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Effect of Cytokinins on *In vitro* Propagation of *Boucerosia umbellata* (Haw.) Wight & Arn. (Syn.: *Caralluma umbellata* Haw.) from Nodal Explants of Field Grown Plants

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors BS and TP designed the entire study and protocols with interpretations of the results, prepared the first draft of the manuscript. Author SSR managed the analyses of the study and computational work. Author TP guided in the entire research and documented the final draft of the manuscript. All the authors have read and approved the final manuscript.

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ABSTRACT

An efficient protocol was described for the rapid *in vitro* multiplication of medicinal plant *Boucerosia umbellata* via enhanced axillary bud proliferation from nodal explants collected from young shoots of three-month-old field grown plants. Effects of cytokinins 6-benzylaminopurine (BAP), Thiadiazuron (TDZ), Kinetin (Kin) and 2-isopentenyl adenine (2iP) individually and in combination have been investigated. MS medium supplemented with BAP (2 mg/l) and TDZ (1 mg/l) gave maximum response for multiple shoot induction with a shoot sprouting frequency of 86% and mean shoot number of 3.9±0.16 per node and 2.04±0.05 cm shoot length. Rooting was best achieved on ½ strength MS (Murashige and Skoog, 1962) medium supplemented with Naphthalene Acetic Acid (NAA) (0.1 mg/l). The regenerated plantlets were successfully established in the garden with 73% survival rate.

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1. INTRODUCTION

The genus *Caralluma* has been included in the family Apocynaceae (sub family Asclepiadoideae) according to APG III classification. The *Caralluma* species found in India are edible plants. A number of phytochemical compounds isolated from *Caralluma* species possess many medicinal properties having antiinflamatory, antiulcer, antidiabetic, carminative, febrifuge, antipyretic and antioxidant activity [1]. The extracts of *Caralluma* have also been found to be appetite suppressant, a property which is well known to Indian tribals and hunters. Indian folklore records its use as a potent appetite suppressant and weight loss promoter. Some *Caralluma* species are used in the treatment of obesity. The extract of *Caralluma* species in the form of capsules has been released under trade name GENASLIM for body weight control [2]. The genus *Boucerosia* was separated from the genus *Caralluma* (*sensu stricto*) mainly by the pseudoumbellate terminal cymes [3]. Genus *Boucerosia* comprises eight species and these are endemic to Peninsular India and Sri Lanka [1].

Boucerosia umbellata (Haw.) Wight & Arn. (Syn: *Caralluma umbellata* Haw.) is a succulent leafless herb, up to 30 cm tall with flowers in terminal umbels. It is distributed in the dry hilly regions of Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. Tender stems of *Caralluma umbellata*, locally known as *Pedda saara* are consumed as vegetable in both the forest areas and plains of Andhra Pradesh [4]. Stems of *Caralluma umbellata* are used for treatment of stomach disorders, abdominal pains [5] and kidney stones by the ethnic practitioners of Nalgonda district in Andhra Pradesh [6]. According to Neelima et al*.* [7], external application of latex of *Caralluma umbellata* heals mouth ulcers. Plenty of pregnane glycosides were identified and isolated from different parts of the plant [1]. Bellamkondi et al*.* [8] reported antihyperglycemic activity of *Caralluma umbellata. In vitro* shoot multiplication from nodal explants of *Boucerosia diffusa* (Syn.: *Caralluma diffusa*) was described by Ramadevi et al*.* [9] and Kalimuthu et al*.* [10]. Naveen and Pullaiah [11] studied *in vitro* propagation of *Boucerosia indica*. Regeneration of multiple shoots from callus of *Boucerosia truncato-coronata* was investigaated by Kalimuthu et al*.* [12]. Influence of aseptic seedling explants on *in vitro* shoot multiplication of *Caralluma adscendens* var. *attenuata* was investigated by Aruna et al*.* [13]. Sreelatha and Pullaiah [14] carried out work on high frequency axillary bud multiplication of *Caralluma stalagmifera*. Natural populations of these plants are declining because of increase in demand in pharmaceutical market [1]. There is no agrotechnology available for controlled cultivation. We have developed protocol for their micropropagation and conservation. The available literature reveals no published reports on the *in vitro* propagation of *Boucerosia umbellata* (Syn.: *Caralluma umbellata*) and hence the present investigation has been undertaken.

2. MATERIALS AND METHODS

The plants of *B. umbellata* were collected along with roots from Penukonda hills, Anantapur district, Andhra Pradesh and maintained in earthen pots in the Botanical garden of Sri Krishnadevaraya University Ananatapur, India. Young sprouts of *B. umbellata* were collected from 3-month old garden plants (Fig. 1A). The chemicals used in the study were Analytical Reagent grade and procured from Qualigens, India, while plant growth regulators were obtained from Sigma Chemical Co., USA. Nodal segments were washed with Tween 20 (5% v/v) for 5 min and then surface sterilized with mercuric chloride (0.1% w/v) solution for 5 min. The segments were washed thoroughly with sterile water before cutting into an appropriate size (1 cm) explants. Murashige and Skoog (1962) (MS) medium containing vitamins and 30 g/l sucrose, solidified with 0.8% (w/v) agar, was used for the culture. The pH of the medium was adjusted to 5.8 before autoclaving. Nodal segments were cultured on MS basal medium supplemented with various cytokinins like BAP, Kin, TDZ and 2iP to obtain the best response for shoot proliferation. All the cultures were incubated at 25±2℃ with 16/8 h photoperiod under white fluorescent tubes (25 µ E m^{-2} S^{-1}). For culture establishment, MS medium was supplemented with various cytokinins viz. BAP (0.5, 1, 2, 3 and 5 mg/l), Kin (0.5, 1, 2, 3 and 5 mg/l), TDZ(0.5, 1, 2, 3 and 5 mg/l) and 2iP (0.5, 1, 2, 3 and 5 mg/l) individually and in combination. *In vitro* produced shoots (each with two nodes) were excised and transferred to full strength and half strength MS medium with Indole-3-Acetic Acid (IAA) (0.1, 0.25, 0.5, 1.0, 2.0

mg/l), Indole-3-Butyric Acid (IBA) (0.1, 0.25, 0.5, 1.0, 2.0 mg/l), and Naphthalene-3-Acetic Acid (NAA) (0.1, 0.25, 0.5, 1.0, 2.0 mg/l), for rooting. The rooted shoots were washed to remove the adhering gel and transferred to pots containing a mixture of sterile sand: soilrite: garden soil in a ratio 1: 2: 1. Potted plantlets were covered with polyethylene bags to maintain humidity. The plants were continued to an exposure of 25±2℃ and 90-95% relative humidity by watering the plants and covering with polythene bags. After 15 days, the covers were removed and the plants were exposed to less humid conditions. The plants were first transferred to pots and kept in greenhouse and gradually shifted to field conditions. The survival rate of the regenerated plants was recorded 1 month after transfer to field. Each experiment was repeated at least three times with 15 replicates for each treatment. Observations were recorded after interval of 4 weeks. The data was statistically analyzed using one-way analysis of variance (ANOVA) and means were compared using the DMR test at 0.05% level of significance.

3. RESULTS AND DISCUSSION

3.1 Influence of Cytokinins on Shoot Regeneration

A good multiplication rate is the most important factor in rendering a propagation protocol cost effective. According to the classical theory of hormonal regeneration of plant growth and development, modulation of hormonal levels and interactions between different hormones are responsible for the regulation of different aspects of plant development [15]. In tissue culture, cytokinins appear to be necessary for plant cell division. It has been suggested that cytokinins might be required to regulate the synthesis of proteins involved in the formation and function of the mitotic spindle apparatus [16]. In order to identify the optimum range of BAP/Kin/2iP/TDZ for shoot proliferation various concentrations from 0.5 to 5 mg/l were tested. The axillary proliferation of the node explants varied depending on the plant growth regulators and concentrations used. However, there were no shoots on MS basal medium without any growth regulator (control). Bud break usually occurred after one week of culture. The percentage response, number of shoots, and average shoot length were controlled by the type and concentration of the growth regulator employed. Table 1 summarizes the response of the nodal explant on MS medium with different concentrations of cytokinins. When a single cytokinin was used, nodes on MS medium with BAP (2mg/l) showed the best results with a shoot sprouting frequency of 84% and a mean number of 4.6±0.18 shoots were produced with a mean length of 2.32±0.02 cm (Fig. 1D, Table 1). When BAP concentration was increased to 3mg/l in MS medium, frequency of shoot sprouting was reduced to 68% with a mean shoot numbers of 2.7±0.21 and shoot length of 2.01±0.09 cm (Fig. 1B, Table 1). Among the various concentrations of TDZ used for regeneration, better response (76%) was observed at 3 mg/l with 3.3±0.23 shoots/explant and a maximum length of 1.89±0.07 cm (Fig. 1F, Table 1). Lower concentration of TDZ i.e., 2 mg/l had a limited response of 62% with a mean shoot number 2.4±0.12 and mean shoot length 1.5±0.08 cm (Fig. 1G, Table 1). In Kin maximum response was observed with 2 mg/l with a shoot sprouting frequency of 65% and a mean number of 2.9±0.22 shoots were produced with a mean length of 1.55±0.05 cm (Fig. 1C, Table 1). 2iP at 2 mg/l concentration gave good results among all the concentrations tried. The shoot sprouting frequency was 78% with a mean number of 3.4±0.17 shoots with a mean length of 2.18±0.06 cm (Fig. 1E, Table 1).

Among cytokinins, best shoot induction capacity with BAP rather than Kin and 2iP is in agreement with findings that BAP is needed for the micropropagation of latex producing plants [17]. BAP was more efficient for shoot induction of nodal explants in many plants such as *Boucerosia indica* [11], *Boucerosia diffusa* (Syn.: *Caralluma diffusa*) [9,10].

Boucerosia umbellata cultures from test tubes after 30 days of incubation, were transferred to culture bottle for further multiplication of shoots as it becomes difficult for the further growth of shoots in test tube. Transfer of cultures to culture bottles enhanced growth of shoots by axillary branching within few days (Fig. 1H). The shoot multiplication at an enhanced pace by subsequent cultures observed in this study is in agreement with the reports of other Asclepiadaceae members such as *Ceropegia bulbosa* [18], *Gymnema sylvestre* [19], *Hemidesmus indicus* [20], *Holostemma ada-kodien* [21], *Tylophora asthmatica* [22]. According to Rathore et al. [23] *Caralluma edulis* produced multiple shoots by activation of axillary meristem and bud proliferation on MS medium containing BAP (2 mg/l) which on repeated transfer and subculture further multiplied shoots.

3.2 Influence of Combination of Cytokinins on Shoot Regeneration

Media containing combination of different cytokinins may increase the number of shoots formed when compared to media with only one cytokinin. Nodal explants of *Boucerosia umbellata* were cultured on different concentrations and combinations of cytokinins and the response of nodal segments with combinations showed relatively increased response than single cytokinin (Table 1 & 2). Addition of more than one cytokinin to the medium resulted in improved shoot production or shoots of better quality. Table 2 depicts the effect of different concentrations of cytokinins in addition to BAP (2 mg/l) added to MS medium. BAP (2 mg/l) was combined with different concentrations of cytokinins like Kin, TDZ and 2iP at various concentrations for optimizing multiple shoot regeneration. When TDZ (1 mg/l) was added in addition to BAP (2 mg/l), response was enhanced in comparison to single cytokinin with a shoot sprouting frequency of 86% and mean shoot number of 3.9±0.16 per node and 2.04±0.05 cm shoot length (Fig. 2H, Table 2). When the concentration of TDZ was increased to 3 mg/l and 5 mg/l, the response decreased to 56% and 49% respectively. The mean shoot number and shoot length also decreased (Fig. 2C & F, Table 2). Among the various concentrations of Kin used with BAP (2 mg/l), Kin (2 mg/l) gave a shoot sprouting frequency of 74% with a mean number of 2.9±0.12 shoots and 2.27±0.03 cm mean shoot length (Fig. 2B, Table 2). As the concentration of Kin was increased to 3 mg/l, the response was relatively reduced (Fig. 2D, Table 2). Nodal explants on MS medium with BAP (2 mg/l) and 2iP showed response slightly lesser than BAP in combination with TDZ, 2iP (1 mg/l) along with BAP (2 mg/l) resulting in 76% shoot sprouting frequency with 2.3±0.24 mean shoot number and 2.98±0.03 cm mean shoot length (Fig. 2A, Table 2). When the concentration of 2iP was increased to 3 mg/l the response declined to 48% shoot sprouting frequency with a mean shoot number of 1.9±0.13 and the mean shoot length of 1.34±0.04 cm (Fig. 2G, Table 2). Least response was observed in MS + BAP (2 mg/l) +2iP (5 mg/l) with a shoot sprouting frequency of 42% and mean shoot number of 1.0±0.11 per node and 1.12±0.02 cm shoot length (Fig. 2E, Table 2).

Growth becomes feasible only on the addition of one or more of these classes of hormones to the medium [24]. This has been reported by Ramadevi et al*.* [9] and Kalimuthu et al*.* [10] in *Boucerosia diffusa* (Syn.: *Caralluma diffusa*), Aruna et al*.* [13] in *Caralluma adscendens* var. *attenuata* and Thomas and Shankar [25] in *Sarcostemma brevistigma*.

3.3 *In vitro* Rooting of Regenerated Shootlets

Rhizogenesis, the formation of roots, is a crucial step in micropropagation for the formation of complete plantlets. Auxins induce root differentiation and their role in root development is well documented [26]. In vitro grown 3-4 cm length microshoots having 4-5 nodes were excised and inoculated on ½ strength having 4-5 nodes length were excised and inoculated on ½ MS and full strength MS medium fortified with various concentrations of auxins. Rooting was successful with different concentrations of IAA, IBA and NAA (0.1 mg/l, 0.25 mg/l, 0.5 mg/l, 1 mg/l and 2 mg/l) as shown in Table 3. The root formation was noticed at the basal ends of the shoots with initiation of roots starting by 18-25 days.

In *Boucerosia umbellata* ½ strength MS medium fortified with NAA (0.1 mg/l) gave the best result with 78% response, 8.9+ 0.4 roots per explant and 3.82+ 0.03 cm root length. Full strength MS medium was not favourable. NAA (0.25 mg/l) when added to MS medium response was 60% frequency producing 6.4±0.19 roots per shoot with a length of 2.48±0.02 cm (Fig. 3A, Table 3). When the concentration of NAA was increased to 0.5 mg/l and 1.0 mg/l the response reduced to 49% and 38% respectively (Fig 3B & E, Table 3). In all experiments roots produced with NAA were thick and short. IBA at 0.1 mg/l gave a response of only 36% giving rise to mean number of 2.6±0.16 roots the length reaching a mean length of 3.16±0.03 cm (Fig. 3F, Table 3). Increase in concentration of IBA to 1.0 mg/l decreased the response (20%) producing 1.7±0.21 roots per shoot with a mean length of 1.96±0.02 cm. (Fig. 3D, Table 3). IAA added to MS medium was not favourable when compared to NAA as far as root induction was concerned (Fig. 3C, Table 3).

When compared to full strength MS medium, ½ strength MS medium gave better result for rooting experiments. Of the various auxins tested, NAA was favourable for for percentage of response and number of roots whereas the length of root was more with IBA. Out of various concentrations of NAA used, best response (78%) was observed with ½ MS + NAA (0.1 mg/l) producing mean number of 8.9±0.14 roots per

shoot with mean root length of 3.82±0.03 cm (Fig. 3G, Table 3). When the concentration of NAA was enhanced to 0.25 mg/l and 0.5 mg/l, the response reduced to 76% and 64% respectively. The mean root number and root length also relatively decreased (Fig. 3H & I, Table 3). IAA when added to ½ strength MS medium showed moderate results. IAA (0.5 mg/l) on addition to ½ strength MS medium gave a response of 52% with a mean root number of 1.7±0.13 and root length of 3.28±0.01 cm (Fig. 3J, Table 3). When the concentration was increased to 1.0 mg/l, both the root number and root length decreased. IBA addition to ½ strength MS medium produced increased length of the roots. When 0.1 mg/l IBA was added to ½ strength MS medium, 61% response was observed with a mean root number of 2.5±0.12 and a length of 4.61±0.02 cm (Fig. 3K, Table 3).

Addition of NAA produced a large number of thick stout roots and was the favourable auxin for root formation in *Boucerosia umbellata*. This is similar to many previous reports like *Caralluma stalagmifera* [14] and *Caralluma adscendens* [27].

3.4 Hardening and Field Establishment of Plantlets

The plantlets were removed from the culture tubes after 30 days of rooting. They were gently extricated from the culture medium so as to make sure the intact roots without any damage. The roots were disentangled and washed under running tap water. Complete removal of medium / agar is mandatory or otherwise, bacterial contamination may set in and rot the root system and even the entire plant in due course. The plantlets after retrieval were transferred to small pots containing a mixture of sandy soil, farmyard manure and soil rite in the ratio 2:1:1 (Fig. 3L). These pots were totally covered with polythene covers to maintain humidity and maintained inside the culture room. The plants were supplied with ½ strength MS medium without any vitamins and sucrose.

This condition was continued for 10-15 days inside the culture room. During the latter part of this treatment (after 8 days) small holes were punched in the polythene cover. After 15 days, the pots were shifted out of the culture room, but retained within the laboratory under shade and later into the garden. Even in the garden, gradual exposure to sunlight intermittently for a few hours in a day was given. Once the plantlets got acclimatized to the garden atmosphere, they were transferred to large earthen pots and watered with tap water (Fig. 3M). Out of a total of 88 plantlets obtained, 65 of them survived with a survival percentage of 73.

Table 1. Effect of different cytokinins added to MS medium for the induction of multiple shoots from axillary buds of nodal explants of field grown plants of *B. umbellata*

Plant growth regulator	(mg/l)	Shoot sprouting frequency (%)	Mean shoot no. per explant ± SE	Mean length of shoots (cm) ± SE
MS+BAP	0.5	64	1.9±0.16^{e}	1.72±0.03^{d}
	1	76	3.1±0.27^{bc}	1.92±0.07^{bc}
	2	84	4.6±0.18^{a}	2.32±0.02^{a}
	3	68	2.7±0.21^{cd}	2.01±0.09^{b}
	5	53	1.6±0.17^{f}	1.53±0.05 ^{de}
Kin	0.5	36	1.6±0.28^{f}	1.05±0.05^{hi}
	1	43	1.8±0.13^{ef}	1.24±0.03^{fg}
	2	65	2.9±0.22^{c}	1.55±0.05^{de}
	3	38	1.8±0.11^{ef}	1.19±0.07^{g}
	5	32	1.4±0.16^{fg}	1.08±0.03^{h}
TDZ	0.5	40	1.2±0.15^{gh}	1.14±0.05^{gh}
	1	53	2.2±0.23^{de}	1.36±0.0^{9f}
	2	62	2.4±0.12^{d}	1.5±0.08^{e}
	3	76	3.3±0.23^{bc}	1.89±0.07^{c}
	5	58	2.2±0.16^{de}	1.52±0.04^{de}
2iP	0.5	49	1.6±0.18	1.82±0.02^{cd}
	1	56	2.2±0.14^{de}	1.94±0.06^{bc}
	2	78	3.4±0.17^{b}	2.18±0.06^{ab}
	3	62	2.6±0.23^{cd}	1.86±0.02^{cd}
	5	43	1.3±0.12^{fg}	1.44±0.03^{ef}

Means ± SE, n = 45. Means followed by the same letter are not significantly different by the DMR test at 0.05% probability level

Table 2. Effect of different concentrations of cytokinins in addition to BAP (2 mg/l) added to MS medium for the induction of multiple shoots from nodal segments of field grown plants of *B. umbellata*

Plant growth regulator (mg/l)		Shoot sprouting frequency (%)	Mean shoot number per explant ± SE	Mean length of shoots (cm) ± SE
Kin	0.5	61	1.9±0.14^{ef}	2.37±0.04^{bc}
	1	68	2.4±0.27^{cd}	2.53±0.06^{b}
	2	74	2.9±0.12^{bc}	2.27±0.03^{cd}
	3	59	2.1±0.06^{de}	2.25±0.07^{cd}
	5	46	1.2±0.26^{fg}	1.89±0.03^{de}
TDZ	0.5	71	3.6±0.22^{b}	2.4±0.06^{bc}
	1	86	3.9±0.16^{a}	2.04±0.05^{d}
	2	68	3.1±0.13^{bc}	2.85±0.02^{ab}
	3	56	2.6±0.26^{c}	2.36±0.06^{bc}
	5	49	2.1±0.19^{de}	2.06±0.04^{d}
2iP	0.5	66	2.1±0.12^{de}	1.92±0.06^{de}
	1	76	2.3±0.24^{cd}	2.98±0.03^{a}
	2	58	2.0±0.22^{e}	1.96±0.02^{de}
	3	48	1.9±0.13^{ef}	1.34±0.04^{ef}
	5	42	1.0±0.11^{fg}	1.12±0.02^{fg}

Means ± SE, n = 45. Means followed by the same letter are not significantly different by the DMR test at 0.05% probability level

Table 3. Root induction of in vitro derived shoots of *Boucerosia umbellata* on ½ strength and full strength MS medium with various concentrations of auxins

Plant growth regulator (mg/ml)		Frequency of response (%)	Mean root number ± SE	Mean length of root (cm) ± SE
MS + IAA	0.1	49	1.8±0.15^{fg}	3.28±0.04^{d}
	0.25	38	1.4±0.28^{hi}	2.82±0.08^{ef}
	0.5	36	1.3±0.18^{ij}	2.43±0.01^{fg}
	1.0	32	1.1±0.12^{j}	1.91±0.08^{hi}
	2.0	30	0.9±0.24^{jk}	1.32±0.04^{jk}
MS + IBA	0.1	36	2.6±0.16^{de}	3.16±0.03^{de}
	0.25	28	2.1±0.13^{ef}	2.89±0.03^{ef}
	0.5	26	1.9±0.17^{fg}	2.21±0.07^{gh}
	1.0	20	1.7±0.12^{g}	1.96±0.02^{hi}
	2.0	20	1.5±0.14^{h}	1.72±0.04^{j}
MS + NAA	0.1	54	7.6±0.21^{b}	2.05±0.04^{h}
	0.25	60	6.4±0.19^{c}	2.48±0.02^{fg}
	0.5	49	4.8±0.14^{cd}	1.94±0.06^{hi}
	1.0	38	2.6±0.12^{de}	1.83±0.04^{ij}
	2.0	32	2.2±0.31^{ef}	1.42±0.01^{k}
½ MS + IAA	0.1	68	2.2±0.13^{ef}	3.72±0.04^{bc}
	0.25	65	2.0±0.15^{f}	3.49±0.05^{c}
	0.5	52	1.7±0.13^{g}	3.28±0.01^{d}
	1.0	48	1.4±0.18^{hi}	2.08±0.09^{h}
	2.0	42	2.1±0.24^{f}	1.09±0.02^{kl}
½ MS + IBA	0.1	61	2.5±0.12^{e}	4.61±0.02^{a}
	0.25	57	2.2±0.18^{ef}	4.46±0.06^{ab}
	0.5	54	1.9±0.19^{fg}	3.32±0.04^{cd}
	1.0	40	1.8±0.15^{fg}	2.94±0.02^{e}
	2.0	38	1.6±0.21^{gh}	1.81±0.04^{i}
½ MS + NAA	0.1	78	8.9±0.14^{a}	3.82±0.03^{b}
	0.25	76	6.4±0.12^{bc}	3.74±0.07^{bc}
	0.5	64	4.7±0.12^{cd}	2.58±0.05^{f}
	1.0	56	5.2±0.18^{c}	2.34±0.01^{g}
	2.0	51	4.9±0.22^{cd}	2.14±0.15^{h}

Means ± SE, n = 45. Means followed by the same letter are not significantly different by the DMR test at 0.05% probability level

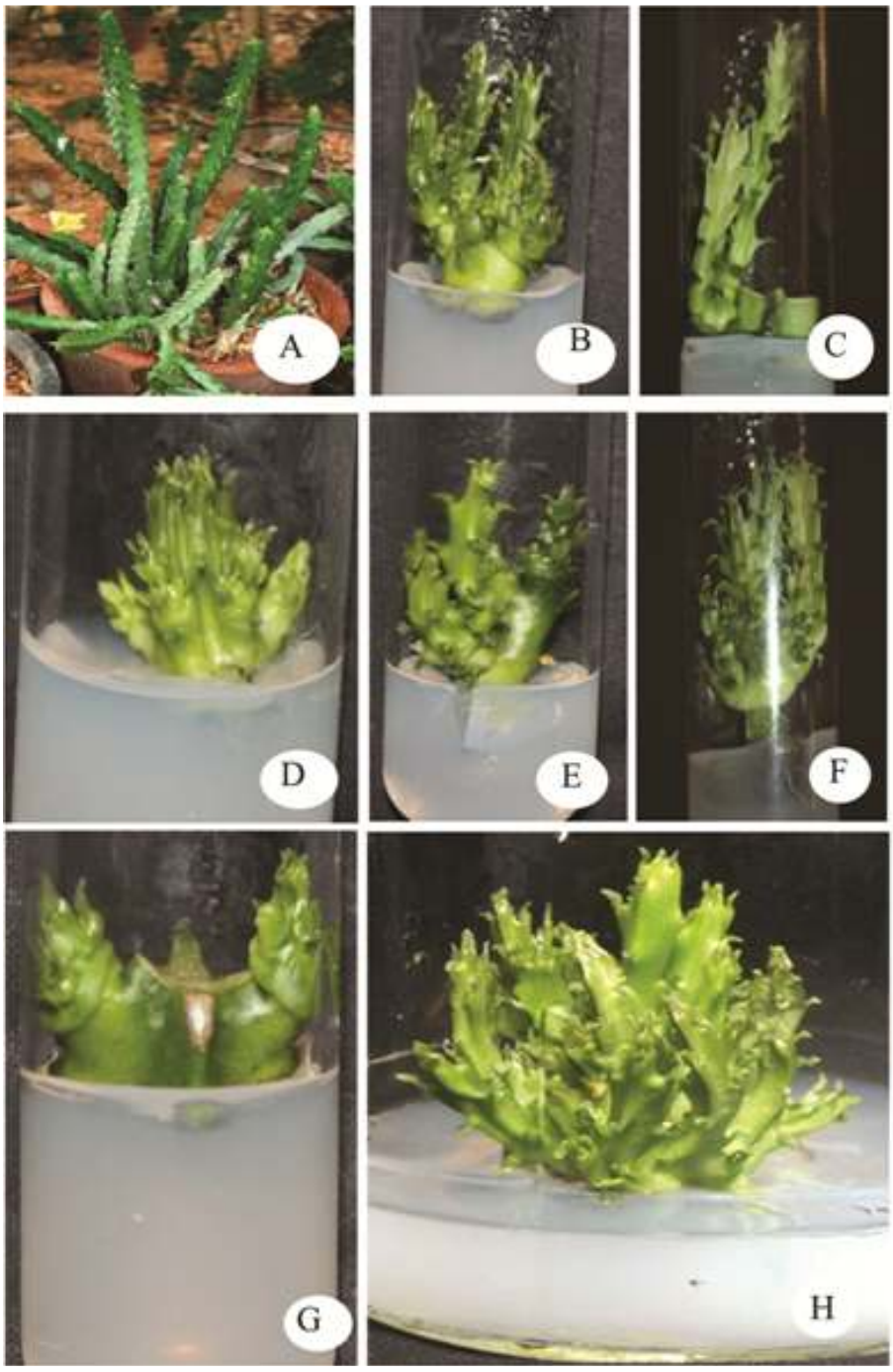

Fig. 1. A- Potted plant of *Caralluma umbellata,* B-MS medium+ BAP (3 mg/l) (68% of sprouting per explant and 2.7 number of shoots per explant), C- MS medium+ Kin (2 mg/l) (65% sprouting per explant and 2.9 number of shoots per explant), D- MS medium + BAP (2 mg/l) (84% sprouting per explant and 4.6 number of shoots per explant), E- MS medium+ 2iP (2 mg/l) (78% sprouting per explant and 3.4 number of shoots per explant), F- MS medium+ TDZ (3 mg/l) (76% sprouting per explant and 3.3 number of shoots per explant), G- MS medium + TDZ (2 mg/l) (62% sprouting per explant and 2.4 number of shoots per explant), H- MS medium + BAP (2 mg/l) Subculture

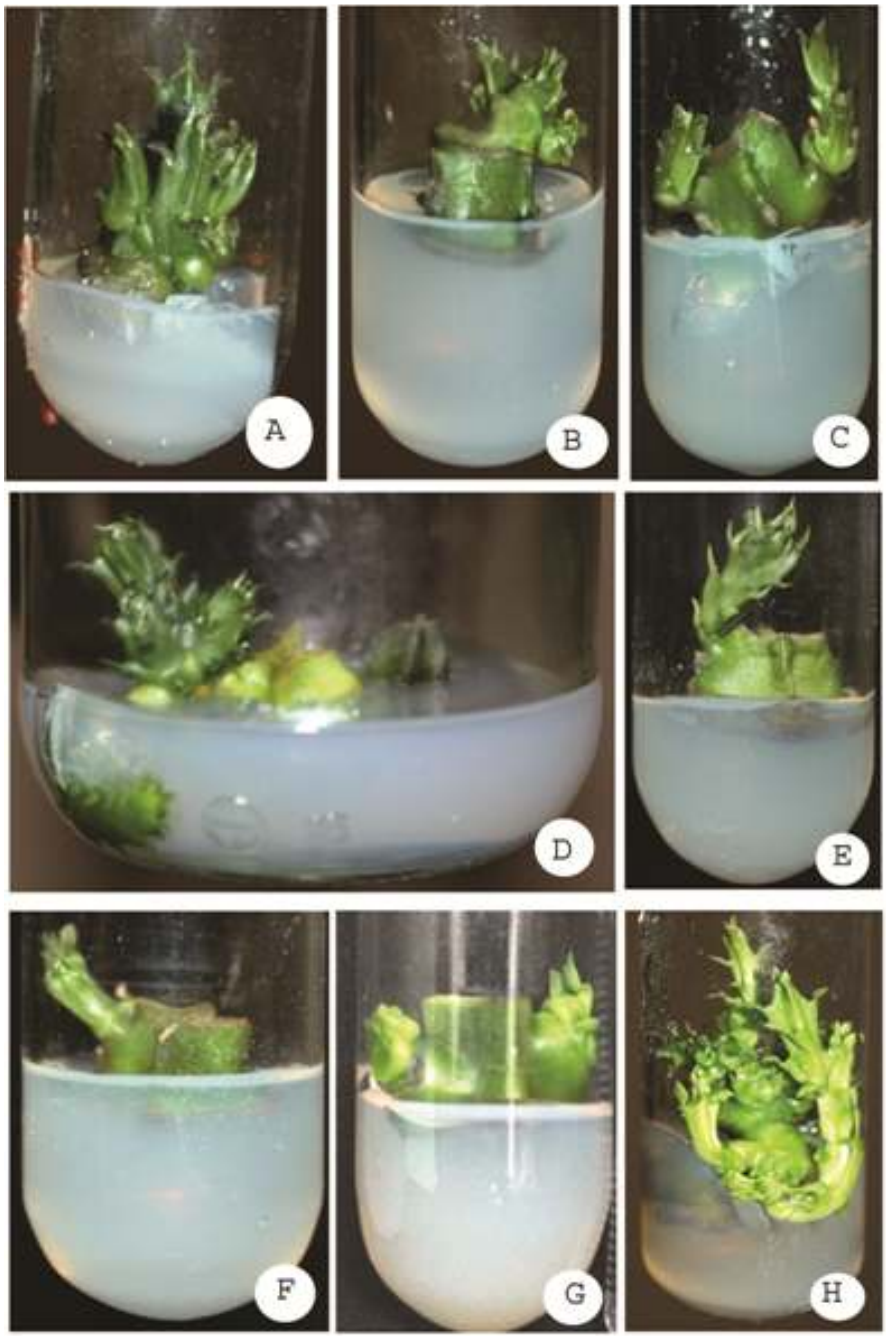

Fig. 2. A- MS medium + BAP 2 mg/l+2iP 1 mg/l (76% sprouting per explant and 2.3 number of shoots per explant), B- MS medium + BAP 2 mg/l+ Kin 2 mg/l (74% of sprouting per explant and 2.9 number of shoots per explant), C- MS medium + BAP 2 mg/l +TDZ 3mg/l (56% of sprouting per explant and 2.6 number of shoots per explant), D- MS medium + BAP 2 mg/l+ Kin 3 mg/l (59% of sprouting per explant and 2.1 number of shoots per explant), E- MS medium + BAP 2 mg/l+2iP 5 mg/l (42% of sprouting per explant and 1.0 number of shoots per explant), F- MS medium + BAP 2 mg/l +TDZ 5 mg/l (49% of sprouting per explant and 2.6 number of shoots per explant), G- MS medium + BAP 2 mg/l+2iP 3 mg/l (48% of sprouting per explant and 1.9 number of shoots per explant), H- MS medium + BAP 2 mg/l +TDZ 1 mg/l (86% of sprouting per explant and 3.9 number of shoots per explant)

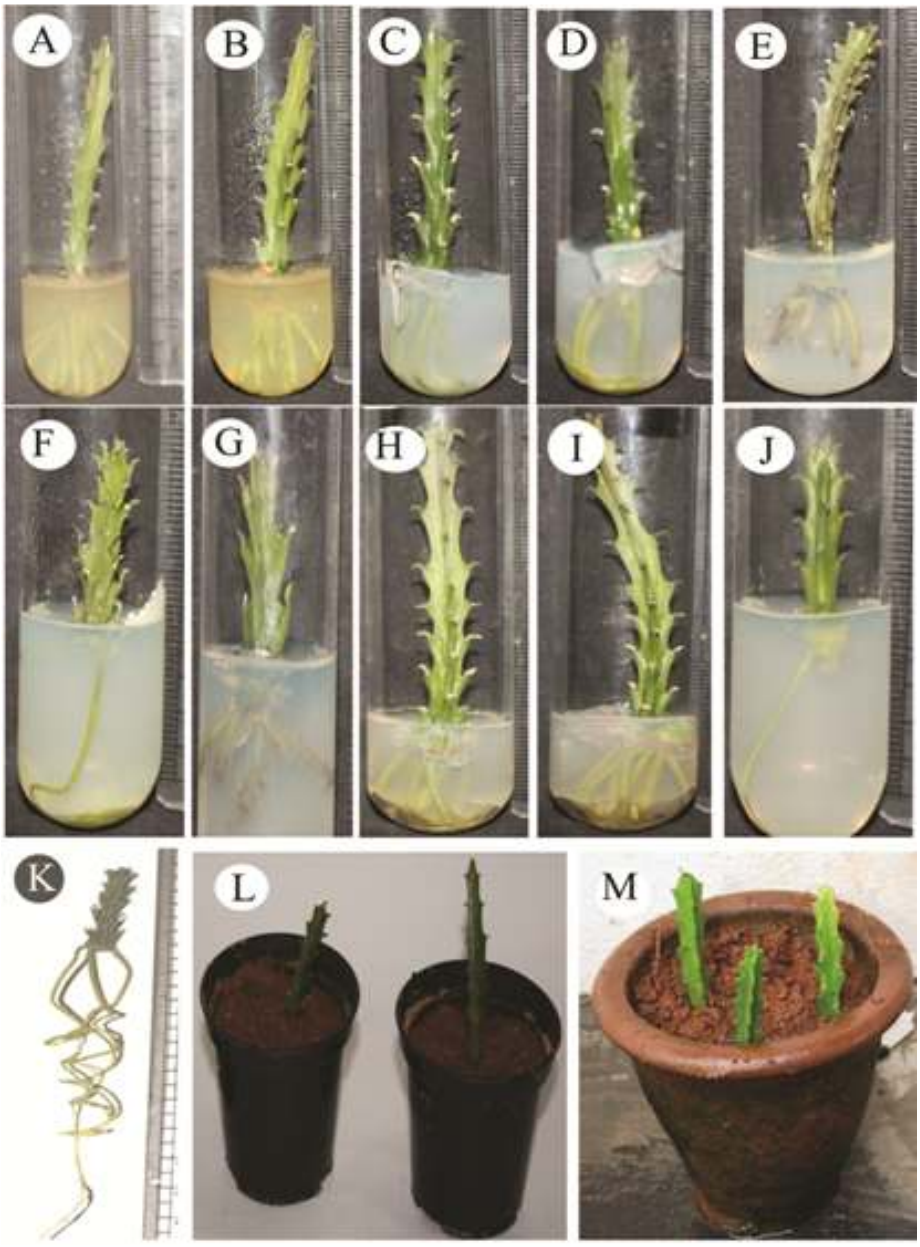

Fig. 3. A- MS medium + NAA (0.25 mg/l) (60% of sprouting per explant and 6.4 number of roots per explant), B- MS medium + NAA (1.0 mg/l) (38% of sprouting per explant and 2.6 number of roots per explant), C- MS medium + IAA (0.25 mg/l) (38% of sprouting per explant and 1.4 number of roots per explant), D- MS medium + IBA (1.0 mg/l) (20% of sprouting per explant and 1.7 number of roots per explant), E- MS medium + NAA (0.5 mg/l) (49% of sprouting per explant and 4.9 number of roots per explant), F- MS medium + IBA (0.1 mg/l) (36% of sprouting per explant and 2.6 number of roots per explant), G- ½ MS medium + NAA (0.1 mg/l) (78% of sprouting per explant and 8.9 number of roots per explant), H- ½ MS medium + NAA (0.25 mg/l) (76% of sprouting per explant and 6.4 number of roots per explant), I- ½ MS medium + NAA (0.5 mg/l) (64% of sprouting per explant and 4.7 number of shoots per explant), J - ½ MS +IAA (0.5 mg/l) (52% of sprouting per explant and 1.7 number of roots per explant), K- ½MS medium + IBA (0.25 mg/l) (57% of sprouting per explant and 2.2 number of roots per explant), L- Plantlet during hardening and M- Plantlet transferred to earthen pot

4. CONCLUSION

Murashige and Skoog (1962) medium supplemented with BAP (2 mg/l) and TDZ (1mg/l) in solid medium under 16-h photoperiod with the best conditions for induction of multiple shots. Rooting was best achieved on ½ MS medium supplemented with Naphthalene Acetic Acid (NAA) (0.1 mg/l).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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