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Synthetic seeds - A novel approach for the conservation of endangered *C. spiralis* wt. and *C. pusilla*

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Abstract

In the present study best suitable explant for encapsulation and effect of nutrient composition of alginate matrix on regrowth performance of encapsulated explants of endangered *C. spiralis* and *C. pusilla* were studied. Among all the explants tested, good sprouting frequency was observed with shoot tips in both the species. Where as, multiple shoot induction with maximum shoot length was noticed with nodes. Sprouting frequency and viability of explants were reported in both the encapsulation mixtures tested. But, the significant increase in sprouting percentage, maximum number of shoots formed for each encapsulated explant and maximum shoot length were achieved with the encapsulation matrix prepared with Murashige and Skoog (MS) + 3 mg/L Benzyle amino purine (BAP) + 3 % sucrose + 3 % sodium alginate in both the species *C. spiralis* and *C. pusilla*.

Keywords: Synthetic seeds; Encapsulation; *C. spiralis; C. pusilla*; Shoot tips; Nodes; Morphogenetic callus

Introduction

Ceropegia spiralis Wt. and *C. pusilla* Wt. & Arn. are endangered and endemic medicinal herbs, belong family Asclepiadaceae. The root tubers contain an alkaloid called ceropegin and consumed after cooking (Mabberely 1987). The root tubers also contain starch, sugars, gum, albuminoids, fats, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against many diseases especially diarrhea and dysentery. The starchy tubers are edible and are useful as a nutritive tonic (Nadkarni, 1976; Reddy *et. al*., 2006). So, conservation of these plants is an important issue because of their high medicinal importance and was in endangered category. Due to some limitations in *in situ* conservation like very low availability of seeds and less reproductive ability the alternative methods were required to conserve these plant species.

Biotechnological approaches have been developed to conserve a number of plant species *in vitro*. Among these synthetic seed technology is the fast growing area of plant biotechnology. In the past artificial seed technology was developed to conserve a number of medicinal plants. Efficient plantlet regeneration from encapsulated somatic embryos and shoot tips in Asclepiadaceae member *Tylophora indica* were reported by Chandrasekhar *et. al.* (2006) and Faisal and Anis (2007). Ramesh *et. al.* (2009) reported the effect of bavistin on *in vitro* plantlet conversion from encapsulated uninodal microcuttings of *Bacopa monnieri.* Srivastava *et. al.* (2009) studied the genetic stability of plants derived from encapsulated microshoots following 6 months of storage in *Cineraria maritime*. Plantlet regeneration from encapsulated nodal segments in *Vitex negundo,* shoot tips in *Solanum nigrum* and micro shoots in *Picrorhiza kurrooa* were respectively reported by many authors (Ahmad and Anis (2010); Verma *et. al*. (2010); Mishra *et. al.* 2011).

The *in vitro* regeneration protocols had already been described in both the species i.e. *Ceropegia spiralis* (Murthy *et. al.,* 2010) and *C. pusilla* (Kondamudi and Murthy, 2011). In the present study encapsulation of different explants of *C. spiralis* and *C. pusilla* was reported and effect of nutrient compositions of encapsulation matrix on regrowth performance of different explants ware evaluated.

Materials and methods

Collection of Plant material and establishment of cultures

The wild grown plants of *Ceropegia spiralis* (Nimmati gadda) and *Ceropegia pusilla* (churning stick) were collected from Akashaganga of Tirumala hills, Andhra Pradesh and

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Shevaroy hills of Tamilnadu, Eastern Ghats, India. The nodal segments derived from *in vivo* grown plants of both the species were surface sterilized and cultured on MS medium supplemented with different concentrations of BAP to induce multiple shoots *in vitro*. Different types of explants like nodes, internodes and leaf were cultured on MS media supplemented with different auxins like Indole-3-acetic acid (IAA), Indole-3 butyric acid (IBA), Naphthalene acetic acid (NAA) and 2, 4-dichlorophynoxy acetic acid (2,4 -D) alone or in combination with different cytokinins to induce morphogenetic callus in both the species.

Encapsulation of explants

The shoot tips, nodal segments excised from the *in vitro* grown cultures and morphogenetic callus developed from different explants were used for encapsulation to know the best explant for production of seed analogues. Sodium alginate solution of different concentrations (1 to 5 % w/v) were prepared by mixing sodium alginate with calcium free liquid MS medium containing 3 % sucrose and at the same time different concentrations of calcium chloride (25 to 100 mM) solution were also prepared. Then, the explants were suspended in sodium alginate solution in laminar air flow for 1 to 2 minutes and dropped one by one through a modified 1000 µL micropipette into a sterile aqueous solution of calcium chloride. Due to the exchange of ions between sodium alginate and calcium chloride, calcium alginate beads were formed with in 20 - 30 minutes. The beads were then collected by discarding the calcium chloride solution, washed with sterilized double distilled water and surface dried by plating them in sterilized petri dishes containing blotting papers. Finally prepared beads were directly cultured on MS medium supplemented with 3 mg/L BAP.

Effect of the nutrient composition of the encapsulation matrix on regrowth of different explants

To confirm the effect of nutrient composition of encapsulation matrix on the regrowth of different explants, the germination percentage of encapsulated explants containing MS basal medium and MS with 3 mg/L BAP as components of capsules were investigated. Along with the germination percentage, shoot number and length were also calculated after one month of the regrowth of encapsulated explants for better understanding. All the media and solutions were sterilized by autoclaving at 108 kpa and 121^{o}C for 20 min. All the cultures were maintained at 24±2^{o}C under 16-h photoperiod with 3000 lux light intensity using fluorescent lights (Philips, India Ltd.) and 80-90 % relative humidity.

Ten cultures were raised for each treatment and all experiments were repeated thrice. The response was observed after every week. The data was recorded periodically and analyzed statistically using one way analysis of variance (ANOVA), and the data means $\pm$ SD of at least three different experiments were represented and compared using Tukey-Kramer multiple comparisons test with the level of significant P=0.05.

Results and discussion

Initiation and establishment of cultures

Large scale *in vitro* grown viable materials are required for the production of artificial seeds. So, standardization of *in vitro* culture systems is required before for the production of synthetic seeds. Among all the concentrations of BAP tested the best response of shoot proliferation with maximum number of shoots and shoot length was observed at 3 mg/L of BAP in both the species *Ceropegia spiralis* and *C. pusilla.* From all the explants tested excellent amount of callus was produced from the nodes of *Ceropegia spiralis* and *C. pusilla*. Among all the concentrations and combinations of auxins and cytokinins tested, medium supplemented with 3 mg/l BAP and 1 mg/L 2,4 - D showed the best callusing ability. The morphology of the callus was friable; cream to yellow colored and nodular in its stricture from where the embryos were also observed (Data was not presented).

Effect of sodium alginate and calcium chloride composition on bead formation

In the present study of all the concentrations of sodium alginate and calcium chloride tested 2.5 to 3 % (w/v) sodium alginate with 50 mM calcium chloride solution showed best results by the formation of identical beads with all the explants tested. The lower concentrations of sodium alginate (1to 2 % w/v) and calcium chloride (25 mM) were not suitable for encapsulation because the resulting beads were showing irregular shape and too soft to handle. Where as at higher concentrations of sodium alginate (4 to 5 % w/v) and calcium chloride (75 to 100 mM) the beads were too hard and cause considerable delay in sprouting. The present finding was similar with the Castillo *et. al.* (1998) who reported that 2.5% sodium alginate solution was optimum for maximum synthetic seed germination (77.5 %) in *Carica papaya* and with Bekheet (2006) coho reported that 3% sodium alginate used for encapsulation of bulblets in *Allium sativium* was optimum for production of more number of shoots.

In vitro syn-seed Germination

Encapsulated explants sprouted with in 20 - 50 days in both *C. spiralis* and *C. pusilla* when cultured on MS medium supplemented with 3.0 mg/L BAP. The quick germination with in short period was noticed with shoot tips (16 - 19 and 21 - 28 days respectively) encapsulated with artificial endosperm containing 3.0 mg/L BAP compared with nodal explants (28 - 33 and 27 - 31 days respectively) in both the species. However the delayed germination period was observed with morphogenetic callus (40 -53 and 44-51 days respectively) encapsulated with MS basal medium as artificial endosperm.

The percentage of germination and number of shoots formed were also varied with the type of species, Explant and nutrient composition of the encapsulation matrix tested.

In both the species *C. spiralis* and *C. pusilla* the maximum percentage of shoot sprouting frequency was observed with shoot tips (90.0 and 86.6% respectively) encapsulated with MS + 3 % sucrose + 3.0 mg/L BAP + 3 % sodium alginate. Where as, maximum number of shoots were induced from nodes (2.80±0.35 and 3.90±0.37 respectively) encapsulated with MS + 3 % sucrose + 3.0 mg/L BAP + 3 % sodium alginate. Efficient shoot proliferation from encapsulated nodal segments was also reported by Refouveiet *et. al.* (1998) in *Syringa vulgaris*. In both the species the lowest percentage of germination (13.3 and 16.6 %) and least number of shoots (0.70±0.26 and 0.60±0.26 respectively) were raised from morphogenetic callus encapsulated with MS + 3 % sucrose + 3 % sodium alginate (Table I).

In the present investigation, effect of nutrient composition of the encapsulation matrix on regrowth of different explants in *Ceropegia spiralis* and *C. pusilla* were tested. In both the species all the explants showed viability upto two months with both the encapsulation mixtures tested. The best germination results were observed by adding BAP 3.0 mg/L to the encapsulation matrix. Good sprouting frequency by adding growth regulators to the artificial endosperm was also reported in *Punica granatum* (Naik and Chand, 2006) and in *Solanum melongena*, (Huda and Bari, 2007). However in contradictory to the present findings Mariani (1992) reported that addition of growth regulators showed negative effect on synthetic seed germination. Where as 100% germination of banana shoot tips encapsulated with MS + 3% sodium alginate alone with out any growth regulators was reported by Ganapathi *et al.* (1992).

Table I. Effect of nutrient composition of encapsulation matrix on regrowth of different Explants

Plant species	Bead composition	Explant	Germination period in days	Percentage of germination	Number of shoots/ explant	Shoot length in cm
C. spiralis	MS +	Shoot tip	20 - 27	83.3	1.10 0.23	2.84 0.50
	Na alginate	Node	32 - 35	70.0	2.40 0.33	4.45 0.24
		Organogenic callus	40 - 53	13.3	0.70 0.26	0.91 0.31
	MS +	Shoot tip	16 -19	90.0	1.90 0.31	3.65 0.45
	3.0 mg/l BAP +	Node	28 - 33	86.6	2.80 0.35	5.70 0.20
	Na alginate	Organogenic callus	41 - 48	20.0	0.90 0.27	1.43 0.46
C. pusilla	MS +	Shoot tip	25 - 31	80.0	1.70 0.30	1.25 0.22
	Na alginate	Node	30 - 38	73.3	3.40 0.47	5.33 0.14
		Organogenic callus	44 - 51	16.6	0.60 0.26	1.28 0.52
	MS +	Shoot tip	21 - 28	86.6	2.10 0.31	2.89 0.07
	3.0 mg/l	Node	27 - 31	83.3	3.90 0.37	6.28 0.21
	BAP + Na alginate	Organogenic callus	40 -51	23.3	1.00 0.33	1.97 0.54

Results represent mean ± SD of three replicated experiments.

Conclusion

In the present study a procedure for the encapsulation of different explants in two medicinally important plant species *C. spiralis* and *C. pusilla* were optimized and their percentage of regrowth were tested. Among all the explants encapsulated the best use one is nodal explant then the shoot tip, because even though the shoot tips have high sprouting frequency the number of shoots formed are less. Where as in case of nodal segments even though they have slight less sprouting frequency, they form more number of shoots. This method may be used for further germplasm conservation studies and to also develop protocols for other *Ceropegia* species and to multiply these plant species *in vitro* in large number.

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References

Ahmad N and Anis M (2010), Direct plant regeneration from encapsulated nodal segments of *Vitex negundo. Biologia plantarum.* **54:** 748 -752

Bekheet SA (2006), A synthetic seed method through encapsulation of *in vitro* proliferated bulblets of garlic (*Allium sativum* L.). *Arab J. biotech.* **9:** 415-26.

Castillo B, Smith MAL and Yadav UL (1998), Plant regeneration from encapsulated somatic embryos of *Carica papaya* L. *Plant Cell Rep.* **17:** *172-176.*

Chandrasekhar T, Hussain MT, Gopal RG and Rao SJV (2006), Somatic embryogenesis of *Tylophora indica* (Burm.f.) Merrill., an important medicinal plant. *Int. J. Appl. Sci. Eng.* **4:** 33-40.

Faisal M and Anis M (2007), Regeneration of plants from alginate encapsulated shoots of *Tylophora indica* (Burm. F) Merrill. An endangered medicinal plant. *J. Hort. Sci Biotechnol.* **82:** 1-4.

Ganapathi TR, Suprasanna P, Bapat VA and Rao PS (1992), propagation of banana through encapsulated shoot tips. *Plant Cell Rep.* **11:** 571-575.

Huda AKMN and Bari MA (2007), Production of synthetic seed by encapsulating asexual embryo in eggplant (*Solanum melongena* L.). *Intern. J Agri. Res.* **2:** 832-837.

Kondamudi R and Murthy KSR (2011), Micropropagation and *in vitro* flowering of *Ceropegia pusilla* Wt. & Arn. - *J. Trop. Med. Plants.* **12:** 41-48.

Mabberely DJ (1987), The plant book. Cambridge University Press, Cambridge, 114-115.

Mariani P (1992), Eggplant somatic embryogenesis combined with synthetic seed technology. *Capsicum news lett*. Pp. 289-294.

Murthy KSR, Kondamudi R and Pullaiah T (2010), High frequency somatic embryogenesis in *Ceropegia spiralis* Wight. - An endemic and endangered medicinal plant, *Indian J. Biotechnol.* **9:** 414 - 418.

Nadkarni KM (1976). Indian Materia medica. Vol.1pp. 303-304. Popular Prakasha, Bombay, India.

Naik SK and Chand PK (2006), Nutrient alginate encapsulation of *in vitro* nodal segments of Pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. *Scientia horticulture,* **108:** 247-252.

Ramesh M, Marx R, Mathan G and Pandian SK (2009), Effect of bavistin on *in vitro* plant conversion from encapsulated uninodal microcuttings of micropropagated *Bacopa monnieri* L - An Ayurvedic herb. *Journal of environmental biology.* **30:** 441-444.

Reddy CS, Reddy KN, Pattanaik C and Raju VS (2006), Ethnobotanical observations on some endemic plants of Eastern Ghats, India. http//:www.siu.edu.beb/leaflets/ghats.htm;

Refouvelet E, Le Nour S, Tallon C and Daguin F (1998), A new method for *in vitro* propagation of lilac (*Syringa vulgaris* L.): Regrowth and storage conditions for axillary buds encapsulated in alginate beads, development of a pre-acclimatization stage. *Sci. Hortic.* **74:** 233-241.

Srivastava V, Khan SA and Banerjee S (2009), An evaluation of genetic fidelity of encapsulated Microshoots of the medicinal plant: *Cineraria maritime* following six months of etorage. *Plant Cell Tissue Organ Cult.* **99:** 193 - 198.

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