European Journal of Biotechnology and Bioscience

Volume: 3, Issue: 4, 20-23
May 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

**Y. Ruby Stella**
Department of Botany, Sri Parasakthi College for Women, Courtallam, Inida

**T. Amutha Priya**
Lab of Developmental Biology and Plant Biotechnology, SPK Centre for Environmental Sciences, Manonmaniam Sundranar University, Alwarkurichi, Tirunelveli, Tamil Nadu, India

**K. Mydeen Fathima Begam**
Department of Botany, Sri Parasakthi College for Women, Courtallam, Inida

**V. Manimekalai**
Assistant Professor
Department of Botany
Sri Parasakthi College for Women, Courtallam,
Tamil Nadu, India

**Correspondence:**
**V. Manimekalai**
Assistant Professor
Department of Botany
Sri Parasakthi College for Women, Courtallam,
Tamil Nadu, India

# *In vitro* seed germination, somatic embryogenesis and protocorm based micro propagation of a terrestrial ornamental orchid - *spathoglottis plicata* blume

**Y.Ruby Stella, T.Amutha Priya,K. Mydeen Fathima Begam, V. Manimekalai**

**Abstract**
*Spathoglottis plicata* Blume, is a beautiful terrestrial orchid species with long grassy leaves. Orchids are commercially important flowers and potted plants with in floriculture trade are valued word wide. It is considered outstanding in the ornamental flowers trade due to its diverse colors, shapes, forms and long lasting blooms. In this investigation an attempt was made to propagate the plant by tissue culture techniques. In vitro regeneration potential of seeds was investigated. These seeds were cultured on Murashige and Skoog's Medium with (or) without hormones. 6 Benzal Amino Purine (BAP) and Kinetin at 10µM concentration was effective in inducing callus on seed explants. Further, periodical subculture and maintenance resulted in the development of somatic embryos which subsequently formed Protocorm like Bodies (PLBs). PLBs developed into complete plantlets forming micro shoots and roots. On an average six micro shoots were formed from every PLB. The developed plantlets were transferred to polycups containing artificial soil – vermiculite. Synthetic seed technology is today an important tool for the *in vitro* propagation and conservation of ornamental plants. PLBs formed *in vitro* were encapsulated in 3% sodium alginate matrix to form synseeds. Encapsulation ensures slow growth and long term preservation. These seeds are being maintained in cold storage and could be used in future for germination to produce new plantlets. The protocol developed in the present investigation can be commercially exploited and used as a means for the rapid regeneration of this terrestrial orchid – *Spathoglottis plicata*.

**Keywords:** Murashige and Skoog's medium, 6 Benzal Amino Purine, Protocorm Like Bodies, Kinetin, Sodium hydroxide

## Introduction
Orchidaceae is a diverse and widespread family of flowering plants with blooms that are often colourful and often fragrant commonly known as the orchid family. Along with the ***Asteraceae*** it is one of the two largest families of flowering plants with between 21,950 and 26,049 currently accepted species distributed among 880 genera [1]. A genus of about 45 species distributed in tropical parts of India, South -east Asia, Malaysia, Philippines, Indonesia, New Guinea, New Caledonia, Polynesia and Australia where there are 2 species distributed in northern tropical areas, one species *Spathoglottis paulinae*, is endemic, State occurrence Queensland, Northern Territory and the other is *Spathoglottis plicata*. Orchids have been used in traditional medicine in an effort to treat many diseases and aliments [2][3]. Synthetic seed technology is an important tool available to breeders and scientists for the *in vitro* propagation and conservation of ornamental plants. Many protocols have been developed for the encapsulation of *in vitro* regenerated somatic embryos and protocorms [4]. More commonly somatic embryos are used in sodium alginate matrix for encapsulation. Synthetic seeds are used today in advanced procedures of cryopreservation with very promising results for long-term preservation of plant germ plasm. The first appearance of synthetic seed also known as artificial seed or syn seed dates back to over 30 years ago and emerged from the idea of encapsulating a single somatic embryo inside an artificial seed coat, thus mimicking the natural seeds [5].

## Material and Methods:
The culture place (culture laboratory) was maintained sterile by periodic fumigation once in 15 days. It was carried out by vaporizing formaldehyde solution along with alcohol for about 8-12 hours. Excess of formalin vapor was absorbed by placing ammonia solution in an open container usually in a petridish. Explants inoculation and sub culturing were carried out in a

laminar air flow system. Prior to this the inner area of system was swabbed with alcohol using tissue paper and followed by exposing to germicidal UV light for about 15 minutes. Murashige and Skoog's (MS) medium was used to culture plants of *Spathoglottis plicata*. Stock solutions were stored in refrigerator, sucrose was added as carbon source at a concentration of 2.5%. The pH was adjusted to 5.6-5.7 with the help of 0.1 N KOH and 0.1 N HCl prior to adding the hormone. Kinetin (k) and BAP (Benzyl Amino Purine) were supplemented at 10µM concentration. 0.8% agar was added as a gelling agent. Media prepared in bulk were boiled till the agar melted and then dispensed to culture bottles and sealed with cotton plug and sterilized as 15 lbs pressure for 15 minutes in an autoclave.

**Tissue culture technique of orchid seeds**

Capsules of *Spathoglottis plicata* were collected from nursery as well as household gardens in and around Courtallam. Immature (green) and healthy capsules were soaked in 100% bleach solution for 30 minutes. Under aseptic conditions, using a sterile knife or scalpel, the capsule was opened and the seeds were scraped out layered over the surface of the culture medium. The culture vessels were then sealed using cotton plugs. The cultures were maintained at 25± 2ºC in light conditions. Cultures were observed for morphogenetic changes and regeneration.

**Preparation of artificial seeds**

The *in vitro* formed somatic embryos and protocorm like bodies were encapsulated in sodium alginate beads. 3% sodium alginate was used as the matrix and the encapsulated beads were formed in 100mM calcium chloride solution [6]. All the responses were photographed using Olympus E 300 SLR digital camera and developmental stages of protocorm were photographed under stereo zoom microscope fitted with Nikon labophot camera**.**

**Results and discussion**

*Spathoglottis plicata* Blume. is a tropical orchid ornamentally valued for its beautiful magenta coloured flowers (Fig.1). *Spathoglottis* is conveniently propagated through separation of pseudobulbs, but the proliferation rate is very low. A more efficient approach for its regeneration is *in vitro* seed culture [7] [8]. Reports on the *in vitro* culture of *Spathoglottis plicata* are limited [9][10]. The available information on *in vitro* propagation of *Spathoglottis plicata*, however does not provide a comprehensive protocol and understanding of regeneration. The present investigation was undertaken to develop a suitable protocol for *in vitro* fast regeneration system of this indigenous terrestrial orchid. Seeds were used as explants and the protocorm like bodies (PLBs) were used for further subculture and regeneration.

Surface sterilized explants (seeds) were cultured aseptically initially on MS medium containing 2.5% sucrose. The medium was supplemented with different concentrations of kinetin and Benzyl Amino Purine for getting maximum number of micro-shoots.

Seeds were dusted on MS basal medium and MS medium supplemented with cytokinins like kinetin and benzyl amino purine (BAP) at 10 µM concentration. Both the PGRs used induced callusing. Callus formed was either white or pale white in colour and friable in nature. The callus masses were composed of compact and loose aggregates of cells.

On subculture the calli differentiated to form protocorm like bodies (Fig.2). [9] Used 3% sucrose in MS medium and obtained PLBs in *Spathoglottis plicata*. Addition of cytokinins - kinetin and BAP enhanced the proliferation of protocorms and stimulated shoot development. Similar observations were made by [11] in *Cymbidium* orchid, when MS medium was supplemented with 10% coconut milk.

On germination orchid seeds give rise to an ephemeral tubercle structure called a protocorm from which a shoot and a root subsequently differentiate [12]. The term protocorm was first coined by Treub, to describe a stage in lycopod development [13]. This term has also been used to describe the corm-like stage of orchid seed germination [13]. The strategy of delaying histo-differentiation is important in maximizing massive seed production upon a single successful pollination event. One event can lead to the formation of a huge number of seeds, e.g., in *Anguloa ruckeri*, a single capsule contains 3.9 million seeds [14]. In order that a proper seedling can develop from each embryo, the shoot apical meristem must form for vegetative development to continue. As [15] wrote in his review, “meristems make a plant”; without the presence of a shoot apical meristem, continuous vegetative development cannot take place. The formation of a protocorm is a solution to vegetative establishment in orchids, i.e., the formation of a functional shoot meristem for postembryonic growth. Chances are the developmental programs for protocorm development are established during embryo maturation. Even though the protocorm is a postembryonic structure, it has been considered as a continuation of embryogeny, an integral part of embryogenesis [16]. *Orchid protocorm-like bodies are somatic embryos* —, PLBs should be regarded as somatic embryos because they show features similar to that of their zygotic counterparts. It was demonstrated by [17] that (1) the early division pattern of embryogenic cells from EC is similar to that in zygotic embryos, (2) soon after PLB formation, the globular shaped PLBs have the ability to synthesize storage product, albeit transient in nature, and (3) wall proteins similar to those in the zygotic embryos are present.

Periodical subcultures (once in two weeks) were made for proliferation of protocorms as well as shoot development. A number of microshoots developed (Fig.3-7) and the clumps of micro shoots were subsequently dissected longitudinally and subculture. On an average 6 shoots were obtained from each of the PLB subcultured.

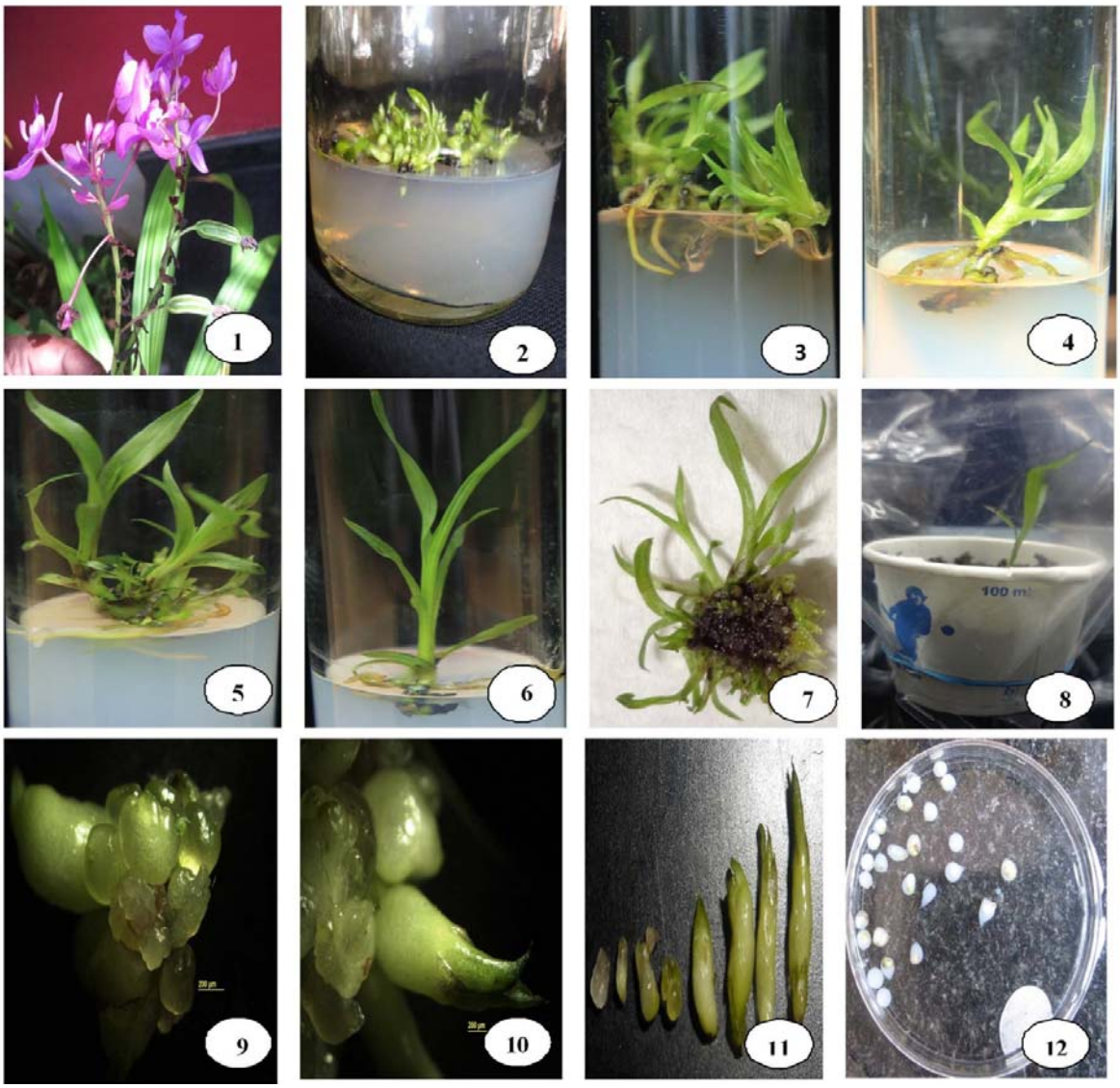
After three weeks of growth in culture the shoots were subcultured on MS medium supplemented with Kinetin and BAP (10 µM). Roots were formed within 18 days. On an average 4 roots were formed in each plantlet. This finding corroborates with that of [10] who reported the regeneration of roots in *Spathoglottis plicata* on MS medium supplemented with IAA 0.5 mg/L.

Rooted plantlets were taken for hardening (Fig.8). Plantlets were planted in polycups containing finely ground vermiculite (artificial soil). As in the present study plants were directly regenerated through protocorms and microshoots without intervention of callus, there was no risk of somaclonal variation. The protocol formulated through present study would be definitely reproducible and could be utilized in high frequency regeneration of *Spathoglottis plicata* for commercial as well as conservation programs.

The *in vitro* regenerated somatic embryos and protocorms were encapsulated in 3% sodium alginate matrix to form synthetic seeds (fig.9-12). This kind of encapsulation ensures

slow growth and helps in long term preservation. This kind of encapsulation of somatic embryos was earlier reported in *Cyclamen persicum* using 1.5% sodium alginate [18]. [19] Used chitosan along with sodium alginate as water –barrier coating agents to encapsulate the protocorms of *Spathoglottis plicata*. In the present study the encapsulation matrix was not supplemented with any hormone. The inclusion of somatic embryos and protocorms in the alginate matrix containing or not growth regulators, produces a sort of artificial endosperm which can be beneficial for synseed germination.

Synthetic seed technology is today an important tool available to breeders and scientists for the *in vitro* propagation of ornamental species, including trees, shrubs and cut flowers [20]. Synthetic seed technology has greatly contributed to further improvement of cryopreservation techniques. Synthetic seed technology approach is important for the long-term preservation of endangered and valuable germplasm from economically important ornamental species.




**Reference:**


1. Pridgeon AM, Cribb PJ, Chase MW, Rasmussen F, Genera and Orchidacearum, 2005,vol.4. Oxford and Newyork: Oxford University press.
2. Dockrill AW, Australian Indigenous Orchids, Vol.I&II. Society for growing Australian plants, 1992, Beatty and Sons Chipping Norton, NSW.
3. David, Jones L, A complete guide to native orchids of Australia Including the Island territories. 2006,Sydney, Read new Holland.
4. Maurizio Lambardi, Carla Benelli, Elif Aylin Ozudogru, Yelda Ozden- Tokatli, Synthetic seed technology in ornamental plants floriculture and ornamental plant biotechnology, 2006,Vol.II, Global Science Books, UK.
5. Murashige T, Plant cell and organ cultures as horticultural practices**,** Acta horticulturae 1977,78**:** 17-30.
6. Murashige T, Skoog F, A revised medium for rapid growth and bioassay with Tobacco tissue cultures, Physiol.Plant, 1962,Vol:15**:** 473-497.
7. Sarker RH, Roy AR, In vitro propagation of local orchids from Bangaladesh, In vitro cell.Dev.Biol. 1993,29 A (part 2):90A.
8. Minea M, Piluek C, Menakanit A, Tantiwiwat S, A

study on Seed Germination and Seedling Development of Spathoglottis Orchid, J. Nat. Sci, 2004, 38:147-156.
9. Teng WL, Nicolson L, Teng M.C, Micropropagation of Spathoglottis plicata, Plant cell Reports 1997,16**:** 831-835**.**
10. Barua AK, Bhadra SK, In vitro micro propagation of Cymbidium atoifolium (L.)SW. and Spathoglottis Plicata BL, Plant tissue culture, 1999**:**133-140.
11. Nayak NR., Tanaka M, Teixeira da Silva, JA, Biotechnology of Cymbidium—an overview of recent progress and future opportunities. In: Teixeira da Silva JA, (Ed.), Floriculture, Ornamental and plant biotechnology: Advances and topical issues, 2006, Vol. IV: 558-562.
12. Cribb PJ, 1999. Morphology. *In*. Pridgeon AM, Cribb PJ, Chase MA and Rasmussen FN [eds.], Genera orchidacearum, vol. 1, General introduction, Apostasioideae, Cypripedioideae, 13–23. Oxford University Press, Oxford, UK.
13. Arditti J, Krikorian A, Orchid micropropagation: The path from laboratory to commercialization and an account of several unappreciated investigators. Botanical Journal of the Linnean Society**,** 1996, 122: 183 – 241.
14. Arditti, J, Fundamentals of orchid biology. John Wiley, 1992, New York, USA.
15. Sussex IM, Developmental programming of the shoot meristem, Cell, 1989,56: 225– 229.
16. Curtis JT, Nicholson MA, Culture of proliferation orchid embryos in vitro. Bulletin of the Torrey Botanical Club, 1948,75 **:** 358 – 373.
17. Lee YI, Yeung EC, Lee N, Chung MC, Embryology of Phalaenopsisamabilis var. formosa : Embryo development, Botanical Studies (Taipei, Taiwan) 2008,49 : 139 – 146.
18. Winklemann T, Meyer L, Serek M, Germination of encapsulated of somatic embryos of somatic embryos of cyclamen persicum, Hort Science, 2004, 39, 1093-1097.
19. Khor E, Loh CS, Two -coat system for encapsulation of Spathoglottis plicata (Orchidaceae) seeds and Protocorms, Biotechnology and Bioengineering, 1998,59: 635-639.
20. Onishi N, Sakamoto Y, Hirosawa T, Synthetic seeds as van application of mass production of Embryos. Plant cell, Tissue and Organ culture,1994, **39:** 137-145.