Orchid Mycorrhizal Fungi: Structure, Function, and Diversity

13

Kullaiyan Sathiyadash, Thangavelu Muthukumar, Velusamy Karthikeyan, and Kuppu Rajendran

Abstract

All orchids are mycoheterotrophic during seed germination and early stages of seedling development. Nevertheless, this dependency on the mycobiont extended into adulthood in many green photosynthetic orchids and is termed as mixotrophy. The fungal hyphae colonize orchids early during seed germination and protocorm development and form highly coiled structures called pelotons. Conventional studies mostly focused on orchid mycorrhizal fungi (OMF) that are saprophytic, but later the role of both ectomycorrhizal and parasitic fungi in orchid mycorrhizal symbiosis were recognized. Although there is enough evidence to believe that OMF is not host-specific, there are also indications which suggest the possible existence of physiological compatibility in orchid-fungal interaction. Current advances in molecular techniques have enabled us to untangle the diversity of fungi involved in the symbiosis and have helped to overcome the bottlenecks associated with the traditional identification of the fungal taxa using morphological characters. OMF symbiosis is shown to assure orchid survival in habitats vulnerable to stressful conditions or habitats with resource limitations. Further, the OMF has been shown to play a key role in the rehabilitation of threatened orchid species in their natural habitats. In spite of this, there is a large gap in our understanding of the fungal diversity associated with the tropical epiphytic and lithophytic orchid taxa.

Keywords

Orchidaceae · Peloton · Nutrient uptake · Growth promotion · Specificity · Seedling recruitment

K. Sathiyadash · V. Karthikeyan · K. Rajendran
Department of Botany, Thiagarajar College, Madurai, Tamil Nadu, India

T. Muthukumar (✉)
Root and Soil Biology Laboratory, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India

S. M. Khasim et al. (eds.), *Orchid Biology: Recent Trends & Challenges*,
https://doi.org/10.1007/978-981-32-9456-1_13

13.1 Introduction

Orchidaceae is one of the largest angiospermic plant families with roughly around 28,484 species (Govaerts et al. 2017). A majority of the orchids are narrowly distributed in a particular region (Lozano et al. 1996) and are highly affected by habitat clearance compared to other plant species (Jacquemyn et al. 2007). The mycorrhizal fungi symbiotically associate with orchids and serve as an important source of nutrients and water to the orchids (Zettler 1997a, b). The specialization in pollination and mycorrhizal association is linked to the size and diversity of taxa in Orchidaceae (Stewart and Kane 2007; Cozzolino and Widmer 2005; Taylor et al. 2003). The floral beauty and attraction of orchids directly contribute to their mass decline in the wild. But, mycorrhizal associations have enabled orchids to survive in diverse habitats around the world. In nature, orchid seeds fail to germinate in the absence of mycorrhizal fungi (Fig. 13.1). *In vitro* culture of orchids was successful using both seeds (Knudson 1922) and explants (Arditti 1984). Many studies have focused on asymbiotic propagation of orchids from seeds (Arditti 1967; Sheehan 1983). As symbiotic propagation and restoration of orchid population in the wild are either totally neglected or not much emphasized, Rasmussen (1995) suggested that a worldwide support and importance should be given to investigations involving symbiotic seed germination. Orchids are economically important as Vanillin from *Vanilla planifolia* is used as flavoring agent for food and drink, and tissues of *Gastrodia* are used in the preparations of natural medicine (Griesbach 2000). Anthropogenic activities including theft of attractive individuals have driven many of the aesthetic orchid species in nature to extinction. Therefore, conservation efforts for these orchid species should involve a thorough understanding of their biology (Dearnaley 2007). In this chapter we discuss not only the structure, diversity, and significance of the mycorrhizal fungi in orchid lifecycle, but also the importance of these fungi in orchid conservation.

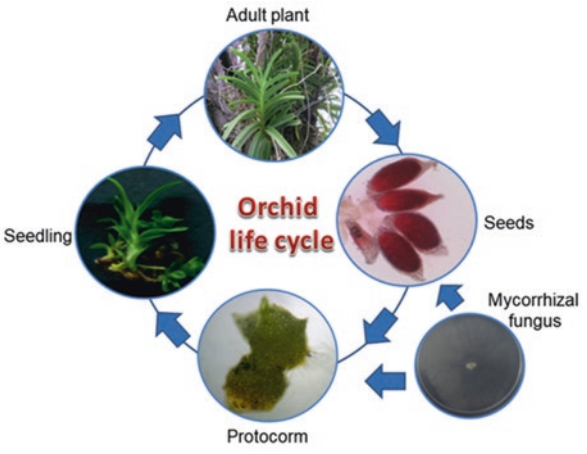

Fig. 13.1 Different stages in the lifecycle of an orchid. Mycorrhizal fungi are mandatory for seed germination and seedling development. Adult green photosynthetic orchid can acquire nutrients through mycorrhizal fungi and even supplement carbon through mixotrophy

13.2 Types of Orchid Roots and Velamen

The root system of orchid life-forms differs in several aspects (Rasmussen 1995). The roots of epiphytic and lithophytic orchids are ecologically similar as the roots are exposed to light and air (Fig. 13.2). The aerial roots of epiphytic and lithophytic orchids are perennial, photosynthetic with a fairly constant growth throughout the year (Dressler 1993; Muthukumar and Shenbagam 2018). In contrast, roots of terrestrial orchids are usually non-photosynthetic, have a limited life span of up to 3 years, and show seasonal changes in growth and architecture (Bayman and Otero 2006).

Roots of terrestrial orchids grow in soil or litter, and certain terrestrial orchids have two distinct types of roots, i.e., mycorrhizal and non-mycorrhizal (Rasmussen 1995). Non-mycorrhizal roots have more xylem and higher amyloplast than mycorrhizal roots (Bayman and Otero 2006). Nevertheless, most of the terrestrial orchids possess mycorrhizal fungi in their roots even in the adult stage of their life cycle (Rasmussen 1995). Compared to the obligate mycorrhizal nature of the terrestrial orchids, roots of most of the epiphytic and lithophytic orchids are facultatively mycorrhizal with frequency of colonization exhibiting variation to certain extent (Zelmer et al. 1996; Bayman et al. 1997; Otero et al. 2002; Rasmussen 2002).

Orchids have multiple epidermis consisting of one to several layers of thin-walled cells called velamen (Porembski and Barthlott 1988). The velamen helps the roots to trap water and nutrients (Dressler 1990; Rasmussen 1995). Roots of epiphytic orchids have more layers of velamen than terrestrial roots (Dressler 1990). In addition, epiphytic orchids growing in exposed or dry microenvironment like the *Acampe* tend to have multilayered velamen compared to those like *Bulbophyllum* which grow in more humid microenvironment (Muthukumar and Kowsalya 2017; Muthukumar and Shenbagam 2018). Based on their observations of orchids from exposed habitats of dry and humid environments, Sanford and Adanlawo (1973) suggested that the size of velamen may be indicative of the prevailing environmental factors such as moisture and temperature of the habitats. Oliveira and Sajo (1999) indicated that the velamen cells with suberized and lignified thickenings provided mechanical support to avoid cellular collapse during dehydration.

13.3 Nature of the Endophytic Fungi

In nature, orchid roots are colonized by diverse group of fungi, some of which may not be of true mycorrhizal in nature (Warcup 1981; Bayman et al. 1997). The endophytic orchid mycorrhizal fungi (OMF) of true mycorrhizal nature should have the potential to stimulate seed germination, enhance protocorms, and/or early seedling development and subsequently improve the growth and reproduction of the adult plants (Liu et al. 2010). OMF forms pelotons in root cortical cells and in seeds and the limitation of the OMF colonization to suspensor cells in the embryo and the epidermal hairs in germinating seeds clearly shows that the colonization process is dictated by the orchid and the symbiosis is adapted to this control (Hadley 1982).

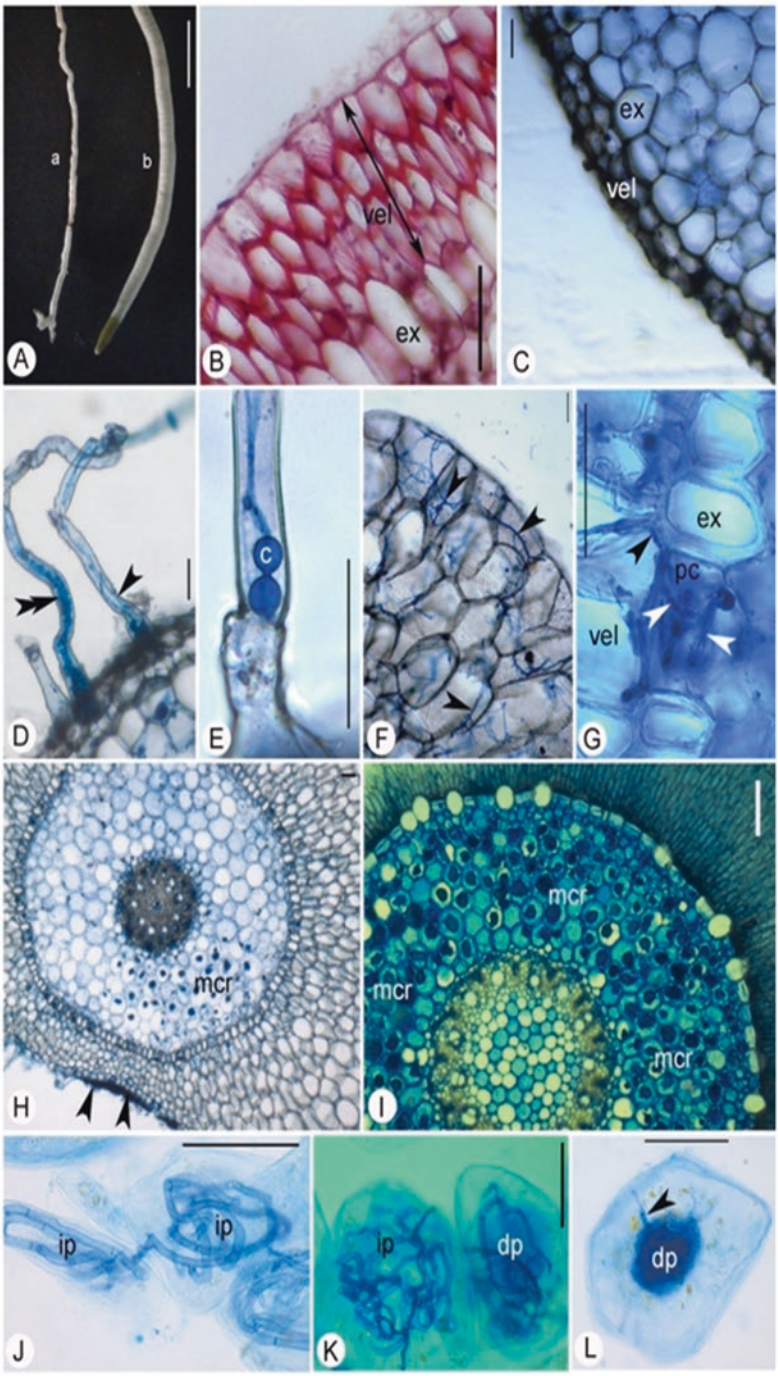

Fig. 13.2 (**a**)–(**l**) Morphology and mycorrhizal anatomy of orchid roots. (**a**), Velamentous roots of *Dendrobium* sp. (*a*) and *Acampe praemorsa* (*b*); (**b**), Velamen (*vel*) and exodermis (*ex*) in aerial roots of *A. praemorsa*; (**c**), Bilayered velamen (*vel*) and exodermis (*ex*) in *Spathoglottis plicata*;

The one way, through which the orchids obtain nutrients from the fungus, is through the digestion of the fungal hyphae. In orchids, the digestion of the pelotons takes place either by tolypophagy or phytophagy. In tolypophagy the complete digestion of pelotons occurs, whereas in phytophagy the fungal cell contents are released into the plant fungus interface through the lysis of the fungal tips (Burgeff 1959). Therefore, at any given point of time, orchid roots contain different proportions of intact and lysed pelotons (Fig. 13.2). Varying levels of intact to lysed or lysing pelotons have been reported in several orchids growing in the Western Ghats of south India (Muthukumar and Sathiyadash 2009; Muthukumar et al. 2011; Sathiyadash et al. 2012). Some components derived during this hyphal digestion play the role of fungal elicitors, which make easier the further colonization of the OMF (Liu et al. 2010). Further, the fungal elicitors of OMF have been shown to promote the development of protocorms and the development of seedling growth in orchids (Dong et al. 2008; Zhao and Liu 2008).

Several fungi that exhibit differential responses as parasites and pathogens are also shown to be symbionts of orchids. For example, *Ceratobasidium* stimulate the germination and seedling growth of *Ionopsis* sp. (Otero et al. 2004), but the same fungal isolate was shown to be pathogenic to *Dendrobium mericlones* (Porras-Alfaro 2004). Similarly, studies have also shown that the fungal symbionts of orchids could cause root rots in pea, soy bean, pine and spruce (Hietala et al. 2001; Yang et al. 2005). *Thanatephorus* isolated from the orchid *Pterostylis acuminata* was moderately pathogenic on lettuce and severely pathogenic on cauliflower and radish (Carling et al. 1999). *Rhizoctonia solani* which act as mycorrhizal symbionts in *V. planifolia* and *Vanilla phaeantha* also cause pathogenic lesions on the same root (Alconero 1969).

13.4 Orchid Mcyorrhizal Fungi (OMF)

13.4.1 Colonization Patterns of OMF in Orchid Roots

The fungal colonization patterns significantly differ both in epiphytic or lithophytic and ground orchids. In all these types the fungus enters the root primarily through the root hairs and occasionally directly through the epidermal cells (Sathiyadash et al.

Fig. 13.2 (continued) (**d**), Root hairs of *S. plicata* with fungal hyphae (black single arrow head) and moniliform cells (black double arrow head); (**e**) Chlamydospore-like cells (*c*) in root hair of *S. plicata*; (**f**), Fungal hyphae (black arrow heads) transversing the velamen in *Dendrobium* sp.; (**g**), Fungal hyphae (black arrow head) in the velamen (*vel*) and passage cell (*pc*) of the exodermis (*ex*) (white arrow heads) in *Dendrobium* sp. root; (**h**), Transverse section (T.S) of *Dendrobium* sp. substrate root with patchy mycorrhizal colonized region (*mcr*). The root portion attached to the substrate is indicated by black arrow heads; (**i**), T.S. of *Eulophia epidendraea* terrestrial root with diffused mycorrhizal colonized regions (*mcr*) throughout the root cortex; (**j**), Intact pelotons (*ip*) in cortical cells of *Luisia pulniana*; (**k**), Intact (*ip*) and degenerating (*dp*) pelotons in *Vanda spathulata*; (**l**), Degenerated peloton (*dp*) with fungal hyphal remnants (black arrow head) in cortical cell of *L. pulniana*. Scale bars: A = 2 cm; B–L = 50 μm

2012). In epiphytic forms, root portions that are only attached to the substrate like the tree bark contain mycorrhizal colonization and those that are not attached to any substrate are free from fungal colonization (Fig. 13.2). Nevertheless, the free aerial root upon entry into soil develops mycorrhizal colonization (Muthukumar and Kowsalya 2017). The fungal colonization therefore is patchy in epiphytic and lithophytic orchids, whereas it is diffused throughout in ground orchids (Sathiyadash et al. 2012; Muthukumar and Kowsalya 2017). The fungal hyphae transverse the velamen tissue and enter the cortex through the passage cells of the exodermis. From the exodermis the fungus spreads through the root cortex intracellularly forming a highly coiled structure called pelotons (Fig. 13.2). The size of the pelotons varies with the orchid species and is determined by the cortical cell size as evidenced by a strong correlation between the peloton and cortical cell dimensions (Sathiyadash et al. 2012). The nucleus of the cortical cells is pushed to the periphery in the invaded cells and like in other mycorrhizal types, the fungus never trespass the endodermis. In the cells of the root cortex and in root hairs chlamydospore-like structures or moniliform cells are present (Sathiyadash et al. 2012).

The relationship between orchid and the mycorrhizal fungi is unique in the plant kingdom. Orchid mycorrhizae may at times involve mycobionts that are pathogenic to other plant species as shown above, suggesting the orchid's specific ability to neutralize the possible virulence pathways of the pathogens (Watkinson 2002). These factors render orchids as an excellent model to study plant-fungal interactions. The establishment of the symbiotic interaction between the mycorrhizal fungi and the process of fungal colonization encompasses a wide range of similarities and differences with pathogenesis. This knowledge could provide an idea in developing new strategies to overcome or reduce the severity of the pathogenic interactions in non-orchidaceous plant species (Watkinson 2002). In the symbiosis between the orchid and the fungi, orchid is a dominant partner which helps to predict the evolution of plant-fungal interactions.

An increased survival of the orchid seedlings was often possible when the seedlings are mycorrhizal (Anderson 1991). Seeds of certain orchids like *Encyclia tampensis, Liparis liliifolia,* and *Taeniophyllum obtusum* germinate only in the presence of OMF under *in vitro* conditions (Irawati 1993; Rasmussen and Whigham 1998; Zettler et al. 1999). Likewise, protocorms of *Eulophia alta* develop more rapidly when mycorrhizal under *in vitro* conditions (Johnson et al. 2007). Symbiotically raised orchid seedlings can serve as both plant material as well as the source for mycorrhizal inoculums in conservation efforts (Batty et al. 2006).

13.4.2 Nutrient Transfer by OMF

Most of the orchids depend on mycorrhizal partners for their nutrients during seed germination and early developmental stages (Rasmussen 1995; Cameron et al. 2006; Rasmussen and Rasmussen 2009; Wu et al. 2013) (Fig. 13.1). The limited food resources in the seeds and the inefficiency of orchids to acquire nutrients from the substrates render orchids highly dependent on mycorrhizal fungi (Leake 1994).

The OMF transfer nutrients from different substrates to their symbiotic orchid host (Dearnaley and Cameron 2017). Numerous studies have demonstrated the mycorrhizal fungi mediated enhancement of nutrient acquisition by orchids in the native ecosystems (Alexander 2007; Smith and Read 2008 and references there in). In mixotrophic orchids, OMF transfer carbon (C), nitrogen (N), phosphorus (P), and other minerals to the adult photosynthesizing green plants (Zimmer et al. 2007). In epiphytic orchids, the OMF mycelium decompose organic matter such as bark and make available the nutrients to the orchids (Zhang et al. 2018). Nevertheless, at times the transfer of nutrients could be unidirectional (Hadley 1989), bidirectional (Cameron et al. 2006) or even mycorrhizal independent (Purves and Hadley 1975).

Carbon Transfer Most of the adult green orchids are autotrophic and fulfill their C requirement by photosynthesis. In addition to these, the orchid might also supplement its C requirement through the transfer organic C from the OMF (Gebauer and Meyer 2003). No difference in the C fixation or distribution was evident among the mycorrhizal and non-mycorrhizal *Goodyera repens* plants exposed to $^{14}CO_2$ (Hadley and Purves 1974). Moreover, the fungal mycelium originating from the colonized roots had no measurable radioactivity. So it could be inferred that *Rhizoctonia goodyerae-repentis* fail to utilize the photosynthates from the host orchids (Hadley and Purves 1974). The absence of C translocation from the host to the fungus could be either due to the inability of the fungus to obtain the host metabolites, or may also be due to the adequate amount of C in the growing media (Hadley and Purves 1974). In *Ceratobasidium cornigerum*, ^{14}C supplied as glucose peaked in the external mycelium within 48 hours, while its accumulation took several days in *G. repens* protocorms (Hadley 1984). Further, when the protocorms, plantlets and plants of *G. repens* were fed with ^{14}C through the external mycelium of the fungus, the plants failed to take up the C even under stressed conditions (Alexander and Hadley 1985). This suggests that the translocation of C movement mostly was unidirectional from the fungus to the host and the translocation of C ceased when the orchids reached certain stage of development (Alexander and Hadley 1985). In contrast to these, the C uptake rate by mycorrhizal *Dactylorhiza purpurella* and *Cymbidium* protocorms was higher than non-mycorrhizal protocorms (Hadley 1984). In the same study, the C uptake by the mycorrhizal *G. repens* protocorms was 50% higher than the non-mycorrhizal protocorms (Hadley 1984). In addition to the mycorrhizal mycelium of *Ceratobasidium cornigerum* transferring C to roots, rhizomes and green shoots of the *G. repens* plants, the C fed to shoots as $^{14}CO_2$ was readily assimilated and transferred to the rhizome and onwards to the extending external mycelium of the OMF (Cameron et al. 2006). This confirms the bidirectional flow of C and the true mutualistic nature of OMF.

Nitrogen Transfer The plants absorb the N in the form of NO_3^- and NH_4^+ (Zhang et al. 2018). Both terrestrial and epiphytic orchid life forms absorb NO_3^- and NH_4^+ from the substrates, but terrestrial orchids tend to absorb more NO_3^- than NH_4^+. In addition, NO_3^- concentration plays a critical role in flower and flower bud formation in *Cymbidium sinense*, whereas NH_4^+ concentration fails to produce any such effects

(Pan and Chen 1994). This clearly shows the orchids preference for different N forms. The inability of the orchids to utilize organic N emphasizes the importance of the mycorrhizal symbiosis in orchid seed germination and development of the seedlings in the natural habitats (Dijk and Eck 1995). In addition, the N nutrition of orchids gets even more complicated as the N sources vary depending on the orchids' requirement and life stages (Dijk and Eck 1995). The mycorrhizal seedlings of *Cymbidium goeringii* also differed in its uptake of N forms from the soil at different depths due to the variation in the distribution patterns of the *Rhizoctonia* spp., hyphae in the soil. Further, the fungal hyphae present in the upper and deeper layers of the soil are responsible for NH_4^+ and organic N uptake respectively (Wu et al. 2013). The transfer of N from the fungus to the orchid was first demonstrated by Cameron et al. (2006) in *G. repens*. Significant amounts of glycine labeled N (^{15}N) was transferred to the roots (2%) and shoots (20%) of the terrestrial orchid *G. repens* by the mycorrhizal fungus (Cameron et al. 2006). This suggests that the rate N transfer from the fungus to the plant is likely to be dependent on the activity and nature of the plant-fungal interfaces in the roots (Cameron et al. 2006).

The foliar concentrations of isotope labeled N (^{15}N) both in green (*Cephalanthera damasonium*) and achlorophyllous (*Dactylorhiza sambucina*) orchids growing in forest and grassland sites in Europe was significantly higher than the co-occurring herbs and trees suggesting the significance of the fungal symbiosis (Gebauer and Meyer 2003). Earlier studies have demonstrated an improved N nutrition in certain autotrophic plants, when glycine and other amino compounds were provided to its mycorrhizal fungal partner (Taylor et al. 2004).

Phosphorus Transfer The P uptake of adult *G. repens* plants colonized by *R. goodyerae-repentis* and grown in different P levels was 100 times more than its non-mycorrhizal counterparts because of OMF mediated P uptake like in other mycorrhizal systems (Alexander and Hadley 1984). Radioactivity could be detected in the protocorms of *D. purpurella* inoculated with orchid endophytes and fed with radioactive P orthophosphate suggesting the transfer of P from the fungus to the host plant (Smith 1966). The translocation of ^{32}P via the fungal hyphae occurs up to a distance of 9 cm from the root, with optimum distance being 2–3 cm (Alexander and Hadley 1984). However, the density of the fungal hyphae decreases with an increasing distance leading to a delay of phosphate reaching the nearest root (Alexander and Hadley 1984). Although the lack of a significant positive correlation between root length and ^{32}P content in mycorrhizal *G. repens* (Alexander and Hadley 1984) casts doubt on the role of OMF in P nutrition, later studies have shown that OMF mediated P transfer. For example, the external mycelium of *C. cornigerum* colonizing *G. repens* was capable of assimilating and transporting the ^{33}P orthophosphate into the plant (Cameron et al. 2007). Further, 7 days after ^{33}P exposure, 6.3% of the 10% of the P transferred over diffusion barrier were detected in the shoots of *G. repens* indicating that the OMF could uptake and translocate significant amount of P to its partner (Cameron et al. 2007).

13.4.3 Plant Growth Stimulation by OMF

Colonization by OMF enhances both the orchids vegetative and reproductive growth, ex vitro survival rates, induces early flowering, improves flower quality, and reduces disease severity in the seedlings (Chang 2008). Similarly, all the *Acampe praemorsa in vitro* raised seedlings inoculated with OMF survived under *ex vitro* conditions (Sathiyadash et al. 2013). Colonization of roots by *Rhizoctonia* sp., increases plant height, biomass, root formation, and root length in *Cymbidium kanran* (Lee et al. 2003), *Cymbidium goeringii* (Wu et al. 2013), and *Cymbidium* sp. (Wu et al. 2010). However, colonization by *Tulasnella repens* only enhanced the plant biomass in these orchids. Similar effects were also reported for *Dendrobium nobile*, *D. loddigesii* (Zaiqi and Yin 2008), *Dendrobium officinale* (Yang et al. 2008a), *Haemaria discolor* (Chang and Chou 2001), and *Anoectochilus formosanus* (Chang and Chou 2007) colonized by different OMF. Orchids like *Anoectochilus roxburghii* (Dan et al. 2012a), *Dendrobium candidum* and *D. nobile* colonized by *Epulorhiza* sp., *Mycena dendrobii*, *Moniliopsis* sp., *Gliocladium* sp., *Mycena anoectochila* were taller and had higher biomass (Dan et al. 2012b). *Cymbidium* colonized by six strains of unidentified OMF endophytes, as well as *Doritaenopsis* and *Phalaenopsis* colonized by *Rhizoctonia* and *Ceratobasidium* had higher biomass than un-inoculated seedlings (Fang et al. 2008; Wu et al. 2009, 2011). The *in vitro* raised *Guarianthe skinneri* when acclimatized with *Trichoderma harzianum* were taller and had more leaf and shoot numbers (Gutierrez-Miceli et al. 2008). Ex vitro raised *Cattleya aurantiaca* and *Brassavola nodasa* colonized by *Epulorhiza* were taller and heavier than their non-mycorrhizal counterparts (Ovando et al. 2005). Colonization of *Dendrobium officinale* roots by *Mycena* sp., increased plant height, biomass, and the number of new buds by two- four folds (Zhang et al. 2012). Further, increment in plant height and biomass are often considered as reliable parameters for the successful establishment of the symbiotic relationship (Jin et al. 2009a, b).

13.4.4 Phytohormone Production by OMF

The beneficial plant growth promoting microorganisms produce phytohormones, which are utilized by the host plants and it is one of the mechanisms for plant growth promotion by the microorganisms (Van Loon 2007; Shoresh et al. 2010). The bioactive compounds like indole-acetic acid (IAA), gibberellic acid (GA), and naphthalene acetic acid (NAA) produced by OMF are suggested to stimulate the development of *D. candidum* and *D. nobile* plantlets (Dan et al. 2012b). Gibberellic acid and NAA synthesized by OMF can also promote the elongation of stems and roots of *D. huoshanense* (Zhang et al. 1999). Isolates of *Tulasnella*, *Epulorhiza* and an unusual orchid endophyte *Colletotrichum gloeosporioides* has been shown to produce IAA under *in vitro* culture conditions (Robinson et al. 1998; Chung et al. 2003). The significant amounts of IAA detected both in the fungal mycelium as well as in the culture medium may be transferred to the orchid hosts which may influence the colonization process (Barroso et al. 1986). In addition, the detection of the presence of indole-3-ethanol

(IEt) in the *Ophrys lutea* culture medium indicates its synthesis and this IEt could act as a precursor for IAA synthesis in the host orchids (Barroso et al. 1986).

Earlier studies have shown that OMF are capable of secreting gibberellins, heteroauxin, dormin, zeatin and zeatin riboside (Wu and Zheng 1994; Wu et al. 2002) and these plant hormones were shown to improve the growth of orchids (Yang et al. 2008a, b). In *Gastrodia elata*, seed germination and cell differentiation process are stimulated by plant hormones or fungal metabolic products (Guo and Xu 1990; Xu 1993). In *Dendrobium hancockii,* the germination of seeds was stimulated by the presence of OMF extracts obtained from the protocorms of other orchids like *Liparis nervosa* and *G. elata* (Guo and Xu 1990). *Trichoderma* sp., can either synthesis phytohormones or alter the internal phytohormone homeostasis of the host plant (Shoresh et al. 2010; Salas-Marina et al. 2011). Extracted compounds and mixtures of fungi, filter concentrates, mycelia extracts, and hydrolytic products of cell walls, peptides and proteins are shown to act as fungal elicitors (Smith 1996; Hahn 1996).

13.4.5 Role of OMF in Disease Resistance

One of the major bottlenecks in commercial orchid cultivation is the disease outbreak, as bacteria, fungi or viruses affect the quality of plants by leaving brown spots or scars, and when the disease is not controlled or eradicated it may cause huge economic loss in orchid production (Wu et al. 2011). Soft rot diseases caused by *Erwinia* spp. is the most devastating disease in commercial orchid production (Liau et al. 2003). *Phalaenopsis* inoculated with *R. solani* and *Ceratobasidium* strains reduced the severity of soft rot, and a correlation also exists between disease symptom reduction and plant growth (Wu et al. 2011). In *Vanilla*, colonization by *Ceratobasidium* was shown to control the root rot caused by *Fusarium* (Bayman et al. 2011). OMF directly inhibit the pathogens through competition for space or nutrients. Further, OMF provides growth promoters or systemic-induced resistance to strengthen the plant defense mechanisms (Burns and Benson 2000; González et al. 2002). The improved nutritional status of orchids due to OMF makes them less susceptible to pathogens (Bayman et al. 2011).

Ceratobasidium isolated from orchids could also protect other type of plants from pathogens (Bayman et al. 2011). Hypovirulent and non-pathogenic *Ceratobasidium* present in soil protect the seedlings of cucumber and other crops from damping-off disease (Sneh et al. 2004; Ichielevich-Auster et al. 1985). *Ceratobasidium* isolated from *Cranichis* sp., and *Maxillaria* sp., decreased the severity of sheath blight in rice caused by *R. solani* (Mosquera-Espinosa et al. 2013).

13.5 OMF Diversity

The presence of compatible mycobionts and different environmental factors plays a major role in the growth and recruitment of orchids in nature (McCormick et al. 2004). The unique feature of orchidaceae as well as its wide diversity may be

attributed to its distinctive relationship with the mycorrhizal fungi (Zettler et al. 2004). The OMF diversity could be separated based on the ecology and photosynthetic ability of the orchid hosts (Taylor et al. 2002). However, information is limited on the role of fungal diversity in orchid distribution, population size, and genetic diversity (McCormick et al. 2004).

13.5.1 OMF Diversity in Terrestrial Photosynthetic Orchids

Terrestrial photosynthetic orchids are generally colonized by fungi belonging to five groups such as Heterobasidiomycetes, Hericianae, Hymenocaetanae, Thelephoranae, and Agaricanae (Rasmussen 2002) (Table 13.1 and Fig. 13.3). Earlier records mainly focused on the saprophytic fungi involved in the mycorrhizal association of orchids, but later both ectomycorrhizal (ECM) and parasitic fungi were also shown to associate with orchids in a mutualistic manner (Rasmussen 2002). Isolation of the ECM fungi belonging to Russulaceae from *Corallorhiza* spp. (Taylor and Bruns 1997) and the colonization of field-grown seedlings of *Corallorhiza trifida* with *Thelephora–Tomentella* complex of Thelephoriaceae, suggests that the ECM fungi can symbiotically associate with orchids (McKendrick et al. 2000). The ECM fungal genera associating with the non-photosynthetic *Lecanorchis* spp. include *Lactarius*, *Russula*, Atheliaceae, and *Sebacina*, of which *Lactarius* and *Russula* were found to be dominant (Okayama et al. 2012). Colonization of the achlorophyllous *Epipogium roseum* by the saprotrophic members of Coprinaceae suggest that the decaying wood materials would be used as a large and persistent C source for the growth of this orchid (Yamato et al. 2005). Mycobionts of *Hexalectris* spp. were found to be members of Sebacinaceae, Ceratobasidiaceae, Russulaceae, and Thelephoraceae (Kennedy et al. 2011). It is hypothesized that achlorophyllous orchids obligately associated with fungi have access to a large and persistent C source supplied by ECM fungi (Taylor and Bruns 1997; Leake 2005), as these fungi are likely to have access to such C supply (Kennedy et al. 2011).

An investigation on the mycobionts of *Caladenia* yielded 103 fungal isolates, most of which belonged to *Sebacina vermifera* and one isolate belonged to *Tulasnella calospora*. In an allied *Caladenia* species, 94% of the 33 isolates belonged to *S. vermifera* and a few to *T. calospora*, indicating that these orchids were associated with specific OMF (Warcup 1971). Further, investigations on mycobionts of terrestrial orchids have resulted in the discovery of new fungal taxa. For example, two new mycorrhizal endophytes, *Ceratorhiza pernacatena* and *Epulorhiza calendulina*, were characterized and identified from the roots of *Platanthera praeclara* and *Amerorachis rotundifolia*, respectively, from Canada (Zelmer and Currah 1995). A phylogenetic analysis of the mycorrhizal diversity from a single peloton of *Disabracteata* (a South African orchid) and *Pyrorchis nigricans* showed the presence of the fungi belonging to distinct groups of the *Rhizoctonia* alliance, like *Epulorhiza*, *Ceratobasidium,* and *Sebacina* (Bonnardeaux et al. 2007). Nevertheless, composite peloton formation by multiple endophytes within a cell further complicates the nutritional basis of OMF relationships

Table 13.1 The diversity of fungal taxa involved in mycorrhizal association with different orchid life-forms

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Epiphytes		
Ceratobasidium cornigerum	*Sarcochilus* sp., *Thrixspermum congestum*	Roberts (1999)
C. endophytica	*Cymbidium sinensis*	Lee and You (2000)
Ceratobasidium sp.	*Taeniophyllum obtusum*	Irawati (1993)
	Campylocentrum fasciola, Campylocentrum filiforme, Ionopsis satyrioides, Ionopsis utricularioides, Tolumnia variegata, Oeceoclades maculata, Oncidium altissimum	Otero et al. (2002)
	I. utricularioides	Otero et al. (2004)
	Sarcochilus weinthalii	Graham and Dearnaley (2012)
	Vanda thwaitesii	Decruse et al. (2018)
	Rhynchostylis retusa, Aerides multiflorum	Hossain et al. (2013)
	Dendrophylax lindenii	Mújica et al. (2018)
Ceratobasidium sp., *Rhizoctonia* sp.	*Erythrodes plantaginea*	Otero et al. (2002)
C. sphaerosporum	*Pomatocalpa macphersonii*	Warcup and Talbot (1971)
	Robiquetia wassellii	Warcup and Talbot (1971)
Ceratorhiza sp.	*Isochilus linearis*, *Oncidium flexuosum*, *Oncidium varicosum*, *Maxillaria marginata*	Pereira et al. (2005)
Epulorhiza calendulina	*Spathoglottis plicata*	Ma et al. (2003)
	Epidendrum rigidum, Polystachya concreta	Pereira et al. (2003)
E. epiphytica	*E. rigidum*, *P. concreta*	Pereira et al. (2005)
E. repens, Tulasnella violea, Trichosporiella multisporum	*Dendrobium friedericksianum*	Khamchatra et al. (2016)
Epulorhiza sp.	*Arachnis* sp., *Arundina graminifolia, Dendrobium crumenatum*, *Diplocaulobium* sp., *Oncidium nanum*, *Vanda* sp.	Ma et al. (2003)
	Coelogyne nervosa	Sathiyadash et al. (2014)
Epulorhiza sp., *Tulanella calopsora*, uncultured *Tulasnella*, *Pluteus seticeps, Ceratobasidium* sp.	*Dendrobium officinale, Dendrobium fimbriatum*	Xing et al. (2013)
Rhizoctonia butinii	*Cyrtochilum myanthum*	Novotna et al. (2018)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Rhizoctonia sp.	*Tolumnia variegata*	Otero et al. (2004)
	P. nutans, Pterostylis longifolia, Pterostylis obtusa	Bougoure et al. (2005)
	Gastrochilus acaulis, P. concreta, Nervilia prainiana	Senthilkumar (2003)
Rhizoctonia zeae	*Vanda coerulea*	Aggarwal et al. (2012)
Thanatephorus sp.	*Acampe praemorsa*	Senthilkumar et al. (2000)
Tremella sp.	*Cymbidium bicolor*	Downing et al. (2017)
Tulasnella calospora	*Laelia autumnalis*	Beltrán-Nambo et al. (2018)
	Cymbidium tracyanum, Dendrobium crystallinum	Nontachaiyapoom et al. (2010)
T. deliquescens	*Diuris, Acianthus, Caladenia, Thelymitra, Lyperanthes* spp., *Dendrobium dicuphum*	Warcup and Talbot (1980)
	Dendrobium sp.	Roberts (1999)
T. irregularis	*D. dicuphum*	Warcup and Talbot (1980)
	Encyclia tampensis	Zettler et al. (2013)
T. pinicola	*Dendrobium* sp.	Warcup and Talbot (1967)
Tulasnella sp.	*Stelis superbiens*	Novotna et al. (2018)
	Stelis concinna, Stelis hallii, Stelis superbiens	Suarez et al. (2006)
	Pleurothallis lilijae	
	Dendrobium speciosum	Boddington and Dearnaley (2008)
	Cymbidium lowianum	Nontachaiyapoom et al. (2010)
	Cymbidium tracyanum	Athipunyakom et al. (2004b)
	C. macranthos	Shimura et al. (2009)
Uncultured *Tulasnella* sp.	*Cymbidium bicolor*	Downing et al. (2017)
Tulasnella violea	*Dendrobium friedericksianum*	Nontachaiyapoom et al. (2010)
Hemiepiphyte		
Scleroderma areolatum	*Vanilla planifolia, Vanilla pompona, Vanilla insignis*	González-Chávez et al. (2018)
Lithophyte		
Uncultured *Tulasnella* sp.	*Paphiopedilum dianthum, Paphiopedilum hirsutissimum*	Downing et al. (2017)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Terrestrial		
Armillaria jezoensis	*Galeola septentrionalis*	Cha and Igarashi (1996)
Armillaria mellea	*Gastrodia elata*	Lan et al.(1994)
Atheliaceae	*Lecanorchis flavicans* var. *acutiloba*	Okayama et al. (2012)
Atheliaceae, *Russula* sp.	*Lecanorchis trachycaula*	Okayama et al. (2012)
Ceratobasidium bicorne	*Prasophyllum macrostachyum*	Warcup and Talbot (1980)
C. cornigerum	*Acianthus reniformis*	Warcup and Talbot (1971)
	Canadian orchids	Currah et al. (1987)
	Platanthera obtusata	Currah et al. (1990)
	Pterostylis sp., *Prasophyllum* sp.	Roberts (1999)
C. cornigerum, Rhizoctonia repens, Ceratobasidium globisporum, Thanatephorus cucumeris, Epulorhiza sp., *Tulasnella* sp.	*Habenaria dentata*, *Eulophia flava, Spiranthes hongkongensis*	Shan et al. (2002)
Ceratobasidium sp., *Sebacina* sp., *Rhizoctonia* sp.	*Anacamptis morio*	Waud et al. (2016)
C. globisporum	*Calanthe triplicata*	Roberts (1999)
C. pseudocor- nigerum	*Pterostylis mutica*	Warcup and Talbot (1980)
Ceratobasidium sp.	*Gymnadenia conopsea*	Stark et al. (2009)
	Pterostylis sanguina, *P. recurva*	Bonnardeaux et al. (2007)
	Dactylorhiza hatagirea	Aggarwal and Zettler (2010)
	Paphiopedilum hirsutissimum	Downing et al. (2017)
Ceratobasidium sp., *Leptodontidium* sp., *Phialophora* sp., *Tulasnella* sp.	*Platanthera chlorantha*	Bidartondo et al. (2004)
Ceratobasidium sp., *Leptodontidium* sp., *Sebacina* sp., *Tulasnella* sp.	*Epipactis palustris*	Bidartondo et al. (2004)
Ceratobasidium sp., *Russula* sp., uncultured *Tricholoma* sp.	*P. nutans*	Irwin et al. (2007)
Ceratobasidium sp., *Sebacina* sp.	*Epipactis helleborine*	Bidartondo et al. (2004)
Ceratobasidium sp., *Sebacina vermifera*	*Cypripedium californicum*	Shefferson et al. (2005)
Ceratobasidium sp., *Tulasnella* sp.	*Dactylorhiza majalis*	Bidartondo et al. (2004)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Ceratobasidium spp.	*Coppensia doniana*	Valadares et al. (2011)
Ceratobasidum albasitensis	*Dactylorhiza baltica*	Shefferson et al. (2008)
	D. baltica	Shefferson et al. (2008)
Ceratorhiza cerealis	*Goodyera procera*	Athipunyakom et al. (2004b)
Ceratorhiza goodyera-repentis	*Ludisia discolor, G. procera*	Athipunyakom et al. (2004b)
Ceratorhiza pernacatena	*Platanthera praeclara*	Zelmer and Currah (1995)
	Calanthe rubens	Athipunyakom et al. (2004b)
Ceratorhiza ramicola	*Paphiopedilum exul*	Athipunyakom et al. (2004b)
	G. procera	Athipunyakom et al. (2004b)
Coprinus spp.	*Epipogium roseum*	Yamato et al. (2005)
Cortinarius, *Hymenogaster*, *Inocybe*, *Thelephora* sp., *Tomentella* sp.	*Cephalanthera damasonium*	Bidartondo et al. (2004)
Epacris pulchella, *Russula mustelina*, *Gymnopus luxurians*	*Erythrorchis cassythoides*	Dearnaley (2006)
Epulorhiza anaticula	*Coeloglossum viride*, *Platanthera obtusata*, *Platanthera hyperborea*	Currah et al. (1990)
E. calendulina	*Amerorchis rotundifolia*	Zelmer and Currah (1995)
	Paphiopedilum concolor	Athipunyakom et al. (2004a, b)
Epulorhiza repens	*Microtis parviflora*	Perkins et al. (1995)
	Oeceoclades maculata	Pereira et al. (2005)
	Spiranthes brevilabris	Johnson et al. (2007)
	Calanthe rosea, Cymbidium sinense, Paphiopedilum concolor, P. exul, P. villosum, Spathoglottis plicata	Athipunyakom et al. (2004a, b)
Epulorhiza sp.	*Eulophia alta*	Johnson et al. (2007)
	Eulophia spectabilis, Pecteilis susannae, Paphiopedilum bellatulum, Spathoglottis affinis	Chutima et al. (2011)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
	C. sinensis, Paphiopedilum callosum, Paphiopedilum charlesworthii, P. exul, Paphiopedilum sukhakulii, P. villosum, Cymbidium (hydrid).	Nontachaiyapoom et al. (2010)
Epulorhiza sp., and *Ceratorhiza* sp.	*P. praeclara*	Sharma et al. (2003)
Erythromyces crocicreas	*Galeola altissima*	Umata (1995)
	Erythrorchis ochobiense	Umata (1998)
Gloeotulasnella sp.	*E. spectabilis*	Chutima et al. (2011)
Inocybe sp., *Leptodontidium* sp., *Phialophora,Sebacinoid* sp., *Tuber* sp., *Tulasnella* sp.,*Wilcoxina* sp.	*Epipactis atrorubens*	Bidartondo et al. (2004)
Lactarius sp.	*Lecanorchis japonica* var. *kiiensis, Lecanorchis kiusiana* var. *suginoana, Lecanorchis virella*	Okayama et al. (2012)
Lactarius sp., and *Russula* sp.	*L. japonica* var. *japonica, L. japonica* var. *kiiensis, L. japonica* var. *hokurikuens, Lecanorchis nigricans*	Okayama et al. (2012)
Leptodontidium sp., *Phialophora* sp., *Tomentella* sp.	*Cephalanthera rubra*	Bidartondo et al. (2004)
Moniliopsis anomala	*Coeloglossum viride, Platanthera hyperborea*	Currah et al. (1990)
Mycena orchidicola	*C. sinense*	Fan et al. (1996)
M. osmundicola	*Gastrodia elata*	Lan et al. (1996)
Oliveonia pauxilla	*G. elata*	Warcup (1975)
Phaeosphaeria phragmiticola	*Acianthus exsertus, A. pusillus*	Bougoure et al. (2005)
Phialophora graminicola	*A. achalensis*	Sebastián et al. (2014)
Phialophora sp.	*Thelymitra crinita*	Bonnardeaux et al. (2007)
Rhizoctonia globularis	*G. procera*, *S. plicata*	Athipunyakom et al. (2004a)
R. repens	*Cymbidium goeringii*	Lee et al. (1998)
	Aerides rosea, Phalaenopsis manii	Saha and Rao (2006)
R. solani	*Phaius tankervilleae*	Saha and Rao (2006)
	Chloraea riojana	Fracchia et al. (2016)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Rhizoctonia sp.	*Aa achalensis*	Sebastián et al. (2014)
	S. plicata	Athipunyakom et al. (2004a)
Rhizoctonia sp., *Ceratobasidium* sp., Helotiales sp., *Naevola minutissima*	*Dactylorhiza sambucina*	Pellegrino and Bellusci (2009)
Rhizoctonia sp., *Thanatephorus cucumeris, Tulasnella danica*	*Pyrorchis nigricans*	Bonnardeaux et al. (2007)
Rhizoctonia sp., unidentified Tulasnellaceae and Ceratobasidiaceae, *Rhizoctonia* sp., *Sebacina* sp., *Thanatephorus* sp.	*Orchis mascula*	Waud et al. (2016)
Rhizoctonia sp.1	*Paphiopedilum niveum*	Athipunyakom et al. (2004b)
Rhizoctonia sp.2	*P. exul*	Athipunyakom et al. (2004b)
Russula sardonia	*Cypripedium parviflorum*	Shefferson et al. (2005)
Russula sp.	*Limodorum abortivum, L. brulloi, L. trabutianum*	Girlanda et al. (2006)
	Corallorhiza sp.	Whitridge and Southworth (2005)
Russula spp.	*Dipodium variegatum*	Bougoure and Dearnaley (2005)
Russulaceae spp.	*Corallorhiza maculata, Corallorhiza mertensiana*	Taylor and Bruns (1999)
Sebacina sp.	*Hexalectris spicata, Hexalectris spicata* var. *arizonica, Hexalectris revoluta*	Taylor et al. (2003)
	S. plicata	Athipunyakom et al. (2004a)
	Neottia nidus-avis	Bidartondo et al. (2004)
	Lecanorchis flavicans var. *flavicans*	Okayama et al. (2012)
Sebacina spp.	*Stigmatodactylus sikokianus*	Yagame and Yamato (2008)
Sebacina spp., *Ceratobasidium* sp., *Cortinarius* sp., *Thelephoraceae*	*Hexalectris* spp.	Kennedy et al. (2011)
Sebacina vermifera	*Caladenia carnea*	Bougoure et al. (2005)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
	Caladenia falcata, *Microtis media*	Bonnardeaux et al. (2007)
Serendipita vermifera	*Acianthus* spp., *Caladenia* spp., *Elythranthera* spp., *Eriochilus* spp., *Glossodia major, Microtis* spp., *Prasophyllum* spp.	Roberts (1999)
Sistotrema sp.	*Platanthera obtusata, Piperiya unalascensis*	Currah et al. (1990)
	Paphiopedilum godefroyae	Athipunyakom et al. (2004b)
Steccherinum ochraceum	*Microtis parviflora*	Bougoure et al. (2005)
Thanatephorus cucumeris	*A. achalensis*	Sebastián et al. (2014)
	Prasophyllum odoratum, Pterostylis foliata	Roberts (1999)
	Goodyera oblongifolia	Shefferson et al. (2005)
	Rhizanthella gardneri	Warcup (1991)
Thanatephorus ochraceus	*Calypso bulbosa*	Currah (1987)
	C. viride, Orchis mascula	Warcup and Talbot (1967)
T. sterigmaticus	*Thelymitra antennifera*	Warcup and Talbot (1967)
Thelephoraceae sp.	*Cephalanthera austinae*	Taylor and Bruns (1997)
Thelephoraceous ectomycorrhiza	*Cypripedium candidum, Cypripedium fasciculatum, Cypripedium montanum*	Shefferson et al. (2005)
Tomentella sp., *Ceratobasidium* sp., and *Tuber* sp.	*Cephalanthera damasonium*	Julou et al. (2005)
Trichosporiella multisporum	*Paphiopedilum niveum*	Athipunyakom et al. (2004b)
Tuber sp. and *Russula* sp.	*Epipactis microphylla*	Selosse et al. (2004)
Tuber sp. and *Sebacina* sp.	*E. microphylla*	Selosse et al. (2004)
Tulansella sp.	*Paphiopedilum micranthum, Paphiopedilum armeniacum, Paphiopedilum dianthum, Cypripedium flavum, Cypripedium guttatum, Cypripedium tibeticum*	Yuan et al. (2010)
Tulasnella asymmetrica	*Cymbidium* (hydrid)	Nontachaiyapoom et al. (2010)
T. calospora	*Prasophyllum giganteum, Diuris magnifica*	Bonnardeaux et al. (2007)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
	Paphiopedilum sukhakulii, P. villosum	Nontachaiyapoom et al. (2010)
	Chloraea collicensis, Chloraea gavilu	Pereira et al. (2014)
T. cruciata	*Acianthus caudatus, Thelymitra* spp.	Warcup and Talbot (1971)
T. deliquescens	*Dactylorhiza purpurella*	Roberts (1999)
T. irregularis	*Cymbidium* (hydrid), *Paphiopedilum charlesworthii*	Nontachaiyapoom et al. (2010)
	Thelymitra luteocilium	Warcup and Talbot (1967)
T. pruinosa	*Cymbidium* (hydrid)	Nontachaiyapoom et al. (2010)
T. repens	*Cymbidium goeringii*	Lee and You (2000)
Rhizoctonia sp., *Ceratobasidium* sp., *Sebacina* sp., unidentified Ceratobasidiaceae and Tulasnellaaceae	*Gymnadenia conopsea*	Waud et al. (2016)
Tulasnella sp.	*Goodyera pubescens, Liparis lilifolia, Tipularia discolor*	McCormick et al. (2004)
	Piperia sp.	Whitridge and Southworth (2005)
	E. spectabilis, S. affinis	Chutima et al. (2011)
Tulasnella sp., and *Ceratobasidium* sp.	*Liparis loeselii*	Illyes et al. (2005)
Tulasnella sp., *Epulorhiza* sp., *Nectria mauritiicola*, *Leptontidium orchidicola*	*Disa bracteata*	Bonnardeaux et al. (2007)
Tulasnella sp., *Sebacina* sp., *Russula* sp., *Lactarius* sp., uncultured *Tomentella* sp.	*Gymnadenia conopsea*	Stark et al. (2009)
Tulasnella sp., and *Thanatephorus* sp.	*Neuwiedia veratrifolia*	Kristiansen et al. (2004)
Tulasnella violea	*Thelymitra aristata*	Warcup and Talbot (1971)
	P. villosum	Nontachaiyapoom et al. (2010)
Uncultured Tulasnellaceae	*Gymnadenia conopsea*	Stark et al. (2009)
Uncultured *Ceratobasidium* sp.	*P. nutans*	Irwin et al. (2007)
Uncultured *Tulasnella* sp.	*Cymbidium* (hydrid), *P. villosum*	Nontachaiyapoom et al. (2010)
	Geodorum eulophioides	Downing et al. (2017)
Uncultured Basidiomycetes, *Tomentella*	*Paphiopedilum dianthum*	Downing et al. (2017)
Unnamed Tulasnellaceae	*Epipactis atrorubens*	Shefferson et al. (2008)

(continued)

Table 13.1 (continued)

Orchid life forms Mycorrhizal fungal taxa	Orchid taxa	References
Unnamed Tulasnellaceae	*Orchis militaris*	Shefferson et al. (2008)
Tulasnellaceae	*Epidendrum* sp., *Stelis superbiens*, *Elleanthus* sp., *Elleanthus virgatus, Maxillaria* sp., *Epidendrum lacustre*	Herrera et al. (2018)
Waitea circinata	*Paphiopedilum niveum*	Athipunyakom et al. (2004b)
Wilcoxina sp.	*Epipactis distans*	Bidartondo et al. (2004)

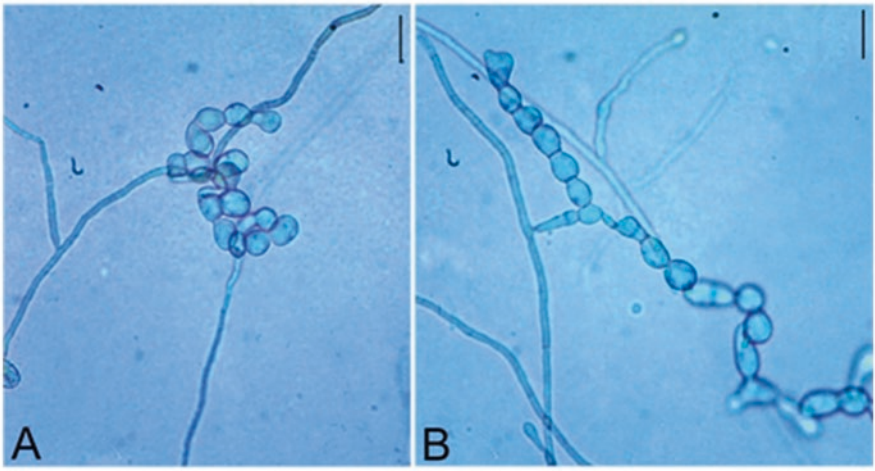

Fig. 13.3 (**a**)–(**b**) Morphology of orchid mycorrhizal fungi isolated from roots of the terrestrial orchid *Tropidia thwaitesii* and cultured in potato dextrose agar medium. (**a**), Hyphae and moniliform cells of *Tulasnella* (**a**) and *Euphlorhiza* (**b**). Scale bars = 20 μm

(Rasmussen 2002). Further, the simultaneous presence of both intact and degraded patches of pelotons in root cortex may represent individual colonization events as adjacent patches may be formed by different fungi (Zelmer et al. 1996). If the nutritional concept is considered over the lifetime of an orchid, different types of fungi could be involved in utilizing different substrates during different periods (Rasmussen 2002). For example, the development of *G. elata* seedlings associated with *Mycena osmundicola* was dependent on its successive association with the parasitic *Armllaria mellea* (Xu and Mu 1990).

The saprotrophs were possibly over represented in previous studies on OMF diversity because of their ability to establish and grow in pure cultures more readily (Rasmussen 2002). Nevertheless, the development of DNA based approaches has

widened the range of endophytic fungi with different nutritional strategies associating with orchids (Rasmussen 2002). In the view of Rasmussen (2002), majority of orchid mycobionts are basidiomycetes. In contrast, 78% of the photosynthetic and non-photosynthetic roots of *Epipactis microphylla* were found to be colonized by the *Tuber* sp., with the remaining roots also being colonized by other ascomycetes and a few basidiomycetes fungi (Selosse et al. 2004). The other potential ascomycetes mycorrhizal endophytes of *Epipactis* spp. in addition to *Tuber* sp., include *Wilcoxina* and *Phialophora* (Bidartondo et al. 2004).

The diversity of OMF associated with photosynthetic terrestrial orchids is generally low due to their high host-mycobiont specificity (McCormick et al. 2004). A phylogenetic analysis of the mycorrhizal association in *Cypripedium* elucidated that, most of the *Cypripedium* fungi formed narrow clades within the family Tulasnellaceae, whereas other root endophytes from Sebacinaceae, Ceratobasidiaceae, and *Phialophora* rarely support the high specificity in photosynthetic orchids (Shefferson et al. 2005). Narrow mycorrhizal specificity was seen in *Stigmatodactylus sikokianus* for *Sebacina* spp. even when the orchid roots were collected from different geographical regions (Yagame and Yamato 2008). The frequent colonization of roots in *Paphiopedilum micranthum*, *Paphiopedilum ameniacum*, *Paphiopedilum dianthum*, *Cypripedium flavum*, *Cypripedium guttatum,* and *Cypripedium tibeticum*, mainly by members of Tulasnellaceae, is a clear proof for the narrow phylogenetic range of mycorrhizal taxa in terrestrial photosynthetic orchids (Yuan et al. 2010). Further, the diverse OMF isolates colonizing these orchids belonged to the four discrete groups of the genus *Epulorhiza*. However, *P. nigricans* was colonized by the widest diversity of fungi including isolates of *Epulorhiza* and *Ceratobasidium* suggesting that this orchid could potentially benefit from this diverse association (Bonnardeaux et al. 2007).

The simultaneous colonization of *D. officinale* by *Tulasnella* spp., *Epulorhiza* sp., and *Plateus seticeps* and association of *Dendrobium fimbriatum* with *Tulasnella* sp., in certain orchid populations (Xing et al. 2013) suggest that co-occurring orchid species could benefit from different mycorrhizal partners (Waterman et al. 2011). However, this needs further confirmation as the small sample size of *Dendrobium* could have led to an underestimation of the fungal diversity (Xing et al. 2013). Twenty seven isolates of *Tulasnella calospora*, *Tulasnella pruinosa*, *Tulasnella asymmetrica*, *Tulasnella irregularis*, *Epulorhiza* sp., *Tulasnella violea,* and uncultured *Tulasnella* were associated with the orchid genera *Paphiopedilum*, *Dendrobium,* and *Cymbidium* (Nontachaiyapoom et al. 2010). In a study on the mycorrhizal diversity of *Coppensia doniana*, all the isolated OMF belonged to the genus *Ceratobasidium* which explains the wide potential habitats of this orchid, as these fungi can gain energy from organic matter in soil, litter, tree barks, and rock surfaces (Valadares et al. 2011).

The diverse group of fungal associates, such as typical mycorrhizal fungi from Tulasnellaceae, Ceratobasidiaceae, and ECM taxa of Pezizales colonizing the terrestrial orchid *Gymnadenia conopsea* contributes to its ability to colonize different habitat types (Stark et al. 2009). Edaphic conditions are also known to play an important role in the distribution of OMF colonizing roots of terrestrial orchids. For

example, *Tulasnella prima* is the major mycobiont colonizing roots of several *Chiloglottis* species growing in soil and sphagnum hammocks over a range of more than 1000 km in southeastern Australia (Ruibal et al. 2017). In contrast *Tulasnella sphagneti* associated with *Chiloglottis* is rather restricted to the wet conditions of alpine sphagnum hammocks (Ruibal et al. 2017).

13.5.2 OMF Diversity Epiphytic Photosynthetic Orchid

In epiphytic orchids, low levels of patchy mycorrhizal colonization could be seen in roots attached to substratum (Bermudes and Benzing 1989; Pereira et al. 2005). Twenty-four *Epulorhiza* isolates were obtained from the roots and protocorms of *Oncidium*, *Vanda*, *Arachnis*, *Dendrobium*, *Arundina*, *Diplocaulobium,* and *Spathoglottis* examined from different sites in Singapore (Ma et al. 2003). The Andean epiphytic orchids like *Stelis concinna*, *Stelis hallii, Stelis superbiens,* and *Pleurothallis lilijae* were found to be colonized by *Tulasnella* spp. based on the septal ultrastructure, whereas the molecular analysis of the roots yielded seven distinct *Tulasnella* clades (Suarez et al. 2006). All the *Tulasnella* sequences were distinct from the already known sequences of mycobionts in certain terrestrial orchids and this indicated the adaptation of these fungi to tree stems and also its importance in orchid growth (Suarez et al. 2006).

Twenty-six isolates of OMF were identified from the tropical epiphytic orchids which formed two fungal lineages, related to *Ceratobasidium* spp., of which majority of orchids hosted more than one fungal lineage suggesting the variation in mycobiont association within related orchid species (Otero et al. 2002). Mycobionts from *Tolumnia variegata* clade with four fungal lineages of which only one lineage include fungi from *Ionopsis utricularioides* (Otero et al. 2002) and in a later study the same orchid was seen colonized with *Ceratobasidium* suggesting that *I. utricularioides* is specialized in effectively exploiting a specific fungal clade (Otero et al. 2004). The broad mycobiont specificity of *I. utricularioides* explains its survival in a broad geographical range and also its large population size (Otero et al. 2004). The rare epiphytic orchid *Sarcochilus weinthalii* was seen associated with single species of *Ceratobasidium* and direct sequencing of colonized root fragments and culture dependent methods also indicated its narrow specificity (Graham and Dearnaley 2012). In China, four epiphytic endemic orchids (*Laelia autumnalis*, *Laelia speciosa*, *Euchile citrina*, and *Prosthechea squalida*) harbored 71 isolates of fungal endophytes belonging to 20 genera of basidiomycetes and ascomycetes indicating the rich diversity of OMF association in these orchid hosts (Beltrán-Nambo et al. 2018). In south Ecuador, the epiphytic orchids colonized by 115 fungal isolates of 49 fungal operational taxonomic units (OTUs,) including four mycorrhizal OTUs belonging to Ceratobasidiaceae and Tulasnellaceae revealed high diversity of fungi colonized with orchid roots (Novotna et al. 2018).

13.6 Taxonomy of OMF

In the past, majority of the peloton forming fungi were classified as a member of anamorphic form-genus *Rhizoctonia* based on morphological characteristics (Athipunyakom et al. 2004b). The genus concept in *Rhizoctonia* was first established by De Candolle (1815). Earlier the form-genus *Rhizoctonia* consisted of a heterogenous assemblage of filamentous fungal taxa that failed to produce sexual spores and only share limited common features in their anamorphic stage (Garcia et al. 2006). However, new morphological criteria include nuclear number, septal nature, and parenthesome perforation, as well as teleomorphic stage for OMF (Moore 1987). Based on these, three new genera such as *Ceratorhiza*, *Epulorhiza*, and *Moniliopsis* were separated from *Rhizoctonia*. Ma et al. (2003) characterized the *Rhizoctonia* isolates using different media and colony hyphal characteristics, and identified them as species belonging to *Epulorhiza*. Basidial morphology was considered to be a reliable criterion for characterizing *Rhizoctonia* species at morphospecies level (Warcup and Talbot 1966, 1971, 1980), as fungal isolates from orchid roots only occasionally forms fruiting bodies in pure culture (Currah et al. 1987, 1990; Milligan and Williams 1988). In addition, the septal ultrastructure characteristics are also considered important for distinguishing the *Rhizoctonia* taxa (Khan and Kimbrough 1982; Marchisio et al. 1985; Currah and Sherburne 1992).

A system of anastomosis grouping based on hyphal fusion was widely adopted until last decade, as the basis for recognizing groups and taxa among the several fungi that constitute form-genus (Sneh et al. 1991). Currently, there are several accepted classifications based on the anastomosis group concept for both multinucleate (*Ceratobasidium*) taxa within the *Rhizoctonia* species complex (Carling 1996). *Rhizoctonia*-like mycobionts from Australian orchids though formed five anastomosing groups; the molecular sequencing showed that most of the anastomosing groups were monophyletic (Ramsay et al. 1987). Therefore, hyphal anastomosis behavior may not be the best indicator of evolutionary relationships between different intraspecific groups (Vilgalys and Cubeta 1994).

Later, the developments of biochemical methods were thought to be better to infer the phylogenetic relationships of fungi in *Rhizoctonia* species complex (Jabaji-Hare 1996). The biochemical methods for characterizing *Rhizoctonia* species include soluble protein patterns, zymograms and isoenzyme profiles which were employed to identify and study the genetic relationships among members of the form-genus (Jabaji-Hare 1996). Damaj et al. (1993) studied the relationship between binucleate *Rhizoctonia* isolates by isoenzyme electrophoresis. Grouping and identification of binucleate *Rhizoctonia* by pectic zymograms distinguished five zymographic groups in *Ceratobasidium cornigerum* (MacNish et al. 1993).

As induction of teleomorphic stages under laboratory conditions has been difficult for *Rhizoctonia*, their characterization is primarily based on the comparison of a limited number of anamorphic features and cytological probes (Moore 1987; Andersen and Stalpers 1994; Roberts 1999). Recently, molecular identification of fungi involving polymerase chain reaction (PCR) amplification of the nuclear ribosomal internal transcribed spacer (ITS) has revolutionized the identification of OMF

(Currah et al. 1995; Gardes and Bruns 1993; Redecker 2000; Vralstad et al. 2002). Internal transcribed spacer region is the most effective single loci for the identification of fungi from species to genus levels (Bruns 2001; Seifert et al. 2007). Gardes and Bruns (1993) suggested that ITS region has certain advantages in fungi, as the whole ITS region has 600–800 bp and could be easily amplified with universal primers (White et al. 1990). Amplification of ITS gene from small, dilute or highly degraded samples is easy because of its multicopy nature of the rDNA repeats. Earlier studies (Gardes and Bruns 1991; Chen et al. 1992; Lee and Taylor 1992) demonstrated that the ITS region is often variable among morphologically distinct fungal species. Gardes and Bruns (1993) also developed a taxon specific primer for amplification of ITS rRNA gene. Based on these developments, the presence of *Tulasnella* and *Laccaria* in roots of *Dactylorhiza majalis* was detected by amplifying the mitochondrial large subunit (Mt-Lt) RNA gene (Kristiansen et al. 2001). The endophytes colonizing *Neuwiedia vetrifolia* roots were identified as belonging to Tulasnellales and Ceratobasidiales by amplification and sequencing of the mitochondrial ribosomal large subunit DNA (Kristiansen et al. 2004). The OMF endophytes of *Acianthus*, *Caladania,* and *Pterostylis* were identified using ITS-restriction fragment length polymorphism (RFLP) analysis from Queensland (Bougoure et al. 2005). Pereira et al. (2005) suggested that random amplification of polymorphic DNA (RAPD) analysis might reveal higher polymorphism between *Epulorhiza epiphytica* and *Epulorhiza repens* than found in the PCR-RFLP analysis. Furthermore, RAPD and morphological analysis indicated a degree of relatedness among the *Ceratorhiza* isolates obtained from the roots of different *Oncidium* species (Pereira et al. 2005).

Though, the presence of *Epulorhiza* in the roots of several tropical orchids was confirmed by Ma et al. (2003) using amplification of rDNA repeat from 3′ end of the 18S rDNA gene and 5′ end of the 28S rDNA gene, the use of PCR based approaches for the amplification of OMF endophyte has certain limitations. For example, taxa belonging to Tulasnellaceae are difficult to characterize using standard PCR primer sets in spite of their common presence in orchid roots. This shortfall arises from the high evolution of the nuclear ribosomal operon (Binder et al. 2005; Moncalvo et al. 2006) and subsequent mutations of the conserved regions to which the primers hybridize (Taylor et al. 2002). Electron microscopic examination of the fungal pelotons of several epiphytic Pleurothalline orchids growing in the Andes revealed the predominance of the fungi belonging to Tulasnellaceae (Suarez et al. 2006). Nevertheless, only few of these fungi could be amplified using standard primers. But, true mycorrhizal symbionts of these orchids started to emerge only when nested PCR and several *Tulasnella* specific primers were used. Therefore, Taylor and McCormick (2008) developed a new set of selective primers with certain advantages like, low amplification of plant sequences and improved amplification of all tested Basidiomycota nuclear ribosomal DNA, including *Tulasnella*. Since then, specific primers have been routinely used to identify the mycobionts of orchids like *Cypripedium*, *Papilopedium*, *Dactylorhiza,* and *Dendrobium*. These types of sequencing analyses have revealed the association of a wide range of fungi belonging to Tulasnellaceae, Pluteaceae, and Ceratobasidiaceae with orchids (Shimura et al. 2009; Yuan et al. 2010; Valadares et al. 2011; Pellegrino and Bellusci 2009;

Xing et al. 2013). The various mycorrhizal fungi isolated from the orchid roots along with their origin are shown in Table 13.1.

13.7 Symbiotic Seed Germination

13.7.1 Fungal Preference

Orchids have been well known for their exclusive association with fungi. Generally, orchids have minute seeds with a very small embryo without an endosperm. Therefore association with fungi is a prerequisite for orchid seed germination, as the germinating seeds are incapable of obtaining nutrients successfully independent of fungi (Arditti 1992; Uetake et al. 1992). Bernard (1899) first described the role of mycobionts in orchid seed germination. Different isolates of fungi are known to differ in their potential to stimulate the germination of orchid seeds which tempted Warcup (1973) to suggest that the most efficient fungal isolates need not have its origin from the same host. This suggestion was also supported by the study of Stewart and Zettler (2002) where the terrestrial orchid *Habenaria macroceratitis* was non-specific in its requirement for the fungal mycobiont. Nevertheless, Stewart and Kane (2006) in a later study speculated that *H. macroceratitis* could have a certain degree of fungal preference as the germination percentage of the seeds was higher in the presence of fungi isolated from orchid populations from which the seeds were collected compared to those isolated from other orchid populations. The existence of fungal preference is also evident in another study (Stewart and Zettler 2002) where *Epulorhiza* strains isolated from *Habenaria quinqueseta* promoted seed germination of *Habenaria repens* better than those isolated from *Epidendrum conspseum*, *Spiranthes brevilabris,* and *H. macroceratitis*. In the same study, Stewart and Zettler (2002) also showed that the *in vitro* raised seedlings of *H. repens* growing in different potting media and colonized by *Epulorhiza* strains isolated from *S. brevilabris* survived better compared to those colonized with *Epulorhiza* strains isolated from *Epidendrum conopseum*. Similarly, *Cymbidium aloifolium* inoculated with OMF *Ceratobasidium* strain RR isolated from distant orchid taxa (*Rhynchostylis retusa*) promoted the number, length, leaf thickness, root number and length, and biomass (Hossain et al. 2013). In addition, *Epulorhiza* sp., isolated from terrestrial orchid *Eulophia epidendraea* promoted the seed germination and seedling development of epiphytic endemic orchid *Coelogyne nervosa* (Sathiyadash et al. 2014). In a recent study *Ceratobasidium* species isolated from the roots of adult ghost orchid *Dendrophylax lindenii* was able to promote seed germination and seedling development of the same orchid (Mújica et al. 2018).

13.7.2 Fungal Specificity

An understanding on the specificity of OMF-orchid interactions is of crucial importance both for ecology and conservation of orchids. Seed germination, protocorm development, and seedling growth are stimulated by digestion of orchid mycobionts

and subsequent uptake of the released nutrients by the immature orchid embryo (Clements 1988; Rasmussen 1995). Widely distributed orchids are expected to be either general in their preferences for mycorrhizal fungi or could be specific in associating with a broadly distributed fungus, as in many mutualistic relationships (Bascompte et al. 2003; Vazquez and Aizen 2003). In contrast, a narrow OMF specificity could be the reason for rarity and vulnerability of certain orchid species. Orchid-mycobiont specificity was considered controversial for many years. Earlier researchers thought that the orchid-fungus relationship to be quite natural and non-specific both under *in vitro* and *in situ* conditions (Knudson 1922; Curtis 1939; Hadley 1970; Masuhara and Katsuya 1989; Masuhara et al. 1993).

To assess the orchid-mycobiont specificity under *in vitro* conditions, symbiotic seed germination techniques are often considered to be more useful (Dixon 1987; Zettler 1997a, b; Stewart and Kane 2006). The seed germination efficiency of terrestrial orchids are often low (Stewart et al. 2003; Zettler et al. 2005; Stewart and Kane 2006) compared to asymbiotic seed germination for the same taxa. This low seed germination efficiency is often attributed to the existence of certain degree of orchid-mycobiont specificity as the mycobionts colonizing terrestrial orchids during seed germination and later stages could be entirely different (Stewart and Kane 2007).

Spiranthes brevilabris although non-specific for mycobionts, fungal isolates Econ 242 (*Epidendrum magnoliae*) and Sbrev-266 (*S. brevilabris*) induced seed germination and development of the seedlings better than the several isolates tested (Stewart et al. 2003). *Spiranthes cernua* seeds successfully developed into leaf bearing stage when colonized by the mycobionts originating from *Platanthera ciliaris* suggesting that the mycobiont specificity could rarely be species-specific (Zettler and McInnis 1993). In contrast, *in vitro* seed germination of *Bipinnula fimbriata* showed the lack of specificity for mycobionts, as the seed germination was stimulated by all the *Rhizoctonia*-like mycobionts isolated from the adult orchids in its habitat (Steinfort et al. 2010). Similarly, *Ceratobasidium* isolated from *Chloraea crispa* of different habitat promoted protocorm development in *B. fimbriata* (Steinfort et al. 2010). Hadley (1970) also observed that the four isolates of *Tulasnella calospora* originating from wide geographical regions exhibited almost similar patterns of symbiotic response in *Coeloglossum*, *Dactylorhiza*, *Goodyera*, *Cymbidium*, *Epidendrum*, *Laeliocattleya,* and *Spathoglottis* (Hadley 1970). Nevertheless, *Thanatephorus orchidicola* originating from *Dactylorhiza elata* and *Coeloglossum viride* failed to form symbiosis with any of the orchids tested (Hadley 1970). As the mycobionts isolated from particular host was equally symbiotic with other orchid hosts, further, the orchid-fungus relationship does not appear to be also completely random, as certain orchids tend to be more receptive to certain endophytes than others (Hadley 1970).

Isolates of *Sebacina vermifera* and *Tulasnella calospora* originating from *Caladenia* and *Diuris* stimulated seed germination of the same host expressing the existence of a genus level specificity (Warcup 1971). Similarly, *Ceratobasidium* sp., (strain VT3) isolated from epiphytic orchid *Vanda thwaitesii* efficiently promoted seed germination, protocorm, leaf, and up to root formation stage in same taxa (Decruse et al. 2018). In addition, the three co-occurring orchid species *Anacamptis morio*, *Gymnadenia conopsea,* and *Orchis mascula* shared a low number of OMF

OTUs, explaining the high specificity in OMF associations (Waud et al. 2016). Further, although *S. vermifera* stimulated the seed germination of *Cladenia*, *Glossodia* and *Elythranthera* (sub tribe Caladeniinae) failed to stimulat seed germination in *Eriochilus*, *Leporella*, *Acianthus* sp., *Microtis,* and *Parsophyllum,* thereby exhibiting specificity at subtribe level (Warcup 1981). The failure of the mycobionts isolated from *Spiranthes floridana* to support the seed germination and seedling development of *S. brevilabris* suggests that the genus *Spiranthes* failed to share the mycobionts during seed germination (Stewart and Kane 2007). Shefferson et al. (2005) also observed high mycobiont specificity at genus level occurring in the terrestrial orchid *Cypripedium*. Similar observation was made in the non-photosynthetic *Hexalectris* spp. colonized by *S. vermifera* (Taylor et al. 2003).

13.7.3 Cold Treatment

A delay in germination due to polymorphism may be an inherent characteristic of orchid seeds of temperate origin preventing the simultaneous germination of the seeds (Baskin and Baskin 1998). In natural habitats, seeds of *Platanthera praeclara* germinate during the first spring following dehiscence of capsule and seed dispersal, but further development of the protocorms were delayed until exposed to one or more winters (Sharma et al. 2003). So seeds subjected to cold treatment appear to be necessary to break seed dormancy in orchids.

The duration of the cold stratification may vary within a genus and may be species specific. Terrestrial orchids may require 3–9 months of cold stratification which may be species specific (Rasmussen 1995). Seeds of *P. leucophaea* require a cold stratification of 2–11 months for germination and the protocorms required chilling to transform into leaf-bearing seedlings (Stoutamire 1996; Zettler et al. 2001). In addition, cold stratification for 4 months combined with colonization by *Ceratorhiza* or *Ceratorhiza* and *Epulorhiza* resulted in higher percentage of protocorms advancing to the next stage (stage 3, top shaped protocorm and appearance of leaf) and proceed further. However, cold treatment for 6 months along with mycorrhization resulted in higher percentage seedlings transforming from stage 3 to 4 (elongation of the leaf promordium and protocorm enlargement) (Sharma et al. 2003). Higher seed germination percentage was observed in *Cypripedium macranthos* inoculated with mycorrhizal fungi after cold treatment (Shimura and Koda 2005).

13.7.4 Light

Orchid seeds possess a hydrophobic testa which maintains the seeds even the soil surface and exposing them to sunlight (Stewart and Kane 2006). Light exposure often initiates nutrient mobilization and therefore may not be directly involved in seed germination (Rasmussen and Rasmussen 1991). The effect of light on orchid seed germination may be genus or species specific (Stewart and Kane 2006). Initiation of photosynthesis occurred in *in vitro* raised *Habenaria repens* seedlings

when exposed to light for 1 week. Similarly, exposure of *Habenaria quinqueseta* seeds to 33 days light increased its germination percentage (Stewart and Zettler 2002). Seedlings of *Platanthera praeclara* in stage 5 (formation of root initial) developed their first green leaf only after exposure to illumination for 30 days. The leaves grew up to 8 cm upon exposure to light for 50 days (Sharma et al. 2003). Transfer of *H. macroceratitis* seeds from dark to a 15 h photoperiod enhanced seed germination and early protocorm development (Stewart and Zettler 2002). The influence of photoperiod on seed germination is evident from the fact that exposure of *H. macroceratitis* seeds to 16–24 h of photoperiod increased the seed germination than those exposed to continuous dark. Further, a 16 h photoperiod appear to be optimum for symbiotic seed germination of *H. macroceratitis* (Stewart and Kane 2006). In contrast, protocorm development tends to peak under dark conditions (Stewart and Kane 2006). However, protocorms of *Eulophia alata* developed into plantlets only on exposure to 16 hour photoperiod (Johnson et al. 2007). Takahashi et al. (2000) failed to find any significant effect of light on the germination of *Habenaria radiata* seeds exposed to either continued darkness or light conditions.

13.8 Role of OMF Specificity in Orchid Rarity

All orchids depend on mycorrhizal fungi during seed germination and adulthood in the wild (Porras-Alfaro and Bayman 2007). As most of the orchid seeds are microscopic (Kull 2002), they lack stored nutrients to support germination (Rasmussen 2002). When the orchid seeds are colonized by compatible fungi, the germination of seed is initiated by utilizing the fungal sugars and this myco-heterotrophic condition is retained into adulthood in many orchids (Gill 1989). Therefore, mycorrhizal associations are a prerequisite for seed germination and seedling growth of orchids in natural habitats.

The rarity of many orchid species around the globe could be attributed to the decline in the occurrence of mycorrhizal fungi, as most of the orchids appear to have certain degree of specificity for certain mycobionts at the time of germination and during later life stages (Stewart and Kane 2007). A study on the specificity and preference of mycorrhizal associations in two species of the genus *Dendrobium* suggested that *D. officinale* associate with a wide range of basidiomycetes, while *D. fimbriatum* had a high degree of specificity toward *Tulasnella* (Xing et al. 2013). The rare status of the Florida terrestrial orchid *Spiranthes brevilabris* could be due to the high mycobiont specificity of this orchid (Stewart and Kane 2007). Tropical epiphytic orchids found that *I. utricularioides* was highly specialized in effectively utilizing a specific fungal clade, *Ceratobasidium* (Otero et al. 2004). In contrast to these, the wide distribution of *S. cernua* can be partially associated with its low-species specificity (Zettler and McInnis 1993).

The orchid-fungal specificity may be at genus or even at species levels. In the terrestrial orchid *Cypripedium*, high mycobiont specificity occurs at generic level (Shefferson et al. 2005). Of the seven species of *Cypripedium* examined, five species had mycobionts belonging to Tulansnellaceae (Shefferson et al. 2005). *Dactylorhiza majalis* predominantly associated with Tulasnellaceae showed

occasional colonization by members of the genus *Laccaria* (Kristiansen et al. 2001). Irrespective of the nutritional mode, genus level specificity was seen in three varieties of non-photosynthetic orchid *Hexalectris spicata* with Sebacinaceae, whereas, species-specific mycobiont specificity was also reported in *Hexalectris* (Taylor and Bruns 1999). The mycorrhizal fungi may limit orchid distribution if they had genus or species specific for certain mycorrhizal fungi.

Fungal specificity is also influenced by certain factors like growth conditions, variety, and life stage of orchids. The fungi that can associate with an orchid in natural habitat may not necessarily germinate orchid seeds *in vitro* (Masuhara and Katsuya 1994). The fungal specificity in natural conditions is termed as ecological specificity and *in vitro* as potential specificity. Fungi may also differ in their ability to grow under different ecological conditions. A study on the tropical orchids, *Tolumnia variegata* and *I. utricularioides,* showed that orchid species with overlapping habitat preferences may differ in mycorrhizal specificity which could influence their distribution (Otero et al. 2004). The varied mycorrhizal diversity and specificity among the different taxa of *Lecanorchis* could be due to the diverse climatic conditions in which they exist (Okayama et al. 2012). Therefore, the patchy distribution of orchids in nature may be due to the presence or absence of the specific mycorrhizal fungi essential for their survival.

The mycorrhizal fungi colonizing the protocorm and the adult roots might not be the same. For example, Milligan and Williams (1988) found that *Epulorhiza* sp., initiated germination and *E. repens* was seen associated in the later stages of *Microtis parviflora.* Pre-infection with one fungus appears not to preclude colonization of a second endophyte. Even though adult *Tipularia discolor* had multiple fungal partners at the adult stage, its protocorm stage is more fungal specific associating with only taxa belonging to Tulasnellaceae (McCormick et al. 2004). But in *Liparis liliifolia*, both protocorms and adult plants fail to associate with multiple mycobionts (McCormick et al. 2012). Bidartondo and Read (2008) hypothesized from their studies on *Cephalanthera* and *Epipactis* that the mycobiont specificity is high during early stages of seedling development compared to more promiscuous germination and mature stages of plants life cycle. Isolation of four distinct types of fungi from *H. spicata* var. *spicata* and *H. spicata* var. a*rizonica* gives evidence for the contribution of mycobiont specificity to the evolutionary diversification in orchids (Taylor et al. 2003). But the orchid *Drakaea* which is widely distributed in different environments, in spite of its preference for *Tulasnella* sp. (Phillips et al. 2011), raises the question of host-fungus specificity within the Orchidaceae which has been a point of contention for many years.

13.9 Importance of OMF in Conservation and Restoration of Orchids

Threat to the survival of orchids in nature is alarming, in spite of the significant advances in our understanding of the ecology of orchids (Gale et al. 2018). It is important to note that only 3.3% of the global natural orchid flora has been subjected to IUCN Red List assessments and 56.5% of these are under one or the other

threat categories (Govaerts et al. 2017; IUCN 2017). Some of the common threats to natural populations of orchids include deforestation, expanding agricultural and forest plantations, and unsustainable exploitation of plants for food, medicine, and ornamental purposes (Gale et al. 2018). Reintroduction of orchids into their natural habitats is a strategy often adopted in the conservation of the threatened species (Vallee et al. 1997). Although orchid seedlings from asymbiotic process have been transferred to ex vitro conditions, they rarely survive due to the biological and ecological specificities of the taxa. The long term survival of orchids in managed or restricted habitats require the presence of appropriate fungi for seedling recruitment and plant nutritional support (Zettler and Piskin 2011).

Asymbiotically raised economically important endangered orchid *Vanda coerulea* exhibited a high mortality (>90%) when introduced into their natural habitat (Aggarwal et al. 2012). But, a successful protocol for the reintroduction of *V. coerulea* seedlings into their natural habitat was developed using *Rhizoctonia* isolated from the same orchid. Anderson (1991) found a 100% survival for all the symbiotic seedlings of *Spiranthes magnicamporum* upon their transfer to soil compared to the 5% survival for the seedlings raised asymbiotically (Anderson 1991). Successful application of symbiotic seed germination using *Ceratobasidium* for reintroduction of endangered terrestrial orchid, *Dactylorhiza hatagirea* has been reported (Aggarwal and Zettler 2010). The reintroduction of endangered orchids, *S. brevilabris* and *H. repens* was highly successful when mycorrhizal with *Epulorhiza* sp. (Stewart et al. 2003). So the symbiotic technique has practical merit for the conservation of highly important and endangered orchids.

Ecological changes at designated orchid locales may destroy the target mycorrhizal species and the compatible fungi may not be present at the site if the orchid itself is not present. So the symbiotically associated seedlings can serve as both plant material and a source of mycorrhizal inoculum for reintroduction efforts. Reintroduction of orchid seedlings from different habitats could be detrimental as this could alter the gene pool of the resident orchids which could eventually initiate anthesis (Zettler et al. 2005). In a recent study, the success of the out planted *in vitro* raised *Cypripedium calceolus* seedlings in the conservation programme in England was shown to be dependent on establishment of mycorrhizal symbiosis by the introduced plants and the presence of suitable mycorrhizal fungi at the introduced sites (Fay et al. 2018).

13.10 Conclusion

OMF colonize orchids and make the orchids survive better in various habitats. Terrestrial orchids show high diversity of OMF fungi, likewise epiphytic orchids are colonized by diverse groups of fungi. OMF is a crucial factor in the reintroduction of orchids and restoration of orchid populations in their natural habitats. So, identification of OMF diversity at specific sites provides the potential OMF which stimulates seed germination and seedling recruitment. This should be used for orchid conservation program in particular habitats.

References

Aggarwal S, Zettler LW (2010) Reintroduction of an endangered terrestrial orchid *Dactylorhiza hatagirea* (D. Don) Soo, assisted by symbiotic seed germination: first report from the Indian subcontinent. Nat Sci 8:139–145

Aggarwal S, Nirmala C, Beri S, Rastogi S, Adholeya S (2012) *In vitro* symbiotic seed germination and molecular characterization of associated endophytic fungi in a commercially important and endangered Indian orchid *Vanda coerulea* Griff. ex Lindl. Eur J Environ Sci 2:33–42

Alconero R (1969) Mycorrhizal synthesis and pathology of *Rhizoctonia solani* in *Vanilla* orchid roots. Phytopathology 59:426–430

Alexander I (2007) A knight of symbiosis. New Phytol 176:499–510

Alexander C, Hadley G (1984) The effect of mycorrhizal infection of *Goodreya repens* and its control by fungicide. New Phytol 97:391–400

Alexander C, Hadley G (1985) Carbon movement between host and mycorrhizal endophyte during the development of the orchid *Goodyera repens* Br. New Phytol 101:657–665

Andersen TP, Stalpers JA (1994) A check-list of *Rhizoctonia* epithets. Mycotaxon 51:437–457

Anderson AB (1991) Symbiotic and asymbiotic germination and growth of *Spiranthes magnicamporum* (Orchidaceae). Lindleyana 6:183–186

Arditti J (1967) Factors affecting the germination of orchid seeds. Bot Rev 33:1–96

Arditti J (1984) An history of orchid hybridization, seed germination and tissue culture. Bot J Linn Soc 89:359–381

Arditti J (1992) Fundamentals of orchid biology. Wiley, New York

Athipunyakom PL, Manoch L, Piluek C, Artjariyasripong G, Tragulrung S (2004a) Mycorrhizal fungi from *Spathoglottis plicata* and the use of these fungi to germinate seeds of *S. plicata in vitro*. Kaset J Nat Sci 38:83–93

Athipunyakom P, Manoch L, Piluek C (2004b) Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Kasetsart J (Nat Sci) 38:216–228

Barroso J, Chaves Neves H, Pair MS (1986) Production of indole-3-ethanol and indole-3-acetic acid by the mycorrhizal fungus of *Ophrys lutea* (Orchidaceae). New Phytol 103:745–749

Bascompte J, Jordano P, Melian CJ, Olesen JM (2003) The nested assembly of plant-animal mutualistic networks. Proc Nat Aca Sci USA 100:9383–9387

Baskin CC, Baskin JA (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Australia Academic Press, Bowen Hills

Batty AL, Brundrett MC, Dixon KW, Sivasithamparam K (2006) *In situ* symbiotic seed germination and propagation of terrestrial orchid seedlings for establishment at field sites. Aus J Bot 54:375–381

Bayman P, Otero JT (2006) Microbial endophytes of orchid roots. In: Schulz B, Boyle C, Sieber TN (eds) Microbial root endophytes, soil biology, vol. 9, part II. Springer, Berlin, pp 153–177

Bayman P, Lebron L, Tremblay R, Lodge J (1997) Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). New Phytol 135:143–149

Bayman P, Mosquera-Espinosa AT, Porras-Alfaro A (2011) Mycorrhizal relationships of *Vanilla* and prospects for biocontrol of root rots. In: Havkin-Frenkel D, Belanger FC (eds) Handbook of *Vanilla* science and technology. Blackwell, West Sussex, pp 266–280

Beltrán-Nambo M, Martínez-Trujillo M, Montero-Castro JC, Salgado-Garciglia R, Otero-Ospina JT, Carreón-Abud Y (2018) Fungal diversity in the roots of four epiphytic orchids endemic to Southwest Mexico is related to the breadth of plant distribution. Rhizosphere 7:49–56

Bermudes D, Benzing DH (1989) Fungi in neotropical epiphyte roots. Biosystems 23:65–73

Bernard N (1899) Sur la germination du *Neottia nidus-avis*. C R Hebd Seances Acad Sci 128:1253–1255

Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Mol Ecol 17:3707–3716

Bidartondo MI, Birghardt B, Gebauer G, Bruns TD, Read DJ (2004) Changing partners in the dark; isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc R Soc Lond B 271:1799–1806

Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of homobasidiomycetes. Syst Biodivers 3:113–157

Boddington M, Dearnaley JDW (2008) Morphological and molecular identification of fungal endophytes from roots of *Dendrobium speciosum*. Proc R Soc Queensl 144:13–17

Bonnardeaux Y, Brundrett M, Batty A, Dixon K, Koch J, Sivasithamparam K (2007) Diversity of mycorrhizal fungi in terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. Mycol Res 111:51–61

Bougoure JJ, Dearnaley JDW (2005) The fungal endophytes of *Dipodium variegatum* (Orchidaceae). Australas Mycol 24:15–19

Bougoure JJ, Bougoure DS, Cairney JWG, Dearnaley JDW (2005) ITS-RFLP and sequence analysis of endophytes from *Acianthus*, *Caladenia* and *Pterostylis* (Orchidaceae) in southeastern Queensland. Mycol Res 109:452–460

Bruns TD (2001) ITS reality. Inoculum 52:2–3

Burgeff H (1959) Mycorrhiza of orchids. In: Withner CL (ed) The orchids: a scientific survey. Ronald Press, New York, pp 361–395

Burns J, Benson M (2000) Biocontrol of damping-off of *Catharanthus roseus* caused by *Pythium ultimum* with *Trichoderma virens* and binucleate *Rhizoctonia* fungi. Plant Dis 84:644–648

Cameron DD, Leake JR, Read DJ (2006) Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfer in the green leaved terrestrial orchid *Goodyera repens*. New Phytol 171:405–416

Cameron DD, Johnson I, Leake JR, Read DJ (2007) Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. Ann Bot 99:831–834

Carling DE (1996) Grouping in *Rhizoctonia solani* by hyphal anastomosis. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology, and disease control. Kluwer Academic, Dordrecht, pp 37–47

Carling DE, Pope EJ, Brainard KA, Carter DA (1999) Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. Phytopathology 89:942–946

Cha JY, Igarashi T (1996) *Armillaria jezoensis*, a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido. Mycoscience 37:21–24

Chang DCN (2008) Research and application of orchid mycorrhiza in Taiwan. Acta Hortic 766:299–305

Chang DCN, Chou LC (2001) Seed germination of *Haemaria discolour* var. *dawsoniana* and the use of mycorrhizae. Symbiosis 30:29–40

Chang DCN, Chou LC (2007) Growth responses, enzyme activities, and component changes as influenced by *Rhizoctonia* orchid mycorrhiza on *Anoectochilus formosanus* Hayata. Botany 48:446–451

Chen W, Hoy JW, Schncider RW (1992) Species specific polymorphism in transcribed ribosomal DNA of five *Pythium* species. Exp Mycol 16:22–34

Chung KR, Shilts T, Ertürk UM, Timmer LW, Ueng PP (2003) Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and post bloom fruit drop of citrus. FEMS Microbiol Lett 226:23–30

Chutima R, Dell B, Vessabutr S, Bussaban B, Lumyong S (2011) Endophytic fungi from *Pecteilis susannae* (L.) Rafin (Orchidaceae), a threatened terrestrial orchid in Thailand. Mycorrhiza 21:221–229

Clements MA (1988) Orchid mycorrhizal associations. Lindleyana 3:73–86

Cozzolino S, Widmer A (2005) Orchid diversity: an evolutionary consequence of deception? Trends Ecol Evol 20:487–494

Currah RS, Sherburne R (1992) Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycol Res 96:583–587

Currah RS, Siegler L, Hamilton S (1987) New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta. Can J Bot 65:2473–2482

Currah RS, Smreciu A, Hambleton S (1990) Mycorrhizae and mycorrhizal fungi of boreal species of *Plantanthera* and *Coeloglossum* (Orchidaceae). Can J Bot 68:1171–1181
Currah RS, Zelmer CD, Hambleton S, Richardson KA (1995) Fungi from orchid mycorrhizas. In: Arditti J, Pridgeon AM (eds) Orchid biology: reviews and perspectives. Kluwer, Dordrecht, pp 117–170
Curtis TJ (1939) The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. Am J Bot 26:390–399
Damaj M, Jabaji-hare S, Charest PM (1993) Isozyme variation and genetic relatedness in binucleate *Rhizoctonia* species. Phytopathology 83:864–871
Dan Y, Meng ZX, Guo SX (2012a) Effects of forty strains of orchidaceae mycorrhizal fungion growth of protocorms and plantlets of *Dendrobium candidum* and *D. nobile*. Afr J Microbiol Res 6:34–39
Dan Y, Yu XM, Guo SX, Meng ZX (2012b) Effects of forty-two strains of orchid mycorrhizal fungi on growth of plantlets of *Anoectochilus roxburghii*. Afr J Microbiol Res 6:1411–1416
De Candolle AP (1815) Me'moire sur les rhizoctones, nouveau genre de champignons qui attaque lesracines des plantes et en particulier celle de laLuzerne cultive'e. Mem Mus Hist Nat 2:209–216
Dearnaley JDW (2006) The fungal endophytes of *Erythrorchis cassythoides*-is this orchid saprophytic or parasitic? Australas Mycol 25:51–57
Dearnaley JDW (2007) Further advances in orchid mycorrhizal research. Mycorrhiza 17:475–486
Dearnaley JD, Cameron DD (2017) Nitrogen transport in the orchid mycorrhizal symbiosis further evidence for a mutualistic association. New Phytol 213:10–12
Decruse SW, Neethu RS, Pradeep NS (2018) Seed germination and seedling growth promoted by a Ceratobasidiaceae clone in *Vanda thwaitesii* Hook. f., an endangered orchid species endemic to South Western Ghats, India and Sri Lanka. S Afr J Bot 116:222–229
Dijk E, Eck N (1995) Axenic *in vitro* nitrogen and phosphorus responses of some Dutch marsh orchids. New Phytol 131:353–359
Dixon KW (1987). Raising terrestrial orchids from seed. In: Harris WK (ed) Modern orchid growing for pleasure and profit. Orchid Club of S. Australia, Inc, Adelaide, pp 47–100
Dong F, Zhao JN, Liu HX (2008) Effects of fungal elicitors on the growth of the tissue culture of *Cymbidium goeringii*. North Hortic 5:194–196
Downing JL, Liu H, Shaoe S, Wange X, McCormick M, Deng R, Gao J (2017) Contrasting changes in biotic interactions of orchid populations subject to conservation introduction vs. conventional translocation in tropical China. Biol Conserv 212:29–38
Dressler RL (1990) The Spiranthoideae: grade or subfamily? Lindleyana 5:110–116
Dressler RL (1993) Phylogeny and classification of the orchid family. Dioscorides Press, Portland
Fan L, Guo S, Cao W, Xiao P, Xu J, Fan L, Guo SX, Cao WQ, Xiao PG, Xu JT (1996) Isolation, culture, identification and biological activity of *Mycena orchidicola* sp. nov. in *Cymbidium sinense* (Orchidaceae). Acta Mycol Sin 15:251–255
Fang D, Hong LX, Hui J, Yi LB (2008) Symbiosis between fungi and the hybrid *Cymbidium* and its mycorrhizal microstructures. For Stud China 10:41–44
Fay MF, Feustel M, Newlands C, Gebauer G (2018) Inferring the mycorrhizal status of introduced plants of *Cypripedium calceolus* (Orchidaceae) in northern England using stable isotope analysis. Bot J Linn Soc 186:587–590
Fracchia S, Rickert AA, Rothen C, Sede S (2016) Associated fungi, symbiotic germination and *in vitro* seedling development of the rare Andean terrestrial orchid *Chloraea riojana*. Flora 224:106–111
Gale SW, Fischer GA, Cribb PJ, Fay MF (2018) Orchid conservation: bridging the gap between science and practice. Bot J Linn Soc 186:425–434
Garcia VG, Onco MAP, Susan VR (2006) Biology and systematics of the form genus *Rhizoctonia*. Span J Agric Res 4:55–79
Gardes M, Bruns TD (1991) Rapid characterization of ectomycorrhizae using RFLP pattern of their PCR amplified- ITS. Mycol Soc Newsl 41:14

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes- application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gebauer G, Meyer M (2003) ^{15}N and ^{13}C natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytol 160:209–223

Gill DE (1989) Fruiting failure, pollinator inefficiency, and speciation in orchids. In: Otte D, Endler JA (eds) Speciation and its consequences. Sinauer Associates, Inc, Sunderland, pp 458–481

Girlanda M, Selosse MA, Cafasso D, Brilli F, Delfine S, Fabbian R, Ghignone S, Pinelli P, Segreto R, Loreto F (2006) Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. Mol Ecol 15:491–504

González V, Salazar O, Julián MC, Acero J, Portal MA, Muñoz R, López-Córcoles H, Gómez-Acebo E, López-Fuster P, Rubio V (2002) *Ceratobasidium albasitensis,* a new *Rhizoctonia*-like fungus isolated in Spain. Persoonia 17:601–614

González-Chávez MDCA, Torres-Cruz TJ, Sánchez SA, Carrillo-González R, Carrillo-López LM, Porras-Alfaro A (2018) Microscopic characterization of orchid mycorrhizal fungi: *Scleroderma* as a putative novel orchid mycorrhizal fungus of *Vanilla* in different crop systems. Mycorrhiza 28:147–157

Govaerts R, Bernet P, Kratochvil K, Gerlach G, Carr G, Alrich P, Pridgeon AM, Pfahl J, Campacci MA, Holland Baptista D, Tigges H, Shaw J, Cribb PJ, George A, Kreuz K, Wood JJ (2017) World checklist of Orchidaceae. The Board of Trustees of the Royal Botanic Gardens, Kew

Graham RR, Dearnaley JDW (2012) The rare Australian epiphytic orchid *Sarcochilus weinthalii* associates with a single species of *Ceratobasidium*. Fungal Divers 54:31–37

Griesbach RJ (2000) Potted *Phalaenopsis* Orchid Production. ASHS–2000 symposium: potted orchid production in the new millennium

Guo SX, Xu JT (1990) Isolation and culture of fungi promoting seed germination of Shihu etc. medicinal plants of orchid family (Orchidaceae). Chin Tradit Herb Drug 21:30–31

Gutierrez-Miceli FA, Ayora-Talavera T, Abud-Archila M, Salvador-Figueroa M, Adriano-Anaya L, Arias-Hernandez ML, Dendooven L (2008) Acclimatization of micropropagated orchid *Guarianthe skinnerii* inoculated with *Trichoderma harzianum*. Asian J Plant Sci 7:327–330

Hadley G (1970) Non-specificity of symbiotic infection in orchid mycorrhiza. New Phytol 69:1015–1023

Hadley G (1982) Orchid mycorrhizal. In: Arditi J (ed) Orchid biology reviews and perspectives II. Cornell University Press, Ithaca, pp 85–118

Hadley G (1984) Uptake of (^{14}C) glucose by asymbiotic and mycorrhizal orchid protocorms. New Phytol 96:263–273

Hadley G (1989) Host-fungus relationships in orchid mycorrhizal systems. In: Pritchard HW (ed) Modern methods in orchid conservation: the role of physiology, ecology and management. Cambridge University Press, Cambridge, pp 5–71

Hadley G, Purves S (1974) Movement of ^{14}carbon from host to fungus in orchid mycorrhiza. New Phytol 73:475–482

Hahn MG (1996) Microbial elicitors and their receptors in plants. Annu Rev Phytopathol 34:387–412

Herrera P, Kottke I, Molina MC, Mendez M, Suarez JP (2018) Generalism in the interaction of Tulasnellaceae mycobionts with orchids characterizes a biodiversity hotspot in the tropical Andes of southern Ecuador. Mycoscience 59:38–48

Hietala AM, Vahala J, Hantula J (2001) Molecular evidence suggests that *Ceratobasidium bicorne* has an anamorph known as a conifer pathogen. Mycol Res 105:555–562

Hossain MM, Rahi P, Gulati A, Sharma M (2013) Improved *ex vitro* survival of asymbiotically raised seedlings of *Cymbidium* using mycorrhizal fungi isolated from distant orchid taxa. Sci Hortic 159:109–112

Ichielevich-Auster M, Sneh B, Koltin Y, Barash I (1985) Pathogenecity, host specificity and anastomosis groups of *Rhizoctonia* spp. isolated from soils in Israel. Phytoparasitica 13:103–112

Illyes Z, Rudnoy S, Bratek Z (2005) Aspects of *in situ, in vitro* germination and mycorrhizal partners of *Liparis loeselii*. Acta Biol Szeged 49:137–139

Irawati I (1993) Orchid mycorrhiza in *Taeniophyllum obtusum* L. J Biol Indones 1:6–16
Irwin MJ, Bougoure JJ, Dearnaley JDW (2007) *Pterostylis nutans* (Orchidaceae) has a specific association with two *Ceratobasidium* root-associated fungi across its range in eastern Australia. Mycoscience 48:231–239
IUCN (2017) The IUCN red list of threatened species. Version 2017-2. Published on the internet at www.iucnredlist.org. Retrieved on 19 Oct 2018
Jabaji-Hare S (1996) Biochemical methods. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic, Dordrecht, pp 65–71
Jacquemyn H, Honnay O, Pailler T (2007) Range size variation, nestedness and species turnover of orchid species along an altitudinal gradient on Réunion Island: implications for conservation. Biol Conserv 136:388–397
Jin H, Xv ZX, Chen JH, Han SF, Ge S, Luo YB (2009a) Interaction between tissue-cultured seedlings of *Dendrobium officinale* and mycorrhizal fungus (*Epulorhiza* sp.) during symbiotic culture. Chin J Plant Ecol 33:433–441
Jin WJ, Li CJ, Nan ZB (2009b) Biological and physiological characteristics of *Neotyphodium* endophyte symbiotic with *Festuca sinensis*. Mycosystema 28:363–369
Johnson TR, Stewart SL, Dutra D, Kane ME, Richardson L (2007) Asymbiotic and symbiotic seed germination of *Eulophia alta* (Orchidaceae) – preliminary evidence for the symbiotic culture advantage. Plant Cell Tissue Organ Cult 90:313–323
Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA (2005) Mixotrophyin orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. New Phytol 166:639–653
Kennedy AH, Taylor DL, Watoson LE (2011) Mycorrhizal specificity in the fully mycoheterotrophic *Hexalectris* Raf. (Orchidaceae: Epidendroideae). Mol Ecol 20:1303–1316
Khamchatra N, Dixon K, Chayamarit K, Apisitwanich S, Tantiwiwat S (2016) Using *in situ* seed baiting technique to isolate and identify endophytic and mycorrhizal fungi from seeds of a threatened epiphytic orchid, *Dendrobium friedericksianum* Rchb.f. (Orchidaceae). Agric Nat Resour 50:8–13
Khan SR, Kimbrough JW (1982) A reevaluation of the basidiomycetes based upon septal and basidial structures. Mycotaxon 15:103–120
Knudson L (1922) Symbiosis and asymbiosis relative to orchids. New Phytol 26:328–336
Kristiansen KA, Taylor DL, Kjøller R, Rasmussen HN, Rosendahl S (2001) Identification of mycorrhizalfungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Mol Ecol 10:2089–2093
Kristiansen KA, Freudenstein JV, Rasmussen FN, Rasmussen HN (2004) Molecular identification of mycorrhizal fungi in *Neuwiedia veratrifolia* (Orchidaceae). Mol Phylogenet Evol 33:251–258
Kull T (2002) Population dynamics of north temperate orchids. In: Kull T, Arditti J (eds) Orchid biology: reviews and perspectives VIII. Kluwer Academic, Dordrecht, pp 139–165
Lan J, Xu JT, Li JS (1994) Study on symbiotic relation between *Gastrodia elata* and *Armillariella mellea* by autoradiography. Acta Mycol Sin 13:219–222
Lan J, Xu J, Li J (1996) Study on the infecting process of *Mycena osmundicola* on *Gastrodia elata* by autoradiography. Acta Mycol Sin 15:197–200
Leake JR (1994) The biology of mycoheterotrophic (saprotrophic) plants. New Phytol 127:171–216
Leake JR (2005) Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the saprophytic plant myth. Mycologist 19:113–122
Lee SB, Taylor JW (1992) Phylogeny of five fungus-like protoctistan *Phytophton* species, inferred from the internal transcribed spacers of ribosomal DNA. Mol Biol Evol 9:636–653
Lee SS, You JY (2000) Identification of the orchid mycorrhizal fungi isolated from the roots of Korean native orchid. Mycobiology 28:17–26

Lee SS, Oh CH, Paek KY, Lee TS (1998) Isolation of the orchid mycorrhizal fungi from the roots of the Korean native orchid and inoculations of the isolates to four different orchids. Korean J Plant Pathol 14:536–542

Lee JK, Lee SS, Eom AH, Paek KY (2003) Interactions of newly isolated orchid mycorrhizal fungi with Korean *Cymbidium kanran* hybrid 'Chungsu'. Mycobiology 31:151–156

Liau CH, Lu JC, Prasad V, Hsiao HH, You SJ, Lee JT, Yang NS, Huang HE, Feng TY, Chen WH, Chan MT (2003) The sweet pepper ferredoxin-like protein (*pflp*) conferred resistance against soft rot disease in *Oncidium* orchid. Transgenic Res 12:329–336

Liu H, Luo Y, Liu H (2010) Studies of mycorrhizal fungi of Chinese orchids and their role in orchid conservation in China – a review. Bot Rev 76:241–262

Lozano FD, Herbada DG, Rivero LM, Saiz JCM, Ollero HS (1996) Threatened plants in peninsular and Balearic Spain: a report based on the EU Habitats Directive. Biol Conserv 76:123–133

Ma M, Koon T, Wong SM (2003) Identification and molecular phylogenyof *Epulorhiza* isolates from tropical orchids. Mycol Res 107:1041–1049

MacNish GC, Carling DE, Brainard KA (1993) Characterization of *Rhizoctonia solani* AG-8 from bare patches by pectic isozyme (zymograms) and anastomosis techniques. Phytopathology 83:922–927

Marchisio VF, Berta G, Fontana A, Mannina FM (1985) Endophytes of wild orchids native to Italy: their morphology, caryology, ultrastructure and cytochemical characterization. New Phytol 100:623–641

Masuhara G, Katsuya K (1989) Effects of mycorrhizal fungi on seed germination and early growth of three Japanese terrestrial orchids. Sci Hortic 37:331–337

Masuhara G, Katsuya K (1994) *In situ* and *in vitro* specificity between *Rhizoctonia* spp. and *Spiranthes sinensis* (Persoon) Ames. var. *amoena* (M. Bieberstein) Hara (Orchidaceae). New Phytol 127:711–718

Masuhara G, Katsuya K, Yamaguchi K (1993) Potential for symbiosis of *Rhizoctonia solani* and binucleate *Rhizoctonia* with seeds of *Spiranthes sinensis* var. *amoena in vitro*. Mycol Res 97:746–752

McCormick MK, Whigham DF, O'Neil J (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytol 163:425–438

McCormick MK, Taylor LD, Juhaszova K, Burnett RK, Whigham DF, O'Neill JP (2012) Limitations on orchid recruitment: not a simple picture. Mol Ecol 21:1511–1523

McKendrick SL, Leake JR, Taylor DL, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of *Corallorhiza trifida* and characterization of its mycorrhizal fungi. New Phytol 145:523–537

Milligan J, Williams PG (1988) The mycorrhizal relationships and multinucleate rhizoctonias from non-orchids with *Microtis* (Orchidaceae). New Phytol 108:205–209

Moncalvo JM, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, Porter TM, Margaritescu S, Weiß M, Garnica S, Danell E, Langer G, Langer E, Larsson E, Larsson KH, Vilgalys R (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia 98:937–948

Moore RT (1987) The genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia*, *Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis*, and *Rhizoctonia*. Mycotaxon 29:91–99

Mosquera-Espinosa AT, Prado BP, Gómez-Carabalí GA, Otero JT (2013) The double life of *Ceratobasidium*: orchid mycorrhizal fungi and their potential for biocontrol of *Rhizoctonia solani* sheath blight of rice. Mycologia 105:141–150

Mújica EB, Mably JJ, Skarha SM, Corey LL, Richardson LW, Danaher MW, González EH, Zettler LW (2018) A comparision of ghost orchid (*Dendrophylax lindenii*) habitats in Florida and Cuba, with particular reference to seedling recruitment and mycorrhizal fungi. Bot J Linn Soc 186:572–586

Muthukumar T, Kowsalya A (2017) Comparative anatomy of aerial and substrate roots of *Acampe praemorsa* (Rox.) Blatt. & McCann. Flora 226:17–28

Muthukumar T, Sathiyadash K (2009) Mycorrhizal morphology of Nun's orchid [*Phaius tankervilliae* (Banks ex L' Herit.) Blume]. Mycorrhiza News 21:9–11

Muthukumar T, Shenbagam M (2018) Vegetative anatomy of the orchid *Bulbophyllum sterile* (Orchidaceae, Epidendroideae). Lankesteriana 18:13–22

Muthukumar T, Uma E, Karthikeyan A, Sathiyadash K, Jaison S, Priyadharsini P, Chongtham I, Muniappan V (2011) Morphology, anatomy and mycorrhizae in subterranean partsof *Zeuxine gracilis* (Orchidaceae). An Biol 33:127–134

Nontachaiyapoom S, Sasirat S, Manoch L (2010) Isolation and identification of *Rhizoctonia*-like fungi from roots of three orchid genera, *Paphiopedilum*, *Dendrobium* and *Cymbidium* collected in Chiang Rai and Chiang Mai provinces of Thailand. Mycorrhiza 20:459–471

Novotna A, Benítez A, Herrera P, Cruz D, Filipczykova E, Suarez JP (2018) High diversity of root-associated fungi isolated from three epiphytic orchids in southern Ecuador. Mycoscience 59:24–32

Okayama M, Yamato M, Yagame T, Iwase K (2012) Mycorrhizal diversity and specificity in *Lecanorchis* (Orchidaceae). Mycorrhiza 22:545–553

Oliveira VC, Sajo MG (1999) Root anatomy of nine Orchidaceae species. Braz Arch Biol Technol 42:405–413

Otero JT, Ackerman JD, Bayman P (2002) Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. Am J Bot 89:1852–1858

Otero JT, Ackerman JC, Bayman P (2004) Differences in mycorrhizal preferences between two tropical orchids. Mol Ecol 13:2393–2404

Ovando I, Damon A, Ambrosio D, Albores V, Bello R, Adriano L, Salvador M (2005) Isolation of endophytic fungi and their mycorrhizal potential for the tropical epiphytic orchids *Cattleya skinneri*, *C. aurantiaca* and *Brassavola nodosa*. Asian J Plant Sci 4:309–315

Pan RC, Chen JX (1994) Effects of nitrate-nitrogen and ammonium-nitrogen on growth and development in *Cymbidium sinense*. Acta Bot Yunnanica 16:285–290

Pellegrino G, Bellusci F (2009) Molecular identification of mycorrhizal fungi in *Dactylorhiza sambucina* (Orchidaceae). Biologia 64:893–897

Pereira OL, Rollemberg CL, Borges AC, Matsuoka K, Kasuya MCM (2003) *Epulorhiza epiphytica* sp. nov. isolated from mycorrhizal roots of epiphytic orchids in Brazil. Mycoscience 44:153–155

Pereira OL, Kasuya MCM, Borges AC, Araújo EF (2005) Morphological and molecular characterization of mycorrhizal fungi isolated from neotropical orchids in Brazil. Can J Bot 83:54–65

Pereira G, Romero C, Suz LM, Atala C (2014) Essential mycorrhizal partners of the endemic Chilean orchids *Chloraea collicensis* and *C. gavilu*. Flora 209:95–99

Perkins AJ, Masuhara G, McGee PA (1995) Specificity of the associations between *Microtis parviflora* (Orchidaceae) and its mycorrhizal fungi. Aust J Bot 43:85–91

Phillips RD, Barrett MD, Dixon KW, Hopper SD (2011) Do mycorrhizal symbioses cause rarity in orchids? J Ecol 99:858–869

Porembski S, Barthlott W (1988) Velamen radicum micromorphology and classification of Orchidaceae. Nord J Bot 8:117–137

Porras-Alfaro A (2004) Mycorrhizal fungi of *Vanilla*: an integral view of the symbiosis. MS thesis, University of Puerto Rico, Rio Piedras

Porras-Alfaro A, Bayman P (2007) Mycorrhizal fungi of *Vanilla*: diversity, specificity and effects on seed germination and plant growth. Mycologia 99:510–525

Purves S, Hadley G (1975) Movement of carbon compounds between the parents in orchid mycorrhizal. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic, London, pp 175–194

Ramsay RR, Sivasithamparam K, Dixon KW (1987) Anastomosis groups among *Rhizoctonia*-like endophytic fungi in south western Australia *Pterostylis* species (Orchidaceae). Lindleyana 2:161–166

Rasmussen HN (1995) Terrestrial orchids. From seed to mycotrophic plant. Cambridge University Press, Cambridge

Rasmussen HN (2002) Recent developments in the study of orchid mycorrhiza. Plant Soil 244:149–163

Rasmussen HN, Rasmussen FN (1991) Climatic and seasonal regulation of seed plant establishment in *Dactylorhiza majalis* inferred from symbiotic experiments *in vitro*. Lindleyana 6:221–227

Rasmussen HN, Rasmussen FN (2009) Orchid mycorrhiza: implications of a mycophagous life cycle. Oikos 118:334–345

Rasmussen HN, Whigham DF (1998) Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae). Am J Bot 85:829–834

Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80

Roberts P (1999) Rhizoctonia-forming fungi. A taxonomic guide. Royal Botanic Gardens, Kew

Robinson M, Riov J, Sharon A (1998) Indole-3-acetic acid biosynthesis in *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. Appl Environ Microbiol 64:5030–5032

Ruibal MR, Triponez Y, Smith LM, Peakall R, Linde CC (2017) Population structure of an orchid mycorrhizal fungus with genus wide specificity. Sci Rep 7:5613. https://doi.org/10.1038/s41598-017-05855-3

Saha D, Rao AN (2006) Studies on endophytic mycorrhiza of some selected orchids of Arunachal Pradesh – 1. Isolation and identification. Bull Arunachal For Res 22:9–16

Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. Eur J Plant Pathol 131:15–26

Sanford WW, Adanlawo I (1973) Velamen and exodermis characters of West African epiphytic orchids in relation to taxonomic grouping and habitat tolerance. Bot J Linn Soc 66:307–321

Sathiyadash K, Muthukumar T, Uma E, Pandey RR (2012) Mycorrhizal association and morphology in orchids. J Plant Int 7:237–248

Sathiyadash K, Muthukumar T, Bala Murugan S, Sathishkumar R, Uma E, Jaison S, Priyadharsini P (2013) *In vitro* asymbiotic seed germination, mycorrhization and seedling development of *Acampae praemorsa* (Roxb.) Blatt. & McCann, a common South Indian orchid. Asian Pac J Reprod 2:114–118

Sathiyadash K, Muthukumar T, Bala Murugan S, Sathishkumar R, Pandey RR (2014) *In vitro* symbiotic seed germination of South Indian endemic orchid *Coelogyne nervosa*. Mycoscience 55:183–189

Sebastián F, Vanesa S, Eduardo F, Graciela T, Silvana S (2014) Symbiotic seed germination and protocorm development of *Aa achalensis* Schltr., a terrestrial orchid endemic from Argentina. Mycorrhiza 24:35–43

Seifert KA, Samson RA, Dewaard JR, Houbraken J, Levesque CA, Moncalvo JM, Louis-Seize G, Hebert PDN (2007) Prospects for fungus identification using C01 DNA barcodes, with *Penicillium* as a test case. Proc Natl Acad Sci USA 104:3901–3906

Selosse MA, Faccio A, Scappaticci G, Bonfante P (2004) Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. Microb Ecol 47:416–426

Senthilkumar S (2003) Mycorrhizal fungi of endangered orchid species in Kolli, a part of Eastern Ghats, South India. Lankesteriana 7:15–156

Senthilkumar S, Britto SJ, Krishnamurthy KV, Hariharan C (2000) Biochemical analysis of mycorrhizal roots of *Aerides maculosum*. Phytomorphology 50:273–279

Shan XC, Liew ECY, Weatherhead MA, Hodgkiss IJ (2002) Characterisation and taxonomic placement of *Rhizoctonia*-like endophytes from orchid roots. Mycologia 94:230–239

Sharma J, Zettler LW, Sambeek JWV, Ellersieck MR, Starbuck CJ (2003) Symbiotic seed germination and mycorrhizae of federally threatened *Platanthera praeclara* (Orchidaceae). Am Midl Nat 149:104–120

Sheehan TJ (1983) Recent advances in botany, propagation and physiology of orchids. In: Janick J (ed) Horticultural reviews, vol 5. AVI Publishing Company, Westport, pp 279–315

Shefferson RP, Wei M, Kull T, Taylor DL (2005) High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. Mol Ecol 14:613–626

Shefferson RP, Kull T, Tali K (2008) Mycorrhizal interactions of orchids colonizing Estonian mine tailings hills. Am J Bot 95:156–164
Shimura H, Koda Y (2005) Enhanced symbiotic seed germination of *Cypripedium macranthos* var *rebunense* following inoculation after cold treatment. Physiol Plant 123:281–287
Shimura H, Sadamoto M, Matsuura M, Kawahara T, Naito S, Koda Y (2009) Characterization of mycorrhizal fungi isolated from the threatened *Cypripedium macranthos* in a northern island of Japan; two phylogenetically distinct fungi associated with the orchid. Mycorrhiza 19:525–534
Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43
Smith SE (1966) Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytol 65:488–499
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London
Smith CJ (1996) Accumulation of phytoalexins: defence mechanism and stimulus response system. New Phytol 132:1–45
Sneh B, Burpee L, Ogoshi A (1991) Identification of *Rhizoctonia* species. American Phytopathology Society, St. Paul
Sneh B, Yamoah E, Stewart A (2004) Hypovirulent *Rhizoctonia* spp. isolates from New Zealand soils protect radish seedlings against damping-off caused by *R. solani*. N Z Plant Protect 57:54–58
Stark C, Abik WB, Durka W (2009) Fungi from the roots of the common terrestrial orchid *Gymnadenia conopsea*. Mycol Res 113:952–959
Steinfort U, Verdugo G, Besoain X, Cisternas MA (2010) Mycorrhizal association and symbiotic germination of the terrestrial orchid *Bipinnula fimbriata* (Poepp.) Johnst (Orchidaceae). Flora 205:811–817
Stewart SL, Kane ME (2006) Symbiotic seed germination of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. Plant Cell Tissue Organ Cult 86:159–167
Stewart SL, Kane ME (2007) Symbiotic seed germination and evidence for *in vitro* mycobiont specificity in *Spiranthes brevilabris* (Orchidaceae) and its implications for species-level conservation. In Vitro Cell Dev Biol Plant 43:178–186
Stewart SL, Zettler LW (2002) Symbiotic germination of three semi-aquatic rein orchids (*Habenaria repens*, *H. quinquiseta*, *H. macroceratitis*) from Florida. Aquat Bot 72:25–35
Stewart SL, Zettler LW, Minso J, Brown PM (2003) Symbiotic germination and reintroduction of *Spiranthes brevilabris* Lindley, an endangered orchid native to Florida. Selbyana 24:64–70
Stoutamire WP (1996) Seeds and seedlings of *Platanthera leucophaea* (*Orchidaceae*). In: Allen C (ed) Proceedings of the North American native terrestrial orchid-propagation and production conference. National Arboretum, Washington, pp 55–61
Suarez JP, Weiß M, Abele A, Garnica S, Oberwinkler F, Kottke I (2006) Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. Mycol Res 110:1257–1270
Takahashi K, Ogiwara I, Hakoda N (2000) Seed germination of *Habenaria* (*pecteilis*) *radiata* (Orchidaceae: Orchideae) *in vitro*. Lindleyana 15:59–63
Taylor DL, Bruns TD (1997) Independent, specialized invasions of ectomycorrhizal mutualism by two non-photosynthetic orchids. Proc Natl Acad Sci USA 94:4510–4515
Taylor DL, Bruns TD (1999) Population, habitat and genetic correlates of mycorrhizal specialization in the cheating orchids *Corallorhiza maculata* and *C. mertensiana*. Mol Ecol 8:1719–1732
Taylor DL, McCormick MK (2008) Internal transcribedspacer primers and sequences for improved characterization of basidiomycetous orchid mycorrhizas. New Phytol 177:1020–1033
Taylor DL, Bruns TD, Leake JR, Read DJ (2002) Mycorrhizal specificity and function in mycoheterotrophic plants. In: Van der Heijden MGA, Sanders I (eds) Mycorrhizal ecology. Springer, Berlin, pp 375–413
Taylor DL, Bruns TD, Szaro TM, Hodges SA (2003) Divergence in mycorrhizal specialization within *Hexalectris spicata* (orchidaceae), a nonphotosynthetic desert orchid. Am J Bot 90:1168–1179
Taylor DL, Bruns TD, Hodges SA (2004) Evidence for mycorrhizal races in a cheating orchid. Proc R Soc Lond 271:35–43

Uetake Y, Kobayashi K, Ogoshi A (1992) Ultrastructural changes during the symbiotic development of *Spiranthes sinensis* (Orchidaceae) protocorms associated with binucleate *Rhizoctonia* anastomosis group. Mycol Res 96:199–209

Umata H (1995) Seed germination of *Galeola altissima*, an achlorophyllous orchid, with aphyllophorales fungi. Mycoscience 36:369–372

Umata H (1998) A new biological function of shiitake mushroom, *Lentinula edodes*, in a mycoheterotrophic orchid, *Erythrorchis ochobiensis*. Mycoscience 38:355–357

Valadares RBS, Pereira MC, Otero JT, Cardoso EJBN (2011) Orchid mycorrhizal diversity in *Coppensia doniana*, a widespread Oncidiinae from Campos do Jordão-SP, Brazil. Biotropica 44:114–122

Vallee L, Hogbin TLM, Monks L, Makinson B, Matthes M, Rossetto M (1997) Guidelines for the translocation of threatened plants in Australia. Australian Network for Plant Conservation, Canberra

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Vazquez DP, Aizen MA (2003) Null model analyses of specialization in plant–pollinator interactions. Ecology 84:2493–2501

Vilgalys R, Cubeta MA (1994) Molecular systematics and population biology of *Rhizoctonia*. Annu Rev Phytopathol 32:135–155

Vralstad T, Schumacher T, Taylor AFS (2002) Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. New Phytol 153:143–152

Warcup JH (1971) Specificity of mycorrhizal association in some Australian terrestrial orchids. New Phytol 70:41–46

Warcup JH (1973) Symbiotic germination of some Australian terrestrial orchids. New Phytol 72:387–392

Warcup JH (1975) Factors affecting symbiotic germination of orchid seed. In: Sanders FK, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic, London/New York, pp 87–104

Warcup JH, Talbot PHB (1966) Perfect states of some rhizoctonias. Trans Br Mycol Soc 49:427–435

Warcup JH, Talbot PHB (1967) Perfect states of rhizoctonias associated with orchid. New Phytol 66:631–641

Warcup JH, Talbot PHB (1971) Perfect states of rhizoctonias associated with orchids. II. New Phytol 70:35–40

Warcup JA, Talbot PHB (1980) Perfect states of rhizoctonias associated with orchids. III. New Phytol 86:267–272

Warcup JH (1981) The mycorrhizal relationships of Australian orchids. New Phytol 87:371–381

Warcup JH (1991) The Rhizoctonia endophytes of Rhizanthella (Orchidaceae). Mycol Res 95:656–659

Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Gebauer G, Savolainen V, Barraclough TG, Pauw A (2011) The effects of above- and below-ground mutualisms on speciation and coexistence. Am Nat 177:54–68

Watkinson JI (2002) Characterization of two genes, trehalose-6-phosphatesynthase/phosphatase and nucleotide binding protein, shown to be differentially regulated in roots of *Cypripedium parviflorum* var. *pubescens* grown with a mycorrhizal fungus *Thanatephorus pennatus*. PhD thesis submitted to Virginia Polytechnic Institute

Waud M, Busschaert P, Lievens B, Jacquemyn H (2016) Specificity and localized distribution of mycorrhizal fungi in the soil may contribute to co-existence of orchid species. Fungal Ecol 20:155–165

White TJ, Bruns T, Lee S, Taylor J (1990) PCR protocols. In: Innis MA, Gelfand DH, Snisky JJ, White TJ (eds) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic, London, pp 315–322

Whitridge H, Southworth D (2005) Mycorrhizal symbionts of the terrestrial orchid *Cypripedium fasciculatum*. Selbyana 26:328–334

Wu JP, Zheng SZ (1994) Isolation and identification of *Fusarium* sp. from mycorrhizal fungus in *Dendrobium densiflorum* and analyses of its metabolites. J Fudan Univ (Nat Sci) 33:547–552
Wu JY, Qian J, Zheng SZ (2002) A preliminary study on ingredient of secretion from fungi of orchid mycorrhiza. Chin J Appl Ecol 13:845–848
Wu JY, Hu T, Yang SZ, Liu L, Wang Q, Wang T, Li LB (2009) rDNA ITS analysis and preliminary study in the specificity for the symbiotic mycorrhizal fungi of *Cymbidium goeringii* and *C. faberi*. Ecol Sci 28:134–138
Wu J, Ma H, Lu M, Han S, Zhu Y, Jin H, Liang J, Liu L, Xu J (2010) *Rhizoctonia* fungi enhance the growth of the endangered orchid *Cymbidium goeringii*. Botany 88:20–29
Wu PH, Huang DD, Chang DCN (2011) Mycorrhizal symbiosis enhances *Phalaenopsis* orchid's growth and resistance to *Erwinia chrysanthemi*. Afr J Biotechnol 10:10095–10100
Wu J, Ma H, Xu X, Qiao N, Guo S, Liu F, Zhang D, Zhou L (2013) Mycorrhizas alter nitrogen acquisition by the terrestrial orchid *Cymbidium goeringii*. Ann Bot 111:1181–1187
Xing X, Ma X, Deng Z, Chen J, Wu F, Guo S (2013) Specificity and preference of mycorrhizal associations in two species of the genus *Dendrobium* (Orchidaceae). Mycorrhiza 23:317–324
Xu JT (1993) The cultivation of *Gastrdia elata* in China, 1st edn. Beijing Medical College & Peking Union Medical College, Beijing
Xu JT, Mu C (1990) The relation between growth of *Gastrodiaelata* protocorms and fungi. Acta Bot Sin 32:26–31
Yagame T, Yamato M (2008) Isolation and identification of mycorrhizal fungi associated with *Stigmatodactylus sikokianus* (Maxim. ex Makino) Rauschert (Orchidaceae). Mycoscience 49:388–391
Yamato M, Yagame T, Suzuki A, Iwase K (2005) Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). Mycoscience 46:73–77
Yang G, Chen H, Naito S, Ogoshi A, Deng L (2005) First report of AG-A of binucleate *Rhizoctonia* in China, pathogenic to soya, bean, pea and snap bean. J Phytopathol 153:333–336
Yang YL, Lai PF, Jiang SP (2008a) Research development in *Dendrobium officinale*. J Shandong Univ TCM 32:82–85
Yang YL, Liu ZY, Zhu GS (2008b) Study on symbiotic seed germination of *Pleione bulbocodioides* (Franch) Rolfe. Microbiology 35:909–912
Yuan L, Yang ZL, Li SY, Hu H, Huang JL (2010) Mycorrhizal specificity, preference, and plasticity of six slipper orchids from south western China. Mycorrhiza 20:559–568
Zaiqi L, Yin Y (2008) Endophytic fungi and their growth effects on *Dendrobium nobile* Lind. and *Dendrobium loddigesii* Rolfe. Guizhou For Sci Technol 1:28–32
Zelmer CD, Currah RS (1995) *Ceratorhiza pernacatena* and *Epulorhiza calendulina* spp. nov. mycorrhizal fungi of terrestrial orchids. Can J Bot 73:1981–1985
Zelmer CD, Cuthbertson L, Currah RS (1996) Fungi associated with terrestrial orchid mycorrhizas, seeds, and protocorms. Mycoscience 37:439–448
Zettler LW (1997a) Orchid fungal symbiosis and its value in conservation. Mcilvaninea 13:40–45
Zettler LW (1997b) Terrestrial orchid conservation by symbiotic seed germination: techniques and perspectives. Selbyana 18:188–194
Zettler LW, McInnis TM (1993) Symbiotic seed germination and development of *Spiranthes cernua* (L.) L.C. Rich. and *Goodyera pubescens* (Willd.) R. Br. (Orchidaceae: Spiranthoideae). Lindleyana 8:155–162
Zettler LW, Piskin KA (2011) Mycorrhizal fungi from protocorms, seedlings and mature plants of the eastern prairie fringed orchid, *Platanthera leucophaea* (Nutt.) Lindley: a comprehensive list to augment conservation. Am Midl Nat 166:29–39
Zettler LW, Burkhead JC, Marshall JA (1999) Use of a mycorrhizal fungus from *Epidendrum conopseum* to germinate seed of *Encyclia tampensis in vitro*. Lindleyana 14:102–105
Zettler LW, Stewart SL, Bowles ML, Jacobs KA (2001) Mycorrhizal fungi and cold-assisted symbiotic germination of the federally threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nuttall) Lindley. Am Midl Nat 145:168–175

Zettler LW, Sharma J, Rasmussen F (2004) Mycorrhizal diversity. In: Dixon K, Cribb P, Kell S, Barrett R (eds) Orchid conservation. Natural History Publication, Kota Kinabalu, pp 185–203

Zettler LW, Piskin KA, Stewart SL, Hartsock JJ, Bowles ML, Bell TJ (2005) Protocorm mycobionts of the federally threatened eastern prairie fringed orchid, *Platanthera leucophaea* (Nutt.) Lindley, and a technique to prompt leaf elongation in seedlings. Stud Mycol 53:163–171

Zettler LW, Corey LL, Jacks AL, Gruender LT, Lopez AM (2013) *Tulasnella irregularis* (Basidiomycota: Tulasnellaceae) from roots of *Encyclia tampensis* in South Florida, and confirmation of its mycorrhizal significance through symbiotic seed germination. Lankesteriana 13:119–128

Zhang JH, Wang CL, Guo SX, Chen JM, Xiao PG (1999) Studies on the plant hormones produced by 5 species of endophytic fungi isolated from medicinal plants (Orchidaceae). Acta Acad Medicinae Sinicae 21:460–465

Zhang L, Chen J, Lv Y, Gao C, Guo S (2012) *Mycena* sp., a mycorrhizal fungus of the orchid *Dendrobium officinale*. Mycol Prog 11:395–401

Zhang SB, Yang Y, Li J, Qin J, Zhang W, Huang W, Hu H (2018) Physiological diversity of orchids. Plant Divers 40:196–208

Zhao JN, Liu HX (2008) Effects of fungal elicitors on the protocorm of *Cymbidium eburneum*. Ecol Sci 27:134–137

Zimmer K, Hynson NA, Gebauer G, Allen EB, Allen MF, Read DJ (2007) Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyroloids and monotropoids (Ericaceae) and in orchids. New Phytol 175:166–175