The Journal of Animal & Plant Sciences, 25 (3 Suppl. 1) 2015 Special Issue Page: 270-277
ISSN: 1018-7081

DIVERSITY, HOST- AND HABITAT- PREFERENCES ON THE FUNGI COMMUNITIES FROM THE ROOTS OF *CYMBIDIUM* SPP. AT TWO SITES IN CHINA

F. Huang and C. Y. Zhang

Shanghai Landscape and Gardening Research Institute, Shanghai 200232, People's Republic of China
*Corresponding Author E-mail: mayzhang55@163.com

ABSTRACT

Three species of Cymbidium spp., Cymbidium faberi, Cymbidium goeringii, and Cymbidium goeringii var. longibracteatum were collected from two different habitats in southwestern China, including a subtropical forest and a transplanted site nearby. Two hundred and ninety isolates, obtained from the roots of the three orchids, were characterized through both morphological and molecular methods. Among a total of 32 taxa, 8 were classified to the level of species and the other 24 to genus. One comprising 205 isolates belonged to ascomycetes (69%), another of 48 basidiomycetes (16%), and the last of 43 belonged to zygomycetes (15%). This revealed high taxonomic diversity in fungal composition, including typical orchid mycorrhiza of the Tulasnella and several other endophytic fungi of the Pezizales and Helotiales. The compositions of the fungal communities correlated with host identify or preference. Because of their relatively closer phylogenetic distance, the fungi communities between Cymbidium goeringii and Cymbidium goeringii var. longibracteatum shared more taxa than any others, whether the comparisons were within wild plants or transplants. Two different regions significantly affected the root-associated fungi assemblages. Besides the predominant one, the overlap, even between the common hosts at two sites, shared extremely limited taxa. Orchids of Cymbidium spp. from the wild habitat had a higher number and taxa of root-associated fungi besides the Tulasnella sp. than the transplanted orchids. For the purpose of propagation and conservation, both the host species and the spatial structure of orchids deserve attention as important factors influencing the compositions of fungal communities. It might be important to pay attention to mycorrhizal associations when transplanting orchids of Cymbidium spp. from the wild habitats.

Keywords: Cymbidium spp., Orchid mycorrhizal fungi, Tulasnella, Diversity, ITS sequences.

INTRODUCTION

The term "Chinese orchids" (*Cymbidium* spp., Orchidaceae) traditionally refer to some terrestrial species of *Cymbidium* that includes five major species, like Chunlan (*Cymbidium goeringii*) and Huilan (*Cymbidium faberi*) (Chen and Tsi, 1998; Huang *et al*., 2010). Besides the two species, ISSR was applied to detect the relationships between 16 *Cymbidium* species, and thus the genetic distance was closest between *Cymbidium goeringii* and *Cymbidium goeringii* var. *longibracteatum* (Wu *et al*., 2008). As the king of fragrant plants, *Cymbidium* spp. has an extremely high economic and ornamental value (Chen and Tang, 1982). It is famous for its magnificent inflorescence and leaves, its wide array of colors, and fascinating varieties of shapes and size (Huang *et al*., 2010). *Cymbidium* spp. is one of the most popular and significant orchids and has been cultivated in China for more than ten centuries (Wang *et al*., 2009). But the number of Chinese orchids has greatly reduced due to over-exploitation and habitat destruction for economic benefit. In addition, many wild orchids have been transplanted from their native habitats for cultivation in human disturbance regions.

Endophytic fungi have a vital influence on the structure of plant communities, fitness, and ecology (Brundrett, 2006). Fungal endophytes, especially mycorrhizal fungi obtained from orchids, may contribute to the vigor of propagations, including seed germination and seedlings (Yam and Arditti, 2009). Dependency on the fungi that form mycorrhizas with terrestrial orchids may persist into adulthood (Gebauer and Meyer, 2003; Abadie *et al*., 2006). A few studies have researched the differentiation of fungi under changed ecological conditions, and the results show that they differ in ability to accommodate new conditions (McCormick *et al*., 2004; Yuan *et al*., 2010). Despite broad and historic transplanting from the plants' natural habitats in China, there have been relatively few studies of mycorrhizal fungi symbionts in *Cymbidium* spp. when in new environments. Identifying the root-associated fungi and the specificity of the relationships is crucial for understanding how orchids interact with their environment; this will help us determine how susceptible orchids are to habitat alterations and assess how to conserve and propagate endangered orchids (Bougoure*et al*., 2009).

In the present study, twenty individual plants were collected from both a tropical forest and a human disturbance region to screen for root-associated fungi in

three species: *Cymbidium* orchids, *Cymbidium faberi*, *Cymbidium goeringii*, and *Cymbidium goeringii* var. *longibracteatum*. The study attempts to solve the following questions: (1) Do the compositions of fungi from *Cymbidium* spp. show richness and diversity? (2) Do the three different terrestrial orchids have identity or preference for their mycorrhizal associations even in the same habitat? (3) Are the fungal communities of orchids shaped by locality between wild and cultured regions?

MATERIALS AND METHODS

Study Site and Sampling Strategy: The study was carried out in SichuanProvince in China. The three orchid species chosen in December 2012 were *Cymbidium faberi*, *Cymbidium goeringii*, and *Cymbidium goeringii* var. *longibracteatum*. The wild plants were collected from a typical subtropical forest, while the cultured individuals had been transplanted from the forest for two years. The transplant orchids were cultured by local residents at the foot of the mountains near their former habitat. Fungal isolation was performed from the rhizomes of the three kinds of orchids, both wild and cultured.

Isolation of the root-associated fungi: The study used Warcup and Talbot's (1967) isolation method of root-associated fungi with slight modifications. The root samples were cleaned in tap water to remove excess soil and litter, and then thoroughly washed under running tap water. Their surfaces were sterilized by consecutive immersion for 60 seconds in 75% ethanol, 60 seconds in 2.0% sodium hypochlorite, and 30 seconds in 75% ethanol. They were then dried with sterile paper towels and cut into segments of about 2-5 mm long. In total, 960 sections (3 species × 20 individuals × 2 sites × 8 segments) were isolated and cultured. Sets of four segments were each evenly placed in 90 mm Petri dish containing malt extract agar (MEA). 50mg/L Streptomycin sulphate was added to suppress bacterial growth. Petri dishes were sealed, incubated for 5-20 days at 28°C, and examined periodically. When colonies developed, they were separated by morphological appearance and characteristics (Rivera-Ordun˜a, 2010) and transferred to new cultured medium.

DNA extraction, PCR amplification and sequencing: Total DNA was extracted from fresh cultures following the protocol of Guo *et al*. (2000). The internal transcribed spacer (ITS1, 5.8 S and ITS2) regions were amplified using primer pairs ITS1 and ITS4 (White *et al*., 1990). The Gene Bank database of National Center for biotechnology information (NCBI) was used to blast the reference sequences of the isolates. Clustal X software aligned and adjusted all the sequences to optimize their sites (Thompson *et al*., 1997). The phylogenetic tree was performed by the neighbor joining method, after the boot stap of 1000 replications (Saitou and Nei, 1987). If the similarities between the new and original sequences were greater than 97%, they were considered to belong to the same species or species complex; others were identified to genus level (O'Brien *et al*., 2005).

Data analysis

Fungi diversity: Relative frequency (Pi) was calculated as the number of particular taxon divided by the total number of all taxa in each host. Fungal dominance was determined by Camargo's index (1/S), where S represents species richness. A species was defined as dominant if Pi>1/S (Camargo, 1992), where Pi is the relative abundance of the species i, defined as the number of competing species present in the community (Camargo, 1992). The Shannon (Shannon and Weaver, 1949) diversity indices were estimated for each tissue and the total population. The Shannon-Weiner index (H′) was calculated according to the formula: $H' = -\sum_{k}^{i} Pi \times \ln Pi$, where k is the total species number of one plot, and *pi* is the relative abundance of the endophytic fungus species of one host (Pielou, 1966).

Similarity index: To describe the taxonomic affinity of fungi among the various parts of the two hosts, Jaccard's coefficient (JI) was used to measure the similarity between pairs of samples (Arnold *et al*., 2000):

$JI = x/(x+y+z)$

In the above formula, *x* represents the number of species occurring in both samples, *y* represents the number of species restricted to sample 1, and z represents the number of species restricted to sample 2. JI ranges from 0 (no taxa shared) to 1 (all taxa shared).

RESULTS

Composition of root-associated fungi and fungal diversity: A total of 296 fungal colonies were isolated from the roots of the three terrestrial orchids at the two different habitats. ITS sequences were obtained for 86 representative isolates by their morphotypes. The phylogenetic analysis (Fig. 1) for these sequences was 32 taxa, which were classified to 8 species with 24 to genus. One comprising 205 isolates belonging belongs to ascomycetes (69%), another of 48 basidiomycetes (16%), and the last of 43 belonging to zygomycetes (15%). All of the taxa were assigned to 15 orders: Cantharellales, Xylariales, Helotiales, Pezizales, and Mucorales. The dominant species included *Ilyonectria*sp., *Xylaria*sp., *Nemaniadiffusa* and others (Fig. 2). Regardless of sites or hosts, the most dominant species in our study (Pi>10%) were *Mortierella* sp. and *Tulasnella* sp..

Effect of habitat: The fungal communities of *Cymbidium* spp. from the two habitats were differentiated. The wild

orchids had approximately fifty more isolates than the hosts from the other site. In addition, the species abundance of the wild orchid fungi was higher than that from the transplanted site. The dominant species varied between the two habitats, except for *Mortierella* sp. (Fig. 2). The strains of *Nemaniadiffusa* and *Xylaria* sp. were most frequently isolated from the wild orchids and seldom isolated from the transplanted orchids. The wild orchids also showed a higher Shannon index of diversity than the transplants (Table 1). The comparison between the root-associated fungal communities recovered from the different hosts and sites was computed using the Jaccard coefficient for any possible pairs. The values of the Jaccard similarity index of the comparison of hosts in the same regions were more than 0.45, except that between *Cymbidium faberi* and *Cymbidium goeringii* var. *longibracteatum* from the transplanted region. Each overlap between the wild and transplanted plants was lower than 0.45, including the comparisons between the common host species (Table 2).

Effect of host species: Although the Chinese orchids were collected from identical environments, the fungal communities between two plants were different. Regardless of the site, the number of strains of *Cymbidium faberi* exceeded the other two species. The Shannon index of diversity of *Cymbidium faberi* was the lowest, with the highest in *Cymbidium goeringii* var. *longibracteatum*. As the most dominant taxa, *Mortierella* sp. was found from the three host species, while *Tulasnella* sp. was only predominantly found in *Cymbidium faberi* (Fig. 2). With 41% shared taxa, the three species compositions of the two regions showed a little overlap. The common taxa up to 53% between *Cymbidium goeringii* and *Cymbidium goeringii* var. *longibracteatum* were most within comparisons of the two different host plants. The Jaccard similarity index was highest between *Cymbidium goeringii*and and *Cymbidium goeringii* var. *longibracteatum* in the same environment. The overlap was less between the wild orchids and the orchids that experienced human disturbance. The taxa of *Tulasnella* sp. were isolated more frequently from the orchids in the wild than the transplanted plants. *Cymbidium faberi* and *Cymbidium goeringii* var. *longibracteatum* shared the lowest overlap among the comparisons within host species.

Table 1. The root-associated fungi isolated from the three kinds of *orchids* in Chinaand their fungal diversity.

	YH	***YL***	***YJ***	***H***	***L***	**J**
No. of total isolates	62	55	53	47	40	39
Species richness(S)	16	15	17	12	14	14
Camargo's index (1/S)	0.063	0.067	0.059	0.083	0.071	0.071
Shannon index of diversity (H′)	2.397	2.399	2.628	2.170	2.180	2.347

Note: YH-*Cymbidium faberi* in wild, YL-*Cymbidium goeringii* in wild,YJ-*Cymbidium goeringii* var. *longibracteatum*, H-*Cymbidium faberi* in the human disturbance region, L-*Cymbidium goeringii*in the human disturbance region J-*Cymbidium goeringii* var. *longibracteatum*in the human disturbance region. The instead of name were all followed by the tables and figures.

Table 2.Jaccard similarity index calculated for the fungal communities isolated from the different host species and sites.

	YH	***YL***	***YJ***	***H***	***L***	***J***
YH	1	0.63	0.57	0.33	0.20	0.38
YL		1	0.60	0.23	0.26	0.35
YJ			1	0.35	0.24	0.43
H				1	0.3	0.47
L					1	0.59
J						1

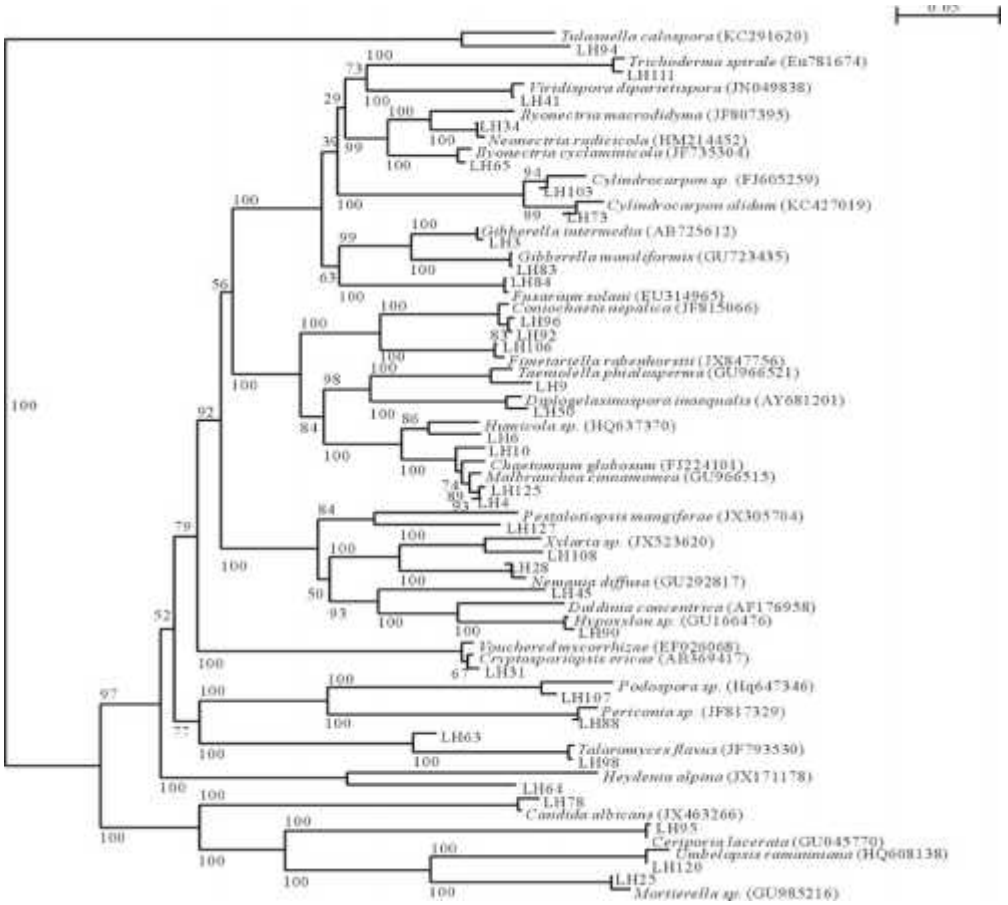

Fig.1 Neighbor-joining tree generated from the ITS 1, 5.8 S gene and ITS 2 sequences showing the relationships of the isolates with reference taxa within the GeneBank database. Bootstrap values greater than 50% (1000 replicates) are shown at branches.

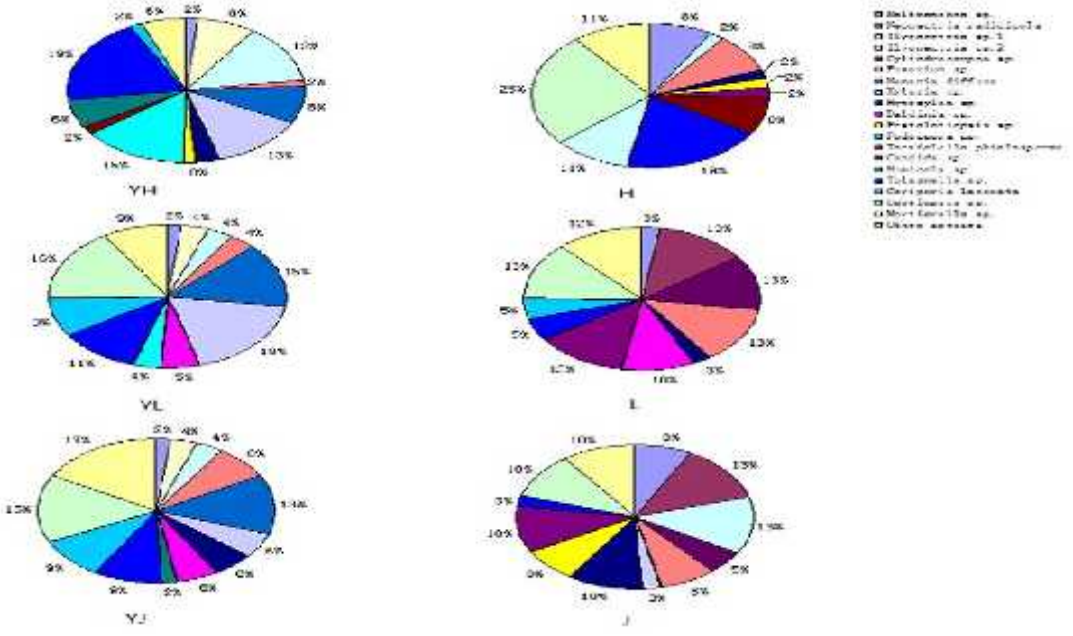

Fig.2 Relative frequencies (Pi) of dominant taxa in the three orchids at two regions.

DISCUSSION

Fungal diversity: In our study, the majority of root-associated fungi from the *Cymbudium* spp. belonged to Ascomycetes, including the orders of Pezizales, Helotiales, Hypocreales, and Xylariales. Previous studies have revealed that the fungi from Pezizales and Helotialeswere also isolated from the orchid roots (Stark *et al*., 2009; Jiang *et al*., 2011). These fungi were to comprise some taxa of the orders interacting as mycorrhizas (Julou *et al*., 2005; Tedersoo *et al*., 2006; Zhang *et al*., 2009). Stark *et al*. thought that the ecological function of the Helotiales was difficult to assess, because they are an ecologically diverse order that includes plant endophytes, both ectomycorrhizal and ericoid fungi, and even plant pathogens (Vralstad *et al*., 2002; Wang *et al*., 2006; Zhang *et al*., 2009).

Fusarium sp. (anamorphic*Gibberella*) within Hypocreales was found among the roots of the three orchids. Many previous studies have claimed that *Fusarium*was recorded as an orchid plant pathogen that could cause root and stem rot, among other problems (Burnett 1975; Benyon *et al*., 1996; Ichikawa and Aoki, 2000; Kim *et al*., 2002). But some authors believed that *Fusarium* was able to promote germination and was beneficial to the host orchid (Rayner, 1927; Vujanovic*et al*., 2000). The possible reason for the argument is that the genus plays a different role in orchids during their growth phase. We suggested that comparable viability techniques should used independently for each orchid species tested. These taxa, *Neonectriaradicicola* (anamorph: *Cylindrocarpondestrustans*) (Jiang *et al*., 2011; Tan *et al*., 2012), *Xylaria*sp. (Tremblay *et al*., 1998; Yuan *et al*., 2009), *Hypoxylon*sp. (Jiang *et al*., 2011), *Nemania*sp. (Jiang *et al*., 2011), *Trichoderma* sp., and *Pestalotiopsis* sp. (Nontachaiyapoom *et al*., 2010), were also found to be associated with the roots of orchids, including Chinese orchids.

On rare occasions, ascomycetous fungi were recovered and reported in Orchidaceae (Jiang *et al*., 2011). The Ascomycetes have been studied less frequently, and so their taxonomy and ecology have not been examined; their importance as mycorrhizas are probably seriously underestimated (Stark *et al*., 2009). Previous studies have identified the orchid mycorrhizas as Basidiomycetes of the *Rhizoctonia* group. The common orchid associated with *Tulasnella* contains many un-described species and some phylogenetically problematic taxa, like *T. calospora,* which require more extensive sequence analysis (Suarez *et al*., 2006). Overall, the main mycobionts found in epiphytic orchids are similar to terrestrial photosynthetic species, including the *Tulasnella* species (Dearnaley and John, 2007). The *Tulasnella* species (anamorph**:***Epulorhiza*) was commonly isolated from terrestrial orchids and may form mycorrhizal associated with the host plant (Pereira *et al*., 2005; Suarez *et al*., 2006; Tan *et al*., 2012). Many mycorrhizal orchid fungi were classified as *Rhizoctonia*-like fungi, including the anamorphic genus *Epulorhiza* and the teleomorph (Rasmussen, 2002; Nontachaiyapoom *et al*., 2010). To our knowledge, *Ceriporia lacerate* within Basidiomycetes is the first time it has been isolated from orchid roots. *Mortierella* sp., belonging to Zygomycetes, have been indentified from the roots of orchids and conformed to the mycorrhizal fungi (Ochora*et al*., 2001; Jiang *et al*., 2011). Only one strain of *Umbelopsis* sp. was isolated in this study and was rarely found in the orchid roots.

Effect of host: From the total of 32 taxa, 11 were widespread and occurred in the different orchids from the natural forest, and nine taxa occurred in the human disturbance site. Regardless of region, ten taxa of fungi were not from the common taxa collected from the three host species, and five dominant fungal taxa were recovered within one of the hosts. The isolates of *Candida* sp. were only found within the root segments of *Cymbidium goeringii* var. *longibracteatum*. Root-associated fungi of *Cymbidium* spp. have been shown to be strongly influenced by the host species. In addition, the general groups of fungi were common at the two sites. Previous studies have also showed that there were preferences for fungal partners on some orchids (Otero *et al*., 2004; Suarez *et al*., 2006). Cox *et al*. (1997) argued that no identical taxa were shared between *Cypripedium* and *Paphiopedilum*. However, on fungal ITS sequence types in common, some shared fungal taxa were isolated either from the *Cypridium* or the *Paphiopedilum* (Yuan *et al*., 2010). Our study revealed that fungal assemblages between *Cymbidium goeringii*, and *Cymbidium goeringii* var. *longibracteatum* with closer phylogenetic relationships shared more taxa, including the predominate one. For example, the most frequent taxa were up to five, with either shared both *Cymbidium goeringii* and *Cymbidium goeringii* var. *longibracteatum*, or only *Cymbidium faberi*. Our findings support the concept that the phylogenetic breadth of mycorrhizal fungi should be evolutionary traits of some orchids (Shefferson *et al*., 2007).

Effect of Locality: The fungal species compositions of *Cymbidium* spp. were greatly influenced by region. A higher diversity of fungal assemblages was collected from the forest than from the transplanted habitat. The rate of overlapping taxa between the wild and transplant habitats was from only 20% to 43%. Except for the taxa of *Mortiella* sp., the most dominant fungi were completely different. Dearnaley (2007) concluded that fungal-orchids associations are sensitive to environmental stimuli, and suggested some strategies to make adjustments in favor of the plant partner's survivor. Of the two closely related genera of slipper orchids transplanted from the wild to the greenhouse, over 80%

plants of *Paphiopedilum* grew well and flowered normally, while most of plants of *Cypripedium* either died or did not flower (Yuan *et al*., 2010). The authors suggested that the reason for the above phenomenon was that there were no mycobionts available for the plants of *Cypripedium* to thrive or the plants had no plasticity to switch to mycorrhizal fungi in the greenhouse.

Despite historic transplant and cultivation from the wild habitats in China, this study is the first to compare the transplanted plants of mycorrhizal fungi symbionts in *Cymbidium* spp. The wild orchids had a greater number and taxa of mycorrhizal associations than those sampled from the transplant region. The roots of the orchids in wild were preferably associated with mycobionts of the *Tulasnella sp.*, while transplanted orchids had mycorrhizal associations of other tulanelliod taxa. The distributions of fungal taxa influenced by sampling sites differed in their geographic and ecological characteristics, such as the immediate climatic conditions and plant associations and so on (Go¨re and Bucak, 2007; Wu *et al*., 2013). In this study, the transplant site was the site with the close longitude and latitude to the forest in the province of Sichuan. Obvious differences between the two regions were the environmental factors, including humidity, temperature, and rainfall. Besides the climate, the orchids obtained from the forest interacted with the rich species variation, including plants and microorganisms. In contrast, the transplanted species may be affected by human activity, thus potential inoculum is lower. It is meaningful to take some measures for in or ex situ conservation and propagation by mycorrhization.

Acknowledgements: We would like to thank Xiao-jun Yang for sampling the orchids. This study is supported by Shanghai Chenshan Botanical Garden (Grand No. JB110226).

REFERENCES

Abadie, J. C., U. Puttsepp, G. Gebauer, A. Faccio, P. Bonfante and M. A. Selosse (2006) *Cephalantheralongifolia* (Neottieae, Orchidaceae) is mixotrophic: a comparative study between green and non-phytosynthetic individuals. Can. J. Bot. 84: 1462-1477.

Arnold, A. E., Z. Maynard, G. S. Gilbert, P. D. Coley, and T. A. Kursar (2000) Are tropical fungal endophytes hyper diverse? Ecol. Let. 3: 267-274.

Benyon, F., B. A. Summerell and L.W. Burgess (1996) Association of *Fusarium* species with root rot of *Cymbidium* orchids. Aust. Plant. Pathol. 25: 226-228.

Bougoure, J., M. Ludwig, M. Brundrett and P. Grierson (2009) Identity and specificity of the fungi forming mycorrhizas with the rare mycoheterotrophic orchid *Rhizanthellagardneri*. Mycol. Res. 113: 1097-1106.

Brundrett. M. C (2006) Understanding the roles of multifunctional mycorrhizal and endophytic fungi. In: Schulz B, Boyle, C., Sieber. T(eds). Microbial root endophyte. Springer-Verlag, Berlin, pp:281-293.

Burnett, H.C (1975) Diseases Caused by Fungi and Bacteria. Handbook of Orchid Pests and Diseases. 1stEdn., American Orchid Society, Cambridge Massachusetts, pp: 15-36.

Camargo, J.A (1992) Can dominance influence stability in competitive interactions? Oikos.64:605–609.

Chen, S.C.and Z. H. Tsi (1998) The Orchids of China. China Forestry Publishing House, Beijing.

Chen, S.C. and T. Tang (1982) A general review of the orchid flora of China. In: Arditti, J. (Ed.), Orchid Biology: Reviews and Perspectives, II. Cornell University Press, New York, pp. 39–87.

Cox, A.V., A. M. Pridgeon, V. A. Albert and M. W. Chase (1997) Phylogenetics of the slipper orchids (Cypripedioideae, Orchidaceae): nuclear rDNA ITS sequeces. Plant. Syst. Evlo. 208:197-223.

Dearnaley, J.Q.W (2007) Further advances in orchid mycorrhizal research. Mycorrhiza. 17:475-486.

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

Gore, M. and C. Bucak (2007) Geographical and seasonal influences on the distribution of fungal endophytes in Laurusnobilis. Forest. Patho. l37 (4): 281–288.

Guo, L.D., K. D. Hyde and E.C.Y. Liew (2000) Identification of endophytic fungi from Livistonachinensis based on morphology and rDNA sequences. New. Phytol., 147:617–630.

Huang, Y., F. Li and K. S. Chen (2010) Analysis of diversity and relationships among Chinese orchid cultivars using EST-SSR makers. Biochem. Syst. . Ecol.38: 93–102,

Ichikawa, K. and T. Aoki (2000) New leaf spot disease of *Cymbidium* species caused *Fusariumsubglutinans* and *Fusariumproliferatum*. J. Gen. Plant. Pathol. 66:213-218.

Jiang, W. M., G. M. Yang., C. L. Zhang and C. X. Fu (2011) Species composition and molecular ananlysis of symbiotic fungi in roots of *Changnieniaamoena* (Orchidaceae). Afr. J. Microbiol. Res.5(3):222-228.

Julou, T., B. Burghardt, G. Gebauer, D. Berveiller, C. Damesin and M. A. Selosse (2005) Mixotrophy in orchids: insights from a comparative study of

green individuals and nonphotosynthetic individuals of Cephalanther adamasonium. New. Phytol. 166: 639–653.

Kim, W.G., B.D. Lee, W.S. Kim and W.D. Cho (2002) Root rot of moth orchid caused by*Fusarium* species. Plant. Pathol. J.,18:225-227.

McCormick, M.K., D. F. Whigham and J. O'Neill (2004) Mycorrhizal diversity in photosynthetic terrestrial orchids. New. Phytol. 163:425-438.

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

O'Brien, H.E., J. L. Parrent., J. A. Jackson, J. Moncalvo and R. Vilgalys (2005) Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbi.71:5544–5550.

Ochora, J., W. D. Stock, H. P. Linder and L. E. Newton (2001) Symbiotic seed germination in twelve Kenyan orchid species. System. Geogr. Plants.71(2): 585-596.

Otero, J.T., J. D. Ackerman and P. Bayman (2004) Differences in mycorrhizal preferences between two tropical orchids. Mol. Ecol. 13: 2393-2404.

Pereira, O.L., M. C. M. Kasuya, A. C. Borges and D. E. Araujo (2005) Morphological and molecular characterization of mycorrhizal fungi isolated from neotropical orchids in Brazil. Can. J. Bot. 83: 54-65.

Pielou E.C (1966) The measurement of diversity in different types of biological collections. J. Theor. Biol. 13: 131-144.

Rasmussen H.N (2002) Resent developments in the study of orchid mycorrhiza. Plant. Soil. 244:149-163.

Rayner M.C (1927) Mycorrhiza: An accuout of non-pathogenic infection by fungi in vascular plants and bryophytes. New. phytol. (Reprint) 15:51-62.

Rivera-Ordun˜a, F.N., R. A. Suarez-Sanchez, Z. R. Flores-Bustamante, J. N. Gracida-Rodriguez and L. B. Flores-Cotera (2010) Diversity of endophytic fungi of *Taxusglobosa* (Mexican yew). Fungal. Divers. 47:65–74. Saitou N., Nei, M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4): 406–425

Shefferson, R.P., D. L. Taylor, B. M. Wei, S. Garnica, L. McCormick, S. Adams, H. M. Gray, J. W. McFarland, K. Tali, T. Yukawa, T. Kawahara, K. Miyoshi and Y. I. Lee (2007) The evolutionary history of mycorrhizal specificity among lady's slipper orchids. Evolution. 61: 1380-1390.

Stark, C., W. Babik and W. Durka (2009) Fungi from the roots of the common terrestrial orchid *Gymnadeniaconopsea*. Mycol. Res. 113:952-959.

Suárez, J.P., M. Wei, A. Abele, S. Garnica, F. Oberwinkler and I. Kottke (2006) Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. Mycol. Res. 110: 1257-1270.

Tan, X.M., X. M. Chen, C. L. Yang, X. H. Jin,J. L. Cui, J. Chen, S. X. Guo and L. F. Zhao (2012) Isolation and Identification of Endophytic Fungi in Roots of Nine *Holcoglossum* Plants(Orchidaceae) Collected from Yunnan, Guangxi, and Hainan Provinces of China. Curr. Microbiol. 64:140-147.

Tedersoo, L., K. Hansen, B. A. Perry and R. Kjoller (2006) Molecular and morphological diversity of pezizaleanectomycorrhiza. New. Phytol. 170: 581–596.

Thompson, J.D., T. J. Gibson, F. Plewniak, F. Jeanmougin and D. G. Higgins (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids. Res. 25(24):4876–4882.

Tremblay, R.L., J.K. Zimmerman, L. Lebron, P. Bayman and I. Sastre (1998) Host specificity and low reproductive success in the rare endemic Puerto Rican orchid *Lepanthescaritensis*. Biol. Conserv. 85: 297-304.

Vralstad, T., E. Myhre and T. Schumacher (2002) Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. New. Phytol. 155: 131–148.

Vujanovic, V., M. St-arnaud, D. Barabe and G. Thibeault (2000) Viability Testing of Orchid seed and the Promotion of Colouration and Germination. Ann. Bot-London.86:79-86.

Wang, H.Z., Z. X. Wu, J. J. Lu, N. N. Shi, Y. Zhao, Z. T. Zhang and J. J. Liu (2009) Molecular diversity and relationships among *Cymbidium goeringii* cultivars based on inter-simple sequence repeat (ISSR) markers. Genetia. 136: 391-399

Wang, Z., P. R. Johnston, S. Takamatsu, J. W. Spatafora and D. S. Hibbett (2006) Toward a phylogenetic classification of the leotiomycetes based on rDNA data. Mycologia. 98: 1065–1075.

Warcup, J.H. and P. H. B. Talbot (1967) Perfect states of *Rhizoctonias*associated with orchids. New. Phytol. 66:631-641.

White, T.J., T. D. Bruns, S.B. Lee and J. Taylor (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: White TJ (ed) PCR protocols: a guide to methods and applications. Academic, London, pp 315–322.

Wu, L.S., T. Han, W. C. Li, M. Jia, L. M. Xue, K. Rahman and L. P. Qin (2013) Geographic and

tissue influences on endophytic fungal communities of *Taxuschinensis* var. *mairei* in China. Curr. Microbiol.66:40-48.

Wu, Z.X., H. Z. Wang, N. N. Shi and Y. Z. Zhao (2008) The genetic diversity of *Cymbidium* by ISSR. Hereditas (Beijing), 30(5):627-632.

Yam, T.W. and J. Arditti (2009) History of orchid propagation: a mirror of the history of biotechnology. Plant. Biotechnol. Rep. 3:1-56.

Yuan, L., Z. L. Yang, S. Y. Li, H. Hu and J. L. Huang (2010) Mycorrhizal specificity, preference, and plasticity of six slipper orchids from South Western China. Mycorrhiza. 20: 559-568.

Yuan, Z.L., Y.C. Chen and Y. Yang (2009) Diverse non-mycorrhizal fungal endophytes inhabiting an epiphytic, medicinal orchid (*Dendrobiumnobile*): estimation and characterization. World. J. of Microb. Biot. 25: 295-303.

Zhang, C.Y., L. J. Yin and S. L. Dai (2009) Diversity of root-associated fungal endophytes in *Rhododendron fortunei* in subtropical forests of China. Mycorrhiza. 19:417–423.