Artificial Seed Production from Encapsulated Foliar Regenerated Protocorm like Bodies of *Rhynchostylis Gigantea*: A Study *in vitro*

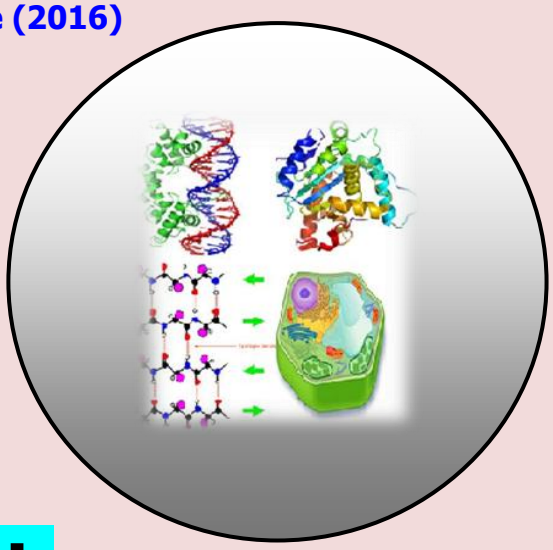
By

Vishal Sharma and Harsh Manchanda

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 35 (2) 2018 Pages No. 861-867



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 35, No. 2: 861-867, 2018
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/25/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

Dr. Vishal Sharma
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER
Received: 14/10/2018 Revised: 26/11/2018 Accepted: 10/12/2018

Artificial Seed Production from Encapsulated Foliar Regenerated Protocorm like Bodies of *Rhynchostylis Gigantea*: A Study *in vitro*

Vishal Sharma and Harsh Manchanda
Botany Department, Post Graduate Government College for Girls-11, Chandigarh, India

ABSTRACT

Somatic embryos (=PLBs) are the most common micropropagule used for the encapsulation purposes in production of artificial seeds. Artificial seed development is an effective and alternative method of clonal propagation in recalcitrant species, its germplasm conservation and prolonged storage without losing viability. The conversion frequency of artificial seeds shows inverse relationship with the period and storage temperature. The artificial seeds stored at 4°C showed better re-growth and prolong preservation than those stored at 25°c, attributed to low metabolic rate. Artificial seeds are easy to handle, ensure economy of space, and also reliable for plant breeders for clonal germplasm conservation. An isodiametric, spherical, firm beads are formed in a gelling matrix of 3% sodium alginate and 100 mM calcium chloride and these artificial seeds stored at 4°c are inoculated on agar gelled nutrient medium (BM) after 0, 15, 30, 45, 60, 120, 150, 180 days, with conversion frequencies 84.38 ± 3.12; 84.38 ± 3.12; 78.13 ± 3.12; 78.13 ± 3.12; 75; 75; 59.4 ± 3.12; 31.25 respectively. However, at 25°C, the re-growth occurs in 81.25 ± 6.25; 81.25 ± 6.25; 75 ± 6.25; 43 ± 6.25 & 37.5% respectively. When artificial seeds are coated with sterilized talcum powder, showed positive effect on shelf-life, however, the conversion frequency is hampered at both temperature regime (4°c and 25°c). The regenerated plantlets are accilimatized and transferred to pots filled with moss, pinebark, brick and charcoal pieces (1:1:1:1) mixture with 90% success.
Keywords: Orchid, Artificial Seeds, Tissue Culture, Protocrom like bodies and in vitro.

INTRODUCTION

The orchids constitute one of the largest & diverse family of flowering with 30,000-35,0000 species in 600-800 genera (Sarmah *et al*., 2017). Orchids are not only important in terms of economical and aesthetic aspects but the ecological specialization contributed to the great species diversity. Despite the fact that orchids produce large number of seeds and are bestowed with an inherent potential of vegetative reproduction ,they do not form dominant vegetation anywhere in the world due to its reliance and complex ecological interaction on insect pollination and suitable mycorrhizal association, hence orchids are the first biological indicators of ecosystem decay and progressively losing its natural habitat and is getting rarer with every passage of time due to poor regeneration and extensive anthropogenic pressures and figures prominently in the red data book prepared by International Union for Conservation of Nature and Natural Resources (IUCN, 1991). Tissue culture technique ias an alternative technology to propage recalcitrant species. Murashige (1978) pioneered the concept of 'artificial seeds' for the clonal propagation by encapsulating embryoids in nutrient gel. Artificial seeds, an analog to natural botanic seed, opened new avenue for multi-clone commercial production.

Synseeds' are easy to store and transport, cost effective and as a cheap alternative to cryopreservation; it contributed in germplasm preservation of recalcitrant species. Earlier the concept of artificial seed production is restricted to somatic embryogenetic plant species. Recently, various plant materials i.e. shoot tips, Axillary buds, nodal segments. microshoots and embryogenic callus are used (Ara *et al*.,2000; Mandal *et al*., 2000; Rai *et al*., 2008; Chand and Singh, 2004; Ahmad and Anis, 2010; Sharma and Shahzad, 2012; Rihan *et al*., 2011; Rihan *et al*., 2017).

Rhynchostylis gigantean (Lindl.) Ridl., native of Thailand, distributed in the tropical climates from India eastwards to Philippines. Its taxonomic identity is often mistaken with *R. retusa* from which it differs in having robust morphology, hence also called giant orchid. *R. gigantean* (Chang Phueak orchids) is most valuable fox tail orchid, which bears beautiful flowers with attractive citrus scent, is prodominatly used as ornamental plant. *R. gigantea* has been used as progenitor of high profile inter specific to inter generic hybrids. Besides being victim of its own beauty and utility *R. gigantea* is progressively losing its natural habitat and is getting rarer with every passage of time and figures prominently in AppendixII of the Convention on International Trade in Endangered species of Wild fauna and flora (CITES, 2012, 2017).The production of artificial seeds is reported in several plant species (Rihan *et al*., 2017), however, in orchids the present study is the pioneer attempt in the encapsulation *of prtocorm like bodies(PLBs)* in *R. gigantea*.

MATERIAL AND METHODS

Explant Material

Somatic embryos (=PLBs) are the most common micropropagule used for the encapsulation purposes in production of artificial seeds (=Synseeds) .Uniform size (5mm) organogenetic PLBs are harvested from the *in vitro* inoculated foliar explants of *R. gigantea* Lindl. plants, which are collected in nature from Garhwal Himalayas eastwards to Arunachal Pradesh(1000-1800m) and grown under greenhouse conditions at Punjab University, Chandigarh.

Sterilization Method

The foliar explants harvested from stock plants are first washed in running tap water with 2% teepol for 15 minutes which is followed with sequentially sterilization with solutions of Streptomycin (0.1%, 20 min). Sodium hypochlo--rite (4%, 15min) and ethanol (70%, 3sec) before rinsing thrice with sterilized distilled water.

Media and culture conditions

Excised foliar explants(<0.75 cm long) are used as explants and inoculated on sucrose (2%) supplemented and agar(0.9%) gelled basal medium(BM: Mitra *et al* 1976) and its various combinations with alone BAP (6-Benzyl amino purine; 0.5-5mg/), KN (Kinetin; 0.5-5mg/l) or in combination with NAA (α-naphthalene acetic acid; 1 mg/l) for production of protocorm like bodies (PLBs). The pre-inoculation medium pH was adjusted at 5.6.In parallel set of experiments 0.2% activated charcoal (AC) is used in the medium. Sixteen replicates for each treatment and the experiments were repeated a four times. All experimental manipulations are done under aseptic conditions and the cultures incubated at 25±2^{0}C under 12 hr photoperiod of 3500 lux light intensity.

Preparation of Artificial Seeds

The preparation of the artificial seeds initiated with the dispersion of uniform size (5mm) PLBs in the sodium alginate (2, 2.5,3 and 4 %) solutions in the BM medium for 10 minute. The mixture is pipette dropped using pasteur pipette individually into the different concentrations of calcium chloride ($CaCl_2 2H_2O$: 50, 75, 100 mM) solutions, which was constantly agitated with Teflon coated magnetic stirrer (Table 1). When sodium alginate drops come in contact with calcium chloride solution, surface complexation begins and firm round beads are formed with one PLB. The blobs are allowed to complex in solution for time period ranging between 10-40 min (Table2). It is observed that the PLBs encapsulated with 2-2.5% alginate and exposed to 50-100mM $CaCl_2$ $2H_2O$ solution produced fragile, leaking and tailed beads. However, the spherical, isodiametric and non-leaky beads are formed by using 3% sodium alginate and 100mM $CaCl_2$ $2H_2O$ with 40 minutes of complexation time (Table2; Fig.1) and subsequently the beads are picked up with the help of spatula dried on a filter paper. After encapsulation, the artificial seeds were preserved at 4^{o}C and 25^{o}c for 15, 30, 45, 60, 120, 150 and 180 days respectively. Agar gelled nutrient (Mitra *et al.,* 1976) medium and sterilized talcum powder are used as the sowing substratum. The liquid medium pH is adjusted at 5.6. All the experimental manipulations are done under aseptic conditions and the cultures incubated at 25± 2^{O}C under 12 hr photoperiod of 3500 lux light intensity, are regularly observed.

Data recording

The cultures are observed on every fifth day and the response of artificial seeds to different chemical stimuli and temperature regime is recorded to study different parameters i.e. regeneration frequency, number of proliferative loci/synseed and the time required for artificial seed initiation, organogenesis and subsequent morphogenesis.

Acclimatization of the Plantlet

Plantlets (3cm) tall are transferred to semisolid medium containing only half strength macro and micro salts of BM (Mitra *et al*, 1976 medium; Fig.5) and subsequently the sucrose and vitamins are elimated. The palntlets are maintained till they are 4-5cm tall and after that washed acclimatized plantlets are transferred to moss, pinebark, brick and charcoal pieces (1:1:1:1) mixture covered with poly bags. The holes of increasing size are made at regular interval onto poly bags, to reduce the humidity level gradually. The bags were removed after 4 weeks and hardened plants are transferred from 90% shade to the sunlight. Fortnightly spray with Bavistin (1%) is done to keep fungus off from the young plants& 90% survival rate is observed.Fig.6shows an acclimatized plantlet.

RESULT

In present investigation, an attempt have been made to produce synthetic seeds by encapsulating protocorm like bodies(PLBs) in *R. gigantea* with a view to save propagules from stresses ,strains and injuries during long distance transport. The synthetic seeds of sodium alginate(3%) and 100 mM calcium chloride was found to be most suitable for formation of firm, clear and isodiametric ideal beads (Fig1;Table 1), however, lower concentrations of sodium alginate (2%) and calcium chloride (50 -75 mM), beads were fragile and tailed beads. The freshly formed 'Synseeds' shows 84.38 ± 3.12 and 81.25 ± 6.25 conversion frequency respectively at 4^{o}C and 25^{o}c (Fig.2). However, with every 15 days passage they showed progressive decline i.e. after 0, 15, 30, 45, 60, 120, 150, 180 days of storage at 4^{o}c in agar gelled nutrient medium(BM),the frequencies to plantlet conversion are 84.38 ± 3.12; 84.38 ± 3.12; 78.13 ± 3.12; 78.13 ±3 .12; 75;75; 59.4 ± 3.12; 31.25 respectively (Table3). However, at 25^{o}C, the re-growth occurs in 81.25 ± 6.25; 81.25 ± 6.25; 75 ± 6.25; 43 ± 6.25 and 37.5% respectively at 0, 15, 30, 45 and 60 days and the regeneration id indirect somatic embryogenesis (Fig. 4). When these artificial seeds are coated with sterilized talcum powder and inoculated on the agar gelled medium after storage at different temperature regime (4^{o}c and 25^{o}c) showed positive effect on shelf-life, however, the conversion frequency is hampered i.e. 84.38 ± 3.12;78.13 ± 3.12; 78.13 ± 3.12; 75;75; 59.4 ± 3.12; 31.25; 31.25 at 4^{o}C, however, at 25^{o}C, despite the fact that the conversion frequency hampered, the conversion ability of 'artificial seeds' is prolonged and showed 81.25 ± 6.25; 75 ± 6.25; 43 ± 6.25; 37.5; 37.5; 25 respectively at 0, 15, 30, 45,60, 120, 150, 180 days, however, talcum treated 'Synseeds' retained viability after 120 days of storage (Fig. 3; Table3).

Table 1. Effect of different concentration of sodium alginate and dihydrate Calcium chloride on beads formation Sodium Alginate.

$CaCl_2.2H_2O$ (mM)	2.0%	2.5%	3%
50	+	+	++
75	+	+++	+++
100	+	+++	+++

*observation taken after a 40 mins complexation period; + refers to bead quality in terms of shape, size and firmness

Table 2. Effect of treatment time for synthetic seeds formation.

Medium	Treatment time (min)	Quality of 'seeds'	Remarks
Na-Alginate (3%) + $CaCl_2$ $2H_2O$ (100Mm)	10	+	Fragile, leaky and tailed seeds
	20	+++	Fragile, leaky and tailed seeds
	30	+++	Isodiametric soft
	40	++++	Isodiametric compact

DISCUSSION

The attempt to produce synthetic seeds have been done on several plant species, however, there were only few reports in Orchids (Mohan raj *et al*., 2009, Sarmah *et al*., 2010, Gupta, 2016, Rihan *et al*., 2017).

Explants such as shoot tips, axillary buds and somatic embryos are encapsulated in cryoprotectant material like hydrogel, sodium alginate, ethyleneglycol and chitosan. Although a variety of natural and synthetic polymers are available for encapsulation, sodium alginate is the most commonly used gel-matrix because of its easy gelling properties, non-toxicity, less viscosity and low cost (Redenbaugh *et al.,* 1987, Nagananda *et al*., 2011, Pradhan *et al*., 2014, Gupta, 2016, Magray, 2017, Rihan *et al*., 2017). The coating protects explants during handling and allows conversion without inducing variations.

Table 3. Effect of temperature, storage period on the conversion frequency of artificial seeds in *R. gigantea.*

Substratum	Storage Temperature	0	15	30	45	60	120	150	180
Agar-gelled nutrient (Mitra *et al.,*1976)medium	4oc	84.38 ± 3.12	84.38 ± 3.12	78.13 ± 3.12	78.13 ± 3.12	75	75	59.4 ± 3.12	31.25
	25oc	81.25 ± 6.25	87.5 ± 6.25	75 ± 6.25	43 ± 6.25	37.5	-	-	-
Talcum powder coated 'Synseeds"	4oc	84.38 ± 3.12	78.13 ± 3.12	78.13 ± 3.12	75	75	59.4 ±3.12	31.25	31.25
	25oc	81.25 ± 6.25	75 ± 6.25	43 ± 6.25	37.5	37.5	25	-	-

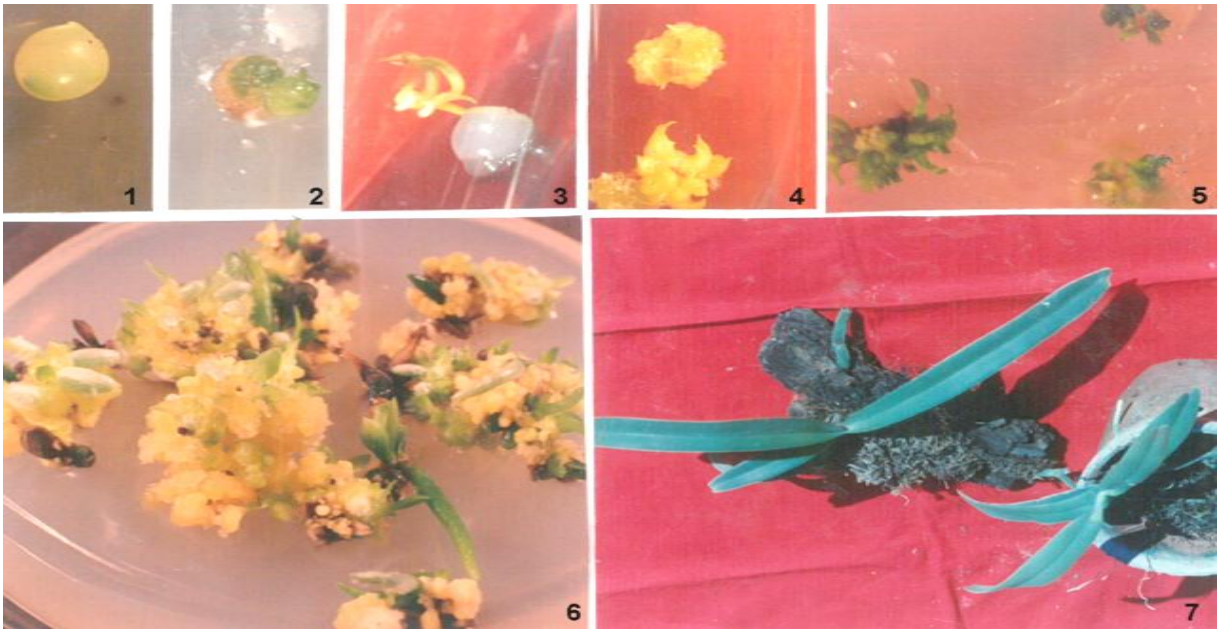


Figure 1. Encapsulation PLBs with 3% Na-alginate in 100mM Cacl2 solution and complexed for 40 min,
Figure 2. Different stages of germination of encapsulated PLBs in BM medium,
Figure 3. Talcum coated 'Synseed' on BM+AC medium,
Figure 4. 'Synseed' showing indirect somatic embryogenesis mediated proliferation,
Figure 5. Acclimatization in half strength BM medium,
Figure 6. Acclimatized plantlet.

In present study, a gelling matrix of 3% sodium alginate and 100 mM calcium chloride was found to be most suitable for formation of firm, clear and isodiametric ideal beads .A similar result have been observed in the encapsulation of PLBs in *Cymbidium giganteum* and *Dendrobium*' Sonia', *Flickingeria Stevia* (Sai Prasad and Polisetty, 2003, Naganada *et al.*, 2011, Ali *et al*., 2012, Gantait *et al*., 2017, Magray *et al*., 2017).

At lower concentrations of sodium alginate (2%) and calcium chloride (50 -75 mM), beads were fragile and tailed beads , too soft to handle and failed to form isodiametric and defined shaped as it did not support the proper ion exchange due to improper ion exchange in compliance with earlier reports (Singh *et al*., 2006, Sarmah *et al*., 2010, Nagananda *et al*., 2011, Asmah *et al*., 2011, however, at higher concentrations of sodium alginate (4% to 5%) and calcium chloride (150 to 200 mM), beads are iso-diametric but were hard enough to cause considerable delay in seed germination, hence not only the concentrations of gelling agents but also complexing duration plays an important role on rigidity and conversion frequency of artificial seeds in compliance with earlier reports (Singh *et al*.,2006,Sarmah *et al*., 2010, Nagananda *et al*., 2011, Asmah *et al*., 2011, Magray *et al*., 2017). The conversion frequency of such 'Synseeds' is observed to vary with the passage of time and their storage i.e. inversely proportional to the period and temperature of storage. The artificial seeds stored at 4^{o}C A showed better re-growth than to those stored at 25^{o}c, attributed to low metabolic rate and 'Synseeds' remained in a quiescent state that prolonged preservation of nutritive reservoir, on contrary artificial seeds stored at room temperature (25^{o}c), showed higher metabolic activities, hence deplete the nutritive endosperm &results in low germination frequency (Table3), in compliance with earlier reports (Bapat and Rao, 1990; Redenbaugh *et al*., 1991; Nieves *et al*., 2001; Ikhaq *et al*., 2010; Rihan *et al*., 2017). In present study, a gelling matrix of 3% sodium alginate and 100 mM calcium chloride was found to be most suitable for formation of firm, clear and isodiametric ideal beads .A similar result have been observed in the encapsulation of PLBs in *Dendrobium*' Sonia', *Flickingeria, Grammatop--hyllum scriptum, Spathoglottis plicata*, *Stevia, Tylophora indica* (Sai Prasad and Polisetty, 2003, Nagananda *et al*., 2011, Ali *et al*., 2012, Gantait *et al*., 2017, Haque *et al*., 2017, Pitoya *et al*., 2017), however, in contrary the higher conversion frequency observed in 4% Sodium Alginate and 75mM dihydrate calcium chloride in *Cymbidium giganteum*, pointed Gourd (Magray *et al*.,2017). Use of talcum powder though pronounced shelf life of 'seeds' but adversely affect the conversion frequency of synthetic seeds (Dave *et al*., 2004; Table3).

CONCLUSION

The encapsulation technique has opened up new avenues in the field of floriculture and became an asset to micropropagation and germplasm conservation of recalcitrant and elite genotypes. The conversion frequency of such 'Synseeds' is observed to vary with the passage of time and their storage i.e. inversely proportional to the period and temperature of storage. An optimal concentration of 3% sodium alginate coated with100 mM calcium chloride dihydrate is essential for formation of firm, clear and isodiametric beads which showed maximum germination 84.38 ± 3.12 after 15 days of storage at 4^{0}C.

ACKNOWLEDGEMENTS

The authors are thankful to Prof (Dr) Anita Kaushal, Principal PGGCG-11, Chandigarh for her constant inspiration and support during the present investigation.

REFERENCE

Ahmad, N. and Anis, M. (2010). Direct plant regeneration from encapsulated nodal segments of Vitex negundo. *Biol. Plant.,* 54, 748–752.

Amir, A., Gill, I., Majid, Sleem, A., Naz, S. and Naveed, N.H. (2012). In vitro conservation and production of vigorous and desiccate tolerant synthetic seeds in *Stevia rebaudiana*. Journal of Medicinal Plant Research, 6 (7), 1327-1333.

Ara, H., Jaiswal, U. and Jaiswal, V. (2000). Synthetic seed: Prospects and limitation. *Curr. Sci.*, *78,* 438–1444.

Bajaj, Y.P.S. (1995). Somatic embryogenesis and synthetic seed.Vol1.Springer-Verlag, Berlin, Germany ISBN-13, 9780387574486, 472.

Bapat, V.A. and Rao, P.S. (1990). *In vivo* growth of encapsulated axillary buds of mulberry (*Morus indica* L.) Plant Cell Tiss. Org. Cult., 20, 69-70.

Chand, S. and Singh, A.K. (2004). Plant regeneration from encapsulated nodal segments of Dalbergia sissoo Roxb, a timber-yielding leguminous tree species. *J. Plant Physiol..161*,237–243.

Corrie, S. and Tandon, P. (1993). Propagation of Cymbidium giganteum wall through high frequency conversion of encapsulated protocorms under in vivo and in vitro conditions. *Indian J. Exp. Biol.,* 31, 61–64.

Dave, A., Joshi, N. and Purohit, S.D. **(2004).** *In vitro* propagation of *Chlorophytum borivilianum* using encapsulated shoot buds. Eur. J. Hort Sci**.**, 69, 37-42.

Daud, N., Taha, R.M. and Hasbullah, N.A. (2008). Artificial seed production from encapsulated micro shoots of *Saintpaulla ionantha* Wendl. (African violet). J. Appl. Sci., 8, 4662-4667.

Dhabhai, R. and Anand, P. (2012). Production and application of artificial seeds: A Review. I. Res. J. Biological. Sci., 195, 74-78.

Gantait, S., Vijayan, J. and Majee, A. (2017). Artificial Seed production of *Tylophora indica* for interim storing and swapping of Germplasm. *Hort. Pl. J*., 3 (1), 41-46.

Haque, S.K.M and Ghosh, B. (2017). Regeneration of cytologically Stable plants through De differentiation, Re differentiation and Artificial seeds in *Spathoglottis plicata* Blum. Orchidaceae). *Hort.J.Pl*., 5 (3), 199-208.

Helal, N.A.S. (2011). The green Revolution via Synthetic (Artificial seeds): A Review. Research J. of Agriculture, 7 (6), 464-471.

IUCN (1991). IUCN Directory of Protected Areas in Oceaia prepared by the World Conservation Monitoring Centre IUCN, Gland, Switzerland and Cambridge, UK,447.

Lawler, L.J. (1984). Ethanobotany of the Orchidaceae-A Manual. In: Orchid Biology: Reviews and Perspectives Cornell Univ. Press, Ithaca, London, 27-149.

Magray, M.M., Wani, K.P., Chatt, M.A. and Ummyiah, H.M. (2017). Synthetic seed technology. Int. J. Curr. Microbiol. App. Sci. 6 (11), 662-674.

Mandal, J., Pattnaik, S. and Chand, P. (**2000)**. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *Basilicum* L. (sweet basil), *Gratissimum* L. (shrubby basil), and *Sanctum* L. *In Vitro Cell. Dev. Biol. Plant*, *36*,287–292.

Mitra, G.C., Prasad, R.N. and Roy, A.C. (1976). Inorganic salts and differentiation of proto corms in seed callus of an orchid and correlated changes in its free amino acid content. Indian J. Exp. Biol., 14, 350-51.

Mohanraj, R. Ananthan, R. and Bai, V.N. (**2009)**. Production and storage of synthetic seeds in *Coelogyne breviscapa* Lindl. Ascan. Journal of Biotechnology, 1,124-128.

Morel, G.M. (1960). Producing virus free cymbidiums. Am. Orchid Soc. Bull., 16,512.

Murashige, T. (**1978)**. Principles of rapid propagation. In : *Propagation of Higher Plant through Tissue Culture*, Ed. Hughes, K. W., Henke, R. and Constantin, M., Technical Information Centre, US. Department of Energy, 14-24.

Naganabda, G.S., Satishchandra, N. and Rajah, S. (2011). Regeneration of encapsulated proto-corm like bodies of medicinally important vulnerable orchid *Flickingeria nodosa* (Dalz.) Seidenf. International Journal of Botany, 4,310-313.

Nieves, N., Martinez, M.E., Castillo, R., Maria Blanco, A. and Gonzalez-Olmedo, J.L. (2001). Effect of abscisic acid and jasmonic acid on partial desiccation of encapsulated somatic embryos of sugarcane. Plant Cell Tissue Organ Cult., 65, 15-21.

Nieves, W., Zambrano, Y., Tapia., Cid, M., Pina, D. and Castillo, R.(**2003).** Field performance of artificial seeds derived sugarcane plants. Plant Cell.Tiss.Org. Cult**.**, 75, 279-282.

Nor, A.H., Nor, H.H., Zaimah, N., A., Noraliza, A., Nadiah, A. and Salmi, N. (**2011).** Synthetic seed technology for encapsulation and regrowth of *in vitro*-derived Acacia hybrid shoot and axillary buds, Afr. J. Biotechnol, 10 (40), 7820-7824.

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

Pitoyo, A., Anggarwulan, E. and Ariza, I. (2017). Effects of encapsulation matrix on physical properties and germination viability of calcium-alginate encapsulated plbs of *Grammatophyllum scriptum.* Cell Biology and Development, 1(1), 36-40.

Rai, M.K., Jaiswal, V.S. and Jaiswal, U. (2008). Encapsulation of shoot tips of guava (*Psidium guajava* L.) for short-term storage and germplasm exchange. *Sci. Horti.*,118,33–38.

Redenbaugh, K.B., Pashi, B.D., Nichol, J., W., Kossler, M.E., Vilas, P.R. and Walke, K.A. (I986). Somatic seeds: encapsulation of sexual plant embryos; *Biotechnology,* 4,797-801.

Redenbaugh, K., Fujii, J.A., Slade, D., Viss, P.R. and Kossler, M. (**1991**). Artificial seeds- encapsulated somatic embryos. In: Bajaj YPS (ed), Biotechnology in Agriculture and Forestry. High-Tech and micropropagation. Springer. Berlin, 2, 395-416.

Redenbaugh, K., Slade, D., Viss, P.R. and Fujii, J. (1987). Encapsulation of somatic embryos in synthetic seed coats. Hort. Sci., 22, 803-809.

Rihan, H.Z., Kareem, F., EL-Mahroukand, M.E. and Fuller, M.P. (**2017)**. Artificial seeds (Principle, Aspects and Applications Agronomy, 7, 71.

Rihan, H.Z., Al-Issawi, M., Burchett, S. and Fuller, M.P. **(2011).** Encapsulation of cauliflower (*Brassica oleracea var botrytis*) micro shoots as artificial seeds and their conversion and growth in commercial substrates. *Plant Cell Tissue Organ Cult.,* 107, 243–250.

Saiprasad, G.V.S. (2008). Artificial seeds and their application. Resonance, 6, 39-47.

Sai Prasad, G.V.S. and Polisetty, R. (2003). Propagation of three orchid genera using encapsulated proto corm like bodies. In vitro cellular and Developmental Biology-Plant., 39, 42-48.

Sarmah, D.K., Borthakur, M. and Borua, P.K. (2010). Artifical seed production from encapsulated PLBs regenerated from leaf base of *Vanda coerulea* Griff, ex Lindl., an endandered orchid. Curr. Sci., 98, 686-690.

Sharma, V. and Vij, S.P. (1997). "Effect of $CuSO_4$. $5H_2O$ on in vitro regenerative capacity on foliar explants excised from mature *Vanda cristata* Lindl. plants. *Phytomorphology,* 147 (2), 203-208.

Sharma, V. (1996). Orchid Propagation for Conservation and Commercialization-A Study *in vitro*. Ph.D. Thesis (dissertation), Panjab University, Chandigarh, India.

Sharma, V. (2017). Regeneration competence of pseudo bulb explants of endangered orchid genera: A study *in vitro*. Int. J. of Recent Sci. Res., 8 (11), 21722-21724.

Sharma, S., Shahzad, A. and daSilva, J.A.T. (2013). Synseed technology—A complete synthesis. *Biotechnol. Adv.*, *31*: 186–207.

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

Sharma, S. and Shahzad, A. (**2012**). Encapsulation technology for short-term storage and conservation of a woody climber, Decalepis hamiltonii Wight and Arn. *Plant Cell Tissue Organ Cult.,* 111, 191–198.

Singh, A.K., Sharma, M., Varshney, R., Agarwal, S.S. and Bansal, K.C. (**2006**). Plant regeneration from alginate encapsulated shoot tips of *Phyllanthus amarus* Schum and Thom. A medicinally important plant species. *In vitro* Cell Dev. Biol. Plant., 42, 109-113.

Wicramasinghe, R.H. (**1992)**. "Lanka's orchids under threat", Malayan Orchid Rev., 26th ed., 23-27.

Corresponding author: Dr. Vishal Sharma, Botany Department, Post Graduate Government College for Girls-11, Chandigarh, India
Email: vishal_2370@yahoo.com

Indexed, Abstracted and Cited in Indexed Copernicus International and 20 other databases of National and International repute