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In vitro Preservation of *Bacopa monnier* (L.) Pennell as a Rare Medicinal Plant in Egypt

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ABSTRACT

Bacopa monnieri (L.) Pennell, commonly known as 'Brahmi', belonging to family Scrophulariaceae, is a rare medicinal herb with global interest, and is one of the natural plants in the flora of Egypt. The plant grows in damp and marshy places of the Eastern Mediterranean coastal region in North Sinai. It is mainly used for the treatment of neurological disorders. With increasing demand for *B. monnieri* in pharmaceutical industries, there is a need to conserve the wild stocks of this plant through biotechnological approaches. A successful protocol for synthetic seeds production is developed in the present study for conservation of *B. monnieri.* Shoot tips excised from *in vitro*-derived multiple shoots were encapsulated in calcium alginate beads. Uniform spherical beads were obtained using 2.5% sodium alginate and 100 mM calcium chloride ($CaCl_2.2H_2O$) solutions. Shoot tips were stored for six months in calcium alginate beads containing different matrixes; water, Murashige and Skoog medium and Murashige and Skoog medium containing 3% sucrose, and were tested for storage at 24±2 and 4ºC. The efficiency of regrowth of *B. monnieri* encapsulated shoot tips was investigated. Hundred percent viability and high regrowth percentage and performance of recovered shoot tips, on Murashige and Skoog medium, were observed in all tested alginate matrixes at 4°C. However, Murashige and Skoog medium with sucrose was significantly superior. Rooting was achieved on the same medium and 93% of regenerated plantlets were successfully hardened under greenhouse conditions. The regeneration ability and regrowth performance of encapsulated shoot tips were declined monthly during storage duration. The protocol derived can be efficiently used for the preservation of encapsulated shoot tips of *B. monnieri* for six months at 4°C without any treatments with growth regulators. It could be cost-effective and useful in germplasm conservation and delivery of tissue cultured *B. monnieri* plants.

KEY WORDS: Brahmi, *in vitro* conservation, encapsulation, synthetic seed, storage, regrowth

INTRODUCTION

Medicinal plants serve as traditional medicine and natural raw material for drug preparations; therefore, they play a key role in world health. According to world health organization (WHO), more than 80% of the world population relies on plants as a source of traditional medicines for their primary health care needs, due to their fewer side effects in comparison to artificial drugs. The conservation of medicinal plants is a must. Natural medicinal plants are facing a combination of factors; such as over-collection, urbanization, pollution, habitat destruction, degradation, spread of invasive alien species and climate change. A large number of medicinal plants are already at the risk of extinction. Plant tissue culture is an emerging tool for the propagation and conservation of plants that are listed as endangered or rare. *In vitro* propagation and preservation of medicinal plants, especially natural plants, and their extracts are efficient and the most suited alternative solution to the problems faced by the phytopharmaceutical industry. They provide a ready source of sterile, uniform and compatible plant material (Banerjee and Shrivastava, 2008).

Synthetic seed technology is an emerging and broadly used technique in the field of plant biotechnology to conserve germplasm of the endangered and economically important plants (Ikhlaq *et al*., 2010). It greatly aids in germplasm exchange programs and storage of viable and productive tissues (Mehrotra *et al*., 2012). Synthetic seeds (synseeds) produced through encapsulation technique have the ability to produce an entire plant after storage at room temperature or under low temperatures; above 0ºC (Gantait *et al*., 2015). They have great advantages in the field of plant conservation, because of their small size, minimum requirements of space, time and care, virus free germplasm, low cost of production, ready

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availability, ease of handling and transporting, and also produce viable and genetically identical plants even after long term storage (Mishra *et al*., 2011). In the beginning, synthetic seeds for germplasm conservation were prepared from somatic embryos. However, recently synthetic seeds were mostly prepared from shoot tip explants, because of their effectiveness for *in vitro* regeneration and having great mitotic activity of the meristem, when compared to other parts of the plants (Ballester *et al*., 1997).

Bacopa monniera (L.) Pennell, commonly known as "Brahmi", is a member of family Scrophulariaceae. It is a rare perennial creeping herb grows in wetlands and muddy shores and found in Egypt; mainly in wet and marshy areas of the eastern Mediterranean coast in North Sinai (Boulos, 2009). *B. monnieri* has a great medicinal value. It is considered the main rejuvenating herb for nerve and brain cells; therefore, has a very important role for the treatment of the neurological and cognitive disorders of aging (Russo and Borrelli 2005 and Aguiar and Borowski, 2013), especially, Alzheimer's disease (Goswami *et al*., 2011). It is the second most important medicinal plant in India according to its medicinal importance, commercial value and potential for further research and development (Anonoymous, 1997). It contains many active compounds; mainly saponins such as bacosides A, B, C and D, which are the active triterpenoid principles that are known as ''memory chemicals'', alkaloids (nicotine, brahmine and herpestine), and other chemicals; such as stigmastanol, bsitosterol and stigmasterol (Russo and Borrelli, 2005).

The plant is extensively investigated for its pharmacological and therapeutic effects; mainly memory, learning and intelligence improvement (Singh *et al*., 2015), and in treatment of neurological diseases like epilepsy, insomnia, asthma, insanity, depression, psychosis and stress (Russo and Borrelli, 2005 and Pandareesh *et al*., 2016). It is reported to have anti-inflammatory, analgesic, antipyretic, sedative (Hossain *et al*., 2014), antioxidant (Simpson *et al*., 2015 and Sabina *et al*., 2016) and hepatoprotective (Sumathi and Devaraj, 2009) effects. The plant has also been used to treat cardiac, digestive and respiratory disorders (Channa *et al*., 2003). Moreover, it is known to have anticancer properties (Patil *et al*., 2014). Beside its various medicinal uses, *B. monnieri* has also been utilized in phytoremediation programs, due to its ability to remove heavy metals (Mehta *et al.*, 2012).

Because of the rapid expansion of the pharmaceutical industry coupled with the popularity of Brahmi based drugs, the pharmaceutical requirement of this plant has increased dramatically. Therefore, it is very important to conserve this already rare herb. Furthermore, their natural regeneration is limited by death at two-leaf stage and submerged shoots can hardly attain the required growth and multiplication. Its *ex situ* conservation in the seed bank is limited due to insufficient seed availability, and propagation through seeds is low due to short seed viability and frequent seedling death. Also, vegetative propagation of *B. monnieri* is slow (Tiwari and Singh, 2010). Therefore, there is a need to conserve the wild stocks of this medicinally important herb to reduce the exploitation of its natural populations and increase the supply of plant materials for pharmaceutical industry.

From the literature, there are many studies that have reported *in vitro* multiple shoot induction and regeneration of *B. monnieri*, using various explants and different culture media and growth regulators; such as the recent studies of Behera *et al*. (2015), Gupta *et al*. (2015), Nagarajan *et al*. (2015), Karatas *et al*. (2016), Pothiaraj *et al*. (2016) and Wangdi and Sarethy (2016). Although attempts towards its *in vitro* conservation are limited. There are few detailed reports on *B. monnieri* synthetic seeds production, although no studies were carried out without the application of growth regulators or other elicitors. *B. monnieri* regeneration was assessed after storage of alginate encapsulated seeds from nodal segments (Sharma *et al.*, 2012 and Gantait *et al*., 2015) and shoot tips (Bansal and Pandey, 2011 and Rency *et al*., 2016) or both (Muthiah *et al*. 2013) with the use of growth regulators or elicitors, either in the alginate matrix or the conversion medium. Attention should be paid to conserve this plant and improve the technology to achieve more success with the least cost, to meet the growing demand of its raw material. Thus, this study aimed to *in vitro* preserve *B. monnieri* through encapsulation technique, to conserve the wild stocks of this medicinally important rare plant in Egypt. It reports the effect of synthetic seeds production without growth regulators, for cost reduction, on viability and regrowth performance of alginate-encapsulated shoot tips for six months.

MATERIALS AND METHODS

1. Plant material

Actively growing shoots of *B. monnieri* were collected from a wild population grown in the eastern Mediterranean coast of North Sinai in Egypt. *In vitro* cultures of *B. monnieri*, from shoot tips and stem nodal segments, were initiated and subcultured every four weeks for multiplication on Murashige and

Skoog (MS) (Murashige and Skoog, 1962) medium (Duchefa, Haarlem, the Netherlands) containing 0.1 mg/L myo-inositol and 3% sucrose and supplemented with 4.44 µM 6-benzylaminopurine (BAP) and 0.53 µM naphthalene acetic acid (NAA) (Sigma Cell Culture, min. 90%, USA). The pH of the medium was adjusted to 5.7-5.8 prior to addition of 2.7 g/L phytagel (Duchefa, Haarlem, the Netherlands) as a solidifying agent. Medium was dispensed into glass jars and autoclaved under a pressure of 1.06 kg/cm^2 and 121°C for 20 min. Cultures were maintained at 24±2°C with a 16 h photoperiod under white cool fluorescent tubes (F140t9d/38, Toshiba).

2. Encapsulation of shoot tips and storage conditions

In vitro-derived shoot tips, obtained from the proliferated shoots, were used for encapsulation. Shoot tips (≤ 5 mm) were encapsulated using 2.5% sodium alginate (Na-alginate; CDH, India) and 100 mM calcium chloride ($CaCl_2.2H_2O$; Merck, Germany) solutions. Encapsulation was carried out by mixing the shoot tips into Na-alginate solution. The alginate-covered shoot tips were then dropped into the $CaCl_2.2H_2O$ solution with an autoclavable micropipette. The beads (each usually containing one or two shoot tips) were left for 20-30 min to ensure polymerization of calcium alginate. After hardening, the encapsulated beads were rinsed with sterilized distilled water for three times. The capsules had an average diameter of about 7 mm (Photo 1). In order to achieve efficient storage, encapsulation solution was prepared using different Na-alginate matrix compositions; 2.5% Na-alginate in distilled water, MS medium and MS medium + 3% sucrose.

Encapsulated shoot tips with different Na-alginate matrixes were stored in Petri dishes containing water agar medium (0.7% w/v agar; Duchefa, Haarlem, the Netherlands) for six months. Two different temperatures of 24±2 and 4°C (in the dark) were tested for storage. Non-encapsulated shoot tips were treated as control.

3. Recovery of encapsulated shoot tips

Every one month, encapsulated shoot tips were cultured on MS medium without growth regulators for the recovery. Percentage of viability, regrowth, mean number and length (cm) of shoots per explant, in addition to rooting percentage were evaluated, for a period of six months, after four weeks from culturing. Rooted plantlets were carefully separated from the medium and washed with distilled water to remove excess medium. The healthy root induced plantlets were transferred to small pots containing a mixture of peatmoss and sand at a 1:1 ratio. The pots were covered with transparent plastic bags with minute holes to maintain humidity and placed inside the greenhouse. After 2 weeks, covers were removed for further growth at natural conditions and plantlets were irrigated with tap water every two days. The survival rate of plantlets was calculated a month after transfer to the greenhouse.

4. Experimental design and data analysis

The experiment was subjected to the completely randomized design. Treatments were represented by twenty replicates and the experiment was repeated twice. Variance analysis of data was carried out using ANOVA program for statistical analysis. Means were compared by using Duncan´s multiple range test (Duncan, 1955) at $p \leq 0.05$.

RESULTS AND DISCUSSION

1. Effect of storage temperature and alginate matrix on the recovery of encapsulated shoot tips

The encapsulation of *in vitro* regenerated shoot tips *via* Na-alginate has been proved to be the most efficient technique for mass propagation of plants as well as short term conservation of important germplasms (Kulus and Zalewska, 2014). Based on the previous reports, shoot tip explants are the best source for *in vitro* regeneration of *B. monnieri* (Rency *et al*., 2016), and are the most effective explants for synthetic seeds preparation, because of the great mitotic activity of the meristem (Ballester *et al*., 1997).

Suitable storage temperature plays a vital role in preservation of synthetic seeds. Two different temperatures of 24±2 and 4°C were tested for storing the encapsulated shoot tips. Results showed that the beads stored at 24±2°C started sprouting within a week, and thus this temperature was found to be inefficient for storage. This observation was not occurred in the beads stored at 4°C. According to this observation, further storage of encapsulated shoot tips was carried out at 4°C for six months. Previously,

4°C was very promising for low-temperature *in vitro* preservation of *B. monnieri* (Muthiah *et al*., 2013), and other plants (Faisal and Anis, 2007; Adhikari *et al*., 2014 and Saha *et al*., 2015).

The composition of the alginate matrix used for encapsulation is a crucial factor in plant regeneration after storage. The proper optimization of the alginate matrix composition improves the regrowth performance of encapsulated shoot tips and consequently the technique could be more efficient and applicable. Non-encapsulated and encapsulated shoot tips of *B. monnieri* in 2.5% Na-alginate and 100 mM $CaCl_2.2H_2O$ exhibited shoot formation within one week after transferring them to regeneration medium (Photo 2). All tested Na-alginate matrix compositions demonstrated 100% viability of encapsulated shoot tips after six months of storage (Table 1). However, regrowth percentage and the number and length of regenerated shoots were lower in encapsulated shoot tips compared to non-encapsulated ones (Photo 3a), because the non-encapsulated shoot tips are directly in contact with the nutrient medium.

The Na-alginate matrix containing MS medium demonstrated significant superiority over that containing distilled water (Photo 3b) with respect to regrowth percentage and shoot number and length. This treatment gave a percentage of regrowth of 85.2% of encapsulated shoot tips, produced 7 shoots per explant with a length of 5.27 cm, after six months of storage (Photo 3c). This result confirms that MS nutrients are essential ingredients of Na-alginate matrix for regrowth and regeneration. Gelling matrix containing nutrient ingredients served as "artificial endosperm", that provides nutrients to the encapsulated explants for regrowth (Bapat and Rao, 1992).

Table 1. Effect Na-alginate matrix composition on the viability and regrowth performance of encapsulated shoot tips of *Bacopa monnieri.* Data was recorded after four weeks from culturing following six months of storage (non-encapsulated shoot tips were served as a control).

Alginate matrix composition	Viability %	Regrowth %	Mean no. of shoots/explant	Mean shoot length (cm)	Rooting %
Non-encapsulated shoot tips	100	100.0[a]	18.00[a]	7.30[a]	100
Distilled water	100	73.7[d]	1.33[d]	4.27[d]	100
MS medium	100	85.2[c]	7.00[c]	5.27[c]	100
MS medium + 3% sucrose	100	93.3[b]	10.00[b]	6.00[b]	100

The regrowth percentage and shoot number and length gave their maximum values when sucrose was supplemented in the nutrient medium (Photo 3d). Using the MS medium containing normal sucrose concentration for encapsulation, 93.3% of encapsulated shoot tips regrew 10 shoots per explant with a mean length of 6 cm, after six months of storage. These findings proved that sucrose is an essential component in the alginate matrix. The nutrient ingredients and sugar of the alginate matrix of the encapsulated explants are of key importance for their storage and regrowth efficiency (Tsvetkov *et al*., 2006). Sucrose is a carbohydrate and energy source in plant tissue culture systems (Bhojwani and Razdan, 1996). It is known to provide a carbon source for *in vitro* propagated tissues, and its incorporation in the alginate matrix of encapsulated explants enhanced plant recovery and regrowth performance (Danso and Ford-Lloyd, 2003).

2. Effect of storage duration on the recovery of encapsulated shoot tips

The most important feature of the encapsulated explants is their ability to remain viable and regrow after storage. Low temperature and high humidity were essential for retention of the viability of encapsulated shoot tips and thus for their storage. Response for the regrowth of encapsulated shoot tips was slightly affected by increasing storage duration at 4˚C, until six months. Regrowth percentage and the mean number and length of shoots produced from the encapsulated shoot tips declined following storage at 4˚C, during the storage duration for each tested Na-alginate matrix composition (Figure 1-4).

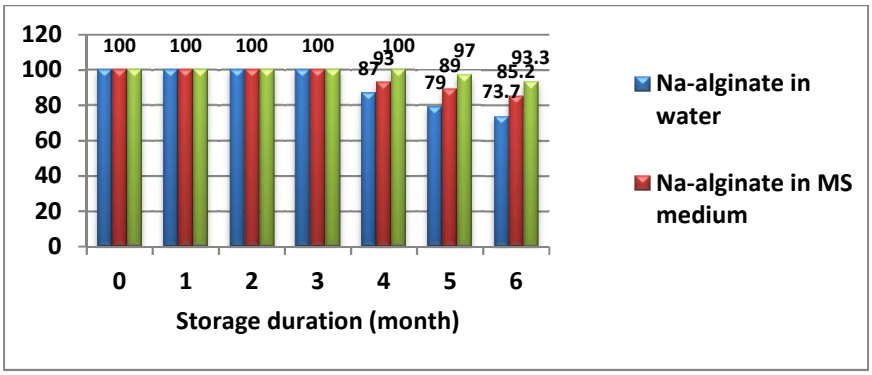

Figure 1. Effect of different storage durations on regrowth percentage of encapsulated shoot tips of *Bacopa monnieri* in Na-alginate of different tested matrixes, after four weeks from culturing.

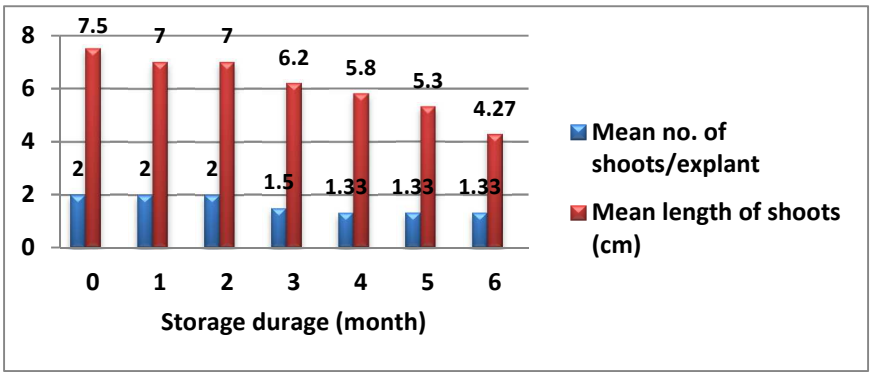

Figure 2. Effect of different storage durations on regrowth performance of encapsulated shoot tips of *Bacopa monnieri* in Na-alginate matrix containing water after four weeks from culturing.

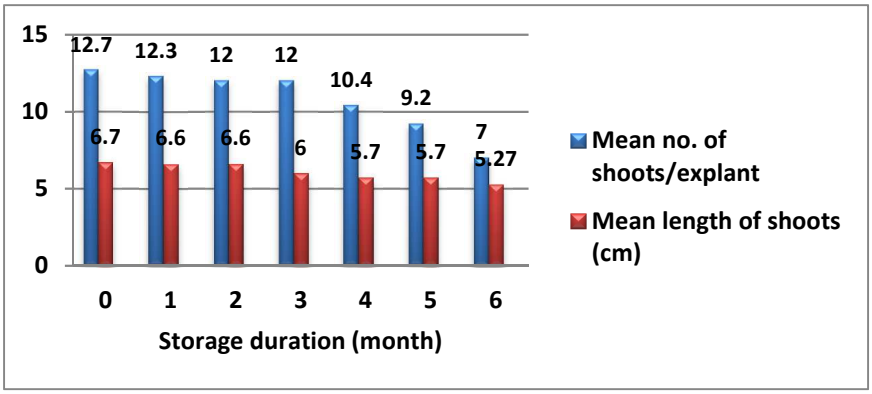

Figure 3. Effect of different storage durations on regrowth performance of encapsulated shoot tips of *Bacopa monnieri* in Na-alginate matrix containing MS medium after four weeks from culturing.

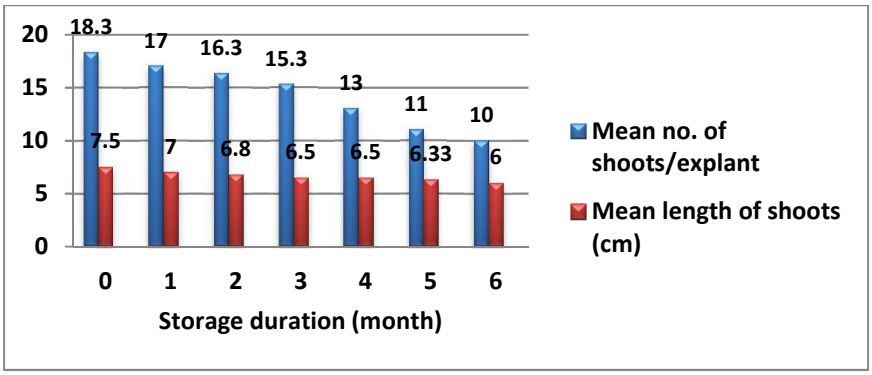

Figure 4. Effect of different storage durations on regrowth performance of encapsulated shoot tips of *Bacopa monnieri* in Na-alginate matrix containing MS medium with 3% sucrose after four weeks from culturing.

It is supposed that the decline in the growth parameters, observed among encapsulated shoot tips during storage for longer durations, may be a result of the inhibition in the respiration of tissues by the alginate cover due to oxygen deficiency; or a loss of moisture content due to partial desiccation of tissues (Danso and Ford-Lloyd, 2003 and Faisal and Anis, 2007). Also, Bazinet *et al*. (1992) found that plant regeneration rate after *in vitro* preservation was reduced by loss of viability of tissues caused by mechanical constraints or diffusional limitation.

All the plantlets had the potential to produce roots and they were not affected by the storage duration. The rooting of the plantlets regrown from encapsulated shoot tips after storage proved the efficiency of the *in vitro* preservation potential. Hardening in the greenhouse was carried out after one month from culturing. About 93% of the acclimatized plantlets remained healthy in natural conditions (Photo 4).

Conclusion

The present study describes a detailed promising and efficient germplasm preservation method for conserving the rare multipurpose *B. monnieri* plant. In order to obtain about 93% of regrown *B. monnieri* plants, with high regrowth performance, from encapsulated shoot tips after storage at 4°C for six months, 2.5% Na-alginate solution (containing MS nutrient medium with 3% sucrose) and 100 mM calcium chloride ($CaCl_2.2H_2O$) should be used as encapsulation matrix.

This protocol can be applied as an effective solution for the mass conservation of the plant with minimum requirements, without any subculturing, for the period of six months. This could facilitate germplasm exchange between laboratories and transportation to different parts of the world, while maintaining their viability and growth can resume immediately after culture. This research opens new prospects for further studies on this valuable Egyptian plant to preserve its elite tissues for longer periods of time.

Photo 1. Calcium alginate encapsulated shoot tips of *Bacopa monnieri.*

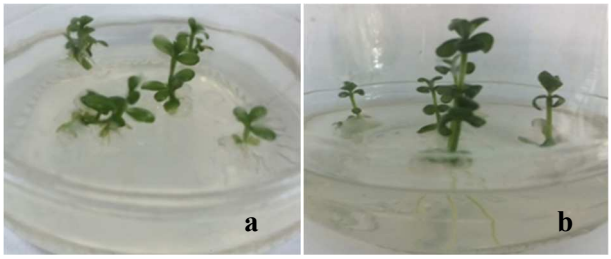

Photo 2. Sprouting of encapsulated shoot tips *Bacopa monnieri* after **a.** one week and **b.** two weeks from culturing.

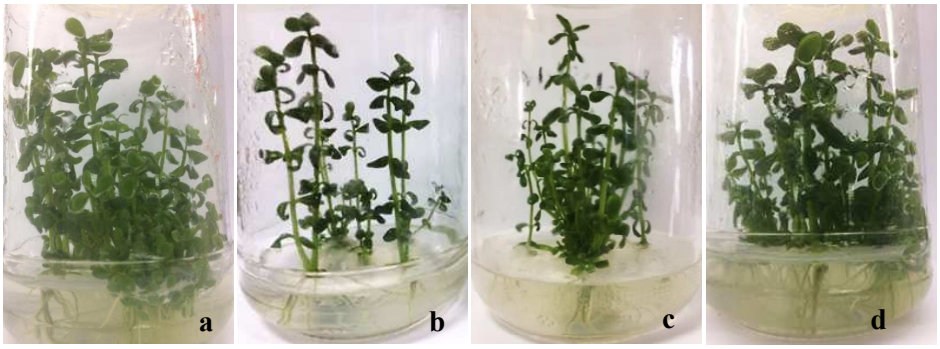

Photo 3. Regrown plantlets with healthy shoots and roots from shoot tip-derived synthetic seeds of *Bacopa monnieri* in different Na-alginate matrix compositions after four weeks from culturing following six months of storage at 4°C. **a.** non-encapsulated shoot tips (control), **b.** encapsulated shoot tips in distilled water, **c.** encapsulated shoot tips in MS medium, **d.** encapsulated shoot tips in MS medium containing 3% sucrose.

Photo 4. Acclimatized plantlets from encapsulated shoot tips of *Bacopa monnieri* after two months from transfer to the greenhouse following six months of storage at 4 °C.

REFERENCES

Adhikari, S., Bandyopadhyay, T.K. and Ghosh, P. (2014). Assessment of genetic stability of *Cucumis sativus* L. regenerated from encapsulated shoot tips. Scienta Horticulturae, 170: 115–122.

Aguiar, S. and Borowski, T. (2013). Neuropharmacological review of the nootropic herb *Bacopa monnieri*. Rejuvenation Research, 16(4): 313-326.

Anonoymous (1997). Indian Medicinal Plants: A Sector Study. Occasional paper No. 54. Export-Import Bank of India, Quest publications, Bombay, India.

Ballester, A., Janeiro, L.V. and Vieitez, A.M. (1997). Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* L. and *Camellia reticulata* Lindley. Scienta Horticulturae, 71: 67‑78.

Banerjee, M. and Shrivastava, S. (2008). An improved protocol for *in vitro* multiplication of *Bacopa monnieri* (L.). World Journal of Microbiology and Biotechnology, 24: 1355‑1359.

Bansal, Y.K. and Pandey, S. (2011). *In vitro* regeneration of 'Brahmi' (*Bacopa monnieri* L. Pennel) and synthetic seed formation. Flora and Fauna (Jhansi), 17(2): 195-201.

Bapat, V.A. and Rao, P.S. (1992). Plantlet regeneration from encapsulated and non-encapsulated desiccated somatic embryos of forest tree: sandalwood (*Santalum album* L.). Journal of Plant Biochemistry and Biotechnology, 1: 109-113.

Bazinet, C., Kersulec, A., Dufrene, V., Timbert, R., Hervagault, J.F. and Barbotin, J.N. (1992). Physiological somatic embryos (*Daucus carota* L.) behaviour depending on the storage conditions. Biotechnology, 92: 139.

Bhojwani, S.S. and Razdan, M.K. (1996). Plant Tissue Culture: Theory and Practice. Elsevier, Amsterdam.

Behera, S., Nayak, N., Shasmita, Barik, D.P., Naik, S.K. (2015). An efficient micropropagation protocol of *Bacopa monnieri* (L.) Pennell through two-stage culture of nodal segments and *ex vitro* acclimatization. Journal of Applied Biology and Biotechnology, 3(3): 16-21.

Boulos, L. (2009). Flora of Egypt Checklist: Revised Annonated Edition, Alhadara Publishing, Egypt.

Channa, S., Dar, A., Yaqoob, M., Anjum, S., Sultani, Z. and Rahman, A. (2003). Broncho-vasodilatory activity of fractions and pure constituents isolated from *Bacopa monnieri.* Journal of Ethnopharmacology, 86: 27–35.

Danso, K.E. and Ford-Lloyd, B.V. (2003). Encapsulation of nodal cuttings and shoot tips for storage and exchange of cassava germplasm. Plant Cell Report, 21: 718-725.

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

Faisal, M. and Anis, M. (2007). Regeneration of plants from alginate-encapsulated shoots of *Tylophora indica* (Burm. f.) Merrill, an endangered medicinal plant. Journal of Horticultural Science and Biotechnology, 82(3): 351–354.

Gantait, S., Kundu, S. and Nasim, M.A. (2015). Influence of encapsulating agent and matrix levels on synseed production of *Bacopa monnieri* (L.) Pennell. Medicinal Plants. International Journal of Phytomedicines and Related Industries, 7(3): 182-187.

Goswami, S., Saoji, A., Kumar, N., Thawani, V., Tiwari, M. and Thawani, M. (2011). Effect of *Bacopa monnieri* on cognitive functions in Alzheimer's disease patients. International Journal of Collaborative Research on Internal Medicine and Public Health, 3 (4).

Gupta, R., Thomas, G. and Kajla S. (2015). Effect of different growth regulators on *in vitro* multiplication of brahmi (*Bacopa monnieri*). Annals of Biology, 31(1): 36-40.

Hossain, H., Al-Mansur, A., Akter, S., Sara, U., Ahmed, M.R. and Jahangir, A.A. (2014). Evaluation of anti-inflammatory activity and total tannin content from the leaves of *Bacopa monnieri* (Linn.). International Journal of Pharmaceutical Sciences and Research, 5(4): 1246-1252.

Ikhlaq, M., Hafiz, I.A., Micheli, M., Ahmad, T., Abbasi, N.A. and Standardi, A. (2010). *In vitro* storage of synthetic seeds: effect of different storage conditions and intervals on their conversion ability. African Journal of Biotechnology, 9: 5712-5721.

Kulus, D. and Zalewska, M. (2014). Cryopreservation as a tool used in long term storage of ornamental species-a review. Scienta Horticulturae, 68: 88-107.

Karatas, M., Aasim, M. and Dazkirli, M. (2016). Influence of light-emitting diodes and benzylaminopurin on adventitious shoot regeneration of water hyssop (*Bacopa monnieri* (L.) Pennell) *in vitro*. Archives of Biological Sciences, 68(3): 501-508.

Mehrotra, S., Khwaja, O., Kukreja, A.K. and Rahman, L. (2012). ISSR and RAPD based evaluation of genetic stability of encapsulated micro shoots of *Glycyrrhiza glabra* following 6 months of storage. Molecular Biotechnology, 52: 262-268.

Mehta, J., Ansari, R., Syedy, M., Khan, S., Sharma, S., Gupta, N., Rathore, R. and Vaishnav, K. (2012). An effective method for high frequency multiple shoots regeneration and callus induction of *Bacopa*

monnieri (L.) Pennel: An important medicinal plant. Asian Journal of Plant Science and Research, 2(5): 620-626.

Mishra, J., Singh, M., Palni. L.M.S. and Nandi, S.K. (2011). Assessment of genetic fidelity of encapsulated microshoots of *Picrorhiza kurrooa*. Plant Cell, Tissue and Organ Culture, 104: 181-186.

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

Muthiah, J.V.L., Shunmugiah, K.P. and Manikandan, R. (2013). Genetic fidelity assessment of encapsulated *in vitro* tissues of *Bacopa monnieri* after 6 months of storage by using ISSR and RAPD markers. Turkish Journal of Botany, 37(6): 1008-1017.

Nagarajan, T., Alagumanian, S., Jahirhussain, G. and Subbaiya, S. (2015). *In vitro* mass propagation of *Bacopa monnieri* (Linn.) Wettst from nodal explant-a multipurpose medicinal plant. World Journal of Pharmaceutical Research, 4(12): 1970-1982.

Pandareesh, M.D., Anand, T. and Khanum F. (2016). Cognition enhancing and neuromodulatory propensity of *Bacopa monniera* extract against scopolamine induced cognitive impairments in rat hippocampus. Neurochemical Research, 41(5): 985-999.

Patil, A., Vadera, K., Patil, D., Phatak, A., Juvekar, A. and Chandra, N. (2014). *In vitro* anticancer activity and phytochemical analysis of *Bacopa monnieri* (L.) Wettst. International Journal of Pharmaceutical Sciences and Research, 5(10): 4432-4438.

Pothiaraj, G., Ebenezer, R.S., Christdas, E.J. and Shakila, H. (2016). Comparative analysis on the effect of seaweed liquid extracts and commercial plant growth regulators on *in vitro* propagation of *Bacopa monnieri*. International Journal of Research in BioSciences, 5(2): 1-9.

Russo, A. and Borrelli, F. (2005). *Bacopa monnieri*, a reputed nootropic plant: an overview. Phytomedicine, 12: 305–317.

Rency, A.S., Satish, L., Pandian, S., Rathinapriya, P. and Ramesh, M. (2016). *In vitro* propagation and genetic fidelity analysis of alginate-encapsulated *Bacopa monnieri* shoot tips using *Gracilaria salicornia* extracts. Journal of Applied Phycology, DOI 10.1007/s10811-016-0918-0

Sabina, E.P., Lavinya, B.U., Nair, A.L., Ajith, G., Ammu, U.M. and Arumugam, G. (2016). Antioxidant potential of indigenous medicinal plant *Bacopa monnieri*: an *in vitro* evaluation. Journal of Chemical and Pharmaceutical Research, 8(5): 371-375.

Saha, S., Sengupta, C. and Ghosh, P. (2015). Encapsulation, short-term storage, conservation and molecular analysis to assess genetic stability in alginate-encapsulated microshoots of *Ocimum kilimandscharicum* Guerke. Plant Cell, Tissue and Organ Culture, 120(2): 519–530.

Sharma, M., Raina, H., Verma, V., Mallubhotla, S. and Ahuja, A. (2012). Synthetic seeds a viable approach for conservation and propagation of phytoremediant herb: *Bacopa monnieri* (L.) Wettst. Journal of Environmental Research and Development, 7(1A): 399-404.

Simpson, T., Pase, M. and Stough, C. (2015). *Bacopa monnieri* as an antioxidant therapy to reduce oxidative stress in the aging brain. Evidence-Based Complementary and Alternative Medicine, Article ID 615384.

Singh, R., Rachumallu, R., Bhateria, M., Panduri, J. and Bhatta, R.S. (2015). *In vitro* effects of standardized extract of *Bacopa monniera* and its five individual active constituents on human P-glycoprotein activity. Xenobiotica, 45(8): 741-749.

Sumathi, T. and Devaraj, S.N. (2009). Effect of *Bacopa monnieri* on liver and kidney toxicity in chronic use of opioids. Phytomedicine, 16: 897–903.

Tiwari, K.N. and Singh J. (2010). Effective organogenesis from different explants of *Bacopa monnieri* L. (Wettst.) - an important medicinal plant. Biological Forum, 2(1): 18-22.

Tsvetkov, I., Jouve, L. and Hausman, J. F. (2006). Effect of alginate matrix composition on regrowth of *in vitro*-derived encapsulated apical microcuttings of hybrid aspen. Biologia Plantarum, 50: 722-724.

Wangdi, K. and Sarethy, I.P. (2016). Evaluation of micropropagation system of *Bacopa monnieri* L. in liquid culture and its effect on antioxidant properties. Journal of Herbs, Spices and Medicinal Plants, 22(1): 69-80.