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Propagation of *Cerbera odollam* Plant by Using Tissue Culture Technique

[1]Salwa S. Sakr, [1]Saad S. Melad, [2]M.A. El-Shamy and [2]Asmaa E. Abd Elhavez

[1]Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Botanical Garden Research, Horticulture Research Institute,
Agriculture Research Center, Giza, Egypt

Abstract: The experimental trial was consummated in Plant Tissue Culture Laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture from 2008 to 2010. The aim of this study was to reach a well defined protocol for *In vitro* propagation of *Cerbera odollam* plant. Shoot tips and auxiliary buds of *Cerbera odollam* were surface sterilized with 20% Clorox (sodium hypochlorite as commercial bleach) for 20 and 15 min, respectively for sterilizing explants gave the best result (92.59 and 88.89%) survival, (7.41 and 7.41%) contamination and (0.00 and 3.70%) mortality of shoot tips and auxiliary buds respectively. Explants were cultured on 1/2 MS medium supplemented with 1.0 mg/l NAA and 200 mg/l polyvinyl pyrrolidone (PVP) to establish effectively. In the multiplication stage, 0.5 mg/l BA plus 1.5 mg/l Kin formed not only the highest number of leaves but also the highest shoots lengths. MS medium plus 1.5 mg/l BA plus 0.5 mg/l Kin was favored for number of shoots. For *In vitro* rooting, 0.1 mg/l IBA was more suitable to form roots on *Cerbera odollam*. After 4 weeks in rooting medium, *Cerbera* plants were transplanted into pots containing peat moss and covered by polythene sheets. After three weeks, they were removed the sheets and leaved for two weeks under plastic house condition where 40 % of the plantlets survived.

Key words: Micropropagation · *In vitro* · Tissue culture · Cerbera · Shoot tips

INTRODUCTION

Cerbera odollam is commonly known as the Suicide tree, Pong-pong and Othalanga, native to India and other parts of Southern Asia It is 6m in height with narrow, leathery leaves and highly fragrant white flower with a pink center held in large bunches. The large, single-seeded, egg-shaped fruits are 5-8 cm long and ripen from green to red. The kernels of *C. odollam* contain cerberin, a potent alkaloid toxin related to digoxin, a poison found in foxglove. The poison blocks the calcium ion channels in heart muscle, causing disruption of the heart beat. The fruits are used for manufacturing bioinsecticides and deodorants.

Many previous researches dealt with that topic. *Aspidosperma polyneuron* efficient apical shoots sterilization was achieved with NaOCl (0.25%-10 minutes) or $HgCl_2$ (0.05%-10 minutes); survival rates were 72.89% and 84, 10%, respectively [1]. Nishi and Bansal [2] found that shoot bud explants derived from Sargapagandha *(Rauvolfia serpentina*) seedlings have shown regenerative response on MS medium containing various concentrations of kin or BA alone or 1.0 mg/l BA + 0.1 mg/l NAA, besides,8-10 (multiple) shoots/explant were formed at MS+5.0 mg/l BA. Apical shoots were elongated to a height of 12 cm within 8 weeks and produced 2-3 cm long axillary buds. Roots induced in the elongated micro shoots (4-5 cm) on MS medium supplemented with 0.5 and 1.0 mg/l NAA.

Mehatre *et al*. [3] recommended the best protocol developed for the *In vitro* culture of Sarpagandha (*Rauvolfia serpentina*). They obtained 100% of developed axillary bud on MS medium + 0.5 mg/l NAA + 0.1 mg/l kin. Sumita *et al*. [4] concluded that growth of callus *Rauwolfia serpentina* [*Rauvolfia serpentina*] had been obtained on MS medium supplemented with 2,4-D at 1 mg/l + BAP at 0.5 mg/l. best shooting was obtained on MS medium supplemented with 2,4-D at 2.0 mg/l + IAA at 0.5 mg/l + BAP at 1.5 mg/l. Rooting was best with the supplementation of IBA at 4.0 mg/l + NAA at 1.0 mg/l.

Corresponding Author: Salwa S. Sakr, Department of Ornamental Horticulture,
Faculty of Agriculture, Cairo University, Giza, Egypt.

Gao *et al*. [5] showed that MS+3.0 mg/l BA +0.1 mg/l NAA was the optimum induction culture media for axillary buds of *Trachelospermum jasminoides*, which gave induction rate up to 90%. Ratna and Misra [6]concluded that multiple shoots of Natal plum (*Carissa carandas*) were initiated on a MS medium containing 2.0 mg/l BAP and 0.2 mg/l IBA. *In vitro*-formed plantlets were hardened in a hardening mix containing vermiculite, sand and soil (1:1:1).

Sahu *et al*. [7] illustrated that shoot buds of *Tabernaemontana divaricata* were induced on MS medium supplemented with 3% sucrose, 0.8% agar and a combination of 1 mg/l IAA + 2 mg/l BA + 5 mg/l kin. The elongated shoots were rooted on MS medium supplemented with IAA or NAA. Gajbhiye *et al*. [8] concluded that MS + BA at 2.0 mg/l showed the highest number of axillary sprouting. They added that BA alone produced better result than kinetin alone in both species: in *Rauvolfia serpentine* 62.24% and in *Rauvolfia tetraphylla* 57.99%.

Salma *et al*. [9] reported that using MS medium supplemented with 1.5 mg/l BA and 0.2 mg/l NAA resulted in the maximum number of shoots (4 multiple shoots) explants for *Rauwolfia serpentina* L. Ratna and Misra[10]obtained the best rooting of *Carissa grandiflora* microshoots was obtained on 1/2 MS plus 0.8 mg/l IBA and 0.2 mg/l NAA. Soares *et al*. [11] recommended that 20% of the shoots developed roots on *Hancornia speciosa* the presence of 3.0 mg/l IBA, while using of NAA was inefficient, in all tested concentrations.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Tissue Culture, Zohria Botantial Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt,

The experiments were carried out during the period from 2008 to 2010 to study the most suitable protocol for micropropagation of *Cerbera odollam* plant.

Plant Material: The mother plants grew naturally at the open field condition at Zohria Botanical Garden. Shoot tips and axiliary buds were used as explants.

Culture Room Condition: Cultures of *Cerbera odollam* were placed in a growth chamber under 25±2 °C and 16-h photoperiod provided with white fluorescent light and 2000 lux.

Experimental Design and Statistical Analysis: A factorial experiment in a complete randomize design was employed in all of the experiments. Analysis of variance was used to show statistical differences between treatments using L.S.D at 5% probability level [12].

Culture Media: The Murashige and Skoog (MS) medium was used for explants of *Cerbera odollam*.Media were solidified and supplemented with 7.0 g/l agar. Sucrose at 30.0 g/l was added as a source of carbohydrate. The pH was adjusted to 5.7. Twenty ml medium were poured in 150 ml jars and sterilized by autoclaving under steam pressure 1.5 bar at 121°C for 20 min. Each treatment consisted of 3 jars. In each jar, three shoots were cultured separately.

Experimental Treatments

Surface Sterilization of Explants: The explants were excised from the mother plants and then washed by a soapy water for 10 min. the explants were rinsed under running water for 2 hours. The explants were soaked in the following sterilization treatments. They were then sterilized by immersion in a Clorox (commercial bleach) solution at the rate of 10, 15, 20 and 25% plus 3-5 drops of Tween 20 for 15, 20 and 25 min. After sterilization treatment the explants were then rinsed in sterilized distilled water (5times) to remove all traces of the disinfectant. All steps of the sterilization method have been done under aseptic condition inside the culture cabinet (Laminar air flow) using sterilized instruments.24 treatments were initiated from the use of two types of explants.

- One drop of Tween 20 (Polyoxyethlene sorbitan monolaurate) was used as a wetting agent per 100 ml of sterilizing solution for each treatment. Each treatment consisted of three jars. In each jar, Three explants were sterilized.

- At the end of the experiments, the collected data included:

- Survival percentage.
- Contamination percentage.
- Mortality percentage.

Establishment Stage: In this part, two sources of tissues (shoot tip and auxiliary buds) were used for studying the effect of different treatments on establishment stage. 36 treatments were initiated from the use of explants.

The media employed at this stage were MS at full, half and quarter strength. MS medium was supplemented with Naphthalene acetic acid (NAA) at 0.0, 0.1, 0.5 and 1.0 mg/l to initiate the shoots, activated charcoal (A.C.) at 3 g/l and Polyvinyl pyrrolidone (P.V.P) at 200 mg/l. Each treatment consisted of three jars.

Data Recorded as Follows:

- Shoot length.
- Number of leaves.

Multiplication Stage: In these experiments both of terminal shoot tip explants and lateral buds explants were cultured together in multiplication stage. Their objective was to study the effect of cytokinines on shoot formation of *Cerbera odollam* using Benzyl adenine (BA) at 0.0, 0.5, 1.0, 1.5, or 2.0 mg/l and Kinetin (Kin) at 0.0, 0.1, 0.5, 1.0, 1.5 or 2.0 mg/l were used separately and their combination.

Data Were Recorded as Follow:

- Shoot length (cm).
- Number of leaves.
- Number of shoot.

Rooting Stage: This experiment was carried out to study the effect of IBA (0.0, 0.1, 0.5, 1.0, 2.0 or 4.0 mg/l) concentrations on growth and root formation of *Cerbera odollam* shoots. Three shoots at length of 3.0 cm, (produced from the multiplication stage were cultured in each jar (350 ml), which contained 50 ml of rooting medium.

- After 30 days on the rooting media the following data were recorded:

- Number of roots
- Root length (cm).
- Number of leaves.
- Shoot length (cm).

Acclimatization Stage: Rooted plantlets were pricked out singly into 10 cm plastic pots filled with 1: 0, 1:1, 1:2 and 1:3 (v/v) peat moss and sand, respectively. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for three weeks. The plastic covers were then gradually removed to reduce humidity and adapt plantlets to greenhouse conditions, after that survival capacity (%) was recorded.

RESULTS AND DISCUSSION

Effect of Clorox Concentrations on Surface Sterilization of *Cerbera* Explants: Results recorded in Table 1 show that Clorox (commercial bleach) at 20% gave the highest value of explants survival (75.31%) as compared with the other treatments on shoot tips. But, the percentage of mortality (12.34%) and the percentage of contaminated explants (12.34%) were recorded when the explants immersed in 20% Clorox. On the other hand, the data indicated that increasing the period of immersion decreased the survival percentage of explants and the percentage of free contaminated explants, while the mortality percentage of explants was increased.The data of the interaction between the concentration of Clorox and immersion time indicated that the best percentage of survival explants (92.59%), the percentage of free contaminated explants (7.41%) and the percentage of mortality (0.00%) were observed when explants immersed for 20 min in 20% Clorox.

For axillary buds, data in Table 2 showed that the increasing Clorox concentrations decreased survival percentage of explants and the percentage of free contaminated explants, while the percentage of mortality was increased.Increasing the period of immersion decreased the survival percentage of explants and the percentage of free contaminated explants at the high concentrations of Clorox (25%), while the best concentration (20%) increased the percentage of mortality explants (at 15 min) on axillary buds.The interaction between Clorox concentrations and period of treatments affected significantly on survival explants giving the highest value (88.89) by using Clorox 20% for 15 min. The obtained results were in harmony with that obtained on *Limonium sinnuatum* "Citron Mountion" by Hosni *et al*. [13].

Effect of MS-Strength, NAA Concentrations and Antioxidants on Establishment and Development Stage of *Cerbera odollam*: For the establishment stage, data in Table 3 showed that different concentrations of NAA (0.0, 0.1, 0.5 and 1.0 mg/l) and MS-strength (full, half or quarter) had a significant effect on shoot length and number of leaves.

Shoot Length (cm): Using MS-medium at half strength produced the longest shoot (1.28cm). But the explants cultured on full MS medium gave the shortest shoot length (0.67 cm).The interaction between MS strength and NAA affected significantly on increasing shoot length, in

Table 1: Effect of Clorox and soaking periods on shoot tips explants of *Cerbera odollam*

Soaking Periods (min)(B)	Survival (%)				Mortality (%)				Contamination (%)			
Clorox% (A)	15	20	25	Mean (A)	15	20	25	Mean (A)	15	20	25	Mean (A)
10	3.70	14.81	18.52	12.34	0.00	0.00	7.41	2.47	96.30	85.19	74.08	85.19
15	14.81	22.22	29.63	22.22	3.70	7.41	7.41	6.17	81.48	70.37	62.97	71.61
20	62.97	92.59	70.37	75.31	11.11	0.00	25.92	12.34	25.92	7.41	3.70	12.34
25	0.00	0.00	0.00	0.00	96.30	100.00	100.00	98.77	3.70	0.00	0.00	1.23
Mean	20.37	32.41	29.63		27.78	26.85	35.18		51.85	40.74	35.19	
$LSD_{0.5}$Clorox(A)		5.98				4.41				5.70		
LSD Periods (B)		5.18				3.82				4.93		
LSD (Ax B)		10.35				7.64				9.87		

Table 2: Effect of Clorox and soaking periods on axillary buds explants of *Cerbera odollam*

Soaking Periods (min)(B)	Survival (%)				Mortality (%)				Contamination (%)			
Clorox% (A)	15	20	25	Mean (A)	15	20	25	Mean (A)	15	20	25	Mean (A)
10	18.52	29.63	44.44	30.86	0.00	0.00	3.70	1.23	81.48	70.37	51.85	67.90
15	48.15	59.26	62.97	56.79	3.70	3.70	7.41	4.94	48.15	37.03	29.63	38.27
20	88.89	40.74	7.41	45.68	3.70	55.56	92.59	50.62	7.41	3.70	0.00	3.70
25	7.41	0.00	0.00	2.47	88.89	100.00	100.00	96.30	3.70	0.00	0.00	1.23
Mean	40.74	32.41	28.70		24.07	39.82	50.93		35.19	27.78	20.37	
$LSD_{0.5}$Clorox(A)		7.43				6.24				5.41		
LSD Periods (B)		6.43				5.41				4.68		
LSD (Ax B)		12.87				10.81				9.36		

Table 3: Effect of MS medium strength and NAA on establishment stage of *Cerbera odollam*

	Shoot length				Number of leaves			
	Antioxidants				Antioxidants			
MS and NAA(mg/l)	Cont.	P.V.P.	A.C.	Mean(A)	Cont.	P.V.P.	A.C.	Mean (A)
Full MS	0.50	0.83	0.67	0.67	3.67	5.33	4.67	4.56
Full MS+0.10	0.67	1.50	1.17	1.11	6.67	8.33	7.33	7.44
Full MS+0.50	0.83	1.50	1.17	1.17	5.33	7.67	6.33	6.44
Full MS+1.00	0.83	2.33	1.67	1.61	4.33	6.33	5.00	5.22
1/2 MS	1.00	1.67	1.17	1.28	4.00	5.33	4.67	4.67
1/2 MS+0.10	1.17	1.83	1.33	1.44	6.33	8.67	7.67	7.56
1/2 MS+0.50	1.50	2.33	1.67	1.83	6.33	7.67	6.67	6.89
1/2 MS+1.00	1.67	2.83	2.17	2.22	5.33	6.67	5.33	5.78
1/4 MS	0.67	1.17	1.00	0.94	4.33	5.67	5.00	5.00
1/4 MS0.10+	1.00	1.67	1.17	1.28	5.67	7.33	6.33	6.44
1/4 MS+0.50	1.33	1.83	1.50	1.56	4.33	6.33	5.33	5.33
1/4 MS+1.00	1.50	2.33	1.83	1.89	4.00	5.33	4.33	4.56
Mean (B)	1.06	1.82	1.38		5.03	6.72	5.72	
LSD MS and NAA(A)	0.2741				0.5209			
LSD Antioxidants (B)	0.1371				0.2605			
LSD (A x B)	0.4748				0.9023			

Table 4: Effect of BA and Kin at different concentrations on multiplication stage of *Cerbera odollam*

	Shoot length (cm)							No. of leaves							No. of shoots						
Kin(mg/l)	----------							----------							----------						
BA(mg/l)	0.0	0.1	0.5	1.0	1.5	2.0	Mean (B)	0.0	0.1	0.5	1.0	1.5	2.0	Mean(B)	0.0	0.1	0.5	1.0	1.5	2.0	Mean (B)
0.00	1.83	2.17	2.33	2.83	2.83	3.17	2.53	4.33	4.67	4.67	5.67	6.33	6.33	5.33	0.00	0.00	0.00	0.67	1.00	1.00	0.45
0.50	2.67	3.17	3.67	4.17	5.67	5.50	4.14	5.33	7.33	8.33	9.00	11.67	11.00	8.78	0.33	0.67	1.33	1.67	2.00	2.33	1.39
1.00	2.67	3.00	3.67	3.83	4.33	4.67	3.70	5.67	7.67	8.00	8.33	9.33	8.67	7.95	1.67	2.33	2.33	2.33	2.67	3.00	2.39
1.50	2.67	2.83	3.17	3.67	3.83	4.17	3.39	4.33	4.67	6.33	6.33	8.33	7.33	6.22	2.33	3.00	5.33	4.67	4.33	4.00	3.94
2.00	2.33	3.17	3.67	3.83	4.33	4.67	3.67	4.33	4.67	5.67	6.00	6.33	6.33	5.56	2.33	3.33	4.33	4.33	3.67	3.33	3.55
Mean(A)	2.43	2.87	3.30	3.67	4.20	4.44		4.79	5.80	6.60	7.07	8.40	7.93		1.33	1.87	2.66	2.73	2.73	2.73	
LSD BA (A)	0.1863							0.3845							0.3428						
LSD Kin (B)	0.2041							0.4212							0.3756						
LSD (A x B)	0.4565							0.9417							0.8398						

Table 5: Effect of IBA on rooting stage of Cerbera *odollam*

IBA (mg/l)	No. of roots	Root length (cm)	No. of leaves	Shoot length (cm)
0.00	0.56	0.67	5.33	4.67
0.10	2.11	2.17	7.78	6.33
0.50	0.67	0.89	5.56	4.78
1.00	0.00	0.00	5.11	3.28
2.00	0.00	0.00	5.22	3.22
4.00	0.00	0.00	5.11	3.11
LSD	0.33	0.21	0.44	0.26

Table 6: Effect of peatmoss and sand on survival percentage during acclimatization stage of *Cerbera odollam*

Peatmoss	Sand	Survival %
1	0	40.00
1	1	10.00
1	2	0.00
1	3	0.00
LSD		10.40

most treatments. The longest shoot length was obtained on 1/2 MS medium plus NAA at 1.0 mg/l. Clear suppression of browning had been observed for the media supplemented with Polyvinyl Pyrrolidone. The highest mean value for shoot length had been obtained on 1/2 MS medium supplemented with 1.0 mg/l NAA and 200 mg/l PolyVinyl Pyrrolidone.

On the other hand, explants treated with antioxidant solution and cultured on agar medium combined with activated charcoal (A.C.), were capable for growth and further shoot proliferation [14]. The addition of P.V.P to the medium (concentration of 250-1000 mg/L), P.V.P is a polymer, which adsorbs phenol-like substances [15].

Number of Leaves: It is clear from the data in Table 3 that the various treatments affected significantly on number of leaves. The highest number of leaves (5.00) was obtained on MS quarter strength. MS at half strength medium supplemented with 0.1 mg/l NAA increased significantly number of leaves (7.56).The presence of Polyvinyl Pyrrolidone at 200 mg/l in culture media resulted in greatest number of leaves.The interaction between MS-strength plus NAA and antioxidant, cleared that 1/2 MS medium supplemented with 0.1 mg/l NAA and 200 mg/l Polyvinyl Pyrrolidone produced the highest number of leaves.

This result agreed with that reported by Mehatre *et al*. [16] who obtained 100% establishment of Sarpagandha (*Rauvolfia serpentine*) explants on MS medium supplemented with NAA at 0.5 mg/l and Kin at 0.1 mg/l.

Effect of BA and Kin on Multiplication Stage of *Cerbera odollam*

Shoot Length (cm): The data in Table 4 show that BA concentrations increased shoot length. The longest shoots (4.14) had been obtained on medium containing 0.5 mg/l BA at the end of fourth subculture. Concerning the effect of Kin concentrations, the data revealed that raising

the concentrations of Kin increased steadily the shoot length to reach its highest value (4.44 cm) at 2.0 mg/l Kin. Regarding the interaction between BA and Kin the shoot length had been affected giving the highest value (5.67) on MS medium supplemented with 0.5 mg/l BA and 1.5 mg/l Kin.

In the same trend, great shoot of *Strelitzia* (*Strelitzia reginae* Banks) development was observed on the MS medium supplemented with 20 g/l sucrose, while BAP resulted the smallest plants [17]. On the other hand, the longest shoots of *Catharanthus roseus* occurred on MS medium containing BA and 1 mg/l NAA [18].

Number of Leaves: The number of leaves as shown in Table 4 revealed that BA at different concentrations increased the number of leaves,giving the highest number of leaves (8.78) at 0.5 mg/l BA as compared with (5.56) at 2.0 mg/l BA. In case of Kin the highest number of leaves (8.40) had been obtained on MS medium supplement with 1.5 mg/l as compared with other concentrations.

Number of Shoots: Results illustrated in Table 4 showed that number of shoots was increased due to raising BA concentrations, giving the highest number of shoots (3.94) at 1.5 mg/l BA. Similar trend was obtained as a result of Kin treatment giving the highest number of shoots (2.73) on the medium containing Kin concentration higher than 0.5 mg/l. As for the interaction between BA and Kin, it was found that the greatest number of shoots (5.33) was obtained at 1.5 mg/l BA and 0.5 mg/l Kin after the fourth subculture.

These results seemed to be in harmony with those obtained on *Holarrhena antidysenterica* cultivated on MS medium containing 0.5 mg/l BA [18].On the other hand, nodal segments collected from *In vitro* germinated seedlings were inoculated in WPM medium supplemented with BAP [11].

Effect of Iba on Rooting of *Cerbera odollam:* Data in Table 5 demonstrated that IBA in the presence of activated charcol (3.00 g/l) significantly affected the rooting stage of *Cerbera odollam*. For IBA level, it was found that 0.1 mg/l IBA gave the highest number of roots (2.11) and root length (2.17 cm). For the number of leaves and shoot length, IBA at 0.1 mg/l produced greater number of leaves and shoot length as compared with the other combinations only 0.1 mg/l IBA, induced rooting on shoots.Similar trend was report by El-shamy *et al.* [20] who obtained shoots of *Magnolia grandiflora* treated with IBA in the culture medium.

Effect of Peat Moss and Sand During Acclimatization Stag of *Cerbera odollam*: Data in Table 6 showed that the plantlets of *Cerbera odollam* successfully lifted when they were transferred to be cultured in a mixture of peatmoss and sand (1:0, 1:1; 1:2 and 1:3, respectively) and covered by polythene sheets in greenhouse. After three weeks, the sheets were removed and left for two weeks under plastic house condition. The survival percentages of plantlets after 5 weeks were calculated.

The best percentage of survival plantlets (40 %) was observed when plantlets cultured in a peatmoss. Sand mixture induced the decrease in percentage of survival plantlets. These findings go in line with those reviewed on *Liquidambar styraciflua* [21].

CONCLUSION

There was no died explants observed when shoot tip explants were surface sterilized, the best percentage of survival explants (92.59%) and the percentage of free contaminated explants (7.41%) were observed when explants were immersed for 20 min in 20% Clorox. Explants cultured on 1/2 MS medium supplemented with 1.0 mg/l NAA and 200 mg/l polyvinyl pyrrolidone (PVP) gave the highest mean value for shoot length and number of leaves. For shoot proliferation, the interaction between BA and Kin had been effective, giving the highest value (5.67 cm) on MS medium supplemented with 0.5 mg/l BA and 1.5 mg/l Kin. The greatest number of shoots (5.33) was obtained at 1.5 mg/l BA and 0.5 mg/l Kin, while the highest number of leaves (8.78) was obtained at 0.5 mg/l BA. Optimal rooting was recommended within one month of transfer to MS medium supplemented with 0.1 mg/l IBA,giving the highest number of roots (2.11) and root length (2.17 cm).The best percentage of survival plantlets (40 %) was recommended when plantlets were cultured in pots containing peat moss and covered by polythene sheets in the greenhouse.

Recommendation: For *In vitro* propagation of *Cerbera odollam* plant, a well defined protocol was reached including:

Surface sterilization of shoot tips and auxiliary buds with 20% Clorox (sodium hypochlorite as commercial bleach) for 20 and 15 min, respectively to give the best survival (92.59 and 88.89%), contamination (7.41 and 7.41%) and mortality of shoot tips and auxiliary buds respectively (0.00 and 3.70%). Explants should be cultured on 1/2 MS medium supplemented with 1.0 mg/l NAA and 200 mg/l polyvinyl pyrrolidone (PVP) to establish

effectively. In the multiplication stage, 0.5 mg/l BA plus 1.5 mg/l Kin form not only the highest number of leaves but also the highest shoots lengths. MS medium plus 1.5 mg/l BA plus 0.5 mg/l Kin is favored for number of shoots. For *In vitro* rooting, 0.1 mg/l IBA is more suitable to form roots on *Cerbera odollam*. After 4 weeks in rooting medium, *Cerbera* plants should be transplanted into pots containing peat moss and covered by polythene sheets. After three weeks, sheets are removed and plantlets are left for two weeks under plastic house condition where 40 % of the plantlets survive.

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