RUHUNA JOURNAL OF SCIENCE
Vol. 5: 16-30, 2014
http://rjs.ruh.ac.lk/
ISSN: 1800-279X

In vitro regeneration of *Hypochaeris radicata* L. from sodium alginate-encapsulated synthetic seeds

Jamuna Senguttuvan and Paulsamy Subramaniam*

Department of Botany, Kongunadu Arts and Science College, Coimbatore-641 029, Tamil Nadu, India

*Correspondence: paulsami@yahoo.com

Received: 01 May 2014, Revised version accepted: 23 October 2014

Abstract. Synthetic seeds were produced from *in vitro* derived leaf, root and callus explants of *Hypochaeris radicata* (hairy cat's-ear) by encapsulating different concentrations of sodium alginate hydrogel (1-6%) containing MS medium. The texture, conversion frequency and the effect of temperature on shoot emergence were evaluated. Among three explants attempted, *in vitro* derived leaf segments encapsulated beads stored at 25°C observed successful shoot regeneration above 80% in the standardized MS medium supplemented with 2mg/L BAP. The regenerated shoots were rooted well (73.54%) on MS medium with 1mg/L NAA. The high frequency of plant re-growth was found in four month old leaf segment encapsulated beads. Generally, four months stored beads of all explants at 25°C found to have higher regeneration rate in comparison with 2 and 6 months old encapsulated beads.

Keywords. Asteraceae, *Hypochaeris radicata*, *in vitro* regeneration, synthetic seeds

1 Introduction

Synthetic seeds are artificially encapsulated plant propagules (somatic embryos, shoot buds, cell aggregates or any other tissues) that can be used for sowing as a seed and possess the ability to convert into a plant under *in vitro* or *ex vitro* conditions, and also retain this potential after storage (Krishna Kumar and Dennis Thomas 2012). Plant propagules are encased in protective coating of gelling agents such as alginate, agar, carrageenan, gellan gum (gerlite), sodium pectate, ethylene glycol, dimethyl sulfoxide and carboxyl

methyl cellulose (Harikrishna and Ong 2002). The coating protects the explants from mechanical damage during handling and allows germination and conversion to occur without inducing undesirable variations. It's potential advantages include stability during handling, potential for long term storage without losing viability, ability to transport and plant directly from *in vitro* to field conditions and low cost production at higher scale (Ghosh and Sen 1994). Synthetic seeds have diverse applications such as: multiplication of non-seed producing plants, ornamental hybrids or polypoid plants, propagation of male or female sterile plants for hybrid seed production, germplasm conservation of recalcitrant species and multiplication of transgenic plants. The concept of artificial seed technology has been applied successfully in commercial settings in cardamom (Ganapathy *et al*. 1994), sugar beet (Dennis *et al*. 1991), sandal wood (Bapat and Rao 1998), banana (Ganapathi *et al*. 2002), garlic (Bekheet 2006), rice (Roy and Mandal 2008), neem (Mithilesh and Rakhi 2013), sweet neem (Aman and Shruti 2013) and also in some Orchidaceae members (Sharma *et al*., 1992; Anju *et al*. 2012).

Hypochaeris radicata commonly called as hairy cat's-ear, is an edible, perennial herb belongs to the family Asteraceae. It is native to South Africa and distributed in forest margins of Nilgirs, the Western Ghats, Tamil Nadu at 2000m above msl. It possesses several medicinal properties like antiinflammatory, anticancer, antioxidant (Jamuna *et al*. 2012, 2014) and antimicrobial (Jamuna *et al*. 2013a; Jamuna *et al*. 2013b). It is being prescribed for the treatment of jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney related problems in traditional medicinal practices of Tamil Nadu, India (Pullaiah 2006). It is used for some medicinal purposes like controlling wound infections in Meghalaya (Tynsong *et al*. 2006) and also used as food for ruminants in British Columbia (Lans *et al*. 2007). Further, *H. radicata* is reported to have many bioactive compounds of medicinal importance like phytol, acetate, hexadecanoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, urs-12-en-3-ol, acetate, (3 beta, 1-benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-(3-oxo-1-butenyl) perhydro-methyl ester (Jamuna and Paulsamy 2013). The leaves are usually blanched, steamed and cooked or used as spices in a range of Western and Eastern culinary preparations and bevarages (www.ifood.tv/network/catsear).

As the seed longevity is poor under natural conditions, the germination and hence the population sizes are affected drastically in the upper reaches of Nilgiris (Paulsamy *et al*. 2008). To overcome this problem, the production of synthetic seeds and sowing them during appropriate period is essential. In

light of this fact, the present study was aimed at determining the optimum concentration of encapsulation matrix (sodium alginate solution) to optimize the texture, storage period and temperature of the alginate beads for effective bud-sprouting and also to assess the germination efficiency of the seeds in terms of multiple shoot formation, rooting and hardening attributes to ascertain the feasibility of their use as an alternative to true seeds by standardizing the Murashige and Skoog (MS) medium.

2 Materials and methods

2.1 Plant materials

Leaf, root and callus (leaf-derived) explants from *in vitro* derived plants of *H. radicata* were used. All these explants were cut into 2 to 3 mm size for further use.

2.2 Preparation of encapsulation matrix

For encapsulation process, various concentrations of sodium alginate at 1, 2, 3, 4, 5 and 6% (w/v) were prepared in 100mL of MS liquid medium without agar. For complexation (an ion exchange reaction between Na^+ and Ca^{2+} resulting in the formation of insoluble calcium alginate), 1.016g of calcium chloride ($CaCl_2.2H_2O$) in 150mL in distilled water was prepared. Both solutions were sterilized at 120°C for 15 min at 1.12kg/cm^2 pressure and allowed to cool.

2.3 Formation of beads

For the formation of beads, the *in vitro* derived organs *viz.,* leaf, root and leaf-derived callus explants, each numbering 50 were aseptically transferred into each concentration of sodium alginate solution and incubated at room temperature for 15-20min. Later, the micropropagules in alginate solution were picked up by Pasteur pipette and dropped into a sterile solution of $CaCl_2.2H_2O$. The drops, each containing a single micropropagule when left in $CaCl_2.2H_2O$ for 30 min was placed on shaker to form round-shaped firm beads as a result of the ion exchange reaction between on Na^+ and Ca^{2+} ions

(Louis *et al*. 1998). The beads were recovered by recycling and decanting $CaCl_2.2H_2O$ solution and washed thrice with autoclaved MS liquid medium. The beads were then transferred to sterile filter paper in petridishes. Blot dried beads were stored in culture bottles sealed with parafilm for 2, 4 and 6 months in two different temperature conditions *viz.,* at 4ºC and 25°C separately.

2.4 Culture medium and condition

The encapsulated explants were cultured on the MS medium (Murashige and Skoog 1962) consists of MS mineral salts and vitamins supplemented with 3% sucrose and 0.8% agar. The pH of the medium was adjusted to 5.6 to 5.8 before sterilization by autoclaving at 121°C with 1.12kg/cm^2 pressure for 15 min. All cultures were maintained under white fluorescent light having 2000 lux light intensity. The incubation temperature was 25±2°C with 16 hours light and 8 hours dark period in every 24 hours cycle.

2.5 Induction of organogenesis

The encapsulated beads were implanted on MS medium containing different growth regulators *viz.,* BAP (0.5-3.0mg/L) alone and it in individual combination with GA_3 (0.5mg/L), Kn (0.5mg/L) and IAA (0.5mg/L) for shoot formation. For root induction, the micro-shoots (2-3cm) were excised and sub-cultured onto the MS medium supplemented with various concentrations of growth regulators *viz.,* IAA, IBA and NAA (0.3, 0.5 and 1.0mg/L respectively).

2.6 Hardening and acclimatization

Well-developed healthy plantlets were removed from the culture flasks and were thoroughly washed in running tap water to remove remnant nutrient medium completely without causing any damage to roots. Then the root portion was soaked in 1% (w/v) fungicide, methyl-2 benzimidizole carbamate (Bavistin) solution for 10min and transferred to small earthen pots of 15×15cm (H×W) size each filled with various types of sterilized potting mixtures garden soil: sand: vermicompost (1:1:1 by volume), red soil: sand: vermicompost (1:1:1 by volume), vermicompost: soil (1:1 by volume), red soil: sand (1:1 by volume) and decomposed coir waste: perlite: vermicompost (1:1:1 by volume).

2.7 Statistical analysis

For encapsulation of synthetic seeds and *in vitro* regeneration, randomly selected 30 individuals of each explant and for this purpose in triplicate were maintained. The data on conversion frequency (after 15 days of incubation of encapsulated beads), shooting frequency (after 25 days of incubation of encapsulated beads), multiple shoot induction (after 25 days of incubation of encapsulated beads), root induction (after 30 days of incubation of *in vitro* derived shoots) and number of plantlets survived (after 60 days of hardening) were statistically analyzed using ANOVA, and means were compared by using Duncan's Multiple Range Test (P<0.05) (Duncan 1955).

3 Results

Table 1. Effect of sodium alginate concentration on texture and conversion frequency of leaf, root and callus explants encapsulated beads of *Hypochaeris radicata* in the standardized MS medium supplemented with 2mg/L BAP.

Explants	Concentration of sodium alginate (%)	Texture of beads	Conversion frequency (%)*
In vitro leaf	1	Too soft	28.49 ± 0.31^{ab}
	2	Soft	41.49 ± 0.31^{c}
	3	Soft	86.64 ± 1.04^{g}
	4	Firm	71.12 ± 0.85^{f}
	5	Hard	47.11 ± 0.02^{c}
	6	Very hard	38.71 ± 0.65^{bc}
In vitro root	1	Too soft	21.94 ± 0.80^{a}
	2	Soft	38.94 ± 0.80^{b}
	3	Soft	42.37 ± 0.40^{c}
	4	Firm	51.33 ± 0.89^{d}
	5	Hard	46.43 ± 0.85^{c}
	6	Very hard	31.00 ± 0.29^{b}
In vitro callus	1	Too soft	43.24 ± 0.28^{c}
	2	Too soft	43.24 ± 0.28^{c}
	3	Soft	63.12 ± 0.79^{e}
	4	Firm	55.10 ± 0.42^{d}
	5	Hard	48.37 ± 0.39^{cd}
	6	Very hard	30.30 ± 0.02^{b}

*Mean of three replicates of 30 explants ± standard deviation.
Values within the column followed by same superscript are not significantly different at P<0.05.

3.1 Effect of different concentrations of sodium alginate on conversion frequency

The encapsulated beads differed morphologically with respect to texture with different concentrations of sodium alginate (1-6%) (Table 1). An encapsulation matrix of 3% sodium alginate with 100mM of $CaCl_2.2H_2O$ was found to be the most suitable for the formation of ideal beads (Figs. 1A and 2A). Among the three explants attempted, the highest conversion frequency (86.64%) was observed in leaf explants encapsulated beads followed by the callus encapsulated beads with 63.12% conversion frequency. Based on these findings, leaf and callus explant encapsulated beads were taken for further sub-culturing experiments.

Table 2. Effect of storage period and temperature on shoot emergence from different explant encapsulated beads of *Hypochaeris radicata* in the standardized MS medium supplemented with 2mg/L BAP.

Beads with explants	Storage period (months)	Temperature (°C)	Shooting frequency (%)*
Encapsulated leaf	2	4	32.23 ± 0.21^{c}
		25	59.71 ± 0.04^{e}
	4	4	61.54 ± 0.33^{f}
		25	82.52 ± 1.06^{g}
	6	4	43.21 ± 0.26^{d}
		25	67.28 ± 0.71^{f}
Encapsulated root	2	4	21.34 ± 0.05^{b}
		25	47.28 ± 0.23^{e}
	4	4	10.33 ± 0.52^{a}
		25	32.94 ± 0.43^{c}
	6	4	11.33 ± 0.29^{a}
		25	29.00 ± 0.05^{b}
Encapsulated callus	2	4	25.23 ± 0.18^{b}
		25	39.46 ± 0.48^{c}
	4	4	32.66 ± 0.29^{c}
		25	51.10 ± 0.31^{e}
	6	4	18.20 ± 0.22^{a}
		25	29.31 ± 0.56^{b}

*Mean of three replicates of 30 explants ± standard deviation.
Values within the column followed by same superscript are not significantly different at $P<0.05$.

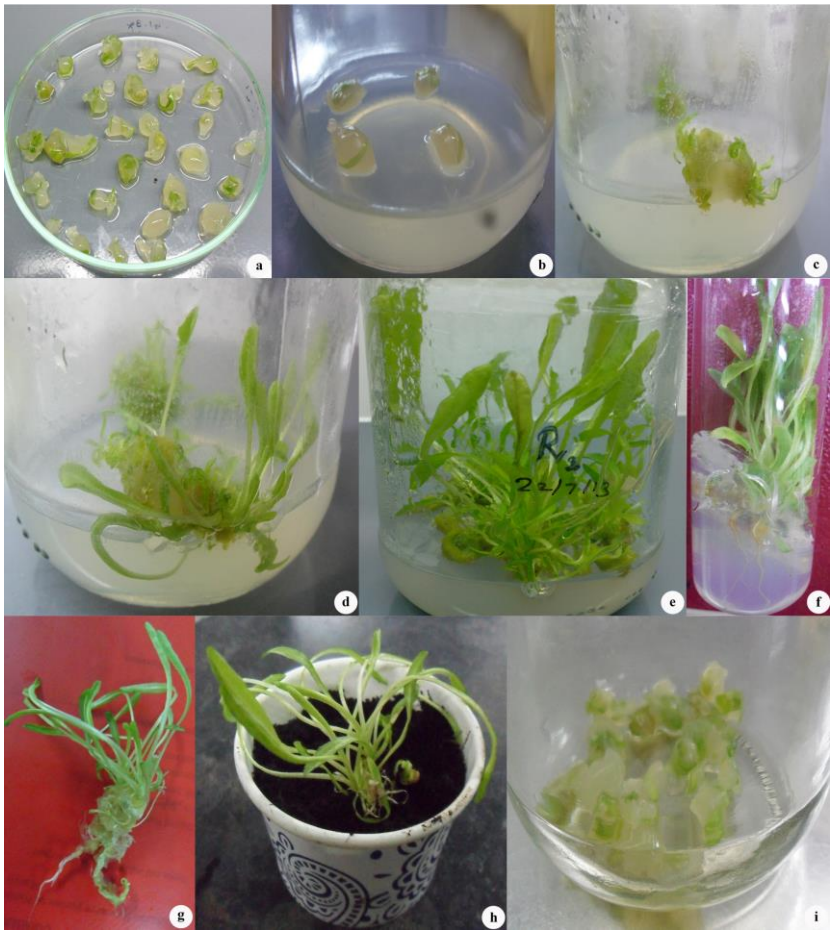

Fig. 1. Stages of regeneration in encapsulated *in vitro* leaf segments of *Hypochaeris radicata*. (A) beads encapsulated with leaf segments in 3% sodium alginate, (B) induction of synthetic seeds on MS medium with BAP at 2mg/L, (C) ruptured beads showing sprouting of shoots on MS medium containing BAP at 2mg/L (D) after 2 weeks of culture (E) well defined multiple shoot emergence on MS medium with BAP at 2mg/L (F) root induction on MS medium fortified with NAA at 1mg/L (G) after 2 weeks of culture (H) regenerated plantlet of *H. radicata* after successful acclimatization (I) storage of synthetic seeds in a culture bottle.

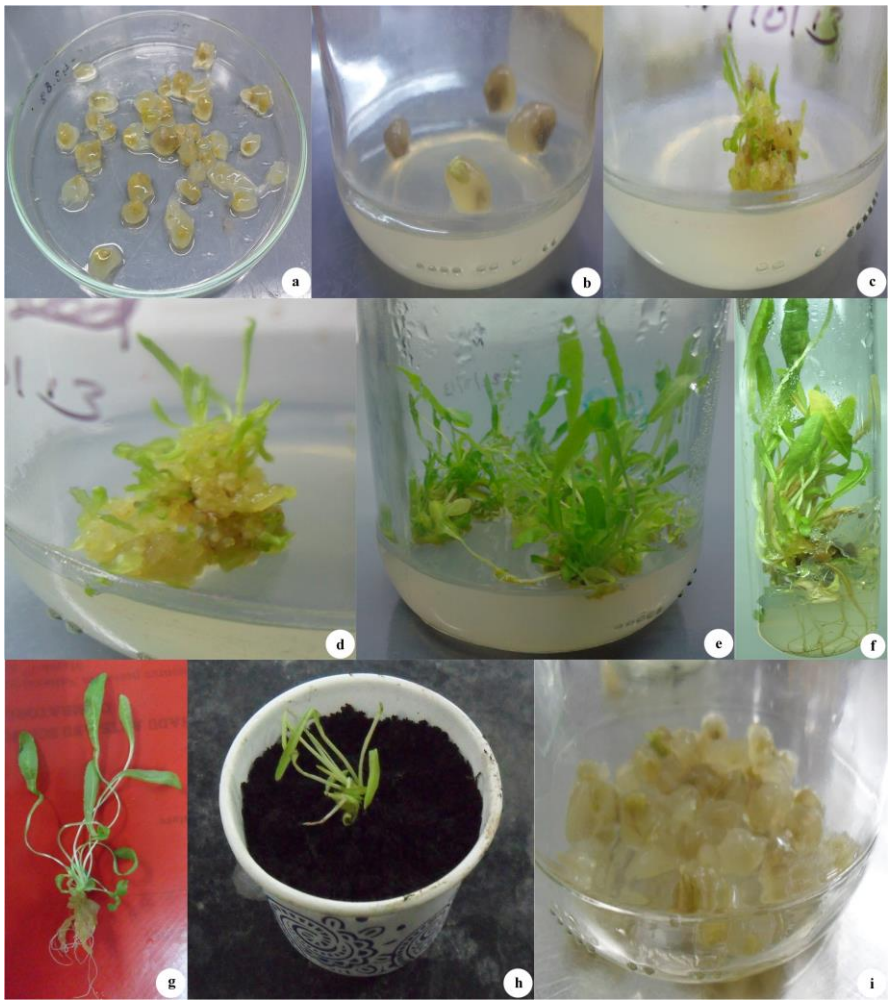

Fig. 2. Stages of regeneration in *in vitro* callus segments of *H. radicata*. (A) synthetic seeds produced by 3% sodium alginate, (B) induction of synthetic seeds on MS medium with BAP at 2mg/L, (C) ruptured beads showing sprouting of shoots on MS medium containing BAP at 2mg/L, (D) after 2 weeks of culture (E), multiple shoots regenerated from an encapsulated callus beads on MS medium containing BAP at 2mg/L, (F) root induction on MS medium fortified with NAA at 1mg/L, (G) after 2 weeks of culture, (H) acclimatized transformed plant, 8 weeks after transferred to soil, (I) storage of synthetic seeds in a culture bottle.

3.2 Effect of storage period and temperature on shoot emergence from encapsulated beads

Table 2 shows the shooting frequency of encapsulated *in vitro* derived leaf, root and callus segments, after 2, 4 and 6 months of storage period at 4 and 25°C. Four months old stored beads at 25°C resulted in high rate of shoot proliferation in all explant segments. Longer storage of 6 months period has significantly decreased the regeneration ability of explants. Higher shooting frequency was noticed in four months stored leaf explant encapsulated beads (82.52%) followed by callus encapsulated beads (51.10%). The root explants encapsulated beads when stored for two months at 25°C produced shoots with lower conversion frequency (47.28%) in comparison to leaf and callus encapsulated beads.

3.3 Effect of various growth regulators on bud sprouting and regeneration of encapsulated beads

Data on certain shooting attributes of 3% sodium alginate encapsulated beads cultured on MS medium with various combinations and concentrations of some plant growth regulators are given in Table 3. The shooting response varied according to growth regulators used in the medium. MS medium supplemented with 2mg/L BAP gave higher frequency (88.55%) (Fig. 1C) for the conversion of leaf encapsulated beads into multiple shoots with a higher number of shoots per explant (15.59shoots/encapsulated bead). When the medium was supplemented by BAP (2.0mg/L) in combination with GA_3 (0.5mg/L) the shoot emergence frequency was 78.54% with shoot length of 7.44cm. However, the shoot proliferation frequency from callus encapsulated beads was noted to be lower (63.87%) than leaf encapsulated beads (Fig. 2C).

3.4 Rooting and acclimatization of plantlets regenerated from encapsulated beads

The rooting characters of *in vitro* derived leaf and callus explants encapsulated synthetic seeds are presented in Table 4. The rooting frequency was significantly higher (73.54%) for leaf encapsulated beads in the MS medium fortified with 1mg/L NAA. At the same time, the number of roots produced (5.30 roos/shoot) and the average root length (6.37cm) were higher in this medium (Fig. 1F). The rooting attributes of *in vitro* derived callus explants ecncapsulated synthetic seeds were lower (59.40%) (Fig. 2F) than

that of leaf encapsulated beads. The *in vitro* regenerated plantlets were acclimatized successfully using garden soil, sand and vermicompost (1:1:1 by volume). The higher survivability rates were 70% for leaf encapsulated beads (Fig. 1H) and 60% for callus encapsulated beads (Fig. 1H) (Table 5).

Table 3. Effect of plant growth regulators on shoot multiplication, shoot number and shoot length of *in vitro* derived leaf, root and callus encapsulated beads of *Hypochaeris radicata* in 3% sodium alginate.

Growth regulators				Multiple shoot induction (%)*		No. of shoots/explant*		Shoot length (cm)*	
BAP	GA_3	Kn	IAA	Leaf	Callus	Leaf	Callus	Leaf	Callus
0.5	0.0	0.0	0.0	-	-	-	-	-	-
1.0	0.0	0.0	0.0	31.15±0.54^{c}	24.43±0.22^{b}	1.23±0.09^{b}	1.03±0.02^{b}	2.54±0.15^{b}	2.05±0.09^{b}
1.5	0.0	0.0	0.0	64.65±0.83^{f}	54.53±0.11^{f}	1.35±0.16^{b}	1.15±0.11^{b}	5.33±0.47^{de}	3.12±0.71^{c}
2.0	0.0	0.0	0.0	88.55±2.82^{h}	63.87±1.86^{g}	15.59±0.51^{g}	10.35±0.41^{e}	6.75±0.23^{e}	4.77±0.38^{d}
2.5	0.0	0.0	0.0	60.23±0.93^{f}	47.85±0.46^{ef}	3.52±0.19^{c}	2.85±0.34^{b}	2.70±0.11^{b}	1.96±0.09^{a}
3.0	0.0	0.0	0.0	32.00±0.51^{c}	33.96±0.46^{c}	1.32±0.55^{b}	1.02±0.09^{b}	1.60±0.06^{a}	1.26±0.76^{a}
0.5	0.5	0.0	0.0	-	-	-	-	-	-
1.0	0.5	0.0	0.0	-	-	-	-	-	-
1.5	0.5	0.0	0.0	34.54±0.62^{c}	31.12±0.54c	1.56±0.91^{b}	1.05±0.34^{b}	2.76±0.09^{b}	2.17±0.76^{b}
2.0	0.5	0.0	0.0	78.54±0.65^{g}	63.87±0.73g	7.11±0.51^{f}	5.10±0.75^{d}	7.44±0.19^{c}	6.44±0.21^{f}
2.5	0.5	0.0	0.0	51.10±0.16^{e}	42.65±0.32e	3.55±0.03^{b}	0.98±0.19^{a}	3.25±0.32^{e}	3.53±0.11^{c}
3.0	0.5	0.0	0.0	30.43±0.22^{bc}	20.53±0.09b	1.41±0.64^{b}	0.75±0.43^{a}	3.10±0.31^{c}	1.36±0.24^{a}
0.5	0.0	0.5	0.0	-	-	-	-	-	-
1.0	0.0	0.5	0.0	-	-	-	-	-	-
1.5	0.0	0.5	0.0	35.22±0.42^{c}	38.32±0.03^{d}	1.22±0.14^{b}	0.96±0.11^{a}	2.13±0.51^{b}	1.32±0.32^{a}
2.0	0.0	0.5	0.0	69.24±0.25^{f}	58.21±0.75^{f}	3.89±0.57^{e}	1.72±0.49^{b}	2.43±0.32^{b}	2.64±0.45^{b}
2.5	0.0	0.5	0.0	47.44±0.27^{d}	17.00±0.51^{b}	1.67±0.83^{b}	1.16±0.27^{b}	4.07±0.33^{d}	3.32±0.12^{c}
3.0	0.0	0.5	0.0	29.31±0.02^{b}	09.53±0.05^{a}	0.78±0.24^{a}	1.01±0.17^{b}	2.30±0.64^{b}	2.11±0.09^{b}
0.5	0.0	0.0	0.5	-	-	-	-	-	-
1.0	0.0	0.0	0.5	-	-	-	-	-	-
1.5	0.0	0.0	0.5	-	-	-	-	-	-
2.0	0.0	0.0	0.5	64.32±0.52^{f}	56.32±0.16^{f}	3.20±0.21^{d}	2.23±0.65^{c}	2.13±0.17^{b}	1.54±0.49^{a}
2.5	0.0	0.0	0.5	38.13±0.63^{c}	35.05±0.02^{cd}	2.74±0.53^{c}	1.52±0.38^{b}	3.98±0.52^{c}	3.12±0.32^{c}
3.0	0.0	0.0	0.5	15.54±0.33^{a}	10.01±0.02^{a}	1.02±0.42^{b}	1.65±0.47^{b}	2.42±0.26^{b}	2.00±0.19^{b}

*Mean of three replicates of 30 explants ± standard deviation.
Values within the column followed by same superscript are not significantly different at $P<0.05$.

4 Discussion

The encapsulation technique for producing synthetic seeds or artificial seeds has become an important asset in micropropagation. Encapsulation of *in vitro* derived vegetative propagules to develop synthetic seeds has been employed in recent years as a suitable alternative to the use of somatic embryos (Nor Asmah *et al.* 2012).

Table 4. Effect of different concentrations of growth regulators on root initiation, root number and root length from *in vitro* derived shoots of *Hypochaeris radicata*.

Growth regulators (mg/L)			Shoots rooted (%)*		No. of roots/shoot*		Root length (cm)*	
IAA	IBA	NAA	Leaf	Callus	Leaf	Callus	Leaf	Callus
0.0	0.3	0.0	12.22±0.56^{a}	10.09±0.18^{a}	1.12±0.37^{a}	1.03±0.49^{a}	0.32±0.02^{a}	0.44±0.10^{a}
0.0	0.5	0.0	24.31±0.71^{b}	18.09±0.07^{ab}	1.55±0.13^{a}	1.32±0.17^{a}	1.64±0.33^{b}	2.00±0.09^{c}
0.0	1.0	0.0	35.44±0.29^{c}	30.32±0.54^{c}	2.63±0.11^{b}	1.93±0.29^{ab}	4.55±0.29^{d}	2.98±0.29^{c}
0.0	0.0	0.3	21.10±0.30^{b}	16.04±0.26^{a}	1.00±0.12^{a}	1.22±0.48^{a}	1.09±0.25^{b}	1.22±0.55^{b}
0.0	0.0	0.5	48.32±0.37^{d}	24.11±0.43^{b}	1.23±0.43^{a}	1.74±0.22^{ab}	2.22±0.75^{c}	2.76±0.06^{c}
0.0	0.0	1.0	73.54±0.19^{e}	59.40±0.33^{d}	5.30±0.64^{c}	5.43±0.48^{c}	6.37±0.33^{e}	6.09±0.43^{d}
0.3	0.0	0.0	-	-	-	-	-	-
0.5	0.0	0.0	10.10±0.03^{a}	25.52±0.04^{b}	1.10±0.22^{a}	1.33±0.29^{a}	1.02±0.32^{b}	1.43±0.05^{b}
1.0	0.0	0.0	39.54±0.09^{c}	31.54±0.32^{c}	2.66±0.26^{b}	2.32±0.40^{b}	2.33±0.11^{c}	2.15±0.45^{c}

*Mean of three replicates of 30 explants ± standard deviation. Values within the column followed by same superscript are not significantly different at $P<0.05$.

Table 5. Effect of various composition of hardening medium on survivability of *Hypochaeris radicata* plantlets.

Hardening medium composition (v/v)	No. of plantlets under hardening	No. of plantlets survived*		Survivability (%)*	
		Leaf	Callus	Leaf	Callus
Garden soil: sand: vermicompost (1:1:1)	30	21±0.12^{d}	18±0.03^{d}	70.00±0.34^{e}	60.00±0.56^{d}
Red soil: sand: vermicompost (1:1:1)	30	16±0.04^{c}	15±0.22^{c}	53.33±0.18^{d}	50.00±0.04^{c}
Vermicompost: soil (1:1)	30	13±0.23^{b}	14±0.13^{b}	43.33±0.61^{b}	46.66±0.19^{b}
Red soil: sand (1:1)	30	09±0.11^{a}	09±0.43^{a}	30.00±0.21^{a}	30.00±0.52^{a}
Decomposed coir waste: perlite: vermicompost (1:1:1)	30	08±0.09^{c}	06±0.57^{c}	26.66±0.21^{c}	20.00±0.49^{c}

*Mean of three replicates of 30 explants ± standard deviation.
Values within the column followed by same superscript are not significantly different at $P<0.05$.

Sodium alginate ($C_6H_7O_6Na$) is a colorless or light yellow filaments, granules, or powder which forms a viscous colloid in water and used in food thickeners and stabilizers, in medicine, textile printing, paper coating and water-base paint. Also it is known as algin; alginic acid sodium salt and sodium polymannuronate. It is a copolymer composed of D-mannuronic acid and L-glucuronic acid units and has been extensively studied because of its biocompatibility, biodegradability and its capacity to form hydrogels in the

presence of divalent cations. The ridge structure and large pore size of these gels which are insoluble in water make them useful for the encapsulation of live cells of plants. Polymer concentration, degree of viscosity of the alginate used, $CaCl_2$ concentration and curing time are important parameters determining the permeability, resistance and hardness of the resulting beads and the subsequent success of the encapsulation method (Block 2003).

In the present study, the polymerizing ability of 3% sodium alginate showed to be the best for encapsulating explants. However, sodium alginate concentration below 3% were not suitable for encapsulation because the resulting beads were without a definite shape and were too soft to handle, whilst at higher concentration the beads became hard causing a considerable delay in regeneration. Sodium alginate preparation at lower concentration became unsuitable for encapsulation, probably because of a reduction in its gelling after exposure to high temperature during autoclaving (Pattnaik *et al*. 1995). The conversion of encapsulated leaf and callus segments into plantlets after considerable period of storage could be attributed to the inclusion of MS salts in encapsulation matrix which serve as an artificial nutrient to the encapsulated explants for regeneration (Ganapathi *et al*. 2001).

The best storage temperature determined was 25°C for all three storage periods: 2, 4 and 6 months for leaf and callus explants encapsulated beads. The morphology and growth of regenerated shoots were not affected at 25°C. The shoots emerged from beads stored at 4ºC exhibited slow growth with necrotic and vitrification symptoms. After 6 months of storage, the per cent frequency of conversion was reduced along with death and decay of the encapsulated explants due to crackings and dehydration of the beads. Saradha and Paulsamy (2013) also reported similar findings in a study with *Hildegardia populifolia*.

The leaf and callus explants encapsulated synthetic seeds produced higher amount of multiple shoots on the MS medium containing 2mg/L BAP compare with other plant growth regulators. It may indicate, that BAP (cytokinin) is an important growth regulator for shoot initiation, as observed by other workers (Gopinath *et al*. 2014; Ummi *et al*. 2014). The regenerated micro-shoots were excised and implanted on to MS medium containing different concentrations of IAA, IBA and NAA for rhizogenesis. The best rooting performance was observed in 1mg/L NAA for encapsulated beads of both explants. Several studies have demonstrated the positive effect of auxins on the root formation and development (Udayakumar *et al*. 2013; Abdel

Hamid *et al*. 2013). The *in vitro* developed plantlets were transferred to potting media containing garden soil, sand and vermicompost (1:1:1) for acclimatization. After one month they were planted in earthen pots containing normal garden soil and maintained in green house condition.

5 Conclusion

The results of the study revealed that the leaf encapsulated beads stored for four months at 25°C could be used to propagate *Hypochaeris radicata* successfully. Therefore, this procedure provides an effective for propagation of this important plant species. In addition, sowing of beads in high hills of Nilgiris in suitable microclimatic sites with the advent of monsoon season (June) will enhance the population of *Hypochaeris radicata* and can reduce the pressure upon the wild habitats where the species being endangered. .

Acknowledgment

The authors are acknowledging Dr. M. Aruchami Research Foundation (ARF/RA-2012/018 dt. 12.02.2012), Coimbatore for financial support to carry out the work.

References

Abdel Hamid, M.H., Rosna Mat, T. and Sadegh, M. 2013. *In vitro* induction and proliferation of adventitious roots in pinapple (*Ananas comosus* L.) cultivars of smooth cayenne and morris. *Aust J crop sci*. 7(7): 1038-1045.

Aman, N. and Shruti, S. 2013. Production of artificial seeds from nodal region of sweet neem (*Murraya Koenigii*). *J Adv Pharmaceu Res Biosci.* 1(2): 71-74.

Anju, P., Suraj, P.V., Promila, P. and Lucky, K.A. 2012. Augmented shelf-life and regeneration competence of activated charcoal (AC) supplemented synthetic seeds in *Cymbidium pendulum* (Roxb.). Sw. *Curr Bot.* 3(5): 30-34.

Bapat, B.A. and Rao, P.S. 1998. Sandalwood plantlets from synthetic seeds. *Plant Cell Rep*. 7: 434-436.

Bekheet, S.A. 2006. A synthetic seed method through encapsulation of *in vitro* proliferation bulblets of garlic (*Allium sativum* L.). *Arab J Biotech.* 9(3): 415-426.

Block, W. 2003. Water status and thermal analysis of alginate beads used in cryopreservation of plant germplasm. *Cryobiology* 47(1): 59-72.

Dennis, J.G., Purohit, A. and Triglano, R.N. 1991. Somatic embryogenesis and development of synthetic seed technology. *Cr Rev Plant Sci.* 10(1): 33-61.

Duncan, D.B. 1955. Multiple range and multiple *F* tests. *Biometrics* 11(1): 1-42.

Ganapathi, T.R., Bapat, V.A. and Rao, P.S. 1994. *In vitro* development of encapsulated shoot tips of cardamom. *Biotechnol Tech.* 8(4): 239-244.

Ganapathi, T.R., Srinivas, L., Suprasanna, P. and Bapat, V.A. 2001. Regeneration of plants from alginate-encapsulated somatic embryos of banana cv. Rasthali (*Musa* spp. AAB group). *In vitro cell Dev Biol* (*Plant*). 37: 178-181.

Ganapathi, T.R., Suprasanna, P., Kulkarni, V.M., Bapat, V.A. and Rao, P.S. 2002. Strategies for *in vitro* propagation and synthetic seeds in Banana. *BARC Newsletter*. Founders Day Special Issue, pp.168-176.

Ghosh, B. and Sen, S. 1994. Plant regeneration from alginate encapsulated somatic embryos of *Asparagus cooperi* Baker. *Plant Cell Rep.* 13(7): 381-385.

Gopinath, B., Gandhi, K. and Saravanan, S. 2014. *In vitro* propagation of an important medicinal plant *Artemisia annua* L. from axillary bud explants. *Adv Appl Sci Res.* 5(1): 254-258.

Harikrishna, K. and Ong, C.H. 2002. *Synthetic seeds In. Basic Principles of Biotechnology and their application in forestry*. Krishnapillay B (ed.). Asian Pacific Association of Forestry Research Institution (APAFRI), Malaysia. pp. 87-91.

Jamuna, S., Paulsamy, S. and Karthika, K. 2013a. *In vitro* antibacterial activity of leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) – a medicinal plant species inhabiting the high hills of Nilgiris, the Western Ghats. *Int J Pharmacy Pharmaceu Sci.* 5(1): 175-178.

Jamuna, S., Paulsamy, S. and Karthika, K. 2013b. *In vitro* antifungal activity of leaf and root extracts of the medicinal plant, *Hypochaeris radicata* L. *Int J Pharmacy Pharmaceu Sci.* 5(3): 758-761.

Jamuna, S., Paulsamy, S. and Karthika, K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pac J Trop Biomed.* 4(Suppl. 1): S359-S367.

Jamuna, S. and Paulsamy, S. 2013. GC-MS analysis for bioactive compounds in the methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae). *Int J Curr Res.* 5(12): 4070-4074.

Jamuna, S., Paulsamy, S. and Karthika, K. 2012. Screening of *in vitro* antioxidant activity of methanolic leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae). *J Appl Pharmaceu Sci.* 2(7): 149-154.

Krishna Kumar, G. and Dennis Thomas, T. 2012. High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. *Plant Cell Tiss Org Cult.* 110: 141-151.

Lans, C., Turner, N., Khan, T., Brauer, G. and Boepple, W. 2007. Ethnoveterinary medicines used for ruminants in British Columbia, Canada. *J Ethnobiol Ethnomed.* 3: 11.

Louis, H.P., Waldman, A.S., Schechinger, L., Govindarajoo, G. and Nowick, J.S. 1998. The alginate demonstration: polymers, food science, and ionexchange. *J Chem Edu.* 75(11): 1430-1431.

Mithilesh, S. and Rakhi, C. 2013. *Somatic embryogenesis in Neem* (*Azadirachta indica* A. Juss.): current status and biotechnological perspectives. *In:* Junaid Aslam, P.S.S. and M.P. Sharma (Editors). *Somatic embryogenesis and Gene expression.* Narosa Publishing House, New Delhi, pp. 35-55.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol Plantarum*15(3): 473-497.

Nor Asmah, H., Nor Hasnida, H., Noraliza, A., Nashatul Zaimah, N.A. and Nadiah Salmi, N. 2012. *In vitro* propagation of *Acacia* hybrid through alginate-encapsulated shoots and axillary buds. *Afr J Biotechnol.* 11(65): 12814-12817.

Pattnaik, S.K., Sahoo, Y. and Chand, P.K. 1995. Efficient plant retrieval from alginate-encapsulated vegetative buds of mature mulberry trees. *Sci Hortic-Amsterdam* 61(3-4): 277-239.

Paulsamy, S., Vijaya Kumar, K.K., Murugesan, M., Sivashanmugam, M., Senthilkumar, P. and Suresh, D. 2008. *Medicinal and other economic plants of Shola understories, the Nilgiris, Western Ghats.* Internationa Book Distributors, Dehradun, Uttarakhand, India. pp. 1-112.

Pullaiah, T. 2006. *Encyclopedia of world medicinal plants*. Regency publication, New Delhi, pp, 1-525.

Roy, B. and Mandal, A.B. 2008. Development of synthetic seeds involving androgenic and pro-embryos in elite *Indica* rice. *Indian J Biotechnol.* 7: 515-519.

Saradha, M. and Paulsamy, S. 2013. Development of propagation strategies and evaluation of medicinal properties for the endangered plant species, *Hildegardia populifolia* (Roxb.) Schott and Endl. (Sterculiaceae). Ph.D., thesis submitted in Bharathiar University.

Sharma, A., Tandon, P. and Kumar, A. 1992. Regeneration of *Dendrobium wardianum* Warner. (Orchidaceae) from synthetic seeds. *Indian J Exp Biol*. 30(8): 747-748.

Tynsong, H., Tiwari, B.K. and Lynser, M.B. 2006. *Medicinal plants of Meghalaya, India. Centre for Environmental studies*. North-Eastern Hill University Shillong, India. Medicinal Plant Network News. News letter of Mappa/ICIMDD, NMPB & MPCN, pp. 7-10.

Udayakumar, R., Choi, C.W., Kim, K.T., Kim, S.C., Kasthurirengan, S., Mariashibu, T.S., Sahaya Rayan, J.J. and Ganapathi, A. 2013. *In vitro* plant regeneration from epicotyl explant of *Withania somnifera* (L.) Dunal. *J Med Plants Res.* 7(1): 43-52.

Ummi Nur, A., Chong Boon, O., Tiew Sing, Y. and Li Kiaw, L. 2014. *In vitro* microporpagation of *Stevia rebaudiana* Bertoni in Malaysia. *Braz Arch Biol Technol.* 57(1): 23-28.