# Bioscene

**Bioscene**
**Volume- 21 Number- 03**
**ISSN: 1539-2422 (P) 2055-1583 (O)**
**www.explorebioscene.com**

# Conservation of *Acampe Praemorsa* (Roxb.) using Protocorm Segments *in Vitro*

**Priyanka Dhiman[1] and Saranjeet Kaur[2*]**
Department of Biosciences, University Institute of Biotechnology, Chandigarh University, District: Mohali, Punjab, India

Corresponding Author: **Saranjeet Kaur**

**Abstract :** The present communication reviews the possibilities and advantages of using protocorms in multiplying the genepool of epiphytic orchid *Acampe praemorsa* (Roxb.) by varying the concentration of Murashige and Skoog (1962) medium i.e. half strength and full strength. Regeneration response was triggered by a chemical stimulation in the nutrient mix. Addition of growth regulators [Naphthalene acetic acid(NAA) and benzyl amino purine (BAP) at 1 mg / l) initiated early response in protocorm segments. Auxins proved best in initiating early response in the cultures as compared to control and BAP supplemented medium. However, BAP favoured protocorm multiplication. The explants invariable followed direct somatic embryogenesis in the cultures. The devised protocol will be helpful in the conservation of *Acampe praemorsa*, a threatened species of medicinal value.

**Keywords:** Auxin; cytokinin; explant; MS medium; protocorm-like body

## Introduction

The orchids belong to highly advanced family Orchidaceae of angiosperms. These are known for their multi-coloured, beautifully architectured long lasting blooms. The Orchidaceae include approx. 28000 encased in 763 genera, is one of the largest families of flowering plants, along with the Asteraceae (Christenhusz and Byng, 2016). The family Orchidaceae comprises around 6-11% of all seeds plants species. *Dendrobium* (1400 species), *Pleurothallis* (1000 species), *Epidendrum* (1500 species), and *Bulbophyllum* (2,000 species) are the largest genera of orchidaceae (Pal *et al*. 2002) and more than 100,000 hybrids and cultivars have been created by horticulturists since tropical plants were first brought into cultivation in the 19th century (Bailes *et al*. 1985; Medhi *et al*.2009).

Orchids are rare, endangered and threatened species all over the world. Several factors are responsible for their present condition such as deforestation, increased use of fertilizers, fragmentation of habitat especially in tropical regions, excessive exploitation of soil, and over collection (Znanieckа*et al*. 2005). They appear as rare, endangered and threatened species (CITES,2024) and *Acampe praemorsa* is no exception among orchids.

*Acampe praemorsa* is a distinctive and ecologically significant orchid species native to the Indian subcontinent and Southeast Asia (Blatt. and McCann, 2007).Renowned for its robust, leathery leaves and striking, long-lasting flowers, this orchid thrives in the arid and semi-arid environments where it often forms part of the region's unique flora. The species is characterized by its impressive adaptability to harsh conditions, including low humidity and high temperatures, which contribute to its survival in less hospitable habitats (Arditti and Ernst,1993). *Acampe praemorsa* holds considerable interest both for its ornamental value and for its potential in conservation and horticultural practices. Understanding its growth habits, reproductive biology, and propagation challenges is crucial for efforts aimed at preserving this orchid in its natural habitat as well as for optimizing its cultivation in controlled environments. *Acampe praemorsa* (Roxb.), an epiphytic species of highly floriferous orchids is the most popular among the leading cut flower crops in the world (Thangavelu and Ayyasamy, 2017). It has tremendous potential as a progenitor of meritorious hybrids of international repute. Being ornamental species for its delightful blossoms it is horticulturally important species, used in international floriculture trade (Arditti *et al*. 1979). It is popularly used in home gardens and landscapes in most parts of the world. The species is also known for its therapeutic uses.

*In vitro* multiplication of *Acampe praemorsa* through protocorm-like bodies (PLBs) represents a sophisticated approach to propagating this valuable orchid species efficiently and sustainably.In orchids, protocorm-like bodies (PLBs) are a pivotal component in modern plant tissue culture, particularly within the realm of orchid propagation. These structures could be artificially induced from cultured plant tissues and closely resemble the early developmental stages of orchids, known as protocorms. PLBs are distinguished by their ability to proliferate rapidly and differentiate into fully functional plants, making them invaluable for large-scale multiplication and genetic improvement. By utilizing PLBs, researchers and horticulturists can efficiently produce numerous clones of a single orchid variety, ensuring both genetic uniformity and high-quality plant material. This method not only enhances the efficiency of orchid propagation but also aids in the conservation of rare and endangered species, contributing to the broader field of plant biotechnology and sustainable horticulture.
It is, therefore, strongly emphasized to multiply the species, through tissue culture techniques. Therefore, present studies were conducted with the aim to multiply the species *in vitro* using protocorm segments and testing the efficacy of growth supplements on *in vitro* multiplication of protocorms and early development of *Acampe praemorsa* plantlets without the use of any growth regulators.

## Material and Mathods

The protocorms (2.0 mm long) were obtained from 30 weeks old aseptic cultures of *Acampe praemorsa.* The protocorms were dissected into two halves and used as

explant. These were inoculated in half strength and full strength Murashige and Skoog (1962) medium (Hi-Media, Mumbai, India). Medium was supplemented with growth regulators such as Auxin-NAA (α-naphthalene acetic acid) and Cytokinin-BAP (6-benzylaminopurine) at 1 mg/l. The medium's pH was adjusted at 5.7 after adding growth regulators.

Inoculations were done under aseptic conditions in a laminar air flow cabinet. Cultures were incubated at 25±2°C under 12 h photoperiod at 3,500 lux light intensity (40W Fluorescent tubes, Philips, India). The experiment was set in a completely randomized manner. Eight replicates were used per treatment. The observations were made regularly and the data was recorded accordingly. The results were recorded on the basis of time taken by the explant to initiate the cultures, pathway of regeneration, and formation of complete plantlets in full and half strength MS medium. The results were expressed as mean ± standard deviation. To check the reproducibility the experiment was repeated twice.

**Results**

The current study involves *in vitro* regeneration of protocorms segments of *Acampe praemorsa* in Murashige and Skoog (1962) medium supplemented in its two versions (Full and half strength) and with growth regulators by varying the salt concentration to half-strength and full-strength version. The results are summarized (Table 1 and Table 2)and illustrated (Figure 1 a-d). In half-strength MS medium the explants regenerated after 5 weeks of culture. Only 8.0 percent explants responded to generation. It took 21 weeks to initiate regeneration response.

In an individual treatment, the explant regenerated after 4 and 2 weeks of culture in BAP and NAA supplemented medium (Table 1). Nearly 71 percent explant responded to regeneration in BAP fortified medium whereas NAA enhanced the regeneration frequency by 10 percent and almost 80 percent explant responded to regeneration and form plantlets within 12 weeks of culture. In full-strength MS medium supplemented with NAA (1 mg/l) and BAP (1 mg/l). Almost 90 percent of the protocorm segments of *Acampe praemorsa* were successfully regenerated. The protocorm segments multiplied profusely in MS + BAP fortified medium (Figure 1a) and formed complete plants in BAP and NAA nutrient mix (Figure 1b, 1c) respectively. In the full-strength basal MS medium, the explants responded after four weeks of culture with less regeneration frequency. Histological sections revealed the neo-formations were being formed through budding (F1gure1d).

**Discussion**

Protocorm like bodies (PLBs) are pivotal in orchid conservation and propagation due to their efficiency in mass-producing plants under controlled conditions. They offer a reliable alternative to traditional seed germination, ensuring rapid and consistent growth of orchid species. PLBs are also instrumental in genetic conservation efforts, allowing for cryopreservation of valuable genetic material.

Furthermore, their ability to be treated for disease and pest management enhances the health and vigor of propagated orchids. PLBs support research by providing a model system for studying orchid development and physiology, thereby advancing both scientific knowledge and practical applications in orchid horticulture and biodiversity conservation.

Protocorm like bodies (PLBs) resemble somatic embryos in structure and development (Lee *et al*. 2013). In an investigation it was observed that during the initial phases of PLB development and discovered that the PLBs are actual somatic embryos (Lee *et al*. 2013) . For the *in vitro* propagation of many orchid species, the type and quantities of plant growth hormones are crucial (Arditti and Ernst, 1993).

Presently the protocorm segments responded to regeneration in basal medium (½ and Full-strength MS medium) regardless of the presence of any kind of growth regulator in the medium. Similar kind of response was earlier reported in *Vanda pumila* protocorm segments (Maharajan *et al*. 2019). The explants in full strength MS medium responded better than the explants inoculated in half strength medium. Such kind of differential response could have occurred due to the dilution of the mineral salt composition which otherwise were required by the explants for early response invocation. Our results differ from earlier findings in *Rhynchostylis retusa* in which mineral salt composition significantly influenced the protocorm development; the protocorms were best formed in ½ strength MS medium and even in 1/4 strength MS medium whereas the shoots developed best in Full strength MS medium. (Oliya*et al*. 2021). In another study, in *Vanda pumila*, 1/2 strength MS medium favoured maximum number of shoot development (Maharajan*et al*. 2019).

Presently, the explants responded better in NAA supplemented ½ and full-strength MS medium. The explants regenerated early even the percent regeneration frequency was also more than those explants cultured in BAP supplemented medium. On the contrary in *Vanilla planifolia* NAA in the medium favoured callus initiation at the base of the shoot buds (Kaur, 2017).PLB formation from protocorm segments can be divided into two categories. The first is direct embryogenesis, which forms PLBs directly from protocorms, shoot tips, root tips, and stem segments (Luo*et al*. 2008; Mayer*et al*. 2010; Naing*et al*. 2011). The second is callus-induced PLB development. In our study, the PLBs of *Acampe praemorsa* emerged straight from the protocorms similar to those reported earlier by (Hong *et al*. 2008; Chung and Huang, 2010; Saleh and Ng, 2011).

**Conclusion**

The present study on the multiplication of *Acampe praemorsa* through protocorm segment culture in half-strength and full-strength MS mediumfortified with NAA

and BAP demonstrates that the growth and multiplication of *Acampe praemorsa* protocorm segments are significantly influenced by the salt strength. The results suggest that optimal multiplication occurs at specific concentrations of NAA and BAP, with differences observed between half-strength and full-strength MS medium. Full-strength medium with NAA and BAP concentrations generally promoted better growth and multiplication rates compared to half-strength medium.

These findings provide valuable insights for optimizing *in vitro* propagation protocols for *Acampe praemorsa*, which can be beneficial for conservation and horticultural purposes.

**Abbreviations**

**PLB**: Protocorm like bodies; **MS medium**: Murashige and Skoog Medium; **PGR**: Plant growth Regulator; **NAA**:α-naphthaleneaceticacid; **BAP**: Benzylaminopurine

**Table 2: *In vitro* growth of protocorms in *Acampe praemorsa* species in ½ MS medium and its combination with growth regulators.**

| Medium | Initiationof response (wks.) | %age of response | Pathway of regeneration | Plantlet development (wks.) | Remarks |
|---|---|---|---|---|---|
| MS | 5.00 ± 0.20 | 8.05 ± 1.7 | Plant mediated | 21.13 ± 0.70 | Low PLB multiplication |
| ½ MS+BAP | 4.00 ± 0.10 | 71.05 ± 1.2 | Plant mediated | 15.01 ± 0.75 | Moderate PLB multiplication |
| ½ MS+NAA | 2.00 ± 0.24 | 80.12 ± 2.3 | Plant mediated | 12.03 ± 0.85 | High PLB multiplication |

Results are expressed as mean ± standard deviation; concentration of plant growth regulators = 1mg/l.

**Table 1*: In vitro* growth of protocorms in *Acampe praemorsa* species in MS medium and its combination with growth regulators.**

| Medium | Initiationof response (wks.) | %age of response | Pathway of regeneration | Plantlet development (wks.) | Remarks |
|---|---|---|---|---|---|
| MS | 4.00 ± 0.28 | 43.00 ± 1.10 | Plant mediated | 14.00 ± 6.4 | Low PLB multiplication |
| MS+BAP | 2.00 ± 0.25 | 80.04 ± 2.1 | Plant mediated | 12.02 ± 1.29 | Moderate PLB multiplication |
| MS+NAA | 2.00 ± 0.47 | 90.15 ± 2.50 | Plant mediated | 10.06 ± 2.85 | High PLB multiplication |

Results are expressed as mean ± standard deviation; concentration of plant growth regulators = 1mg/l.


Figure: - 1 a-d. *In vitro* multiplication of *A. praemorsa* protocorms in MS medium. a. Profuse PLB multiplicationfull-strength of MS medium (arrow);b. Development of healthy plantlets in BAP supplemented medium; c. Development of healthy plantlets in NAA supplemented medium; d.Hand section revel multiplication of PLBs through budding (40x) arrow.

**References:**


1. Arditti, J. and Ernst, R., 1993. Micropropagation of orchids. John Willey & Sons. Inc., NY.
2. Arditti, J., 1967. Factors affecting the germination of orchid seeds. The Botanical Review, 33, pp.1-97.
3. Arditti, J., 1977. Clonal propagation of orchids by means of leaf culture in vitro: a short review. The Orchid Review, 85, 202-203.
4. Arditti, J., 1980. Aspects of the physiology of orchids. In Advances in botanical research (Vol. 7, pp. 421-655).
5. Bailes, C.P., 1985. Orchids in Nepal, the conservation and development of a natural resource, advisory report and recommendations. Richmond, UK: Royal Botanic Gardens Kew.
6. Christenhusz, M.J. and Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa, 261(3), pp.201-217.
7. Chung, H.H., Shi, S.K., Huang, B. and Chen, J.T., 2017. Enhanced agronomic traits and medicinal constituents of autotetraploids in Anoectochilusformosanus Hayata, a top-grade medicinal orchid. Molecules, 22(11), p.1907.
8. Chung, M.Y., López-Pujol, J., Son, S., Suh, G.U., Yukawa, T. and Chung, M.G., 2018. Patterns of genetic diversity in rare and common orchids focusing on the Korean Peninsula: implications for conservation. The Botanical Review, 84, pp.1-25.
9. Hong, P.I., Chen, J.T. and Chang, W.C., 2008. Plant regeneration via protocorm-like body formation and shoot multiplication from seed-derived callus of a maudiae type slipper orchid. Acta Physiologiae Plantarum, 30, pp.755-759.
10. Huang, C.H. and Chung, J.P., 2011. Efficient indirect induction of protocorm-like bodies and shoot proliferation using field-grown axillary buds of a Lycaste hybrid. Plant Cell, Tissue and Organ Culture (PCTOC), 106, pp.31-38.
11. Huang, X.Y., Hu, A.Q., Hsu, T.C. and Liu, Y., 2015. Gastrodiahuapingensis (Orchidaceae: Epidendroideae: Gastrodieae): a remarkable new mycoheterotrophic orchid with dimorphic columns from China. Phytotaxa, 222(4), pp.290-294.
12. Kaur, S. and Vij, S.P., 2000. Regeneration potential of Saccolabiumpapillosum leaf segments. The Journal of the Orchid Society of India, 14: p. 67-73.
13. Kaur, S.,2017. In vitro regeneration of shoots from nodal explants of Dendrobium chrysotoxumLindl. Journal of Horticultural Research, 25(1), pp.27-34.
14. Kaur, S., 2018. In vitro conservation of rare, medicinally important species DendrobumnobileLindl. (Orchidaceae). Annals of Plant Sciences, 7(3): 2121.
15. Kaur, S., 2022. In VitroFlorigenesis with Special Reference to Orchids- A Review. Recent Patents on Biotechnology, 16(4): 311–318.
16. Kaur, S., 2022. In vitro Propagation of Vanda testacea (Lindl.) Reichb. F, a Medicinally Important Threatened Orchid. Plant Tissue Culture and Biotechnology, 31(2): 153–160.

17. Lee, Y., & Yeung, E. C., 2018. Orchid propagation: From laboratories to Greenhouses—Methods and Protocol29s. Humana Press
18. Luo, J., Hou, B.W., Niu, Z.T., Liu, W., Xue, Q.Y. and Ding, X.Y., 2014. Comparative chloroplast genomes of photosynthetic orchids: insights into evolution of the Orchidaceae and development of molecular markers for phylogenetic applications. PloS one, 9(6), p.e99016.
19. Luo, L., Molthoff, J., Li, Q., Liu, Y., Luo, S., Li, N., Xuan, S., Wang, Y., Shen, S., Bovy, A.G. and Zhao, J., 2023. Identification of candidate genes associated with less-photosensitive anthocyanin phenotype using an EMS mutant (pind) in eggplant (Solanum melongena L.). Frontiers in Plant Science, 14, p.1282661.
20. Maharajan, T., Antony Ceasar, S., Ajeesh Krishna, T.P. and Ignacimuthu, S., 2021. Finger millet [Eleusine coracana (L.) Gaertn]: an orphan crop with a potential to alleviate the calcium deficiency in the semi-arid tropics of Asia and Africa. Frontiers in Sustainable Food Systems, 5, p.684447.
21. Maharajan, T., Ceasar, S.A., Krishna, T.P.A. and Ignacimuthu, S., 2021. Management of phosphorus nutrient amid climate change for sustainable agriculture. Journal of Environmental Quality, 50(6), pp.1303-1324.
22. Mayer, J.L.S., Cardoso-Gustavson, P. and Appezzato-da-Glória, B., 2011. Colleters in monocots: new record for Orchidaceae. Flora-Morphology, Distribution, Functional Ecology of Plants, 206(3), pp.185-190.
23. Mayer, J.L.S., Stancato, G.C. and Appezzato-Da-Glória, B., 2010. Direct regeneration of protocorm-like bodies (PLBs) from leaf apices of Oncidium flexuosum Sims (Orchidaceae). Plant Cell, Tissue and Organ Culture (PCTOC), 103, pp.411-416.
24. Medhi, R.P. and Chakraborti, M., 2012. Orchid biodiversity in India: conservation and utilization. Indian Journal of Genetics and Plant Breeding, 72(02), pp.148-156.
25. Naing, A.H. and Kim, C.K., 2019. A brief review of applications of antifreeze proteins in cryopreservation and metabolic genetic engineering. 3 Biotech, 9(9), p.329.
26. Ng, C.Y. and Saleh, N.M., 2011. In vitro propagation of Paphiopedilum orchid through formation of protocorm-like bodies. Plant Cell, Tissue and Organ Culture (PCTOC), 105, pp.193-202.
27. Oliya, B.K., Chand, K., Thakuri, L.S., Baniya, M.K., Sah, A.K. and Pant, B., 2021. Assessment of genetic stability of micropropagated plants of Rhynchostylis retusa (L.) using RAPD markers. Scientia Horticulturae, 281, p.110008.
28. Pal, R., Babu, P.K. and Dayamma, M., 2020. Indian Orchid Germplasm: Conservation and Utilization. Floriculture and Ornamental Plants, pp.1-30.
29. Saleh, N.W., Hodgson, K., Pokorny, T., Mullins, A., Chouvenc, T., Eltz, T. and Ramírez, S.R., 2021. Social behavior, ovary size, and population of origin influence cuticular hydrocarbons in the orchid bee Euglossa dilemma. The American Naturalist, 198(5), pp.E136-E151.
30. Saranjeet Kaur., 1994. Foliar explants and orchid micropropagation: Vanda Kasem's Delight "Tom Boykin". Journal of the Orchid Society of India, Vol. 8, No. 1/2, 79-83 Ref. 10, 8: 79–83.

31. Thangavelu, M. and Ayyasamy, K., 2017. Comparative anatomy of aerial and substrate roots of Acampe praemorsa (Rox.) Blatt. & McCann. Flora, 226, pp.17-28.
32. Vibha, S., Hebbar, S.S., Mahalakshmi, S.N. and Kekuda, T.P., 2019. A comprehensive review on ethnobotanical applications and pharmacological activities of Acampe praemorsa (Roxb.) Blatt. & McCann (Orchidaceae). Journal of Drug Delivery and Therapeutics, 9(1), pp.331-336.
33. Yin, M. and Hong, S., 2009. Cryopreservation of Dendrobium candidum Wall. ex Lindl. protocorm-like bodies by encapsulation-vitrification. Plant Cell, Tissue and Organ Culture (PCTOC), 98, pp.179-185.
34. Znaniecka, J.O.A.N.N.A., Królicka, A., Sidwa-Gorycka, M., Rybczynski, J.J., Szlachetko, D.L. and Lojkowska, E., 2005. Asymbiotic germination, seedling development and plantlet propagation of Encyclia aff. oncidioides-an endangered orchid. Acta SocietatisBotanicorumPoloniae, 74(3).