

Condensed node proliferation technique (CNPT): a better low cost macro-propagation approach through mini-cuttings of *Commiphora wightii* (Arn.) Bhandari an endangered plant of Indian Thar Desert

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Abstract

Commiphora wightii (Arn.) Bhandari (Synonym: *Commiphora mukul*) commonly known as Guggal is a perennial shrub/small medium-sized tree of Burseraceae family. It is valued for its moist, fragrant, golden oleo-resin-gum and its medicinal properties. Prevalent oleo-resin-gum tapping techniques and ruthless exploitation of this species has led to decline in natural population bringing it under Data Deficient (DD) category ver. 2.3 (1994) of the Red Data Book of IUCN. Its natural regeneration through seeds is poor in nature. Propagation by stem cutting has been tried with poor establishment. Propagation by air-layering has been tried with appreciable success but with the loss of potential branches, leaving the mother plant in injured state and open to infections. To overcome this problem, an approach by proliferating condensed nodes present at the base of tender shoot branches (TSBs) of plant has been tried. This technique, called condensed node proliferation technique (CNPT) had shown appreciable success with comparative very less injury and loss of potential branches from mother plant. Earlier efforts with the propagation of *C. wightii* form thin cuttings/explants through tissue culture had been either expensive or unsuccessful. The per plant cost of production through CNPT comes around INR 13.50* (With approximately 3000 plantlets production at the end of 08 weeks from plantation day of TSB; and 1000 TSBs are planted/day for 30 days with rooting success rate of 30%). If the plant production protocol of CNPT is extended as per the availability of TSBs the cost of unit plant can be further reduced to INR 3-4.

Key words: Propagation, Tender shoot branches, Condensed Node Proliferation Technique.

Introduction

Commiphora wightii (Arn.) Bhandari (Synonym: *Commiphora mukul*) commonly known as Guggal is a perennial shrub/small medium-sized tree of Burseraceae family. It is a slow growing plant with crooked and knotty branches ending in sharp spines. In India it occurs in Rajasthan, Gujarat and Madhya Pradesh states. In Rajasthan it grows on the foothills of the Aravalli range and in arid/semiarid land including desert areas. The plant exudes an oleo-resin-gum "Guggal" which has been valued in the Indian system of medicine (Ayurveda) for over 2,500 years (Dev 1999). The oleo-resin-gum is moist, fragrant, golden colored mixture of diterpenes, aliphatic esters, steroids, carbohydrates, inorganic ions, essential oils and other compounds (Chadha 2001). The oleoresin is an indigenous drug known to be highly effective in the treatment of obesity, arthritis and several other diseases in Indian system of medicine. Oleo-resin-gum possess hypocholesterolaemic, antiseptic, antipathogenic, antiparasitic, appetizing,

carminative, antispasmodic, diaphoretic, ebolic, anti-suppurative, aphrodisiac and emmenagogue properties (Rout et al. 2012). The gum solution is used as a gargle for spongy gums, chronic tonsillitis and caries in the teeth (Ragunathan and Mitra 1999).

The prevailing techniques used for tapping the oleo-resin-gum have led to decline in its natural population and the species is struggling for its survival. Ruthless exploitation of this species has led to decline in population making this plant vulnerable and has been categorized as Data Deficient in assemblage of International Union for Conservation of Nature (IUCN) in 2008. Though, *C. wightii* is assigned the Data Deficient (DD) category ver. 2.3 (1994) of the Red Data Book of IUCN, the Government of India has included it under Rare, Endangered, Threatened (RET) category (Samantaray et al. 2010; Kulloli and Kumar 2013). Its natural regeneration through seeds is poor in nature. Propagation by stem cutting had been tried with poor establishment (Puri and Kaul 1972; Shah et al. 1983; Singh et al. 1989; Mertia and Nagarajan 2000). Propagation by air-layering had been tried with appreciable success (Dalal and Patel 1995; Kasera et al. 2001). Though with appreciable success, air-layering takes away a lot of potential branches from mother plant leaving the plant in injured state and open to outside infections. To overcome this problem a different approach by proliferating condensed nodes of mini-cuttings of plant called condensed node proliferation technique (CNPT) has been tried. These mini-cuttings can be harvested from those mother plants in which potential branches for air-layering are not available.

Mini-cuttings are very thin newly arisen tender shoot branches (TSBs). These branches remain dormant throughout the unfavorable growing conditions of desert and start growing or arise *de-novo* with the commencement of favorable season. CNP technique utilizes the inherent potential of lateral meristem i.e. vascular cambium present in the condensed nodes at the basal portion of TSBs. The natural way of adventitious root formation in stem cutting takes place by two means i.e. by preformed (latent) root initials and secondly by wound-induced roots which develop only after the cutting is made in response to wounding (Fabbri et al. 2011). The preformed roots are not found in *C. wightii*, however lateral meristem of newly formed TSBs can be induced in appropriate conditions to form root. These roots are considered to be formed *de novo* (Davies and Joiner 1982; Jackson 1986). In the present investigation TSB harvested by special measures are induced to develop root in response to wounding. In wound induced rooting system outer injured cells die, a necrotic plate of suberin seals the wound followed by formation of parenchymatous cells in the form of callus behind necrotic plate and cells near vascular cambium divide and produce adventitious

roots. Earlier efforts with the propagation of *C. wightii* form thin cuttings/explants through tissue culture had all been either very costlier or unsuccessful.

Methodology

Harvesting of TSBs

For CNP techniques four or five mother plants in the age group of two to five years were selected from experimental field nursery of Arid Forest Research Institute, Jodhpur during monsoon in the first week of July. Healthy plants which showed the emergence of new tender shoot branches TSBs were selected. Newly emerging 6-10 cm long TSBs was harvested from the main and tertiary branches of mother plants. The main technique lied in the harvesting mechanism of the TSBs. The newly emerged TSBs were plucked instead of cutting from the main and tertiary branches with utmost taking following precautions: (a) there should not be any pulling effect on the soft bark with its phloem layer beneath, (b) the TSBs should bring out along with it, the condensed accumulated nodes present at the basal portion of point of attachment to the main or tertiary branch and (c) The detachment of TSB should be so that it should break from the natural weak point at the place of attachment with the main or tertiary branch. The diameter of TSBs at basal portion just above the condensed accumulated nodes ranged from 2 to 4 mm. The TSBs along with leaves were immediately vertically immersed in distilled water with lower half portion dipped in water and upper half flanking above the water.

Treatment imposition

TSBs were brought to the poly house conditions and leaves from lower half portion of TSBs were completely removed while keeping leaves in upper half intact. These were treated with 0.05% Bavistin solution for 10 minutes by dissolving weighted amount of dry Bavistin powder in distilled water. Bavistin treated TSBs were rewashed with distilled water and immersed in different concentrations of IAA (indole-3-acetic acid) and IBA (indole-3-butyric acid) (10, 50, 100, 200, 300, 500 and 1,000 mg.L⁻¹ for 10 minutes where as distilled water was used as control. Per treatment thirty replicates with 1 TSB as replicate was used.

Planting of TSBs

Treated TSBs were planted in plastic trays (35x24x8 cm) containing pre water soaked sun sterilized dune sand for rooting. Before planting of TSBs the trays were filled with sun sterilized dune sand up to 7 cm and saturated with water. The floating debris out of sand was washed away 3 to 4 times. The dripping water form sand was completely decanted by tilting the tray at about 45° angle for about 10 minutes. Hole in wet sand was made with the help of glass rod and treated TSBs were planted in same at approximate distance of 3cm plant to plant and 4 cm line to line. 30 TSBs per tray were planted. After planting of TSBs, holes of approximately 2 mm diameter were made in the base of tray with the help of hot needle to drain excess water if any. 5 holes per tray were made out of which 4 were in corner and 1 in the centre. Trays were arranged on iron wire meshed racks in randomized block design and 70% humidity was maintained in poly house condition with natural light.

Parameters observed

TSBs were observed after 30 days of planting for percentage and number of shoot sprouting points, percentage and number of primary and secondary roots, maximum length of primary and secondary root and callus induction at basal portion of TSBs. Quantification of callusing at basal portion of TSBs was done by assigning digits as "1" for low callusing with callus at 1 or 2 points having size of 1-2 mm diameter; "2" for moderate callusing with callus at 1, 2 or more points having size of 2-3 mm diameter and "3" for high callusing with 1, 2 or more points having size of more than 3 mm diameter. The results obtained were analyzed for significant variation as per Gomez and Gomez (1984).

Results and discussion

Data from table 1 reveal that shoot sprouting and callus formation was observed in all treatments which ranged from 13.33 to 66.66% and 6.66 to 63.33%, respectively. Maximum shoot sprouting was observed in IBA 1000 ppm followed by 63.3% in IAA 500 ppm while minimum in IBA 50 ppm. Mean number of shoot sprouting points were maximum 4.95 in IAA 500 ppm followed by 4.23 in IAA 300 ppm while minimum 2.13 in IAA 100 ppm treated TSBs.

Table 1. Performance of tender shoot branches (TSBs) of *C. wightii* under condensed node proliferation technique (CNPT)

		Control	IAA (ppm)							IBA (ppm)							CD
			10	50	100	200	300	500	1000	10	50	100	200	300	500	1000	
Shoot sprouting	%	56.67	20.00	36.66	26.66	50.00	43.33	63.33	30.00	30.00	13.33	23.33	56.66	46.66	46.66	66.66	0.84**
	Number	3.94 ± 1.78	3.33 ± 1.2	2.91 ± 1.1	2.13 ± 1.1	3.60 ± 1.1	4.23 ± 1.7	4.95 ± 1.1	3.78 ± 1.2	2.22 ± 0.8	2.75 ± 1.2	2.86 ± 0.6	3.06 ± 1.2	3.21 ± 1.4	3.14 ± 1.2	2.45 ± 1.0	
Primary root	%		13.33	1.25	26.66	16.66	13.33	13.33	10.00	10.00	10.00	33.33	30.00	13.33	23.33		1.019**
	Number		±	±	±	±	±	±	1.0 ± 0.00	±	±	±	±	±	±		
	Length (cm)		0.50 ± 1.63 ± 1.1	0.46 ± 4.61 ± 2.2	0.84 ± 4.61 ± 2.2	0.50 ± 2.72 ± 1.4	0.58 ± 3.45 ± 2.3	2.80 ± 5.86 ± 2.6	± 1.1	± 2.4 ± 1.7	± 1.6 ± 1.3	± 1.5 ± 1.5					
Secondary root	%		6.66	23.33	10.00	10.00	10.00	10.00	10.00	10.00	30.00	16.66	10.00	23.33			NS
	Number		±	±	±	±	±	±	±	±	±	±	±	±	±		
	Length (cm)		0.00 ± 0.25 ± 0.1	1.22 ± 0.97 ± 0.6	2.08 ± 1.13 ± 0.7	2.65 ± 1.93 ± 1.1	2.08 ± 1.67 ± 1.2	± 1.2	± 0.7 ± 1.1	± 1.2 ± 0.5	± 1.6 ± 1.6						
Callus	%	36.66	6.66	30.00	16.66	46.66	26.66	46.66	16.66	23.33	10.00	23.33	56.66	40.00	43.33	63.33	0.37**
	Mean	1.00 ± 0.00	1.00 ± 0.0	1.11 ± 0.3	1.20 ± 0.4	1.21 ± 0.4	1.25 ± 0.4	1.14 ± 0.3	1.20 ± 0.4	1.71 ± 0.5	1.33 ± 0.5	1.29 ± 0.4	1.65 ± 0.7	1.42 ± 0.5	1.92 ± 0.5	1.95 ± 0.7	

As per observation maximum callusing 63.33% was observed in IBA 1000 ppm with digital assigned value of 1.95 followed by 56.66% in IBA 200 ppm with digital assigned value of 1.65. The least callusing 6.66% with digital assigned value of 1.0 was observed in IAA 10 ppm treated TSBs. The data for both number of shoot sprouting points and callus formation were highly significant. Primary and secondary rooting percentage ranged from 10 to 33.33% and 6.66 to 30.0%, respectively. Highest rooting percentage both primary and secondary was observed in IBA 200 ppm followed by 26.66 and 23.33% respectively in IAA 200 ppm, where as lowest 10% rooting with no secondary root formation was observed in IBA 10 ppm treated TSBs. Lowest 6.66% secondary root formation was observed in IAA 50 ppm; however no root formation was observed in control, IAA 10 & 100 ppm and IBA 50 ppm treated TSBs. Mean number of primary and secondary root ranged from 1.0 to 6.29 and 2.78 to 10.29, respectively. Maximum number of both primary and secondary root was observed in IBA 1000 ppm followed by 3.0 and 8.67, respectively in IBA 500 ppm, whereas lowest only 1 primary root with no secondary root formation was observed in IBA 10 ppm treated TSBs (Table 1, Figure 1).



Figure 1. Response of tender shoot branches of *Commiphora wightii* under condensed node proliferation technique (CNPT): control (extreme left) followed by IBA (10, 50, 100, 200, 300, 500 and 1000 ppm from left to right) treatments

Mean length of primary and secondary root ranged from 1.63 cm in IAA 50 ppm to 5.86 cm in IAA 1000 ppm and 0.25 cm in IAA 50 ppm to 2.24 cm in IBA 1000 ppm treated TSBs, respectively. Data for number of primary roots was significant at 1% probability level but non-significant for length of primary root and number & length of secondary root (Table1).

C. wightii is under Rare, Endangered, Threatened (RET) category, because of its over exploitation for oleo-resin-gum, fire wood besides slower plant growth-rate, poor quality of seed set and excessive tapping (Ramawat et al. 1991; Reddy et al. 2012; Jain and Nadgauda 2013). Moreover, there is no critical information on the reproductive biology, natural means of seed dispersal and seedling establishment of this plant (Gupta et al. 1996). It is uncommon to see seedlings in the vicinity of wild adult plants. Kulloli and Kumar (2013) emphasized the in-depth understanding of reproductive biology and pollination ecology to enhance its seed setting. Propagation through seeds is advantageous as seedlings have a deeper root system compared to the plants established through cuttings (Yadav et al. 1999). Fruit set and yield of fruits per plant are very low in natural conditions. The plant is also apomictic

(Gupta et al. 1996) and polyembryonic in nature (Gupta et al. 1996; Prakash et al. 2000). Kaseera and Prakash (2005) reported two types of seeds, viz. black (viable) and yellowish-white (non-viable) in *C. wightii* from which, black seeds showed 35-40 percent germination under controlled laboratory and nursery conditions. Poor seed set, very poor seed germination (5%) and harsh arid conditions are responsible for failure of plant establishment from seeds in nature. Natural regeneration had been rarely observed below the parent plants in the forests (Yadav et al. 1999; Reddy et al. 2012). *C. wightii* had been successfully propagated through stem cuttings (Mertia and Nagrajan 2000; Chandra et al. 2001; Kumar et al. 2002; Soni 2010). However a major woody part remain exposed at lower portion of cutting in soil, which often lead to termite attack in arid conditions and failure of plantation up to three years of growth period. Earlier, 1 m long and 10 mm thick woody stem cuttings were reported suitable for raising of *C. wightii* (Dalal and Patel 1995), thus requiring a larger amount of planting material for propagation. IBA has been the widely used auxin for rooting in stem cuttings and for success of cuttings in field (Al-Saqri and Alderson 1996). In the present investigation also both number and length of primary and secondary roots were highest in IBA 1000 ppm treated TSBs along with callusing percentage and digital assigned value for callusing. In earlier studies treatment of IBA had been found better than corresponding IAA concentrations. Effect of callusing on rooting is likely to have some effects on rooting which needs further confirmation (Kulloli et al. 2011). Kaseera et al (2001) raise *C. wightii* plants by air layering techniques, in which treatments of guggul solution (500 and 1000 ppm) was found suitable for root initiation, root length and survival under field conditions. Clonal propagation of *C. wightii* through stem cuttings for large scale plantation has been met with many bottlenecks, termite attack being the major cause of the death of the plants. Requirements of manure, fertilizers and irrigation are yet to be understood and standardized for its successful transplantation. Biotechnological tools had also been utilized to develop protocol for in vitro generation of plantlets, however there has been no outcome from different programs on propagation of *C. wightii* since 1979 (Kumar et al. 2003; Sharma et al. 1998). Sterilization of explants collected from the field grown plants remains a major limitation in establishment of *C. wightii* in vitro due to the presence of oleo-resin-gum in explants (stem, leaf and petiole) and bacteria in the resin canals (Ramawat et al. 2008). Organogenesis has been induced through axillary shoot proliferation from nodal segments (Barve and Mehta 1993; Soni 2010), seedling explants (Yusuf et al. 1999; Kant et al. 2010) and shoot tips, nodes, internodes and leaves (Singh et al. 2010). However, Kant et al. (2010) reported efficient rooting on White's medium without any hormones and high concentration of charcoal. Kumar et al. (2003) achieved somatic embryogenesis by repetitive reciprocal transfer of callus cultures of *C. wightii* between basal medium and MS medium containing 2, 4, 5-trichlorophenoxy acetic acid (2, 4, 5 T) and kinetin. They found that immature zygotic embryos as only suitable explants for somatic embryogenesis. The somatic embryos germinated into plantlets which were successfully transferred to field conditions. Kumar et al. (2006) reported secondary somatic embryogenesis in *C. wightii* on basal medium. Thus, a standardized, viable and low cost protocol for large scale production of plantlets/saplings from tissue culture is yet to come out for commercial adoption. The present study is the first attempt of utilizing the condensed nodes of TSBs, which can be used for large scale production of *C. wightii*

plantlets/saplings at very low cost, with minimal input and labor. Up till now air-layering had been a viable and successful technique for vegetative propagation of *C. wightii* (Prakash et al. 2001), as it provide large sized plantlets/saplings which are successful in field conditions in terms of survival and growth (Kasera et al. 2011). Shukla et al. (2001) reported air-layering as quick and economical method of propagation in case of *Leptadaenia reticulata*. However, air-layering method is also restricted by some limitations like it takes away a major portion of aerial branches of mother plant. Secondly, air-layering is not feasible in plant in which potential branches for layering are limited or absent. Thirdly, after successful rooting, cutting and removal of air-layers leaves mother plant exposed to external infection, whereas unsuccessful air-layered branches dries up. The CNPT is perhaps a better solution to the above three major limitations of air-layering. Cost wise if we look at the scenario, the raising of plant through somatic embryogenesis pathway, is equivalent to Indian Rupees (INR) 19, while that produced through cotyledonary node based protocol is INR 27 (Kant et al. 2010) and INR 80 by the in vitro method (Soni 2010). Tomar et al. (2007) calculated the production cost of plant through tissue culture. Applying the parameters used by Tomar et al. (2007), the per plant cost of production through CNPT comes around INR 13.50* (With approximately 3000 plantlets production at the end of 08 weeks from day of plantation of TSB; if 1000 TSBs are planted/day for 30 days with rooting success rate of 30%). The unit cost of plant mainly depends on the efficacy of the procedure followed specially during the plucking of TSBs. Rooting percentage influences the cost inversely. Higher the rooting percentage lower will be the cost. Less number of stages and time duration in CNPT as compared to propagation lowers the plant cost. A tissue culture protocol requires at least two stages, viz. shoot multiplication, rooting cum hardening (ex vitro rooting) which is very difficult to achieve in case of tree species (Tomar et al. 2007). The percentage of plants showing rooting contributes immensely to the unit cost of a plant raised through CNPT. In the present case TSBs has shown maximum 33% rooting in preliminary study. If the rooting percentage is bettered even up to 60% the cost per unit of plant will be reduce to half i.e. nearly INR 7. Cost analysis of plants reveals that labor constitutes a major portion. Better management of manpower can also help in cost reduction of the plants. In place of high cost mist chambers low cost poly tunnels can be employed thus cutting the electric city consumption and cost reduction of plants. In the present investigation the protocol has been mentioned for 08 weeks; however the TSBs keep on arising throughout the monsoon season till the commencement of winter (July to October), as per the availability of rain/irrigation water. If the plant production protocol of CNPT is extended as per the availability of TSBs the cost of unit plant can be further reduced to INR 3-4. The TSB used in the present investigation showed a wide range of variability and differential growth patterns. Therefore, early performance may not always mean similar performance levels in subsequent experiments and vice-versa. More research introspections are needed as physiological state of mother plant, phenological condition, other morphological indicator and minimum number of condensed nodes present at the basal portion of TSB, affects the rooting percentage. The selection of TSBs based on above criteria, so as to show relatively better and consistent performance throughout the experimental trial may be beneficial for large scale low cost clonal production of plantlets/saplings in plantations programs.

Conclusion

CNP technique was successful with comparatively very less injury and minimum loss of potential branches from mother plant. The present study is the first attempt of utilizing the condensed nodes of TSBs, which can be used for large scale production of *C. wightii* plantlets/saplings with minimal input, labor and at very low cost. The CNPT is a better solution to the limitations of air-layering.

*Cost include Electricity expenses (INR 3920) 560 unit (Watt Hour) @ INR 7 per unit; Labor charge (INR 26000) one skilled @ INR 8000 per month and one unskilled @ INR 5000 per month both for two months; Chemicals (INR 1000) Bavistin and IBA; Glass/plastic ware (INR 9375) (Plastic Beaker, Plastic trays, Measuring cylinder, Polythene bags, etc); Total INR 40295 and total number of plants produced through CNPT is 3000 at the end of 08 weeks. Cost of construction of Mist chamber and shade house is not included.

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