horticulturae

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Review

Medicinal Use, Flower Trade, Preservation and Mass Propagation Techniques of Cymbidium Orchids—An Overview

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Abstract: Cymbidium is an economically important genus in the orchid family (Orchidaceae) that has a pronounced medicinal and ornamental value. Medicinally, the plant is employed as a tonic to treat weakness in chronic diseases, dizziness, eye problems, burns, and wounds, etc. Cymbidiums are highly prized for their graceful flowers and sweet fragrance and are among the top ten most popular cut flowers. They are one of the most important commercial orchid groups and account for 3% of cut flowers in floriculture. Some orchid species in this genus are particularly threatened by excessive harvesting, so conservation measures are needed. Several enthusiastic organizations (e.g., The Cymbidiums Society of America, The Cymbidiums Club in Australia, The Golden Gate Cymbidiums Society, Alameda, CA, etc.) are dedicated to propagating, conserving, promoting, appreciating, and disseminating information about these beautiful and charming orchids. Through organogenesis (direct and indirect) and somatic embryogenesis, extensive propagation techniques for Cymbidiums have been developed to create protocols for synthetic seed production leading to large-scale propagation and long-term ex situ and in vitro conservation. This review highlights the medicinal uses, flower trade, conservation, and massive propagation techniques of Cymbidium orchids.

Keywords: Orchidaceae; traditional uses; health protection; conservation; micropropagation; horticulture

Citation: Balilashaki, K.; Martinez-Montero, M.E.; Vahedi, M.; Cardoso, J.C.; Silva Agurto, C.L.; Leiva-Mora, M.; Feizi, F.; Musharof Hossain, M. Medicinal Use, Flower Trade, Preservation and Mass Propagation Techniques of Cymbidium Orchids—An Overview. *Horticulturae* **2023**, *9*, 690. https://doi.org/10.3390/horticulturae9060690

Academic Editor: Wajid Zaman

Received: 29 April 2023
Revised: 30 May 2023
Accepted: 4 June 2023
Published: 11 June 2023

1. Introduction

The genus Cymbidium, also known as Boat Orchids, includes 75–80 species. As flowering plants in the Orchidaceae family, they are evergreens that bloom in winter and spring. They grow as epiphytes, terrestrial or lithophytes [1] in tropical and subtropical regions such as northeast India, eastern Asia and northern Australia.

Figure 1 shows multiple species of the genus Cymbidium that are predominantly epiphytic, but some species are also lithophytic and terrestrial or rarely leafless, saprophytic herbs, usually with pseudobulbs. Among orchids, Cymbidiums rank first, and in

floricultural crops they account for 2.7% of the total cut flower production [2]. This genus has had medical applications for many years, particularly in the eastern part of Asia. Thus it serves as an important medicinal plant in the pharmaceutical industry [3].

In the next sections, we describe these properties separately, evaluating the plant's medicinal use, its part in the flower trade, its preservation, and mass propagation techniques. We introduce this genus as a medicinal, economic and ornamental plant.

Figure 1. Some natural and hybrid cymbidium orchids: (**A**) *C. giganteum*; (**B**) *C. iridioides* D. Don. (the iris-like cymbidium); (**C**) *C. insigne* 'Alba'; (**D**) Cymbidium 'Maluka Baby Pink'; (**E**) *C. lowianum*, (the Low's boat orchid, (**F**) *C. tracyanum* L. Castle; (**G**) *C. aloifolium* (L.) Sw. (the aloe-leafed cymbidium/boat orchid); (**H**) *C. bicolor* (L.) Sw. (the two-colored cymbidium); (**I**) Cymbidium 'Foxfire Mini Pharaoh Malcome'; (**J**) *C. tigrinum* C.S.P. Parish ex Hook. (the tiger-striped Cymbidium)[Photo plate prepared from Mohammad Musharof Hossain's unpublished photographs].

2. Medicinal Value of Cymbidiums

Orchids have a long history of traditional medicinal use. Some orchids have been utilized by Indians since the Vedic period (2000 BCE–600 BCE) for their healing and aphrodisiac properties [3]; the Chinese and Japanese also have an ancient cultural history with orchids. In legend, they advocated the medicinal fuction of some orchid species in the 28th century BCE [4].

Several Cymbidium species, namely *C. canaliculatum* R. Br, *C. madidum* Lindley, *C. eburneum* Lindl., *C. aloifolium* (L.) Sw., *C. devonianium* Lindl. ex Paxton, *C. iridioides* D. Don, *C. giganteum* Wall. Ex Lindl. and *C. sinense* Willd., are used as medicinal plants in the traditional medicine of many Asian countries [5]. So far, different compounds such as phenols, alkaloids, phenanthrenes, stilbenoid derivatives, and steroids have been extracted and identified from the Orchidaceae, and the molecular structures have been elucidated by various spectroscopic methods [3].

Of the various Cymbidium species, only a few have been critically studied for their ethnomedicinal, glycosidic, and other pharmaceutical properties. The *C. aloifolium* plant, for example, is said to have emetic and laxative properties. It yields salep, a nutritious drink enriched with starchy polysaccharides, which is consumed in traditional drinks and desserts. The root powder can relieve paralysis symptoms, and boiled-down aerial roots are used to bandage broken bones. The extract of the leaves is applied to treat fevers and boils. It is also used as an emetic, tonic, laxative; it can treat burns, wounds, and earaches.

Crushed plant extracts with ginger are administered to treat eye weakness, dizziness, chronic diseases, and paralysis. It has two substituted bibenzyls, a phenanthraquinone (cymbinodin-A) and a dihy-drophenanthrene [3]. A decoction from the rhizome of *C. ensifolium* is used to treat gonorrhea, and a flower extract is used for eye inflammation [6]. The extract of leaves of Cymbidium (*C. giganteum*) has unique blood-clotting properties [7]. The pseudobulbs of *C. longifolium* are employed as a sedative, while an aqueous solution of dried and powdered pseudobulbs produces emesis when taken orally on an empty stomach [8]. The roots of *C. faberi* Rolfe. have been used in China for decades as an important herbal folk medicine to loosen phlegm and relieve cough, etc. [9]. The available literature demonstrates that phenanthrene compounds isolated from various orchids have shown various promising biological and antioxidant activities [10].

Recent reports state that extracts of Cymbidium roots have a high antimicrobial activity against *Staphylococcus aureus,* and the stem extracts contain phenolic compounds that exhibit a high antioxidant activity and cell cytotoxicity [11]. In another study, ephemeranthoquinone B, two phenanthrenes, and a phenanthrene glucoside were isolated from the roots of Cymbidiums along with six known phenanthrenes 5–10 [12]. The extracts from *C. kanran* Makino are enriched in flavone C-glycosides, including vicenin-2, vicenin-3, shaftoside, vitexin, and isovitexin [13]. The compound of 7-(4-hydroxybenzyl)-8-methoxy-9,10-dihydrophenane-threne-2,5-diol (HMD) was synthesized, together with five studied compounds [coelonin, 5,7-dimethoxy-phenanthrene-2,6-diol (DD), shancidin, 1-(4-hydroxybenzyl)-5,7-dimethoxy-phenanthrene-2,6-diol (HDP), and 2-methoxy-9,10-dihydro-phenanthrene-4,5-diol (MDD)], from the roots of *C. faberi*, as reported by Lv et al. [14]. Except for HDP, other compounds dose-dependently suppressed the production of NO, tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL -6) in lipopolysaccharide (LPS)-induced primary mouse peritoneal macrophages. Gigantol, a bibenzyl compound, has been isolated from *C. goeringii*, *C. aloifolium* and some other orchids and has shown anticancer activity [15]. It is a potent inhibitor of TNF-α, IL-6 and IL-1 and affects the mRNA expression levels of these cytokines in a dose-dependent manner. The qualitative analysis of various organic extracts of *C. aloifolium* revealed eight different photochemical compounds, namely n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z), 9,12,15-octadecatrienoic acid, (Z,Z,Z), octadecanoic acid, phytol, 2-butyn, 2-cyclopenten-1-one, and 1,4-benzenedicarboxylic acid [16]. Most of the identified compounds are biologically significant. In addition to the medicinal uses of Cymbidiums, endophytic fungi from orchid plants have been reported to secrete secondary metabolites containing bioactive antimicrobial siderophores [17].

3. Floristic Significance of Cymbidiums

Cymbidiums are a highly valued flower-growing plant. Because of their long-lasting inflorescences and large, attractive flowers, Cymbidiums are among the top ten commercial orchids. Among the 75–80 species, not counting the natural hybrids, are *C. floribundum* Lindl. (Golden-Edged Orchid), *C. devonianum* Lindl. ex Paxt, *C. elegans* Lindl. (Elegant Cymbidium), *C. eburneum* (Ivory-Edged Cymbidium), *C. mastersii* Griff. & Lindl, (Master Cymbidium), *C. erythraeum* Lindl., *C. iridioides* (Iris-Like Cymbidium), *C. lowianum* (Rchb.f.) Rchb.f. (Low's Boat Orchid) and *C. tracyanum* Rolfe. (Tracy's Cymbidium), *C. dayanum* (L.) Sw. (Day's Cymbidium or Phoenix Orchid), *C. suave* Sw. (Snake Orchid or Grassy Boat-Lipped Orchid), are the most beautiful, popular, and floristically important Cymbidium species. The greatest commercial use of this genus is associated with the splendor of its flowers and the splendor of its flowers and inflorescences. In floriculture worldwide, Cymbidiums hybrids are divided into three groups: miniature hybrids (e.g., Cymbidiums Autumn Emerald 'Royale'), large-flowered hybrids (e.g., Cymbidiums 'Kirby Lesh') and another commercial group called 'pending Cymbidiums'. Some Cymbidiums hybrids form clusters of up to 30 extravagant, multicolored flowers, including white, green, cream, mauve, and yellow [18]. The Tropical Cymbidiums Orchid is a well-known 'winter flower' with a flowering period of about two months, showing about 15

exquisitely beautiful and magnificent epiphytic flowers on the first inflorescence [19]. Undoubtedly, the commercial planting of this plant and the use of hybrid cultivars, despite having the advantages of a breeding cultivar, will lead to the elimination of native species and the reduction of genetic diversity and gene pool.

4. Reproductive Biology in Cymbidiums

Cymbidiums take 4–7 years to flower, but they are capable of triggering early flowering. This was demonstrated by Kostenyuk et al. [20] and suggests that the concerted action of phytohormone, as well as nutrient concentration and putative promoters/suppressors, determine the timing of the transition of the Cymbidium orchid from the vegetative to the reproductive stage [20]. The application of 6-benzylaminopurine, restricted nitrogen supplies with phosphorus enrichment, and root removal early induced the transition of a Cymbidium shoot from the vegetative to the reproductive stage for 90 days [20]. Furthermore, according to preview reports, the increase in leaf starch content during vegetative growth and soluble sugar in pseudobulbs and roots during the reproductive growth of Cymbidiums is critical for increasing the growth of the plant and thus promoting flowering [11]. Plants cultured at a high light intensity of photosynthetic photon flux density (PPFD) exhibited a lower time to flowering induction and development, alsoincreasing the number of inflorescences and flowers, in comparison with plants cultured at a low light intensity (PPFD) [11]. Moreover, shading treatments can significantly increase different inflorescence traits, such as quality, height, ratio, and spacing. The study by Zhou et al. [21] showed that inflorescence height, inflorescence ratio, and petal spacing of *Cymbidium* spp. increased significantly by 7.892 cm, 13.125 cm, and 0.484 cm, respectively, after shading [21].

The flowering of adult plants was influenced by several factors, such as fertilizer, light duration and quality, temperature, and plant growth regulators (PGRs), which affect flower induction, development, and flower characteristics. Flower diameter as well as the inflorescence length increased in response to increasing nitrogen and potassium fertilization during the adult vegetative stage in Cymbidiums grown at low light intensity and artificially induced inflorescence, while flower quality decreased in those grown at high light intensity [22]. Barman et al. [23] maintain that water-soluble fertilizers significantly affected the growth and reproductive stages of Cymbidiums. Their results showed that a maximum number of shoots (4.54), length of spikes (54.59 cm) and number of flowers per spike (10.19) were obtained when water-soluble fertilizer (N19P19K19 at 1 g/L concentration) was sprayed fortnightly. Day/night temperatures of 30/25 °C and 25/20 °C are best for vegetative shoot growth and flower bud formation in Cymbidiums, and intermediate flowers form most frequently in the just-growing young pseudobulbs and in the 1 to 2-year-old pseudobulbs [24]. Shoot growth rates and inflorescence numbers were lower at lower temperatures, such as 20/15 °C.

The molecular mechanisms underlying the regulation of the reproductive stage have been extensively studied in the long-day plant models such as Arabidopsis and the short-day plant such as Rice, and many processes of gene regulation during development, especially the reproductive biology of flowering, have been elucidated [25]. However, in orchids such as Cymbidiums, the understanding of the molecular mechanisms controlling flowering is just emerging [26]. Many genes are involved in the transition from the vegetative to the reproductive stage, so inducing these genes may be the best method for inducing the reproductive stage and flowering. In this sequence, researchers discovered some miRNAs, for example, the differential expression of two miRNAs, miR160 and miR396, targeting ARFs and the growth-regulatory factor (GRF), respectively [27]. Thus, genetic engineering is another approach to manipulate the switch from the vegetative to the reproductive stage of Cymbidiums.

The knowledge of reproductive biology is also limited in Cymbidiums. Four types of pollination are known: autonomous self-pollination, reward-based pollination, generalized food deception, and Batesian mimicry of the food source [28].

To date, autonomous self-pollination has been described in *C. macrorhizon*, *C. nipponicum*, and *C. nagifolium* Masam, all of which lack a rostellum that acts as a physical barrier between the anthers and the stigma [29]. In *Cym. mandidum*, pollination occurs by reward. The flowers are pollinated by the stingless bee *Trigona kockingsi* Cockerell., which collects viscous substance on the labellar surface [30]. The substance is probably used as nest-building material. A similar method is also thought to occur in *C. lowianum*, in which the labellar surface has proteinaceous papillae that may function as food hairs [31]. In some species, including *C. lancifolium* [32], *C. goeringii* Reichenbach Ill. [33], and *C. kanran* [33], a general feeding illusion has been observed. The nectarless flowers attract pollinating bees by visual and olfactory stimuli. Finally, a Batesian imitation of the food source occurs in *C. insigne*. The plant depends exclusively on the bumblebee (*Bombus eximius* Smith.), which also pollinates the nectar-producing flowers of *Rhododendron ciliicalyx* [34].

Pollination experiments were conducted on *C. macrorhizon*, *C. aberrans*, and *C. lancifolium* to study the breeding system. It was found that some rewarding myco-heterotrophic plants depend (at least in part) on an insect-mediated pollination system, and some myco-heterotrophic plants can attract pollinators without attractive materials [29].

5. Seed Biology of Cymbidiums

Orchid seeds are very small, extremely light, and produced in large numbers with the length range of from 0.05 to 6.0 mm, the range of longest and shortest known seeds in the family being 120 times. Known 1000-seed weights range from 310 μg to 24 mg (a 78-fold difference). The number of known seeds ranges from 20–50 to 4 million per fruit. The Testa are usually transparent, with outer cell walls that may be smooth or reticulate (Figure 2A). Embryos consist of 100–400 disorganized parenchymatous cells and seeds have no endosperm. The volume of the embryo is much smaller compared to the size of the seed. Orchid seeds are balloon-like with large internal air spaces and difficult to moisten so they can float in the air and on water for long periods of time, a characteristic that facilitates dispersal over long distances [35]. Orchid seeds can also be transported in and on terrestrial animals and birds (in fur, feathers or hair, mud on feet, and perhaps after ingestion) [2].

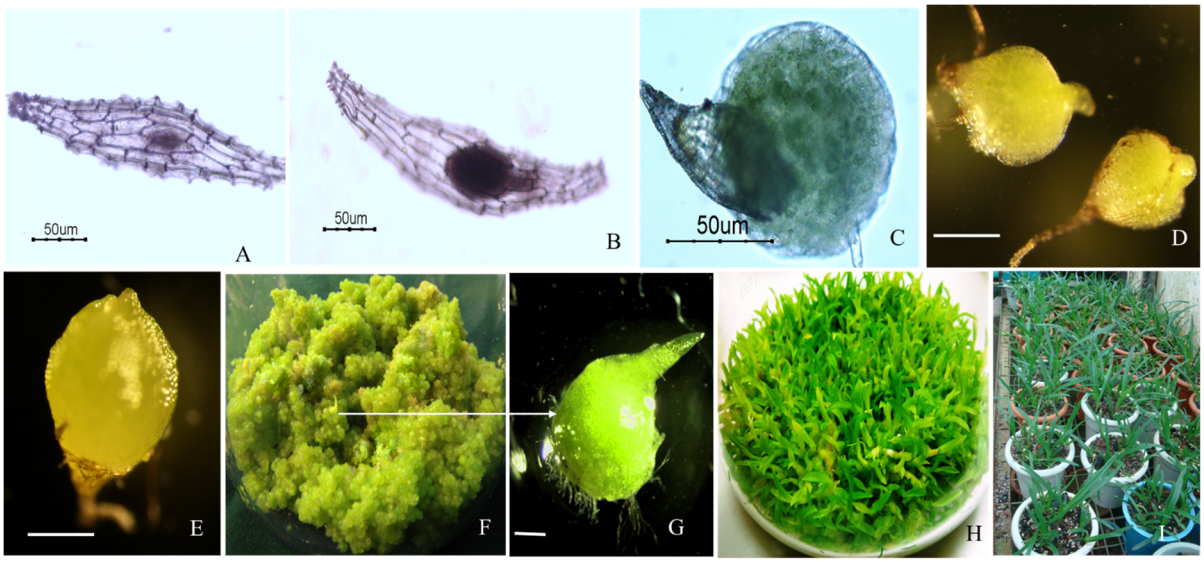

Figure 2. Embryo morphogenesis and seedling development in *C. aloifolium*: (**A**) seed with transparent and reticulate testa and small embryo, (**B**) embryos swell by uptake of nutrients and water, (**C**) cell number increases by repeated anticlinal and periclinal cell divisions, producing a parenchymatous cell mass called spherulite, (**D**,**E**) spherulites accumulated dense chloroplasts and formed a compact structure with bipolar character, (**F**) spherulites developed into protocorm, (**G**) germinated seeds, (**H**) young seedlings, (**I**) in vitro seedlings acclimatized in the outside environment. [Bar = 1 mm].

Cymbidiums produce large capsules that carry thousands to millions of long and fuzzy seeds with pointed ends [2,36].

The germination of orchid seeds under in vitro conditions depends mainly on their viability, which is determined by the pollination and fertilization process and the culture media used for germination. Direct and indirect methods are applicated for testing seed viability [6]. In the direct methods, the germinated orchid seeds on artificial media are counted [37], while in the indirect method, the metabolic activity of embryos is examined using chemicals such as fluorescein diacetate (FDA) ortetrazolium salts. To determine seed quality, germination tests are most commonly adopted to assess seed viability in plant production [38].

The seeds of orchids are orthodox, meaning they must be air dried to extend their longevity. The shelf life of the seeds can be extended by reducing the water content, temperature, and oxygen level in the storage room. Reducing the water content by up to 5 percent and then storing the seeds at a freezing temperature (−20 °C) is used in seed banks of orchids [39,40]. Orchid seeds stored at a low humidity and low temperature can survive for decades. On the other hand, they are resistant to sterilizing agents such as sulfuric acid or hypochlorite, which are used in the sterilization of seeds for in vitro germination [41].

Seed banks are recognized as the most efficient way to store large quantities of living plants in one location [42]. Orchid seed banks have the potential to make an invaluable contribution to orchid conservation [36]. According to studies, storing orchid seeds under cold conditions was the best conventional method to prolong seed viability.

6. Propagation of Cymbidiums

6.1. Conventional Methods

Cymbidiums can be propagated by dividing the plant into parts, by taking cuttings from the mother plant, or by taking dorsal or pseudobulbs and placing them in clay pots or plastic bags with potting substrate. The most used substrates are small bricks/chunks of charcoal, moist sphagnum moss, coconut moss, or coconut peat. Interestingly, the conventional propagation methods are slow and laborious, and the propagation rate is very low [43], with only a few plants produced per year, making this method impractical for large-scale propagation to provide clonal plantlets or plants for commercial purposes.

6.2. Clonal Propagation of Cymbidiums

In vitro methods of propagation are reliable, feasible and reproducible for the large-scale production of seedlings obtained by in vitro seed germination methods, or plantlets for clonal propagation of Cymbidiums using micropropagation. However, while seeds are most used for conservation or breeding , actual large-scale propagation of floricultural orchid varieties is realized preferentially by micropropagation due the clonal origin and industrialization of cultivation that aims at the scale-up and intensification of the cultivation [44].

6.3. Asymbiotic Seed Germination in Cymbidiums

Although symbiotic germination is an important tool for the conservation and restoration of natural species, asymbiotic in vitro germination is the primary method for seedling production and the genetic improvement of commercial orchids, including many Cymbidium spp. and their hybrids. The success of seed germination by in vitro culture is influenced by several factors, including seed maturity and age [45], type of nutrient solution [46,47], type of carbohydrate additives [48], organic additives [49], and PGRs [43].

In nature, only 2–5% of Cymbidium seeds germinate because they require symbiosis with a mycorrhizal fungus and germinate only after infection with a mycorrhizal fungus [50]. Almost all Cymbidiums must form a mycorrhizal and symbiotic relationship with a partner corresponding to a particular fungus during their growth and development [48]. The nutrients stored by the cells for seed germination are very low, making germination

very difficult [51]. Under certain conditions, different strains can significantly promote the germination of Cymbidiums. Further studies may be needed to verify the differences of this symbiosis under different ecological conditions [52].

Induced seed germination can be asymbiotic under in vitro conditions in a special culture medium or using symbiotic methods, which combines seed germination with the presence of a symbiont fungus [53]. Seed germination and propagation by symbiotic approaches are the preferred options for Cymbidiums' reintroduction into native habitats [52].

On the other hand, in vitro cultivation methods or asymbiotic orchid germination provides an interesting platform for the large-scale propagation of orchids without the requisite of fungal symbiosis. These methods facilitate the seeding and cultivation of seedlings on a different culture medium formulation, basically containing nutrients, carbohydrate sources, and agar, cultivated under aseptically controlled conditions [53].

The asymbiotic seed germination of orchids is a multipurpose technique that results in the production of high-quality seedlings and can be used for conservation, propagation, restoration, and breeding aiming at developing new hybrid cultivars [54,55]. In vitro germination and seedling development is the one of the most common methods also used for orchid propagation, mainly because it is technically less complicated than clonal propagation and allows for obtaining a large number of seedlings in a single culture medium, which contains only nutrients and a soluble carbohydrate source such as sucrose. Still, the clonal propagation by shoot propagation or protocorm-like bodies (PLBs) depends on a knowledge of the types, concentrations, and balance of phyto-regulators in the culture media used for each stage of plantlet induction, regeneration, proliferation and rooting/elongation [56]. As can be seen, there are opportunities for improvement in the successful propagation of Cymbidiums. These include selection of the optimal sterilant, improved seed recovery after sterilization, and selection of the optimal culture medium [57].

6.4. Ex Vitro Seed Germination

Seed propagation and seedling development is essential for the continuation of any plant population and Cymbidiums in particular. Due to the tiny seeds and undeveloped embryos, it has long been virtually impossible to observe the germination of Cymbidiums in vivo, which has been an obstacle to understanding seedling site requirements and fluctuations in Cymbidiums populations [58]. Because Cymbidium seeds lack an endosperm, they contain either low levels of food reserves or forms that are unlikely to be metabolized by Cymbidium embryos [58]. In nature, orchid seeds rely on Cymbidium mycorrhizal fungi to provide the necessary nutrients for germination, a process referred to as symbiotic seed germination [59]. Symbiosis, in its broadest sense, means the coexistence of two dissimilar organisms. A wide range of associations can be understood under this definition. The term germination mycobiont is used in this context to mean that a fungus participates in the mycorrhiza of orchid seedlings and supports seedling development. Seedlings and established plants may subsequently be attacked by other mycobionts and endophytes not involved in mycorrhiza. The function and effects of these endophytes in the adult stage on orchid physiology are often unclear [58].

The symbiotic view of germination can be summarized in six points: orchid roots are generally infected with a characteristic fungus that is not considered harmful; different orchid genera may have different strains or species of this fungus; seeds sown in pure culture on different growing media, especially soils containing starch or other insoluble organic matter, do not germinate unless the fungus is present; germination is apparently induced by some strains of fungus and not by others; a certain equilibrium between fungus and host must be maintained; although it is known that the germination of orchid seeds can be achieved by using sugar in the culture medium, germination under these conditions is abnormal and not common in nature [60,61].

A protocorm led to form shoots and roots at the beginning of germination in seeds without an endosperm [41]. Symbiotic germination, especially *in vitro*, is becoming increasingly popular because in many studies it promotes higher germination rates [62,63].

A study on the dependence of Cymbidiums on fungi during seed germination and seedling development provides a means of understanding the role of fungi in the developmental process [64]. Symbiotic seed germination is widely regarded as an effective tool for Cymbidium conservation and could be a cost-effective method to maintain the genetic diversity of reintroduced Cymbidium populations [65].

One of the major challenges is to obtain compatible fungi for symbiotic seed germination [26]. Nevertheless, the presence of compatible symbiotic fungi in Cymbidiums seedlings leads to greater adaptation to the environment, resulting in higher survival and faster growth than with asymbiotic seedlings [66].

To be recognized as an orchid mycobiont, it is generally necessary for the fungus to colonize orchid tissues with the formation of mycorrhizal features such as peloton formation (certain cells form cyclically dense hyphae), i.e., intracellular hyphae coils, and the absence of cortical tissue necrosis [58,67]. Many studies have been conducted to obtain compatible fungi for the germination of symbiotic seeds. On this subject, researchers reported that symbiotic seedlings with many strains show a higher stability than asymbiotic seedlings. For instance, Mahendran et al. [28] noted that mycorrhizal fungi significantly increases the number of roots and shoots [28]. Dong et al. [68] reported that the average increase in fresh weight of Cymbidium seedlings inoculated with strains CF1, CF3, and CF12 was 130.26%, 345.65%, and 153.34%, respectively, while the increase in the control was only 88.40%. The differences among the three treatments and the control are statistically significant. Liu et al. [69] investigated the effects of inoculated mycorrhizal fungi and non-mycorrhizal beneficial microorganisms on the plant characteristics, nutrient uptake, and root-associated fungal community composition of Cymbidium Hybrids Hort. They reported that all inoculants significantly increased the total dry weight of Cymbidiums. The mycorrhizal fungus positively affected the P, K, Ca, and Mg content in the shoots and the Zn content in the roots, suggesting that both mycorrhizal fungi and endophytic fungi have the potential to create a favorable symbiosis in orchid roots and stimulate growth [70]. Chand et al. [56] evaluated twelve isolated instances of the plant growth-promoting activity on *C. aloifolium* protocorms (84 days old). All of them showed growth-promoting activity [56].

As noted above, obtaining compatible fungi is a crucial step for symbiotic seed germination because mycorrhizal fungi have the ability to induce seed germination with varying efficiency and low specificity [71]. To this end, in situ/ex situ seed-baiting techniques have been proposed as an effective method to obtain efficient symbiotic seed germination-promoting fungi [72].

Ex-situ seed-baiting techniques developed in recent years are effective methods to study the compatible mycorrhizal fungi of Cymbidiums. Sheng et al. [73] noted that after 58 days of cultivation, seeds inoculated with a fungal strain showed a high germination rate, while seeds without the fungus did not germinate. Germination and protocorm production were higher in the dark (0/24 h light/dark) than under light conditions (12/12 h light/dark), while subsequent protocorm development was better under light [73].

After germination, the orchid seedling undergoes a long or short non-photosynthetic phase in which it is completely dependent on organic carbon from a mycobiont, so a suitable organic carbon source for the mycobiont is another important requirement.

6.5. Seedling Development

Cymbidiums produce dust-like seeds of microscopic structure The cellular organization of the seeds is simple and consists of an undifferentiated mass of embryonic cells and a rudimentary endosperm covered by a transparent seed coat [74]. According to Arditti and Ghani [2], Cymbidium embryos are relatively small and simple, generally oval or

spherical, and sometimes composed of only a few cells, usually without an endosperm (Figure 2A).

Regarding seed maturation, immature seeds from 3- to 5-month-old capsules could be successfully germinated on a culture medium. Immature seeds from 4- and 3-month-old capsules showed the highest and lowest germination rate [45]. In another study in relation to planting times, younger seeds germinated slowly in early planting but grew rapidly and formed more rhizomes in later planting. In contrast, older seeds germinated rapidly in early culture but formed fewer rhizomes the later planting [75].

In vitro germination in an artificial culture medium is one of the best solutions for increased germination rates and rapid seedling development. During seed germination, four to five different developmental stages are observed in Cymbidiums, and this is also more or less common in other Orchidaceae. Orchid seeds undergo a typical metamorphogenetic process during germination. Stage 1: In this stage, viable embryos swell only by the uptake of nutrients and water (Figure 2B); Stage 2: The number of cells increases by repeated anticlinal and periclinal cell divisions, resulting in the formation of irregularly shaped parenchymatous cell masses that emerge when the seed coat breaks open; this stage is called the globular stage (Figure 2C); Stage 3: The parenchymatous cell mass is enriched with dense chloroplasts and exhibits a bipolar character; the compact structures are referred to as spherulites; some rhizoids emerge from the posterior/basal part of the spherulites, while an appendage appears from the anterior/upper part (Figure 2D); Stage 4: In this stage, the spherulite enlarges and a protuberance appears at the anterior part, demarcating the meristematic zone for the development of a leaf primordium; this stage is called the protocorm stage, and is thought to be the effective germination of orchid seeds (Figure 2E); Stage 5: This is the final stage of germination, where roots emerge from the posterior part of the protocorm and gradually develop into young seedlings (Figure 2F). Protocorm development is considered a characteristic feature of post-seminal development in orchids, and the shape of protocorms is taxon-specific. They may be oval, round, disc-shaped, elongated, branched, spindle-shaped, or thorn-shaped. In Cymbidiums, the early protocorm is usually round, radially symmetrical, green, and gradually assumes an oval shape.

6.6. Culture Media

In terms of culture media, there are a variety of formulas that have different effects on seed germination [76]. Mohanty et al. [77] noted that the percentage of seed germination varied with the composition of the culture media and was highest in a full-strength MS basal medium; the number of secondary protocorms that developed from seed-derived protocorms increased with the addition of 5.0 μM 6-benzilaminopurine (6-BAP) and 2.5 μM α-naphthaleneacetic acid (NAA) [77]. Dep and Pongener [78] reported that immature embryos were successfully germinated 9 months after pollination on an MS medium (sucrose 2% (*w*/*v*) + NAA and benzyladenine (BA) (3 and 6 μM in combination, respectively) within 7 weeks of culture, recording 90% germination [78].

Mahendran et al. [28] cultured immature seeds of *C. bicolor* Lindl. on four basal media, namely Murashige and Skoog (MS), Knudson C (KC), Knudson C modified Morel (KCM), and Lindemann orchid (LO). The results were significantly higher and lower on the LO medium (96.6%) and KC (62.7%) media after 8 weeks, respectively [28].

Gogoi et al. [70] cultured *C. eburneum* Lindl. asymbiotically in different basal media, namely MS, Mitra, B5 and Nitsch. It was found that the medium MS, nourished with 15 μM each of BAP and NAA in combination, increased the number and length of shoots and the number and length of roots in the seedlings [70].

It is believed that nutrient requirements for orchid seed germination are species specific and that the nitrogen source plays an important role in orchid seed germination, which may explain the superior germination of seeds on a Murashige & Skoog medium [79]. It has been reported that nitrogen in the medium MS strongly influences cell growth and differentiation, and that ammonium nitrate in the medium MS is the most suitable

source for seed germination and plantlet development. Other reduced nitrogen forms such as pyridoxine, thiamine and nicotinic acid as vitamins are also absent in KC and VW media but present in MS media [80].

The addition of different types and concentration of organic substances to in vitro culture should stimulate seed germination. These organic additives are natural sources of amino acids, vitamins, minerals, organic acids, sugars, proteins, and natural growth regulators that help in orchid propagation by stimulating development and morphogenesis in the asymbiotic seed culture [81].

For example, the additional presence of riboflavin, biotin and folic acid in a Mitra medium may have further promoted seed germination. Mitra medium has high concentration of phosphate ions that affects the seed germination of asymbiotic orchids [82]. Dutra et al. [82] also showed that nitrogen does not play an important role in the seed germination of asymbiotic orchids compared to other nutrients, especially those in a Mitra medium [82]. Hajong et al. [83] reported that the low response of orchid seeds to nitrogen in the B5 and Nitsch media could be due to the inhibitory effect of nitrogen in the form of ammonium sulfate on seedling growth in B5 media or vitamin mixtures present in both B5 and Nitsch media.

With respect to PGRs, the modification of free PGR culture media by the addition of specific PGRs and other components such as activated charcoal, as well as changes in culture conditions, have been reported to improve the germination percentage and subsequent protocorm development in many Cymbidium genotypes [84]. For the development of efficient germination and micropropagation protocols, the conventional tissue culture media must be modified by adding specific PGRs and various complex additives (peptone, yeast extract, banana pulp, etc.), and plant production must be automated by adjusting the bioreactor system and culture conditions. Recently, orchids have become the focus of new research areas, including genetic engineering, functional genomics, proteomics, and metabolomics, all of which require standardized micropropagation techniques. The successful application of new approaches will contribute to the further improvement of orchids and orchid products [85].

7. Clonal Propagation of Cymbidiums

The clonal propagation of Cymbidiums can be practically realized by division of rhizomes or pseudobulbs, but this conventional technique generates 2–4 plants per year and is applied for amateur cultivation. This technique not practicable in the large-scale production of cut and pot flowers. The actual and realistic technique used in large-scale floriculture is micropropagation by shoot proliferation or by the induction, proliferation and regeneration of protocorm-like bodies, also called IPR-PLBs [86].

7.1. Micropropagation of Cymbidiums

Cymbidiums were the first orchid to be micropropagated by tissue culture. The first success of clonal propagation of *Cymbidium* sp. was reported by Morel [87] through the culture of shoot tips, which led to the in vitro production of millions of plantlets from tiny plant parts [87,88]. The first detailed protocol for in vitro propagation of Cymbidiums, which began with meristem culture, was published by Wimber [89]. The use of explants from plants grown outdoors is generally associated with the problem of a high contamination rate. Different types of explants can be used to start micropropagation, e.g., shoot tips [5,90], leaf segments [30,91], thin cross-sections of PLB [91,92], shoot segments [3], whole proto-buds [3], PLB segments [93], flower buds [94], leaf bases of axenic seedlings [95]; rhizome segments [94,95]; complete seedlings [96], transverse thin cell layers *(t*TCL*)* cut from stem internodes and nodes from the base of the shoot apex [92], and root tips [97]. Nevertheless, for each species or hybrid, a particular explant has been shown to be suitable for efficient micropropagation.

Plantlet propagation in orchids can be accomplished by direct shoot bud formation (direct organogenesis), formation of shoot buds via callus formation (indirect organogenesis), formation of secondary proto-buds or PLBs without an intervening callus phase (direct embryogenesis), and formation of PLBs via callus (indirect embryogenesis) [98–100]. Proto-buds (secondary embryogenesis) can be regenerated from the outer tissues of protocorms [99]. This is an independent pathway in which they develop without an intervening callus phase. The cells of young protocorms are highly meristematic in nature and can be used to rejuvenate and enhance plant regeneration in Cymbidiums [38].

The type of morphogenetic differentiation (organogenesis or embryogenesis, directly or indirectly) depends on a number of plant endogenous and exogenous factors, such as type and origin of explants, the culture media, culture media consistency, cultivation conditions, PGRs, complex additives, and even culture duration [3].

The use of liquid or gelled culture media influences the morphogenic formation. As an example, the new formation of PLBs (also called secondary protocorms) from seed-originated protocorms was observed in *C. aloifolium* and *C. giganteum* in either a liquid or gelled agar medium [96]. Although the liquid medium was more efficient for propagation of PLBs, this method failed to develop plantlets unless transferred to a gelled agar medium. The non-uniformity and hyperhydricity of the PLBs were also observed [3]. Hyperhydricity (development of physiologically abnormal tissues), high contamination rate, and production of etiolated plantlets are the common disadvantages of in vitro propagation using the liquid media system [93]. In general, hyperhydric tissues are unable to regenerate physiologically true plantlets. The failure of hyperhydric tissues to regenerate in a liquid medium could be related to tropisms or perturbations in polarity [92]. There is much evidence that the reduction of the agar concentration or the absence of agar in the culture medium leads to vitrification during tissue growth. However, a number of studies suggest that not all plant species exhibit vitrification when cultured in a liquid medium. Although regeneration of plantlets could be achieved in both a liquid and semisolid culture medium, a liquid medium generally proved to be better for propagation and growth of newly developed plantlets because the cultures are maintained under constant agitation.

PLBs were induced from protocorm sections, pseudostem segments, and even complete seedlings of *C. aloifolium* and *C. giganteum* on a semisolid PM agar medium supplemented with BAP and NAA. The frequency of PLB regeneration was strongly influenced by the concentrations and combinations of the two PGRs. Although histological and cytological analyses suggest that a PLB is indeed equivalent to a somatic embryo, this claim has never been made in the Orchidaceae [91,98].

7.2. Propagation by Artificial Seeds

Nowadays, the encapsulation technique to produce artificial seeds has become an important part of micropropagation [101]. Artificial seeds are artificially encapsulated vegetative parts that can be sown as seeds and transformed into a plant under in vitro or in vivo conditions. These parts include somatic embryos (usually), cell aggregates, shoot buds, auxiliary buds or other micropropagules [102]. Another study on in vitro germination and propagation of *C. aloifolium* (L.) Sw. was conducted by Pradhan et al. [101] using artificial seeds prepared in vitro by encapsulating PLBs with 4% sodium alginate and 0.2 mol·L^{-1} calcium chloride solution. This study showed that artificial seeds are a good alternative for in vitro mass propagation and the short-term preservation of *C. aloifolium* [101]. Artificial seeds are also an excellent way to store orchid material at room temperature, under refrigeration, or even in cryopreservation for weeks, months, or even years while maintaining the clonal stability of the material [103]. Table 1 summarizes the research on some orchids with emphasis on Cymbidiums.

Table 1. Summary of research on some orchids with emphasis on Cymbidiums.

Researchers	Year	Subject	Results
Deb and Pongener [104]	2002	Studies on the in vitro regenerative competence of aerial roots of two horticulturally important Cymbidium species	Of the three basal media tested, MS medium supported optimum regeneration and culture proliferation in both the species. In *C. aloifolium* ~12, shoot buds developed on medium nourished with sucrose 3% and benzyl adenine (BA) 3 μM but in *C. iridioides* optimum regeneration was achieved when medium supplemented with sucrose 3%, coconut water (CW) 15%, casein hydrolysate (CH) 100 mg/L and ~20 shoot buds formed per subculture
Hossain et al. [105]	2009	Cost-effective protocol for in vitro mass propagation of *C. aloifolium* (L.) Sw.–a medicinally important orchid	Mitra medium supplemented with 2.0 g/L activated charcoal (AC) showed 100% seed germination and effective for induction of significantly large-size protocorms (1.64 mm in dia.)
Hossain et al. [106]	2010	Seed germination and tissue culture of *C. giganteum* Wall. ex Lindl	The effects of peptone, AC and two-plant growth regulators [6-benzylaminopurine (BAP) and 2,4-Dichlorophenoxyacetic acid (2,4-D)] were also studied. Both M and PM supplemented with 2.0 g/L peptone or 1.0 mg/L BAP resulted in ~100% seed germination.
Deb and Pongener [78]	2011	Asymbiotic seed germination and in vitro seedling development of *C. aloifolium* (L.) Sw.: a multipurpose orchid	Immature embryos of 9 months after pollination were successfully germinated on MS medium containing sucrose (2%) (*w*/*v*) and α-naphthalene acetic acid (NAA) and BA (3 and 6 μM, respectively, in combination) within 45 days of culture where 90% germination was recorded. The germinated seeds formed PLBs on the optimum germination medium within two passages.
Nahar et al. [107]	2012	Effect of different light and two polysaccharides on the proliferation of protocorm-like bodies of Cymbidiums cultured in vitro	The highest protocorm-like bodies (PLBs) formation, shoot formation rate (90%) and root formation rate (50%) were found among explants cultured on medium supplemented with 0.1 mg/L Chitosan H under green light. After 11 weeks of culture, fresh weight of PLBs was higher (241.3 mg) at HA9 (1 mg/L) treatment with green light. The average number of PLBs (5.7) was higher under green light at HA9 treatment. PLBs under white light showed the highest number of shoot (1.2) at Chitosan H treatment.
Parmar and Pant [108]	2016	In vitro seed germination and seedling development of the orchid *Coelogyne stricta* (D. Don) Schltr	MS medium supplemented with 1 mg/L BAP and 1 mg/L NAA was found to be the best condition for the development. The germination started after 7 weeks of culture and complete seedlings were obtained after 23 weeks of culture on the medium supplemented with 1 mg/L BAP and 1 mg/L NAA suggesting the usefulness of both hormones in root induction. In the hormone, free MS medium germination started after 5 weeks, but root initials were not developed even after 32 weeks of culture.
Pradhan et al. [109]	2016	Efficient plant regeneration of *C. aloifolium* (L.) Sw., a threatened orchid of Nepal through artificial seed technology	Full strength of MS medium without plant growth regulators was found to be the most favourable condition for efficient plantlet regeneration of *C. aloifolium* (9.83 shoot and 2.66 roots per culture).
Bhowmik and Rahman [84]	2017	Effect of different basal media and PGRs on in vitro seed germination and seedling development of medicinally important orchid *C. aloifolium* (L.)	Medium supplemented with hormones favored optimum condition for the germination (approx. 95%) of seeds followed by full strength and half strength on KC, MS, PM and VW media. MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA showed comparatively better response within 6 weeks of culture than other conditions of MS medium as well as KC, PM and VW media.
Philip Robinson et al. [110]	2017	In vitro seed germination of *C. aloifolium* (L.) Sw., a potential medicinal orchid from Eastern Ghats of Tamil Nadu, India	The highest seed germination of 90% was observed KC basal media after30th days whereas germination percentages were 40% and 30% on 1/2 MS and VW media respectively.
Paul et al. [111]	2019	In vitro mass propagation of *C. aloifolium* (L.) Sw	Developing an efficient protocol for rapid propagation of *C. aloifolium* starting with in vitro asymbiotic seed germination, leading to protocorm induction followed by plantlet development and successful ex vitro acclimation.

8. Conclusions

Cymbidium orchids are the most important floricultural plants in the world. They are a potential source of special metabolites used in alternative and conventional medicine. The large number of species and their origin from different habitats allowed the development of varieties that flower in different parts of the world. However, in some tropical regions, the production of flowering plants continues to be concentrated in the autumn/winter because they require low temperatures for flowering. Breeding programs could use new genotypes to obtain hybrids that bridge this period and allow production of commercial plants at other times of the year. Propagation by seed is reliable and simple and can be done under in vitro and ex vitro conditions. However, the large-scale production of seedlings in industrial floriculture requires the development of efficient micropropagation protocols applied to a significant number of important cultivars on the market, producing millions of plants each year to match the number of seedlings commercially distributed in all of the world.

Author Contributions: Conceptualization, methodology and writing—original draft preparation, K.B. and M.V.; validation, investigation and formal analysis, K.B., M.V. and M.M.H.; resources, M.V. and M.M.H.; writing—review and editing, M.E.M.-M., J.C.C., C.L.S.A., M.L.-M., F.F. and M.M.H.; visualization, M.M.H.; supervision, M.E.M.-M. and M.V.; project administration, M.E.M.-M.; funding acquisition, M.E.M.-M., C.L.S.A. and M.L.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within this article.

Conflicts of Interest: The authors declare no conflict of interest.

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