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EFFICIENT MICROPROPAGATION FROM COTYLEDONARY NODE CULTURES OF *COMMIPHORA WIGHTII* (ARN.) BHANDARI, AN ENDANGERED MEDICINALLY IMPORTANT DESERT PLANT

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Abstract: *Commiphora wightii* (Arn.) Bhandari, is a medicinal important desert species of the family *Burseraceae*. It is a well known for its valuable active principle found in its oleo-gum-resin (guggulsterone E and Z), which are used in drugs preparation for lowering the cholesterol level in human body. Due to its overexploitation, poor natural regeneration this valuable plant is on the verge of extinction and thus a IUCN Red listed species. In the present study we report development of an efficient micropropagation protocol from cotyledonary node of *Commiphora wightii*. Cotyledonary nodes were used as an explants and multiple microshoots were obtained on Murashige & Skoog (MS) medium supplemented with 2.68 μM α-Naphthalene acetic acid (NAA) and 4.44 μM 6-Benzylamino purine (BAP) and on 2.68 μM NAA; 4.44 μM BAP with additives (glutamine 684.2 μM; thiamine 29.65 μM; activated charcoal 0.3%) and various other hormonal combinations. Elongation of microshoot was significantly observed on the 2.46 μM Indole-3-butyric acid (IBA) and 2.22 μM BAP supplemented MS medium. Efficient rooting was obtained on pretreated microshoot (4.92 μM IBA for 24 hours) and followed by transfer to White's medium without Plant Growth Regulators (PGR) and high concentration of activated charcoal (AC). Rooted micro-shoots were transferred to vermiculite and wetted with Hoagland's solution for primary hardening for 4-5 weeks and then finally transferred to plastic cups containing vermiculite, placed in mist chamber. Plantlets were transferred to soil: FYM 1:1 containing poly-bags, then to green shade house for complete acclimatization and finally transplanted to the experimental field.

Keywords: Medicinal plant, *Commiphora wightii*, guggulsterone, budbreak, acclimatization

Introduction

Commiphora wightii (Arn.) Bhandari (*Burseraceae*), a small tree having arid natural habitat grows in North-Western arid tracts of the Thar desert in India and Eastern parts of Pakistan. It is an important medicinal plant of the Indian Ayurvedic system of medicine for over 3000 years and also having a nice status in modern drug system. It is the source of valuable oleo-gum-resin popularly known in the Indian sub-continent as 'guggul gum' or 'guglu', has commercial importance and is extracted through tapping of main stem. Guggul gum is a source of guggulsterones and has many medicinal properties. It lowers hepatic cholesterol levels by acting as an antagonist of the FXR bile acid receptor, important in metabolism of cholesterol [URIZAR, 2002]. It takes 8 to 15 years to become commercially exploitable through tapping and yields 700 to 900 g resin. After which the plant invariably dies [SABINSA CORP., 2000]. Moreover, seeds are a result of apomixes so their formation is very irregular. Apomixis is non-pseudogamous [GUPTA & al. 1996, 1998]. According to one report, the germination rate is as low as 1.4% [YADAV & al. 1999) and it is a slow growing

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species [SONI, 2010]. Hence, natural regeneration rate of this species is very low. Coupled with its commercial importance resulting in over exploitation, its natural population is dwindling fast. It has been listed in the IUCN Red List of threatened species [IUCN, 2010].

Hence tissue culture holds promise to mass-multiply this valuable species which is on the verge of extinction. Here we report the results of a study leading to the development of a micropropagation protocol of *Commiphora wightii* (Arn.) Bhandari, from cotyledonary nodes (Fig. 1).

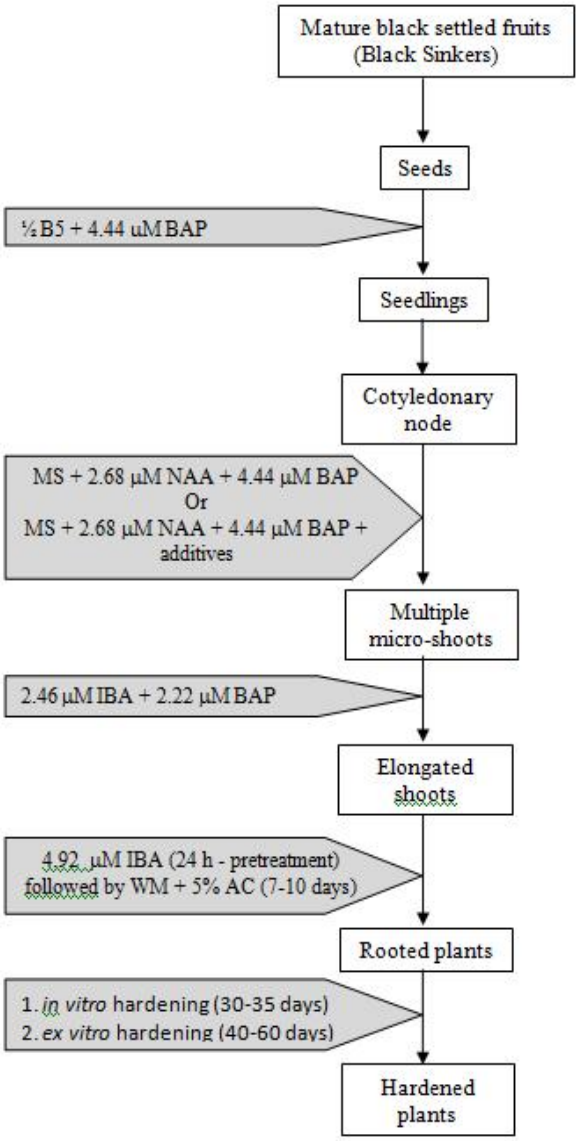

Fig. 1. Complete micropropagation protocol for *Commiphora wightii* from cotyledonary node

Materials and methods

Plant Material.

Commiphora wightii seeds were collected from marked visibly healthy trees growing in the natural population cluster in Kaylana region (Fig. 2A) and plantations growing at the ecology field of JNV University, Jodhpur. The plant flowers twice a year (March and September) and hence fruits (Fig. 2B) were collected twice (summer collection: April-June and winter collection: October-December). Fruits endocarp were either black or white (Fig. 2C). Ripe fruits had red mesocarp.

Fruit selection process. The mature fruits collected (both white and black and from winter and summer season) were subjected to a '*water submergence selection process*'. The fruits either remained submerged or floated and were accordingly classified as sinkers and floaters. Floaters were rejected as they were mostly found to have empty locules with small, improperly developed or no seed. This also explains the reason for their floatation. Sinkers were further categorized into three classes based on their weight. Higher fruit weight is an indicator of healthy and bigger seed. Seeds were isolated from fruits and total number of seeds was counted for all categories.

Surface sterilization. The fruits were de-pulped to remove exo- and mesocarp. The fruit having seeds (category C) were subjected to surface sterilization procedure. The selected fruits were washed in running tap water for 2 minutes to remove dirt and superfluous impurities. They were then shaken in 100 ml. RO water (Millipore RiOS5) having 2 drops of tween-80 for 10 minutes, rinsed 3 times with sRO water (sterilized water from Reverse Osmosis). The cleaned fruits were then treated for 10 minutes with a solution of 200 mg Bavestein and 50 mg streptomycin in 100 ml sRO water with gentle shaking at 50 rpm and rinsed with sRO water once in a laminar flow clean air cabinet. The fruits were finally treated with NaOCl solution (providing 5% available chlorine) for 5 minutes and rinsed with sRO water thrice. The endocarp was now broken open carefully with a sharp sterile scalpel and seeds were scooped out and inoculated on germination medium.

Nutrient Medium. MS, B5 and Whites media were used in various experiments. Media were prepared by re-suspension of readmix nutrient salts (HiMedia Laboratories, India). For germination, full and half strength MS and Gamborg's B5 medium were tested. In one experiment the germination medium was supplemented with BAP (4.44 μM). Different experiments were carried out to test the effect various PGRs on bud break response of the cotyledonary node explants. MS medium supplemented with (4.44, 11.09 and 17.76 μM) BAP alone; MS medium supplemented with Kinetin (4.65, 11.62 and 18.58 μM) alone; MS Medium supplemented with a combination of auxins and cytokinins such that different auxin – NAA (2.68 μM), IAA (2.85 μM), 2,4-D (2.26 μM) and IBA (2.46 μM) was combined with BAP (4.44 and 11.09 μM). To see their effect on bud break response three additives were also tested in the bud break medium. These were glutamine (684.2 μM), thiamine (29.65 μM) and activated charcoal (AC) (0.3% w/v). MS media supplemented with IBA (2.56 μM) + BAP (4.44 μM) and MS media supplemented with IBA (2.46 μM) and BAP (2.22 μM) were used for elongation of micro-shoots.

Root induction. Proliferated shoots were excised from the established cultures and transferred on full strength MS media supplemented with IBA (4.92 μM) for 24 hours treatment. After treatment micro-shoots were subcultured on PGR-free (Plant Growth Regulators free) White's medium, PGR-free White's medium with AC (5% w/v) and PGR-free half strength MS medium for rooting response.

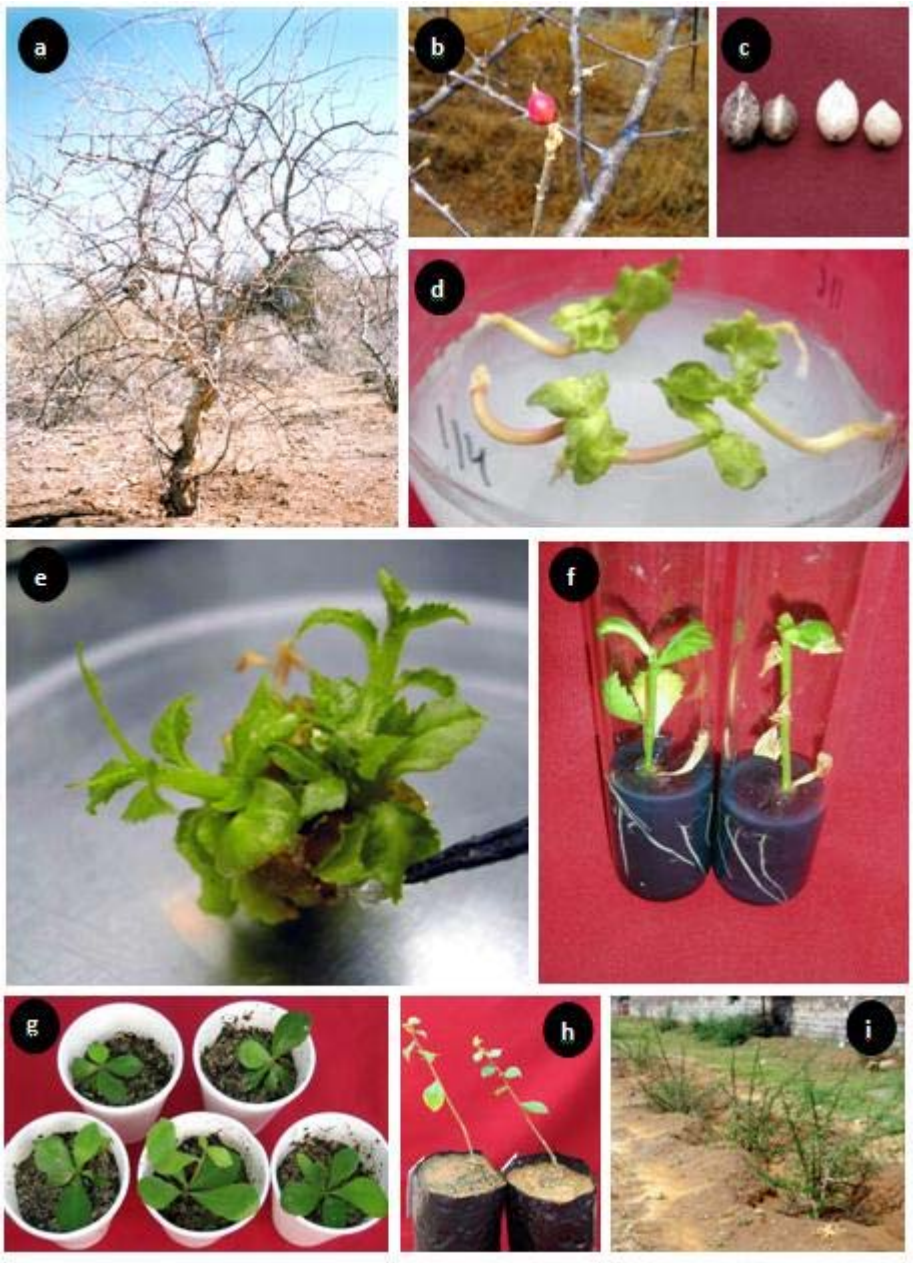

Fig. 2. Cotyledonary node derived micropropagation in *Commiphora wightii*. *A,* a mature tree in May; *B*, mature fruit; *C*, seeds with black and white endocarp; *D*, Germination after BAP pretreatment; *E*, micro-shoot multiplication; *F*, *in vitro* rooting; *G*, *ex vitro* hardening; *H*, hardened plantlets ready for field trial; *I*, plants growing under field conditions (after 1 yr. of transplantation)

Acclimatization and field transfer. Well-rooted plantlets were transferred to glass jam jars filled quarter level with vermiculite and wetted with Hoagland's solution [HOAGLAND, 1950]. After 4-5 weeks when plantlets showed new growth the plastic cap of the glass jar was unscrewed gradually over a period of 2-3 days to reduce relative humidity in the jar, then finally the caps were removed completely from the jars on the third day**.** The plantlets were then transferred to thermocol cups containing vermiculite wetted with Hoagland's solution at one-week interval (Fig. 2G). These plantlets were placed in mist chamber. After two weeks these were then transferred to soil: FYM 1:1 mixture in plastic plantation bags (poly-bags) of size 9x9x36 cm (2916 cm^3). In mist chamber, 90 second misting at ten minutes interval was given to maintain RH between 85 to 95%. The temperature of mist chamber was maintained between 28-30°C. After one month of transfer to poly-bags plantlets were transferred under green-50% agronet shade (Fig. 2H) and after two week transferred to field (Fig. 2I) where they are growing well and have started to flower and set seeds. Growth data of the field grown plants is being collected (data not shown).

*Statistical analysis***.** Experiments were set up in completely randomized design (CRD). Each treatment consisted of 5 replicates and each replicate with five explants. Experiments were repeated thrice. The frequency of bud break and micro-shoot elongation, expressed as percentage, was calculated as the proportion of the number of explants giving positive response. The data were analyzed statistically using SPSS ver 8.0 (SPSS, Chicago, IL). Significance of differences among means was compared using two way analysis of variance and least significant difference at P=0.05.

Results and discussion

Selection of mature fruits for seed isolation. Mature fruits when dipped in water segregate into two types – floaters (F) and sinkers (S). Black fruits collected from different locations during different seasons were dipped in water and it was observed that maximum number of settled black fruits came from winter collection of Kaylana region natural populations. Further selection was based on the weight of the fruits (Tab. 1). Mature fruits could be separated into three categories - floating black fruit, floating white fruit and settled black fruit and were categorized as A, B and C respectively. Settled white fruits were not included in the experiments because their number was very low. Fruits of category C were heavier than other two categories. Therefore, category C fruits were selected to isolate mature seeds. Observations showed no remarkable difference in number of seeds isolated from settled black fruits collected from plantations of JNV University campus and natural plantations of Kaylana region during winter. Further, weight of the isolated seeds from floating and settled fruits was examined**.** No difference was found in weight of the seeds isolated from seed containing floating and settled black fruits collected from Kaylana. Hence, seed present in floating and settled fruits were considered equally good. However, selection was essential to save time and to get maximum seeds in minimum time duration because most of the floating fruits were empty.

Tab. 1. Selection of mature fruits for seed categorization using water dip test

Fruit Category	Fruit colour	Water dip test	Weight (g)
A	White	F	0.02 ± 0.00
B	Black	F	0.04 ± 0.02
C	Black	S	0.07 ± 0.03

Note: F = floater; S = sinker

Seed germination. Isolated seeds from category C fruits, after surface sterilization, were inoculated on full and half strength salt concentrations of MS [MURASHIGE & SKOOG, 1962] and B5 media [GAMBORG'S, 1968]. Half strength B5 media was found to be the most suitable medium for *in vitro* germination of seeds (Tab. 2).

Tab. 2. *In vitro* seed germination response

Treatment	No. of germinated seeds	No. of robust seedlings
MS –fs	10.0 ± 1.92	2.22 ± 1.11
MS –hs	16.5 ± 1.50	16.50 ± 1.41
B5 –fs	19.0 ± 1.0	8.0 ± 0.03
B5 –hs	20.0 ± 0.24	18.0 ± 0.21
MS-hs-1B	21.75 ± 0.93	8.25 ± 1.31
B5-hs-1B	**24.00 ± 0.20**	**17.5 ± 0.32**

Note: fs = full strength; hs = half strength; 1B = 4.44 μM BAP
data scored after 4 weeks of culture inoculation. values indicate Mean ± SE.

Effect of BAP pretreatment. Isolated seeds were treated with BAP during germination. Half strength B5 medium supplemented with BAP (0 and 4.44 μM) was tried. Half strength B5 medium supplemented with BAP (4.44 μM) was found to be best for seed germination and healthy seedling production (Tab. 2). Seed germination was 100% and nearly 72% seedlings were healthy on B5 medium supplemented with BAP (4.44 μM) (Fig. 2D). Positive effect of pretreatment on bud break and multiplication was clearly observed (Tab. 3). Bud multiplication response was also better in BAP treated explants.

Tab. 3. Effect of pretreatment (4.44 μM BAP) on bud break and micro-shoot multiplication through cotyledonary node explants cultured on MS + 2.68 μM NAA + 4.44 μM BAP.

BAP pretreatment (μM)	Percentage explants showing bud break	No. of micro-shoots per sprouted bud (= Fold multiplication)	
		After 20 days	After 45 days
0 ©	58.33 ± 1.10	1 ± 0.32	0
4.44	66.66 ± 1.15	1.75 ± 0.12	**2.25 ± 0.21**

Note: Data scored after 4 weeks of culture inoculation for bud break response.
values indicate Mean ± SE.
© = control

Effect of different hormones on bud break.

1. BAP: Pretreated cotyledonary node exlants were harvested from *in vitro* raised seedlings. These exlants were cultured on MS medium supplemented with BAP (4.44, 11.09 and 17.76 μM). Full strength MS medium supplemented with BAP (4.44 μM) responded best as compare to others (Tab. 4). Increasing concentration of BAP impose

negative effect on bud break and multiplication. Quality of shoot buds was good on BAP (4.44 μM) and BAP (11.09 μM) with very little callusing at the base of cotyledonary node, whereas on higher concentration callusing was more and swelling of micro-shoots was observed. It was also observed that on higher concentration (17.76 μM BAP) proper shoots were not produced.

2. Kinetin: Pretreated cotyledonary node exlants were harvested from *in vitro* raised seedlings. These exlants were cultured on MS medium supplemented with Kinetin (Kn) (4.65, 11.62 and 18.58 μM). Full strength MS medium supplemented with Kinetin (4.65 μM) responded best as compare to others (Tab. 4). Increasing concentration of Kinetin impose negative effect on bud break and multiplication. Quality of shoot buds was good on Kinetin (4.65 μM) but comparatively micro-shoots produced on BAP (4.44 μM) were looking better.

Tab. 4. Effect of BAP and Kn used separately on bud break and micro-shoot multiplication

BAP (μM)	Kn (μM)	Percentage explants showing bud break	No. of micro-shoots per sprouted bud (= Fold multiplication)	
			After 20 days	After 45 days
0 ©	0 ©	11.08 ± 1.33	0	0
4.44	-	**72.25 ± 0.81**	1.19 ± 0.53	**1.49 ± 0.46**
11.09	-	66.66 ± 0.45	**1.29 ± 0.33**	1.42 ± 0.10
17.76	-	63.92 ± 0.34	1.22 ± 0.16	1.35 ± 0.12
-	4.65	69.42 ± 1.17	1.16 ± 0.23	1.20 ± 0.00
-	11.62	63.91 ± 0.72	1.09 ± 0.51	1.13 ± 0.36
-	18.58	58.33 ± 0.85	1.14 ± 0.11	1.19 ± 0.13

Note: Data scored after 4 weeks of culture inoculation for bud break response.
values indicate Mean ± SE.
© = control

3. Auxin and cytokinin: With the aim to study the bud break response on various hormone combinations different auxin NAA (2.68 μM), IAA (2.85 μM), 2,4-D (2.26 μM) and IBA (2.46 μM) were combined with BAP (4.44 and 11.09 μM). On the basis of previous results BAP (4.44 and 11.09 μM) were producing good micro-shoots hence only these were combined here with auxins. Two combinations (2.68 μM NAA + 4.44 μM BAP and 2.26 μM 2,4-D + 11.09 μM BAP) were showing best and similar quantitative results but quality of the microshoot was better on 2.68 μM NAA + 4.44μM BAP (Tab. 5). On 2.26 μM 2,4-D + 11.09 μM BAP, however, microshoot here were not elongating. Later on they turned pale green and died. Qualitatively very nice growth of microshoot was observed on both the BAP combinations with IBA. Micro-shoots were green, shiny and robust on 2.46 μM IBA + 4.44 μM BAP and 2.46 μM IBA + 11.09 μM BAP.

Tab. 5. Effect of auxin and cytokinin used in combination on bud break and micro-shoot multiplication response

Treatment		Percentage explants showing bud break	No. of micro-shoots per sprouted bud (= Fold multiplication)	
Auxin	BAP (μM)		After 20 days	After 45 days
0 ©	0 ©	11.08 ± 1.33	0	0
NAA [2.68 μM]	4.44	**86.7 ± 1.24**	**1.58 ± 1.04**	**2.04 ± 1.22**
	11.09	73.3 ± 1.22	1.36 ± 0.37	1.27 ± 0.67
IAA [2.85 μM]	4.44	76.7 ± 1.89	1.13 ± 0.13	1.09 ± 0.52

	11.09	66.7 ± 2.56	1.29 ± 0.66	1.35 ± 0.39
IBA [2.46 μM]	4.44	73.3 ± 0.89	1.13 ± 0.35	1.68 ± 0.91
	11.09	80.0 ± 1.15	1.17 ± 0.67	1.50 ± 0.72
2,4-D [2.26 μM]	4.44	83.3 ± 1.01	1.28 ± 1.05	1.40 ± 1.04
	11.09	**86.7 ± 1.17**	1.11 ± 0.78	1.19 ± 1.11

Note: Data scored after 4 weeks of culture inoculation for bud break response.
values indicate Mean ± SE.
© = control

Effect of additives on bud break. Glutamine, thiamine and activated charcoal were used as additives. Results show no positive effect of additives. Number of explants showing bud break and number of micro-shoots produced were almost equal in both the experiments (Tab. 5 and 6). Qualitative observation showed that in presence of additives micro-shoots looks healthier with dark green leaves and thick stem.

Tab. 6. Effect of additives on bud break and micro-shoot multiplication response

Treatment (MS Medium + additives)		**Percentage explants showing bud break**	**No. of micro-shoots per sprouted bud (= Fold multiplication)**	
Auxin	**BAP (μM)**		**After 20 days**	**After 45 days**
0 ©	0 ©	73.3 ± 1.26	1.00 ± 0.0	1.00 ± 0.0
0	4.44	80.0 ± 3.15	1.00 ± 0.0	1.00 ± 0.0
0	11.09	80.0 ± 2.11	1.00 ± 0.0	1.00 ± 0.0
NAA [2.68 μM]	4.44	**96.7 ± 1.59**	**1.31 ± 0.51**	**1.69 ± 0.26**
	11.09	90.0 ± 0.66	1.18 ± 0.11	1.15 ± 0.31
IBA [2.46 μM]	4.44	80.0 ± 0.57	1.33 ± 0.23	1.33 ± 0.13
	11.09	83.3 ± 0.89	1.44 ± 0.22	1.44 ± 0.08

Note: Additives (add): glutamine (684.20 μM), thiamine (29.65 μM) and 0.3% (w/v) AC
data scored after 4 weeks of culture inoculation for bud break response.
values indicate Mean ± SE.
© = control

Elongation of micro-shoots.

1. On bud break medium. Observations showed that micro-shoots were showing poor elongation (between 1.5 to 2 cm.) on MS media supplemented with 2.49 μM IBA + 4.44 μM BAP (bud break medium). At the rate of 26.66% after 20 days and 36.66% after 45 days (Tab. 7).

Tab. 7. Elongation of micro-shoots

A. Treatment (bud break medium)	**B. Percentage elongation on bud break medium (A.)**		**C. Percentage elongation of micro-shoots from treatment (A.), upon subculture on EM**
Duration of treatment	**After 20 days**	**After 45 days**	**After 45 days**
Control	0	0	0
MS + add	0	0	0
MS + 4.44 μM BAP	23.33 ± 2.33	23.33 ± 2.33	55.00 ± 2.33
MS + 4.44 μM BAP + add	23.33 ± 1.79	26.66 ± 1.79	55.00 ± 1.79

MS + 11.09 μM BAP	23.33 ± 3.11	20.00 ± 2.84	60.00 ± 1.15
MS + 11.09 μM BAP + add	20.00 ± 1.14	23.33 ± 2.24	55.00 ± 2.88
MS + 2.68 μM NAA +1 BAP	10.00 ± 3.02	10.00 ± 3.02	**75.00 ± 2.56**
MS + 2.68 μM NAA + 4.44 μM BAP + add	13.33 ± 0.89	13.33 ± 0.89	**70.00 ± 1.26**
MS + 2.68 μM NAA +11.09 μM BAP	00.00 ± 0	00.00 ± 0	50.00 ± 3.88
MS + 2.68 μM NAA +11.09 μM BAP + add	13.33 ± 0.78	10.00 ± 0.56	65.00 ± 2.41
MS + 2.46 μM IBA + 4.44μM BAP	**26.66 ± 3.33**	**36.66 ± 3.86**	**70.00 ± 3.23**
MS + 2.46 μM IBA + 4.44μM BAP + add	20.00 ± 2.94	23.33 ± 2.99	75.00 ± 4.18
MS + 2.46 μM IBA + 11.09 μM BAP	20.00 ± 2.13	23.33 ± 2.26	55.00 ± 3.33
MS + 2.46 μM IBA + 11.09 μM + add	20.00 ± 1.68	26.00 ± 2.15	65.00 ± 3.31

Note: EM = elongation medium (MS + 2.46 μM IBA + 2.22 μM BAP)
additives (add): glutamine (684.20 μM), thiamine (29.65 μM) and 0.3% (w/v) AC
values indicate Mean ± SE

2. On elongation media. Since micro-shoot elongation was poor on the bud break medium, BAP concentration was reduced to 2.22 μM. It was also observed in previous experiments that qualitatively better micro-shoots were produced on the medium supplemented with IBA as compare to other tried auxins. Therefore, a new hormonal combination (MS media supplemented with 2.46 μM IBA and 2.22 μM BAP) was tested for elongation of micro-shoots. All the micro-shoots were further subcultured on this medium. Within a period of a month substantial elongation was observed, hence considered as elongation medium (Tab. 7). Best responses was observed on micro-shoot isolated from MS + 2.68 μM NAA + 4.44 μM BAP and MS + 2.46 μM IBA + 4.44 μM BAP + additives followed by MS + 2.46 μM IBA + 4.44 μM BAP and MS + 2.68 μM NAA + 4.44 μM BAP + additives (Fig. 2E).

Rooting of micro-shoots. Low frequency rooting was initiated *sua sponte* on elongation medium. However, White's medium with 5% activated charcoal was found to be best for rooting. A maximum of 43.33% rooting was observed on White's medium with 5% AC (Tab. 8). Positive effect of activated charcoal was clearly visible here (Fig. 2F). In absence of activated charcoal on White's medium, rooting recorded was only 16.67%.

Tab. 8. Rooting response of micro-shoots on different rooting media

Treatment	% Rooting	No. of roots/shoot	Root length (cm)
WM	16.67 ± 3.33	2.5 ± 0.5	2.8 ± 0.1
WM + 5% (w/v) AC	**43.33 ± 3.33**	**5.5 ± 0.5**	**3.7 ± 0.2**
½ MS + 5% (w/v) AC	6.7 ± 0.0	2.0 ± 0.0	1.75 ± 0.05

Note: Data scored after 4 weeks of culture inoculation for rooting response.
values indicate Mean ± SE.

Cotyledonary node explants harvested from BAP-preconditioned seedlings responded better as compare to those without the treatment. Multiple shoots were produced only on preconditioned explants. It showed the necessity of BAP treatment to seeds during germination. Similar observations were recorded in *Pterocarpus marsupium* Roxb. where multiple shoots were induced from cotyledonary nodes derived from 20-d-old axenic seedlings grown on Murashige and Skoog (MS) medium containing 2.22–13.32 μM benzyladenine (BAP) [SINGH & CHAND, 2004]. Better response of explants was observed in *Pithecellobium saman* (Jacq.) Benth, when explants were cultured on half strength MS medium containing 26.63 μM BAP [LISSETTE & al., 1997]. A similar response has been reported in case of *Acacia catechu* Willd. (*Mimosaceae*), [KAUR, 1996]. Best bud break and multiplication was observed on 4.44 μM BAP supplemented medium, while higher concentrations were found to be imposing negative impact in terms of increased callusing and vetrification. Similar observations were also made by JACKSON & HOBBS, 1990 who reported multiple shoots production, from cotyledonary node explants of pea (*Pisum sativum* L., *Fabaceae*) cultured on MS medium containing low concentrations of BAP (4.44 μM).

Excellent growth of micro-shoots was observed on both the BAP combinations with IBA. Micro-shoots were green, shiny and very healthy on 2.46 μM IBA + 4.44 μM BAP and 2.46 μM IBA + 11.09 μM BAP. But overall 2.68 μM NAA + 4.44 μM BAP combination was the best for bud break as well as for multiplication (an average 2-3 shoots per explant) along with good microshoot quality on this combination. A maximum of upto six micro-shoots per cotyledonary node explant was observed. Similarly, 2-3 shoots per explant were obtained in 75% of the cultures of *Commiphora wightii* on MS medium supplemented with 18.58 μM Kn + 17.76 μM BAP [BARVE & MEHTA, 1993].

Shoot buds after bud break showed poor elongation response on same bud break medium. Similar results were reported by BARVE & MEHTA, 1993. It is a known phenomenon in tissue culture that reduction in cytokinin concentration after bud break promotes elongation of micro-shoots. Hence 2.22 μM BAP was used in place of 4.44 μM and 11.09 μM. This also explains the positive responses that were observed by micro-shoots isolated from MS + 2.68 μM NAA + 4.44 μM BAP and MS + 2.46 μM IBA + 4.44 μM BAP + additives followed by MS + 2.46 μM IBA + 4.44 μM BAP and MS + 2.68 μM NAA + 4.44 μM BAP + additives. Positive effect of reduced cytokinin concentration in elongation was also reported by BARVE & MEHTA, 1993 where a maximum of 2-3 cm. elongation was recorded. LI-HUA & al., 2005 also reported that the best shoot production in terms of shoot number and shoot quality was obtained using 4.44 μM BAP and 0.49 μM IBA during the shoot multiplication phase and 1.00 μM BAP and 0.25 μM IBA during the shoot elongation phase.

Pretreatment of IBA with NAA and IAA was also found beneficial in *Commiphora wightii* as reported by BARVE & MEHTA, 1993. Proliferated shoots were excised from the established cultures and cultured on three different media, White's medium [WHITE, 1954], White's medium with 5% AC (w/v) and half strength MS medium with 5% AC (w/v). A maximum of 43.33% rooting was observed on White's medium with 5% activated charcoal. Positive effect of activated charcoal was clearly visible here. In absence of activated charcoal on White's medium rooting recorded was only 16.67%. BARVE & MEHTA, 1993 also reported 40% rooting in *Commiphora wightii* on White's medium.

Glutamine (684.2 μM), thiamine (29.65 μM) and 0.3% AC (w/v) found to be best for *Commiphora wightii* nodal explant and *in vitro* raised shoot apices according to BARVE & MEHTA, 1993. Hence, effects of only these concentrations were studied with cotyledonary node explants in the present study.

Half strength MS and White's root culture media, both with 5% AC were compared and White's root culture media with 5% AC found to be good for rooting. BARVE & MEHTA, 1993 also compared the same media for rooting response and reported 60% rooting on MS half strength with 5% AC. This value greatly differed from present observation. Present study showed only 6.7% rooting on same media. Therefore White's root culture media with 5% AC was selected. Roots were white and produce very few numbers of secondary roots. No callusing was observed with rooting.

Conclusions

Commiphora wightii is on the verge of extinction and need immediate interventions for a sound propagation technology. The protocol described here is suitable to mass multiply the plant from its seeds which are hard to germinate naturally. This can be useful in production of plants that can be utilized in restoration of its degraded habitat and prove helpful in both *in situ* and *ex situ* conservation efforts for this valuable plant.

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