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RESEARCH ARTICLE

INDIRECT ORGANOGENESIS OF *BOUCERSIA PROCUMBENS* (GAMBLE) PLOWES - A RARE SUCCULENT PLANT

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ABSTRACT

Efficient protocols of callus culture indirect organogenesis were established for mature internodal segments of *Boucerosia procumbens* (Asclepiadoideae). When MS medium was supplemented with different concentration of auxins, the texture of the callus varied according to the nature of auxin. Optimum callus was developed on MS medium supplemented with 3mg/l IAA. Best response (65%) of callus proliferation was obtained when MS medium fortified with 2iP 2mg/l + Zeatin 0.5 mg/l. IAA was most effective in producing the highest frequency of organogenic culture. Regeneration of callus into plantlets could not be achieved in the present study. The regenerated shoots were rooted on half strength MS medium fortified with 0.1 mg/l NAA. 57% of the rooted shootlets survived in the field.

Keywords: Indirect organogenesis, high frequency callus, *Boucerosia procumbens*.

1. INTRODUCTION

Boucerosia Plowes (Section *Caralluma*; Apocynaceae; Asclepiadoideae) is a genus of succulent herbs, which comprises about 200 genera and 2500 species world over. Out of 18 species reported from India, 5 species and 7 varieties are endemic to Peninsular India (1). *Boucerosia procumbens* is one such species which is rare and endemic to Southern Peninsular India. *Boucerosia procumbens* is growing wild on rocky areas in Maruthuvamalai hills of Kanyakuamri district, Tamil Nadu, India. No chemical investigation has been reported on this species, but plants belonging to this genus are rich in esterified polyhydroxy pregnane glycosides, some of which showed antitumor activity and others were postulated as precursors of cardenolides (2,3). Most of the species from this genus is also characterized by the presence of flavone glycosides (4,5). Young shoots of *Boucerosia* though very bitter, are commonly eaten raw in South India. But *Boucerosia procumbens* are particularly liked, for instead of being bitter they are pleasantly acid to the taste. Due to endemic nature of this plant, it has become imperative to establish a suitable protocol for its micropropagation. Present investigation therefore aims to develop a rapid and high frequency shoot regeneration system from shoot tip of *Boucerosia procumbens* for providing continuous supply of a better source of elite plants to be used as standard material.

2. MATERIALS AND METHODS

Fresh young and juvenile shoots of *Boucerosia procumbens* were collected from Maruthuvamalai hills, Kanyakumari District, Tamil Nadu. These shoots were washed thoroughly under running tap water to remove dust particles. The explants were then surface disinfected by agitating gently in 2% Tween-20 (v/v) for 15 minutes and washed in running tap water. Then the explants were taken inside the inoculation chamber for further sterilization. The materials were kept in 70% ethanol for 60 seconds, followed by repeated washings (3-4 times) in sterilized distilled water. These were then surface sterilized with 0.1% mercuric chloride for 4 minutes followed by 3-4 with sterile distill water.

Different types of media were used in the present study such as Murashige and Skoog's (MS) medium (6), B_5 medium (7) and Woody Plant medium (8). The culture medium consisted of MS salts supplemented with 2% (w/v) sucrose and various auxins (2,4-D, 2,4,5-T, NAA, IAA, IBA) and cytokinins(BAP, KN, 2iP,and Zeatin) at appropriate concentrations both individually and in combinations. All plant growth regulators were added to the medium before autoclaving. The pH of the medium was adjusted to 5.8 and autoclaved at 108KPa and 121^{o}C for 15 minutes. A quantity of 20 ml medium was dispensed in culture tubes closed with aluminium foil. All the cultures were maintained at a temperature of 25±2^{o}C under a light intensity of 2000lux provided by cool white fluorescent lamps. Subculturing was periodically carried out at 4 week interval. The nature and percentage of response were also recorded at an interval of 4 weeks. The regenerated shoots were rooted on half strength MS medium supplemented with different concentrations of auxins (IAA, IBA, and NAA).

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All the experiments were repeated thrice with 15 replicates each. The data was statistically analyzed using one way analysis of variance and means were compared using the Tukey test at the 0.05% level of significance.

3. RESULTS AND DISCUSSION

3.1. Medium Evaluation

Various types of media such as MS, B_5 and WPM fortified with optimum concentration of 2,4-D 3mg/l and 2% sucrose were tested for callus induction of mature internodal segments of *Boucerosia procumbens*. Among the 3 different media tested, MS was found to be the best basal medium with maximum fresh and dry weights of 614.00 ±81.10 mg and 33.50 ±1.22 mg respectively (Table 1). Internodes failed to induce callus on any media with out auxin but remained green and fresh. MS medium was found to be effective for *in vitro* induction of callus in several other Asclepiadaceae members such as *Decalepis hamiltonii* (9), *Leptadenia reticulata* (10), *Gymnema sylvestre* (11) and *Holostemma ada-kodien* (12).

3.2. Callus studies

The texture of the callus varied according to the nature of auxin. The internodal segments of *Boucerosia procumbens* cultured on MS medium at lower concentration (0.1mg/l) of 2,4-D, 2,4,5-T, NAA, IBA and IBA had failed to respond. When 2,4,5-T fortified individually with MS medium embryogenic callus produced. The texture of callus varied when the internodal segments cultured on MS medium fortified with 2,4,5-TP (0.1- 7mg/l) at 0.1 mg/l the texture of callus was white friable (Fig. 1a), at 2mg/l and 3mg/l green nodular callus was observed. At all concentrations of 2,4-D yellow compact callus was observed. Maximum percent of response (80%) was observed at 3 mg/l IAA and the nature of callus is nodular yellow (Fig.1 e). Callus developed on MS medium fortified with NAA was embryogenic and pale green in color. IBA gave the lowest and slowest callus production.

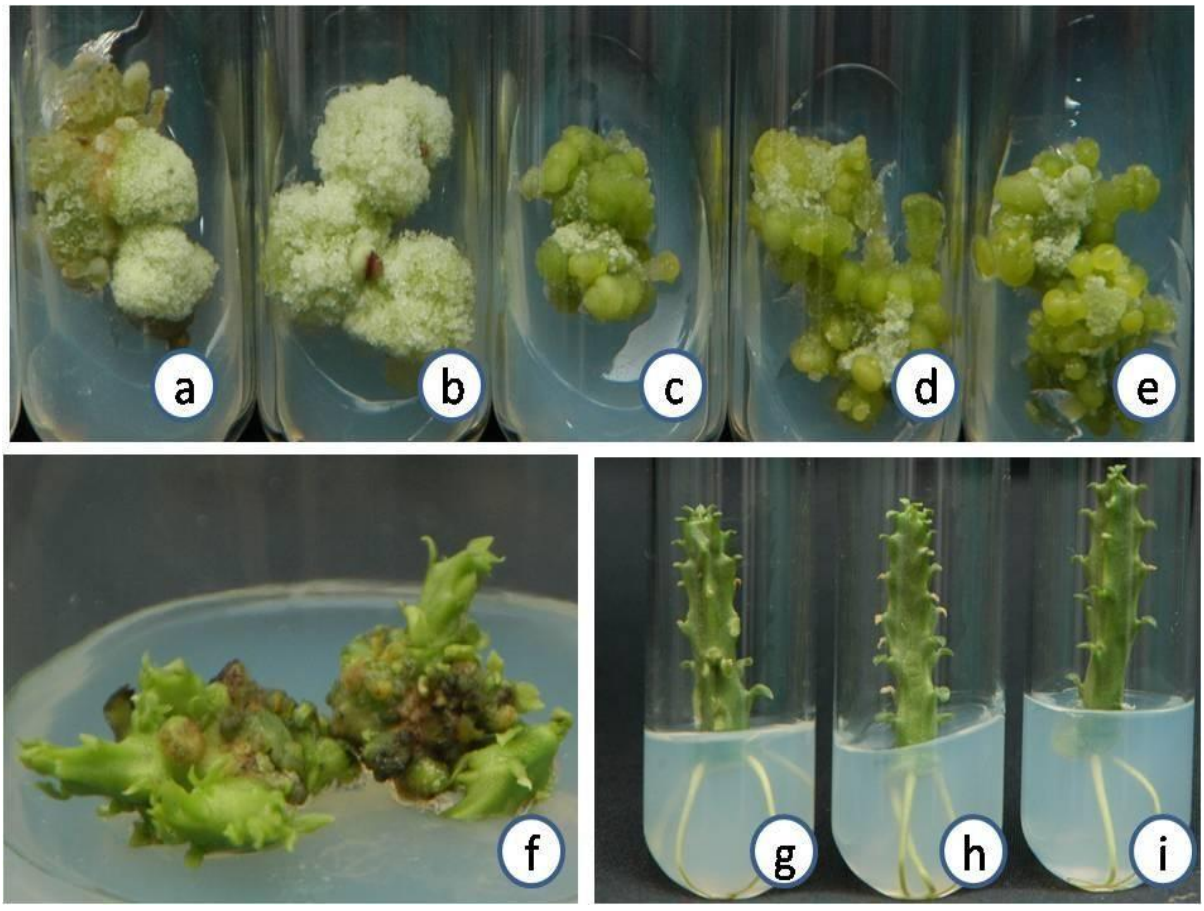

Fig. 1. Callus types of *Boucerosia procumbens* internode culture on MS medium containing various types of auxins. a & b. Friable callus; c-d. Nodular callus; f. Callus regeneration; g-i. *In vitro* rooting of regenerated shootlets on NAA containing medium.

Table 1. Effect of various types of media on callus induction from Internodal mature explants of *Boucerosia procumbens* cultured with 3mg/l 2,4-D.

Plant growth regulator (mg/l)	Type of medium	Internode		
		% of response	Fresh weight (mg) Mean + SE	Dry weight (mg) Mean + SE
2,4-D 3mg/l	MS	73	614.00 ± 81.10[a]	33.50 ± 1.22[a]
	B_5	62	420.00 ± 42.36[ab]	31.10 ± 0.84[ab]
	WPM	61	304.00 + 45.25[b]	23.10 + 0.87[b]

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

Table 2. Effect of various auxins on callus induction from mature internodal explants of *Boucerosia procumbens* cultured on MS medium.

Auxins	Conc. (mg/l)	Internode		
		% of response	Degree of callusing	Nature of response
2,4-D	0.1	0	-	NR
	1.0	30	+	YCC
	2.0	50	++	YCC
	3.0	75	+++	YCC
	5.0	60	++	YCC
	7.0	40	+	YCC
NAA	0.1	0	-	NR
	0.5	20	+	BCC
	1.0	40	++	PGEC
	2.0	55	+++	PGEC
	3.0	70	+++	PGEC
	5.0	60	+++	PGEC
IAA	0.1	0	-	NR
	0.5	10	+	BCC
	1.0	25	++	BCC
	2.0	40	++	WFC
	3.0	60	+++	WFC
	5.0	45	++	BFC
IBA	0.1	0	-	NR
	0.5	10	+	BCC
	1.0	35	++	WFC
	2.0	45	++	WFC
	3.0	55	+++	WFC
	5.0	40	++	BFC

YCC - Yellow compact callus; WEC - White embryogenic callus; BCC - Brown compact callus; WFC - White friable callus; NYC - Nodualr yellow callus; GNC - Green nodular callus; GCC - Green compact callus; PYEC - Pale yellow embryogenic callus; BEC - Brown embryogenic callus; PGNC - Pale green nodular callus; PGEC - Pale green embryogenic callus; BFC - Brown Friable callus; NR - No Response; + Scanty; ++ Less; +++ Moderate; ++++ Profuse; Experiments were repeated thrice with 15 replicated each.

3.3. Indirect organogenesis

The different growth regulators inducing the callus exhibited a significant influence on organogenesis. Callus obtained on MS medium fortified with IAA 3mg/l was selected for morphogenesis. The callus was subcultured on MS medium supplemented with different concentrations of cytokinins. Shoot buds are initiated from the surface of callus within 6 weeks of culture (Fig. 1g).

Among the various concentrations of cytokinins tested, the highest shoot regeneration frequency (65%) and highest number of shoots (2.20 ± 0.20) were recorded at 2iP 2mg/l + Zeatin 0.5 mg/l (Table 3). Auxins and cytokinins are able to bring shoot or root formation from callus but the effective concentrations of these regulators may vary. Effectiveness of each treatment generally depends on the nature and origin of the explant, its endogenous hormone content and the conditions used for *in vitro* culture (13).

Table 3. Effect of various plant growth regulators on morphogenic response of callus induced from internodal mature explants of *Boucerosia procumbens* cultured on MS medium.

Plant growth regulator used for the callus induction	Media used for morphogenesis				Type of explant (leaf)	
	BAP	2iP	KN	Zeatin	% of response	No. of shoot buds / explant Mean + S.E
IAA 3 mg/l	0.1	-	-	-	0	NR
	0.5	-	-	-	15	0.60 ± 0.24[b]
	1.0	-	0.1	-	40	1.60 ± 0.25[ab]
	2.0	-	0.5	-	60	1.80 ± 0.20[ab]
	-	0.1	-	-	0	NR
	-	0.5	-	-	20	1.20 ± 0.20[b]
	-	1.0	-	0.1	50	1.60 ± 0.25[ab]
	-	2.0	-	0.5	65	2.20 ± 0.20[a]

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

3.4. Rooting and plantlet establishment

The shoots were regenerated from callus which are excised and transferred to half strength MS medium fortified with auxins for *in vitro* rooting. Half strength MS medium supplemented with auxins at different concentrations showed varied effect on *in vitro* rooting (Table 4). Maximum number of roots was observed on NAA0.1mg/l, 3.4 ± 0.13 roots per shoot with 2.08 ± 0.14 cm of root length (Fig. 1 h). Increase in concentration of NAA decreases the root number and length. With IBA treatments rooting was very slow and less effective. Lower concentration of IAA failed to induce rooting. In *Boucerosia procumbens* root formation is much better with NAA

than IAA and IBA. It was also proved in other Asclepiadaceae members such as *Decalepis hamiltonii* (9) and *Decalepis arayalpathra* (14).

Rooted plantlets were taken out carefully from culture tube and washed thoroughly to remove all the traces of agar. These plantlets were transferred to paper cups pots containing sterilized peatmoss and sand (3:1) and covered with polythene bags. Potted shootlets were first placed in culture room at 25±2°C, 16h photo period and 85% relative humidity. The potted plants were irrigated with MS basal salts solution (1/4 strength) devoid of sucrose every 5 days for 3 weeks. The hardened plants were transferred to earthen pots and kept under shade and finally acclimatized. Nearly 57% plants of *Boucerosia procumbens* were successfully acclimatized to field conditions.

Table 4. Effect of various auxins on rooting response from *in vitro* regenerated shoots of *Boucerosia procumbens* cultured on MS half strength medium after 30 days.

Auxins (mg/l)	% of response	No. of roots / shoots Mean ± SE	Length of roots (cm) Mean ± SE	Degree of callusing
IAA 0.1	-	NR	NR	-
IAA 0.5	20	1.06 ± 0.11^{c}	0.50 ± 0.05^{d}	-
IAA 1.0	40	1.86 ± 0.21^{bc}	1.70 ±0.08^{ab}	-
IBA 0.1	-	NR	NR	-
IBA 0.5	35	1.13 ± 0.16^{c}	1.02 ± 0.14^{cd}	-
IBA 1.0	20	0.73 ± 0.11^{c}	0.58 ± 0.05^{d}	-
NAA 0.1	75	2.06 ± 0.24^{a}	2.14 ± 0.14^{a}	-
NAA 0.5	60	1.13 ± 0.21^{b}	1.68 ± 0.08^{b}	-
NAA 1.0	50	1.20 ± 0.14^{c}	0.16 ± 0.09^{c}	+

Means followed by the same letter not significantly different by the Tukey test at 0.05% probability level; NR-No Response

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