Journal of Plant Sciences
2024, Vol. 12, No. 4, pp. 90-94
https://doi.org/10.11648/j.jps.20241204.11

Review Article

Complexities and Innovations in Orchid Germination: A Review of Symbiotic and Asymbiotic Techniques

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Abstract

Orchidaceae is the second-largest family among flowering plants; one of the most fascinating characteristics is the morphology of the seed. One of the most important complications is that orchid seeds are tiny and lack endosperm, meaning they almost entirely depend on outside sources for nutrients while germinating or developing. The main objective of this review is to give a summary of the present methods being used in orchid germination, highlighting symbiotic and asymbiotic methods. Symbiotic germination requires interaction with mycorrhizal fungi, which provide the necessary nutrients and support for seedling growth. However, in asymbiotic approaches, controlled conditions are provided to promote germination by using nutrient-rich media. The article also highlights some of these advances that have taken place recently and ways that could be adopted to improve their effectiveness in the future. For example, the effectiveness and ability to grow orchids have improved due to recent developments in tissue culture and biotechnology. This study relies on applying the analytical approach to previous relevant studies on the subject. Nevertheless, further research is needed to refine these techniques and improve their usability in orchid conservation. To summarize, while significant progress has been achieved in understanding and enhancing orchid germination processes, further study and innovation are required. By resolving remaining issues and investigating new approaches, it may be possible to better assist orchid conservation and encourage the long-term cultivation of these wonderful plants.

Keywords

Orchid, Seed Germination, Symbiotic, Asymbiotic, *Orchidaceae*

1. Introduction

As one of the largest families of flowering plants, Orchidaceae is well known for its high diversity and complex life cycles [17]. The use of orchids as an economically important plant reduces their native population, which highlights the need for conservation [17]. As a result of increased demand for medicinal and horticultural uses, the Orchidaceae needs innovative reproduction techniques that are appropriate for the market and conservation [21]. The seeds of orchids are very small, the shape of the seed is 'dust-like', and the seed coat is usually very thin, ranging from one to several cell layers in thickness [13]. One of the factors that contribute to poor germination is the seed's tiny size. Since seeds are directly responsible for the regeneration and distribution of species, they hold a pivotal role in orchid conservation efforts [24]. Furthermore, other parameters that impact orchid seed germination include mycorrhizal fungus association, light conditions, temperature, moisture, substrate composition, gas exchange, seed viability and age, hormonal signals, and

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Received: 14 June 2024; **Accepted:** 1 July 2024; **Published:** 15 July 2024

dormancy mechanisms [3, 20], all of which are described in this paper.

The studies of the germination of orchid seeds have been done by using both symbiotic and asymbiotic methods of research [4]. Symbiotic techniques include placing the orchid seeds in contact with mycorrhiza fungus, which provides important nutrients for seed germination. Symbiotic approaches on the other hand involve the use of an artificial nutrition medium under a controlled environment to deliver the necessary nutrients for seed germination.

Understanding the ecology of orchids and their fungus companions in their native environments may improve methods of management and assist in translocation [7]. The issues and achievements in germinating orchids are very challenging and offer some great prospects in the field of orchid conservation. Symbiotic and asymbiotic techniques are used in these efforts and more studies are needed to improve the management and preservation of these plants.

Nevertheless, the efficiency and applicability of these strategies may vary depending on the orchid species and the surrounding conditions. This study suggests that a complete understanding of these strategies and their underlying mechanisms might lead to more effective conservation efforts for endangered orchid species.

2. Biology of Orchid Seed

Orchids are known for producing the smallest seeds among seed-bearing plants, with sizes ranging from 0.05 to 6 millimeters or weights ranging from 0.31 to 24 micrograms [19]. The orchid produces large numbers of seeds, but the germination rates of these seeds are low, typically less than 1% [24]. Orchid seeds do not have endosperm, which is the nutritive tissue responsible for embryo development and seed germination [18]. Hence, all Orchidaceae members produce seeds that depend on mycorrhizal fungi to complete their life cycle in their natural habitat [19]. Seeds have a very simple cellular architecture. They made up of an undifferentiated mass of embryonic cells covered with a seed coat that is either transparent or visible [24].

The species also showed seed shape variations such as fusiform, spathulate, ovoid, and filiform, the color of seeds also varies including orange yellow, brownish yellow, yellow, white, pale yellow, golden yellow, and light yellow [23].

3. Symbiotic Germination

Symbiotic germination is a natural process in which orchid seeds create a symbiotic association with a certain species of fungus. Many experiments conducted that demonstrate the relationship between fungi and orchid germination found that the external changes induced by the fungus are sufficient to explain germination when the fungus is present. These changes include the digestion of starch, the formation of sugar, and the production of a favorable hydrogen ion concentration; these changes alone are adequate to induce germination [12].

Mycorrhizae perform an essential role in plant development and growth [27]. In mycorrhizal symbioses, fungi provide soil mineral nutrients like nitrogen and phosphorus to their host plants as a trade for carbon resources [27].

3.1. Mechanism of Symbiotic Germination

The symbiotic process begins with the development of protocorms [2]. Which is the embryonic form of orchids. It has a unique structure designed for mycorrhizal establishment and aims to form a shoot apical meristem for plantlet establishment [28]. Orchid relies on symbiotic fungi for nutrition, including carbon compounds, during the initial seedling stage [25].

Mycorrhizal fungi, such as Arbuscular mycorrhizal fungi (AMF), play an important role in orchid development and seed germination. Most terrestrial plants have symbiotic relationships with AMF [5]. By expanding the root's absorbing region, these soil organisms help the plant uptake nutrients and plant carbohydrates given to the symbiont in exchange for ending its life cycle [5].

A study about the seed-fungus effect in orchid germination found that these fungus have great potential for use as propagules in orchid conservation due to their practical advantages such as low-cost mass seedling production, convenient transportation, controllable seedling quantity and density, ease of use in the field, and environmentally friendly biodegradable materials [26].

3.2. In-vitro Versus In-situ Symbiotic Germination

The in-vitro process involves cultivating orchid seeds in a nutrient medium containing the necessary mineral nutrients and some plant growth regulators under controlled conditions. To perform this symbiotic process in the laboratory, the seeds and the appropriate fungus were inoculated on a solid agar medium [17]. This method could be combined with other factors for germination, including nutrients, photoperiod, temperature, and plant hormones [27]. A study about in-vitro germination for orchid seeds found that S. brevilabris maintains a high degree of mycobiont specificity under in vitro symbiotic seed germination conditions [22]. The in-vitro symbiotic seed germination method should be useful for both commercial and conservation purposes [1].

In-situ means that the orchid seed germination is done in their natural habitat. In their native habitat, orchids rely on endophytic mycorrhizal fungus to provide them with minerals and carbohydrates [22]. It was utilized as an innovative restoration-friendly technique for endangered orchid species [21]. This method was found to be more cost-effective and resulted in higher survival rates in the field compared to the in-vitro technique [21]. One study suggests that advanced in situ germination techniques should include a secure sowing site that fosters a warm microhabitat with high and consistent humidity while allowing light penetration to promote germination [21].

A study aimed at assessing the effectiveness of in vitro and in situ symbiotic seed germination revealed that in situ seed germination, extending up to the seedling stage, achieved the highest success rate at 86.85%. In contrast, the in vitro seed germination method demonstrated comparatively lower germination rates, reaching 60.34% up to the protocorm formation stage [7].

4. Asymbiotic Germination

Asymbiotic germination is a technique used to propagate orchids in vitro without the involvement of their typical fungal symbionts. This method substitutes the role of mycorrhizal fungi with an asymbiotic medium, enabling effective germination and propagation of orchid seeds [14].

Asymbiotic seed germination is a more straightforward process because mycobionts do not need to be isolated to germinate orchid seeds [10]. Populations of orchids established with asymbiotic seedlings remain dependent on naturally occurring fungal symbionts for seedling recruitment [10]. That is because even after germination, orchid seedlings still rely on fungus for development and survival [2]. Thus, orchids and their mycorrhizal fungus have a complicated and crucial interaction, which influences not only seed germination but also the continued health and survival of orchid populations.

4.1. Mechanism of Asymbiotic Germination

Orchids produce seeds that have a hard testa [8]. This lignified testa plays a crucial role in orchid seed germination; it serves as a barrier to water absorption and embryo growth [9]. To prevent this, the seeds are often treated with sodium hypochlorite (NaOCl), which softens and removes the testa. In addition, this method sterilizes the seeds [2].

Asymbiotic seed germination is the technique in which nutrients are delivered to seeds from the growing medium, replacing mycorrhizae [11]. During germination, the embryo within the seed begins to take in water and nutrients from the surrounding environment. The medium contains essential nutrients for the germination and growth of orchid seeds, enabling them to mature into fully developed plants [15]. After the seedlings have grown, they can be moved to their native environment.

One study investigated the asymbiotic seed germination and in vitro seedling development of Bletia purpurea, a threatened North American native terrestrial orchid. Six different asymbiotic orchid seed germination media were tested for their efficiency in seed germination. The resulting seedlings were effectively acclimatized to greenhouse conditions, helping to conserve B. purpurea by providing a viable seed propagation strategy [6].

4.2. Advantages and Drawbacks of Asymbiotic Germination

The advantages of asymbiotic germination processes include easier management than symbiotic methods [2]. In addition to their ease of management, asymbiotic germination methods enable rapid and large-scale in vitro plantlet development. Furthermore, they enable direct research into significant factors influencing several biological aspects of orchid life [2].

An in vitro study on orchid seeds revealed that treating the seeds with 0.1% hydrogen peroxide (H_2O_2) and 0.1% potassium nitrate (KNO_3) significantly increased germination rates [16].

The disadvantages of asymbiotic germination include species-specific requirements, as orchids' developmental demands vary widely within the family, notably between tropical and temperate species, requiring the use of different techniques [11]. Furthermore, there are acclimatization concerns since in vitro asymbiotic germination may result in seedlings treated with readily available nutrients and optimal growth conditions. This might cause issues.

5. Conclusion

In conclusion, the conservation of orchids involves the need for new and efficient means of propagation due to the challenges posed by the dropping population and increasing demand. Symbiotic and asymbiotic germination strategies are involved in this process and they offer advantages but also pose new challenges.

Although using symbiotic germination, which is based on mutualistic mycorrhiza, is a natural way, in the given conditions, more convenient propagation techniques are asymbiotic ones. However, both strategies still require more research to improve them for other kinds of orchids as well as the widespread climatically different conditions. The knowledge learned in the present status of orchid biology and the development of propagation Technologies shall help secure these wonderful and useful plant species for future generations.

Abbreviations

AMF	Arbuscular Mycorrhizal Fungi

Author Contributions

Leen Al Zoubi is the sole author. The author read and approved the final manuscript.

Funding

This work is not supported by any external funding.

Data Availability Statement

Not applicable.

Conflicts of Interest

The author declares no conflicts of interest.

References

[1] Aewsakul, N., Maneesorn, D., Serivichyaswat, P., Taluengjit, A., & Nontachaiyapoom, S. Ex vitro symbiotic seed germination of Spathoglottis plicata Blume on common orchid cultivation substrates. Scientia Horticulturae. 2013, 160, 238–242. https://doi.org/10.1016/j.scienta.2013.05.034

[2] Anghelescu, N. E., Vafaee, Y., Ahmadzadeh, K., & Chen, J. T. Asymbiotic Seed Germination in Terrestrial Orchids: Problems, Progress, and Prospects. In: Tiwari, P., Chen, JT. In Advances in Orchid Biology, Biotechnology and Omics. 1st Ed., Singapore: Springer; 2023, pp. 221–60.

[3] Arditti, J. Factors affecting the germination of orchid seeds. The Botanical Review. 1967, *33*(1), 1–97. https://doi.org/10.1007/bf02858656

[4] Arditti, J. Aspects of the Physiology of Orchids. In Advances in botanical research. 1980, (pp. 421–655). https://doi.org/10.1016/s0065-2296

[5] Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hocher, V., & Svistoonoff, S. Roles of Arbuscular Mycorrhizal Fungi on Plant Growth and Performance: Importance in Biotic and Abiotic Stressed Regulation. Diversity. 2020, *12*(10), 370. https://doi.org/10.3390/d12100370

[6] Dutra, D., Johnson, T. R., Kauth, P. J., Stewart, S. L., Kane, M. E., & Richardson, L. Asymbiotic seed germination, in vitro seedling development, and greenhouse acclimatization of the threatened terrestrial orchid Bletia purpurea. Plant Cell, Tissue and Organ Culture. 2008, *94*(1), 11–21. https://doi.org/10.1007/s11240-008-9382-0

[7] Harzli, I., & Kömpe, Y. Z. Assessing the effectiveness of in vitro and in situ symbiotic seed germination: case of *Anacamptis papilionacea* (L.) R. M. Bateman, Pridgeon & M. W. Chase. Symbiosis. 2023, *91*(1–3), 91–100. https://doi.org/10.1007/s13199-023-00950-8

[8] Heslop-Harrison, J. AN INTERPRETATION OF THE HYDRODYNAMICS OF POLLEN. American Journal of Botany. 1979, *66*(6), 737–743. https://doi.org/10.1002/j.1537-2197.1979.tb06277.x

[9] Ho, H. H. The Taxonomy and Biology of Phytophthora and Pythium. Journal of Bacteriology & Mycology. 2018, *6*(1). https://doi.org/10.15406/jbmoa.2018.06.00174

[10] Johnson, T. R., Stewart, S. L., Dutra, D., Kane, M. E., & Richardson, L. Asymbiotic and symbiotic seed germination of Eulophia Alta (Orchidaceae)—preliminary evidence for the symbiotic culture advantage. Plant Cell, Tissue and Organ Culture. 2007, *90*(3), 313–323. https://doi.org/10.1007/s11240-007-9270-z

[11] Jolman, D., Batalla, M. I., Hungerford, A., Norwood, P., Tait, N., & Wallace, L. E. The challenges of growing orchids from seeds for conservation: An assessment of asymbiotic techniques. Applications in Plant Sciences. 2022, *10*(5). https://doi.org/10.1002/aps3.11496

[12] Knudson, L. Physiological Study of the Symbiotic Germination of Orchid Seeds. Botanical Gazette. 1925, *79*(4), 345–379. https://doi.org/10.1086/333488

[13] Lee, Y. I., & Yeung, E. C. The orchid seed coat: a developmental and functional perspective. Botanical Studies. 2023, *64*(1). https://doi.org/10.1186/s40529-023-00400-0

[14] Park, H. B., An, J., Bae, K. H., Hong, S. H., Park, H. J., Kim, S., Lee, C. W., Lee, B. D., Baek, J. H., Kim, N. Y., & Hwang, J. E. Asymbiotic Seed Germination and In Vitro Seedling Development of the Endangered Orchid Species Cypripedium guttatum. Plants. 2023, *12*(22), 3788. https://doi.org/10.3390/plants12223788

[15] Parthibhan, S. Influence of nutritional media and photoperiods on in vitro asymbiotic seed germination and seedling development of Dendrobium aqueum Lindley. African Journal of Plant Science. 2012, *6*(14), 383–393. https://doi.org/10.5897/ajps12.132

[16] Patavardhan, S. S., Ignatius, S., Thiyam, R., Lasrado, Q., Karkala, S., D'Souza, L., & Nivas, S. K. Asymbiotic seed germination and in vitro development of orchid Papilionanthe Miss Joaquim. Ornamental Horticulture. 2022, *28*(2), 246–255. https://doi.org/10.1590/2447-536x.v28i2.2431

[17] Pujasatria, G. C., Miura, C., & Kaminaka, H. In Vitro Symbiotic Germination: A Revitalized Heuristic Approach for Orchid Species Conservation. Plants. 2020, *9*(12), 1742. https://doi.org/10.3390/plants9121742

[18] Ray, H., & Vendrame, W. Orchid Pollination Biology. EDIS. 2015, *15*(6), 6. https://doi.org/10.32473/edis-ep521-2015

[19] Roberts, D. L., & Dixon, K. W. Orchids. CB/Current Biology. 2008, *18*(8), R325–R329. https://doi.org/10.1016/j.cub.2008.02.026

[20] Schwabe, W. W. Book Reviews. Journal of Experimental Botany. 1981, *32*(5), 1117–1119. https://doi.org/10.1093/jxb/32.5.1117

[21] Shao, S. C., Burgess, K. S., Cruse-Sanders, J. M., Liu, Q., Fan, X. L., Huang, H., & Gao, J. Y. Using In Situ Symbiotic Seed Germination to Restore Over-collected Medicinal Orchids in Southwest China. Frontiers in Plant Science. 2017, *8*. https://doi.org/10.3389/fpls.2017.00888

[22] Stewart, S. L., & Kane, M. E. Symbiotic seed germination and evidence for in vitro mycobiont specificity in Spiranthes brevilabris (Orchidaceae) and its implications for species-level conservation. In Vitro Cellular & Developmental Biology. Plant. 2007, *43*(3), 178–186. https://doi.org/10.1007/s11627-006-9023-4

[23] Utami, E. S. W., & Hariyanto, S. Organic Compounds: Contents and Their Role in Improving Seed Germination and Protocorm Development in Orchids. International Journal of Agronomy. 2020, 1–12. https://doi.org/10.1155/2020/2795108

[24] Verma, J., Sharma, K., Thakur, K., Sembi, J. K., & Vij, S. P. Study on seed morphometry of some threatened Western Himalayan orchids. Turkish Journal of Botany. 2014, *38*, 234–251. https://doi.org/10.3906/bot-1307-14

[25] Yamamoto, T., Miura, C., Fuji, M., Nagata, S., Otani, Y., Yagame, T., Yamato, M., & Kaminaka, H. Quantitative evaluation of protocorm growth and fungal colonization in Bletilla striata (Orchidaceae) reveals less-productive symbiosis with a non-native symbiotic fungus. BMC Plant Biology. 2017, *17*(1). https://doi.org/10.1186/s12870-017-1002-x

[26] Yang, H., Li, N. Q., & Gao, J. Y. A novel method to produce massive seedlings via symbiotic seed germination in orchids. Frontiers in Plant Science. 2023, *14*. https://doi.org/10.3389/fpls.2023.1114105

[27] Yeh, C. M., Chung, K. M., Liang, C. K., & Tsai, W. C. New Insights into the Symbiotic Relationship between Orchids and Fungi. Applied Sciences. 2019, *9*(3), 585. https://doi.org/10.3390/app9030585

[28] Yeung, E. C. A perspective on orchid seed and protocorm development. Botanical Studies. 2017, *58*(1). https://doi.org/10.1186/s40529-017-0188-4

Biography

Leen Al Zoubi is a master's student at the University of Jordan, specializing in Horticulture and Crop Science. She holds a Bachelor's degree in Landscaping and Floriculture from the same institution, graduating with distinction. Currently maintaining a 4.0 GPA, her master's thesis focuses on tissue culture and the secondary metabolism of medicinal plants. With one year of professional experience in horticulture and landscaping, Leen works part-time as a teaching assistant, recognized for her dedication. Actively engaged in research projects and workshops, she strives to advance her expertise in horticulture, showing her passion and commitment to the field.

Research Field

Leen Emad Al Zoubi: Horticulture, Landscaping, Tissue culture, Floriculture, Plant Production.