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Synseed Production in *Spilanthes mauritiana* DC. for Short-Term Storage and Germplasm Exchange

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Authors' contributions

This work was carried out in collaboration between both authors. Author AS suggested the study and extended over all guidance during the experimentation. Author SS performed the experimental work, analyzed data and wrote the first draft of the manuscript. Both the authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The present study provides an efficient protocol for short-term storage and germplasm exchange of a potent medicinal herb, *Spilanthes mauritiana* using encapsulated nodal segments.
Study Design: For in vitro conversion of synseeds, 5 beads were placed in each flask having Murashige and Skoog (MS) medium supplemented with different combinations of plant growth regulators (PGRs). While for ex vitro conversion, 5 synseeds per thermocol cups having different planting substrates were directly sown. The data for each experiment were collected after 6 weeks. All the experiments were conducted with a minimum of 20 replicates per treatment and each experiment was repeated thrice.
Place and Duration of Study: Plant Biotechnology Lab, Department of Botany, AMU, July 2012 to November 2013.
Methodology: Concentration of two different manufacture grade of Na-alginate (purchased from Central Drug House and Loba Chemie) were compared for the production of ideal synseeds. Conversion of synseeds was tested under in vitro and ex vitro conditions. A low temperature storage (4ºC) experiment was also carried out to understand the explants' ability to revive physiological activity leading to plantlet development.

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Results: A gelling matrix of 4% Na-alginate (CDH) or 3% (Loba Chemie) with 100 mM calcium chloride ($CaCl_2 \cdot 2H_2O$) was found most suitable for the production of ideal Ca-alginate bead. However, CDH grade Na-alginate (74.4% conversion) was found to be better than Loba Chemie (62% conversion) in terms of in vitro conversion of synseeds into complete plantlets when cultured on MS basal medium. Supplementation of Plant Growth Regulators (PGRs) to the MS basal medium further enhanced the conversion frequency of the synseeds. Maximum conversion (83%) was recorded on MS basal medium supplemented with 5.0 µM 6-benzyladenine (BA) and 0.5 µM indole-3-acetic acid (IAA). Synseeds, stored at 4ºC for 1-8 weeks showed successful sprouting with variable percent in successive weeks of transfer, followed by development into complete plantlets when returned to regeneration medium. Ex vitro conversion of synseeds also occurred when synseeds were sown directly into Soilrite™ moistened with quarter-strength MS salts. Plantlets regenerated from non-stored and stored synseeds were successfully hardened, acclimatized and established in soil with a success of 90%. While plantlets recovered from direct sowing of synseeds exhibited 80% survivability.
Conclusion: Being small in size, synseeds provide an effective tool for conservation, storage and exchange of this valuable medicinal plant species, potentially overcoming many of the difficulties associated with long-distance transport of plant germplasm.

Keywords: Acclimatization; conversion; germplasm conservation; hydrogel encapsulation; toothache plant.

1. INTRODUCTION

Production of synthetic seed using encapsulation technology is highly promising for conservation, storage and exchange of valuable rare hybrids, elite genotypes, sterile unstable genotypes and genetically engineered plant species among laboratories. Limited requirement of space, easy-to-handle during transport, and potential for storage are some of the key features of encapsulation technology which make it attractive to researchers for germplasm conservation and plant material exchange [1-5]. Originally synseed technology was limited to only those plant species where somatic embryogenesis was possible. In response to this shortcoming, possibility of using non-embryogenic vegetative propagules such as shoot tips, nodal segments/axillary buds, protocorm like bodies (PLBs) has been explored as a suitable alternative to somatic embryos. Thus, a synseed which is referred to as artificially encapsulated somatic embryo, shoot bud or any other meristematic tissue that can be used as functional mimic seed for sowing, possesses the ability to convert into a plant under in vitro or ex vitro conditions, and can be stored [6, 7]. This definition extends the concept of synthetic seed from its bonds to somatic embryogenesis and links the term to its use (storage, sowing) and product (plantlet). A wide range of plant species has been re-grown from encapsulated non-embryogenic propagules [8].

Spilanthes mauritiana DC., an endangered herb belonging to the Asteraceae family, is a native of Eastern Africa and used in the local pharmacopeia to cure infections of the throat and mouth [4]. Kamba tribes in Kenya chew the flowers of *S. mauritiana* for the relief of toothache and the treatment of pyorrhea [9], and an infusion of the herb is used as a febrifuge [10]. In Cameroons the plant is used as a snake-bite remedy and in the treatment of articular rheumatism [10]. In India the plant has been used as a remedy for kidney stones and bladder [11]. So far the only isolated active principle in *S. mauritiana* is an antiseptic alkaloid, spilanthol, present at a concentration of as much as 1.25% in the flowers [9].

Spilanthol is effective against blood parasites at extremely low concentrations and indeed is a poison to most invertebrates, while remaining harmless to the majority of vertebrates [4]. Researchers have shown preliminary antimicrobial activity in the crude extract from roots and flower heads of *S. mauritiana* [12,13]. This plant is also used for the control of *Anopheles* and *Aedes* mosquito [14,15].

Conventional vegetative propagation by stem cuttings is arduous and inadequate to meet the need for Ayurvedic drug properties. Micropropagation through plant tissue culture has been reported by Bais et al. [16] and Sharma et al. [17]. Although in our previous report we have also reported for synseed preparation in *S. mauritaiana*. Besides, synseed production has also been reported for the other species of *Spilanthes* i.e., *S. acmella* [1,18].

This study aimed to optimize (1) The best manufacturer grade and concentration of Na-alginate for ideal synseed formation in respect to shape, rigidity, transparency and their conversion; (2) Suitable treatment of plant growth regulators for the conversion of synseeds (shoot and root formation) into complete plantlets; (3) Possible duration of short-term storage of encapsulated nodal segments (with MS nutrient and DDW gel matrix) and compared it with non-encapsulated nodal segments; (4) The best suited substrate for ex vitro conversion through direct sowing of synseeds.

2. MATERIALS AND METHODS

2.1 Plant Material and Culture Conditions

Six month-old in vitro raised microshoots, cultured on Murashige and Skoog (MS) medium [19] supplemented with BA (1.0 µM) and IAA (0.5 µM) (Fig. 1A) were used as the explant source. From each microshoot 4-5 nodal segments (0.7-0.5 cm) from the terminal bud were taken for encapsulation. The 100 ml wide mouth flask (Borosil), each containing 5 synseeds (5 replicates) were placed in controlled environment of culture room at 25±2ºC temperature under 16 h photoperiod with 50 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPFD) provided by cool white fluorescent tubes (40 W; Philips, India) and 55 ± 5% relative humidity, for 6 weeks to determine the conversion percentage. Four flasks per treatment were maintained in culture room, thus there were 20 replicates per treatment (5×1×4=20) and each experiment was repeated thrice.

2.2 Effect of Different Types and Concentrations of Na-Alginate on Conversion

For encapsulating the nodal segments, different concentrations (1, 2, 3, 4 and 5%) of Na-alginate were added in liquid MS medium with 3% (w/v) sucrose. Na-alginate was purchased from two different manufacturer grades i.e., Central Drug House (CDH, India) and Loba Chemie (India). For complexion, 100 mM $CaCl_2 \cdot 2H_2O$ (Qualigens, India) solution was prepared in liquid MS medium. Both the gel matrix and complexing agent were autoclaved at 121ºC and 15 Psi (1.06 kg cm^{-2}) for 15 min after adjusting the pH to 5.8 using 1 N NaOH or 1 N HCl. Droplets of Na-alginate, each containing one nodal segment, were then collected using a sterile glass pipette (10 mm diameter) and dropped immediately into autoclaved $CaCl_2 \cdot 2H_2O$ solution for 20-25 min for polymerization and formation of capsules (0.8-1.0 cm). Ca-alginate beads or synseeds were then collected, rinsed with sterile liquid MS medium for 10 min and transferred to sterilized filter paper placed in Petri-dishes for 5 min under the laminar airflow hood to absorb the excess of MS medium. Synseeds were cultured

in flask containing 20ml of semi-solid MS nutrient medium and conversion frequency was noticed after 6 weeks of culture.

Fig. 1

Fig. 1. Plantlet recovery from synseeds of *Spilanthes mauritiana*; A. An in vitro proliferated culture-6 month old, B. Shoot sprouting from synseeds on MS basal medium-1 week old culture. C. Conversion of synseeds into complete plantlets (shoot and root formation) on MS medium supplemented with BA (1.0 µM) + IAA (0.5 µM)-6 week old culture. D. Healthy and complete plantlets recovered from synseeds, after 6 weeks. E. Acclimatized plantlets maintained in Soilrite™. F. A twig of regenerated plantlet showing flowering

2.3 Effect of Different PGRs on Conversion, Root Number and Shoot and Root Length

In the second experiment, different PGR combinations were tested to improve the conversion frequency, shoot length, root number per synseed and their overall growth. For complete plantlet recovery (conversion), synseeds (prepared with 4% CDH Na-alginate) were cultured in flask containing 20 ml of semi-solid MS nutrient medium supplemented with or without BA (1.0 µM) and α-naphthalene acetic acid (NAA)/indole-3-butyric acid

(IBA)/indole-3-actetic acid (IAA) (0.5 µM). MS basal medium without any PGR was served as control. Above mentioned parameters were evaluated after 6 weeks of culture.

2.4 Effect of Different Storage Durations on Conversion of Encapsulated (Synseeds) and Naked Nodal Segments

This experiment compared complete plantlets recovery (shoot and root formation) from encapsulated (having gel matrix of either MS or DDW) and non-encapsulated nodal segments after short-term storage. Nodal segments with (encapsulation in 4% CDH Na-alginate) or without encapsulation, transferred to sterilized 100 ml beakers moistened with 2 ml of sterile DDW and tightly sealed with two layers of Para Film to prevent desiccation and stored in a laboratory refrigerator at 4°C for 0, 1, 2, 4, 6 and 8 weeks. After each storage period, encapsulated and non-encapsulated nodal segments were placed on semi-solid MS medium supplemented with BA (1.0 µM) + IAA (0.5 µM) and conversion frequency was noticed after 6 weeks of culture.

2.5 Acclimatization and Establishment of Plantlets

Rooted plantlets recovered from non-stored and stored synseeds were removed from the culture medium, washed gently under running tap water to remove agar and gel matrix from the roots, transplanted into thermocol cups (expanded polystyrene) containing sterile Soilrite^{TM} (75% Irish peat moss and 25% horticulture grade expanded perlite) (Keltech Energies Ltd., India). Potted plantlets were covered with a transparent polythene membrane and kept in a culture room at 25±2ºC temperature and 16 h photoperiod with 50 µmol $m^{-2}s^{-1}$ PPFD provided by cool white fluorescent tubes (40 W; Philips, India) and 55±5% relative humidity for 4 weeks and watered every three days. Polythene membranes were gradually opened after two weeks in order to acclimatize the plantlets to field conditions. After 4 weeks, the plantlets were transferred to pots containing normal garden soil and green manure (2:1) and maintained in a greenhouse under normal day length conditions.

2.6 Effect of Different Sowing Substrates on *Ex vitro* Conversion

Various planting substrates were also assessed for the direct conversion of synseeds into complete plantlets under green house conditions at 25±2ºC with a 16 h light photoperiod. Non-stored synseeds were directly sown in Soilrite^{TM} moistened with tap water and quarter-strength MS salts, Soilrite^{TM} and soil mixture (1:1) moistened with tap water and quarter-strength MS salts for ex vitro conversion into plantlets. For initial 4 weeks of sowing, acclimatization procedure was followed to maintain 55±5% relative humidity, as mentioned above. Conversion frequency was noticed after 6 weeks of sowing.

2.7 Data Analysis

The data were analyzed statistically using SPSS version 12 (SPSS Inc., Chicago, IL, USA). The significance of differences among means was analyzed using Tukey's test at P=5% and data represented as mean±standard error (SE).

3. RESULTS AND DISCUSSION

3.1 Effect of Different Types and Concentrations of Na-Alginate on Conversion

Na-alginate 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 capability to form hydrogels in the presence of divalent cations. The rigid 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, $CaCl_2 \cdot 2H_2O$ concentration, and curing time are important parameters for determining the permeability, resistance and hardness of the resulting beads and the subsequent success of the encapsulation method [20].

Encapsulation of nodal segments was affected by the concentration and manufacture grade of Na-alginate. Synseeds were differed qualitatively with respect to texture, shape and transparency with different concentrations and types of Na-alginate. The presence of 4 and 3% Na-alginate purchased from CDH and Loba Chemie, respectively with 100 mM $CaCl_2 \cdot 2H_2O$ were found best composition for gel complexation. This formulation produced more or less sphere-shaped isodiameretic, firm and clear beads which disseminated like a monodisperse populations (Fig. 1B). These concentrations facilitated an optimum ion exchange amid Ca^{++} and Na^{+}, producing isodiametric, clear and firm synseeds. Although 4% CDH and 3% Loba Chemie Na-alginate produced similar kinds of synseeds in respect to shape, rigidity and transparency but conversion frequency was better with CDH grade (74.4%) than Loba Chemie (62%) on MS basal medium (Table 1). This variation might be due to the difference in pore size of Na-alginate. In both cases, synseed sprouting occurred within 8-10 days of incubation by breaking the Ca-alginate matrix resulting in the emergence of shoot. The development of shoot and root was simultaneous and resulted in rapid growth of plantlets within 5-6 weeks. Some of the synseeds failed to sprout and nodal segments within the matrix turned brown after 10-15 days of incubation.

Lower levels of Na-alginate (1-3% CDH and 1-2% Loba Chemie) were not suitable since synseeds were of irregular shape (distorted to oval) and extremely squashy to grip. These synseeds were fragile and difficult to handle during transfer to re-growth medium. The reduction in the gelling ability of lower concentrations of Na-alginate after exposure to high temperature during autoclaving has already been reported by Larkin et al. [21]. On the other hand, at higher concentration of Na-alginate (5% CDH and 4-5% Loba Chemie), synseeds were spherical but hard and opaque, resulted in significant loss in conversion frequency (Table 1). Similar to the present investigation, Mandal et al. [22] and Sharma et al. [18] have also reported variation in the optimum concentration of Na-alginate according to commercial source while working on *Ocimum* species and *S. acmella* accordingly.

3.2 Effect of Different PGRs on Conversion on Root Number and on of Shoot and Root Length

It was important to note that on MS medium simultaneous shoot and root formation was occurred that lead to the conversion of synseeds into complete plantlets, but the induced roots were very thin and less in number which resulted into poor survivability during acclimatization in Soilrite™. On each treatment of PGR, single shoot sprouted from a synseed.

Table 1. Effect of different types and concentrations of Na-alginate on conversion after 6 weeks of culture on MS basal medium

Na-alginate (% w/v)	Conversion (%)	
	Central Drug House (CDH)	Loba Chemie
1.0	Fragile beads	Fragile beads
2.0	Fragile beads	12.6±1.25^{d} (but soft to handle)
3.0	73.0±2.00^{a} (but soft to handle)	62.0±1.52^{a} (ideal beads)
4.0	74.4±1.93^{a} (ideal beads)	53.8±0.97^{b} (hard beads)
5.0	46.6±1.88^{b} (hard beads)	34.2±1.24^{c} (hardest beads)

Different concentrations of Na-alginate and 100 mM $CaCl_2 \cdot 2H_2O$ were prepared in MS basal medium. Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments. Mean value followed by the same alphabets are not significantly different according to Tukey's Test at 5% probability

Amongst the cytokinin (BA) and auxin (NAA/IAA/IBA) combinations tested, MS medium supplemented with BA (1.0 µM) and IAA (0.5 µM) was the best for maximum conversion (83%), highest shoot length (5.0 cm), maximum root number per synseed (4.4) and highest root length (5.0 cm) after 6 weeks of incubation. With this treatment, shoots were healthiest and rooting occurred directly from the basal end of the sprouted shoots without any intervening callus (Fig. 1C, D). While on MS medium supplemented with BA (1.0 µM) + NAA (0.5 µM) and BA (1.0 µM) + IBA (0.5 µM), rooting was associated with moderate to intense callusing respectively that adversely affected the conversion frequency of synseeds (Table 2).

Table 2. Effect of different PGRs on conversion, root number and shoot and root length after 6 weeks of culture

Treatment (µM)	Conversion (%)	Shoot length (cm)	Root no./ synseed	Root length (cm)
MS (control)	74.4±1.93^{b}	2.1±0.13^{c}	1.2±0.20^{b}	1.9±0.13^{b}
MS + BA (1.0) + NAA (0.5)	79.0±1.97^{ab}	3.0±0.16^{b}	2.0±0.31^{b}	2.6±0.18^{b}
MS + BA (1.0) + IAA (0.5)	83.0±2.09^{a}	5.0±0.16^{a}	4.4±0.40^{a}	5.0±0.25^{a}
MS + BA (1.0) + IBA (0.5)	64.0±1.37^{c}	2.5±0.27^{bc}	1.4±0.24^{b}	2.2±0.22^{b}

Synseeds were prepared with 4% Na-alginate (CDH) and 100mM $CaCl_2 \cdot 2H_2O$ using MS basal medium Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments. Mean value followed by the same alphabets are not significantly different according to Tukey's Test at 5% probability

3.3 Effect of Different Storage Durations on Conversion of Encapsulated (Synseeds) and Naked Nodal Segments

Synseed technology also acts as a tool of germplasm exchange between countries without any quarantine check. For this purpose synseed storage is a critical factor which determines their successful conversion into complete plantlets after transportation abroad. During cold storage, synseeds require no transfer to fresh medium, thus reduces the cost of maintaining germplasm cultures [23]. Therefore, appropriate storage conditions and definite storage period are prerequisites to maintain the viability of synseeds during transportation that leads to successful commercialization of encapsulation technology.

Na-alginate combined with MS nutrients demonstrated significant superiority over DDW with respect to synseed conversion (Table 3). With an increase in storage time up to 4 weeks, conversion frequency decreased gradually, thereafter a drastic loss in conversion frequency was noticed for synseeds having encapsulation matrix of MS nutrients. Decline in conversion frequency 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 [24,25]. After 4 weeks of storage, 75.4% sprouting was noticed for synseeds prepared with MS gel matrix. On the other hand, synseeds prepared with DDW failed to store. These findings suggest that the MS nutrients are essential ingredients of Na-alginate matrix for plantlet conversion.

Table 3. Effect of different storage durations on conversion of encapsulated and naked nodal segments after 6 weeks of culture on MS medium supplemented with BA (1.0 µM) + IAA (0.5 µM)

Storage duration (weeks)	Conversion (%)		
	Encapsulated nodal segments (encapsulation matrix prepared in MS basal medium)	Encapsulated nodal segments (encapsulation matrix prepared in distilled water)	Naked nodal segments
0	83.0±2.09[a]	17.2±0.96[a]	97.2±0.96[a]
1	82.0±2.07[ab]	10.6±0.60[b]	70.2±3.55[b]
2	77.4±1.77[ab]	04.0±0.63[c]	42.6±1.66[c]
4	75.4±1.43[b]	00.0±0.00[d]	16.0±1.37[d]
6	57.4±1.12[c]	00.0±0.00[d]	08.8±0.58[de]
8	41.0±1.18[d]	00.0±0.00[d]	04.2±0.58[e]

Synseeds were prepared with 4% Na-alginate (CDH) and 100 mM $CaCl_2 \cdot 2H_2O$ using MS basal medium and DDW. Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments. Mean value followed by the same alphabets are not significantly different according to Tukey's Test at 5% probability

An average of 41% synseeds prepared with MS nutrients remained viable even after 8 weeks of cold dark storage while only 4.2% sprouting (only shoot formation) was noticed for non-encapsulated nodal segments. The observation with cold stored synseeds of this endangered species is in accordance with the previous reports on other species. However, the temperature requirement for optimum viability varies from plant to plant. Generally, 4°C temperature is found to be most suitable for alginate bead storage [26,27]. Whereas few investigations revealed the requirement of higher temperature (25ºC) rather than low temperature for amenable storage of alginate beads in certain tropical and sub-tropical crops. Sundararaj et al. [28] observed 100% re-growth ability for *Zingiber officinale* encapsulated microshoots incubated at 25ºC while no re-growth was observed for encapsulated microshoots stored at 4ºC in dark. Encapsulated microshoots of *Cineraria maritima* and *Picrorhiza kurrooa* could be stored for 6 and 3 months, respectively at 25±2ºC [29,30]. While, Hung and Trueman [31,32] successfully stored encapsulated shoot tips of *Corymbia torelliana* × *Corymbia citriodora* and *Khaya senegalensis* up to 12 months storage under 14ºC and zero-irradiance.

3.4 Acclimatization and Establishment of Plantlets

The plantlets with well developed shoot and root systems were removed from culture medium, washed carefully to remove remnant of culture medium and gel-matrix before transferred to thermocol cups containing sterile Soilrite™. They were covered with

transparent polybags and acclimatized successfully (Fig. 1E). There was no significant difference in the survival percentage of plantlets recovered from non-stored and stored synseeds when transferred to field where they grew well and flowered (Fig. 1F) and exhibited 90% survivability.

3.5 Effect of Different Sowing Substrate on Ex vitro Conversion

After sowing of synseeds in different planting substrates, shoot sprouting was noticed after 2 weeks of sowing followed by root emergence after 3 weeks of sowing. Protection for initial 4 weeks was needed for all the plantlets obtained from direct sowing of synseeds. Thereafter polythene membranes were gradually removed. Among different planting substrates used for direct sowing of synseeds, Soilrite™ moistened with quarter-strength MS salts was found to be the best with 63.4% conversion frequency. Soilrite™ moistened with tap water reduced conversion frequency (34.6%) probably because of reduced nutrient availability in tap water as compared to the MS salts. Soil was not found suitable in both cases either moistened with MS salts or with tap water and this might be due to compact texture of soil as compared to the Soilrite™ (Table 4). During field transfer 80% survivability was noticed for such plantlets.

Table 4. Effect of different sowing substrates on ex vitro conversion of non-stored synseeds after 6 weeks of sowing

Planting substrate	Conversion (%)
Soilrite + ¼ MS salts	63.4±1.02[a]
Soilrite + tap water	34.6±2.03[b]
Soilrite + soil + ¼ MS salts	28.6±0.97[c]
Soilrite + soil + tap water	13.0±1.54[d]
Soil + ¼ MS Salts	6.8±0.96[e]
Soil + tap water	0.0±0.00[f]

Synseeds were prepared with 4% Na-alginate (CDH) and 100 mM $CaCl_2 \cdot 2H_2O$ using MS basal medium. Data represents Mean ± SE of 20 replicates per treatment in three repeated experiments. Mean value followed by the same alphabets are not significantly different according to Tukey's Test at 5% probability

4. CONCLUSIONS

Although a number of plantlets can be procured from in vitro multiplication of vegetative propagules but, the process is tedious. Besides, the multiple shoots have to be separated individually at the time of rooting. By employing the procedure of encapsulation of shoot buds, the intermediary phases of elongation and rooting can be eliminated. In this regards, the present study provides a simple, efficient, highly reproducible and practicable protocol for synseed formation in *S. mauritiana* using nodal segments. Simultaneous shoot and root formation was achieved in all the planting treatments of PGRs. Preservation of encapsulated explants is also simpler than cryopreservation and less labor-intensive than conventional storage of non-encapsulated propagules under minimal growth conditions [33]. In the present study, synseeds could be stored up to 8 weeks at low temperature (4ºC). Recovery of complete plantlets from synseeds in Soilrite™ moistened with quarter strength MS salts shows that this method could be useful in developing a cost-effective propagation system for *S. mauritiana*. Using the protocol described in the present study out of 300 plantlets transferred to the soil, 278 plantlets were survived successfully. Being small in size, synseeds therefore, provide an effective tool for storage and exchange of this valuable medicinal plant species, potentially overcoming many of the difficulties associated with long-

distance transport of plant germplasm. Thus, synseed can be treated like a seed having additional advantages of handling, transportaion, efficient deilvery of plants and would mininize the cost of production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh SK, Rai MK, Asthana P, Pandey S, Jaiswal VS, Jaiswal U. Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) Murr. A medicinally important and herbal pesticidal plant species. Acta Physiol Plant. 2009;31:649-653.
2. Kumar S, Rai MK, Singh N, Mangal M. Alginate-encapsulation of shoot tips of jojoba [*Simmondsia chinensis* (Link) Schneider] for germplasm exchange and distribution. Physiol Mol Biol Plants. 2010;16:379-382.
3. Verma SK, Rai MK, Asthana P, Jaiswal VS, Jaiswal U. In vitro plantlets from alginate-encapsulated shoot tips of *Solanum nigrum* L. Sci Hort. 2010;124:517-521.
4. Gantait S, Sinniah UR. Storability, post-storage conversion and genetic stability assessment of alginate-encapsulated shoot tips of monopodial orchid hybrid *Aranda* Wan Chark Kuan 'Blue' × *Vanda coerulea* Grift. Ex. Lindl. Plant Biotechnol Rep. 2013;7:257-266.
5. Parveen S, Shahzad A. Encapsulation of nodal segments of *Cassia angustifolia* Vahl. For short-term storage and germplasm exchange. Acta Physiol Plant. 2014;36:635-640.
6. Ara H, Jaiswal U, Jaiswal VS. Synthetic seed: Prospects and limitations. Curr Sci. 2000;78:1438-1444.
7. Capuano G, Piccioni E, Standardi A. Effect of different treatments on the conversion of M. 26 apple rootstock synthetic seeds obtained from encapsulated apical and axillary micropropagated buds. J Hortic Sci Biotechnol. 1998;73:299-305.
8. Sharma S, Shahzad A, Teixeira da Silva JA. Synseed technology- A complete synthesis. Biotechnol Adv. 2013;31:186-207.
9. Watt PM, Brayer-Brandwijk MC. The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edn. Edinburgh: E and S Livingstone; 1962.
10. Dalziel JM. The useful plants of West Tropical Africa. London: London: Academic Press, Crown Agents for the Colonies. 1937;206-242.
11. Dragendorff D. He Heilpflazen de volker and different times. Stuttgart: Ferdinand Enke; 1898.

12. Fabry W, Okemo PO, Ansong R. Fungistatic and fungicidal activity of East Africa medicinal plants. Mycoses. 1996;39:67-70.
13. Fabry W, Okemo PO, Ansong R. Antibacterial activity of East African plants. J Ethnopharmacol. 1998;60:79-84.
14. Jacobson M. USDA Agr. Handbook, Washington, DC. 1975;461.
15. Jondiko IJO. A mosquito larvicide in *Spilanthes mauritiana*. Phytochem. 1986;25:2289-2290.
16. Bais HP, Green JB, Walker TS, Okemo PO, Vivanco JM. In vitro propagation of *Spilanthes mauritiana* DC. An endangered medicinal herb, through axillary bud cultures. In Vitro Cell Dev Biol-Plant. 2002;38:598-601.
17. Sharma S, Shahzad A, Jan N, Sahai A. In vitro studies on shoot regeneration through various explants and alginate-encapsulated nodal segments of *Spilanthes mauritiana* DC., an endangered medicinal herb. Int J Plant Dev Biol. 2009a;3:56-61.
18. Sharma S, Shahzad A, Sahai A. Artificial seeds for propagation and preservation of *Spilanthes acmella* (L.) Murr. A threatened pesticidal plant species. Int J Plant Dev Biol. 2009b;3:62-65.
19. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473-497.
20. Black J. Biological performance of materials: fundamentals of biocompatibility, 2nd ed. New York: Marcel Dekker Inc. 1992;73-78.
21. Larkin PJ, Davies PA, Tanner GJ. Nurse culture of low number of *Medicago* and *Nicotiana* protoplasts using calcium alginate beads. Plant Sci. 1998;58:203-210.
22. Mandal J, Pattnaik S, Chand PK. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (Hoary Basil), *O. basilicum* (Sweet Basil), *O. gratissium* (Shrubby Basil) and *O. sanctum* (Sacred Basil). In vitro Cell Dev Biol-Plant. 2000;36:287-292.
23. West TP, Ravindra MB, Preece JP. Encapsulation, cold storage and growth of *Hibiscus moscheutos* nodal segments. Plant Cell Tiss Organ Cult. 2006;87:223-231.
24. Danso KE, Ford-Llyod BV. Encapsulation of nodal cuttings and shoot tips for storage and exchange of cassava germplasm. Plant Cell Rep. 2003;21:718-725.
25. Sharma S, Shahzad A. Encapsulation technology for short-term storage and conservation of a woody climber, *Decalepis hamiltonii* Wight and Arn. Plant Cell Tiss Organ Cult. 2012;111:191-198.
26. Faisal M, Anis M. Regeneration of plants from alginate encapsulated shoots of *Tylophora indica* (Burm. F.) Merrill., an endangered medicinal plant. J Hort Sci Biotech. 2007;82:351-354.
27. Tabassum B, Nasir IA, Farooq AM, Rehman Z, Latif Z, Husnain T. Viability assessment of in vitro produced synthetic seeds of cucumber. African J Biotech. 2010;9:7026-7032.
28. Sundararaj SG, Agrawal A, Tyagi RK. Encapsulation for in vitro short-term storage and exchange of ginger (*Zingiber officinale* Rosc.) germplasm. Sci Hort. 2010;125:761-766.
29. Srivastava V, Khan SA, Banerjee S. An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following 6 months of storage. Plant Cell Tiss Organ Cult. 2009;99:193-198.

30. Mishra J, Singh M, Palni LMS, Nandi SK. Assessment of genetic fidelity of encapsulated microshoots of *Picrorhiza kurrooa*. Plant Cell Tiss Organ Cult. 2011;104:181-186.
31. Hung CD, Trueman SJ. Alginate encapsulation of shoot tips and nodal segments for short-term storage and distribution of the eucalypt *Corymbia torelliana* × *Corymbia citriodora*. Acta Physiol Plant. 2012a;34:117-128.
32. Hung CD, Trueman SJ. Preservation of encapsulated shoot tips and nodes of the tropical hardwoods *Corymbia torelliana* × *Corymbia citriodora* and *Khaya senegalensis*. Plant Cell Tiss Organ Cult. 2012b;109:341-352.
33. Rai MK, Asthana P, Singh SK, Jaiswal VS, Jaiswal U. The encapsulation technology in fruit plants-a review. Biotechnol Adv. 2009;27:671-679.

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