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In Vitro Propagation of Commonly Used Medicinal Trees in Korea

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Abstract

Forest medicinal resources, which constitute one of the non-timber forest products, have been regarded as healthy and highly valued products. To meet the increasing demand of the medicinal resources, it is necessary to improve the propagation methods of medicinal plants. *In vitro* propagation not only allows an opportunity for propagating plants in large numbers but also allows for enhancing the quality and quantity of the desired functional component of a plant by altering the growth factors, such as medium, carbon source, and plant growth regulators influence plant. There have been several studies of *in vitro* propagation methods, such as axillary bud culture, shooting, and embryogenesis, on *Kalopanax septemlobus*, *Eleutherococcus sessiliflorus*, *Hovenia dulcis*, and *Schisandra chinensis* in Korea between from 2000 through 2010. Furthermore, there have been attempts to proliferate callus and plantlets for producing useful natural compounds by using bioreactors. Here, we provide an account of the *in vitro* propagation methods of medicinal trees in Korea based on a review of several micropropagation studies.

Key Words: embryogenesis, *in vitro* propagation, medicinal tree, micropropagation, regeneration

Introduction

Globally, the biotechnology industry has emerged asa future industry, and its domestic and foreign market is on an increasing trend. Efforts to establish the sovereignty of bio-resources that are the source of the bioindustry and utilize them as useful materials are continuing (Jin 2015). Forests are an important source of Korea's bioindustries , and the number of published papers related to the component analysis and plant biotechnology in the Journal of Korean Forest Society has considerably increased since 2010 (Lee et al. 2015). The increasing interest in health for improving the life quality has led to increasing demand for forest medicinal resources, especially, the environmentally friendly natural materials.

The first step to improve the quality of forest medicinal resources is to improve the quality of seeds and seedlings. Besides, seeds (and seedlings) have low propagation rates that affect their production and supply, and hence, the plants are mostly vegetatively propagated (Jang et al. 2013; Kim 2017; Cheong 2018). However, in vegetative propagation, it is difficult to flexibly defend to the demand for new varieties. Further, vegetatively propagated plants (e.g., *Cnidium officinale* and *Paeonia suffruticosa*) that are infected with pathogens such as viruses and bacteria have weak vitality and are less robust; thus, it is important to produce and propagate healthy seedlings (Fisher 2012; Yoo et al. 2015). Paek et al. (1998), while studying a viral infection in tissue-cultured and vegetatively propagated plants, reported that the infection affected 100% of the vegetatively

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propagated plant compared to 0–45% of the tissue-cultured plants. To tackle the problem of infection in vegetatively propagated species, studies for the production of disease-free seedlings in crops such as potato and garlic were also carried out (Conci et al. 2003; Clack and Hoy 2006). Thus, studies are required on *in vitro* propagation technologies of forest medicinal resources, based on clonal propagation and virus verification.

In vitro culture technology for mass propagation has been used in various species particularly in the case of vegetatively propagated ones (Kim et al. 2013; Lee et al. 2015, Cheong 2019). In forests, plant tissue culture was mainly performed on woody plants, including poplar, *Quercus* spp., and *Tilia* spp. (Hyun et al. 1991; Moon and Youn 1996; Cheong and Moon 1999). However, tissue culture studies in medicinal plants are still insignificant when compared to those on economic trees.

Therefore, in this study, we review the trends in the *in vitro* culture research of the commonly used medicinal trees in Korea including the items supported by income sources of forestry, healthy seedlings of forests and medicinal herbs, and research on the production of healthy seedlings. Through this review, we also aim to improve the quality of materials available for the production of healthy seedlings.

Current Status

Lycium chinense Mill.

Vegetative and sexual propagation methods are commonly applied in *L. chinense*. *In vitro* culture studies on the multiplication, callus induction, adventitious shoot induction, embryogenesis and artificial seeds have been performed on this species (Kim et al. 1996; Kim et al. 2002; Lee et al. 2002; Jo et al. 2004; Yoon et al. 2006); multiple shoots were obtained from its cotyledon and hypocotyl on MS medium with 1 mg L^{-1} kinetin and 0.05 mg L^{-1} indole-3-butyric acid (IBA) (Lee et al. 2001).

Cudrania tricuspidata (Carr.) Bureau ex Lavallée

Generally, *C. tricuspidata* is propagated by sowing the seeds either directly after being removed from the fruits or the next year after storage; it is also propagated from the roots and shoots. *In vitro* culture studies were able to induce and regenerate *C. tricuspidata* plantlets, via, shooting and rooting. Cell culture conditions has been studied to mass-produce the active substance *in vitro*. The maximum number of shoots was obtained from axillary shoot explants in MS medium with 1 mg L^{-1} 6-benzylaminopurine (BA) (Lee et al. 2007); the roots were induced from the shoots in MS medium with IBA, and the plantlets were transferred to the soil. To efficiently produce gericudrain, a novel active substance found in the plant in a trace amount, through cell culture, cell proliferation conditions and gericudrain content in callus cultures had been examined (Choi et al. 2001).

Eucommia ulmoides Oliv.

E. ulmoides bears fruit after 10 years of planting. Cuttings of this species have a low rooting rate, and therefore, for the propagation of superior clones, alternative propagation methods are needed. *In vitro* plant regeneration of *E. ulmoides* was achieved through adventitious shoot induction from the *in vitro* germinated seeds; the adventitious shoots were induced from hypocotyl on MS medium with 10 μM BA, and roots were induced on 1/2 MS medium with 1-naphthaleneacetic acid (NAA), subsequently, leading to the regeneration of normal plantlets (Chen et al. 2008).

Sorbus commixta Hedl.

Few studies have discussed the *in vitro* propagation of *S. commixta* as it can be easily propagated via sexual and asexual methods. Although mass production of its superior clones can be done with the available methods, studies on the shoot and root induction from its stem explants are being carried out. In a study by Shung (2010), shoots were induced from stem explants on MS medium with BA and IBA combined, and these were transferred to 1/2 MS medium with IBA and NAA for rooting.

Paeonia suffruticosa Andrews

The callus was induced from the rhizomes, leaves, and stem of *P. suffruticosa*; callus induction rate was found to depend on the physiological and genetic factors of the plant, such as variety and explant type (Wang and Stden 2001). And effect of light emitting diode on callus formation was studied (Wang et al. 2010).

Calcium, citric acid, and ascorbic acid were added to the McCown Woody Plant medium (WPM) to induce shoots;

the genetic factors of the plant were found to affect its propagation ability and contribute to necrosis and hyperhydricity of the *in vitro* cultures (Beruto et al. 2004). Also activated charcoal into the medium was efficient to develop roots, which led to decrease almost 3-fold IAA contents in roots (Bouza et al. 1994).

Acer tegmentosum Maxim

It is not difficult to propagate *A. tegmentosum* through sexual propagation, but asexual propagation of this species is not that successful. Multiple shoots were induced on WPM with 1 mg L^{-1} zeatin, and they were further grown into plantlets on WPM with 2,4-dichlorophenoxyacetic acid (2,4-D) (Seo et al. 2016).

Cornus officinalis Siebold & Zucc.

In practice breeding, seeds of *C. officinalis* without pulp are sown in soil either immediately or after storage in autumn. Axillary shoots were found to be more responsive than the seeds in the initial culture stage. Murashige and Skoog medium (MS) and Driver & Kuniyuki and McGranahan, et al medium (DKW) formed shoots in over 76% of the explants (Park et al. 1993). Xue et al. (2003) found that the *in vitro* shoots of *C. officinalis* rooted in 1/2 MS medium with 1 mg L^{-1} IBA and 0.1 mg L^{-1} BA and subsequently differentiated into normal plantlets. They also found that five cultivars of this species could be propagated *in vitro* using WPM with 2 mg L^{-1} BA, 0.1 mg L^{-1} zeatin, and 0.1 mg L^{-1} NAA (Xue et al. 2003).

Eleutherococcus sessiliflorus (Rupr. & Maxim.) SY Hu

It takes two years for the *E. sessiliflorus* seeds to germinate and requires scarification to break the seed dormancy. Embryogenic callus was induced from petioles, stems, and roots explants, and the induced callus was regenerated into plantlets. Embryogenic callus was induced from root explants in MS medium with 2,4-D; it was regenerated into normal plantlets in MS medium without any PGR (Yang et al. 2012). Direct somatic embryos were induced in 2,4-D containing medium, which later on developed into normal plantlets (Choi et al. 2002). Bioreactor studies have been performed to increase the proliferation efficiency of the *in vitro* cultures and to produce useful substance through embryogenic callus and somatic embryo germination in regenerating *E. sessiliflorus* seedlings (Shohael et al. 2005, 2006, 2007; Yang et al. 2012; Shohael et al. 2013).

Schisandra chinensis (Turcz.) Baill

Somatic embryos of *S. chinensis* were developed from zygotic embryos using abscisic acid and polyethyleneglycol (PEG); the concentration of IBA in the medium was successively increased until the development of somatic embryos was noticed (Smiskova et al. 2005). In other studies, thidiazuron (TDZ) and 2,4-D were found to be effective for callus formation from zygotic embryos, anther (Lee and Lee 1997), and zeatin promoted the embryogenic callus formation (Sun et al. 2013). Besides studying the conditions required for embryogenic callus proliferation, Sun et al. (2013) also studied somatic embryo induction and plant regeneration in *S. chinensis*. The maximum number of somatic embryos were found in 1/3 MS medium with 1% sucrose and 0.5 mg L^{-1} BA, and it resulted in 52% regeneration of the normal plantlets (Chen et al. 2010).

Rhus verniciflua Stokes

R. verniciflua seeds must break the impermeability of seeds coat to germinate. In the *in vitro* culture studies, after sterilization, the seeds were treated with sulfuric acid to remove the lateral seed coat. They were then kept for germination on a BA and NAA combined medium, and the germination rate of approximately 10% was obtained (Doo et al. 2000).

Kalopanax septemlobus (Thunb.) Koidz.

Cytokinin treatment was needed to induce multiple shoots from the newly developed axillary buds in 2-year-old *K. septemlobus* seedlings. Each explant formed 2-3 shoots on WPM with 0.5 mg L^{-1} kinetin (Moon et al. 2002). Embryogenesis was reported to obtain normal plantlets through embryogenic callus and somatic embryo formation; embryogenic callus was induced from the leaf and petiole explants on 1/2 MS medium with 2 mg L^{-1} 2,4-D and 0.1 mg L^{-1} BA, and the same medium with 1 mg L^{-1} 2,4-D was found to be suitable for suspension cultures (Kim et al. 2008). The culture period and density were controlled and improved by Kim and Moon (2009) to obtain torpedo-stage somatic embryos, and they were able to ob-

tain up to 65% of torpedo-stage embryos in suspension culture. The effects of carbon source, PEG, and coconut water on the induction of somatic embryos in *K. septemlobus* have been reported (Moon et al. 2008). The cultures were subjected to osmotic stress, high concentration of 2,4-D, low temperature, and malnutrition to improve the induction rate of somatic embryos, and it was found that 23% of somatic embryos were induced through the immersion treatment with 1M sucrose solution for 6 hr (Moon et al. 2015). There are reports on artificial seed production (Kim et al. 2007) and on the effect of low temperature on the somatic embryos of *K. septemlobus* (Lee et al. 2015); studies employing temperature immersion bioreactor (Kim et al. 2011) and genotypic variation and age (Park et al. 2011) autotrophism (Park et al. 2011) for regenerating plantlets have also been reported.

Cedrela sinensis Juss.

A light green color embryogenic callus was induced from young internodal explants of *C. sinensis* on MS medium with 2,4-D (Choi et al. 1986; Soh et al. 1990). It was developed to a large number of globular somatic embryos on an IAA and kinetin-containing medium. Almost all somatic embryos differentiated into normal plantlets on MS medium without any plant growth regulators (PGRs), but some developed callus and did not have cotyledon or normal leaf (Choi et al. 1986). Additionally, studies on artificial seeds have been performed using somatic embryos by coating the seeds with sodium alginate (Soh et al. 1990).

Zanthoxylum piperitum (L.) DC.

Multiple shoots were obtained up to 98% of *Z. piperitum* explants on MS medium with 0.5 mg L^{-1} BA (Hwang and Hwang 2003). Yellow-green callus was induced in MS and Gamborg's B5 medium (B5) combined medium with 2 mg L^{-1} 2,4-D, BA, and NAA, which later developed shoots (Lim et al. 1993). Adventitious embryos were found to develop through embryogenic callus induction (Song et al. 1991), and malt extract, activated charcoal, sucrose, 2,4-D, ammonium nitrate, and potassium nitrate were found to be effective in increasing the adventitious embryo development (Song et al. 1991; Song and Chi 1995).

Hovenia dulcis Thunb.

During sexual propagation, the seeds of *H. dulcis* have to be scarified and the seed coats have to be removed for germination. In the *in vitro* propagation studies, fruits without pericarp were dipped in sulfuric acid for 20-40 min before surface sterilizing them with ethanol and sodium hypochlorite. The embryos were then removed and used as explants (Eom et al. 2002; Park et al. 2012). The axillary buds from mature trees and seedlings have also been used as explants in the initial culture studies on *H. dulcis* (Echeverrigaray et al. 1998). Propagation of axillary bud was found to be effective on WPM with 1 mg L^{-1} kinetin, or MS medium combined with 0.1 mg L^{-1} NAA and 0.1 mg L^{-1} TDZ (Park et al. 2012). In another study, the number of shoots was highest on MS medium with 1 mg L^{-1} BA and shoot elongation was obtained in 2X concentrated MS medium without any PGR (Park et al. 2006); a considerable amount of roots was induced in 1 mg L^{-1}in a short period (Park et al. 2012). Li et al. (2006) have reported induction of shoots from leaf explants with 2,4-D and kinetin treatments. A growth medium enriched with GA_3 was found to regenerate normal plantlets from zygotic embryo explants while studying embryogenesis in *H. dulcis* (Eom et al. 2002). Also plant regeneration protocol for shoot organogenesis from callus cultures was established (Jeong et al. 2009). The concentration of total polyphenols and flavonoids were found to be higher in the callus than in the wild type plant, however, the DPPH radical scavenging ability was higher in the extracts of the wild type plant than in the callus (Ribeiro et al. 2015).

Euonymus alatus (Thunb.) Siebold

The hypocotyl explants obtained from the *in vitro* germinated seeds of *E. alatus* were used to regenerate plantlets through callusing and adventitious shoot formation; also, its young leaves and floral organs were used to study the suitability of the medium for *in vitro* propagation. The embryogenic callus that was induced from leaf explants was differentiated into somatic embryos; adventitious shoots were induced from the hypocotyl explants of *E. alatus* on MS medium with 2% sucrose, 0.01 mg L^{-1} NAA and 0.1-0.5 mg L^{-1} BA, and it was developed into plantlets through multiple shooting and rooting processes (Smith

Table 1. Review of *in vitro* propagation of medicinal trees

Species	Explants	Regeneration method	Reference
Lycium chinense	*In vitro* plant	Adventitious shoots	Yoon et al. (2006)
	In vitro plant, Young leaf	Callus, Shooting	Kim et al. (2002)
	In vitro plant	Adventitious shoot	Jo et al. (2004)
	In vitro plant	Shooting	Lee et al. (2001)
	In vitro plant	Somatic embryo, Artificial seed	Kim et al. (1996)
	Zygotic embryo	Somatic embryo, Adventitious root	Lee et al. (2002)
	In vitro plant (leaf)	Hairy root, Bioreactor	Bae et al. (2004)
Cudrania tricuspidata (Carr.) Bureau ex Lavallee	*In vitro* plant	Shooting, Rooting	Lee et al. (2007)
Eucommia ulmoides Oliv.	*In vitro* plant (hypocotyl)	Adventitious shoot	Yoon et al. (2006)
Sorbus commixta Hedl	Stem	Shooting, Rooting	Shung (2010)
Paeonia suffruticosa Andrews	Axillary buds, Filament, Petal	Shooting, Adventitious shoot	Beruto et al. (2004)
	Petiole, Leaf	Callus	Wang and Staden (2001)
	Petiole, Leaf	Callus	Wang et al. (2010)
	Axillary bud	Shooting, Rooting	Bouza et al. (1994)
Acer tegmentosum Maxim	Internode	Shooting, Rooting	Seo et al. (2016)
Cornus officinalis Siebold & Zucc	Axillary bud	Shooting	Park et al. (1993)
	Stem	Adventitious shoot	Xue et al. (2003)
Eleutherococcussessiliflorus (Rupr.& Macim.) S.Y.Hu	Embryogenesis	Production of substance	Shohael et al. (2003; 2006, 2007)
	Leaf	Somatic embryo	Choi et al. (2002), Shohael et al. (2005) Yang et al. (2012)
Schisandra chinensis (Turcz.) Baill.	Zygotic embryo	Somatic embryo	Smiskova et al. (2005)
	Zygotic embryo	Somatic embryo	Sun et al. (2013)
	Zygotic embryo	Somatic embryo	Chen et al. (2010)
	anther	Callus	Lee and Lee (1997)
Rhus verniciflua Stokes	Seeds	Germination	Doo et al. (2000)
Kalopanax septemlobus (Thunb.) Koidz.	Axillary buds	Shooting, Regeneration	Moon et al. (2002)
	In vitro plants (leave, petiole)	Embryogenic callus	Kim et al. (2008)
	Zygotic embryos	Embryogeinc callus, Somatic embryo	Kim and Moon. (2009)
	Shoots of grafted nursery plant	Somatic embryo, regeneration	Moon et al. (2008)
	Somatic embryo	Regeneration	Kim et al. (2011)
	Embryogenic callus	Somatic embryo	Park et al. (2011)
	In vitro plants	Somatic embryo, regeneration	Moon et al. (2015)
	In vitro plants from somatic embryos	*In vitro* plants growth on autotrophism	Park et al. (2011)
	Embryogenic callus	Somatic embryo	Lee et al. (2015)
	Somatic embryos	Artificial seeds	Kim et al. (2007)
Cedrela sinensis Juss.	Internode	Embryogeneic callus, Somatic embryos, Artificial seeds	Soh et al. (1990)
	Stem	Somatic embryos, Regeneration	Choi et al. (1986)

Table 1. Continued

Species	Explants	Regeneration method	Reference
Zanthoxylum piperitum (L.) DC.	*In vitro* plants (shoot tip)	Shooting, Rooting	Hwang and Hwang (2003)
	In vitro plants (shoot tip),Internode	Callus, adventitious shoot	Lim et al. (1993)
	Apical meristem, leave	Embryogenic callus, Somatic embryo	Song et al. (1991)
	Apical meristem, leave	Embryogenic callus, Somatic embryo	Song and Chi (1995)
Hovenia dulcis Thunb.	Axillary buds	Regeneration	Echeverrigaray et al. (1998)
	Zygotic embryos	Somatic embryo, Regeneration	Eom et al. (2002)
	In vitro plant (leave)	Callus, regeneration	Jeong et al. (2009)
	Embryogenic callus	Callus proliferation, Somatic embryo	Li et al. (2006)
	Callus	Antioxidant activity test	Ribeiro et al. (2015)
	Zygotic embryo	Embryogenic callus, Somatic embryo	Yang et al. (2012)
Euonymus alatus (Thunb.) Siebold	Zygotic embryo	Callus, Regeneration	Thammina et al. (2011)
	In vitro plant (hypocotyl)	Adventitious shoot	Smith and Jernstedt (1989)
Dendropanax morbiferus H. Lev	*In vitro* plant (apical meristem)	Shooting, Rooting	Choi and Yun (2001)
	In vitro plant	Adventitious root	Bae et al. (2009)

and Jernstedt 1989). Also in vitro regeneration protocol for prodution of triploid E. alatus using endosperm was successfully established (Thammina et al. 2011).

Dendropanax morbiferus H. Lév.

The *in vitro* propagation of *D. morbiferus* from seeds requires them to be stored at low temperature and soaked, similar to the treatment given to the seeds in sexual propagation, before surface sterilizing them by using ethanol and sodium hypochlorite (Bae et al. 2009). MS medium with 0.1-1 mg L^{-1} BA and 0.5-1 mg L^{-1} NAA was found to be suitable for shooting; and the shoots were rooted on MS medium with 1 mg L^{-1} NAA (Choi and Yun 2001). To induce adventitious roots, bioreactor studies were carried out by using the *in vitro* grown shoots as explants and by using different types and concentrations of auxins (Bae et al. 2009).

Conclusion

In vitro propagation techniques are used as alternative methods for the propagation of plant species that are difficult to propagate by conventional methods (Table 1). Further, studies on eliminating viruses in plants are being carried out to produce virus-free plants in crop and fruit trees, including citrus, potato, and grape. Tree species need a long time to mature and flower from the seedling stage and spend a considerable amount of their life in the juvenile stage; this can affect breeding programs. The juvenile stage is regulated by environmental and genetic factors, and it is important to achieve maturity from the juvenile stage as soon as possible. In this respect, clonal propagation using *in vitro* culture is the preferred method to screen for and obtain plants that express the desired characteristics. Various factors including PGRs, carbon source, osmotic stress, and explant type affect the growth of the *in vitro* propagated plants. Therefore, it is necessary to understand the *in vitro* culture conditions that have already been investigated based on the physiological requirements of the plants and the tissue culture techniques.

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