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REVIEW ARTICLE

Plant tissue culture: a biological tool for solving the problem of propagation of medicinally important woody plants- A review.

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The world has a very rich biodiversity of woody plants, many of which are medicinally important. Because of the use in medicine, woody plants require rapid and reliable methods of propagation. The conventional methods of propagation such as cuttings, graftings and layering are very slow. The rapid loss of rooting ability with age of woody plants makes them difficult to propagate. So, they require alternative method. Plant propagation by tissue culture is the possible approach to overcome the problem. Plant tissue culture technique allows mass multiplication and propagation under aseptic conditions and it is not dependent on the season for the availability of plant material. Also, it offers a viable tool for meeting the pharmaceutical needs. In the present report, efforts are made to solve the problems of propagation of medicinally important woody plants through tissue culture technique.

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INTRODUCTION

Medicinal plants play an important role in human life to fight diseases since time immemorial. The World Health Organization has estimated that up to 80% of people still rely on herbal remedies for their health care (Afolayan and Adebola, 2004). All the major system of medicine, such as Allopathy, Homeopathy, Unani and Ayurveda, use most of the drugs obtained from plants. Most of these medicines are actually the byproducts of various processes of plants and each plant species produces its own characteristic chemicals. Medicinal properties of various woody plants have been described in ancient manuscripts. In India the earliest reference of woody medicinal plants is available in the Rigveda, Atharavaveda, Charak Samhita and Sushruta Samhita. Over the past few years, the medicinal plants have received a wide acceptance due to the faith in herbal medicine in view of its lower side-effects as compared to allopathic medicine. Consumption of herbal medicines is widespread and increasing. According to World Health Organization, Global market size for herbal and medicinal plants is estimated at 14 billions US dollars per year and likely to increase more than 5 trillion US dollars in 2050 (Kala *et al*., 2006). The article emphasize on woody medicinal plants, some of which are shown in the Table 1.

Woody plants are more difficult to propagate than herbaceous species. The currently followed methods of propagation of woody species are cuttings, graftings and layering. But these methods have been less successful with woody plants. It is due to the rapid loss of rooting ability with age of woody plant and the limited number of propagules that can be obtained in a reasonable time (Thorpe and Harry, 1990). Therefore, the conventional methods of propagation of woody plants have limited potential for large scale production. But, due to their commercial importance and extensive use in medicine there is a need to develop rapid and reliable methods of propagation of woody plant species.

Plant propagation by tissue culture is possible approach to overcome the problem. Plant tissue culture is the technique of growing plant cell, tissue and organ in an artificially prepared nutrient medium, semi-solid or liquid under aseptic conditions. It is based on the principle of totipotency. Plant tissue culture technique allows mass

multiplication and propagation under aseptic conditions. It is not dependent on the season for the availability of plant material. Tissue culture technique allows obtaining a large number of plants from limited source available. Also, it offers a viable tool for meeting the pharmaceutical needs. In the present report, efforts are made to solve the problems of propagation of medicinally important woody plants through tissue culture technique.

1. Tools of Plant tissue culture technique

2a. Explants

Explant is a piece of tissue used to initiate tissue culture. It may be in the form of shoot tips, nodes, internodes, leaf tissues, petioles, root tips, anther, embryo etc. But explants from actively growing region, having the meristematic tissues, at the beginning of the growing season generally give best results. Different workers used different types of explants to propagate the woody plant species (Table 1).

2b. Sterilization

Sterilization is a procedure used for elimination of microorganism. The maintenance of aseptic conditions is essential for successful *in vitro* propagation. The glassware are sterilized in dry heat for 3 hours at 180ºC in an oven. The explant is washed in running tap water to remove all the dust particles. It is followed by washing with liquid detergent and again, explants were washed several times with tap water to remove all the traces of detergent. Then explant is subjected to 0.2% streptomycin solution for 15-20 minutes before taking them to the sterile airflow chamber. In laminar air flow chamber surface sterilization is carried out by treating with 0.1% (w/v) mercuric chloride solution and subsequently washed 3-4 times with sterile double distilled water to remove all the traces of mercuric chloride. Again explant is disinfected in a 70 % (v/v) ethyl alcohol for 1 min. The nutrient medium is sterilized by using an autoclave at 121°C temperature and 15 psi pressure for 20 minutes.

2c. Nutrient medium

A number of basic nutrient media are used for *in vitro* culture works. Generally MS medium (Murashige and Skoog, 1962) with 3 % (w/v) sucrose and 0.8 % (w/v) agar is used for most of *in vitro* culture studies. But many woody plant species do not respond well in the usual salt concentrations of MS medium. Therefore, many workers used woody plant medium (WPM) in their investigation (Thomas et al., 2003; Sharma and Vashistha, 2010; Siwach and Gill, 2011; Sharma and Vashistha, 2015). The suitability of WPM over MS medium may be due to its low ionic strength which counteracts salt sensitivity of woody species (Lloyd and McCown, 1980). Moreover, Lisowska and Wysokinska (2000) achieved *in vitro* propagation of *Catalpa ovate* on Schenk & Hildebrandt (SH) medium while Mathew and Philip (2000) used MS, White's, Branton and Blake's medium for the culture of *Areca catechu*.

2d. Growth regulators

Among the growth regulators, cytokinins and auxins are of special importance in plant tissue culture. Generally, cytokinins -BAP, Kn, TDZ, Zeitin and auxins- IAA, IBA, NAA; 2,4-D at different concentrations are used in *in vitro* studies. Al-Safadi and Elias (2011) used GA_3 for the growth of *Capparisspinosa.* The combination of cytokinin and auxin was also preferred by Kumar and Seeni, 1998 and Balaraju et al., 2008.

2e. Culture conditions

The plant propagation under *in vitro* condition is exposed to a unique set of growth conditions like low light, high humidity and poor gaseous exchange which may support rapid growth and multiplication. The cultures are maintained at 25$\pm$2ºC under a 16 hours photoperiod with 30 μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes.

3. Shoot multiplication

Broadly direct and indirect approaches have been followed to achieve *in vitro* shoot multiplication in woody plant species. Direct approach followed the proliferation of apical and axillary bud while indirect approach involved the multiplication of shoots through callus initiation and somatic embryogenesis.

3a. Apical and axillary bud proliferation:

In this approach, shoot tips (apical bud) having 0.5 cm in length and nodal explants (axillary bud) having 2 cm in length are excised and cultured on nutrient medium supplemented with different concentrations of cytokinins individually or in combination with auxins. After some days shoots are sub-cultured on same medium for multiplication. This method is most popular approach for enhancing multiple shoots in woody plants because the apical and axillary buds have the potential to develop in to a shoot. Also, the cells of shoot apex and axillary bud are least susceptible to genotypic changes under cultural conditions. The axillary buds have been found to be suitable for micropropagation in several woody species like *Tinospora cordifolia* (Gururaj et al.,

2007),*Asparagus racemosus* (Bopana and Saxena, 2008), *Ficusreligiosa* (Siwach and Gill, 2011) and *Morindacitrifolia* (Sreeranjini and Siril, 2014). While, Kozomara et al., 2008; Sharma and Vashistha (2010) used apical buds for *in vitro* propagation of *Chimonanthus praecox* and *Cinnamomumcamphora*, respectively. A number of workers achieved *in vitro* plant multiplication by using both apical and axillary bud (Babu et al., 2003; Balaraju et al., 2008 and Balaraju et al., 2011).

3b. Callus induction and organogenesis

Callus is an unorganized mass of loosely arranged parenchymatous cells. It is the dedifferentiation of a plant cell in to callus. For callus induction, different explants are cultured on nutrient medium supplemented with different concentrations of auxins individually or in combinations with cytokinins. Callus developed from the explants on induction medium is separated and cut into small pieces and transferred to basal medium supplemented with different concentrations of cytokinins individually for shoot initiation. But, the most serious problem against the use of callus culture for shoot multiplication is the genetic instability of their cells. Gopi and Vatsala (2006) studied callus and suspension culture from nodal and leaf explants of *Gymnemasylvestre*. Prakash et al. (2014) induced callus from nodal and internodal segments of *Crataevareligiosa*. Sharma and Vashistha (2015) developed a protocol for the regeneration of complete plantlets of *Tinospora cordifolia* from the callus induced from leaf explants. Multiple shoot regeneration through a callus phase has been demonstrated in many other woody plants such as *Helicteresisora* (Shriram et al., 2008); *Moringaoleifera* (Kumar et al., 2009); *Gmelinaarborea* (Kumar et al., 2010) and *Morusalba* (Lee et al., 2011).

3c. Somatic embryogenesis

Somatic embryos are those which are formed from the somatic tissue under *in vitro* condition and resemble the zygotic embryos of intact seeds. The embryos initiated either directly from the explant or *via* callus formation and can grow in to seedling on suitable medium. Sarasan et al., 1994 established shoot multiplication via somatic embryogenesis in *Hemidesmusindicus*. Rout (2005) noted the development of somatic embryos from zygotic embryos of *Azadirachtaindica*. Somatic embryogenesis has been achieved by many other workers (Su et al. 1997; Nugent et al. 2001 and Dai et al., 2011).

4. Rooting of *in vitro* regenerated shoots

After *in vitro* regenerated shoots attained a height of 2-3 cm, they are excised and planted on half strength basal medium supplemented with different concentrations of auxins individually for rooting. In *Pterocarpussantalinus* (Arockiasamy et al., 2000) and *Tinospora cordifolia* (Raghu et al., 2006), IAA induced rooting. In other woody species like *Mallotusrepandus* (Prathanturarug et al., 2007) NAA was effective in inducing root under *in vitro* conditions. However, the promotive effect of IBA on rooting has been reported in *Pterocarpus marsupium* (Chand and Singh, 2004) and *Cinnamomumcamphora* (Sharma and Vashistha, 2010). Further, the low salt medium (half strength MS medium) was effective in root formation in *Aegle marmelos* (Nayak et al., 2007) and *Emblicaofficinalis* (Nayak et al., 2010).

5. Hardening and acclimatization of plantlets in Soil

The ultimate success of commercial *in vitro* propagation depends on the ability to transfer plants out of the culture on a large scale and with high survival rate. Hardening refers to the preparation of the *in vitro* regenerated plants for a natural growth environment. For successful acclimatization to natural conditions and normal growth a careful and gradual transfer of *in vitro* regenerated plantlets is necessary. Using similar approach successful acclimatization and field transfer of *in vitro* regenerated plantlets have been achieved inmany medicinally important woody plant species listed in Table 1. The rooted plantlets are gently pulled out of the medium and washed in running tap water. Medium sticking to the root is carefully removed. The plantlets with well-developed roots are transferred to sterilized soil and sand mixture (1:1) in small plastic pots. To maintain high humidity around the plants, for initial some days is covered them with transparent polythene bags and made small holes in them for air circulation. Plants are watered with ½ to ¼ strength salt solution of the nutrient medium on alternate days. Then pots are transferred in Polyhouse.

Table 1. List of some important *in vitro* propagated woody medicinal plants grown under field conditions.

Woody Medicinal Plants	Family	Explants	References
Acacia catechu	Mimosaceae	Node	Kaur et al., 1998
Adhatodavasica	Acanthaceae	Internode	Azad and Amine, 1998
Aegle marmelos	Rutaceae	Cotyledonary nodes, nodal and root segments, nucellar tissue	Bhati et al., 1992; Hossain et al., 1993; Kumar and Seeni, 1998; Nayak et al., 2007
Albizialebbeck	Mimosaceae	Seedling explants, leaf, cotyledonary leaf and root explants	Perveen et al., 2013; Chakravarthy and Negi, 2014
Aralia elata	Araliaceae	Leaf disc, petiole and root segments	Dai et al., 2011
Areca catechu	Arecaceae	Cotyledon and leaf explant	Mathew and Philip, 2000, Karun et al., 2004
Asparagus racemosus	Liliaceae	Nodal segments	Bopana and Saxena, 2008
Azadirachtaindica	Meliaceae	Zygotic embryos, Leaf disc	Ramesh and Padhya, 1990; Su et al., 1997; Quraishi et al., 2004; Rout, 2005
Berberisbuxifolia	Berberidaceae	Node	Pitta-Alvarez et al., 2008
Boswellia serrate	Burseraceae	Cotyledonary nodes	Suthar et al., 2011
Buddleja cordata	Buddlejaceae	Leaf explants	Estrada-Zuniga et al., 2009
Capparisspinosa	Capparaceae	Seeds, stem cuttings, immature fruits and floral explants	Al-Safadi and Elias, 2011; Carra et al., 2012
Catalpa ovata	Bignoniaceae	Shoot tips and nodes	Lisowska and Wysokinska, 2000
Celastruspaniculatus	Celastraceae	Shoot tip, node, internode and leaves	Nair and Seeni, 2001; Martin et al., 2006; Rao and Purohit, 2006; Lal and Singh, 2010
Chimonanthus praecox	Calycantaceae	Shoot tips	Kozomara et al., 2008
Cinnamomumtamala	Lauraceae	Embryos from seeds	Deb et al., 2014
Cinnamomumcamphora	Lauraceae	Shoot tip and nodal segments	Huang et al., 1998; Babu et al., 2003; Sharma and Vashistha, 2010
Cinnamomumzeylanicum	Lauraceae	Seeds and seedling explants	Rai and Jagadishchandra, 1987
Commiphoramukul	Burseraceae	Apical, nodal and leaf segments	Singh et al., 2010
Crataevareligiosa* or *Crataevanurvala	Capparaceae	Nodal and internodal segments	Inamdar et al., 1990; Walia et al., 2003; Shirin and Maravi, 2006 and Prakash et al., 2014
Emblicaofficinalis	Euphorbiaceae	Epicotyl, seedling derived root explants and nodes	Verma and Kant, 1996; Rahman et al., 1999; Tyagi and Govil, 1999; Gour and Kant, 2009 and Nayak et al., 2010
Eucalyptus globulus	Myrtaceae	Cotyledons, hypocotyls and zygotic embryos	Wilson, 1996; Azmi et al., 1997; Nugent et al., 2001; Pinto et al., 2002
Ficusreligiosa	Moraceae	Nodal segments	Siwach and Gill, 2011
Glycyrrhizaglabra	Papilionaceae	Axillary bud	Kohjyouma et al., 1995
Gmelinaarborea	Verbanaceae	Shoot tip, node and internode	Kumar et al., 2010
Gymnemasylvestre	Asclepiadaceae	Nodal and leaf explants	Komalavalli and Rao, 2000 ; Gopi and Vatsala, 2006
Helicteresisora	Sterculiaceae	Nodal explants	Shriram et al., 2008
Hemidesmusindicus	Asclepiadaceae	Leaf and nodes	Sarasan et al., 1994; Patnaik and Debata, 1997
Holostemmaada-kodien	Asclepiadaceae	Leaves, shoot tip and nodes	Pushparanjan and Surendran, 2014
Lawsoniainermis	Lythraceae	Node	Rout et al., 2001; Ram and Shekhawat, 2011
Maesaperlarius	Myrsinaceae	Seedling node and leaf	Faisal et al., 2011

Mallotusrepandus	Euphorbiaceae	Node and internode	Prathanturarug et al., 2007
Morindacitrifolia	Rubiaceae	Nodal segments	Sreeranjini and Siril, 2014
Moringaoleifera	Moringaceae	Cotyledon explants	Kumar et al., 2009
Morus alba	Moraceae	Leaf and nodal segments	Balakrishnan et al., 2009; Lee et al., 2011
Murrayakoenigii	Rutaceae	Internode	Rajendra and D'Suja, 1998
Pongamiapinnata	Leguminoseae	Nodal meristem	Sujatha and Hazra, 2007; Sugla et al., 2007
Pseudarthriaviscida	Papilionaceae	Cotyledonary node and young leaves	Cheruvathur et al., 2011
Pterocarpus marsupium	Fabaceae	Seed, Cotyledonary node	Anuradha and Pullaiah, 1999; Chand and Singh, 2004; Tiwari et al., 2004; Anis et al., 2005; Husain et al., 2007
Pterocarpussantalinus	Fabaceae	Shoot tip and nodes	Anuradha and Pullaiah, 1999; Arockiasamy et al., 2000; Balaraju et al., 2011
Sapindusmukorossi	Sapindaceae	Apical, node and leaf segments	Philomina and Rao, 2000; Singh et al., 2010
Sapindustrifoliatus	Sapindaceae	Seedling node	Asthana et al., 2011
Searsiadentata	Anacardiaceae	Shoot tip and nodes	Prakash and Staden, 2008
Terminalia arjuna	Combretaceae	Leaf, apical and nodal explants	Kumari et al., 1998; Thomas et al., 2003; Pandey et al., 2006
Tinospora cordifolia	Menispermaceae	Shoot tip, nodal and leaf segments	Kumar et al., 2003; Raghu et al., 2006; Gururaj et al., 2007; Sharma and Vashistha, 2014, 2015
Tylophoraindica	Asclepiadaceae	Node, leaf and petioles	Sharma and Chandel, 1992; Faisal and Anis, 2003; Faisal et al., 2005
Vitexagnus-castus	Verbanaceae	Apical and nodal explants	Balaraju et al., 2008
Vitexnegundo	Verbenaceae	Node	Sahoo and Chand, 1998

A. ***Albizialebbeck*** **C.** ***Cinnamomumcamphora***

B. ***Buddleja madagascariensis*** **D.** ***Tinospora cordifolia***

Figure 1. Someimportant woody medicinal plants.

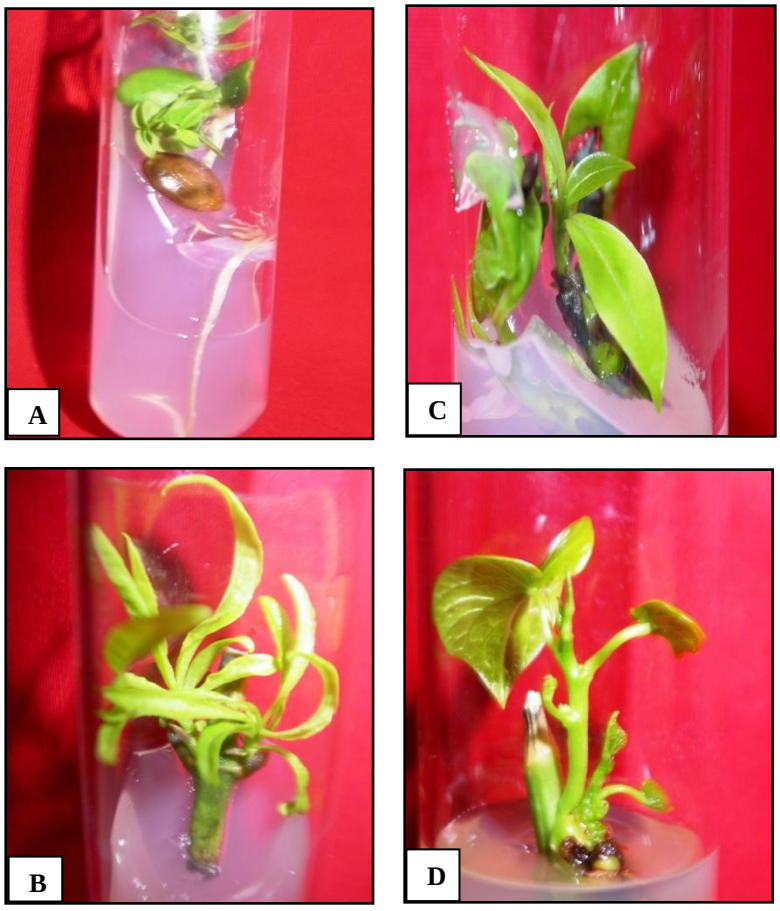

Figure 2. Woody medicinal plants under *in vitro* condition: **(A)** Seed of *Albizialebbeck* germinated on MS basal medium **(B)** Nodal segment of *Buddleja madagascariensis* on MS medium + 0.5 mg/l BAP **(C)** Nodal segment of *Cinnamomumcamphora* on woody plant medium + 1.0 mg/l BAP + 1.0 mg/l KIN **(D)** Nodal segment of *Tinospora cordifolia* on woody plant medium + 2.0 mg/l KIN.

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