic callus, somatic embryogenesis and plantlet conversion	Moon et al. (2006)
Oroxylum indicum	Apical and axillary bud	Shoot multiplication, in vitro rooting and plant regeneration	Gokhale and Bansal (2009)
Phellodendron amurense	Hypocotyl explants	Induction of embryogenic calluses, somatic embryogenesis and plant regeneration	Azad et al. (2009)
Pittosporum eriocarpum	Nodal explants	Shoot multiplication, plant regeneration and genetic fidelity analysis using SCoT, ISSR and RAPD markers	Thakur et al. (2016)
Pterocarpus marsupium	Seedling nodal explants	Shoot proliferation, in vitro rooting and plant regeneration	Tiwari et al. (2004)
	Hypocotyl segments	Induction of callus, somatic embryogenesis and plant regeneration	Husain et al. (2010)
	Shoot tips from seedling	Direct shoot organogenesis and plant regeneration	Ahmad et al. (2021)
Salvadora persica	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration	Phulwaria et al. (2011)
Santalum album	Nodal shoot segments	Shoot multiplication, ex vitro rooting and genetic fidelity analysis using SCoT marker	Manokari et al. (2021)
Sapindus mukorossi	Leaf explant	Indirect somatic embryogenesis and plant regeneration	Singh et al. (2015)
Sapindus trifoliatus	Seedling nodal explants	Shoot multiplication, plant regeneration and genetic fidelity analysis using RAPD	Asthana et al. (2011)
	Sepal explants	Indirect somatic embryogenesis and plant regeneration	Asthana et al. (2017)
Shorea tumbuggaia	Shoot apex from seedlings	Shoot multiplication, plant regeneration and genetic fidelity analysis using ISSR marker	Shukla and Sharma (2017)
Spondias mangifera	Seedling nodal explants	Shoot multiplication, in vitro rooting and plant regeneration	Tripathi and Kumari (2010)
Spondias pinnata	Leaf from in vitro derived shoots	Callus induction, shoot proliferation, in vitro rooting and assessment of genetic fidelity using ISSR and SCoT markers	Jaiswal et al. (2021)
Stereospermum personatum	Shoots excised from seedlings	Shoot multiplication, in vitro rooting and plant regeneration	Shukla et al. (2009)
Syzygium cumini	Cotyledonary node	Callus induction, shoot proliferation and ex vitro rooting	Naaz et al. (2019)
Taxus wallichiana	Zygotic embryos	Indirect somatic embryogenesis and plant regeneration	Datta and Jha (2008)
Tecomella undulata	Nodal segments	Micropropagation and assessment of genetic fidelity using ISSR and SCoT markers	Chhajer and Kalia (2017)

Table 1 (continued)

Plant name	Explant	Mode of regeneration and morphogenic responses	References
Terminalia arjuna	Cotyledonary node	Shoot multiplication, in vitro rooting and plant regeneration	Pandey and Jaiswal (2002)
	Nodal segments	Shoot multiplication, in vitro rooting and plant regeneration	Pandey et al. (2006)
	Shoot segments	In vitro propagation, synthetic seed production and genetic fidelity analysis	Gupta et al. (2014)
Terminalia bellirica	Seedling nodal explants	Axillary bud proliferation and plantlet production	Ramesh et al. (2005)
	Nodal segments	Shoot multiplication, in vitro and ex vitro rooting and plant regeneration	Phulwaria et al. (2012a)
	Nodal segments	Micropropagation and assessment of genetic fidelity using ISSR and RAPD markers	Dangi et al. (2014)
Terminalia catappa	Nodal segments	Shoot multiplication, ex vitro rooting and plant regeneration	Phulwaria et al. (2012b)
Terminalia chebula	Cotyledons and mature zygotic embryo	Induction of callus, somatic embryogenesis and plant regeneration	Anjaneyulu et al. (2004)
Uncaria rhynchophylla	Stem segments	Shoot proliferation, in vitro rooting and plant regeneration	Ishii et al. (2013)
Zanthoxylum armatum	Nodal segments	Micropropagation and assessment of genetic fidelity using RAPD and ISSR markers	Purohit et al. (2017)

Table included only the references of 2000 to 2022

found ineffective for shoot proliferation but showed promising results when used in combination with two cytokinins or cytokinin with low concentrations of auxins (George et al. 2008; Bhojwani and Dantu 2013). The synergistic effect of two cytokinins with auxin has also been studied in some species (Phulwaria et al. 2012a; Patel et al. 2020). In a recent study, Shekhawat et al. (2021) reported the synergistic effect of two cytokinins (mT and Kin) and one auxin indole-3-acetic acid (IAA) on shoot multiplication in *Maytenus emarginata*. They observed that mT or BAP alone or in combination with IAA did not respond reasonably, but the synergism of mT with Kin and IAA greatly enhanced shoot multiplication with more than 80 shoots per culture. In most medicinal tree species, an agar solidified medium has been used for in vitro propagation. However, few studies also reported the use of liquid medium for shoot proliferation and growth (Rathore et al. 2014, 2015; De Carlo et al. 2021). Some growth additives and additional nitrogen and carbon sources like glutamine, proline, arginine and citric acid also enhanced the shoot proliferation in some medicinal trees (Phulwaria et al. 2011, 2012a, b; Gupta et al. 2014; Rathore et al. 2014, 2015; Chhajer and Kalia 2017; Shekhawat et al. 2021).

Adventitious organogenesis

In certain plant species, including medicinal trees, the formation of adventitious shoots or roots from the cultured cells or tissues may provide a reliable in vitro propagation system. Adventitious shoot or root formation is accompanied either directly from explants (direct organogenesis) or from an intermediate callus, i.e., an unorganized mass of cells (indirect organogenesis) (George et al. 2008). Induction of direct organogenesis highly depends on the explant types, the source of explants and the requirement for exogenous PGRs in the process. Although this method is particularly suitable for herbaceous species, several papers have also been published on direct adventitious shoot organogenesis in medicinal tree species (Table 1). In medicinal tree species, the formation of adventitious shoots *in vitro* has been reported from the tissues derived from leaves, stems, roots, or seedling explants. In *Neolamarkia cadamba*, Huang et al. (2014) reported direct adventitious shoot organogenesis from cotyledon explants, and they found that shoots were raised directly at the cut edges of the cotyledonary petioles. In another study, direct adventitious shoot organogenesis was observed in *Millettia pinnata* using hypocotyl explant (Nagar et al. 2015).

Indirect organogenesis involves the induction of callus from explants and further shoot bud differentiation.

Although, the explants from mature or immature plants can form callus, explants with mitotically active cells are usually most suitable for callus induction and further plant regeneration (George et al. 2008). Mostly explants derived from seedlings have been used for callus-mediated organogenesis in medicinal tree species (Table 1). For instance, Jeong et al. (2009) reported callus-mediated organogenesis in *Hovenia dulcis* from leaf explants obtained from seedlings. More recently, Jaiswal et al. (2021) used leaf explants excised from in vitro derived shoots for callus induction, shoot proliferation and plant regeneration in *Spondias pinnata*. In some cases, cotyledon, hypocotyl or cotyledonary nodes have also been used for indirect organogenesis (Naaz et al. 2019; Huang et al. 2020).

In adventitious organogenesis, somatic cells of explants form new meristems and meristematic tissues in the presence of exogenous PGRs (De Klerk 2009). The involvement of exogenous PGRs is very crucial in the induction of adventitious shoot buds either directly from explants or via callus (George et al. 2008). In most tree species, medium containing BAP alone or in combination with Kin or IAA was most effective for direct adventitious shoot organogenesis. TDZ or mT also reported to have beneficial for direct adventitious shoot organogenesis in some species (Gupta et al. 2020a; Ahmad et al. 2021). While medium supplemented with 2,4-D either alone or in combination with low concentrations of cytokinin, preferably Kin or BAP, was advantageous for callus induction. Further, cytokinin in medium leads to the formation of shoot buds from callus (Giri et al. 2004; Bhojwani and Dantu 2013).

Rooting of in vitro regenerated shoots

The successful rooting of microshoots is a necessary prerequisite for developing an in vitro propagation system for any plant species either through axillary shoot proliferation or via adventitious shoot organogenesis. Cytokinin added in growth medium to promote axillary shoot proliferation or adventitious shoot organogenesis usually inhibits root formation. Therefore, it is necessary to excise a single shoot from shoot clusters and transfer it to a different medium containing auxins for in vitro rooting (George et al. 2008; Bhojwani and Dantu 2013). In vitro rooting in microshoots has been encouraged in many plant species, including medicinal trees, by incorporating auxin indole-3-butyric acid (IBA) in root-inducing medium. In the last two decades, many workers emphasized on ex vitro rooting over in vitro rooting. The ex-vitro rooted plants perform well during acclimatization because they have root hairs and well-developed vascular connections in ex vitro root and shoot, which make them more efficient in adaptation during acclimatization. In addition, unlike in vitro rooting, ex vitro rooted plants usually lack callus formation at the root-shoot junction, one of the main reasons for the survival of plants with low frequency when rooted through in vitro rooting technique. Moreover, the ex-vitro rooting technique does not require additional acclimatization as rooting and acclimatization occur simultaneously, reducing the cost and time of micropropagation (Bhojwani and Dantu 2013; Patel et al. 2020). Ex vitro rooting technique could be advantageous, particularly in those tree species where rooting and acclimatization are major constraints during micropropagation. In recent years, ex vitro rooting technique has been applied in many medicinal trees, including *Acacia nilotica* (Rathore et al. 2014), *Bauhinia racemosa* (Sharma et al. 2017), *Couroupita guianensis* (Shekhawat and Manokari 2016), *Hildegardia populifolia* (Upadhyay et al. 2020), *Melia azedarach* (Husain and Anis 2009), *Mitragyna parvifolia* (Patel et al. 2020), *Morinda citrifolia* Shekhawat et al. (2015b), *Salvadora persica* (Phulwaria et al. 2011) and *Terminalia bellirica* (Phulwaria et al. 2012a).

Somatic embryogenesis

The main applied goal of tissue culture of trees is mass multiplication and large-scale clonal propagation. Owing to bipolar nature having both root and shoot meristem, high multiplication rate, scale-up by bioreactor technology, the potential for in vitro storage and suitable target for gene transfer, somatic embryogenesis is recognized as a powerful tool for propagation, conservation and genetic improvement of forest and medicinally important trees (Guan et al. 2016; Isah 2016).

Several factors affect the process of somatic embryogenesis in medicinal tree species, like, types of explants, physiological status of explant, PGRs, and genotypes. In general, a routine practice for inducing somatic embryogenesis in a particular plant species is the selection of suitable explant and culturing them in a nutrient medium containing PGRs, most preferably auxin for the induction of somatic embryogenesis either directly from explant or indirectly via callus (Arnold et al. 2002; Jiménez 2005). In most woody plant species, mature or immature zygotic embryos have been used as a primary explant for somatic embryogenesis. However, other less differentiated tissues, like, hypocotyl, leaf segments, cotyledons, floral parts or shoot apex, etc. have also been proved to be effective for induction of somatic embryogenesis in some medicinal trees (Table 1). The high responsiveness of zygotic embryos towards somatic embryogenesis is mainly due to the presence of pre-embryogenic determined cells (PEDCs) in zygotic embryos, which possess embryogenic competence (Bhojwani and Dantu 2013). Depending on the types, concentrations and treatment duration of auxin or other PGRs, zygotic embryo follows the direct or indirect embryogenic pathways. For instance, zygotic embryos of *Melia azedarach* cultured on medium

supplemented with TDZ exhibit direct somatic embryogenesis (Vila et al. 2003), while in some medicinal trees, somatic embryos induced indirectly from zygotic embryo explant through callus (Anjaneyulu et al. 2004; Moon et al. 2005, 2006; Datta and Jha 2008; Shi et al. 2009). The callus mediated indirect formation of somatic embryos is also observed from other explants, like, hypocotyl, leaf, cotyledon, or young floral parts (Azad et al. 2009; Husain et al. 2010; Singh et al. 2015; Asthana et al. 2017).

In general, auxin, mainly 2, 4-D, is required only for the induction of somatic embryogenesis as cells of plant tissues initially became competent for embryogenic induction. Continuous auxin treatment is usually inhibitory for the development of somatic embryos. Therefore, somatic embryos will only develop when auxin-treated explants or auxin-induced callus are transferred to a medium devoid of auxin or very low auxin concentration (Arnold et al. 2002; Rai et al. 2007; George et al. 2008; Bhojwani and Dantu 2013). In some cases, a combination of high concentration of auxin with low concentrations of cytokinin is necessary for the induction of somatic embryogenesis. In *Sapindus trifoliatus*, embryogenic callus was induced from sepal explants on medium containing 5.0 mg l^{-1} 2, 4-D and 0.1 mg l^{-1} Kin, which further induced nodular embryogenic structures. Later, somatic embryos of different developmental stages were formed from these nodular embryogenic structures when transferred to a medium devoid of PGRs and containing glutamine (Asthana et al. 2017). The use of cytokinin in the induction and development of somatic embryos has also been reported in a few medicinal trees (Vila et al. 2003; Singh et al. 2015). For instance, leaf explants of *Sapindus mukorossi* cultured on medium supplemented with BAP exhibit the induction of callus and further development of somatic embryos (Singh et al. 2015). Improper maturation and development of poor-quality somatic embryos are the main hurdles that limit the plantlet conversion rate of somatic embryos. Incorporation of some additional adjuvants in medium helps in the maturation of somatic embryos. Some such adjuvants include abscisic acid (ABA), L-glutamine, proline, polyethylene glycol (PEG) and, high sucrose concentrations (Rai et al. 2011; Bhojwani and Dantu 2013). For example, inclusion of ABA and L-glutamine in the medium promoted maturation of somatic embryos of *Taxus wallichiana* and *Sapindus trifoliatus*, respectively (Datta and Jha 2008; Asthana et al. 2017).

Genetic stability of tissue culture raised plants

The maintenance of clonal uniformity of micropropagated plants is one of the important requirements for holding certain desirable traits, particularly when using elite genotypes of medicinal trees for pharmaceutical industries. However, the regeneration mode, mainly through callus phase, culture medium, PGRs, and culture conditions, sometimes causes genetic instability in in vitro propagated plants (Rani and Raina 2000; Rai et al. 2012). Hence, genetic fidelity analysis of micropropagated plants is essential before exploiting in vitro regeneration protocol of any plant species. In recent years, polymerase chain reaction (PCR) based molecular techniques have widely been used to analyze genetic fidelity of in vitro regenerated plantlets in many medicinal tree species (Table 1). Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers have been reported as useful molecular markers for the analysis of genetic fidelity of in vitro regenerated plants of many medicinal trees such as *Aegle marmelos* (Pati et al. 2008), *Azadirachta indica* (Arora et al. 2011), *Crataeva magna* (Bopana and Saxena 2009), *Elaeocarpus serratus* (Raji and Siril 2021), *Hildegardia populifolia* (Upadhyay et al. 2020), *Sapindus trifoliatus* (Asthana et al. 2011), *Shorea tumbuggaia* (Shukla and Sharma 2017), *Terminalia bellirica* (Dangi et al. 2014) and *Zanthoxylum armatum* (Purohit et al. 2017). Nowadays RAPD and/or ISSR markers are replaced by highly reproducible and gene-targeted i.e., start codon targeted (SCoT) polymorphism marker for genetic fidelity analysis. During the last decade, many workers assessed clonal fidelity of tissue culture raised plants using SCoT marker in some medicinal trees like *Bauhinia racemosa* (Sharma et al. 2019a), *Pittosporum eriocarpum* (Thakur et al. 2016), *Santalum album* (Manokari et al. 2021), *Spondias pinnata* (Jaiswal et al. 2021) and *Tecomella undulata* (Chhajer and Kalia 2017). In *Bauhinia racemosa*, Sharma et al. (2019a) used another gene-targeted CAAT box-derived polymorphism (CBDP) marker and SCoT marker to analyze the genetic stability of in vitro propagated plants. Both CBDP and SCoT markers can detect somaclonal variations in tissue culture-raised plantlets, particularly the genetic variations in a specific genomic region that is linked with a useful trait (Shekhawat et al. 2018; Rai 2021).

Production of haploid, triploid and polyploid plants

In the last few decades, the production of haploid plants using in vitro culture technique has gained immense importance because it allows the generation of double haploids (DH) homozygous lines from heterozygous parents. Haploid and double haploid plants have remarkable applications in the field of plant breeding and genetics (Germana 2011). The main strategies for obtaining haploid plants under in vitro conditions are androgenesis and gynogenesis. Anther or microspore culture is a technically efficient and straightforward approach for obtaining haploids. Ovary or ovule culture can also prove to be an alternative technique in those species where anther culture is not successful (Bhojwani and Dantu 2013).

Considerable progress has been made on haploids and double haploid plant production in many agricultural crops but restricted to a few tree species (Srivastava and Chaturvedi 2008). In *Azadirachta indica*, Chaturvedi et al. (2003a) successfully achieved haploid plant production by anther culture. Callus was induced on medium containing 2,4-D + NAA + BAP and high sucrose (9%) concentration and proliferated on medium supplemented with 2,4-D and Kin. Further, shoots were differentiated from calli when transferred to a medium containing BAP. They also determined the ploidy level of plants derived from anther culture and found that 60% plants were haploid. In another study, Srivastava et al. (2009) cultured an unpollinated ovary of *A. indica* to obtain haploid plants. However, they could not get haploid plants, and all the regenerated plants from ovary culture in this study were diploid.

Traditionally, triploid plants are produced by crossing a diploid and an induced tetraploid. However, endosperm, a naturally occurring triploid tissue, offers an efficient method of triploid plant production when culturing in vitro. Since, triploids are seed sterile, the induction of triploid plants would be beneficial in those plant species where seed lessness is applied for commercial importance. In addition, triploids have more vigorous growth than diploids. Moreover, triploid plants represent a significant resource for plant breeding of many commercially important plants (Thomas and Chaturvedi 2008). There are a few examples of triploid plant production in medicinal trees. Chaturvedi et al. (2003b) have reported triploid plant production in *Azadirachta indica* by immature endosperm culture. A total of 66% plants regenerated from endosperm callus in *A. indica* were found triploid. In another study, Thang et al. (2018) reported callus induction from immature endosperm culture and triploid plant production in *Melia azedarach*. They also found that all the plantlets (100%) regenerated from endosperms were triploid.

Artificial induction of polyploidization using in vitro culture technique is considered a significant plant breeding asset for crop improvement. Polyploids are usually superior to diploids in terms of productivity, adaptability against biotic and abiotic stresses and higher content of secondary metabolites (Bennici et al. 2006; Niazian and Nalousi 2020). Applying colchicine for artificial chromosome doubling (ACD) has been successfully adopted to generate polyploids in many agricultural, horticultural, medicinal and ornamental plants (Eng and Ho 2019; Niazian and Nalousi 2020). However, only a few reports are available on the induction of polyploidy in medicinal trees using in vitro culture technique. For instance, Zhang et al. (2020) reported tetraploidy induction in *Moringa oleifera* by treating leaf segments with colchicine. They also found that the colchicine-induced tetraploid plants exhibited superior agronomical traits when compared to diploid plants. Recently, Eng et al. (2021) obtained octoploid plants of *Neolamarckia cadamba* from colchicine-treated nodal explants. In comparison to natural tetraploid plants of *N. cadamba*, colchicine-induced octoploid plants showed superior morphological characteristics with thicker leaf blades, thicker midrib, lower stomata density and bigger stomata size.

In vitro conservation of medicinal trees

During the last two to three decades, in vitro culture technique played a vital role in plant germplasm conservation by reducing the growth rate of in vitro cultures for short- to medium-term storage and through cryopreservation for the storage in long-term (Lambardi and De Carlo 2003; Engelmann 2011). The application of tissue culture-based ex-situ conservation of genetic resources of medicinal trees has widely progressed in recent years.

Short- to- medium term conservation by slow growth storage

Slow growth storage is a tissue culture-based in vitro conservation technique in which plant tissues or germplasm can be stored under growth-limiting conditions (Chauhan et al. 2019). The main idea of this approach is to extend the subculture durations up to a few months to 1–2 years without affecting their survival and regeneration potential after storage. The strategies adopted under slow growth storage include low-temperature storage, use of minimal growth medium, applying growth retardants or osmoticum for limiting the growth of cultures (Engelmann 2011; Rajasekharan and Sahijram 2015; Chauhan et al. 2019; Rai 2022). In the case of medicinal trees, most reports are available on the short-term storage of germplasm at low temperatures, mostly at 4 °C (Table 2). For example, Scocchi and Mroginski (2004) successfully stored the apical meristem of *Melia azedarach* for 12 months at a low temperature (4 °C). In another study, shoot tips of *Pistacia lentiscus* could be stored at 4 °C for 12 months only in dark conditions (Koc et al. 2014). In recent years, synthetic seeds have been employed successfully for short- to medium-term conservation of germplasms of several medicinal, fruit and many other commercially important plant species by adopting different slow growth storage strategies. (Ara et al. 2000; Rai et al. 2009, 2021a; Sharma et al. 2013; Gantait et al. 2015; Faisal and Alatar 2019). More recently, Padilla et al. (2021) successfully stored encapsulated nodal segments of *Azadirachta indica* for four weeks at 12 °C when explants were pretreated with acetylsalicylic acid (ASA). They observed that recovery of cold-stored synthetic seeds was higher in ASA treated explants than the non-treated explants.

Table 2 In vitro conservation of some selected medicinal trees

Plant name	In vitro conservation method	Explants	Strategies adopted for in vitro conservation	References
Aesculus hippocastanum	Cryopreservation	Embryogenic callus	Vitrification	Lambardi et al. (2005)
Azadirachta indica	Synthetic seed and slow growth method	Nodal segments	Storage of encapsulated nodal segments at 12 °C with medium containing acetylsalicylic acid	Padilla et al. (2021)
Crateva nurvala	Cryopreservation	Axillary shoot tips	Vitrification	Sanayaima et al. (2006)
Garcinia indica	Normal growth	In vitro raised shoots	Storage under normal growth conditions at reduced BAP concentrations	Malik et al. (2005)
Hancornia speciosa	Cryopreservation	Shoot tips	Vitrification and droplet vitrification	Santos et al. (2015)
Hovenia dulcis	Cryopreservation	Shoot tips	V Cryo-plate technique	Saavedra et al. (2021)
Kalopanax septemlobus	Cryopreservation	Embryogenic callus	Vitrification and droplet-vitrification techniques	Shin et al. (2012)
Lepisanthes fruticosa	Cryopreservation	Embryonic axes	Vitrification and encapsulation vitrification	Suryanti et al. (2021)
Melia azedarach	Slow growth method	Apical meristem-tips	Low temperature storage at 4 °C on minimal growth medium	Scocchi and Mroginski (2004)
	Cryopreservation	Apical meristem-tips	Encapsulation-dehydration method	Scocchi et al. (2004)
	Cryopreservation	Somatic embryos	Encapsulation-dehydration and pregrowth-dehydration method	Scocchi et al. (2007)
Mimusops elengi	Cryopreservation	Embryonic axes	Desiccation of seeds followed by cryopreservation	Wen et al. (2013)
Nothapodytes nimmoniana	Cryopreservation	Embryonic axes with cotyledons	Dehydration under laminar airflow for 120 min before freezing in liquid nitrogen	Radha et al. (2010)
Parkia speciosa	Cryopreservation	Shoot tips	Encapsulation-vitrification in combination with trehalose preculture	Nadarajan et al. (2008)
	Cryopreservation	Zygotic embryonic axes	desiccation or vitrification	Sinniah and Gantait (2013)
Pistacia lentiscus	Slow growth method	Shoot tips	Low temperature storage at 4 °C in dark	Koc et al. (2014)

Long-term conservation by cryopreservation

Cryopreservation is one of the most viable and most acceptable techniques for conserving plant genetic resources for the long-term (Engelmann 2011; Reed et al. 2011; Rai 2022). In this technique, biological materials are stored at −196 °C, usually in liquid nitrogen. The ultra-low temperature suspends the plant cells' metabolic activities and cellular divisions, assuring the viability of germplasm for a longer duration (Engelmann, 2011; Sharma et al. 2019b).

During 1990s, cryopreservation primarily relied on slow cooling method. However, ice crystal formation in the cells of explants was a major hurdle in applying this technique. With the advancement in techniques, mainly based on encapsulation of explants, dehydration and vitrifying solutions, many cryopreservation techniques evolved and were used to conserve various plant species. The most applicable cryopreservation techniques in medicinal trees include vitrification, encapsulation-vitrification, and encapsulation-dehydration (Table 2). These techniques have been applied for the cryopreservation of several medicinal tree species, like, *Aesculus hippocastanum* (Lambardi et al. 2005), *Crateva nurvala* (Sanayaima et al. 2006), *Lepisanthes fruticosa* (Suryanti et al. 2021), *Melia azedarach* (Scocchi et al. 2004, 2007), *Nothapodytes nimmoniana* (Radha et al. 2010) and *Parkia speciosa* (Nadarajan et al. 2008; Sinniah

and Gantait 2013). Two new cryopreservation techniques, namely droplet vitrification and cryo-plate methods, have been evolved during the last two decades (Panis et al. 2005; Yamamoto et al. 2011; Niino et al. 2013). Both the methods are advanced and are now preferred due to ease in handlings, high regrowth rate and reduced risk of damage of explants during cryo-procedure (Wang et al. 2021). Droplet vitrification and cryo-plate methods developed in some medicinal tree species include *Hancornia speciosa* (Santos et al. 2015), *Hovenia dulcis* (Saavedra et al. 2021) and *Kalopanax septemlobus* (Shin et al. 2012).

Production of secondary metabolites from medicinal trees through in vitro culture technique

Many tree species are an excellent source of a wide range of secondary metabolites. These secondary metabolites, i.e., bioactive compounds, are pharmaceutically important and can be extracted directly from plants. Some secondary metabolites have high commercial values in pharmaceutical industries (Rao and Ravishankar 2002). However, low yield and production of secondary metabolites only at a specific developmental stage of specific parts are some major bottlenecks when isolated directly from a plant (Murthy et al. 2014). For example, taxol, an important secondary metabolite, produced from a tree, *Taxus* sp., can be harvested only from a mature tree. Its high yield can only be achieved from a tree that reaches about 60 years of age (Isah et al. 2018). In such a condition, in vitro culture technique plays an alternative and promising role in producing pharmaceutical bioactive compounds commercially and alleviating the overexploitation of plant sources (Chandran et al. 2020). This technique offers an opportunity to exploit cells, tissues and organs for the controlled production of numerous secondary metabolites. In addition, the secondary metabolites produced by in vitro culture technique are similar to those produced by whole plants. Moreover, the production of secondary metabolites by tissue culture technique is not affected by seasons or any environmental fluctuations (Rao and Ravishankar 2002; Murthy et al. 2014; Isah et al. 2018; Shasmita et al. 2018; Silpa et al. 2018; Chandran et al. 2020).

Different types of culture system have been adopted for the production of secondary metabolites in medicinal trees. In many cases, cell suspension and callus culture have been established for the production of secondary metabolites (Table 3). In a few medicinal trees, secondary metabolites are produced by hairy root culture or organ culture (Hussain et al. 2022). Several reports on medicinal trees indicate that the production of secondary metabolites through in vitro culture technique highly depends on the source of explants, types of medium, alteration in nutritional composition of medium, types and concentrations of PGRs or use of biotic and abiotic elicitors (Table 3).

Production of some valuable bioactive compounds from medicinal trees: case studies

Azadirachtin

Azadirachtin is tetraterpenoid and one of the most prominent bioactive compounds of neem tree (*Azadirachta indica*). Azadirachtin is well known for its antimalarial and insecticidal activity and many pharmacological applications. In recent years, the potential of azadirachtin as an effective insecticide has gained attention, especially due to its insecticidal activity against more than 500 insect species (Thakore and Srivastava 2017). The potentiality of in vitro culture technology in the production of azadirachtin is much explored as several papers published on the production of azadirachtin using callus, cell suspension or hairy root culture in recent two decades. A wide range of bioreactors have also been designed for scale up of cell suspension or hairy root culture for the production of azadirachtin (Prakash and Srivastava 2007; Srivastava and Srivastava 2013). Different factors affecting azadirachtin production in vitro using callus and cell suspension culture were studied by many workers. Sujanya et al. (2008) selected an elite variety of neem crida-8 for azadirachtin production using cell suspensions culture. They demonstrated the effect of nutritional alteration, i.e., altered medium with different nitrate: ammonium ratio on azadirachtin production. Srivastava and Chaturvedi (2011) quantified high levels of azadirachtin from leaves of in vitro grown haploid plantlets derived from anther callus. In another study, Singh and Chaturvedi (2013) examined the azadirachtin accumulation in callus induced from different explants i.e., zygotic embryo, leaf, and ovary. They found that zygotic embryos accumulated the highest amount of azadirachtin. Rodrigues et al. (2014) studied the effect of different factors on the production of azadirachtin in calli induced from cotyledons and observed the highest azadirachtin production on agitated liquid woody plant medium (WPM) medium supplemented with glucose, hydrolysed casein and an elicitor, methyl jasmonate. More recently, Ashokhan et al. (2020) demonstrated the effect of two PGRs TDZ and 2,4-D on azadirachtin production in callus induced from leaf and petiole.

Plants produce a wide range of secondary metabolites in response to biotic and abiotic stresses. Nowadays, many biotic and abiotic elicitors are also used to stimulate secondary metabolites in cells, callus, or organ cultures (Rao and Ravishankar 2002). The addition of a number of biotic and abiotic elicitors, i.e., fungal culture filtrate, jasmonic acid, salicylic acid, yeast extract and chitosan in medium

Table 3 Production of bioactive compounds from medicinal trees through in vitro culture technique

Plant name	Name of bioactive compound	Nature of culture	Factors effecting the secondary metabolite contents	References
Azadirachta indica	Azadirachtin	Cell suspension culture	Azadirachtin production in stirred tank reactors	Prakash and Srivastava (2007)
		Hairy root culture	Media optimization, biotic elicitors and abiotic elicitors jasmonic acid and salicylic acid	Satdive et al. (2007)
		Cell suspension culture	An altered medium with a nitrate: ammonium ratio	Sujanya et al. (2008)
		Callus culture & leaves of in vitro grown plantlets	Azadirachtin estimation from haploid cell line	Srivastava and Chaturvedi (2011)
		Callus culture	Azadirachtin accumulation in callus induced from different explants	Singh and Chaturvedi (2013)
		Hairy root culture	Liquid-phase bioreactors	Srivastava and Srivastava (2013)
		Hairy root culture	Abiotic and biotic elicitors salicylic acid and fungal culture filtrate of *Curvularia lunata* and biosynthetic precursors cholesterol	Srivastava and Srivastava (2014)
		Callus culture	Agitated woody plant medium (WPM) liquid medium supplemented with glucose (Gl), hydrolyzed casein (HC) and methyl jasmonate (MeJ)	Rodrigues et al. (2014)
		Colored callus culture	Effect of two PGRs TDZ and 2,4-D	Ashokhan et al. (2020)
	Azadirachtin, nimbin, salannin, 3-acetyl-1-tigloylazadirachtinin and 3-tigloylazadirachtol	Hairy root cultures from stem and leaf explants	–	Allan et al. (2002)
	Azadirachtin-A, nimbin and salannin	Callus and cell suspension cultures	Source of different explants for callus and cell suspension and accumulation of secondary metabolite	Babu et al. (2006)
	Azadirachtin -related limonoid	Cell suspension culture	Effect of sucrose, nitrate and phosphate and three elicitors chitosan, salicylic acid, and jasmonic acid	Vásquez-Rivera et al. (2015)
	Azadirachtin, mevalonic acid and squalene	Cell suspension culture	Yeast extract and chitosan elicitation	Farjaminezhad and Garoosi (2021a, b)
Balanites aegyptiaca	Saponins	Callus culture	Larvicidal effect of saponins obtained from root derived callus	Chapagain et al. (2008)
Camptotheca acuminata	Camptothecin	Callus culture	Light and culture conditions	Park et al. (2003)
	Camptothecin and 10-hydroxycamptothecin	Shoots and leaves from shoot culture, callus and somatic embryos and cell suspension cultures	Different types of cultures grown either in a temporary immersion system or on solid medium	Sankar-Thomas and Lieberei (2011)
Melia Azedarach	Limonoid	Callus and cell suspension cultures	Source of different explants for callus and cell suspension and accumulation of secondary metabolite	Rind et al. (2021)

Table 3 (continued)

Plant name	Name of bioactive compound	Nature of culture	Factors effecting the secondary metabolite contents	References
Nothapodytes foetida syn *N. nimmoniana*	Camptothecin	Callus culture	Influence of medium composition	Thengane et al. (2003)
		In vitro and in vivo plantlets	Different parts of in vitro and in vivo plantlets	Dandin and Murthy (2012)
		Cell culture	Culture medium optimization	Karwasara and Dixit (2013)
		Callus culture	Yeast extract and vanadyl sulfate elicitors	Isah (2017)
		Cell suspension cultures	Use of five different elicitors chitin, chitosan, pullulan, glutathione, and jasmonic acid	Keshavan et al. (2022)
Taxus baccata	Taxol and baccatin	Cell suspension cultures	Effect of elicitor squalestatin	Amini et al. (2014)
	Taxol	Callus culture	Effects of the basal medium, auxin and antioxidants	Toulabi et al. (2015)
	Taxane	Callus culture	Salicylic acid and glucose	Sarmadi et al. (2018)
	Taxane	Callus culture	Polyethylene glycol	Sarmadi et al. (2019)
Taxus chinensis	Taxol	Cell suspension cultures	Oxidative stress and fungal elicitor	Yu et al. (2002)
	Taxol	Cell suspension cultures	Salicylic acid elicitation	Wang et al. (2007)
	Taxol	Cell suspension cultures	Chitosan elicitation	Zhang et al. (2007)
Taxus cuspidata	Taxol	Cell suspension cultures	Selection of stable and high taxol producing cell lines	Wang et al. (2018)
Taxus media	Taxol	Cell culture	Use of elicitor coronatine and calix[8]arenes	Escrich et al. (2021)

improved the production of azadirachtin in callus, cell suspension or hairy root culture of *A. indica* (Satdive et al. 2007; Srivastava and Srivastava 2014; Vásquez-Rivera et al. 2015; Farjaminezhad and Garoosi 2021a, b). In many plant species, hairy root culture is a promising technique for secondary metabolite production, especially in the case of root-specific secondary metabolites (Silpa et al. 2018). Some researchers have also reported the enhanced production of azadirachtin by hairy root culture (Allan et al. 2002; Satdive et al. 2007; Srivastava and Srivastava 2014). Apart from azadirachtin, some other bioactive compounds such as nimbin, salannin, 3-acetyl-1-tigloylazadirachtinin and 3-tigloylazadirachtol, etc., have also been produced from *A. indica* using in vitro culture technique (Allan et al. 2002; Babu et al. 2006; Vásquez-Rivera et al. 2015; Farjaminezhad and Garoosi 2021a, b).

Camptothecin

Camptothecin, a monoterpene indole alkaloid and cytotoxic compound, is a potent anticancerous drug and very popular in pharmaceutical industries after taxol and vinca alkaloids.

Camptothecin was first isolated in Camptotheca acuminata and later in other tree species, like, *Nothapodytes foetida* syn *N. nimmoniana* (Isah et al. 2018). Using in vitro culture technique, camptothecin was produced in two tree species *C. acuminata* and *N. foetida*. Park et al. (2003) optimized light and culture conditions for enhanced production of camptothecin in callus culture of *C. acuminata*. Sankar-Thomas and Lieberei (2011) determined the camptothecin content in different cultures of *C. acuminata*, i.e., ex situ and in vitro seedlings and different stages of somatic embryos grown on solid medium or in a temporary immersion system. They found highest camptothecin content in shoots grown in temporary immersion system. In *N. foetida*, few studies have also shown enhanced production of camptothecin by the influence of medium composition (Thengane et al. 2003; Karwasara and Dixit 2013). In another study, Dandin and Murthy (2012) compared the camptothecin content in acclimatized (ex vitro) plants developed on solid and liquid medium and in vivo plants. This study has shown the highest camptothecin content in leaves of ex vitro plants developed on solid medium. In order to increase camptothecin production in the callus and cell suspension culture of *N. nimmoniana*, several elicitors were employed by Isah (2017) and Keshavan et al. (2022). Isah (2017) reported the increased production of camptothecin in callus culture of *N. nimmoniana* by yeast extract and vanadyl sulfate elicitors. In another study, cell suspension culture of *N. nimmoniana* treated with five different biotic and abiotic elicitors for enhanced camptothecin production showed that the biotic elicitor chitin treated cell suspension culture produced the highest camptothecin (Keshavan et al. 2022).

Taxol

Taxol (generic name—paclitaxel), a tetracyclic diterpene, is considered as the most well-known anticancer drug isolated from plants. Many species of conifer, *Taxus*, are the natural source of taxol. Many pharmacological studies revealed that taxol can directly kill the tumour cells (Sabzehzari et al. 2020). Owing to prominent anticancerous activity, taxol has huge commercial value in pharmaceutical industries and its demand is increasing day by day. No other plant-based anticancer drug has generated as much public interest as taxol (Liu et al. 2016). During the last three decades, considerable progress has been made on the taxol production from different *Taxus* species, including *T. cuspidata*, *T. brevifolia*, *T. baccata, T. chinensis*, *T. cuspidate*, etc. using in vitro culture technique (Liu et al. 2016). Other than taxol, another secondary metabolite 'taxane' has also been isolated from *Taxus* sp. using cell culture technique. Fett-Neto et al. (1992) extracted taxol first time from callus tissues of *T. cuspidata*. Taxol and taxane production enhanced in callus or cell suspension culture of different *Taxus* species by selection of high-yield cell lines (Wang et al. 2018), optimization of culture medium and PGRs (Toulabi et al. 2015), carbohydrate source (Sarmadi et al. 2018) and use of a number of biotic and abiotic elicitors, like, salicylic acid (Wang et al. 2007; Sarmadi et al. 2018), chitosan (Zhang et al. 2007), polyethylene glycol (Sarmadi et al. 2019), squalestatin (Amini et al. 2014), coronatine and calix[8]arenes (Escrich et al. 2021) or by oxidative stress and fungal elicitor (Yu et al. 2002). In vitro culture technique is a promising alternative taxol production method as taxol can be produced only from the bark of the tree and its high yield is achieved only from a mature tree (Liu et al. 2016).

Conclusions

During the last three to four decades, considerable progress has been made on the propagation, conservation and secondary metabolite production in several medicinally important tree species using in vitro culture approaches. Despite significant progress, several factors limit the use of this technology for its commercialization. In recent years, many problems associated with the tissue culture of tree species have been addressed by many modifications and refinements in technologies. For example, replacement of in vitro rooting technique by ex vitro rooting helps in better acclimatization and higher percent survival of tissue culture raised plants. Similarly, more advanced droplet-vitrification and cryo-plate methods are now preferred over traditional slow cooling or vitrification method of cryopreservation for the conservation of many tree species, which eradicate the risk

of damage and loss of regeneration potential after storage. Instead of optimizing PGRs and medium composition, the use of many biotic and abiotic elicitors, hairy root culture, or scale-up of bioreactor technologies have now gained interest in secondary metabolite production at a commercial scale. The production of some secondary metabolites i.e., taxol, camptothecin and azadirachtin, from trees at a commercial scale through cell and tissue culture is highly appreciated. Such success will also encourage exploiting in vitro culture technology for large-scale propagation, conservation, and secondary metabolite production in other medicinal trees.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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