Review

Perspective and challenges of mycorrhizal symbiosis in orchid medicinal plants

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ARTICLE INFO

ABSTRACT

The family Orchidaceae is of the most diverse taxon in the plant kingdom, and most of its members are highly valuable herbal medicines. Orchids have a unique mycorrhizal symbiotic relationship with specific fungi for carbohydrate and nutrient supplies in their whole lifecycle. The large-scale cultivation of the medicinal plant *Gastodia elata* is a successful example of using mycorrhizal symbiotic technology. In this review, we adopted *G. elata* and *Dendrobium officinale* as examples to describe the characteristics of orchid mycorrhiza and mycorrhizal benefits for host plants' growth and health (e.g. biotic and abiotic stress and secondary metabolite accumulation). The challenges in applying mycorrhizal technology to the cultivation of orchid medicinal plants in the future were also discussed. This review aims to serve as a theoretical guide for the cultivation of mycorrhizal technology in medicinal orchid plants.
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1. Introduction

The family Orchidaceae is of the most diverse taxon in the plant kingdom, and most of its members are highly valuable herbal medicines. This family has several unique characteristics, such as high species diversity (28 000 + species), complex pollination mechanism of deceit (food mimicry, sexual mimicry, brood-site mimicry or territorial antagonism) (Nilsson, 1992; Liu et al., 2022a), diverse ecological style (terrestrial, epiphytic or lithophytic) and nutritional type (photoautotrophy, mycoheterotrophy and mixotrophy) (Zhang et al., 2018) and intimate mycorrhizal symbiotic association with compatible fungi (Perotto & Balestrini, 2023). Thus, Orchidaceae is regarded as an excellent model for evolutionary ecology because of its tight biological interactions from head (pollination by insects) to toe (mycorrhizal symbiosis with fungi) (Selosse, 2014). However, many orchid species are endangered because of overcollection and habitat loss. All orchids are included in the Convention on International Trade in Endangered Species (CITES) (Roberts & Dixon, 2008).

Mycorrhiza is one of the most important interactions between biological worlds because it involves about 340 000 species of land plants and about 50 000 species of soil fungi. Orchidaceae plants can form mycorrhizal relationships with some fungi, such as Tulasnellaceae and Serendipitaceae, and these relationships are called orchid mycorrhiza (ORM). ORM is one of the four main mycorrhizal types, the three other mycorrhiza types are arbuscular mycorrhiza (AM), ectomycorrhiza (ECM) and ericoid mycorrhiza (ERM) (Genre, Lanfranco, Perotto, & Bonfante, 2020). AM, an ancient mycorrhizal type that is recognised as the mother of plant root endosymbiosis, is the most widespread amongst terrestrial symbioses and formed by 70%–90% of land plant species. AM symbiosis contributes significantly to global phosphate and carbon cycling and influences primary productivity in terrestrial ecosystems (Van der Heijden, Martin, Selosse, & Sanders, 2015). AM has considerable usage potential in sustainable agriculture because it can functionally promote crop growth and development, thereby increasing the resistance and productivity of biofertilisers (Salvioli di Fossalunga & Bonfante, 2023). ECM, which is predominant in trees in forest ecosystems, has massive potential in forest regeneration and ecological restoration of mine sites (Corrales, Henkel, & Smith, 2018). ERM is similar to ORM, it is a mycorrhiza relationship formed by fungi and ericoid plants and belongs to endophytic mycorrhiza. ERM symbiosis contributes considerably to bioremediation and flower production. Orchidaceae plants and their mutualistic relationship with mycorrhizal fungi have elicited increasing scientific attention because of their unique evolutionary characteristics and medicinal, economic and ornamental value. Studies have discovered that ORM shows potential in the conservation and in-situ habitat restoration of wild resources. Thus, in this review, we adopt Gastodia elata Blume (a completely heterotrophic orchid plant that has been artificially cultivated using mycorrhizal symbiosis techniques) and Dendrobium officinale Kimura & Migo (a plant in which mycorrhizal symbiosis has been well studied but not yet used in practice) as examples to describe ORM characteristics, the benefits of mycorrhizal symbiosis to host plants and the potential application of the mycorrhizal approach in the ecological cultivation and medicinal quality improvement of orchid medicinal plants. Several crucial challenges are also discussed.

2. What are ORM and orchid mycorrhizal fungi?

ORM refers to a symbiotic relationship formed by the roots of Orchidaceae plants and soil fungi. ORM fungi can form a symbiotic relationship with Orchidaceae plants and generate intracellular fungal pelotons belonging to root endophytic fungi (Dai, 2011).

Considerable attention has been given to ORM and the application of molecular biology approaches, such as high-throughput sequencing application for mycobiont identification, which have shown that saprophytic fungi belonging to the families of Tulasnellaceae, Serendipitaceae and Ceratobasidiacea are the most common amongst orchid mycorrhizal fungi (Li, Yang, Wu, Selosse, & Gao, 2021). In addition, studies have shown that many mycoheterotrophic orchids are associated with ectomycorrhizal fungi (Jacquemyn, Waud, Merckx, Lievens, & Brys, 2015) or root endophytes (Selosse et al., 2022). The diversity and composition of ORM fungi are related to plant species, geographical distribution, nutrition type, ecotype and even developmental stages (Jacquemyn et al., 2017; Xing, Gai, Liu, Hart, & Guo, 2015). ORM association starts from the recognition of plants and fungi. Then, fungal hyphae invade the embryo from the suspensor or root hair, colonise into orchid cortical cells and form elaborate coiled structures known as pelotons, which are symbolic structures of symbiotic interaction establishment and a site for nutrient exchange. Afterwards, the pelotons degrade. The whole process of mycorrhizal establishment involves complex physiological and biochemistry reactions and is regulated by a number of genes and proteins (Ghirardo et al., 2020). However, studies on the signal transduction pathway and the role of each partner of ORM symbiosis are limited compared with AM studies (Favre-Godal et al., 2020).

In recent years, various omics technologies, such as genome, transcriptome, proteome and metabolome, have been applied to elucidate the complexity of ORM interaction and provide a preliminary understanding of the establishment and signalling of mycorrhizal symbiosis in Orchidaceae. The common symbiotic signalling pathways (CSSP) involved in AM symbiosis are believed to exist in ORM (Miura et al., 2018; Xing et al., 2020). Plant roots exude hormone or flavoid compounds, and these compounds stimulate fungi to release signalling molecules; the two partners recognise each other through the receptor protein, promote successful fungal colonisation inside plant roots and further trigger the transcription regulation of mycorrhizal symbiosis-related genes (Favre-Godal et al., 2020). Although the signalling pathway in AM is clear, the understanding of the symbiotic process of ORM is insufficient. Extant studies did not find key presymbiotic signals, such as chitin derivatives (or the Myc factor) or plant hormone strigolactones (SLs), in ORM but found multiple copies of SL-related genes in G. elata; these genes may increase the ability of G. elata to establish symbiotic relationships with ORM fungi (Genre, Lanfranco, Perotto, & Bonfante, 2020). Thus, studies on the signal molecules produced by orchid plants and/or mycorrhizal fungi and the recognition mechanism need to be performed in the near future.

3. How does ORM benefit host plants?

Studies have revealed that land plants can provide up to 20 % of photosynthetically fixed organic carbon to their mycobionts, and in exchange, mycorrhizal fungi provide their plant partners with up to 80% of the nitrogen (N) and 100% of the phosphorus (P) required for plant growth and proliferation (Hoysted et al., 2018). Thus, mycorrhizal fungi offer various benefits to host plants, including orchids. ORM fungi can stimulate seed germination and seedling growth, improve the host plant's resistance to biological and abiotic stresses, promote the production and accumulation of effective components of medicinal plants and produce bioactive metabolites (Bowles, Jackson, & Cavagnaro, 2018) (Fig. 1).

3.1. Stimulating seed germination and seedling growth

Germination is a determining factor in the propagation of plant species. Fungi play an important role in the seed germination of
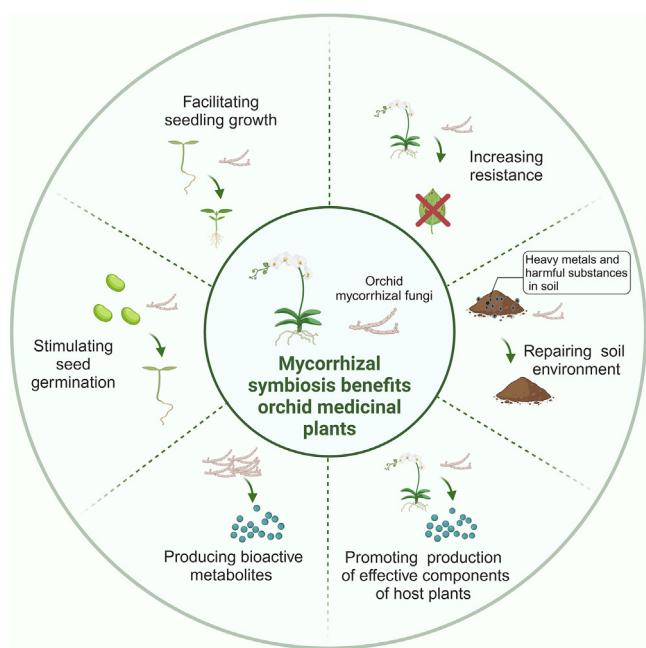
Fig. 1. Potential application of mycorrhizal symbiosis in medicinal orchid plants.

orchids. Orchidaceae seeds are undifferentiated embryogenic structures without endosperm, and the seed germination of almost all orchid plants depends on compatible fungi in nature. Seed germination in orchids needs to go through a unique developmental stage, which is called the protocorm stage. The protocorm stage is essential for the establishment of seedlings and mycorrhizal symbiosis and determines whether or not the seeds will develop smoothly into seedlings (Gao et al., 2023; Smith & Read, 2008).

G. elata is a completely heterotrophic medicinal orchid plant that completely relies on *Armillaria mella* and *Mycena osmundicola* J.E. Lange to provide nutrition during its life history. In the 1980 s, Xu et al. established a symbiotic cultivation system that uses *G. elata* and its mycobiont *A. mella* and revealed the symbiotic nutritional relationship between the two fungi in the life history of *G. elata*. At the sexual stage, seeds must be symbiotic with fungi belonging to *Mycena* spp. to obtain nutrients and launch germination. Then, a symbiotic relationship is established with *Armillaria* members after the seeds produce a vegetative reproductive stem. *Armillaria* spp. fungi provide nutrients for the growth and development of *G. elata* tuber and obtain nutrients from it (Xu, 2013). The bilateral flow of nutrients is the material basis of symbiotic partnerships between *G. elata* and fungi (Liu et al., 2024). Xu et al. isolated *M. osmundicola* from the protocorms of *G. elata* and confirmed that the fungi can effectively increase *G. elata* seed germination to up to 70% (Xu et al., 1980; Xu & Guo, 1989). These studies contributed to the successful large-scale artificial cultivation of *G. elata* by seeds. Afterwards, Guo et al. continued to isolate mycorrhizal fungi belonging to *Mycena* (including *M. anoectochila*, *M. orchidicola* and *M. dendrobii*), members of *Epulorhiza* spp. and *Ceratorhiza* spp. and some endophytic fungi from various orchid plants in different geological regions and development stages. These fungi were found to stimulate the seed germination and seedling growth of *D. officinale* and *Dendrobium nobile* Lindl. effectively (Guo & Xu, 1990a,b, 1991). In recent years, with increasing research on ORM, a number of mycorrhizal fungi have been isolated and confirmed to promote the seed germination or seedling development of other orchid medicinal plants, such as *Dendrobium* spp. (Tang et al., 2022; Zhang, Li, Chen, Guo, & Lee, 2020; Zhang, Li, Chen, Meng, & Guo, 2020), *Bletilla striata* (Thunb. ex A. Murray) Rchb. f. (Xi et al., 2020; Shi et al., 2023), *Cremastra appendiculata* (D. Don) Makino (Gao et al., 2022) and *Gymnadenia conopsea* (L.) R. Br. (Gao et al.,2020) (Fig. 2).
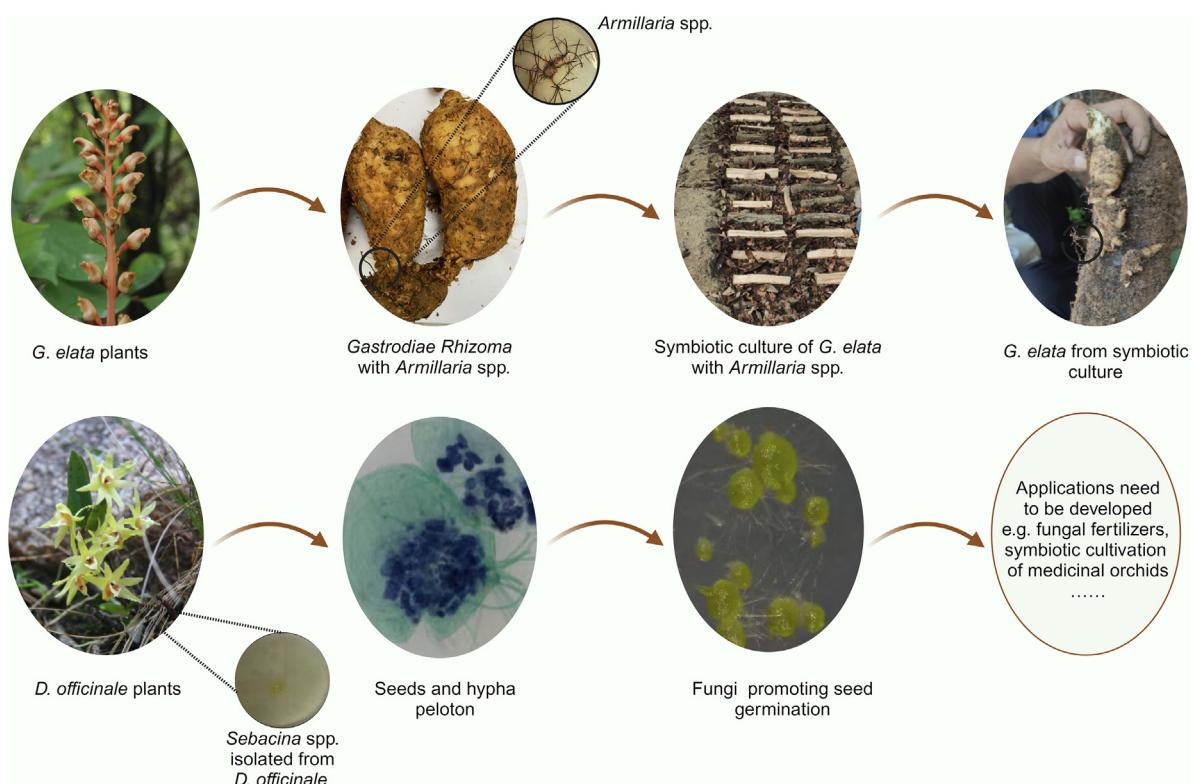

Fig. 2. Practical application of mycorrhizal symbiosis in medicinal orchid plants. The upper part shows the application of *Armillaria* spp. in *G. elata* (the first artificial cultivation of *G. elata*). The lower part shows that fungi isolated from *D. officinale* roots can promote seed germination.

Studies on the mechanism through which mycorrhizal fungi promote seed germination have focused on the effects of the immune defence response, nutrient supply and the role of plant hormones. Zeng et al. conducted transcriptome and proteome analyses of the symbiotic germination system of *G. elata* and found that fungi can change the metabolism and defence of *G. elata* seeds during symbiotic germination, modify the genes related to the seed oligosaccharide elicitor and remarkably increase the gene expression levels of fungal cell wall degradation and plant cell wall xylanase and pectase (Zeng, Li, Ling, Chen, & Guo, 2018; Zeng, Yang, Ling, Zhang, & Guo, 2018). Proteins related to carbon metabolism and endocytosis are also up-regulated in the seeds of *G. elata*. Our comparative transcriptomic and proteomic analyses showed that compared with asymbiotic germination, fungal colonisation in *D. officinale* seeds induces higher and earlier expression of some key genes involved in lipid and carbohydrate metabolism and thus improves the utilisation efficiency of stored substances in the embryo (Chen et al., 2017). In addition, isotope-labelling experiments have been conducted to demonstrate that orchid plants acquire carbon, nitrogen and phosphorus from their fungal partners and that carbon and nitrogen are transferred from symbiotic fungi to host plants by living pelotons or digested hyphae (Cameron, Leake, & Read, 2006; Zahn et al., 2023). Several genes that encode sugar and amino acid transports, such as the sucrose transporter (SUT)-like genes *GeSUT4* and *GeSUT3*, have been found to increase ORM expression; the high expression of *GeSUT4* in symbiotic and large cells of *G. elata* tubers has been verified, thus revealing the role of *GeSUT4* in regulating sugar allocation in *Armillaria*-colonised tubers of *G. elata* (Ho et al., 2021). Shan et al. reported that the expression of gene-encoding N transporters, such as *DoNAR2.1* (nitrate transporter-activating protein), *DoAMT11* (ammonium transporter), *DoATFs* (amino acid transporters) and *DoOPTs* (oligopeptide transporters), is up-regulated in mycorrhizal *D. officinale*, indicating that the mycorrhizal fungus MF23 promotes the production of *D. officinale* by increasing N uptake and NH_4^+ assimilation capacity (Shan et al., 2021). A recent study showed that hypoxia-responsive genes (e.g. encoding alcohol dehydrogenase) and related hypoxia-responsive pathways help to establish successful symbiotic relationships in germinating orchids (Xu et al., 2023).

Mycorrhizal symbiosis also changes plant endogenous hormone levels, such as GA_3, ABA and JA. Miura et al. studied the effects of GA on mycorrhizal symbiosis and seed germination in *B. striata* through a transcriptome analysis, found that a symbiotically germinated seeds share some common genes (e.g. genes related to GA metabolism and signalling and homologous genes with AM-specific markers) and proposed the hypothesis that the mycorrhizal symbiosis pathway is automatically activated by GA inactivation (Miura et al., 2023). Transcriptomic analysis of the symbiotic interactions between *C. appendiculata* and the mycorrhizal fungus *Coprinellus disseminatus* (Pers.:Fr.) Kuhner. also showed that zeaxanthoxygenase (ZEP), 9-cy-epoxide carotenoid dioxygenase NCED3 and beta-carotene hydroxylase involved in ABA biosynthesis are considerably down-regulated after mycorrhizal fungal invasion (Gao, Ji, Zhang, Yang, & Zhang, 2022). In the symbiotic interaction between *D. officinale* and *Tulasnella calospora*, the differential expression of genes related to JA and ABA biosynthesis is also considerably up-regulated relative to uninoculated mycorrhizal fungi (Wang, Song, Wang, Xu, Sun, & Li, 2018). The partially putative genes involved in ORM formation are listed in Fig. 3.

Although a preliminary understanding of the mechanisms through which fungi promote seed germination has been achieved, many questions remain. For example, why are some seeds selective to fungi at different stages of germination, and are there some differences in the nutritional relationships between fungi and seeds at different stages of germination, which resulting in some fungi maintaining symbiotic relationships only at a specific stage of seed germination? And why the promoting effects of fungi on seed germination are unstable (between laboratory and field), are these phenomena related to the signal recognition and establishment of symbiotic relationship between
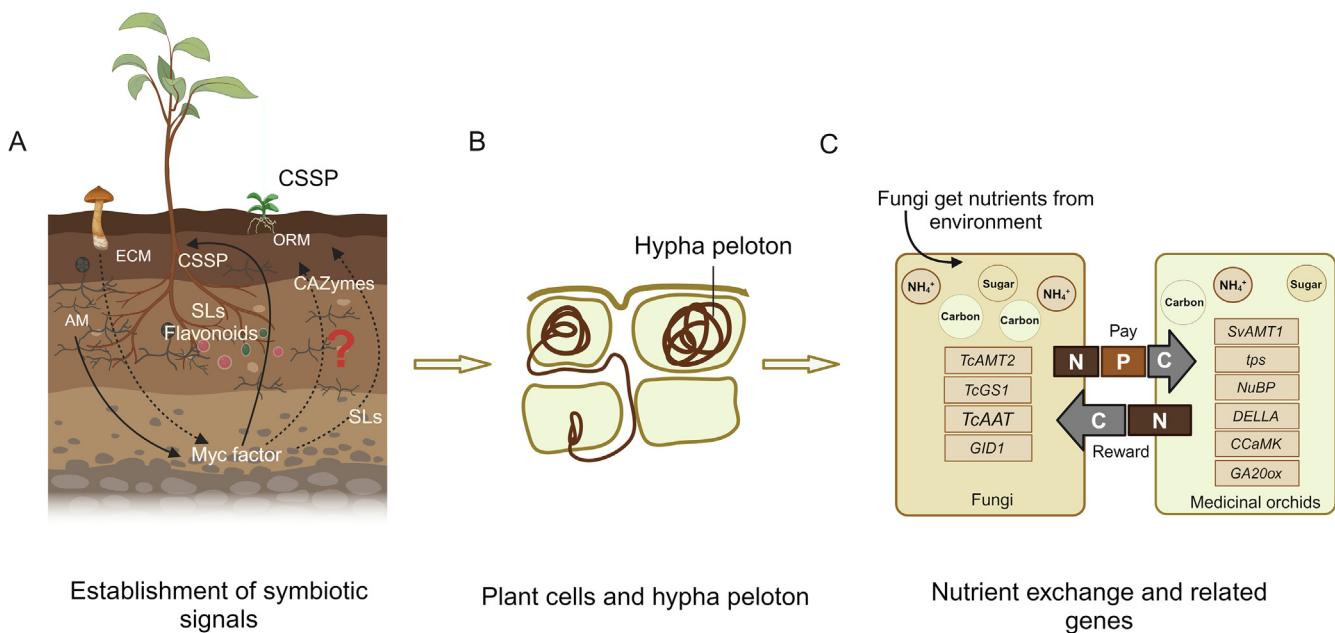
Fig. 3. Symbiosis mechanism between fungi and plants. (A) Fungi secrete bioactive signals such as Mycorrhizal factor (Myc factor) and plants secrete signaling factors such as strigolactone (SLs), flavonoids, which activate the common symbiotic signaling pathway (CSSP) in the host plant. (B) Hyphae infects plant cells. (C) Nutrient exchange between plants and fungi and related genes (*TcAMT2*: ammonium transporter; *TcGS1*: glutamine synthetase; *TcAAT*: amino acid transporter; *GID1*: gibberellin receptor; *SvAMT1*: ammonium transporter; tps: trehalose-6-phosphate synthase; *NuBP*: nucleotide-binding protein; *DELLA, GA20ox*: genes about the biosynthesis of gibberellin; *CCaMK*: calcium-and calmodulin-dependent protein kinase).

fungi and seeds under the "poor" symbiotic germination conditions in the laboratory? Is the specificity of fungi for seed germination related to the establishment of a symbiotic relationship between fungi and plant seeds and signal recognition (Huang, Zi, Lin, & Gao, 2018; Tang et al., 2022; Yamamoto et al., 2017)? Further study of the symbiosis mechanism between fungi and seeds is important for addressing these questions and applying mycorrhizal symbiosis.

3.2. Increasing host plants' resistance

The growth of medicinal plants is often threatened by abiotic stresses, such as high temperature, high salt content and drought, which pose a serious threat to the biomass and quality of medicinal herbs. Mycorrhizal symbiosis can improve the response ability of host plants to environmental stress and the survival rate of plants under conditions involving drought, low temperatures, high salt contents and heavy metal pollution by increasing photosynthetic activity, water use efficiency, osmoprotectant production, antioxidant activity and gene expression (Santander et al., 2017; Zhang et al., 2024). Herrera et al. comparatively studied nonmycorrhizal and mycorrhizal *Bipinnula fimbriata* (Poepp.) I.M. Johnst. under heavy metal stress and found that mycorrhizal *B. fimbriata* has high protein levels, which are mainly related to cell growth, membrane transport, resistance to pathogens and oxidative stress control (Herrera et al., 2018). Previous studies have shown that the medicinal orchid plant *Coelogyne viscosa* Rchb. f. inoculated with dark septate endophyte (DSE) and mycorrhizal fungi can considerably enhance the biomass of seedlings and increase the activities of drought resistance (Liu et al., 2022b).

In addition to abiotic stress, pathogenic bacteria, fungi, insect pests, virus and other biological stresses disturb orchid medicinal plants during their growth and development and affect the normal growth of plants and quality of herbs (Jain, Sarsaiya, Chen, Wu, Lu, & Shi, 2021). Studies have shown that mycorrhizal fungi can improve the disease resistance of host plants by inducing plant defence systems, including improving phytohormone concentrations, inducing signal substrate production, regulating gene expression and enhancing protein production (Weng et al., 2022; Pu et al., 2022). Zhou et al. showed that endophytic or mycorrhizal fungi can reduce the incidence rate of root rot disease in *D. officinale* and enhance the host resistance to *Sclerotium delphinii* (Zhou et al., 2020). Fungi also increase the activities of disease-resistant enzymes, including β-1, 3-glucanase, chitinase and superoxide dismutase, of host plants; they enhance disease resistance and alleviate the effects of pathogen stress on host growth. Some fungi can regulate or even reverse the expression of certain genes under pathogenic stress induced by *Fusarium oxysporum* (Ye, 2019). In addition to influencing the plant's response to pathogenic microorganisms, research indicates that AMF can improve the rhizosphere environment and enhance plant resistance to diseases by promoting the growth of beneficial microorganisms and competing with pathogenic microorganisms (Weng et al., 2022). In orchid mycorrhizal symbiosis, can beneficial fungi similarly enhance host plant resistance by competing for ecological niches with pathogenic microorganisms? Mycorrhizal fungi have great potential for cultivating the medicinal plants of the Orchidaceae family, developing new biological control agents and improving the safety of medicinal plants.

3.3. Promoting production and accumulation of effective components of medicinal plants

Secondary metabolites are the material basis of the quality evaluation of traditional Chinese medicinal plants. Studies have revealed that AM fungi (AMF) can promote host plant growth and increase the accumulation of secondary metabolites by the response mechanisms of plant hormones, nutritional elements and expression of key enzymes (Ran et al., 2022; Zhao, Cartabia, Lalaymia, & Declerck, 2022). Zhu et al. found that symbiotic fungi can affect the accumulation of sugars and alcohol-soluble components in stems of *D. officinale* (Zhu et al., 2016). Experiment showed that the polysaccharide yield is increased by 42.69 % in mycorrhizal plants compared with that in nonmycorrhizal *D. officinale* (Zhang et al., 2022). In addition, mycorrhizal fungi stimulate the production of dendrobine (Chen et al., 2021).

Comparative metabolic and transcriptional profiling analyses between mycorrhizal and nonmycorrhizal *Anoectochilus formosanus* Hayata have revealed that the mycorrhizal fungus *Ceratobasidium* sp. AR2 can increase the accumulation of flavonol-glycosides, flavonols and flavones to some degree (Zhang, Li, Chen, Guo, & Lee, 2020; Zhang, Li, Chen, Meng, & Guo, 2020). Modern molecular biology studies have found that fungi may enhance the accumulation of active components by increasing the expression of genes related to secondary metabolites in plants. For example, the fungus MF23 may stimulate the biosynthesis of dendrobium alkaloids in *Dendrobium* sp. by regulating the expression of related genes in the mevalonate pathway (MVA) (Li, Ding, Li, & Guo, 2017). Tsai et al. identified the genes related to gastrodin biosynthesis by conducting a comparative transcriptome analysis of the symbiosis between *G. elata* and fungi (Tsai et al., 2016). These studies facilitated the conservation and sustainable utilisation of this highly valued medicinal orchid by the mycorrhizal symbiotic relationship.

3.4. Mycorrhizal fungi can produce bioactive metabolites

During the interaction with plants, mycorrhizal fungi not only stimulate the host plant to produce secondary metabolites but also produce active metabolites, they can even generate the same chemical components as their hosts (Huang, Cai, Hyde, Corke, & Sun, 2008). Researchers have isolated and identified nearly 100 compounds, such as terpenoids, sterols, adenosines, organic acids, polysaccharides and other secondary metabolites, from *A. mellea* and its fermentation broth, these compounds have various biological activities (Guo, Xu, Xiao, & Yang, 1996; Yuan, Liu, & Cai, 2008). Studies have also indicated that *Tulasnella* sp. fungi isolated from the roots of the orchid plant *B. striata* can produce plant hormones to promote seed germination and seedling growth (Liu et al., 2022c). Fungi isolated from the roots of *Dendrobium* can synthesise indoleacetic acid (IAA) in the presence of tryptophan (Shah et al., 2022), thus promoting plant growth and development. In addition, *Tulasnella* sp. S7 can produce vanillic acid, benzyl alcohol, indole-3-formic acid and indole-3-formaldehyde and promote seed germination and protocorm development (Yang et al., 2020; 2023). These results indicate that the promotion of the seed germination of orchids by fungi is related to exogenous hormonal changes. An increasing number of recent studies have also revealed that endophytes can biosynthesize medicinally important phytochemicals (Ancheeva, Daletos, & Proksch, 2020).

Plant endophytic fungi can produce the same chemical components as their hosts. Li et al. analysed alcoholic extracts from the fermentation broth of the endophytic fungi in *Spiranthes sinensis* (Pers.) Ames and found that the endophytic fungi *Penicillium pinophilum* and *Curvularia inaequalis* can produce flavonoids. Therefore, the claim that fungi can promote the accumulation of active ingredients in medicinal orchids needs to be further explored regardless of whether the fungi themselves produce active ingredient compounds or can promote the production of host plants (or both). Mycorrhizal fungi can produce bioactive substances, and the structural analysis, activity determination and modern biological technology (such as synthetic biology) can be conducted to provide

references for human to seek new sources of active ingredients of medicinal plants, and it is of great significance to promote the research and development of medicinal plants and the sustainable utilization of traditional Chinese medicine resources.

4. Challenges in applying ORM to actual cultivation of orchid medicinal plants

The symbiotic relationship between *G. elata* and fungi has been successfully established, and the artificial cultivation technology of *G. elata* has solved the cultivation and resource problem of *G. elata* and created certain economic benefits and social value. Fungal symbiosis can promote the seed germination and plant growth of other medicinal orchids, such as *D. officinale*, but no relevant studies have been conducted on the application of fungi in the actual cultivation of medicinal orchids, except *G. elata*. Research on the application of the mycorrhizal symbiosis of orchid plants needs to be further strengthened. Effective and stable strains must be screened to develop microbial and biocontrol agents and realize the transformation of results. However, several key questions in ORM need to be addressed in the near future.

4.1. Acquisition of mycorrhizal fungi

Mycobiont isolation, culture and identification are important for mycorrhizal technology application in orchid plants. Although the members of Tulasnellaceae, Ceratobasidiaceae and Serendipitaceae families are acknowledged as major symbiotic fungi, some orchid species have a preference or exhibit specificity for mycobionts; for example, the green medicinal orchid *C. appendiculata* shows specificity for Psathyrellaceae fungi (Yagame, Funabiki, Nagasawa, Fukiharu, & Iwase, 2013). The application of high-throughput sequencing in the investigation of mycorrhizal fungi contributes to knowledge on global mycorrhizal fungal diversity and the potential ecological function of fungi related to orchid plant distribution and evolution. However, the isolation of cultivable symbiotic fungi from orchid root or protocorm is difficult and requires targeted isolation methods, such as bait seed packages (Brundrett, Scade, Batty, Dixon, & Sivasithamparam, 2003; Shao et al., 2017). In addition, compared with other endophytic fungi (e.g. *Fusarium* sp.), the mycorrhizal fungi of orchid plants usually grow more slowly in artificial media under laboratory conditions and are easily contaminated by other fungi.

Moreover, the taxonomy of the mycorrhizal fungi of orchids is challenging. Most fungal strains isolated from orchids are colourless colonies and do not easily produce spores or fruiting bodies. Thus, classifying and identifying them is difficult. Nevertheless, DNA sequencing can be used as an alternative. Useful molecular markers can be developed based on genome sequencing of some mycorrhizal fungi, such as *Tulasnella* or *Sebacina* strains.

Aside from the identification problem of orchid mycorrhizal fungi, another issue is the application and management of fungal strains used for *G. elata* cultivation in the field. Mycelial subculture is an indispensable part of the cultivation and production of all kinds of edible fungi or cultivable orchid mycorrhizal fungi, but successive subcultures usually lead to strain degeneration (Zhu et al., 2020). Currently, the fungal strains for *G. elata* cultivation (e.g. *Armillariella* spp. and *Mycena* spp.) available in the market are confusing and lack scientific management. High-quality strains are important because they are related to the yield and quality of *G. elata*, so exploring the mechanism of fungal strain degeneration and screening fungal strains with excellent bioactivities (e.g. rapid growth and strong resistance) are valuable work directions in the future.

4.2. Mycorrhizal specificity and preference for orchid medicinal plants

Orchid species differ considerably in terms of lifestyle. Epiphylic (e.g. *Dendrobium* spp.), terrestrial (*C. appendiculata)* and saprophytic (e.g. *G. elata*) orchid plants have different mycorrhizal fungal species, and even the same orchid plant has different mycobionts at different development stages (e.g. seed germination stage vs. adult stage) (Chen, Wang, & Guo, 2012). Some fungi can promote seed germination but not seedling growth. Further research is needed to understand the symbiosis between fungi and seed germination. In some orchid species, several fungal strains isolated from adult plants can stimulate germination *in vitro*, but the extent to which they enhance subsequent growth of the same species may vary greatly depending on the strain used even in the short term of four weeks (Rasmussen & Whigham, 1998). The long-term effects of fungi on orchids need to be assessed. Notably, germination is not the only indicator of fungal compatibility. Research has shown that fungi isolated from host protocorms usually lead to accelerated germination and seedling formation. Therefore, the fungal source should be considered when seeds or young seedlings are used for propagation and *in-situ* reintroduction (Shao, Wang, Beng, Zhao, & Jacquemyn, 2020).

4.3. Symbiotic mechanism research needs to be strengthened

Transcriptome sequencing is widely used in nonmodel plants, but due to the lack of detailed genome information in most orchid plants, gene identification remains limited, making transcriptome assembly a challenge. Given the lack of orchid genome sequences, direct comparison of the short reads of the transcript with those of the reference genome is impossible, resulting in incomplete gene identification. Therefore, a high-quality orchid genome and transcriptome database must be established. In recent years, the genomes of several important medicinal plants, such as *G. elata* and *D. officinale*, and some horticultural species have been sequenced. The next step should be to explore the transcriptome databases of other orchids (Chen et al., 2022; Yuan et al., 2018).

Given the lack of a genetic transformation system, verifying the function of genes related to mycorrhizal symbiosis via transcriptome analysis is difficult. Although advanced transformation systems for *Oncidium* and *Phalaenopsis* orchids have been established to facilitate orchid breeding research (Li, Liao, Huang, & Chan, 2015), no effective fungal or plant genetic transformation system has been developed to analyse the functional genes of ORM. Thus, a model study system for ORM symbiosis needs to be constructed.

4.4. Interaction between mycorrhizal fungi and other root microbes

The root system of plants exists in a complex ecological environment, and the rhizosphere environment comprises plant roots, soil and root microbes. The dynamic equilibrium in the rhizosphere is the result of collaborative and antagonistic relationships amongst roots, bacteria, fungi and nematodes. In the rhizosphere environment of *D. nobile,* bacteria play an important role. A previous study showed that some bacterial taxa are involved in the recruitment of genera from the same phylum (Zhao et al., 2023). Orchid root-associated beneficial bacteria can facilitate plant growth, fitness and a number of other ecological or evolutionary processes in orchids (Kaur & Sharma, 2021). However, experimental studies focused on specific relationships, such as that between orchid plants and their mycorrhizal fungi, and overlooked the influence of other environmental microorganisms, such as mycorrhizal helper bacteria (MHBs) and plant growth-promoting rhizobacteria (PGPR) (Berrios et al., 2023). Although studying the symbiotic relationship between a single type of fungus and plants

can provide insights into plant–microbe interactions, it cannot fully elucidate the broad, intricate ecological effects and functions within the rhizosphere. To comprehensively understand the ecological balance in the rhizosphere, we need to employ integrated approaches (e.g. metacommunity analysis, metagenomics and metatranscriptomics) to investigate the composition and functions of different microorganisms. These approaches can reveal the complex microbial networks in the plant root environment and enhance our understanding of the interactions between plants and rhizospheric microorganisms. Moreover, the techniques related to synthetic microbiomes can be adopted to realise the ecological cultivation of traditional Chinese medicinal plants.

CRediT authorship contribution statement

Chunyan Leng: Conceptualization, Writing – review & editing, Visualization. **Mengyan Hou:** Writing – review & editing. **Yongmei Xing:** Writing – review & editing. **Juan Chen:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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