Research Article

BanaNexus Medium: Masspropagation of *Bulbophyllum biflorum* using Banana-Enriched Medium

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Abstract: Bulbophyllum biflorum is a rare and endangered orchid species from the Bulbophyllum genus in the family Orchidaceae. Highly sought after by collectors, its survival is increasingly threatened by rapid urbanization, habitat destruction, and environmental changes. Additionally, its reliance on certain environmental conditions makes natural seed propagation incredibly inefficient, with a survival rate of less than 1%, further compounding this difficulty. To address this critical issue, this project introduces an innovative tissue culture method tailored for B. biflorum propagation. A key breakthrough in this approach is the incorporation of bananas as natural plant growth enhancers, replacing synthetic hormones. This not only supplies essential nutrients but also significantly reduces costs, making large-scale propagation more feasible. Additionally, the culture medium is formulated as a user-friendly 3-in-1 powder, ensuring accessibility for orchid enthusiasts, researchers, and conservationists. The impact of this innovation is far-reaching. It provides a cost-effective and sustainable solution for large-scale propagation, directly supporting conservation efforts and reducing the risk of extinction. Moreover, it enhances accessibility for growers and researchers, fostering wider participation in orchid preservation. With strong commercial potential, this solution is poised for adoption in the horticultural industry, bridging the gap between scientific advancement and sustainability. By combining biotechnology with ecological responsibility, this project presents a practical and scalable solution for the conservation of B. biflorum, ensuring its survival while delivering both commercial and environmental benefits.

Keywords: BanaNexus Medium; Bulbophyllum biflorum; orchid; masspropagation; plant tissue culture.

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1. INTRODUCTION

The *Bulbophyllum* genus is one of the largest in the orchid family, comprising over 2,000 species across 78 sections. Among them, *Bulbophyllum biflorum*, or the Two-Flowered *Bulbophyllum*, is a miniature-sized epiphytic orchid distinguished by its Single leaf, growing at the tip of the pseudobulb, ovate, four-angled yellow pseudobulbs, each supporting a single leaf (None Miswarti et al., 2021). Thriving in warm to cool climates, this rare species is primarily found in Malaysia, with some populations in Indonesia and the Philippines. However, its survival is increasingly at risk due to habitat

destruction and overcollection. Alarmingly, the conservation status of *Bulbophyllum* species is steadily declining. According to the IUCN Red List (2022), 18 species are classified as Critically Endangered, 51 as Endangered, and 39 as Vulnerable (Mihin Targu et al., 2023). *B. biflorum* is also of conservation concern, as it is listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II, which safeguards species that could become endangered if their trade is not carefully managed (Kumar et al., 2024). Given these threats, immediate action is necessary to prevent this species from extinction. One of the biggest challenges in preserving *B. biflorum* lies in its propagation techniques. Traditional methods, such as seed germination and vegetative division, are highly inefficient due to slow growth rates and the species' reliance on symbiotic fungi. Natural seed germination rates are typically below 1%, making large-scale cultivation nearly impossible. Fortunately, tissue culture presents a promising alternative, offering a controlled, sterile environment that significantly enhances propagation success (Imtinene Hamdeni et al., 2022). To take this approach a step further, we have developed BanaNexus Medium, which is an innovative formulation designed to improve the masspropagation of *B. biflorum*. Not only does this user-friendly medium enhance success rates and promote healthier plantlets, but it also makes the propagation process easier and more efficient. Ultimately, BanaNexus Medium has the potential to revolutionize both orchid conservation and commercial cultivation, ensuring a sustainable future for this rare species.

2. METHOD & MATERIAL

2.1 Development of BanaNexus Medium

The medium was precisely crafted, incorporating a modified Murashige and Skoog (MS) medium (Duchefa Biochemie), as the base which included essentials such as nitrogen (ammonium nitrate and potassium nitrate), phosphorus (potassium phosphate), potassium (potassium nitrate), calcium (calcium chloride), magnesium (magnesium sulfate), and sulfur. To ensure comprehensive nutrient availability, micronutrients like iron (iron EDTA), manganese (manganese sulfate), zinc (zinc sulfate), boron (boric acid), copper (copper sulfate), and molybdenum (ammonium molybdate) were added. Vitamins (thiamine, nicotinic acid, and pyridoxine) and amino acids (glycine) were included to support robust plant development. Additionally, a banana, which acts as a natural plant growth enhancer, was added to stimulate plant defense mechanisms and promote vigorous growth.

2.2 Preparation Process

The medium comes in three easy-to-use sachets. Sachets 1 and 2 are poured into a container, and distilled water is added to make 1 liter of solution. The pH is adjusted to 5.6-5.8 using a pH meter and appropriate pH adjustment solutions. Sachet 3 is then added, mixed thoroughly, and the medium is sterilized by autoclaving at 120 psi for 20 minutes. Once sterilized, the medium is ready to be poured into jars for micropropagation.

2.3 Testing and Validation

Bulbophyllum biflorum seeds were sterilized and inoculated onto the prepared BanaNexus Medium in a controlled laboratory environment, with control groups using traditional propagation media set up for comparison. Over 6 months, seed germination rates, root development, plantlet health, and overall growth were monitored. Data were collected on growth rate and plantlet height to assess the effectiveness of BanaNexus Medium compared to traditional methods.

3. FINDINGS

Figure 1 demonstrates the growth performance of *Bulbophyllum biflorum* seed into plantlets that were cultivated for six months in various media, including Control medium, Medium A, Medium B, Medium C, Medium D, and BanaNexus medium. The control medium exhibits the least plantlet development with low germination and little biomass accumulation.

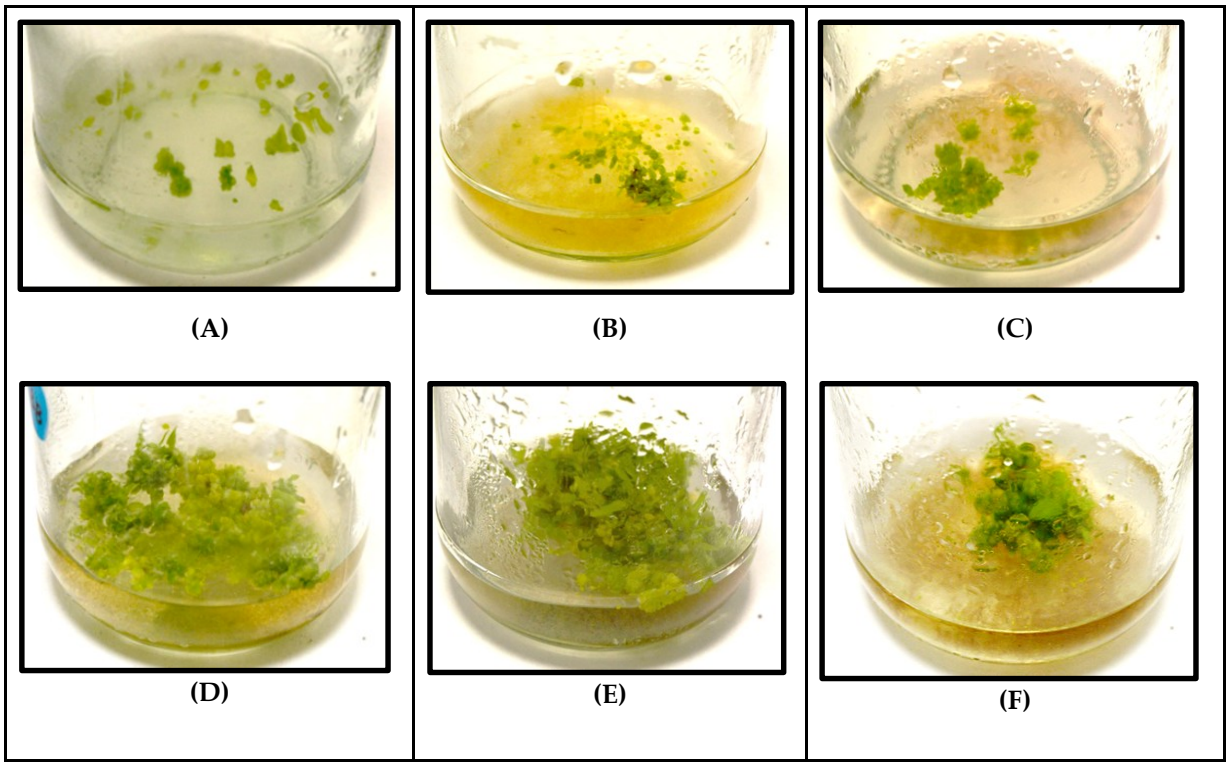


Figure 1. Growth Comparison of *Bulbophyllum biflorum* treated with different type of culture media: **(A)** Control; **(B)** Medium A; **(C)** Medium B; **(D)** Medium C; **(E)** BanaNexus Medium; **(F)** Medium D

Growth of shoots in vitro was significantly influenced by banana extract-enriched medium. The maximum growth rate, 90%, was attained by *Bulbophyllum biflorum* plantlets cultivated on BanaNexus medium, followed by Medium D (80%) and Medium C (70%) according to growth rate analysis presented in Figure 2.

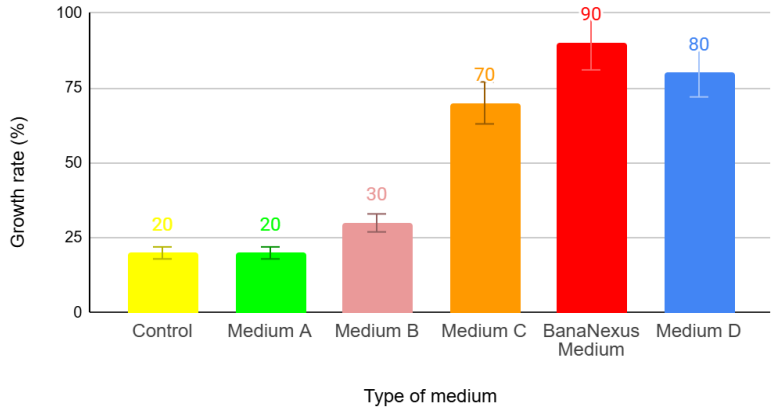


Figure 2. Germination Success Rate of *Bulbophyllum biflorum* in Different Culture Media

Similarly, shoot length measurements in Figure 3 further confirmed the effectiveness of BanaNexus Medium. Out of all the studied media, orchid plantlets cultivated on BanaNexus medium had the greatest average shoot length, measuring 2.4 cm.

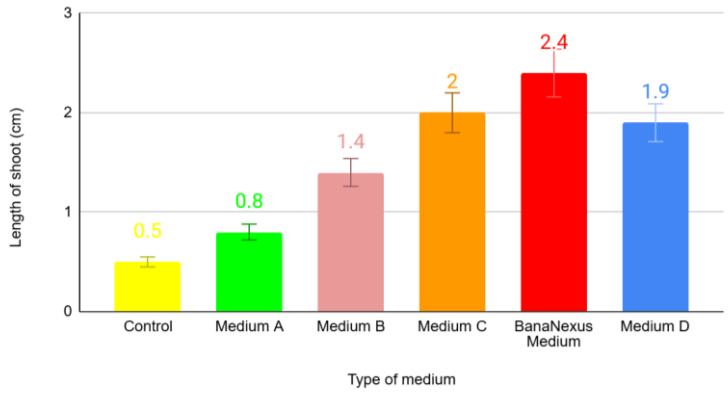


Figure 3. Shoot Length of *Bulbophyllum biflorum* in Different Culture Media

4. DISCUSSION

The superior performance of BanaNexus medium in promoting growth rate and shoot elongation highlights the potential of banana supplementation as a natural growth enhancer in orchid tissue culture. The presence of banana in the medium likely contributed essential phytohormones such as cytokinins, auxins and gibberellin. which stimulate cell division, elongation, bud formation, and root development (Agbadje et al., 2021; Apensa & Mastuti, 2018). Additionally, banana contains natural sugars, carbohydrates, proteins, amino acids vitamins, and antioxidants which may have improved nutrient balance, resistance to oxidative stress and supported the plantlets' general growth and well-being (Islam et al., 2016).

The comparison with conventional Murashige and Skoog-based media shows that effective *Bulbophyllum biflorum* propagation requires more than just normal nutrients, as evidenced by the limited growth observed in the control medium. This indicates that conventional formulations and other mediums tested may lack certain bioactive compounds necessary for promoting efficient orchid

tissue culture. Furthermore, the higher growth rates seen in the BanaNexus medium group imply that the medium promotes long-term plantlet development in addition to improving germination success.

One of the key advantages of BanaNexus medium (figure 4) is its ease of use and efficiency. This medium is designed to be user-friendly, allowing both experts and beginners in plant tissue culture to successfully propagate orchids, specifically *Bulbophyllum Biflorum,* without the need for extensive technical knowledge about tissue culture. Moreover, its ability to simplify the propagation process while maintaining high success rates enhances its potential for widespread use, therefore improving overall efficiency and reliability in both commercial and research applications.



Figure 4. BanaNexus Medium packaging

BanaNexus medium also provides an economical and adaptable alternative for orchid propagation. The formulation can support large-scale production while maintaining affordability, making it an attractive choice for nurseries, research institutions, and commercial growers. BanaNexus medium utilized a natural ingredient, which is banana, that reduced production cost. This is because bananas can be easily obtained and considered cheap as compared to another conventional medium that requires expensive synthetic plant growth regulators as one of the key ingredients.

Apart from commercial use, BanaNexus medium also has the potential for conservation efforts. Nowadays, many rare and endangered species, such as *Bulbophyllum biflorum*, face challenges growing in nature due to habitat loss, overcollection, and low germination rate. The ability of BanaNexus medium to improve seed germination, shoot elongation, and overall plantlet health makes it a valuable tool for conservation programs seeking to propagate and reintroduce threatened orchid species into their natural habitats.

As the global orchid market continues to expand, the need for efficient and sustainable orchid supply will rise. BanaNexus medium stands out as an innovative approach that offers a reliable and effective medium for orchid tissue culture while also supporting environmental sustainability and conservation efforts. Its ability to enhance growth rates, improve survival rates, and provide an accessible solution for plant propagation underscores its significance as a breakthrough innovation in the field of orchid micropropagation.

5. CONCLUSION

BanaNexus medium project has proven to be a highly effective and innovative solution for the micropropagation of the *Bulbophyllum biflorum* orchid. The addition of banana as a natural growth enhancer to the culture medium helps in providing essential nutrients that significantly improve the plantlet growth and germination rate. Due to its user-friendly and cheap formulation, it ensures accessibility for both experts and beginners, making it suitable for widespread adoption in commercial

orchid cultivation. Moving forward, BanaNexus medium may stands out as a reliable alternative in both commercial and conservation efforts to preserve other rare orchid species.

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References

Agbadje, E. T. a. E., Agbidinoukoun, A., Zandjanakou-Tachin, M., Cacaï, G. T. H., & Ahanhanzo, C. (2021). Mass production of bananas and plantains (*Musa spp.)* plantlets through in vitro tissue culture partway: A review. *European Journal of Biology and Biotechnology*, *2*(4), 1–8.

Apensa, V., & Mastuti, R. (2018). Effect of banana homogenate on shoot regeneration of *Ciplukan (Physalis angulata L.). The Journal of Experimental Life Science*, *8*(1), 53-60.

Imtinene, H., Mounir, L., Slim, S., Abdennacer, B., & Taoufik, B. (2022). Incorporation of organic growth additives to enhance in vitro tissue culture for producing genetically stable plants. *Plants*, *11*(22), 3087–3087.

Islam, M. O., Islam, M. S., & Saleh, M. A. (2016). Effect of banana extract on growth and development of protocorm like bodies in *Dendrobium sp. orchid*. *The Agriculturists*, *13*(1), 101–108.

Kumar, J., Diksha Katoch, Thakur, A., Arpit Pathania, Anand, A., Choudhary, K., & Shelja Shelja. (2024). A comprehensive review on threats and conservation status of orchids. *Journal of Applied Biology and Biotechnology*.

Mihin, T., Debnath, S., & Suman, K. (2023). Biotechnological approaches for in vitro propagation, conservation and secondary metabolites production in Bulbophyllum, an endangered orchid genus: a review. *3 Biotech*, *13*(10).

Miswarti, Calista, I., Putra, W. E., Oktavia, Y., Yuliasari, S., Musaddad, D., & Sastro, Y. (2021). Morphology characteristics of orchids species in Bukit Barisan, Bengkulu province. *IOP Conference Series: Earth and Environmental Science*, *653*(1), 012149.