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RESEARCH ARTICLE

ROLE OF GROWTH REGULATORS IN MICROPROPAGATION OF WOODY PLANTS-A REVIEW

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Abstract

The success of micropropagation technique depends upon the use of growth regulators in the culture medium. Growth regulators regulate the growth and developmental processes. These are the key factors in initiating the process of regeneration in tissue culture. In most of *in vitro* studies, explants do not respond well on culture media without growth regulators. An interactive balance of auxins and cytokinins controlled the *in vitro* growth and differentiation response in plant tissues. This review highlights on the role of growth regulators in micropropagation of woody plants.

Abbreviations

BAP: 6 benzylaminopurine
Kn: Kinetin
TDZ: Thidiazuron
2iP: 2-isopentenyl adenine
GA_3: Gibberellic acid
IAA: Indole-3-acetic acid
IBA: Indole-3-butyric acid
NAA: α-Naphthalene acetic acid
2,4-D: 2,4-Dichloro phenoxy acetic acid
ABA: Abscisic acid

Introduction

The world has a very rich biodiversity of plant species. Many of which are herbaceous and many others are woody in nature. In the view of propagation, woody plants are difficult to propagate than herbaceous species. The difficulty in propagation is due to their poor seed germination capacity, as seeds are not viable in most of the time. In this case the favorable season is a very important criterion for the successful germination. Moreover, the slow growth is also a barrier, because apical and axillary buds become dormant during specific time periods. Therefore, woody plants require favorable season for the germination of seeds and buds. Further, some more conventional methods of propagation such as cuttings and graftings are also used for woody plants. But these are not much effective methods for their large scale production. As, for a wide population, woody plants are important source of timber, medicines, fruits, dyes etc. Therefore, there is a need to propagate them wisely as well as in large amount to fulfill the requirements of the population. The possible approach to overcome the problem is micropropagation.

Micropropagation is the technique of growing the plants from seeds or small pieces of tissues under sterile condition in a laboratory on a specially selected medium. It allows mass multiplication of a species from a small piece of tissue. One of the important aspects of this technique is that it is not dependent on the season for the propagation. Through micropropagation, a number of woody plant species have been propagated successfully during past years. But, the success of micropropagation technique depends upon the use of plant growth regulators in the culture medium. Growth regulators regulate the growth and developmental processes, which are present in various concentrations in different plant parts. These are the key factors in initiating the process of regeneration in tissue

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culture. Miller and Skoog (1953) reported that *in vitro* root and shoot differentiation is regulated by exogenous hormonal interaction. In most of *in vitro* studies, explants do not respond well on culture media without growth regulators (Kumar, 1992; Kumari *et al*., 1998; Kumar *et al*., 2003; Walia *et al*., 2003; Gururaj *et al*., 2007; Sharma and Vashistha, 2010b; 2015c). Growth regulators apply externally might disturb the internal polarity and change the genetically programmed physiology of explants resulting in organogenesis from the explants.

Basically, plant growth regulators come under two broad categories- auxins and cytokinins. Commonly used auxins are- IAA, NAA, IBA and 2,4-D and cytokinins are- BAP, Kn, TDZ, Zeatin and 2-iP. An interactive balance of auxins and cytokinins controlled the *in vitro* growth and differentiation response in plant tissues. During indirect organogenesis, the formation of callus or somatic embryo from explants is stimulated by altering the levels of exogenously applied auxins or combinations of auxins and cytokinins. Similarly, direct organogenesis from apical or axillary buds is stimulated by the cytokinins or combinations of cytokinins and auxins in the culture medium. A variety of plant growth regulators have been used individually as well as in combinations to achieve the micropropagation of a number of woody plant species (Table 1). During past years, some reviews have been published on micropropagation of trees and woody plants (Sharma and Vashistha, 2015a; Sharma, 2016). In these reviews, problems of propagation of woody plants and their possible solution through micropropagation technique have been taken in to consideration. But, this review highlights on the role of growth regulators in micropropagation of woody plants, which categories under following headings:

***In vitro* seed germination and shoot elongation**

Many woody plants are propagated through seeds under *in vitro* condition. GA_3 is a growth regulator effective for seed germination as well shoot elongation. A number of workers used GA_3 in their culture media and found useful results (Isogai *et al*, 2008; Balaraju *et al*., 2011; Joseph *et al*., 2011 and Al-Safadi and Elias, 2011). Ghimire *et al*. (2016) reported that among different growth regulators tried in *Melastoma malabatricum*, GA_3 was the most effective for shoot elongation. But in *Pyrus boissieriana*, GA_3 negatively affected number and length of shoots (Zakavi *et al*., 2016).

***In vitro* shoot regeneration**

In case of most of woody plants, basal nutrient medium without growth regulator is not much effective in inducing shoot buds. Similarly no shoot buds developed in *Crataeva nurvala* (Walia *et al*., 2003) and *Cinnamomum camphora* (Sharma and Vashistha, 2010c) on basal medium. Growth regulators applied exogenously have variable effects which varied with the type of growth regulator, its concentration and nature of explants. In woody species, *in vitro* shoot regeneration is achieved by two methods: direct and indirect organogenesis.

Direct organogenesis

Direct method involves the proliferation of apical and axillary buds. This method is most popular in woody plants for shoot multiplication because the apical (shoot tips) and axillary buds (nodes) have the potential to develop in to a shoot. In contrast to basal medium, it is observed that when the medium is supplemented with cytokinins individually or in combination with auxins shoot tips and nodal explants produced multiple shoots. The number and frequency of shoot induction is mainly dependent on the concentration of cytokinin used in the culture medium. The past studies showed that among the cytokinins (BAP, Kn, TDZ and Zeatin) tested in different woody species, BAP and Kn were most common. Further, in many woody species, BAP is more effective than Kn for shoot induction and multiplication (George, 1993; Sharma and Vashistha, 2010b; Sharma *et al*., 2015). Bunn (2005) and Asthana *et al*. (2011) reported that BAP resulted in the highest shoot multiplication rates when compared to Kn and Zeatin. The superiority of BAP over other cytokinins has also been reported in *Capparis decidua* (Tyagi and Kothari, 2001) and *Pterocarpus marsupium* (Chand and Singh, 2004). In contrast, Shahzad and Siddiqui (2001) in *Melia azedarach* reported that Kn proved more effective than BAP for direct shoot regeneration. Similar observation is reported in *Gmelina arborea* (Kumar *et al*., 2010). Further, it is reported that higher concentration of BAP and Kn are inhibitory in some woody plants (Anuradha and Pullaiah, 1999; Tornero *et al*., 2000; Nair and Seeni, 2001; Balaraju *et al*., 2011 and Sharma *et al*., 2015). Besides these growth regulators, many workers used TDZ in their culture medium and found positive effects on shoot induction and multiplication as in *Pterocarpus marsupium* (Husain *et al*., 2007), *Fraxinus pennsylvanica* (Du and Pijut, 2008), *Medusagyne oppositifolia* (Marriott and Sarasan, 2010) *Pterocarpus santalinus* (Balaraju *et al*., 2011). In spite of these findings, a combination of cytokinin and auxin also used for shoot proliferation in *Salvadora persica* (Mathur *et al.*, 2002) and *Ficus religiosa* (Siwach and Gill, 2011).

Indirect organogenesis

Indirect method involves the shoot regeneration through callus induction and somatic embryogenesis. For callus induction, different explants are cultured on nutrient medium supplemented with different concentrations of auxins individually or in combinations with cytokinins. Callus so obtained is further separated and transferred in to fresh medium supplemented with different concentrations of cytokinins individually for shoot generation. The somatic embryos initiate either directly from the explants or *via* callus formation and can grow in to seedling on suitable medium.

Callus induction and plantlet regeneration

It is reported in most of woody species that basal medium without growth regulator failed to induce callus (Sharma and Vashistha, 2010a; 2011b). This is probably due to the insufficient level of endogenous growth regulators in explants to induce callus and therefore it requires an exogenous supply. In woody plants, commonly used auxins are IAA, NAA, IBA and 2,4-D. In some investigations, 2,4-D has been essential for callus formation, as in *Thevetia peruviana* (Kumar, 1992) and *Terminalia arjuna* (Kumari *et al*., 1998). In addition to this, 2,4-D is effective in inducing callus in *Moringa oleifera* (Kumar *et al*., 2009), *Citrus jambhiri* (Savita *et al*., 2011) and *Simmondsia chinensis* (Bala *et al*., 2015). According to Murashige (1974) 2,4-D is a most potent auxin and it stimulates callus formation and strongly antagonizes organized development. In contrast, NAA played an important role in callus formation in *Cinnamomum camphora* (Sharma and Vashistha, 2010a), *Pseudarthria viscid* (Cheruvathur and Thomas, 2011) and *Tinospora cordifolia* (Sharma and Vashistha, 2011a; 2014). Similarly, IAA has been used in some *in vitro* culture studies to initiate callus (Isah and Mujib, 2015; Sharma and Vashistha, 2015b).

Table 1: Role of growth regulators in micropropagation of some woody plant species

Woody plant species	Explants used	Growth regulators	Role of growth regulators	References
Abies cephalonica	Embryo	ABA	Somatic embryogenesis	Krajnakova *et al*. (2009)
Aegle marmelos	Cotyledonary node and Nodal explants	BAP, Kn, IAA and IBA	Direct shoot regeneration	Kumar and Seeni (1998), Nayak *et al*. (2007)
Ailanthus altissima	Stem segments	BAP & IBA	Direct shoot regeneration	Gatti (2008)
Azadirachta indica	Zygotic embryos	BAP; 2,4-D and ABA	Somatic embryogenesis	Rout (2005)
Bixa orellana	Nodal segments	GA_3, BAP and IBA	Direct shoot regeneration	Joseph *et al*. (2011)
Boehmeria nivea	Cotyledon, hypocotyl, leaf, petiole and stem explants	TDZ + NAA	Direct and indirect (callus) shoot regeneration	Wang *et al*. (2008)
Capparis spinosa	Seeds, immature fruits and stem cuttings	GA_3, BAP, IAA and NAA	Callus induction and plantlet regeneration	Al-Safadi and Elias (2011)
Cinnamomum camphora	Shoot tip, nodal and internodal explants	BAP, Kn, IBA, NAA and 2,4-D	Direct and indirect (callus) shoot regeneration	Sharma and Vashistha (2010a, 2010b and 2010c)
Citrus jambhiri	Cotyledon explants	2,4-D; BAP and NAA	Callus induction and plantlet regeneration	Savita *et al*. (2011)
Couroupita guianensis	Seeds and nodal explants	BAP, Kn, NAA and IBA	Direct shoot regeneration	Shekhawat and Manokari (2016)
Crataeva nurvala	Shoot tips	2,4-D	Somatic embryogenesis	Inamdar *et al*. (1990)
Elaeocarpus sphaericus	Nodal explants	BAP+Kn, NAA	Direct shoot regeneration	Saklani *et al*. (2015)
Emblica officinalis	Juvenile roots and Epicotyl explants	BAP, IAA, NAA and IBA	Direct and Indirect (callus) shoot regeneration	Gour and Kant (2009), Nayak *et al*. (2010)
Eucalyptus camaldulensis	Nodal explants	Bap, NAA and IBA	Direct organogenesis and Somatic embryogenesis	Girijashankar (2012)
Ficus religiosa	Nodal explants	BAP, TDZ, 2iP,	Direct shoot	Siwach and Gill (2011)

		IAA and NAA	regeneration	
Fraxinus americana	Hypocotyls & cotyledons	BAP, TDZ, IAA and IBA	Direct shoot regeneration	Palla and Pijut (2011)
Gmelina arborea	Shoot tip, node and internode explants	Kn, BAP, NAA and 2,4-D	Direct and indirect (callus) shoot regeneration	Kumar *et al*. (2010)
Juglans nigra	Shoot tips and nodal segments	Zeatin, TDZ , BAP and IBA	Direct shoot regeneration	Bosela & Michler (2008)
Lawsonia inermis	Nodal explants	BAP, Kn, IAA and IBA	Direct shoot regeneration	Ram and Shekhawat (2011)
Moringa oleifera	Cotyledons	NAA; 2,4-D and BAP	Callus induction and plantlet regeneration	Kumar *et al*. (2009)
Morus alba	Leaf explants	2,4-D; IAA, NAA, BAP and Kn	Callus induction and plantlets regeneration	Lee *et al*. (2011)
Pongamia pinnata	Nodal explants	BAP, Kn, Zeatin and TDZ	Direct shoot regeneration	Sujatha and Hazra (2007)
Populus tremula	Axillary buds	BAP, IAA, NAA and IBA	Callus induction and plantlet regeneration	Peternel *et al.* (2009)
Prosopis laevigata	Cotyledonary node and Zygotic embryos	2,4-D + BAP and NAA	Direct and indirect (callus) shoot regeneration, Somatic embryogenesis	Buendia-Gonzalez *et al*. (2007), Buendia-Gonzalea *et al*. (2012)
Prunus domestica	Hypocotyl explants	TDZ, IBA and NAA	Direct shoot regeneration	Tian *et al*. (2007)
Pseudarthria viscida	Cotyledonary node and leaf pieces	2,4-D; NAA, BAP , IAA & IBA	Callus induction and plantlet regeneration	Cheruvathur and Thomas (2011)
Psidium guajava	Zygotic embryos	2,4-D	Somatic embryogenesis	Rai *et al*. (2007)
Pterocarpus marsupium	Cotyledonary node	TDZ, BAP and IBA	Direct shoot regeneration	Husain *et al*. (2007)
Pterocarpus santalinus	Shoot tip explants	GA3, BAP, TDZ and IBA	Direct shoot regeneration	Balaraju *et al*. (2011)
Salvadora persica	Cotyledonary node explants	BAP & IAA	Direct shoot regeneration	Mathur *et al*. (2002)
Sapindus trifoliatus	Nodal explants	BAP & IBA	Direct shoot regeneration	Asthana *et al*. (2011)
Simmondsia chinensis	Leaf explants	2,4-D; IBA and BAP	Callus induction and plantlets regeneration	Bala *et al*. (2015)
Tabebuia donnell-smithii	Stem segments	Zeatin & IBA	Direct shoot regeneration	Gonzalez-Rodriguez (2010)
Tinospora cordifolia	Shoot tip, Node, Internode, leaf and petioles	BAP, Kn, 2iP, IAA, NAA, IBA and 2,4-D	Direct and indirect (callus) shoot regeneration	Gururaj *et al*. (2007), Sharma and Vashistha (2011b; 2015b; 2015c) Sharma *et al*. (2015)
Vitex agnus-castus	Apical & nodal explants	GA_3, BAP, Kn, NAA and IBA	Direct shoot regeneration	Balaraju *et al*. (2008)
Ziziphus jujuba	Leaf explant	TDZ & NAA	Direct shoot regeneration	Feng *et al*. (2010)
Zygophyllum xanthoxylon	Cotyledons, hypocotyl and radicles	BAP, NAA and IAA	Callus induction and plantlet regeneration	Sun *et al.* (2008)

Somatic embryogenesis

Somatic embryogenesis and plant regeneration studies has been reported in many woody plant species such as *Crataeva nurvala* (Inamdar *et al.*,1990), *Thevetia peruviana* (Kumar, 1992), *Emblica officinalis* (Tyagi and Govil, 1999), *Pinus roxburghii* (Arya *et al.*, 2000 and Mathur *et al.*, 2000), *Acacia farnesiana* and *A. schaffneri* (Ortiz *et al.*, 2000), *Eucalyptus globulus* (Nugent *et al.*, 2001 and Pinto *et al.*, 2002), *Areca catechu* (Karun *et al.*, 2004),

Acacia arabica (Rout and Nanda, 2005) and *Psidium guajava* (Rai *et al*., 2007). In *Azadirachta indica* (Rout, 2005) used a combination of BAP and 2,4-D for plantlets generation from zygotic embryo and ABA for maturation and germination of embryo.

***In vitro* rooting**

In few cases, *in vitro* formed shoots develop roots when transfer in basal nutrient medium (without growth regulators). Otherwise, in most of studies auxins are known to induce rooting. In some species IAA induced rooting (Anuradha and Pullaiah, 1999; Arockiasamy *et al*., 2000; Raghu *et al*., 2006). In other species NAA was effective in inducing roots under *in vitro* condition (Kumar and Kumar, 1995; Savita *et al*., 2011). However, in many investigations, maximum roots were formed when medium was supplemented with IBA (Ndoye *et al*., 2003; Babu *et al*., 2003; Chand and Singh, 2004; Sharma and Vashistha, 2010b; 2015c). Babu *et al*., 2003 in *Cinnamomum camphora* reported that although roots were developed on basal medium without any growth regulator yet maximum *in vitro* rooting observed on 0.5 mg/l IBA in the medium.

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