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RESEARCH ARTICLE

In vitro plant regeneration from nodal segments of *Commiphora wightii*- an endangered medicinally important desert plant.

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Abstract

Experiments were conducted for micropropagation of endangered medicinal plant ***Commiphorawightii*** using Nodal explants by modified M.S medium.For nodal explants on the MS medium containingBAP 3mg/l and IAA 0.5 mg/l with additives, 82% of cultures showed maximum bud break with 2.40±0.28 no.of shoots with shoot length of 1.82±0.19 within 30 days.A low concentration of auxin along with a high concentration of cytokinin was most promising for the induction and multiplication of shoots in*Commiphorawightii*. The explants cultured on these media showed their first response by initial enlargement of the existing axillary buds followed by bud break.In vitro rooting was achieved on half strength M.S. medium.

INTRODUCTION

Commiphorawightii(Ar.) Bhandari, commonly known as guggul, belongs to family Burseraceae. Commiphorawightii is a shrub, 1.2-1.8 m. high; young parts glandularpubescent;branches knotty and crooked, usually endingin a sharp spine (Kirtikar and Vasu,1987).*Commiphorawightii*(Arn.) Bhandari (family Burseraceae) is well known by its vernacular names guggal (Hindi), guggul (English), guggulu (Sanskrit), etc. *C. wightii*(Arnott) Bhandari (Burseraceae) is an endangered, that grows in arid, rocky tracts of the Aravalli range ,Gujarat,Maharashtra, Karnataka and Madhya Pradesh in India.It is a slow growing plant with a long dormant phase and deciduous nature. The plant is dimorphic in nature, *i.e.*, one having bisexual and male flowers and other having female flowers withstaminodes (Dalal,1995).Two types of seeds-black and white are observed in maturfruits . While the black onesare viable, the whitish-black ones are non-viable due to absence of embryo (Prakash et al.,2000).It is now on the verge of extinction and predominant reasons for its fastdiminishing population are over-exploitation, poor natural germination rate and slow. It has been listed in IUCN Red Data Book under Data Deficient Category IUCN, 2012 (Parmar and Kant, 2012).The plant yields medicinally important natural gum resin. Guggul was first introduced to the scientific world by an Indian Medical Researcher, G. V. Satyavati, in 1966 (Deng, 2007). *Commiphorawightii*(Arnott) Bhandari, a highly valuable medicinal plant yields an oleo-gum resin important inAyurvedic medicines (Chakravarty, 1975).Guggul-gum is known to be hy- polipidemic, hypocholestrolemic and anti-obesity (Singh et al.,1994.,Urizar et al.,2003) astringent and antiseptic (Kaser et al., 2002), anti-inflammatory (Gupta et al., 1974), and anti- cancerous (Xiao and Singh, 2008). It is also reported for the treatment of thrombosis (Tripathi et al.,1968) and chronic bronchitis (Sinha and Sinha, 2001), nodulocystic acne (Thappa and Dogra, 1994), spongy gums, chronic tonsillitis and teeth carries (Raghunathan and Mitra, 1999). The gum is also used in perfumery, calico- printing, fumigation, dyeing silk and cotton and as incense (Reddy et al.,2012).The oleo-gum resin of *C. wightii*(locally known as gum guggul), is mentioned inthe classic Ayurvedic literature as anefficacious treatment for bone fractures,arthritis, inflammation, obesity, cardiovasculardisease, and lipid disorders (Urizar and Moore, 2003., Wang et al., 2004)). The gum is extractedfrom the bark by resin tapping . Aplant generally takes 10 years to reach tappingmaturity under the prevailing dry climaticconditions. Thick branches are incised duringthe

winter to extract the gum resin.Overexploitation, slow-growth and associatedpoor seed set has led to this plant becoming an endangered species (Kumar et al, 2003).*Commiphorawightii* (Arnott) Bhandari is a plant of immense medicinal importance and this plant is becoming endangered and demands severe measures for its conservation.The plant produce a dense oily resin identified as guggul, the gumresin, is used in many medicinal preparations. It is a paleyellow or brown in color, aromatic complex mixture ofsteroids, diterpenoid, aliphatic esters, carbohydrates andvarieties of inorganic ions (Pullaiah, 2006). Guggul gumhas been employed as a traditional remedy in the practiceof Ayurvedic medicine. It induces relief from epilepsy,ulcer, obesity and rheumatoid arthritis (Gujral et al., 1960). It also has anti-inflammatory, antimicrobial activity (Kasera and Mohammed, 2007.,Tuchila et al., 2008),hypolipidemic and hypocholesterolemic activity (Nityaanand and Kapoor,1971., Satyavati,1991., Nohr et al., 2009).

Considerable efforts are still required to find out efficient in vitro methods for the regeneration of this critically endangered medicinal plant. The *in vitro* propagation method can be used for clonal propagation of selected germplasm, genetic improvement, production of active compound in cell culture. Therefore, in vitro propagation method would be thepromising option for multiplication and conservation of*Commiphorawightii*. The *in vitro* propagation method can be used for clonal propagation of selected germplasm, genetic improvement, production of active compound in cell culture. *In vitro* propagation in *C. wightii*has been attempted through organogenesis and somatic embryogenesis methods by various researchers. Organogenesis has been induced through axillary shoot proliferation from nodal segments, seedling explants, shoot tips, internodes and leaves, by various workers. Organogenesis has been induced through axillary shoot proliferation from nodal segments (Barve and Mehta, 1993., Soni, 2010., Parmar and Kant, 2012), seedling explants (Yusuf et al., 1999., Kant et al., 2010) and shoot tips, nodes, internodes and leaves (Singh et al ., 2010), while (Kumar et al., 2006) reported somatic embryo development from immature zygotic embryo and leaf explants.Considerableefforts are still required to find out efficient in vitromethods for the regeneration of this critically endangeredmedicinal plant.Therefore, there is need to develop reliable and rapid protocol for its micropropagationthrough *in vitro.*Our present study demonstrates that tissue culture protocols through cotyledonary node cultures for in vitro mass multiplication and conservation of *C. wightii*- an endangered medicinally important desert plant.

MATERIALS AND METHODS

Plant Material

*Commiphorawightii*nodal segments were collected from healthy trees growing in AFRI Nursery,Jodhpur district. Nodal segments from young juvenile branch of mature *C. wightii*treeused as explant having 1-2 nodes. Nodal segments measuring about 1.0-1.5 cm were cut from the shoots and rinsed thoroughly with tap water for 5 min to remove dust. The segments were subjected to surface sterilization using tween 20 for 10 minutes, then with distilled water 4-5 times.After it were sterilized with bavistin for 5-10 minutes, then with distilled water 4-5 times.In laminar air flow bench it were surface sterilized with 70% ethanol for 30 seconds ,then with 0.1% (wt/vol) mercuric chloride (BDH, India) for 3-6 min depending upon the maturation of explants. Thereafter, the segments were washed 5 - 7 times with autoclaved distilled water.

Culture medium and conditions

The most responsive treatments for improvement in terms of bud break response, length and numbers of shoots were tried on different media types M.S. media (Murashige and Skoog, 1962),Gamborg's B5 media (Gamborg's et al., 1968).Murashige and Skoog's medium supplemented with sucrose (3%) + 100 mg of Trimethoprim antibiotic and various growth regulators used for our current study. The pH of the medium was adjusted to 5.8 before gelling with 0.8% agar .Molten medium was dispensed into culture tubes or jam bottles depending upon the requirement. The culture tubes containing media were autoclaved at 20 psi pressure (104kPa)and 121°C for 15 min. The processed explants were inoculated vertically on the culture medium. All the cultures were grown under 16-h photoperiod maintained by florescent light at 25±2°C and 1600 lux intensity light (via cool florescent lamps and incandescent light bulbs). The number of explants cultured in each treatment varied from 20-30 depending upon the experiments.

Effect of Growth Regulators on shoot proliferation

For shoot proliferation, nodal segments were collected from actively growing shoots and inoculated on M.S. medium supplemented with different concentrations of BAP (1.0 ,2.0, 3.0, 4.0. 5.0 mg/l)in combination withIAA(0.5mg/l)with additives (50 mg/L ascorbic acid, 25 mg l/Lcitric acid and 25 mg l/Larginine) ,activated charcoal0.3% reported earlier by (Parmar and Kant, 2012). For each experiment 20 replicates were taken and repeated twice. Explants cultured for 30 days were observed and data on bud break and shoot regeneration was recorded and analyzed. Once the culture conditions for actively growing shoots from explants were established,the isolated shoots were multiplied on same medium and additives as in the initiation mediumfor shoot proliferation for

obtaining adequate no. of shoots and grown for another 30 days, bringing the total culture period to 60 days. Data on bud break and shoot regeneration was recorded and analyzed.

In vitro rooting and complete plant regeneration

Micro- shoots with 2-3 pairs of leaves regenerated on MS medium were taken out from the culture vessel and agar which is adhering on the stem of micro shoots were removed under running tap water,initially micro shoots given a treatment in liquid M.S. media and White's medium fortified with 0.5 mg/L IBA under dark condition for 2 days, then they are transfer to light in semi-solid half-strength hormone-free MS media and White's medium supplemented with 2% sucrose and 1% activated charcoal.These shoots were retained on the same medium for 40 days and observation was recorded on root and shoot development. Plantlets with well- developed root and shoot system were removed and transferred to small plastic pots having a sterile 'soilrite' (soilless compost and soil conditioner) and acclimatize.Plantlets initially covered with the a transparent polythene bags were maintained at 25 ± 10^0C under white fluorescent light to ensure high humidity around the plants. The polythene bags weregradually removed, to expose the plants to theconditions of natural humidity for acclimatization. .Finally, in vitro regeneratedplants were transferred to the green house for hardening.

RESULTS AND DISCUSSION

Effect of different sterilants on disinfection nodal segments

Effect of different sterilants on disinfection of nodal explants of Commiphorawightii Following the selection of explants, the next effort was to initiate maximum contamination free cultures, which is usually difficult and problematic, due to high rate of contamination, when the explants are collected from the field grown plants. The fungicide Bavistin at various time durations was found to be effective, when used along with $HgCl_2$. However explants surface sterilized with NaOCl(1%) and $HgCl_2$ (0.1%) for different time intervals showed varying level of % of contamination.However sterilization of apical bud and nodal explant with .1% $HgCl_2$ for 4 minutes was more effective than NaOCl (1%).Maximum contamination free cultures were obtained when the explants were disinfected with 0.1% $HgCl_2$ for 4 minutes along with Bavistin (Table-1). In general $HgCl_2$was reported to be an effective disinfectants and widely used in micropropagation of several woody species such as coffee (Rajasekaran and Mohankumar, 1993), tea (Rajakumar and Ayyappan,1992). $HgCl_2$ is better than NaOClwas reported in*A. cadamba* by (Kavitha et al., 2012).

Table-1 Effect of different sterilants on disinfection of nodal segmentsof *Commiphorawightii*.

Bavistin	.1%$HgCl_2$	% of contamination
5 min.	2 min.	70
6 min.	4 min.	30
7 min.	5 min.	40

Initiation of shoot cultures

The frequency of bud break and no. of shoots developed per explant was highest inM.S. medium followed by B5 medium. Cosequently, M.S. medium is used in all our experiments. Nodal explants collected from actively growing shoots when cultured on MS medium supplemented with different conc .of BAP (1.0- 5.0 mg/l) in combination with IAA (0.5 mg/l) withadditives (50 mg/L ascorbic acid, 25 mg l/L citric acid and 25 mg l/L arginine and activated charcoal 0.3%) reported earlier by (Parmar and Kant, 2012., Barve and Mehta, 1993), percentage of explant sprouted, mean no. of shoots and mean length of shoots were varied in different concentrations and also promoted multiple shoot formation.

For nodal explants on the M.S. medium containingBAP 3mg/l and IAA 0.5 mg/l with additives, 82% of cultures showed maximum bud break with 2.40±0.28 no.of shoots with shoot length of 1.82±0.19 within 30 days.A low concentration of auxin along with a high concentration of cytokinin was most promising for the induction and multiplication of shoots in *Commiphorawightii*. The explants cultured on these media showed their first response by initial enlargement of the existing axillary buds followed by bud break (Table-2.).These results showed the positive effect of additives on bud break response and increase in the shoot number and shoot length . The positive effects of different additives on bud break response and shootmultiplication were reported earlier by(Parmar and Kant, 2012., Barve and Mehta, 1993 and Shekhawat et al., 1993). However combination of auxin and cytokinine promoted elongation of shoots. The addition of auxin promotes shoot elongation was reported earlier in *Eucalyptus grandis* (Luis et al., 1999). Direct multiple shoot regeneration from nodal explants has been reported in many medicinal plants (Nobre et al., 2000., Tiwari et al., 2000).The synergistic effect of BAP in combination with an auxin has been demonstrated in many medicinal plants *Leptadeniareticulata* (Arya et al., 2003), *C. candelabrum* (Beena et al., 2003), *Tylophoraindica* (Faisal et al., 2007), *Huerniahystrix* (Amoo et al., 2009)and *Sarcostemmabrevistigma* (Thomas and Shankar, 2009). Beneficial effects of BAP in combination with IAA on shoot induction have been

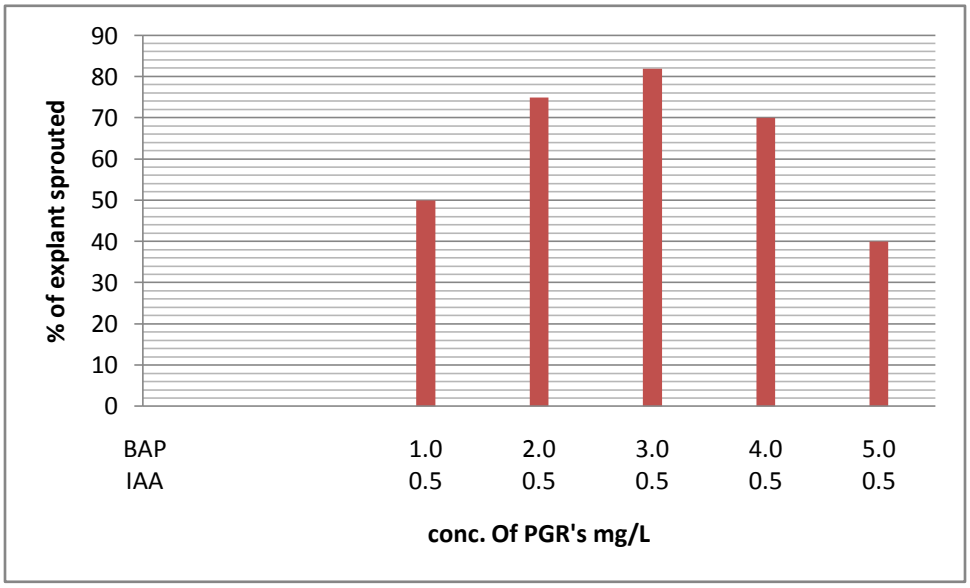
observed in Commiphorawightii (Yusuf et al., 1999.,Parmar and Kant, 2012), *Leptadeniareticulata* (Arya et al., 2003).

After initial proliferation of shoots in medium containing BAP 3mg/l with IAA 0.5 mg/l, shoots along with mother explant subcultured on medium containing BAP 3mg/l for 30 days results in 3 fold multiplication of shoots from basal end of explants of*Commiphorawightii*.Each explant produce 3-4 shoot clumps.

Table-2 Effect of various conc. Of PGR's on shoot proliferation from stem nodaleplants of*Commiphorawightii*

MS + Conc.of PGR'S (mgl^{-1}) + Additives		Percentage of explant sprouted	Mean no. of shoots ± SD	Mean length of shoots ± SD(cm)
BAP	IAA			
1.0	0.5	50	1.54± 0.49	0.64±0.17
2.0	0.5	75	1.76± 0.37	0.88±0.26
3.0	0.5	82	2.40± 0.28	1.82±0.19
4.0	0.5	70	1.64± 0.33	0.70±0.18
5.0	0.5	40	1.26± 0.12	0.52±0.13

Data from 20 replicates in two experirnents(Mean±SD)
Growth period 30 days



***In vitro* rooting**

For root induction regenerated individual shoots of 1-2 cm of Commiphorawightiiwere initially given a treatment in liquid MS media and White's medium (White, 1954)fortified with 0.5 mg/L IBA under dark condition for 2 days, then they are transfer to light in semi-solid half-strength hormone-free MS media and White's medium supplemented with 2% sucrose and 1% activated charcoal.It was observed that continuous darkening during the rooting inductive phase, increases peroxidase activity which resulted in higher rooting rate (Druart et al., 1982).On half-strength hormone-free MS medium with 1% activated charcoal 85 percent rooting was achieved within 40 days with 3.21 ± 0.21 mean no. of roots of 6.04 ± 0.24 cm length while 54% rooting was observed on White's medium

with 1% AC (Table-3). Earlier reported by (Parmar and Kant, 2012.,Barve and Mehta, 1993) for rooting of micro-shoots of C. wightii. The promoting effect low conc. of auxin and darkness on adventitious root formation in Micro-shoots of different species were reported by (Sharma et al., 2007., George et al., 1993 and Monteuuis and Bon, 2000). The effect of low conc. of salts, sucrose and activated charcoal on adventitious root formation in micro-shoots were reported by (Sharma et al., 2007) in apple rootstocks. The enhancing role of IBA on *in vitro* root induction has been reported earlier by (Asghar et al., 2011.,Rafique et al., 2012).

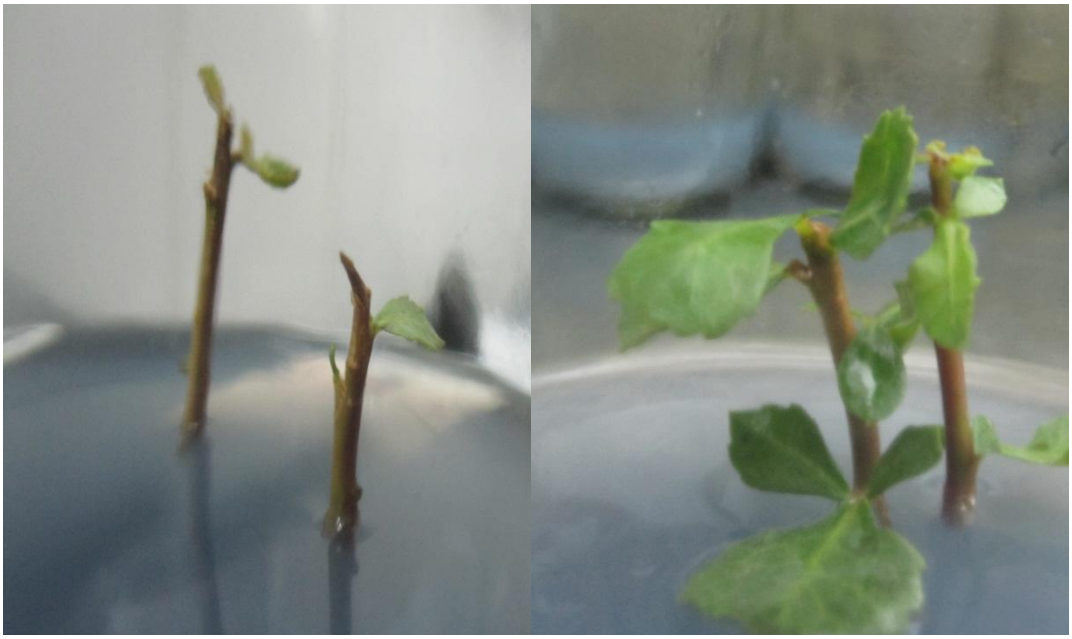
Table-3.Effect of different media on *in vitro* rooting of *Commiphorawightii*..

Medium	% of rooting	Mean no. of roots ±SD	Mean length of roots ± SD(cm)
MS ½ Medium +2% Sucrose + 1% activated charcoal	85%	3.21±0.21	6.04±0.24
White's Medium + 2% Sucrose + 1% activated charcoal	54%	2.76±0.18	5.87±0.22

Data from 20 replicates in two experirnents(Mean±SD)
Growth period 40 days

Acclimatization and transfer of plantlets to the soil

After development of sufficient roots, the plantlets were taken out from the medium,washed gently under running tap water to remove any adherent gel and then washed for 5-10 min in autoclaved distilled water (Thiruvengadam, 2006)and transferred to small plastic pots having a sterile 'soilrite' (soilless compost and soil conditioner) and acclimatize.Each pot was covered with a transparent polythene bag to ensure high humidity. The plantlets were irrigated with half strength MS salt solution on alternate days. Polythene bag were gradually removed after 2 weeks to acclimatize theplantlets to field condition. The plantlets after gradual acclimatization were transferred to the natural conditions.Gradual acclimatization and hardening reported earlier by (George, 1996). Successful acclimatization and field transfer of the *in vitro* regenerated plantlets have also been reported in *Peganumharmala*(Goel et al., 2009) *Celastruspaniculatus* (Lal and Singh, 2010).



A- Bud break from explants. **B- Shoot initiation**


C--Multiple shoot production **D- *IN VITRO* rooting of Commiphorawightii**


E- *IN VITRO* rooted plantlet. **F-Hardening of *IN VITRO* raised plants.**

PLATE-1

Conclusion

C.wightii is medicinally a very important plant especially, as a source of guggulsterone, having tremendous medicinal importance. Therefore, it becomes very important to save this species from the danger of becoming extinct. The plant faces a high risk of endangerment due to various factors like over-exploitation, narrow extent of occurrence, small area of occupancy, severe fragmentation of populations, very low regeneration, slow growth, excessive and unscientific tapping method (Jain and Nadgauda, 2013).

Thus in vitro cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species. Tissue culture protocols have beendeveloped for several plants which are overexploited in pharmaceutical industries and need conservation. This protocol would provide an effective strategy for the conservation of this overexploitedmedicinal plant.

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