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In vitro propagation of endangered medicinal plant-*Commiphora wightii*

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Abstract

Present study reports *in vitro* multiplication and conservation of *Commiphora wightii* and the influence of growth regulators, location of the explant on mother plant, influence of apical dominance and the size of explant on regeneration ability. Out of the 40, single as well as combinations of BAP, Kn, NAA, IBA and GA_3 used, only three combination treatments of BAP (3mg/l) with IBA (0.1-0.3mg/l) induced shoot development in node and shoot tip cultures. Further, among the three nodes, 1st node (shoot tip) performed better on BAP, IBA and GA_3 supplemented medium. *Commiphora wightii* shows strong apical dominance, the influence of dominance was tested with four explants- shoot tip, simple node, axillary bud and node -AD. Shoot tip, simple node, and axillary bud result no shoot bud development on any concentration or combinations of BAP and/or GA_3 tested. Whereas, the node -ADs alone showed shoots development. Best percentage response of node-AD was observed on 2mg/l BAP+0.5mg/l GA_3 (27%) followed by 1mg/l BAP+1.5mg/l GA_3 (25%) medium. Further, node-AD with two nodes and two internodes performed better and survived for more than 6 weeks on the same medium without subculture as compared to node-AD of 0.5-0.8 cm in size with single node. Regenerated shoots transferred to MS + activated charcoal medium after dipping in NAA, IAA or IBA (200ppm) solutions , showed root development within 20 days of culture with 24 - 26% response. All the regenerated plants were successfully transferred to field conditions. Thus, the paper reports the successful *in vitro* propagation of *Commiphora wightii.*

Keywords: *Commiphora wightii,* Endangered, Medicinal plant, *In vitro* propagation, Tissue culture, India

Introduction

Commiphora wightii (Ar.) Bhandari, commonly known as guggul, belongs to family Burseraceae. *Commiphora wightii* is a shrub, 1.2-1.8 m. high; young parts glandular-pubescent; branches knotty and crooked, usually ending in a sharp spine (Kirtikar & Basu, 1987). The plant produce a dense oily resin identified as guggul, the gum resin, is used in many medicinal preparations. It is a pale yellow or brown in color, aromatic complex mixture of steroids, diterpenoid, aliphatic esters, carbohydrates and varieties of inorganic ions (Pullaiah, 2006). Guggul gum has been employed as a traditional remedy in the practice of Ayurvedic medicine. It induces relief from epilepsy, ulcer, obesity and rheumatoid arthritis (Gujral *et al.*, 1960). It also has anti-inflammatory, antimicrobial activity (Kasera & Mohammed, 2007; Tuchila *et al.*, 2008), hypolipidemic and hypocholesterolemic activity (Nityanand & Kapoor, 1971; Satyavati, 1991; Nohr *et al*., 2009).

According to the studies of FRLHT (2006), *Commiphora* has been listed as **critically endangered** species in Madhya Pradesh and it also has been kept under **Data Deficient** category (IUCN, 2010). Slow growth, poor seed germination ability and excessive tapping for gum resin made this plant endangered. Therefore, *in vitro* propagation method would be the promising option for multiplication and conservation of *Commiphora.* Reports in this area, however, are limited. So far, direct plant regeneration from cotyledonary node segments (Kant *et al*., 2010), shoot tips and nodal explants (Barve & Mehta, 1993) and from stem cuttings (Soni, 2010) has been reported. While Kumar *et al*. (2004, 2006) reported somatic embryo development from immature zygotic embryo and leaf explants. Considerable efforts are still required to find out efficient *in vitro* methods for the regeneration of this critically endangered medicinal plant. Present study reports *in vitro* propagation of *Commiphora* of Madhya Pradesh using nodal segments and further successful establishment in the natural habitat.

Material and method

In the present study, we have used node, shoot tip, and axillary bud as initial plant material. Small healthy twigs from green house plants were collected and initially washed for 5-10 minutes thoroughly under running tap water. Twigs were then surface sterilized as per the procedure of Tejovathi *et al*. (1996).

The node, shoot tip, axillary bud were cut from the surface sterilized twigs and inoculated on Murashige and Skoog's (1964) medium with various concentrations and combinations of growth regulators, and 0.8% Agar-Agar Type-I (Hi Media, India) as gelling agent. All the cultures were incubated under white fluorescent light (2000 Lux.) with 24 hours photoperiod at 25±1°C temperature.

In the present study, effect of various parameters such as- growth regulators, location of the explant on mother plant, influence of apical dominance and size of the explant, on *in vitro* regeneration ability of *Commiphora* has been studied.

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Effect of growth regulators

Influence of auxins, cytokinins and gibberellic acid, their concentrations and combinations, on node, shoot tip and axillary bud cultures was studied using BAP (1.0 - 4.0 mg/l), Kn (1.0-4.0 mg/l), IBA (0.1-4.0 mg/l), NAA (1.0-4.0 mg/l) and GA_3 (0.5-1.5mg/l).

Effect of location of the explant on mother plant

To study the effect of location of the explant on mother plant , 1st three nodes of each twig i.e. shoot tip, 1st & 2nd node were cultured on MS medium supplemented with BAP (1-3mg/l), IBA (0.05-0.3mg/l) and GA_3(0.5-1.5mg/l).

Effect of apical dominance and the size of the explant

Commiphora wightii shows strong apical dominance. Influence of apical dominance on plant regeneration was studied using various explants such as- shoot tip, simple node, axillary bud and node-ADs (nodes from branches with shoot tip and leaves removed 2-3 days prior to inoculation). To investigate the effect of explant size, two types of node-ADs were used (1) Single node-AD of 0.5-0.8 cm in size. (2) Node-AD with two nodes and two internodes.

Rooting and plantlet regeneration

Different concentrations of NAA, IAA, IBA (0.5-5.0mg/l) were used to induce rooting. Regenerated shoots were transferred directly onto root inducing - MS+auxin+2% sucrose medium or onto MS basal +activated charcoal medium after dipping in NAA, IAA, IBA (200ppm) solutions.

Primarily *in vitro* regenerated plantlets were placed on wick culture for better root development, later transferred to root trainers containing the mixture of autoclaved soil and manure. Plantlets initially covered with the polythene bags were maintained at 25±1^{0}C under white fluorescent light. The polythene bags were gradually removed, as plantlets acclimatized to *in vivo* conditions. Finally the established *in vitro* regenerated plants were transfer to the green house for hardening.

All the experiments, each of 20 replicates/ experiment/ treatment, were repeated thrice. The data was recorded as- percentage response, no. of plantlets/explant, from 1 month old cultures.

Result and discussion

Effect of growth regulators

In this study, 40 single as well as combinations of NAA (1.0-4mg/l), IBA (0.1-4mg/l), BAP, Kn (1-4mg/l) and gibberellic acid- GA_3 (0.5-1.5mg/l) were tested. The data, presented in Table 1, shows no shoot development on any cytokinin, auxin or GA_3 supplemented medium. Similarly, in the combinations- lower concentrations of BAP with IBA, also failed to induce shoot development from any of the explant. However, at higher concentrations of BAP (3.0mg/l) with IBA, shoot development was observed from both the explants. MS with BAP (3.0mg/l) + IBA (0.2mg/l) gave better response (20%) followed by 3mg/l BAP + 0.3mg/l IBA (13%) (Table1).

Table 1. Effect of growth regulators, on shoot bud development from shoot tip and node culture of Commiphora sp.

Plant growth regulator (mg/l)					% Response ± S.E.	
BAP	KN	NAA	IBA	GA_3	Shoot Tip	Node
-	1	-	-		NR*	NR
-	2	-	-		NR	NR
-	3	-	-		NR	NR
-	4	-	-		NR	NR
1	-	-	-		NR	NR
2	-	-	-		NR	NR
3	-	-	-		NR	NR
4	-	-	-		NR	NR
1	1	-	-		NR	NR
2	2	-	-		NR	NR
3	3	-	-		NR	NR
4	4	-	-		NR	NR
-	-	1.0	-		NR	NR
-	-	2.0	-		NR	NR
-	-	3.0	-		NR	NR
-	-	4.0	-		NR	NR
-	-	-	1.0		NR	NR
-	-	-	2.0		NR	NR
-	-	-	3.0		NR	NR
-	-	-	4.0		NR	NR
-	-	-	-	0.5	NR	NR
-	-	-	-	1.0	NR	NR
-	-	-	-	1.5	NR	NR
1		-	-	0.5	NR	NR
1		-	-	1	NR	NR
1		-	-	1.5	NR	NR
2		-	-	0.5	NR	NR
2		-	-	1	NR	NR
2		-	-	1.5	NR	NR
1	-	-	0.1	-	NR	NR
1	-	-	0.2	-	NR	NR
1	-	-	0.3	-	NR	NR
2	-	-	0.1	-	NR	NR
2	-	-	0.2	-	NR	NR
2	-	-	0.3	-	NR	NR
3	-	-	0.1	-	6.6±0.01	6.6±0.01
3	-	-	0.2	-	20.0±0.06	-
3	-	-	0.3	-	13.4±0.0	6.6±0.01

NR- no response*

Earlier studies of Kumar *et al*. (2004; 2006); Mathur *et al*. (2007) reported callus development from shoot tip and node cultures on various auxins and cytokinins supplemented media. While, Soni (2010) and Kant *et al*. (2010) reported shoot development response of nodal segments from immature fruit and immature fruit seedlings on auxin and cytokinins supplemented medium, which are in accordance with our results.

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Effect of Location of the explant on mother plant

When, shoot tip and its two successive nodes of each twig were cultured on 15 combinations of BAP, IBA and GA_3 supplemented medium, percentage response, was highest in shoot tips followed by 1st and 2nd nodes (Table 2). Influence of explant location on mother plant on plant regeneration has been well documented in number of plants (Mok & Norzulaani, 2007; Mitsukuria *et al*., 2009; Pereira *et al*., 1995; Sharma & Rajam, 1995; Christopher & Rajam, 1996; Fari, & Czako, 1981). No such work, however, has been reported so far in *Commiphora.*

Table 2. Effect of location of the explant on mother plant in C. wightii

Concentrations of Growth regulator			Shoot development % Response ± S.E.		
BAP	IBA	GA_3	Shoot tip	1st node	2nd node
1	-	-	NR	NR	NR
1	0.05	-	NR	NR	NR
1	0.1	-	NR	NR	NR
1	0.3	-	NR	NR	NR
2	-	-	NR	NR	NR
2	0.05	-	NR	NR	NR
2	0.1	-	NR	NR	NR
2	0.3	-	NR	NR	NR
3	-	-	NR	NR	NR
3	0.05	-	8.2±0.01	8.1±0.05	5.1±0.01
3	0.1	-	12.2±0.03	7.2±0.02	3.3±0.02
3	0.3	-	19.8±0.03	7.9±0.02	callus
3	0.05	0.5	16.0±0.04	9.2±0.03	6.0±0.03
3	0.1	1.0	15.23±0.02	8.3±0.06	5.6±0.07
3	0.3	1.5	13.33±0.05	8.0±0.05	3.3±0.04

Effect of apical dominance and size of the explant

Commiphora wightii shows strong apical dominance, due to which axillary buds stay dormant for long period of time. Decapitation of shoot tip and cutting of leaves from the branches 2-3 days prior to inoculation facilitates axillary bud activation (Barve & Mehta, 1993).

Table 3. Influence of BAP and GA_3 on shoot development from different explants: node, shoot tip, axillary bud and node-AD in Commiphora wightii

Plant growth regulator (mg\l)		Response			Node -AD % Response ± S.E.
BAP	GA_3	Node	Shoot tip	Axillary bud	
1	-	Callus	Callus	Callus	15.0±0.04
2	-	Callus	Callus	Callus	18.8±0.06
1	0.5	Callus	Callus	Callus	21±0.05
1	1	Callus	Callus	Callus	22.0±0.23
1	1.5	Callus	Callus	Callus	25.0±0.02
2	0.5	Callus	Callus	Callus	27.4±0.10
2	1	Callus	Callus	Callus	23.5±0.12
2	1.5	Callus	Callus	Callus	20.0±0.03

Table 3 shows the response of shoot tip, simple node, axillary bud and node- AD on MS+ BAP(1-2mg/l) + GA_3 (0.5-1.5mg/l) medium. Three explants- shoot tip, simple node, axillary bud resulted no shoot bud development on any concentration or combinations of BAP and/or GA_3 tested (Table 3), while node -AD alone showed shoot elongation (Fig. 1A). Best percentage response of node-AD was observed on 2mg/l BAP+0.5mg/l GA_3 (27%) followed by 1mg/l BAP+1.5mg/l GA_3 (25%) medium (Table 3).

Effect of explant size was investigated by using two types of node-ADs: 1. Single node of 0.5-0.8 cm in size. 2. Node-AD with two nodes and two internodes. The results obtained clearly indicate positive response on all the six combinations used (Table 4).

Table 4. Effect of explant size on shoot development

Plant growth regulator (mg/l)		% Response ± S.E.	
BAP	GA_3	Single node-AD	Node-AD with two nodes & internodes
1	0.5	21.62	22.14
1	1	23.33	22.58
1	1.5	20.0	19.23
2	0.5	28.0	26.92
2	1	24.13	24.00
2	1.5	21.42	23.33

However, in single node-AD the leaves dropped and callus initiation was noticed from the same position (Fig.1B,C).While shoots developed from 2nd node-AD with two nodes and internodes were healthy and survived for more than 6 weeks on same medium (Fig.1D), indicating the influence of explants size on the shoot development. Prajapati *et al*. (2010) reported better performance of nodal explant with 2-3 nodes than single node culture in *C. wightii,* which is in agreement with our results.

Rooting and plantlet regeneration

Table.5. Effect of IAA, IBA and NAA solutions on root induction in regenerated shoot of Commiphora wightii

Growth regulators			No. of shoots inoculated for rooting	Root initiation % Response ± S.E.
IAA			45	NR
	IBA		45	NR
		NAA	45	NR
IAA	IBA		45	25.55±0.02
IAA		NAA	45	24.55±0.04
	IBA	NAA	45	26.22±0.01

In vitro shoots (1-2 cm long), cultured on MS supplemented with NAA, IAA and IBA at 0.5-5.0mg/l concentration, failed to induce rooting. Rooting and *in vitro* plantlet regeneration studies in *C. wightii,* are scanty. MS basal medium supplemented with IAA and NAA was reported to induce rooting from *in vitro* shoots (Soni, 2010). While Barve & Mehta (1993), obtained better root

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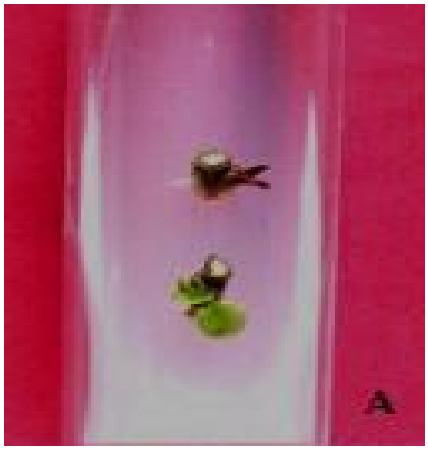
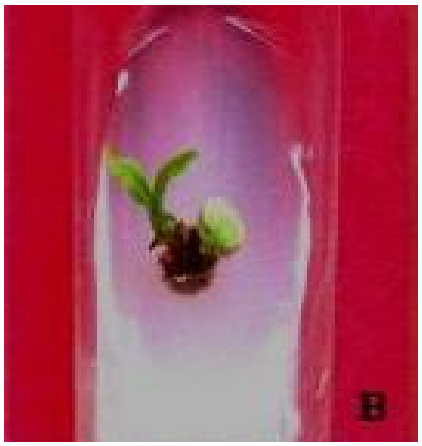
Fig.1. In vitro multiplication of Commiphora wightii

A-Node-AD showing shoot development

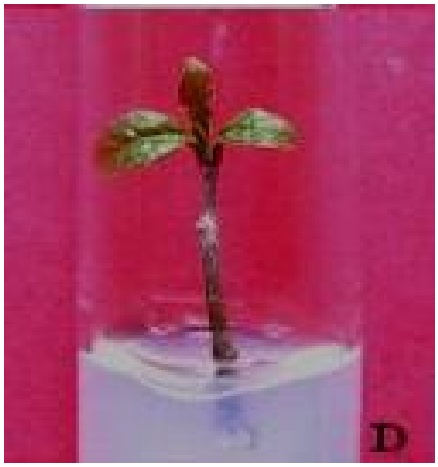


B-Node-AD showing shoot development



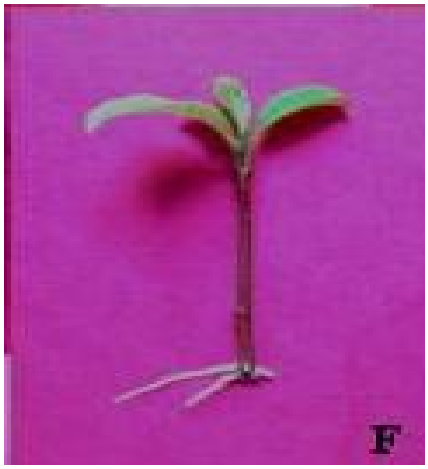
C-Node-AD with shoot showing callus development from leaf

E-Node-AD with two nodes and internodes showing shoot development



F-Node-AD with two nodes and internodes showing shoot with root development

D-Plantlet regenerated from Node-AD with two nodes and internodes



induction on combinations of IAA, IBA, and NAA. Kant *et al*. (2010) achieved rooting by transferring of regenerated shoots to White's medium without hormones and high concentration of activated charcoal. In our studies we have attempted all earlier combinations used, but the response was not favorable. Shoots transferred to MS + activated charcoal medium after dipping in NAA, IAA, and IBA (200ppm) solutions (Table 5), surprisingly shoots showed root development within 20 days of culture (Fig.1E,F). The response was positive in 24-26% cultures dipped in two auxin combinations (Table 5).

About 24-26% plantlet regeneration from nodal segments of *Commiphora*, has been recorded in the present *in vitro* conservation studies. All the regenerated plants survived further on transferring to wick culture and successfully maintained in the field pots.

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