

## ***IN VITRO* PRESERVATION OF THE EGYPTIAN ENDEMIC *SILENE SCHIMPERIANA* BOISS. PLANT VIA ENCAPSULATION**

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**S** *ilene schimperiana* (Caryophyllaceae) is an endemic plant of Saint Katherine Protectorate, Sinai, Egypt. This genus has various medicinal properties. Due to over exploitation, *S. schimperiana* is at risk of extinction at the vulnerable stage in Egypt. Therefore, its conservation is important. An efficient protocol for *S. schimperiana* synthetic seeds production was developed in the present study. Shoot tips and nodal segments obtained from *in vitro* proliferated shoots were encapsulated in calcium alginate beads for medium term conservation and germplasm exchange. Encapsulated propagules were stored for five months at 4°C in three different matrixes of calcium alginate; water, Murashige and Skoog (MS) medium and MS medium containing 3% sucrose. The most efficient gel complex for formation of identical beads was 3% Na-alginate and 100 mM CaCl<sub>2</sub>.2H<sub>2</sub>O. The optimum temperature for storage was 4°C with 100% viability during the period of five months of storage with all tested alginate matrix compositions. However, the matrix containing MS medium with 3% sucrose was the best for the encapsulated propagules. The regrowth frequency of encapsulated propagules and mean number of shoots were decreased by increasing in storage duration. Rooting was achieved on ¼ MS medium supplemented with 1.0 mg L<sup>-1</sup> indole-3-butyric acid (IBA) and 2 mg L<sup>-1</sup> α-naphthalene acetic acid (NAA). About 90% of regenerated plantlets were successfully acclimatized under greenhouse conditions. This method can be successfully applied for *in vitro* preservation of *S. schimperiana* propagules for five months at 4°C to conserve this valuable plant.

**Keywords:** Caryophyllaceae, ornamental, synthetic seed, synseeds, *in vitro* conservation, regrowth, storage

The 1<sup>st</sup> Scientific Conference of Plant Genetic Resources Department, Ecology and Dry Lands Agriculture Division, Desert Research Center “Plant Genetic Resources and Sustainable Development Under Egyptian Desert Conditions” 13-16 November, 2019, Sharm El-Sheikh, South Sinai, Egypt

Wild plants consider a natural source for food, fuel, clothing and treating human diseases since ancient civilizations. World Health Organization (WHO) estimated that about 70–80% of people worldwide depend on traditional medicine to meet their health demands (Chandra and Rawat, 2015). Also, a large number of plant species are used in pharmaceutical industry either directly or indirectly. So, they are under the threat of extinction due to many parameters such as climatic changes, fuel usage, over grazing, habitat loss, indiscriminate collection and unsustainable utilization of plant natural resources. Therefore, there is an urgent need to follow the conservation strategies and adopt an alternative means of production to safeguard these natural plants either *in situ* or *ex situ*.

Tissue culture techniques are used to conserve the genetic material of the rare and threatened natural medicinal plants. The most important application for plant tissue culture is the synthetic seeds technology using encapsulation technique (Sidhu, 2010).

Synthetic seeds (synseeds) can be defined as; artificially encapsulated somatic embryos or any non-embryogenic *in vitro* derived propagules, which are able to convert into a plantlet after sowing under *in vitro* or *ex vitro* condition (Chandana et al., 2018). Synthetic seed or artificial seeds merges the advantages of *in vitro* mass propagation and seed propagation, which include small space needed for storage, easy transportation, handling ability of the *in vitro* material, saving time, genetically identical, and low cost. The most important factor that limits the practical use of the synseed technology, is the regrowth percentage from encapsulated propagules into plantlet (Adriani et al., 2000).

*Silene* is the largest genus in Caryophyllaceae family and known for its ornamental flowering plants. This genus comprises 700 species worldwide and growing in Northern Africa, Asia and Northern Europe (El-Ghamery et al., 2016). This genus contains a wide range of valuable, interesting and pharmaceutically promising plants that are rich in secondary metabolites and used in folk medicine to treat cold, bronchitis, infections and inflammations or as analgesic, emetic, diuretic and antipyretic (Mamadalieva et al., 2014). There are numerous reports about the medicinal properties of the species belonging to *Silene* genus; they have hepatoprotection activity since their extracts relieve fibrotic liver damage induced by carbon tetrachloride and inhibited hepatic fibrosis (Shin et al., 2006). Mamadalieva et al. (2010) and Kritskaya et al. (2016) reported that, some of them have antifungal, anabolic, tonic hypoglycemic and hepatoprotective properties. Also, the genus has immunomodulatory (Ghonime et al., 2011) and antitumor (Chandra and Rawat, 2015) activities. Moreover, this genus has antiviral (Mamadalieva et al., 2011; Karamian and Ghasemlou, 2013 and Taskin and Bitis, 2013) and insecticidal (Mosaddegh et al., 2012 and Behzad et al., 2014) activities.

*Silene schimperiana* Boiss. is one of the vulnerable and an endemic plants of Saint Katherine Protectorate, Sinai, Egypt (El-Hadidi et al., 1991 and

Boulos, 2009). It is known in Arabic as Wesbi or Losseiq (Täckholm, 1974). The plant is a perennial glaucous herb, woody at the base (El-Hadidi et al., 1991 and Boulos, 2009). The vulnerability of the plant is due to over collection either by Bedouins or researchers for scientific research, over grazing especially by goats as well as limited geographical distribution (El-Hadidi et al., 1991).

Because of the vulnerability of this species, its ornamental value and pharmaceutical importance, there is a need to conserve this endemic vulnerable plant. So, this study is aimed to test the viability and regrowth ability from encapsulated *S. schimperiana* shoot tips and nodal segments after five months storage as a tool for germplasm preservation.

## MATERIALS AND METHODS

### 1. Plant material, culture medium and conditions

Seeds of *S. schimperiana* were collected from mother plants growing at Wadi Gebal, Saint Katherine Protectorate, Sinai, Egypt (Fig. 1). They were sterilized by soaking in 1% sodium hypochlorite solution for 15 min, then washed five times with sterilized distilled water. Seeds were placed on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose and 100 mg L<sup>-1</sup> myo-inositol. The pH of the medium was adjusted to 5.7 – 5.8 before adding 3 g L<sup>-1</sup> phytagel as a gelling agent. The medium was poured into glass jars and then autoclaved under a pressure of 1.06 kg cm<sup>-2</sup> and 121°C for 20 min. Shoot tips and nodal segments were dissected from *in vitro* grown seedlings and cultured on MS medium supplemented with 1 mg L<sup>-1</sup> benzyl-6- adenine (BA) for multiplication. The cultures were maintained at 24±2°C in a growth chamber under 16 h photoperiod with white cool fluorescent tubes. *In vitro* proliferated stock culture of the plant was used as plant material.

### 2. Encapsulation

#### 2.1. Encapsulation procedure

*In vitro* derived shoot tips (2 – 3 mm) and nodal segments (3 – 4 mm), dissected from *in vitro* proliferated healthy cultures, were used for encapsulation.

Calcium alginate beads were formed by Sodium alginate (Na-alginate) as a gelling matrix and calcium chloride (CaCl<sub>2</sub>.2H<sub>2</sub>O) as a complexing agent. Na-alginate solution was prepared in the range of 1.0, 2.0, 3.0, 4.0 and 5.0% (w/v) and the pH was adjusted at 5.7 – 5.8. Whereas CaCl<sub>2</sub>.2H<sub>2</sub>O solutions were prepared in the range of 50, 100, and 150 mM in distilled water to test the production of uniform calcium alginate beads.

To study the role of the alginate matrix composition on the beads regrowth, the propagules were coated with three different matrixes of Na-

alginate; 3% Na-alginate in distilled water, MS medium and MS medium supplemented with 3% sucrose.

To form beads, the alginate-covered propagules were dropped into  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solutions using autoclavable micropipette and sterile plastic tips. Beads were left in  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution for 25-30 min to achieve polymerization of Na-alginate and maintain uniform texture beads. To remove  $\text{CaCl}_2$  traces, the encapsulated beads were washed three times with sterilized distilled water. After polymerization, the beads were about 5 – 6 mm in diameter.



**Fig. (1).** *S. schimperiana* plant growing at Wadi Gebal, Saint Katherine Protectorate, Sinai, Egypt. **(a)** Whole plant; **(b)** Flower.

## 2.2. Storage conditions

Encapsulated shoot tips and nodal segments of *S. schimperiana* with different matrixes of Na-alginate were placed in sterilized Petri dishes (10 cm diameter) containing water agar medium (0.7% w/v) and held for five months of storage at 4°C and 24°C in darkness. Non-encapsulated propagules were served as control.

## 2.3. Regrowth ability of encapsulated propagules

In order to evaluate the regrowth percentage of the encapsulated propagules, they were transferred every month to the multiplication medium (MS medium supplemented with 1 mg L<sup>-1</sup> BA) under standard conditions for the regrowth. Percentage of viability (explants with a green appearance, without necrosis or yellowing), regrowth (encapsulated propagules that produced shoots) and mean number of shoots per explant were recorded monthly for a period of five months after four weeks of culturing.

The converted shoots were transferred to the rooting medium (¼ MS medium supplemented with 1.0 mg L<sup>-1</sup> IBA and 2 mg L<sup>-1</sup> NAA) to study their potential to induce roots after each storage month.

Rooted shoots were isolated carefully from the rooted medium, then washed with distilled water. They were transferred to a mixture of sand and peatmoss at a 1:1 ratio in small pots. The survival percentage of the rooted shoots was recovered after two months of transferring to the green house.

## 3. Experimental Design and Data Analysis

All the treatments were arranged using the completely randomized design. The experiments were repeated two times and fifteen replicates were

used in each experiment. Variance analysis of data was carried out using ANOVA program for statistical analysis. Duncan's multiple range test (Duncan, 1955) at  $p \leq 0.05$  was used for comparing the means.

## RESULTS AND DISCUSSION

Using the correct composition of Na-alginate and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  controls the good quality of the capsule and leads to the successful production of the synthetic seeds. Sodium alginate has several advantages than other encapsulating agents (agar, carrageenan and carboxy methyl cellulose, etc.) such as, low toxicity to the propagules, rapid gelation, moderate viscosity and low cost. Calcium chloride solution is used to give hardness to the beads by dropping the alginate-coated propagules into this solution for a period of 25 – 30 min to allow the ion exchange between  $\text{Na}^+$  and  $\text{Ca}^{++}$  to take place, forming calcium alginate (Standardi and Micheli, 2012 and Gantait et al, 2017a).

Regarding all tested concentrations and combinations of Na-alginate and calcium chloride in this experiment, the concentration of 3% Na -alginate and 100 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  was the most effective combination for the formation of identical capsules (Fig. 2). Beads showed different morphology regarding shape, texture, diameter and transparent appearance with different combinations and concentrations of Na-alginate (1.0 – 5.0%) and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (50 –150 mM). Low concentrations of Na-alginate (1.0 or 2.0%) and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (50 mM) gave undefined shape, too soft and fragile beads to handle. Whereas the higher concentrations of Na-alginate (4.0 – 5.0%) or  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (150 mM) produced isodiametric but hard beads. So, 3% Na-alginate and 100 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  was applied for the encapsulation of *S. schimperiana* propagules throughout experiments. Similar finding has been observed in *Eclipta alba* (Singh et al., 2010), *Capparis orientalis* (Hegazi, 2011), *Ruta graveolens* (Ahmad et al. 2012), *Rauwolfia serpentine* (Gantait et al., 2017a), *Tylophora indica* (Gantait et al., 2017b) and *Saccharum offinarum* (Badr-Elden, 2018).



**Fig. (2).** *S. schimperiana* shoot tips encapsulated in 3% Na-alginate and 100 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

### 1. Effect of Storage Temperature and Alginate Matrix Composition on the Regrowth and Shoot Number of Encapsulated Propagules

Storage temperature for the encapsulated propagules is one of the crucial factors, which determine the success of the synthetic seeds preservation. In this experiment, two different temperatures of 4 and 24±2°C with three different matrix compositions of Na - alginate were tested for storing the encapsulated *S. schimperiana* propagules. After each month of storage, the encapsulated propagules were cultured on MS basal medium supplemented with 1 mg L<sup>-1</sup> BA to induce shooting (Fig. 7a and 8a). Either with 4 or 24±2°C, the encapsulated propagules showed 100% viability with all tested matrixes during the first month of storage. Results in table (1) clearly shows that, regrowth percentage of the encapsulated shoot tip and nodal segment propagules at 4°C was higher than 24°C with distilled water and MS medium matrixes. After one month, by increasing the storage duration at 24°C, the majority of stored beads of both types of propagules completely lost their viability. For the stored beads at 4°C, they remain viable and complete for another four months of storage in darkness. Akhtar and Shahzad (2017) reported that, low temperature storage (4°C) help in lowering pathogenic activities and reducing beads dehydration than storage at room temperature (24±2°C). It was also noticed that, alginate matrix containing MS medium with 3% sucrose was better than other matrixes compositions. This matrix produced 100% regrowth with the two tested temperatures during the first month of storage. For efficient regrowth, further storage of encapsulated *S. schimperiana* propagules were achieved at 4°C for other four months. Sujatha and Kumari (2008) found that cold storing (5°C) of the encapsulated nodal segments of *Artemisia vulgaris* did not develop any shoots during storage than others stored at 25°C. Also, Hegazi (2016) reported that 24±2°C was inefficient for storage of encapsulated *Bacopa monnieri* shoot tips compared to 4°C, which was optimal for *in vitro* preservation of the plant. *In vitro* preservation at low temperature was encouraged in other plants too such as *Salvia splendens* (Sharma et al., 2014) and *Glycyrrhiza glabra* L. (Akhtar and Shahzad, 2017).

The alginate matrix composition considers the key factor in the regrowth frequency of the encapsulated propagules after storage that determines the efficiency and successes of the technique (Singh, 2006). The non-encapsulated propagules formed shoots within a week after transferring them to the culture medium (MS medium supplemented with 1 mg L<sup>-1</sup> BA) (Fig. 7b and 8b).

**Table (1).** Effect of storage temperature and alginate matrix composition on the regrowth of encapsulated *S. schimperiana* propagules after one month of storage. Data were taken after four weeks of culturing on MS medium supplemented with 1 mg L<sup>-1</sup> BA.

Alginate matrix composition	Regrowth (%)			
	Shoot tips		Nodal segments	
	4°C	24±2°C	4°C	24±2°C
Distilled water	88 <sup>c</sup>	50 <sup>c</sup>	81 <sup>c</sup>	30 <sup>c</sup>
MS medium	93 <sup>b</sup>	70 <sup>b</sup>	85 <sup>b</sup>	40 <sup>b</sup>
MS medium + 3% Sucrose	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>

After five months of storage, the three tested matrixes compositions showed 100% viability of the encapsulated propagules. However, the regrowth percentage was higher in the non-encapsulated propagules (control) compared to the encapsulated ones (Table 2 and Fig. 7c and 8c). Alginate matrix contains MS medium with 3% sucrose showed good results as compared to other matrixes with respect to regrowth percentage (Fig. 7d, e and f and 8d, e and f) and shoot number for both types of propagules. This demonstrates that, MS medium plays a vital role and considers an important ingredient in Na-alginate matrix for regrowth. Piccioni and Standardi (1995) and Grzegorzcyk and Wysokinska (2011) reported that; the addition of MS to the alginate matrix led to reduction in the gel viscosity. This explains increasing the regrowth percentage of propagules coated with MS containing matrixes. The inclusion of sucrose in the alginate matrix also improved the regrowth percentage from encapsulated shoot tips of *Camelia japonica* and axillary buds of *Betula pendula* (Grzegorzcyk and Wysokinska, 2011). Rai et al. (2008) proved that; the emergence of shoots from encapsulated shoot tips of *Psidium guajava* was completely inhibited in medium lacking sucrose.

**Table (2).** Effect of alginate matrix composition on the regrowth and mean number of shoots of encapsulated *S. schimperiana* propagules after five months of storage. Data were taken after four weeks of culturing on MS medium supplemented with 1 mg L<sup>-1</sup> BA. Non-encapsulated propagules were served as control.

Alginate matrix composition	Shoot tips		Nodal segments	
	Regrowth (%)	Mean no of shoots/explant	Regrowth (%)	Mean no of shoots/explant
Non-encapsulated	100 <sup>a</sup>	13 <sup>a</sup>	100 <sup>a</sup>	19 <sup>a</sup>
Distilled water	45 <sup>d</sup>	1.9 <sup>d</sup>	41 <sup>d</sup>	2.9 <sup>d</sup>
MS medium	60 <sup>c</sup>	2.8 <sup>c</sup>	59 <sup>c</sup>	4.0 <sup>c</sup>
MS medium + 3% sucrose	74 <sup>b</sup>	3.5 <sup>b</sup>	70 <sup>b</sup>	4.5 <sup>b</sup>

## 2. Effect of Storage Duration at 4°C on the Regrowth and the Shoot Number of Encapsulated Propagules

Storage of the synthetic seeds consider the most critical factor for germplasm exchange and conservation that determines achieving of this technology, since remaining viable after a considerable period of time. Storage at low temperatures results in lowering the metabolic activities of the synthetic seeds, allowing them to remain in a stationary state that aids in saving the nutritive reservoir in the encapsulated propagules (Gantait et al., 2017a).

Encapsulated propagules were further stored at 4°C in darkness for five months. As shown in table (3), the regrowth percentage of the encapsulated (in alginate matrix containing MS medium with 3% sucrose) propagules that were cultured immediately without storage was 100%. However, with the increase in storage time at 4°C, the regrowth percentage of the encapsulated propagules gradually decline until reach 74 and 70% for the encapsulated shoot tips and nodal segments, respectively, after five months of storage (Fig. 3 and 4). This decrease may be due to oxygen reduction and drying in calcium alginate beads (Singh et al., 2006) or as a result to the accumulation of secondary metabolites in the matrix and various metabolic events that happened to the encapsulated propagules. The regrowth percentage of encapsulated propagules was decreased by increasing in storage durations in many plants such as *Phyllanthus amarus* (Singh et al., 2006), *Capparis orientalis* (Hegazi, 2011), *Bacopa monnier* (Hegazi, 2016) *Tylophora indica* (Gantait et al., 2017b).

Also, with the increase in storage time at 4°C, shoot number of the encapsulated propagules gradually decreased until reach 3.5 and 4.5 shoot/explant for the encapsulated shoot tips and nodal segments, respectively, after five months of storage (Fig. 5 and 6).

**Table (3).** Effect of storage duration at 4°C on the regrowth and mean number of shoots of encapsulated *S. schimperiana* propagules in alginate matrix containing MS medium with 3% sucrose. Data were taken after four weeks of culturing on MS medium supplemented with 1 mg L<sup>-1</sup> BA.

Storage duration (month)	Shoot tips		Nodal segments	
	Regrowth (%)	Mean no of shoots/explant	Regrowth (%)	Mean no of shoots/explant
0	100 <sup>a</sup>	6.2 <sup>a</sup>	100 <sup>a</sup>	7.3 <sup>a</sup>
1	100 <sup>a</sup>	6.0 <sup>a</sup>	100 <sup>a</sup>	7.0 <sup>a</sup>
2	97 <sup>b</sup>	5.4 <sup>a</sup>	91 <sup>b</sup>	6.5 <sup>a</sup>
3	94 <sup>c</sup>	4.5 <sup>ab</sup>	75 <sup>c</sup>	5.3 <sup>ab</sup>
4	83 <sup>d</sup>	3.6 <sup>b</sup>	73 <sup>d</sup>	4.7 <sup>b</sup>
5	74 <sup>e</sup>	3.5 <sup>b</sup>	70 <sup>e</sup>	4.5 <sup>b</sup>

All the converted shoots had the ability to induce roots at ¼ MS medium containing 1.0 mg L<sup>-1</sup> indole-3-butyric acid and 2 mg L<sup>-1</sup> α-naphthalene acetic acid and they were not affected by the period of storage (Fig. 9). Rooted plantlets were transferred to green house and about 90% of the acclimatized plantlets remain healthy in natural conditions (Fig. 10).

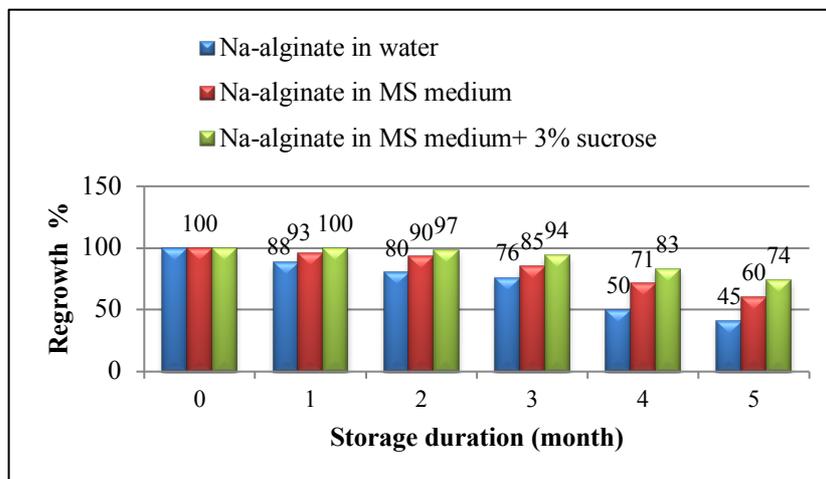


Fig. (3). Effect of alginate matrix composition and storage duration at 4°C on regrowth percentage of encapsulated *S. schimperiana* shoot tips, after four weeks of culturing.

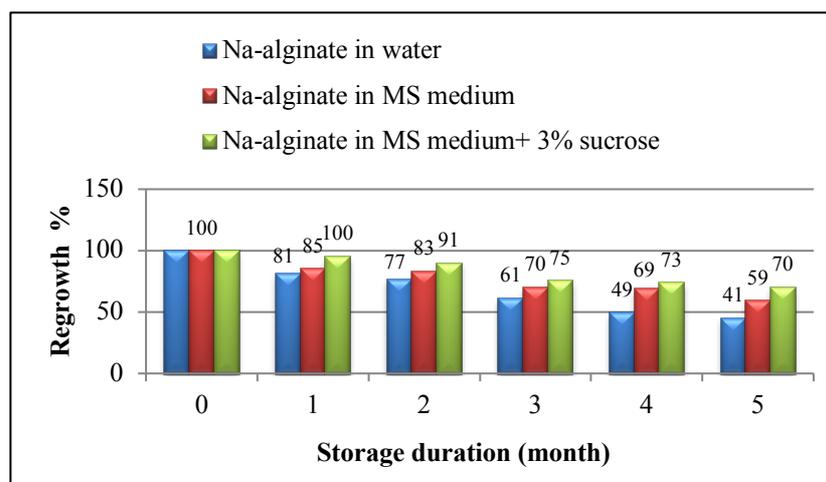
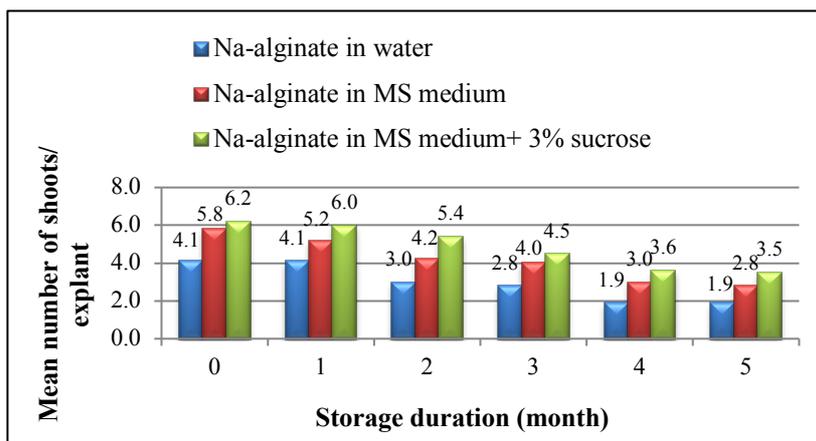
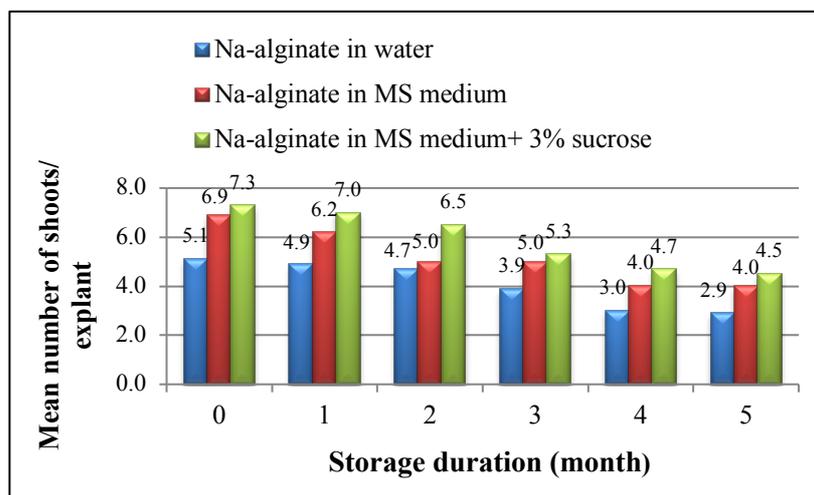


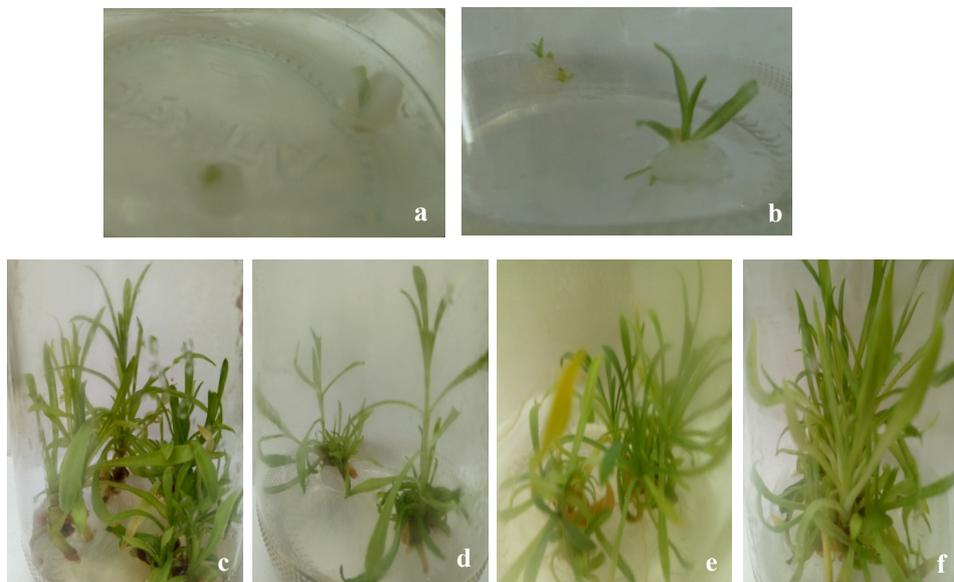
Fig. (4). Effect of alginate matrix composition and storage duration at 4°C on regrowth percentage of encapsulated *S. schimperiana* nodal segments, after four weeks of culturing.



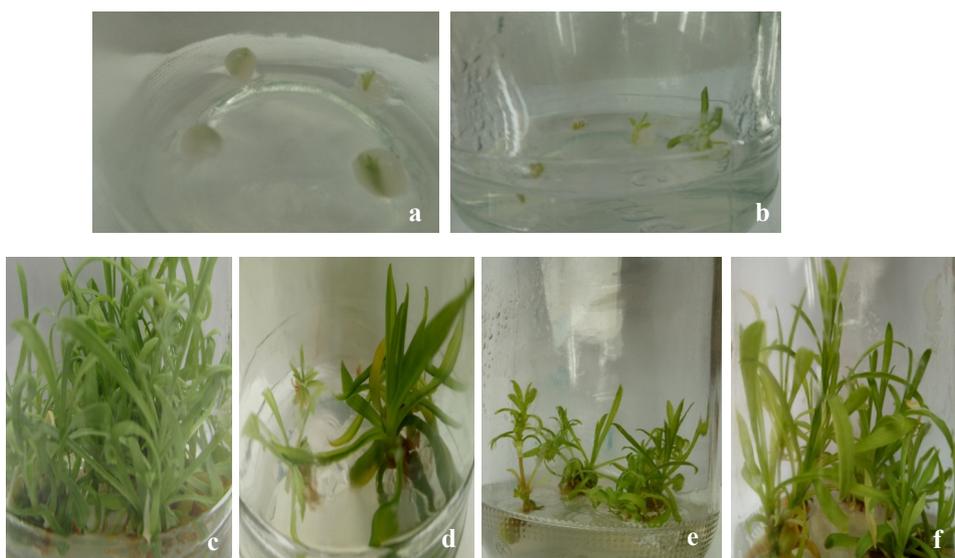
**Fig. (5).** Effect of alginate matrix composition and storage durations at 4°C on the mean number of shoots/explant of encapsulated *S. schimperiana* shoot tips, after four weeks of culturing.



**Fig. (6).** Effect of alginate matrix composition and storage durations at 4°C on the mean number of shoots/explant of encapsulated *S. schimperiana* nodal segments, after four weeks of culturing.



**Fig. (7).** Converted plantlets from encapsulated shoot tips of *S. schimperiana*. **a.** Encapsulated shoot tip beads; **b.** Emergence of shoots from encapsulated shoot tip after one week of culture on MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BA; **c.** Non-encapsulated shoot tips cultured on MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BA; **d.** Encapsulated shoot tips in distilled water after four weeks of culturing following five months of storage; **e.** Encapsulated shoot tips in MS medium after four weeks of culturing following five months of storage; **f.** Encapsulated shoot tips in MS medium containing 3% sucrose after four weeks of culturing following five months of storage.



**Fig. (8).** Converted plantlets from encapsulated nodal segments of *S. schimperiana*. **a.** Encapsulated nodal segments beads; **b.** Emergence of shoots from encapsulated nodal segment after one week of culture on MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BA; **c.** Non-encapsulated nodal segments cultured on MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  BA; **d.** Encapsulated nodal segments in distilled water after four weeks of culturing following five months of storage; **e.** Encapsulated nodal segments in MS medium after four weeks of culturing following five months of storage; **f.** Encapsulated nodal segments in MS medium containing 3% sucrose after four weeks of culturing following five months of storage.



**Fig. (9).** Plantlet of *S. schimperiana* with well-developed roots on  $\frac{1}{4}$  MS medium supplemented with  $1.0 \text{ mg L}^{-1}$  IBA and  $2 \text{ mg L}^{-1}$  NAA.



**Fig. (10).** Acclimatized plantlet from encapsulated propagules of *S. schimperiana* after two months from transfer to the greenhouse following five months of storage at 4°C.

In conclusion, this study presents the first promising germplasm preservation method developed by synthetic seeds production for successful conservation of *S. schimperiana* plant. The most effective combination for the formation of identical capsules was 3% Na -alginate and 100 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . The best suitable temperature for beads coated propagules storage was 4°C. After five months of storage, the highest regrowth percentage and the highest mean number of shoots of the encapsulated propagules was obtained with alginate matrix containing MS medium with 3% sucrose. Success in forming whole plant after acclimatization proves the efficiency of the *in vitro* preservation of *S. schimperiana* by encapsulation. This study provides the efficiency of synthetic seeds as a mean for reducing subculture periods, reducing cost and effort, germplasm exchange from place to another all over the world and conservation of the vulnerable and endemic Egyptian plants for longer periods of time especially with medicinal values.

#### ACKNOWLEDGMENT

The author is thankful and appreciated the effort of Prof. Dr. Ghada A. Hegazi, Professor of Plant Biotechnology, Department of Genetic Resources, Desert Research Center, in reviewing this paper.

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## الحفظ المعملّي لنبات اللصيق المتوطن بمصر عن طريق الكبسولة

هبة السيد غريب

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يتبع نبات السيلينية عائلة الـ Caryophyllaceae وهو نبات متوطن في محمية سانت كاترين، سيناء، مصر. هذا الجنس يمتلك العديد من الخصائص الطبية. يصنف هذا النبات في مصر على أنه مهدد بالانقراض نتيجة الاستغلال الجائر لذلك يجب الحفاظ عليه. تم في هذه الدراسة إجراء بروتوكول لإنتاج البذور الصناعية synthetic seeds لهذا النبات، فتم عمل كبسولات من ألجينات الكالسيوم calcium alginate beads بداخلها القمم النامية والأجزاء الساقية البرعمية التي تم الحصول عليها من الأفرع المتضاعفة معملياً وذلك لحفظ النبات على المدى المتوسط وسهولة تداوله. تم تخزين الكبسولات لمدة خمسة أشهر عند درجة 4° م والمتكونة من ثلاث مكونات مختلفة من ألجينات الكالسيوم وهي الماء وبيئة موراشيجي وسكوج (MS) Murashige and Skoog وبيئة موراشيجي وسكوج (MS) مضاف لها 3٪ سكروز. وقد كانت بيئة الكبسولة المكونة من 3٪ ألجينات صوديوم Na-alginate و 100 ملي مول من كلوريد الكالسيوم CaCl<sub>2</sub>.2H<sub>2</sub>O أفضل توليفة لتكوين كبسولات متماثلة. كما كانت درجة 4° م أنسب درجة لتخزين الكبسولات حيث أعطت 100٪ حيوية مع جميع البيئات المستخدمة في تكوين الكبسولة أثناء فترة الخمس أشهر من التخزين. وقد كانت الكبسولة المحتوية على بيئة موراشيجي وسكوج (MS) بالإضافة إلى 3٪ سكروز هي الأفضل. وقد أدت زيادة مدة الحفظ إلى تقليل معدل النمو ومتوسط عدد الأفرع للأجزاء النباتية المكبسلة. وقد كونت هذه الأجزاء النباتية المحفوظة جذوراً على ربع قوة بيئة موراشيجي وسكوج مضاف إليها 1 مجم/لتر إندول حامض البيوتريك (IBA) indole-3-butyric acid و 2 مجم/لتر نفثالين حامض الخليك  $\alpha$ -naphthalene acetic acid (NAA). استجاب حوالي 90٪ من هذه النباتات الناتجة للأقلمة تحت ظروف الصوبة. من خلال هذه الطريقة يمكن إجراء الحفظ المعملّي لنبات السيلينية لمدة خمسة أشهر على درجة 4° م وذلك كوسيلة لحفظ هذا النبات الهام والمهدد بالانقراض.