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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Abstract

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.

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 121ºC 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 GA3 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±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.,
Asparagus racemosus (Bopana and Saxena, 2008), Ficus religiosa (Siwach and Gill, 2011) and Morinda citrifolia (Srereanjini and Siril, 2014). While, Kozomara et al., 2008; Sharma and Vashistha (2010) used apical buds for in vitro propagation of Chimonanthus praecox and Cinnamomum camphora, 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 Vashishtha (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 Helicteresis orara (Shriram et al., 2008); Moringa oleifera (Kumar et al., 2009); Gmelina arborea (Kumar et al., 2010) and Morus alba (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 Hemidesmus indicus. Rout (2005) noted the development of somatic embryos from zygotic embryos of Azadirachta indica. 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 Pterocarpus santalinus (Arockiasamy et al., 2000) and Tinospora cordifolia (Raghu et al., 2006), IAA induced rooting. In other woody species like Mallotus repandus (Prathanturarag 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 Cinnamomum camphora (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 Emblica officinalis (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 in many 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.

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A. Albizia lebbeck  
B. Buddleja madagascariensis  
C. Cinnamomum camphora  
D. Tinospora cordifolia
Figure 1. Some important woody medicinal plants.

Figure 2. Woody medicinal plants under in vitro condition: (A) Seed of Albizia lebeck germinated on MS basal medium (B) Nodal segment of Buddleja madagascariensis on MS medium + 0.5 mg/l BAP (C) Nodal segment of Cinnamomum camphora 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.

REFERENCES


