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Standarization of cloning in *Commiphora wightii*

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Abstract
Indian bdellium [*Commiphora wightii* (Arnott.) Bhandari] is a large shrub of the family Burseraceae wildly growing in arid and semi arid tracts of Rajasthan and Gujarat in India and included in the Red Data Book (IUCN 2011). The over exploitation and unscientific methods of gum tapping led to destruction of its natural population. Here, studies were conducted for standardization of macro-propagation protocols through stem cuttings to develop a suitable macro-propagation technique for cost effective production at large scale. Experiments were imposed to elucidate the effect of cutting diameter (0.50 cm to > 1.50 cm) in combination with growing conditions (sunlight, shade house and mist chamber) on shoot sprouting and adventitious rooting. Results revealed that the cutting diameter of 0.75-1.00 cm in mist chamber performed best for sprouting (90.00%) and rooting (73.33%), number of primary root (6.67) and number of secondary root (16.67) followed, by 1.00-1.51 cm in mist chamber conditions. Less performance of sprouting (40.00%), rooting (33.33%), number of shoot (1.33), primary root (1.00) and number of secondary root (1.00) was recorded in cutting size of > 1.50 cm diameter in sunlight. Another experiment was performed with different concentrations (100, 200, 500 and 1,000 mg L^{-1}) of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) on adventitious root formation on cuttings of 0.25-0.50 cm of diameter in comparison to control (without application of plant growth regulator). Maximum rooting percentage was recorded in 200 mg L^{-1} IBA (93.33%) followed by 500 mg L^{-1} IBA (86.66%) as compared to control which showed sprouting (60.00%) only. Third experiment was performed with newly formed mini-cuttings treated with concentrations of IAA and IBA. The propagules of less than 8-10 cm in length and basal diameter less than 0.25 cm were selected as mini-cuttings. The mini-cuttings treated with IBA (500 mg L^{-1}), showed 64.30% rooting as compared to other treatments. Thus, the main finding of these experiment was that *Commiphora wightii* can also be propagated through mini-cuttings technique having diameter of < 0.25 cm in mass level and using plant growth regulator.
Key words: Stem cutting; Diameter; Mini-cuttings; Plant growth regulator; Adventitious root.

Introduction

The Indian bdellium [*Commiphora wightii* (Arnott.) Bhandari] is a herbal plant of Burseraceae family. It is well documented for its use-values and associated anatomical characteristics and reported to be an important component of the tropical arid ecosystem flora (Kumar and Shankar 1982; Dixit and Rao 2000). The market demand of this plant is due to the presence of secondary metabolite called guggulsterone. Guggulsterone is a plant sterol derived from the gum resin (i.e., guggulu) present in the ducts of secondary phloem of *C. wightii* (Dennis et al. 1980). It has been safely used for thousands of years in the Allopathic, Ayurvedic and Unani systems of medicines for the treatment of different disorders including bone fracture, arthritis, inflammation, rheumatic, hypercholesterolemia, hypocholesteremic and to inhibit angiogenesis (Satyavati 1991; Xiao and Singh 2008). The pharmaceutical industry is largely dependent upon the populations of this plant to supply the extraction of their intrinsic bioactive components. This plant has become endangered and has been reported in Data Deficient category of IUCN's Red Data list (IUCN 2011) because of its slow growing nature, low seed production and germination rate, lack of cultivation and without adequate research method of its gum tapping by pharmaceutical industries and religious prophets.

Inadequate availability of quality planting material is a main difficult in commercial cultivation of guggal. Therefore, it is required to develop a rapid, convenient and economically viable method for the establishment of germplasm bank of rare medicinal plant for domestication at large scale. It is propagated through stem cuttings (Mertia and Nagarajan 2000; Chandra et al. 2001; Kumar et al. 2002; Kumar et al. 2006). Vegetative propagation has been recognised as an important way for the multiplication of this plant and is suggested for quick multiplication and perpetuation to achieve conservation target and/or commercial plantation. Moreover, the use of plant growth regulators (PGRs) plays a vital role influencing the sprouting and rooting of stem cuttings. Indole-3-butyric acid (IBA) is still the most widely used auxin for rooting in stem cuttings and to increase the root percentage of cuttings due to its weak toxicity and great stability (Weisman et al. 1988; Kumar et al. 2006; Hartmann et al. 2011). Effect of different IBA concentration on the dry matter accumulation of leaves, sprouts and diameter of stem cuttings and roots of *C. wightii* were reported earlier (Kumar et al. 2006). It has been established in the literature that environment factors, seasons, age and size of cuttings influence rooting in the tree species (Palanisamy and Kumar 1997; Soundy et al. 2008).

Use of mini-cuttings for propagation of different perennial plant species recently emerged as a popular technique (Assis et al. 2004) and has been equally effective and reliable for mass propagation of desired plant. This technique supports that besides use of conventional propagation methods, endemic, threatened and rare plants can efficiently be conserved with various *ex vitro* strategies (Fay 1992), which have low impact on natural populations with minimum of plant materials (Ozel et al. 2006). Such type of work has not been reported earlier for guggal and it was assumed that the propagation is not possible from cuttings of < 0.50 cm diameter through classical methods.

The present investigation was aimed to evaluate the effect of cutting diameter under different growing conditions and to study the effect of IBA and IAA on the regeneration of roots in propagules having varying diameter.

Materials and methods

Study was carried out at Arid Forest Research Institute, Jodhpur, Rajasthan, India during July to September of 2010 and 2011. Healthy experimental propagules were collected from vegetative multiplication garden of the institute. The cuttings were prepared using sterile pruning scissors. In the first experiment, hardwood cuttings (leafless) of same length (15-20 cm, by using scale) but different diameter were collected (0.50-0.75 cm, 0.75-1.00 cm, 1.00-1.50 cm and >1.50 cm, by using Vernier caliper) from single 5-year-old mother shrubs. While preparing the cuttings a straight cut at 0.5 cm below a bud on the proximal region was performed. Cuttings were treated for 5 min in freshly prepared 0.1% aqueous solution of bavistin (systemic fungicide, BASF India Ltd, Bombay) and then washed two times with distilled and autoclaved water to remove excess fungicide.

The experiment was laid out in a randomized block design (RBD) with three replications, having 25 cuttings per replication. Cuttings were planted in black polybags (1 litre volume) containing vermiculite and placed under the following experimental conditions that are sunlight, shade house and mist chamber. The cuttings were placed in sunlight and shade and were watered regularly while those in the mist chamber were given intermittent mist and all the cuttings received 12 h natural photoperiod.

For regeneration of softwood leafless cuttings (0.25-0.50 cm length), another experiment was designed in which the healthy dormant apical shoots (20-25 cm length) from 8-10 month old branch of 5 year old plant were selected during first week of July 2010. The lower portion (5-7 cm) of the softwood cuttings were dipped for 10 min in freshly prepared aqueous solution of two different plant growth regulators (PGRs) indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) from (company- SD fine chemical, Mumbai, India) at 100, 200, 500 and 1,000 mg L^{-1}, respectively.

Cuttings dipped in distilled water for 10 min were considered as control. All the cuttings were treated with 0.01% bavistin (systemic fungicide) for 5 min subsequently distilled water washed then transferred to trays containing fresh vermiculite with randomized block design in triplicate having 25 cutting in each replication and kept in mist chamber at temperature 28±2^{o}C and 70-80% relative humidity. Light intensity was reduced to 22% of ambient sunlight and day-length was 12±1 h throughout. Intermittent mist was supplied for 30 s at 15 min intervals.

In the third experiment juvenile branches (20-30 days old) having 6-10 cm length and 3-5 nodes with diameter of ≥ 0.25 cm were collected using a sharp knife from healthy mother plants of *C. wightii* (5-years) in the first week of July 2011. The propugles (i.e., mini-cutting) of plant were kept in wet cloth for preventing the dehydration. Mini-cuttings were defoliated before establishment for reducing the evapotranspiration rate. Mini-cuttings were treated for 5 min in freshly prepared 0.01% aqueous solution of bavistin and washed properly. In total twenty five mini-cuttings for every replication were selected for treatment with different concentrations of IAA and IBA (i.e., 10, 50, 200, 300 and 500 mg L^{-1}) at room temperature (24°C) and treated for 10 min. Thirty mini-cuttings were used as control (dipped for 10 min in plain tap water).

The treated mini-cuttings were transferred to trays made up from polystyrene containing sand in three replication of 25 mini-cuttings each and kept in mist chamber at temperature 28±2^{o}C and 70-80% relative humidity. Light intensity was reduced to 22% of ambient sunlight and day-length was 12±1 h throughout. Intermittent mist was supplied for 30 s at 15 min intervals.

A hole was thumbed on surface side of trays, so the excess water was passing out. The propagation unit was supplemented with 24 h a day misting and fogging system which worked automatically based on the humidity of the mist chamber.

Data were recorded for shoot and root sprouting, number and length of shoots, number and length of primary roots and secondary roots. The data were analyzed using general linear model of SPSS 8.0 version program. Analysis of variance and F-test for significance and the critical difference (CD) value calculated for comparing the treatment means.

Results

The succession rate of mini-cuttings proportionally depends on their stored energy source and as well as environmental condition. For the studied experiment the sprouting and rooting performance of cuttings were significantly ($p \leq 0.01$) affected by their diameter (0.50-0.75 cm, 0.75-1.00 cm, 1.00-1.50 cm and > 1.50 cm), as well as by their environmental conditions showed in Table 1. Cutting with diameter > 1.50 cm in all environmental conditions and cuttings of 1.00-1.50 cm diameter in shade house condition were sprouted early (after 7 days of establishment) while rest were sprouted after 14-21 d of incubation. Independent of environmental conditions the mean value of sprouting percentage (68.89%), rooting percentage (57.78%), number of shoot (10.11) and primary root (4.78) was greater for cuttings of 0.75-1.00 cm of diameter.

Whereas the maximum value of shoot length (21.78 cm), primary root length (15.58 cm), number (14.67) and length of secondary root (4.93 cm) were registered by cuttings of 1.00-1.50 cm of diameter. The least percent of sprout (56.67%), percent of root (43.46%), number of shoot (3.67), length of primary root (3.52 cm), number of secondary root (1.11) and length of secondary root (0.69 cm) was registered by cutting having diameter of > 1.50 cm. Independent of cutting diameter the maximum values for the percent sprouting (80.00%) and rooting (65.09), number (8.67) and length of shoot (15.63 cm), number (4.67) and length of primary root (10.88 cm), number (11.67) and length of secondary root (4.08 cm) was observed for cuttings placed in mist chamber condition.

Minimum percent of shoot (52.50%) and root (45.00), shoot length (6.83 cm), number (2.25) and length (8.89 cm) of primary root and secondary root length (2.47 cm) was observed in mini-cuttings placed in sunlight conditions. The interaction between diameter of mini-cutting and environmental cultivation conditions was also found to be significant ($p \leq 0.01$). Between interactions the maximum percent of sprouting (90.00%), rooting (73.33%), number of shoots (13.33), number of primary root (6.67), and number of secondary root (16.67) was registered by cutting of 0.75-1.00 cm diameter in mist chamber conditions while the values of rest parameters showed approximate of the mean value.

The minimum percent of sprouting (40.00%), rooting (33.33%), number of shoots (1.33), number of primary roots (1.00), length of primary root (2.33 cm), number of secondary roots (1.00) and length of secondary root (0.50 cm) was observed in cutting of > 1.50 cm in sunlight conditions.

Finally it is observed that the cuttings with 0.75-1.00 cm diameter places under mist chamber condition showed good response compared to mini-cuttings of other diameter and their environmental condition (Fig. 1A-B). It suggests that for multiplication (macropropagation) of *C. wightii* without using auxins the cutting of 0.75-1.00 cm diameter and mist chamber are appropriate condition.

Table 1. Stem cuttings of *Commiphora wightii* performance of various diameters under different growing condition.

Stem cutting condition (SCC)	0.50-0.75 (cm)	0.75-1.00 (cm)	1.00-1.50 (cm)	>1.50 (cm)	Mean (cm)	0.50-0.75 (cm)	0.75-1.00 (cm)	1.00-1.50 (cm)	>1.50 (cm)	Mean (cm)	0.50-0.75 (cm)	0.75-1.00 (cm)	1.00-1.50 (cm)	>1.50 (cm)	Mean (cm)
	Sprouting days					Sprouting percentage					Rooting percentage				
Sunlight	21.00	21.00	14.00	7.00	15.75	53.33	53.33	63.33	40.00	52.50	43.33	50.00	53.33	33.33	45.00
Shade house	14.00	21.00	7.00	7.00	12.25	60.00	63.33	53.33	53.33	57.50	50.00	50.00	43.33	36.67	45.00
Mist chamber	7.00	7.00	7.00	7.00	7.00	70.00	90.00	83.33	76.67	80.00	63.33	73.33	63.33	60.37	65.09
Mean	14.00	16.33	9.33	7.00		61.11	68.89	66.67	56.67		52.22	57.78	53.33	43.46	
CD size ($p \leq 0.01$)					3.01*					5.34**					10.36**
CD condition ($p \leq 0.01$)					2.15*					11.86**					5.72**
CD size × condition ($p \leq 0.01$)					4.18*					10.67**					9.80**
SCC	Number of shoots					Length of shoots (cm)					Number of primary roots				
Sunlight	5.00	11.33	11.33	1.33	7.25	0.50	4.17	18.83	3.83	6.83	1.00	3.00	4.00	1.00	2.25
Shade house	6.00	5.67	8.67	4.00	6.08	0.67	4.50	23.17	11.50	9.96	1.00	4.67	4.33	2.00	3.00
Mist chamber	6.00	13.33	9.67	5.67	8.67	5.50	11.17	23.33	22.50	15.63	2.00	6.67	5.67	4.33	4.67
Mean	5.67	10.11	9.89	3.67		2.22	6.61	21.78	12.61		1.33	4.78	4.67	2.44	
CD size ($p \leq 0.01$)					0.42**					2.12**					0.89**
CD condition ($p \leq 0.01$)					1.06**					1.93**					1.61**
CD size × condition ($p \leq 0.01$)					0.40*					5.78**					2.99**
SCC	Length of primary roots (cm)					Number of secondary roots					Length of secondary roots (cm)				
Sunlight	5.67	10.00	17.57	11.67	8.89	3.67	7.00	15.33	1.00	6.75	1.80	3.17	4.40	0.50	2.47
Shade house	6.20	12.17	13.17	12.17	9.03	3.67	5.00	13.33	1.33	5.83	1.80	4.67	3.90	0.58	2.74
Mist chamber	11.67	12.17	16.00	16.00	10.88	13.67	16.67	15.33	1.00	11.67	4.50	4.33	6.50	1.00	4.08
Mean	7.84	11.44	15.58	3.67		7.00	9.56	14.67	1.11		2.70	4.06	4.93	0.69	
CD size ($p \leq 0.01$)					0.65**					2.07**					0.42**
CD condition ($p \leq 0.01$)					3.60**					4.60**					1.16**
CD size × condition ($p \leq 0.01$)					8.10**					7.13**					2.26**

*significant at 0.05% probability, **significant at 0.01% probability. CD - critical difference.

The regeneration potential of cutting depends on the level of endogenous auxins which might be varying species to species. Effects of various concentrations of IAA and IBA (100, 200, 500 and 1,000 mg L^{-1}) were studied for initiation of rooting in stem cuttings having diameter of 0.25-0.50 cm. The results presented in Table 2 show that the highest percent of both sprouting and rooting were observed in 200 mg L^{-1} IBA (93.33%), followed by 500 mg L^{-1} IBA (89.29% and 86.66%, respectively) while these were lowest in control.

Number of shoots of varied from 1.72 (control-distilled water only) to 2.76 (100 mg L^{-1} IAA) whereas length of shoots from 0.82 cm (200 mg L^{-1} IAA) to 19.16 cm (1,000 mg L^{-1} IBA). The maximum numbers of primary roots were observed of 8.54 (500 mg L^{-1} IBA) and minimum was 1.33 (100 mg L^{-1} IAA). Cuttings without hormonal pretreatment did not able to induce adventitious rooting. Maximum length of primary root (22.41 cm) was recorded in 1,000 mg L^{-1} IBA and closely followed by 22.20 cm (500 mg L^{-1} IAA) and minimum length was 12.50 cm (100 mg L^{-1} IAA). Highest numbers of secondary roots (14.50) were observed in 1,000 mg L^{-1} IBA and least was 5.00 (200 mg L^{-1} IAA). Length of secondary root varied from 3.80 cm (500 mg L^{-1} IBA) to 18.50 cm (100 mg L^{-1} IAA). The analysis of variance revealed that the response of cutting (0.25-0.50 cm) for sprouting and rooting was significantly affected by pretreatment of auxins (i.e., IAA and IBA) in various concentration (i.e., 100, 200, 500 and 1,000 mg L^{-1}). Study suggest that in *C. wightii* the endogenous auxin level is not at appropriate level for regeneration of softwood cutting and is dose-dependent hence multiplication through softwood cutting (25-0.50 cm) can be increase at par level by using moderate concentration of IBA, i.e., 200 mg L^{-1}.

The smaller stem here refers to cuttings those are having diameter below 0.25 cm responded well towards pretreatment of auxins (Table 3). No visible changes were detected in all the treatments after first four days of incubation. After 45 d, 11 treatments only nine treatments were able to initiate adventitious rooting.

The IBA treatments were superior when compared at IAA. Untreated cuttings showed no roots initiation. In IBA treatment the adventitious rooting percentage was maximum in 500 mg L^{-1} IBA (64.30%) and minimum in 10 mg L^{-1} IBA (33.3%). The treatment with 10 mg L^{-1} IAA and the control were not able to induce adventitious roots (Fig. 2). Maximum number of primary root (3.03) was observed in 500 mg L^{-1} IBA and minimum was (1.02) in 50 mg L^{-1} IAA. The IAA treatment of 50 and 300 mg L^{-1} IAA were statistically similar in term of rooting (36.4% and 38.5%, respectively).

The maximum length of primary root (4.59 cm) was observed in 200 mg L^{-1} IAA followed by mini-cuttings treated with in 500 mg L^{-1} IBA (4.29) and minimum in 50 mg L^{-1} IAA (2.15 cm). Number of secondary roots varied of 2.80 (200 mg L^{-1} IBA) to 8.00 (500 mg L^{-1} IBA) (Fig. 3A-C). Maximum length of secondary root was 1.87 cm (500 mg L^{-1} IBA) while the minimum was 0.30 cm (10 mg L^{-1} IBA). Overall the best performance occurred in mini-cuttings treated with IBA in concentration of 500 mg L^{-1}. The result of present investigation suggested that the success rate of micro-cutting in *C. wightii* can be increase by using of 500 mg L^{-1} IBA.

Table 2: Effect of auxins on sprouting and rooting of stem cuttings (0.25-0.50 cm) of *Commiphora wightii* under mist chamber condition.

Treatments (mg L^{-1})	Percent sprouting	Percent rooting	No of shoot	Length of shoot (cm)	No of primary root	Length of primary root (cm)	No of secondary root	Length of secondary root (cm)
Control	60.00	-	1.72	1.20	-	-	-	-
IAA 100	63.33	6.67	2.76	3.54	1.33	12.50	6.33	18.50
IAA 200	66.67	10.00	2.60	0.82	1.50	21.00	5.00	9.00
IAA 500	77.27	22.73	2.65	5.77	5.40	22.20	7.40	11.8
IAA 1,000	88.89	59.26	2.67	14.64	2.13	19.00	8.93	7.33
IBA 100	83.33	73.33	2.64	7.46	3.41	14.61	5.41	5.98
IBA 200	93.33	93.33	2.25	9.79	4.32	14.91	8.44	4.54
IBA 500	89.29	86.66	2.36	10.97	8.54	13.03	14.46	3.80
IBA 1,000	60.00	60.00	2.22	19.16	5.44	22.41	14.50	4.76
CD $p \leq 0.01\%$	3.25**	2.84**	1.04*	11.36**	3.21**	8.41**	4.84**	2.99**

*significant at 0.05% probability; **significant at 0.01% probability; IAA- indole-3-acitic acid; IBA- indole-3-butyric acid; CD - critical difference

Table 3: Performance of *Commiphora wightii* mini-cuttings in response to concentrations of IAA and IBA under mist chamber condition.

Treatment (mg L^{-1})	Number of primary root	Length of primary root (cm)	Number of secondary root	Length of secondary root (cm)
Control	-	-	-	-
IAA 10	-	-	-	-
IAA 50	1.02	2.15	2.97	1.65
IAA 200	1.25	4.59	4.07	0.92
IAA 300	1.84	2.74	3.67	1.14
IAA 500	1.33	3.93	6.02	1.04
IBA 10	1.02	2.83	4.02	0.30
IBA 50	1.38	3.02	3.34	0.94
IBA 200	1.74	4.03	2.80	1.66
IBA 300	2.13	2.46	4.08	1.58
IBA 500	3.03	4.29	8.00	1.87
CD $p \leq 0.01\%$	0.10**	0.11**	0.14**	0.16**

*significant at 0.05% probability; **significant at 0.01% probability; IAA- indole-3-acitic acid; IBA- indole-3-butyric acid; CD - critical difference.

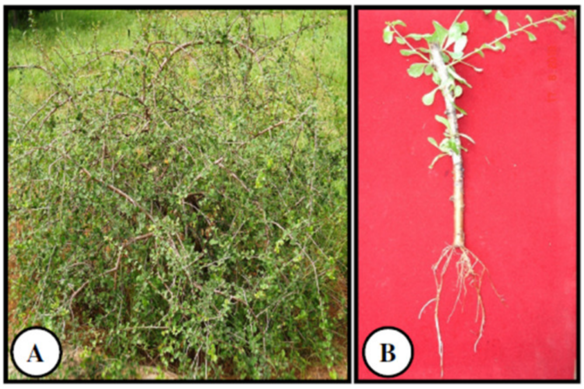

Figure 1. Detail of *Commiphora wightii* plants. (A) Full grown plant. (B) Rooting in cutting size 0.75-1.00 cm of diameter under mist chamber condition.

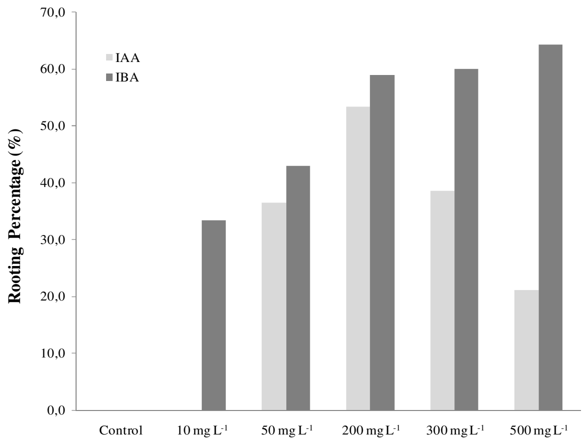

Figure 2. Effect of plant growth regulator (IAA and IBA) in pre-treatment on rooting percentage of mini-cuttings of *Commiphora wightii.*

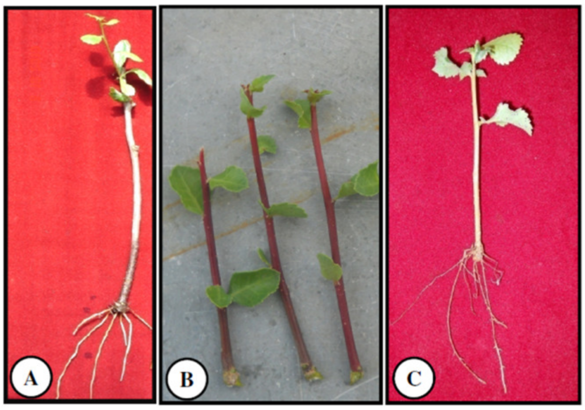

Figure 3. Detail of propagules of *Commiphora wightii.* (A) Rooting in cutting size 0.25-0.50 cm of diameter in 200 mg L^{-1} IBA. (B) Mini-cuttings containing the leaves. (C) Rooting in mini-cutting in 250 mg L^{-1} IBA.

Discussion

The formation of adventitious roots required a high energy process, which involves cell division, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia; hence need more reserve food material for root initiation (Aeschabacher et al. 1994). The most probable reason for good sprouting and rooting of 0.75-1.00 cm mini-cuttings of *Commiphora wightii* might be due to more reserved food material, level of inducing factors and quantity of permanent tissues as compared to higher diameter cuttings. The cuttings of 1.00 cm to > 1.50 cm diameter have more stored food material as well as it have more permanent tissues and lower amount of root initiation factors resulting the sprouting and rooting capacity reduced gradually from 1.00-1.50 cm to > 1.50 cm mini-cuttings. It has been reported in woody tree species that the rooting potential of the mini-cuttings is a juvenile characteristic and that the rooting capacity declines after maturation (House et al. 1996; Kibbler et al. 2004).

In addition to reserve food material other inducing factor such as growth regulator plays an important role on adventitious root formation in plant (Hartmann et al. 2011). Efficiency for root induction depends upon the presence of endogenous level of auxin. Jacobs (1979) reported that the endogenous auxin content was higher in the shoot tip portion and decrease as the distance increase from the apices in the same plant, in *C. wightii* the endogenous auxin level in the branch is presumably in the order of 0.50-0.75 cm > 0.75-1.00 cm > 1.00-1.50 cm > more than 1.51 cm but the permanent tissue and reserve material in order of > 1.51 cm > 1.00-1.51 cm > 0.75-1.00 cm > 0.50-0.75 cm resulting in the best combination of auxin and reserve material are found in 0.75-1.00 cm cuttings. Stem cuttings which have appropriate level of auxins and carbohydrates are especially suitable for adventitious rooting. A number of researchers have shown that rooting is facilitated when the carbohydrate reserve food material is in abundance (Haissig 1974).

Auxin-induced effect on rooting of cuttings is presumed to be mediated through its effect in mobilizing the reserve food material by enhancing the activity of hydrolytic enzymes (Nanda 1975).

On the other study, Kesari et al. (2009) reported that mature stem cuttings which have high level of auxins and carbohydrates are especially suitable for adventitious rooting in *Pongamia pinnata*. Similarly, result was obtained by Singh et al. (2009) the author reported that sprouting percentage increased with increasing stem diameter they also observed maximum sprouting in stem diameter of 2.0 cm and above (38.8%) where as minimum was observed in 0.5 cm (16.10%) in *Commiphora*. The good response of 0.75-1.00 cutting size is might be due to the presence of intermediate compound and suitable physiological conditions of cutting (Table 1). Cutting having diameter of > 1.50 cm and 1.00-1.50 cm presented fast sprouting due to the presence of reserve material (e.g., fructose, glucose and sucrose) but it does not able to produce best rooting due to the lack of sufficient concentration of auxins.

The cutting of 0.50-0.75 cm does not sprouted fast and also not gives sufficient rooting suggests that the level of carbohydrate and other root-inducing factors (i.e., co-factor of adventitious rooting) may be low for rooting in small cuttings. Palanisamy and Kumar (1997) stated that in *Azadirachta indica* the cuttings from distal end gave better rooting than proximal end supporting our result.

Among different environmental factors, temperature, light intensity and relative humidity are the main factors which influence the growth and development of plants considerably (Ozturk and Serdar 2011).

The cuttings planted in the mist chamber, shade house and sunlight conditions received same watering and temperature but have got different percent of humidity and radiation. During present study rooting was marginally effected by external environmental conditions because cuttings were raised under controlled humidity and temperature. However, relatively poor root formation recorded during extremely hot and cold conditions was due to low activity of cambium to proliferate in unfavorable and adverse environmental conditions. The variation in rooting and shooting response may be attributed to the physiological condition of the plant cuttings. Cellular activities during root initiation require availability of sugars which are synthesized due to activity of various hydrolytic enzymes (Nanda 1975). The activity of these enzymes might have been at the highest level during monsoon and post-monsoon months. The failure of cuttings to produce good root system in non-monsoon months may be due to a high rate of metabolism, low enzymatic activity and increased inhibitor/promoter ratio. The cuttings of 0.75-1.00 cm diameter placed in mist chamber gave best results may be due to the availability of monsoon type surrounding condition (Table 1).

The humidity levels in the area surrounding the plants influence all important processes of plant growth including, transpiration, water balance and cooling. Sufficient light is needed to maintain minimal endogenous auxin for rooting. On the contrary high light intensity can cause photo destruction of auxins (Hartmann et al. 2011). The percentage of rooting was significantly higher in the mist chamber (65.09%) than in shade house and sunlight conditions (45.00%) as well as number of primary roots were high in mist chamber of all sized cuttings than shade house and sunlight conditions (Table 1). The results indicated that low radiation and high humidity may have had a positive role on enhancement of rooting in *C. wightii.* In mist conditions, due to evaporative cooling of an intermittent mist system, heat load is reduced on the cuttings, thereby permitting the utilization of light conditions to increase photosynthesis and encourage the root development (Prolongs and Therios 1976).

Same finding was reported by Srisvastava et al. (2007) in *C. wightii* plant. In sunlight and shade house conditions, the relative humidity is less as compared to mist conditions due to transpiration losses is more as compare to mist, which the possible reason for less result in growth parameters of *C. wightii* cuttings compared to mist environment. In open condition the higher light intensity coupled with higher temperature might causes the loss of moisture from cuttings, thereby causing adverse effect on growth and development of root.

On the basis of results it has been cleared that hormone (e.g., auxin group) promotes the tissue of cutting to produce more number of roots than control. The sprouting percentage for all the treatments was above 60.00% including control but the rooting percent varied from 0.00% (control) to 93.33% (200 mg L^{-1} IBA) (Table 2.). Kesari et al. (2009) find relatively poor rooting with IAA treated stem cuttings of *Pongamia pinnata* in comparison to IBA, production of more ethylene which is known to inhibit the root production (Mullins 1972) and could also be due to the higher metabolic turnover. IBA is less sensitive than IAA to non-biological degradation such as photo-oxidation (Epstein and Ludwig-Müller 1993; De Klerk et al. 1997). In the present study, the maximum rooting percent was 93.33% in IBA and 59.26% in IAA treatments. It might be due to the low availability of endogenous auxins. Higher concentration of IBA (> 500 mg L^{-1}) is inhibiting for root formation. Ozel et al. (2006) studied on *Centaurea tchihatcheffii tchihatcheffii* and find that the highest frequency of rooting, mean number of roots per plant, and root length along with normal flowering were determined from juvenile cuttings treated with 500 mg L^{-1} IBA and then reduced with higher concentration of IBA.

Different portion of a single branch vary in their rooting and sprouting response depending on the seasonal, physiological conditions and age factor, thus their response will differ under same environmental conditions. Propagation techniques of mini-cuttings by using auxins provide an opportunity for the rapid and large scale production of plant with cost effective purpose especially in tree species which are difficult to produce by traditional methods. Propagation through mini-cutting in guggal is an efficient technique without harming mother plant in *Commiphora wightii*. The results revealed that the percent of rooting was higher and increased in IBA treatment as compared to similar concentration of IAA.

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