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Plant Regeneration from Alginate Encapsulated Shoot Tips of *Boucerosia umbellata* (Haw.) Wight & Arn. (Syn.: *Caralluma umbellata* Haw.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author TP designed the study. Author BS carried out the experimental work and wrote the first draft of the manuscript. Authors SSR and TP managed literature searches and made final corrections to the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The shoot apices of multiple shoots of *Boucerosia umbellata* under *in vitro* conditions were selected for encapsulation studies. Gel matrix Sodium Alginate at different concentrations was exposed to complexing agent Calcium Chloride for different durations. The seeds thus produced were stored at 4℃ under aseptic conditions to study their viability and were inoculated on nutrient medium to study their morphogenetic response. The germination percentage decreased after storage of these synthetic seeds. Immediate germination gave a germination percentage of 98 and gradually reduced during storage. After one month of storage, the germination percentage decreased to 74%. After 6 months of storage at 4℃ the germination was as low as 36%.

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Keywords: Boucerosia umbellata; Caralluma umbellata; encapsulation; synthetic seeds.

1. INTRODUCTION

The genus *Caralluma* has been included in the family Apocynaceae (sub family Asclepiadoideae) in APG III classification. The plants of species of *Caralluma* found in India are edible. The medicinal properties of *Caralluma* includes anti-inflammatory, anti-ulcer, anti-diabetic, carminative, febrifugal, anti-pyretic and anti-oxidant effects, besides a number of phytochemical compounds were isolated from the genus *Caralluma* [1]. *Caralluma* extracts have also been found to be appetite suppressant, a property well known to Indian tribals and hunters [1]. Indian folklore records its use as a potent appetite suppressant and weight loss promoter [1]. Some *Caralluma* species are used in the treatment of obesity [1]. 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*, which was earlier included in the genus *Caralluma*, is now separated from the genus *Caralluma* (*sensu stricto*) mainly by the pseudoumbellate terminal cymes (in *Boucerosia*) [3].

Boucerosia umbellata (Haw.) Wight & Arn. (Syn: *Caralluma umbellata* Haw.) is a succulent leafless herb, 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 in stomach disorders and abdominal pains [5], for the treatment of kidney stones by the ethnic practitioners of Nalgonda district [6]. According to Neelima et al. [7], external applications of latex of *Caralluma umbellata* heals mouth ulcers. Ramesh et al. [8] reported antinociceptive and anti-inflammatory activity of carumbelloside-I isolated from *Caralluma umbellata*. The literature survey indicates pregnane glycosides were isolated from various parts of the plant [1]. Bellamkondi et al. [9] reported antihyperglycemic activity of *Caralluma umbellata.*

The encapsulated vegetative propagules could be used for mass clonal propagation of high economically important plants when somatic embryos are not available for encapsulation [10-13]. Synthetic seed technology studies have several advantages such as ease of handling and exchange of plant material, genetic uniformity of plantlets, delivery to the soil, shortening the breeding cycle and reduction of storage space. During the last four decades, synthetic seed technology has gained considerable importance in plant biotechnology as a potential viable and valuable system for *ex situ* conservation of commercially important plants [14]. Available reports indicate that there is no work on encapsulation in any species of the genus *Caralluma* and hence the present investigation has been undertaken.

We reported earlier enhanced axillary proliferation of *Boucerosia umbellata* from nodal explants; on MS medium supplemented with BAP (2 mg/l) and TDZ (1 mg/l) gave maximum response [15]. We report here encapsulation of shoot tips for development of synthetic seeds in *Boucerosia umbellata.*

2. MATERIALS AND METHODS

The plants of *B. umbellata* were collected from Penukonda hills, Anantapur district and maintained in earthen pots. Young sprouts were collected from 3-month old garden plants for *In vitro* propagation to obtain multiple shoots. The possibility of using aseptic propagules like shoot apices obtained during direct shoot organogenesis for synthetic seed production was undertaken. Shoot tips measuring 0.5-1 cm were excised from proliferated *In vitro* multiple shoots (Fig. 1 A). The following variables were tested to study encapsulation (firm transparent bead formation).

1. Concentration of hydrogel Sodium Alginate (2%, 3%, 4% and 5%)
2. Concentration of complexing agent Calcium Chloride (25 mM, 50 mM, 75 mM and 100 mM)
3. Duration of exposure to Calcium Chloride (5 min, 10 min, 20 min and 30 min).

The excised aseptic propagules such as shoot apices were mixed in MS basal medium containing Sodium Alginate under aseptic conditions. The Sodium Alginate coated buds were individually dropped into solution of $CaCl_2$ one by one with the help of pipette or glass tube. This resulted in the formation of Calcium alginate encapsulated beads. The encapsulated beads thus prepared were washed with sterile distilled water and stored at 4℃ in sterilized half strength MS basal medium. The synthetic seeds were

germinated by inoculating them on MS + Benzylaminopurine (BAP) (2 mg/l) + Naphthalene Acetic Acid (NAA) (2 mg/l) at different storage time intervals to test their viability.

All the experiments were conducted with a minimum of 10 replicates per treatment. The experiments were repeated three times. Standard error of means was calculated in each experiment. The data was subjected to statistical analysis using one-way analysis of variance (ANOVA) and means compared using the DMR test at 0.05% level of significance.

3. RESULTS AND DISCUSSION

Gel matrix Sodium Alginate at different concentrations was exposed to complexing agent Calcium Chloride for different durations. Propagules in 3% Sodium Alginate exposed to 50 mM $CaCl_2$ for duration of 20 minutes produced firm transparent beads (Table 1, Fig. 1 B & C). Lower concentrations of sodium alginate and calcium chloride (2% and 25 mM respectively) resulted in very soft beads irrespective of the duration of exposure. The very soft beads were very difficult to handle. Increase in the concentrations of sodium alginate and calcium chloride resulted in hard beads and these were unfavourable for the germination purpose even though they were comfortable to handle. These beads were collected in sterilized tea strainer and rinsed several times in sterile water to remove traces of calcium chloride. The seeds thus produced were stored at 4℃ under aseptic conditions to study their viability and inoculated on nutrient medium to study their morphogenetic response. They were germinated on MS+BAP (2 mg/l) at different time intervals. On germination, the encapsulated shoot apex started proliferating by increasing its axis. The apex as it grew, slit open the gel matrix of the Alginate and emerged to the exterior (Fig. 1 D). Later on the shoot apex produced multiple shoots more profusely than *in vivo* field grown explants (Fig. 1E & F). The germination percentage decreased on storage of these synthetic seeds (Table 2). Immediate germination gave a germination percentage of 98 and gradually reduced during storage. After one month of storage, the germination percentage decreased to 74%. After 6 months of storage at 4℃ the germination was as low as 36%.

Table 1. Effect of different concentrations of sodium alginate and the duration of exposure to different concentrations of calcium chloride for synthetic seed formation of shoot apex explant of *Boucerosia umbellata* (Haw.) Wight & Arnott

	Concentration of sodium alginate %	Concentration of calcium chloride mM	Duration in minutes	No of seeds produced	Quality of seeds
1	2.0	25	5	20	Very soft beads
2	2.0	25	10	20	Very soft beads
3	2.0	25	20	20	Very soft beads
4	2.0	25	30	20	Very soft beads
5	2.0	50	5	20	Very soft beads
6	2.0	50	10	20	Soft beads
7	2.0	50	20	20	Soft beads
8	2.0	50	30	20	Soft beads
9	2.0	75	5	20	Very soft beads
10	2.0	75	10	20	Soft beads
11	2.0	75	20	20	Soft beads
12	2.0	75	30	20	Soft beads
13	2.0	100	5	20	Very soft beads
14	2.0	100	10	20	Soft delicate beads
15	2.0	100	20	20	Soft beads
16	2.0	100	30	20	Soft beads
17	3.0	25	5	20	Very soft beads
18	3.0	25	10	20	Soft beads
19	3.0	25	20	20	Soft beads
20	3.0	25	30	20	Soft beads
21	3.0	50	5	20	Soft beads
22	3.0	50	10	20	Firm delicate beads
23	3.0	50	20	20	Firm transparent beads

	Concentration of sodium alginate %	Concentration of calcium chloride mM	Duration in minutes	No of seeds produced	Quality of seeds
24	3.0	50	30	20	Hard beads
25	3.0	75	5	20	Slightly hard beads
26	3.0	75	10	20	Hard beads
27	3.0	75	20	20	Hard beads
28	3.0	75	30	20	Hard beads
29	3.0	100	5	20	Slightly hard beads
30	3.0	100	10	20	Hard beads
31	3.0	100	20	20	Hard beads
32	3.0	100	30	20	Hard beads
33	4.0	25	5	20	Slightly hard beads
34	4.0	25	10	20	Hard beads
35	4.0	25	20	20	Hard beads
36	4.0	25	30	20	Hard beads
37	4.0	50	5	20	Slightly hard beads
38	4.0	50	10	20	Hard beads
39	4.0	50	20	20	Hard beads
40	4.0	50	30	20	Very hard beads
41	4.0	75	5	20	Hard beads
42	4.0	75	10	20	Hard beads
43	4.0	75	20	20	Hard beads
44	4.0	75	30	20	Hard beads
45	4.0	100	5	20	Hard beads
46	4.0	100	10	20	Hard beads
47	4.0	100	20	20	Hard beads
48	4.0	100	30	20	Very hard beads
49	5.0	25	5	20	Hard beads
50	5.0	25	10	20	Very hard beads
51	5.0	25	20	20	Very hard beads
52	5.0	25	30	20	Very hard beads
53	5.0	50	5	20	Hard beads
54	5.0	50	10	20	Very hard beads
55	5.0	50	20	20	Very hard beads
56	5.0	50	30	20	Very hard beads
57	5.0	75	5	20	Very hard beads
58	5.0	75	10	20	Very hard beads
59	5.0	75	20	20	Very hard beads
60	5.0	75	30	20	Very hard beads
61	5.0	100	5	20	Very hard beads
62	5.0	100	10	20	Very hard beads
63	5.0	100	20	20	Very hard beads
64	5.0	100	30	20	Very hard beads
1	2.0	25	5	20	Very soft beads

Table 2. Percentage germination response for encapsulated aseptic shoot apex explant of *Boucerosia umbellata* on MS+BAP (2 mg/l)+NAA (2 mg/l)

Storage duration (months)	No of beads Inoculated	No of beads Responded (germinated)	Germination response (days)	Germination percentage	Mean number of shoots produced ± SE
0	50	49	5	98	4.2±0.18
0.25	50	47	5	94	4.8±0.23
0.50	50	43	6	86	4.6±0.02
1	50	37	7	74	4.0±0.11
2	50	29	10	58	3.7±0.08
4	50	21	12	42	3.4±0.27
6	50	18	18	36	3.4±0.12

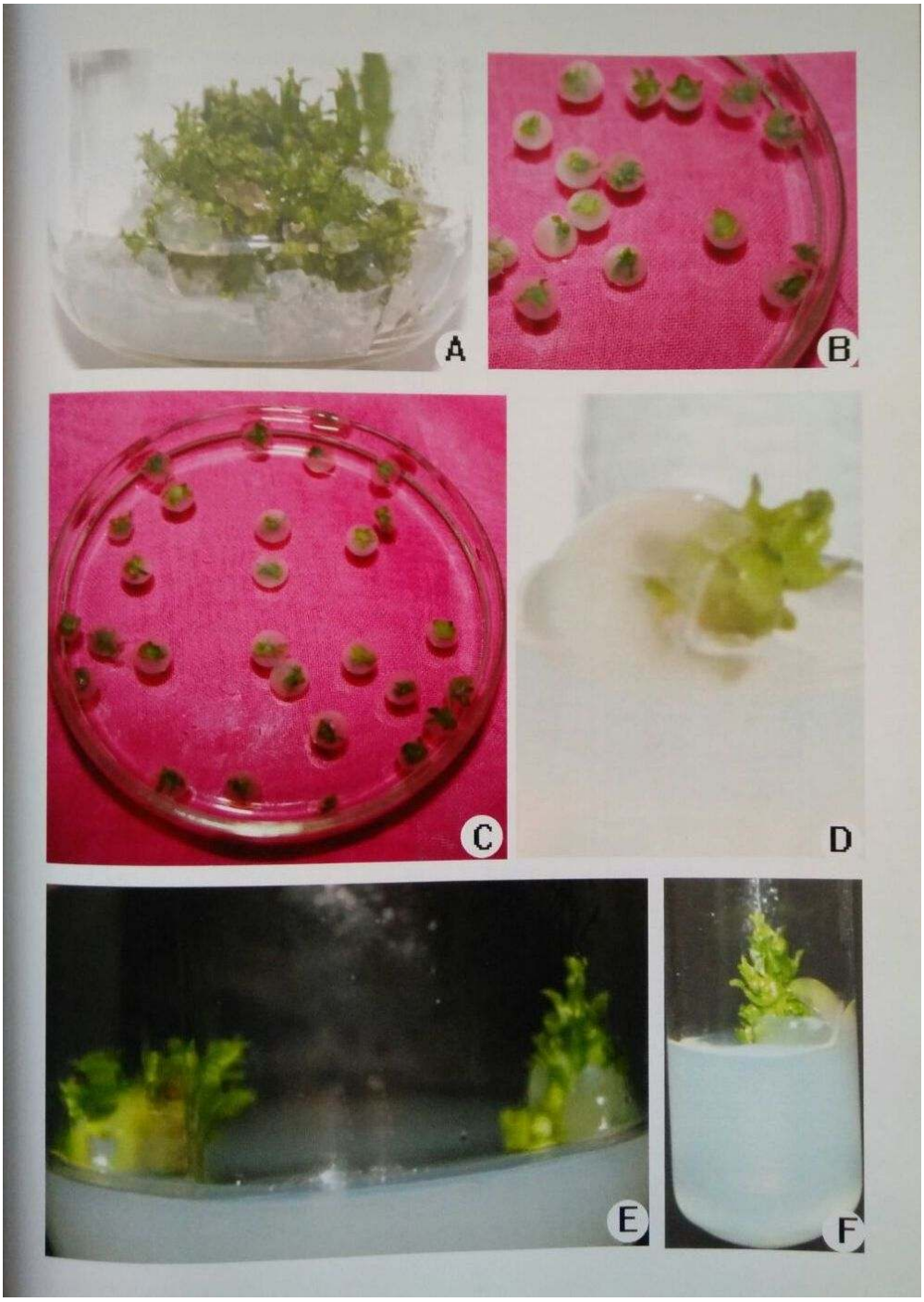

Fig. 1. Encapsulation of shoot apical buds in *Boucerosia umbellata*

A. In vitro grown multiple shoots on MS + BAP (2 mg/l) subculture. B, C. Encapsulated shoot tips in sodium alginate. D. Germinating synthetic seed on MS + BAP (2 mg/l) + NAA (2 mg/l). E. 60 days old culture of synthetic seeds on MS + BAP (3 mg/l) + NAA (2 mg/l)

In some species the unipolar axillary shoot buds and/ or apical shoot tips have been encapsulated to produce synthetic seeds. *In vitro* shoot apex was also very commonly utilized to produce synthetic seeds through encapsulation. Ganapathi *and collaborates* [12] produced

synthetic seeds of Banana while Nyende et al. [16] encapsulated the shoot apices of four cultivars of Potato. Encapsulation of shoot apices was earlier reported in *Actinidia deliciosa* [17], *Phyllanthus amarus* [18], *Rhododendron* [19], *Rauvolfia serpentina* [20], *Trichosanthes dioica* [21], *Solanum nigrum* [22], *Salvia officinalis* [23], *Brassica oleracea* var. *botrytis* [24] and *Oxalis triangularis* [25].

In *Salvia officinalis* Izabela and Halina [23] reported that an encapsulation was better with 3% Sodium alginate and 50 mM $CaCl_2$ H_2O was ideal for the formation of microshoot beads compared with other concentrations while Daud et al. [26] and Bukhari et al. [27] used 3% Sodium alginate with 100 mM $CaCl_2$ H_2O and Siong et al. [24] used 4% Sodium alginate with 100 mM $CaCl_2$ H_2O to obtain satisfactory synthetic seeds. In our present study 3% Sodium Alginate exposed to 50 mM $CaCl_2$ for 20 minutes produced firm transparent beads.

Decrease in conversion response during storage could be attributed to inhibition of tissue respiration by the alginate matrix, or a loss of moisture due to partial desiccation during storage as reported earlier by Danso and Llyod [28], Faisal et al. [29], and Faisal and Anis [30].

4. CONCLUSION

Three percent of Sodium alginate with 50 mM $CaCl_2$ for 20 minutes was optimum for producing firm transparent beads. These synthetic seeds can be stored for one month with 74% of germination. The present study describes a simple, reproducible and efficient protocol for synthetic seed production. This may facilitate cold storage of encapsulated shoot tips, offers possibility for germplasm conservation and exchange between laboratories.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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