

Review Article

## Application of tissue culture in afforestation and wasteland development: A mini review

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Population growth influences the environmental balance in multiple ways. The alarming rate with which the forest cover getting disappeared from the earth makes decisive menace to the living world. The present century witness a number of initiatives to protect the existing vegetation and to improve the green mass through tree plantation programmes. Compared to any other group of plants, trees possess some unique features like, perennial nature and robust physiological mechanisms, which help them to survive in adverse conditions. To employ trees in afforestation and waste land development programmes needs a continuous supply of huge number of plantlets, which will be possible only through *in vitro* propagation methods. This review focused on the advancement in the area of *in vitro* propagation of trees mainly used in afforestation and wasteland development programmes.

**Keywords:** *In vitro* propagation, woody trees, afforestation.

### 1. Introduction:

Unpredictable and hazardous changes happening in the climatic conditions all over the globe indicate the existence of an imbalanced equilibrium in terms of the destructive and constructive phenomenon. As the population increases, the exploitation of natural resources also increases. This may lead to severe consequences, if the equilibrium is not restored. With the elaboration of domestic existence to the land, the forest cover is disappearing in an alarming rate. As an option to regain, at least partially the forest cover, extensive programmes are being planned world wide. Compared to any small plants, trees hold the major stake in these programmes. In order to supply huge number of plantlets in these programmes, well established *in vitro* propagation machinery is needed.

Even though, tree species are considered to be the toughest group of plants

for *in vitro* propagation, there is considerable development in this field during the past few decades. In general most of the *in vitro* propagation studies were carried out on tree species of agronomic or economic importance like fruit trees, medicinal trees, ornamental trees and trees for wood and paper industry (McDonnel *et al.*, 2010). *In vitro* techniques like anther culture, embryo culture and meristem culture have been used for specific applications like production of haploids, shortening of breeding cycle, to overcome dormancy, recovery of plants from intra-specific crosses, production of virus free plants, somatic cell hybridization etc. There are nearly 40 media formulations used successfully for the *in vitro* propagation of different tree species. The recalcitrant nature of the explants from many of the tree species has been modified to convert them to totipotent cells made way to multiply those using *in vitro* techniques (Bonga *et al.*, 2010).

**Table 1. Few reports on the tissue culture studies on trees used for afforestation and wasteland development.**

Tree species	Explant	Media & Phytohormone(s)	Effect	Reference
<i>Acacia crasycarpa</i>	Phyllode	MS+TDZ+NAA	Green nodules-shoots	Yang <i>et al.</i> , 2006
		1/2MS+IBA	Rooting	
<i>A. senegal</i>	Nodal segments	MS+ BAP+ Zeatin	Multiple shoots	Badji <i>et al.</i> , (1993)
		Modified JN+ NAA	Rooting	
<i>A. nilotica</i>	Cotyledonary nodes	B5+ BAP+ Kin/Zeatin	Multiple shoots	Dewan <i>et al.</i> , (1992)
		B5+ IAA	Rooting	
<i>A. nilotica</i>	Epicotyl seedlings	NAA+BA+GA	Shoot regeneration	Samake <i>et al.</i> , 2011
		IBA	Rooting	
<i>Litsea cubeba</i>	Shoot tip/ nodal/ leaf/petiole	MS+ BAP/ WPM/BAP	Multiple shoots	Mao <i>et al.</i> , (2000)
		MS basal	Rooting	
<i>Casuarina hybrid</i>	Epicotyl segments	MS+BA	Shoot formation	Shen <i>et al.</i> , 2009
		MS+IBA	Rooting	
<i>C. equisetifolia</i>	Axillary shoot s	MS+NAA+BAP	Multiple shoots	Satheeshkumar <i>et al.</i> , 2009
		MS+IBA	Rooting	
<i>Eucalyptus tereticornis</i>	Nodal	MS+BA+2,4-D	Shoot proliferation	Aggarwal <i>et al.</i> , 2010
<i>E. camaldulensis</i>	Cotyledon, ZE	MS+NAA	Calli	Prakash and Gurumurthy, 2010
		MS+ NAA+BA	Indirect SE	
		MS+BA	Direct SE	
<i>Sesbania grandiflora</i>	Cotyledon	MS+BAP+NAA	Adventitious shoots	Detrez <i>et al.</i> , (1994)
		MS+ NAA	Rooting	
<i>Robinia pseudoacacia</i>	Protoplast (callus and meshphyll)	WPM+NAA+BAP	Calli	Kanwar <i>et al.</i> , 2009
		MS+NAA+BAP	Shoots	
		MS+IBA	Rooting	
<i>R. ambigua</i>	Axillary buds	MS+BA+NAA+GA	Bud induction	Guo <i>et al.</i> , 2006
		1/4MS+IAA+IBA	Rooting	
<i>R. psuedoacacia</i>	Immature seeds	MS+ 2,4-D+BAP		Arrillaga <i>et al.</i> , (1994)
<i>Juglans regia</i>	Immature cotyledons	DKW+ABA	SE	Vahdati <i>et al.</i> , 2008
<i>Alnus cremastogyne</i>	Epicotyl and hypocotyl	WPM+ BAP	Shoots	Tang <i>et al.</i> , 1996
		WPM basal/ +IBA	Rooting	
<i>A. glutinosa</i>	Apical shoots	WPM+BAP	Shoot multiplication	Lall <i>et al.</i> , 2005

Abbreviations used: 2, 4-D- 2, 4-Dichlorophenoxy acetic acid, IAA- Indole-3-acetic acid, IBA- Indole-3-butyric acid, NAA- Naphthalene acetic acid, S.E- Somatic embryogenesis, MS- Murashige and Skoog's (1962) nutrient medium, B5- Gamborg's medium (Gamborg et al., 1968), JN-Jordan's medium (Jordan et al., 1979), GA3- Gibberellic acid, ABA- Absciscic acid, BAP-6-benzyl amino purine, TDZ- Thiadiazuron, WPM, Woody plant medium (Loyd and McCown,1981), DKW -Driver and Kuniyuki medium (Driver and Kuniyuki, 1984), Kin-Kinetin.

## 2. *In vitro* propagation:

Trees are being used for the afforestation and wasteland development programmes, mainly because of their robust survival mechanisms in adverse conditions. Most of the trees used for this purpose belong to the family Leguminosae or those having the property of fixing atmospheric nitrogen to increase the soil fertility (Detrez *et al.*, 1994). The *in vitro* multiplication programmes on these tree species have been given priority in many of the government funded projects to have enough saplings for plantation in the deforested areas. Important afforestation tree species include *Alnus* sps, *Robinia* sps, *Eucalyptus* sps, *Acacia* sps, *Sesbania* sps, *Litsea* sps, *Casuarina* sps, *Leucaena* sps and *Juglans* sps (Table-1).

### 2a. *Alnus*:

*Alnus* is a genus (common name- Alder) belonging to the birch family (Betulaceae) composed of 30 species. The plants are known for its medicinal properties and are being used in the traditional medical practices. *Alnus* sps inhabits the N<sub>2</sub> fixing filamentous ascomycetean fungi, *Frankia alni*, in its root system, which forms root nodules, sometimes as big as a human fist. Because of the N<sub>2</sub> fixing properties these plants have been employed in the tree plantations extensively. Tang *et al* reported the *in vitro* propagation of *Alnus cremastogyne* using WPM (Woody Plant Medium) supplemented with BAP (Benzylaminopurine) (callus /regeneration) and IBA (Indole butyric acid) (rooting). Epicotyl and hypocotyls explants were found to be efficient in giving an indirect plantlet formation (Tang *et al.*, 1996). Another species, *Alnus glutinosa* was propagated *in vitro* from shoot tip explants. Multiple shoots induced with BAP in combination with 1-N- naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA), was rooted when transferred to WPM basal medium without any phytohormones.

Another reports from the research groups, Garton *et al.*, 1981; Perinet and Lalonde, 1983; Tremblay and Lalonde, 1984, Lall *et al.*, 2005 etc also showed successful *in vitro* plant regeneration in different species of *Alnus*.

### 2b. *Robinia*:

Among different species of *Robinia*, *Robinia psuedoacacia* (Black locust) is the one underwent much investigations related to *in vitro* propagation (Kanwar *et al.*, 2009). Black locust is considered for its potential use in environmental restoration. It is widely valued for reclamation of sites which are difficult to restore for vegetative cover, such as surface mined lands, mine waste dumps, smelter soils, seaboards etc (Han and Park, 1999). Black locust's rapidly developing root system stabilizes the soil and increases soil permeability. Plant regeneration of *Robinia* sps has been reported from leaf discs, cambial tissue, shoot tips seedling derived callus etc (Davis and Keathley, 1985, Arrillaga *et al.*, 1995, Guo *et al.*, 2006, Chalupa, 1983). Auxillary buds and nodal segments were also been used for this purpose. In many of the cases a modified Murashigue and Skoog (MS), Litvey medium or WPM was used (Han and Park, 1999). Han and Keathly reported the isolation and culture of protoplasts from the calli derived explants of *R. psuedoacacia* (Han and Keathly, 1998).

### 2c. *Eucalyptus*:

*Eucalyptus* sps are used extensively in the afforestation programmes due to its highly adaptable nature under varied environmental conditions. The physiological peculiarities make these plants utilize the nutrients from a highly adverse environment. *In vitro* propagation methods for different species of *Eucalyptus* have been reported earlier (Gupta and Mascarenhas, 1987). Muralidharan *et al* used long term callus cultures of *E. citriodora* to induce somatic embryogenesis using Gamborg's B5 medium

supplemented with NAA, glutamine and casein hydrolysates. A hormone free medium induced rooting and the plants were survived in soil (Muralidharan *et al.*, 1989). Subbaiah and Minocha used leaf and stem explants to induce multiple shoots using B5 medium with BA and NAA (0.1 and 5mg/l respectively). Hypocotyl segments induced roots when cultured on B5 medium containing BA. Shoots were rooted on a modified WPM medium containing IBA (Subbaiah and Minocha, 1990). Other species like *E. marginata*, *E. camaldulensis*, *E. leichow*, *E. sideroxylon* etc also were reported to be propagated through *in vitro* methods (Benet and McComb, 1982, Burger 1987, Muralidharan and Mascarenhas, 1987). Two recent reports on the *in vitro* propagation of *Eucalyptus* sps (Prakash and Gurusurthi, 2010, *E. camaldulensis*, Aggarwal *et al.*, 2010, *E. tereticornis*) used MS medium with different combinations of phytohormones to establish a regeneration protocol.

## 2d. Acacia:

Leguminous trees are significant components in any afforestation or wasteland development programmes due to their property to restore soil fertility through atmospheric nitrogen fixation. Since the regeneration rate of leguminous trees are very low in nature, it is essential to find ways to multiply them. There are many reports on the *in vitro* propagation of Acacia species like, *A. koa* (Skolmen and Mapes, 1976), *A. Senegal* (Dave *et al.*, 1980), *A. albida* (Dohoux and Davis, 1985), *A. catechu* (Kaur *et al.*, 1998, Kulneet and Kant, 2000), *A. nilotica* (Dewan *et al.*, 1991, Samake *et al.*, 2011), *A. chundra* (Rout *et al.*, 2008), *A. mearnsii* (Sascha *et al.*, 1998) etc. Cotyledonary explants are used widely as the starting material for tissue culture along with other sources like shoot tip, immature embryos and phyllodes. MS or B5 medium with phytohormone combinations found to be capable of inducing organogenesis in many of the Acacia sps. Yang *et al* reported

the plant regeneration from phyllode explants cultured on MS media supplemented with different combination of NAA and TDZ (Yang *et al.*, 2006).

## 2e. Casuarina:

Genus Casuarina is indigenous to north and north east Australia, some pacific islands and from Indonesia and Malaya to India and Srilanka. These monaceous or dioecious trees belong to the family Casuarinaceae, mainly propagated by seeds. Vegetative propagation by means of cuttings or gootee, air-layering of lower side of branches was also reported to be successful. Casuarina sps are used commonly for the afforestation programmes due to the following facts. It grows in a wide range of soils from volcanic, sandy to compact clay (Kondas S, 1983) and can tolerate water logging, saline and drought conditions (NAS, 1984, Djogo, 1989). It is an actinorhizal woody plant, fix atmospheric nitrogen when nodulated by an ascomycete known as *Frankia* (Diem *et al.*, 1982). The traditional seed propagation usually results in plantlets exhibit inbreeding depression. It is very important to have suitable *in vitro* plant regeneration protocol to propagate superior biotypes to have a continuous supply of healthy and vigorous plantlets. Only a few studies on *in vitro* propagation of Casuarina have been reported due to its recalcitrant nature (Duhoux *et al.* 1986; Parthiban *et al.* 1997; Seth *et al.* 2007, Satheeshkumar *et al*, 2009, Shen *et al*, 2009). Shoot tips, needles, axillary buds and cotyledonary explants were used to develop *in vitro* propagation protocols. Satheeshkumar *et al* used axillary shoots developed aseptically to induce organogenesis through callusing under MS medium supplemented with BAP and IAA. The shoots were rooted with NAA or IAA. A Casuarina hybrid, (*Casuarina equisetifolia* L. x *Casuarina glauca*, Sieber ex Spreng) was given multiple shoot induction when seeds were cultured on MS medium with different

concentrations of BA. The shoots were rooted on IBA containing media (Shen *et al.*, 2009).

## 2f. Other plants:

There are other tree species employed in the afforestation programmes also studied extensively to establish *in vitro* plant regeneration protocols. They include *Sesbania* sps (Subhan *et al.*, 1998), *Litsea* sps (Mao *et al.*, 2000, Chongjian *et al.*, 2005), *Leucaena leucocephala* (Jube and Borthakur, 2010) etc (Table-1). One of the major problems faced during the acclimatization process of the leguminous plantlets in the green houses was that, those which were having a natural symbiotic partner like *Alnus*, *Robinia* and *Casuarina* failed to survive in the green house pots (Subhan *et al.*, 1998, McClusky and Fisher, 1986, Arrillaga *et al.*, 1994). It was observed that once the microorganisms were inoculated in the soil, it colonized in the root system and helped the plantlets to grow normally.

## 3. Conclusions:

With the advent of newer technologies, the scientific knowledge on plant development and the factors controlling different aspects of development has been increased. This has opened up new avenues to improve the existing protocols of plant propagation through *in vitro* methods. Tissue culture experiments holds great promise to the nature lovers as it supply a continuous unlimited number of plantlets for the afforestation programmes. Many of the government agencies are funding to institutions focused of tree propagation including *in vitro* methods. With the ever increased rate in which the forest cover is getting disappeared from the earth, it is an urgent call to restore at least a few percentage of the lost plantation to avoid severe climatic changes, which may become detrimental to the existence of living beings.

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