2.3-1.9
16.11.00
ARO

In vitro encapsulation technique for conservation of *Dendrobium friedericksianum*

P. Sukhumpinij[a] and N. Chanasit

Faculty of Agricultural Technology, Rambhai Barni Rajabhat University, Chanthaburi, 22000, Thailand.

Abstract

Efficient artificial seed protocol for the orchid *Dendrobium friedericksianum* Rchb.f. was developed for in vitro conservation study. *Dendrobium friedericksianum* Rchb.f. is a beautiful native Thai wild orchid found in the eastern provinces of Thailand especially in Chanthaburi province. Currently, illegal forest harvests are impacting this orchid. Meanwhile, environmental changes due to wood-cutting and the decreasing rate of natural breeding cause decreasing numbers of this orchid and lead to a higher risk of extinction. For the reasons mentioned, we have been trying to either keep or preserve the orchid strains. The present study was to develop an effective and applicable protocol for short term in vitro storage of protocorm-like bodies (PLBs) of this orchid species. PLBs were encapsulated in 3% sodium alginate (Na-alginate) and 100 mM calcium chloride ($CaCl_2.2H_2O$). The efficiency of survival declined with both increasing storage duration and storage temperature. The encapsulated PLBs were stored at 4 and 25°C for 30, 60, 90, 150 and 180 days. After storage, PLBs were transferred to modified VW medium to determine the time taken for germination, the germination percentage, and the morphological categorization of regenerated PLBs. Encapsulated PLBs stored at 4°C had rapid deterioration and complete death within 150 days, while those stored at 25°C were more tolerant to storage. Encapsulated PLBs survived longer when stored at 25°C compared to 4°C. All plantlets survived after acclimatization when transferred to greenhouse.

Keywords: artificial seed, Thai wild orchid, in vitro preservation, short term storage

INTRODUCTION

"Leaung chanthaboon" is the name of local orchid that was found in the eastern region of Thailand, especially Chanthaburi province. It is in the genus *Dendrobium*, from the family *Orchidaceae*, and the scientific name is *Dendrobium friedericksianum* Rchb.f. (Boonkead et al., 1982). The scientific name for the flower strain with a red brown spot at the lip is *D. friedericksianum* Rchb.f. var. *oculatum* S&S. (Paireepairit, 1978). Nowadays, illegal forest cutting has nearly wiped out this orchid. Meanwhile, the changes in the environment from wood-cutting and the decreasing rate of natural breeding have resulted in decreasing numbers of orchids, which leads to a much higher risk of extinction. For the reasons mentioned, we have made an effort to keep and preserve the orchid strains.

Tissue culture techniques of great interest for the collecting, multiplication and storage of plant germplasm are aseptic systems, the production of pathogen-free stocks, the reduction of space requirements, genetic erosion reduced to zero under optimal, storage conditions, and the reduction of the expenses in labor costs (Engelmann, 1991). There are several in vitro conservation techniques such as reducing the growth, artificial seed technique, or cryopreservation. Alginate-encapsulation of plant organs has been used to produce artificial seeds or synthetic seeds which can serve as an efficient tool for storage, germplasm conservation, and direct distribution of planting materials for propagation (Germanà et al., 2011). Germplasm conservation using alginate encapsulation techniques has been reported for regeneration of low temperature stored encapsulated protocorms of orchid, with most concluding 4°C to be optimum (Datta et al., 1999; Saiprasad and Polisetty,

[a]E-mail: sukhumpinij@hotmail.com

Acta Hortic. 1104. ISHS 2015. DOI 10.17660/ActaHortic.2015.1104.37
XXIX IHC – Proc. Int. Symp. on Ornamental Horticulture in the Global Greenhouse
Ed.: R.A. Criley

2003; Mohanraj et al., 2009). However, the response of synthetic seeds to storage temperature appears to be species specific. Interestingly, a few reports exist that demonstrate success when stored at higher temperatures such as 22±2°C (Divakaran et al., 2006) or 25°C (Bustam et al., 2013). The aim of this present study was to develop an effective and applicable protocol for short term in vitro storage of protocorm-like bodies of *Dendrobium friedericksianum* Rchb.f.

MATERIALS AND METHODS

Protocorm-like bodies (PLBs) of *Dendrobium friedericksianum* Rchb.f., were induced from seed-derived protocorms. These PLBs were used as the starting material for multiplication on modified Vacin and Went (1949) medium supplemented with coconut water (150 mL L^{-1}), sucrose (20 g L^{-1}) and agar (8 g L^{-1}). The pH of the medium was adjusted at 5.0 with 0.1 M NaOH and 0.1 M HCl and autoclaved at 121°C and 15 p.s.i. for 15 min. Cultures were kept under a 16-h photoperiod provides by cool-white fluorescent lamps with a light intensity of 40 µmol m^{-2} s^{-1} at 25°C. PLBs of *Dendrobium friedericksianum* Rchb.f. were encapsulated with 3% sodium alginate and 100 mM $CaCl_2.2H_2O$ (Sarmah et al., 2010). The alginate beads containing the PLBs were held for 30 min with continuous shaking to complete polymerization of sodium alginate. The calcium chloride solution was decanted and encapsulated PLBs were retrieved by washing with sterilized distilled water for 3 times, and surface dried with sterilized filter paper in petri dishes for 5 min. Encapsulated PLBs were transferred to screw capped air-tight containers. Ten beads were kept in a single container and were replicated four times for all storage temperature and storage period. The containers were stored for 30, 60, 90, 150 and 180 days at 4 and 25°C. After storage period, encapsulated PLBs were cultured into culture bottles containing 20 mL of modified Vacin and Went (1949) medium supplemented with coconut water (150 mL L^{-1}), banana (50 g L^{-1}), potato (50 g L^{-1}), sucrose (20 g L^{-1}) and agar (8 g L^{-1}). The pH of the medium was adjusted at 5.0. PLB growth was observed to determine the time taken for germination, germination percentage, and morphological categorization of regenerated PLBs.

Each experiment consisted of four replicates, with ten encapsulated PLBs in each tube. All data were analyzed as factorial in completely randomized design which used analysis of variance (ANOVA) and least significant difference (LSD) tests at $P<0.05$ to determine differences among treatments.

RESULTS AND DISCUSSION

Germination of stored encapsulated PLBs and their subsequent conversion into plantlets occurred sequentially on modified Vacin and Went (1949) medium. Initially, encapsulated PLBs were observed on the surface of the beads in all treatments. PLBs were considered to have survived upon emergence from the encapsulation matrix on regeneration medium. The present study has revealed that encapsulated PLBs storage at 25°C survived longer when compared to PLBs stored at 4°C. Encapsulated PLBs stored at 25°C maintained 100% of germination percentage without loss of viability up to 180 days of storage whereas when stored at 4°C decreased in germination percentage with 75, 62.5 and 40% after storage for 30, 60 and 90 days, respectively. The conversion percentage after 90 days of storage at 4°C was lower when compared to storage at 25°C (Table 1). Similar results were found in *Aranda* Wan Chark Kuan 'Blue' × *Vanda coerulea* Grifft. ex. Lindl.; a monopodial orchid was achieved at 25°C for 180 days (Gantait et al., 2012).

After 8 weeks of culture all of the encapsulated PLBs stored at 4°C for more than 90 days turned white when cultured and were not able to germinate. An earlier observation of *Dendrobium* 'Shavin White' reported that the decline in the viability of the long-stored encapsulated PLBs at 4°C may be due to chilling injury inside the cell when stored long in low temperature (Bustam et al., 2013). PLB has high moisture content and could not tolerate below a critical level to assure post-storage viability. Thus, storage at low temperature was not possible because it requires low moisture content which could not be achieved without losing the viability. Stored at 25°C, encapsulated PLBs converted to plantlets at 85 to 95% (Table 2). The time required for encapsulated PLBs to germinate and produce plantlets at

each storage temperature differed for the different storage periods. With increasing storage duration, at each storage temperature treatment, the number of PLBs that survived declined, and the time taken to produce both leaves and roots increased. Similar results were found with encapsulated PLBs of *Cymbidium devonianum* (Das et al., 2011) and *Dendrobium* 'White Fairy' (Siew et al., 2014). The regenerated plantlets were transferred to non-aseptic conditions and acclimatized by progressively reducing humidity. The acclimatized plantlets were transferred to the greenhouse, and showed no morphological abnormalities.

Table 1. The effect of different storage temperatures and duration on germination and conversion percentage of encapsulated PLBs.

Storage temperature (°C)	Storage time (days)	Germination date (days)	Germination percentage after 4 weeks (%)
4	30	21.3±1.5 cd	75.0±2.9 b
	60	27.0±1.2 bc	62.5±4.3 c
	90	40.8±5.8 a	40.0±3.5 d
	150	0.0±0.0 f	0.0±0.0 e
	180	0.0±0.0 f	0.0±0.0 e
25	30	9.8±0.9 e	100±0.0 a
	60	15.8±0.5 d	100±0.0 a
	90	18.5±1.3 d	100±0.0 a
	150	21.8±1.1 cd	100±0.0 a
	180	32.8±1.4 b	100±0.0 a

Means with different letters within column are significantly different at P≤0.05.

Table 2. The effect of different storage temperature and duration on morphological categorization of encapsulated PLBs of *Dendrobium friedericksianum* Rchb.f. after culture on modified Vacin and Went (1949) supplemented media.

Storage temperature (°C)	Storage time (days)	Morphological categorization after 8 weeks (%)		
		Plantlet	Shoot/proliferation	Whitening
4	30	25.0±1.2	50.0±4.0	25.0±1.2
	60	10.0±0.8	52.0±4.9	38.0±2.4
	90	10.0±0.8	30.0±1.4	60.0±3.3
	150	0.0±0.0	0.0±0.0	100±0.0
	180	0.0±0.0	0.0±0.0	100±0.0
25	30	95.0±4.9	5±4.0	0.0±0.0
	60	90.0±4.0	10.0±0.8	0.0±0.0
	90	90.0±4.0	10.0±4.0	0.0±0.0
	150	85.0±6.3	15.0±0.5	0.0±0.0
	180	85.0±4.9	15.0±1.6	0.0±0.0

CONCLUSIONS

In conclusion, this study determined the suitable short-term storage for protocorm-like bodies of *Dendrobium friedericksianum* Rchb.f. Encapsulated PLBs can be stored up to 180 days at 25°C with 100% of germination.

ACKNOWLEDGEMENTS

Authors are thankful to Rambhai Barni Rajabhat University, Chanthaburi, Thailand for financial support for conducting the present research.

Literature cited

Boonkead, S., Sadakorn, C., and Sadakorn, T. (1982). Botanical Name of Plants in Thailand. (Thailand: Forestry Book Printing Fund, Faculty of Forestry, Kasetsart University).

Bustam, S., Sinniah, U.R., Kadir, M.A., Zaman, F.Q., and Subramaniam, S. (2013). Selection of optimal stage for protocorm-like bodies and production of artificial seeds for direct regeneration on different media and short term storage of *Dendrobium* Shavin White. Plant Growth Regul. *69*, 215–224 http://dx.doi.org/10.1007/s10725-012-9763-6.

Das, M.C., Kumaria, S., and Tandon, P. (2011). Storage and high conversion frequency of encapsulated protocorm like bodies of *Cymbidium devonianum* (orchid). J. Hortic. Sci. Biotechnol. *86*, 611–615.

Datta, K.B., Kanjilal, B., and De Sarker, D. (1999). Artificial seed technology: development of a protocol in *Geodorum densiflorum* (Lam) Schltr. An endangered orchid. Curr. Sci. *76*, 1142–1145.

Divakaran, M., Babu, K.N., and Peter, K.V. (2006). Conservation of *Vanilla* species in vitro. Sci. Hortic. (Amsterdam) *110*, 175–180 http://dx.doi.org/10.1016/j.scienta.2006.07.003.

Engelmann, F. (1991). In vitro conservation of tropical plant germplasm – a review. Euphytica *57*, 227–243 http://dx.doi.org/10.1007/BF00039669.

Gantait, S., Bustam, S., and Sinniah, U.R. (2012). Alginate-encapsulation, short-term storage and plant regeneration from protocorm-like bodies of *Aranda* Wan Chark Kuan 'Blue' × *Vanda* coerulea Grifft. ex. Lindl. (*Orchidaceae*). Plant Growth Regul. *68*, 303–311 http://dx.doi.org/10.1007/s10725-012-9699-x.

Germanà, M.A., Micheli, M., Chiancone, B., Macaluso, L., and Standardi, A. (2011). Organogenesis and encapsulation of in vitro-derived propagules of *Carrizo citrange* (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf). Plant Cell Tissue Organ Cult. *106*, 299–307 http://dx.doi.org/10.1007/s11240-011-9921-y.

Mohanraj, R., Ananthan, R., and Bai, V.N. (2009). Production and storage of synthetic seeds in *Coelogyne breviscapa* Lindl. Asian J. Biotechnol. *1*, 124–128 http://dx.doi.org/10.3923/ajbkr.2009.124.128.

Paireepairit, P. (1978). Orchid Book for the Beginner (Bangkok, Thailand: Artorn Printing).

Saiprasad, G.V.S., and Polisetty, R. (2003). Propagation of three orchid genera using encapsulated protocorm-like bodies. In Vitro Cell. Dev. Biol. Plant *39*, 42–48 http://dx.doi.org/10.1079/IVP2002360.

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

Siew, W.L., Kwok, M.Y., Ong, Y.M., Liew, H.P., and Yew, B.K. (2014). Effective use of synthetic seed technology in the regeneration of *Dendrobium* White Fairy orchid. J. Orn. Plants *4*, 1–7.

Vacin, E.F., and Went, E.W. (1949). Some pH changes in nutrient solutions. Bot. Gaz. *110*, 605–613 http://dx.doi.org/10.1086/335561.

ISHS Acta Horticulturae 1104

XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposium on Ornamental Horticulture in the Global Greenhouse

List price € 112 **Buy this book**

This title is available both in print and ActaHort CD-rom format

Conveners R.A. Criley, V. Loges, D.M. Marcsik, E. Morgan, M. Serek

Editor R.A. Criley

Publication date 1 December 2015

ISBN 978-94-62610-90-3

ISSN 0567-7572 (print) 2406-6168 (electronic)

Number of articles 75

Volumes 1

Place Brisbane, Australia

Online articles:

NLM Catalog "Acta Hortic"[ta]

Full

Acta horticulturae

Author(s): International Society for Horticultural Science
NLM Title Abbreviation: Acta Hortic
ISO Abbreviation: Acta Hortic.
Title(s): Acta horticulturae.
Publication Start Year: 1963
Country of Publication: Netherlands
Publisher: The Hague, International Society for Horticultural Science.
Description: no. ill. 25 cm.
Language: English, French, Multiple languages
ISSN: 0567-7572 (Print)
0567-7572 (Linking)
Coden: AHORA2
LCCN: sn 79005714
In: MEDLINE: Selected citations only
PubMed: Selected citations only
Current Indexing Status: Not currently indexed for MEDLINE. Only articles related to space life sciences were indexed.
Version Indexed: Print
Notes: "Technical communications of the International Society for Horticultural Science."
No. 12 never published.
In English, French and various other languages.
Other ID: (OCoLC)01772773
NLM ID: 100955313 [Serial]

เอกสารแนบท้ายประกาศ

วารสารทางวิชาการที่เป็นไปตามหลักเกณฑ์ที่ ก.พ.อ. กำหนด ได้แก่ วารสารที่มีชื่ออยู่ในฐานข้อมูลที่เป็นที่ยอมรับในระดับชาติและระดับนานาชาติ ดังต่อไปนี้

๑. ฐานข้อมูลระดับนานาชาติ

- Academic Search Premier (http://www.ebsco.com/home)
  (select ebscohost and then academic search premier)
- Agricola (http://agricola.nal.usda.gov)
- BIOSIS (http://www.biosis.org)
- CINAHL (http://www.ebscohost.com/academic/cinahl-plus-with-full-text)
- EiCOMPENDEX (http://www.ei.org)
- ERIC (http://www.eric.ed.gov/)
- H.W.Wilson (http://www.ebscohost.com)
  (select ebscohost and then H.W.Wilson)
- Infotrieve (http://www.infotrieve.com)
- Ingenta Connect (http://www.ingentaconnect.com)
- INSPEC (http://www.theiet.org/publishing/inspec)
- MathSciNet (http://www.ams.org/mathscinet)
- MEDLINE/Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/)
- PsycINFO (http://www.apa.org/pubs/databases/psycinfo/index.aspx)
- Pubmed (http://www.ncbi.nlm.nih.gov/pubmed/)
- ScienceDirect (http://www.sciencedirect.com)
- SciFinder (https://scifinder.cas.org/)
- Scopus (http://www.info.scopus.com)
- Social Science Research Network
  (http://papers.ssrn.com/sol3/DisplayAbstractSearch.cfm)
- Web of Knowledge (http://wokinfo.com)

๒. ฐานข้อมูลระดับชาติ ได้แก่ ศูนย์ดัชนีการอ้างอิงวารสารไทย (Thai Journal Citation Index -- TCI) เฉพาะวารสารที่มีชื่ออยู่ในกลุ่มที่ ๑ และกลุ่มที่ ๒ (http://www.kmutt.ac.th/jif/public_html/list%20journal.php)

หมายเหตุ กรณีที่มีการเปลี่ยนแปลงชื่อฐานข้อมูล ก.พ.อ. จะได้มีการประกาศเพิ่มเติมต่อไป

SJR SCImago Journal & Country Rank

LST MODUS IN RLBUS

Home

Journal Rankings

Journal Search

Country Rankings

Country Search

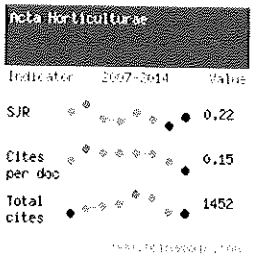
Compare

Map Generator

Help

About Us

Show this information in your own website



☑ Display journal title

Just copy the code below and paste within your html page:

<a href="http://www.scimagojr

Related product

SCIMAGO INSTITUTIONS RANKINGS

@scimago

SJR is developed by:

Scimago

Scopus

How to cite this website?

Journal Search

Search query

in Journal Title Search

☐ Exact phrase

Acta Horticulturae

Country: Belgium

Subject Area: Agricultural and Biological Sciences

Subject Category:

Category	Quartile (Q1 means highest values and Q4 lowest values) 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014
Horticulture	Q2 Q2 Q1 Q1 Q3 Q3 Q4 Q3 Q3 Q3 Q3 Q3 Q3 Q3 Q3 Q3

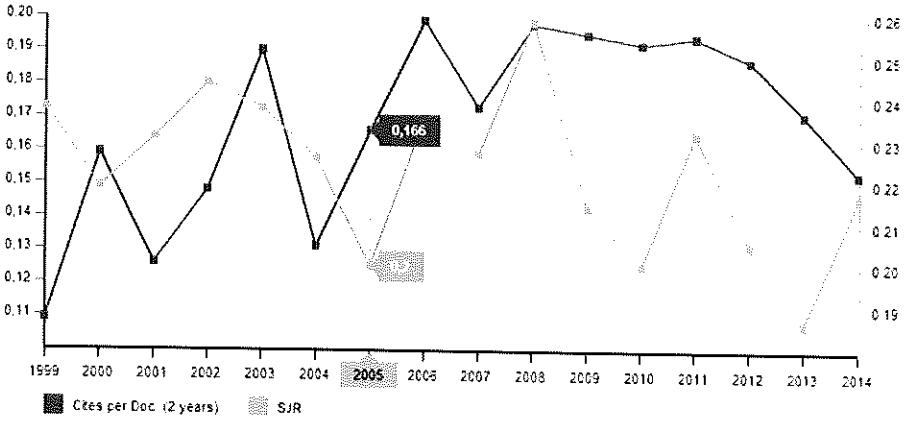
Publisher: International Society for Horticultural Science. **Publication type:** Book Series. **ISSN:** 05677572

Coverage: 1976, 1988, 1996-2014

H Index: 39

Charts Data

SJR indicator vs. Cites per Doc (2y)



The SJR indicator measures the scientific influence of the average article in a journal, it expresses how central to the global scientific discussion an average article of the journal is. Cites per Doc. (2y) measures the scientific impact of an average article published in the journal, it is computed using the same formula that journal impact factor ™ (Thomson Reuters).

Citation vs. Self-Citation