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Clonal Propagation in *Commiphora Wightii* (Arnott.) Bhandari

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Abstract

Studies were carried out to standardize and develop a suitable macro-propagation technology for large scale production of superior clonal stock through stem cuttings in *Commiphora wightii* Arnott (Bhandari), a data deficient medicinal plant of arid region. For the purpose, three experiments were conducted. The first experiment was tried to elucidate the impact of various cutting diameters (0.50-0.75 cm, 0.75-1.00 cm, 1.00-1.50 cm, and >1.50 cm) in combination with varying growing conditions (sunlight, shade house and mist chamber) on shoot sprouting and rooting without using exogenous plant growth regulators. Cutting diameter (size 0.75-1.00 cm) in mist chamber has shown maximum sprouting (90.00%) and rooting (73.33%), primary root (6.67) and secondary root (16.67) followed by 1.00-1.51 cm in mist chamber. Minimum 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 diameter (size >1.50 cm) in sunlight. Second experiment was performed to find out optimum growth regulator concentration of rooting hormone (100, 200, 500 and 1000 ppm) of Indole-3-acetic acid (IAA) and Indole-3-butyric Acid (IBA) on adventitious root formation on cuttings diameter (size 0.25-0.50 cm) in comparison to control. Maximum rooting percentage (93.33%) was recorded in 200 ppm followed by 500 ppm (86.66%) of IBA as compared to control, which showed only 60 per cent sprouting. Third experiment was performed with newly formed juvenile micro-cuttings treated with varying concentrations of IAA and IBA. The juvenile cuttings (size 6-10 cm, basal dia <0.25 cm) were selected as micro-cuttings. The cuttings treated with IBA (500 ppm) showed 64.30% rooting as compared to other treatments. Results of above experiments indicate that cuttings (size 0.75-1.00 cm dia) may be developed in mist chamber for better performance. While using heavier cuttings, no growth promoting hormones is required however; growth regulator 200 ppm concentration of IBA rooting hormone was observed optimum for promoting macro-propagation in stem cuttings of lower diameter class (0.25-0.50 cm).

Key Words: *Commiphora wightii*, stem cutting, thickness, plant growth regulators, adventitious roots

Introduction

The Indian bdellium (*Commiphora wightii* (Arnott.) Bhandari) is a well known herbal plant of Burseraceae family. Guggulsterone is a plant sterol derived from the gum resin (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, and inflammation, rheumatic, hypercholesterolemia, hypocholesteremic and to inhibit angiogenesis (Satayavti 1991; Tajuddin et al. 1997; Urizar and Moore 2003; Xiao and Singh 2008). The pharmaceutical industry is largely dependent upon the populations of this plant to supply for the extraction of their intrinsic bioactive components. This plant has become endan-

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gered and reported in Data Deficient category of IUCN's Red Data list (IUCN 2011). Due to its slow growth, poor seed setting and lower germination rate, and unscientifically exploiting of its gum tapping method by pharmaceutical industries and religious prophets (Tajuddin et al. 1997). Tapping is carried out during December- February. Plant attaining 7.5 cm diameter is suitable for tapping. Well grown mature plants of six to eight years or more old are suitable for gum extraction. Tapping is usually done by giving 3-4" long and 1.5 cm bark deep circular incisions in circular or horizontal type on the main trunk and branches at a same distance with the knife dipped in guggal solution. The gum is collected after 15-20 days of incision and subsequent collection after an interval of 10-15 days. The yield of gum varies from 50-500 g/plant (Yadav et al. 1999). However, the total yield 1000-2000 gm per plant corresponding to 3200 kg/ha after 5 years for healthy plants was also reported (http://www.banajata.org/guggul.htm).

Insufficient availability of quality planting material is a major bottleneck in commercial cultivation of guggal. Therefore, it is required to develop a rapid, convenient and economically viable method for raising planting material of rare medicinal plant for domestication at large scale. Regeneration through seed is very poor. In nature few seedlings develop from seeds. It can be propagated by seed and vegetative method. Germination through seed is very poor. Vegetative propagation through stem cutting is most common and successful method (Mertia and Nagrajan 2000; Chandra et al. 2001; Kumar et al. 2002; Kumar et al. 2006; Thosar and Yande 2009). Diwakar (2011) has also studied the vegetative propagation of *C. wightii*. The rooting starts after 21 days. The 25 to 30 cm long in size cuttings are placed at a depth of 15 cm for raising.

Vegetative propagation has been recognised as an important way for the multiplication of this plant and thus vegetative propagation of *C. wightii* is suggested for quick multiplication and perpetuation to achieve conservation target. Moreover, the use of plant growth regulators (PGRs) plays a vital role in 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 success percentage of cuttings due to its weak toxicity and great stability (Weisman et al. 1988; Mertia and Nagrajan 2000; Chandra et al. 2001; Kumar et al. 2002; Kumar et al. 2006; Hartmann et al. 2007). Effect of different concentration of indole-3-butyric acid on the dry matter accumulation of leaves, twigs and thickness of stem cuttings and roots of *C. Wightii* were recorded earlier (Kumar et al. 2006). It has been established in the literature that environmental factors, seasons, age and size of cuttings influence rooting in the tree species (Soundy et al. 2008; Palanisamy and Kumar 1997).

Use of micro-cuttings for propagation of different perennial plant species emerged as a popular technique recently (Titon et al. 2006) and has been equally effective and reliable for mass propagation of desired plant. This technique support 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 wild populations with minimum of plant materials (Ozel et al. 2006). Such type of work has not been reported earlier in guggal and it was assumed that the propagation is not possible in cuttings of <0.50 cm diameter through classical methods.

Therefore, the present investigations were designed to evaluate the effect of cutting diameter with different growing conditions provided to it and to study the effect of IBA and IAA on the regeneration of roots in cuttings having varying diameters. The aim of this study was to develop a suitable technique for large scale production of superior clonal stock with maximum success and also to meet the required demand for high-quality planting material at commercial scale in guggal.

Materials and Methods

Studies were carried out at Arid Forest Research Institute, Jodhpur, Rajasthan during July-September of 2010. Healthy material for experiment was taken from vegetative multiplication garden (VMG) of the institute. The cuttings were prepared using sterile pruning scissors. There were tried three experiments using above materials. In the first experiment, hardwood cuttings (leafless) size 15-20 cm long using four different diameters viz. 0.50-0.75 cm, 0.75-1.00 cm, 1.00-1.50 cm and >1.50 cm were taken from single 5-year-old mother shrub during first week of July 2010. While preparing the cuttings, care was taken to make a straight cut at 0.5 cm below a bud on the proximal end.

Cuttings were treated for 5 minute in freshly prepared 0.01% aqueous solution of Bavistin (fungicide) and then washed properly with distilled 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 (size 12x20 cm) containing vermiculite and placed under different experimental conditions viz. sunlight, Net/shade house and mist chamber. The cuttings placed in sunlight and nethouse/shade were watered regularly while those in the mist chamber were given intermittent mist and all the cuttings received 12 h natural photoperiod.

Softwood leafless cuttings size 20-25 cm long with diameter of 0.25-0.50 cm were taken from 8-10 months old branches of 5 year old shrub during first week of July 2010 for second experiment. All the cuttings were treated with 0.01% Bavistin fungicide as mentioned in first experiment. The lower portion of the softwood cuttings (up to 5-7 cm) was dipped for 10 minutes in a freshly prepared aqueous solution of Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA) at 100, 200, 500 and 1000 ppm respectively. Whereas, cuttings dipped in distilled water for 10 minutes were considered as control. All the cuttings were shifted in trays containing fresh vermiculite in randomized block design in triplicates having 25 cuttings in each and kept in mist chamber at 35 ± 2^{o}C temperature and 70-80% relative humidity.

In the third experiment, juvenile branches (20-30 days old) having length 6-10 cm and diameter of less than 0.25 cm with 3-5 nodes were harvested by using a sharp knife from healthy 5-years old mother shrub of *Commiphora*. These detached micro-cuttings were kept in wet cloth for preventing from dehydration. The lower portion of the cuttings was defoliated before establishment for reducing the transpiration rate. Cuttings were treated with 0.01% aqueous solution of Bavistin as mentioned in first experiment. 25 cuttings for each replicate were selected for treatment of different concentrations of IAA and IBA viz., 10, 50, 200, 300 and 500 ppm, respectively at room temperature and treated for 10 minutes. Treated cuttings were transferred in trays made up from polystyrene containing sand and were kept in mist chamber. A hole was thumbed on surface side of trays, for passing out excess water. 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 collection and statistical analyses

Data were recorded daily for sprouting initiation and number for shoots (cumulative). Length of shoots and length of primary and secondary roots, rooting percentage were taken at the time of termination of experiment (after 45/60 days). Shoot and length were measured with measuring scale. The collected data were analyzed using general linear model of SPSS 8.0 version program. All the data were collected and analyzed through ANOVA using SPSS computer package. Critical difference (CD) values were calculated for comparing the treatment means. The P values < 0.05 were taken as significant.

Results

Table 1 depicts the Performance of stem cuttings of various diameters (cm) under different growing conditions in *C. wightii*. Cuttings size >1.50 cm diameter in all environmental conditions and cuttings size 1.00-1.50 cm diameter in shade house conditions were sprouted early (after 7 days) while rest were sprouted after 14-21 days of establishment. Mean sprouting percentage (68.89%), rooting percentage (57.78%), number of shoots (10.11) and primary roots (4.78) were observed maximum for cuttings having size 0.75-1.00 cm of diameter irrespective of environmental conditions. Maximum shoot length (21.78 cm), primary root length (15.58 cm), number (14.67) and length of secondary roots (4.93 cm) were recorded in cuttings size 1.00-1.50 cm diameter. Minimum sprouting (56.67%), percent rooting (43.46%), number of shoots (3.67), length of primary root (3.52 cm), number of secondary roots (1.11) and length of secondary root (0.69 cm) were recorded in cuttings size >1.50 cm diameter. Irrespective of cuttings size, the maximum values for percent sprouting (80.00) and rooting (65.09), numbers (8.67) and length of shoots (15.63 cm), numbers (4.67) and length of primary roots (10.88 cm) and numbers (11.67) and length of secondary roots (4.08 cm) were observed for cuttings placed in mist chamber and minimum percentage of shoots (52.50) and roots (45.00), shoot length (6.83 cm), numbers (2.25) and length (8.89 cm) of primary and secondary root length

Table 1. Performance of *Commiphora wightii* stems cuttings of various diameters (cm) under different growing conditions

Stem cutting conditions	0.50-0.75	0.75-1.00	1.00-1.50	>1.50	Mean	0.50-0.75	0.75-1.00	1.00-1.50	>1.50	Mean	0.50-0.75	0.75-1.00	1.00-1.50	>1.50	Mean
	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					3.01*					5.34**					10.36**
CD condition					2.15*					11.86**					5.72**
CD size X condition					4.18*					10.67**					9.80**
	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					0.42**					2.12**					0.89**
CD condition					1.06**					1.93**					1.61**
CD size X condition					0.40*					5.78**					2.99**
	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					0.65**					2.07**					0.42**
CD condition					3.60**					4.60**					1.16**
CD size X condition					8.10**					7.13**					2.26**

*Significant at 5% probability; **Significant at 1% probability.

(2.47 cm) were observed in cuttings placed testing under sunlight. The interaction between diameter of cuttings and environmental growing conditions were also found significant ($p \leq 0.01$). Between interactions the maximum percentage of sprouting (90.00%), rooting (73.33%), number of shoots (13.33), number of primary roots (6.67), and number of secondary roots (16.67) were registered by cuttings having 0.75-1.00 cm diameter in mist chamber conditions while the values of rest parameters showed at par of the mean value. The minimum percentage 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) were observed in cutting size > 1.50 cm under sunlight conditions.

Effects of different concentrations of IAA and IBA (100, 200, 500 and 1000 ppm) have also been studied for initiation of rooting in stem cuttings having diameter 0.25-0.50 cm. Results indicated that the higher percentage of both sprouting and rooting were observed in 200 ppm of IBA (93.33%) followed by 500 ppm of IBA (89.29% and 86.66%, respectively). Number of shoots varied from 1.72 in control to 2.76 in 100 ppm of IAA. Whereas length of shoots from 0.82 cm in IAA (200 ppm) to 19.16 cm in IBA (1000 ppm). The maximum number of primary roots (8.54) were observed in 500 ppm of IBA and minimum (1.33) was in 100 ppm of IAA. Cuttings without hormonal pretreatment did not able to induce rooting. Maximum length (22.41 cm) of primary root was recorded in 1000 ppm of IBA followed closely by 500 ppm of IAA (22.20 cm). Minimum length was 12.50 cm in IAA (100 ppm). Higher numbers of secondary roots (14.50) were observed in IBA (1000 ppm) and lower numbers was 5.00 in IAA (200 ppm). Length of secondary root varied from 3.80 cm (IBA 500 ppm) to 18.50 cm (IAA 100 ppm). 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 (IAA and IBA) given in various concentrations (Table 2).

Smaller stem cuttings (micro-stem-cuttings) responded well towards pretreatment of auxins (Table 3). No visible changes were detected in all the treatments after first four days of incubation. Out of 11 treatments only 9 were able to initiate rooting after 45 days of incubation. Among the treatments, IBA was observed best in all the concentrations tested than IAA. Untreated cuttings showed no root initiation. Within IBA treatment, the rooting percentage was maximum (64.30%) in IBA (500 ppm) while minimum (33.3%) was in 10 ppm of IBA. Two treatments control and IAA 10 ppm was not able to induce roots (Fig. 1). Maximum number of primary roots (3.03) were observed in 500 ppm of IBA and minimum were (1.02) in 50 ppm of IAA. The maximum length (4.59 cm) of primary root was observed in IAA (200 ppm) followed by 4.29 cm in IBA (500 ppm) and minimum was (2.15 cm) in 50 ppm of IAA. Number of secondary roots varied from 2.80 in IBA (200

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

Treatments (ppm)	Percent sprouting	Percent rooting	No of shoots	Length of shoots (cm)	No of primary roots	Length of primary roots (cm)	No of secondary roots	Length of secondary roots (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 1000	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 1000	60.00	60.00	2.22	19.16	5.44	22.41	14.50	4.76
CD	3.25**	2.84**	1.04*	11.36**	3.21**	8.41**	4.84**	2.99**

ppm, Part Per Million; IAA, Indole-3-acitic acid; IBA, Indole-3-butyric acid; cm, centimeter.
*Significant at 5% probability; **Significant at 1% probability.

Table 3. Performance of micro-cuttings in response to various concentrations of IAA and IBA in *C. wightii* under mist chamber

Treatment (ppm)	Number of primary roots	Length of primary roots (cm)	Number of secondary roots	Length of secondary roots (cm)
Control	-	-	-	-
IAA 10	-	-	-	-
IAA 50	1.02	2.15	2.97	1.65
IAA 200	1.25	4.59	4.07	.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	.30
IBA 50	1.38	3.02	3.34	.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	0.10**	0.11**	0.14**	0.16**

ppm, Part Per Million; IAA, Indole-3-acitic acid; IBA, Indole-3-butyric acid; cm, centimeter.
*Significant at 5% probability; **Significant at 1% probability.

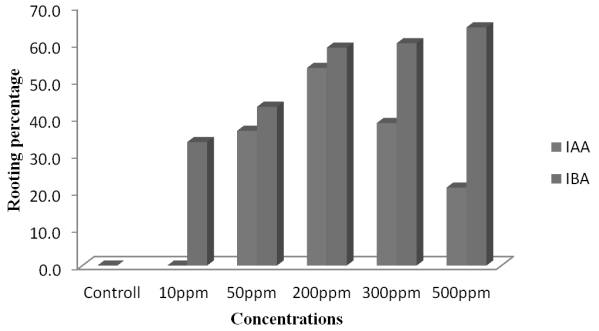

Fig. 1. Effect of various concentrations of auxins (IAA/IBA) on rooting percentage of micro cuttings in *C. wightii*.

ppm) to 8.00 in IBA (500 ppm). Maximum length of secondary root was observed 1.87 cm in IBA (500 ppm) while minimum was 0.30 cm in IBA (10 ppm). Overall cuttings treated with 500 ppm of IBA showed best performance.

Discussion

The formation of adventitious roots is a high energy requiring 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). Apart from reserve food material, other inducing factors such as growth regulators play an important role for adventitious root formation in plants. Efficiency for root induction depends upon the presence of endogenous level of auxins. The most probable reason for good sprouting and rooting of 0.75-1.00 cm cuttings might be due to more reserved food material, level of inducing factors and less quantity of permanent tissues as compared to higher diameters of cuttings. The cuttings size 1.00 cm to > 1.50 cm diameter have more stored food material as well as it also 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 diameter cuttings. It has been reported in woody tree species that the rooting potential of the cuttings is a juvenile characteristic and that the rooting capacity declines after maturation (House et al. 1996; Kibbler et al. 2004). Jacobs (1979) reported that the endogenous auxin content was higher in the shoot tip portion and decreased as the distance increase from the apices in the same plant. Hence the endogenous auxin level in the branch of *Commiphora* is presumably in the order of 0.50-0.75 cm > 0.75-1.00 cm > 1.00-1.50 cm > 1.51 cm but the permanent tissues and reserved food material in order of > 1.51 cm > 1.00-1.51 cm > 0.75-1.00 cm > 0.50-0.75 cm was resulted in the best combination of sufficient auxin and reserved food material. It was found in 0.75-1.00 cm diameter cuttings. This finding is in accordance to the findings of Kesari et al., 2009. They reported that mature stem cuttings which having

higher level of auxins and carbohydrates were specifically suitable for adventitious rooting in *Pongamia pinnata*. Similar results were also obtained by Singh et al. (1989). The cuttings sizes 0.50-0.75 cm diameter did not sprouted faster and also not had sufficient rooting. It is suggested that the level of carbohydrate and other root-inducing factors might be lower for rooting. Palanisamy and Kumar (1997) stated that in neem the cuttings from distal end gave better rooting than proximal end and their finding were in accordance of our results. Among different environmental factors, temperature, light intensity and relative humidity are the main factors which influence the growth and development of plants considerably. The cuttings size 0.75-1.00 cm diameter placed in mist chamber were shown better results. The humidity levels in the area surrounding the plants influence all important processes of plant growth i.e., transpiration, water balance and cooling of plants hence sufficient humidity plays an important role in the growth and development of cuttings to initiate good roots. Sufficient light is needed to maintain minimal endogenous auxin for rooting. On the contrary higher light intensity can cause photo destruction of auxins (Hartmann et al. 2002). The percentage of rooting was significantly higher in the mist chamber (65.09%) than in shade house and in sunlight conditions (45.00%) as well as number of primary roots were also high in mist chamber of all sized cuttings than shade house and sunlight conditions (Table 1). This is indicated that lower radiation and higher humidity may have a positive role on enhancement of rooting in *Commiphora*. 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). In sunlight and shade house conditions, the relative humidity was lower as compared to mist chamber conditions due to which the transpiration losses was more as compared to mist chamber. In open conditions, the higher light intensity coupled with higher temperature might be caused the loss of moisture from cuttings, thereby causing adverse effect on growth and development of roots.

On the basis of above experiments it may recommend that cuttings size (0.75-1.00 cm) diameter may be developed in mist chamber for better performance. While using heavier cuttings, no growth promoting hormones is required however; growth regulator 200 ppm concentration of IBA rooting hormone was observed optimum for promoting macro-propagation in stem cuttings of lower diameter class (0.25-0.50 cm). This lower diameter class is very much needed for propagation; otherwise a lot of precious material will be wasted. The juvenile cuttings treated with 500 ppm IBA showed better performance in terms of number and length of primary and secondary roots.

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