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Studies of Mycorrhizal Fungi of Chinese Orchids and Their Role in Orchid Conservation in China—A Review

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Abstract China has over 1,200 species of native orchids in nearly 173 genera. About one fourth of native species are of horticultural merit. Some species are of Chinese medicinal value. In fact, the demand on orchid species with high Chinese medicinal values such as *Gastrodia elata*, *Dendrobium offcinale*, along with demands on species of cultural importance, such as those in the genus of *Cymbidium*, is a major factor causing wild populations to diminish and in some cases, drive wild populations to the brink of extinction. These market demands have also driven studies on the role of mycorrhizal fungi in orchid seed germination, seedling and adult growth, and reproduction. Most of these mycorrhizal studies of Chinese orchids, however, are published in Chinese, some in medical journals, and thus overlooked by the mainstream orchid mycorrhizal publications. Yet some of these studies contained interesting discoveries on the nature of the mycorrhizal relationships between orchids and fungi. We present a review of some of these neglected publications. The most important discovery comes from the mycorrhizal studies on *G. elata*, in which the researchers concluded that those fungi species required to stimulate seed germination are different from those that facilitate the growth of *G. elata* beyond seedling stages. In addition, presence of the mycorrhizal fungi associated with vegetative growth of post-seedling *G. elata* hindered the germination of seeds. These phenomena were unreported prior to these studies. Furthermore, orchid mycorrhizal studies in China differ from the mainstream orchid studies in that many epiphytic species (in the genus of *Dendrobium*, as medicinal herbs) were investigated as well as terrestrial orchids (mostly in the genus *Cymbidium*, as traditional horticultural species). The different responses between epiphytic and terrestrial orchid seeds to fungi derived from roots suggest that epiphytic orchids may have a more general mycorrhizal relationship with fungi than do terrestrial orchid species during the seed germination stage. To date, orchid mycorrhizal research in China has had a strongly commercial purpose. We suggest that this continuing research on orchid mycorrhizal relationships are a solid foundation for further research that includes more rare and endangered taxa, and more in-situ studies to assist conservation and restoration of the endangered orchids. Knowledge on the identities and roles of mycorrhizal fungi of orchids holds one of the keys to successful restoration and sustainable use of Chinese orchids.

Keywords Biodiversity conservation · Non-timber forest products · Orchid mycorrhiza · Plant-fungus interactions · Restoration ecology · Sustainable harvest

Introduction

The Orchidaceae is one of the largest families with close to 25,000 species (Cribb et al., 2003), roughly one tenth of all flowering plants. China is considered to have over 1,200 species of native orchids in nearly 173 genera (Chen, 1996). About one fourth of native species are of horticultural merit. Some species also have high medicinal value, such as *Gastrodia elata*, *Dendrobium offcinale, Ludisia discolor* (Luo et al., 2003a). In recent years, the orchid industry and natural medicine industry developed rapidly and more and more orchid species have been introduced into commercial trade. Many wild orchids are threatened or endangered due to over collection or orchid habitat destruction by human-related factors. Protection, restoration and sustainable use of orchids are an urgent task facing us today. Efforts to propagate orchids from seeds on nutrient media have been largely successfully for some orchid species (Luo et al., 2003b). Up to now only a few native species have been widely cultivated in China, e.g. *Gastrodia elata*, *Anoectochilus roxburghii* as well as the Chinese traditional *Cymbidium* species. Seed germination and seedling development of many of horticultural and medicinal orchids, especially endangered native terrestrial orchids, are still impossible (Luo et al., 2003a).

Orchids are myco-heterotropic. Most orchids are photosynthetic at maturity and therefore their dependency on mycorrhizal fungi may be reduced once photosynthetic ability is gained. However, more than 100 species of orchid are achlorophyllous, which means that they are completely dependent on their fungal partners throughout their lifetime (Leake, 1994, 2005; Bidartondo, 2005). The best known example is *Gastrodia elata* which we will discuss in detail later. Thus, to successfully conserve orchids like *G. elata* and restore orchid habitats, a pressing need arises to isolate, screen, and preserve compatible mycorrhizal fungi (Zettler, 1997). Based on a comprehensive understanding of orchid mycorrhiza, it should be possible to employ orchid mycorrhizal fungi to develop artificial propagation, and reintroduction of selected endangered species to the wild habitats (Luo et al., 2003a).

In this paper, we primarily focused on mycorrhizal studies of Chinese orchids, most of which are published in Chinese, many are in Chinese medicinal research journals and thus are overlooked by the mainstream English-based orchid mycorrhizal literature. We placed these Chinese studies in the context of current knowledge presented in the mainstream literatures to determine what these Chinese studies can contribute to our understanding of orchid mycorrhiza in general. We then suggest ways to extend studies of orchid mycorrhiza to serve orchid conservation in China.

The History of Orchid Mycorrhiza Research in China

In China, the study of orchid mycorrhiza started on *G. elata* in the 1960s, prompted by the complete collapse of wild *G. elata* populations on the national scale after the unsustainable harvest (more than 100 tons/year nation-wide) in the 1950s (Chinese Medicinal Material, INC. 1995; Jingtang Xu, Chinese Academy of Medicine, pers. comm.). Studies on *G. elata* set the Chinese national pattern on orchid mycorrhizal studies, that is, studies were driven, until recently, entirely by needs for commercial cultivation of Chinese medicinal orchid species, mostly in the genus *Dendrobium*, and of Chinese traditional horticultural species, mostly in the genus of *Cymbidium*.

A Case Study of *Gastrodia elata* and its Mycorrhizal Fungi

Gastrodia elata is a prominent traditional Chinese herbal medicine that has been used for over 2,000 years. It is used in the treatment of headache, vertigo, hemiplegia and infantile convulsions (Xu & Guo, 2000). *Gastrodia elata* is a holomycotrophic perennial herb. For more than 2,000 years, this medicinal herb was obtained only from wild habitats because knowledge on how to propagate the plants was lacking. Kusano (1911) reported the symbiosis between *G. elata* and *Armillaria mellea*, but he was not able to cultivate *G. elata* (Xu & Dixon, 2006). In 1965, the research group headed by Jintang Xu of the Chinese Academy of Medicine was the first to successfully use the *A. mellea* infected timber to cultivate *G. elata*. He then initiated a method of clonal propagation for large scale production by dividing a large tuber into many small parts and each at least with a bud (Xu & Guo, 2000). Since then, many studies of *G. elata* have focused on the sexual reproduction, the life cycle of *G. elata*, and the relationship with the purported symbiont, *Armillaria mellea* (e.g. Xu et al., 1980; Xu & Guo, 1989). These studies were undertaken in order to eliminate degeneration of *G. elata* tuber and reduction of yield associated with multiple re-generation via vegetative propagation.

After numerous failed attempts to germinate *G. elata* seeds symbiotically with *A. mellea*, Xu and colleagues eventually found that seeds of *G. elata* successfully germinated on fallen leaves of *Quercus* spp. (Fagaceae) in the field without *A. mellea* (Xu & Guo, 2000). Afterwards they isolated 12 kinds of fungal strains from the protocorms that developed from seeds of *G. elata* sown on leaves of an oak (*Quercus* sp.) under laboratory conditions. Seed germination rates under controlled conditions were close to 80% after inoculation with one of the isolated fungal strains, which was later identified as *Mycena osmundicola* (Xu et al., 1981; Xu & Guo, 1989, 1991). Several other studies also found *M. dendrobii* isolated from the roots of *Dendrobium offcinale*, *M. anoectchila* from *Anoectochilus roxburghii* and fungi in six other genera were able to stimulate seed germination of *G. elata* effectively (Fan & Guo, 1999; Fan et al., 2001) (Table 1). Careful experiments showed that seeds of *G. elata* sprouted when they were able to obtain nutrition by

Table 1 Isolations, Cultivation and Effects of Mycorrhizal Fungi of Chinese Orchid Species

Orchid Species	Habit	Use/Status[a]	Number of tested[b] (effective) fungus spp.	Fungi source species[c]	Effects[d]			Effective fungi	Reference
					SG	SDLG	VG		
Aerides rosea	Epiphyte	Wild/CR	2	*Aerides rosea*				N/A	Fan et al., 1998
Anoectochilus roxburghii	Terrestrial	Med/NT	21 (4)	*Anoectochilus roxburghii*		+		*Chromosporium sp. Epulorhiza sp. Gliocladium sp. Mycena anoectochila*	Guo et al., 2000a, b
			3 (3)	*Anoectochilus roxburghii*; *Cymbidium sinese*; *Dendrobium officinale*		+		*Mycena anoectochila; M. orchidicola; M. dendrobii*	Guo & Guo, 2002
Bletilla striata	Terrestrial	Med/VU	1 (0)	*Anoectochilus roxburghii*	0			*Mycena anoectochila*	Guo et al., 1997
			1 (0)	*Cymbidium sinense*	0			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*M. dendrobii*	Guo et al., 1999
Bulbophyllum affine	Epiphyte	Wild/VU	1	*Bulbophyllum affine*				*Epulorhiza albertaensis*[e]	Fan et al., 1998
Calanthe triplicate	Terrestrial	Hort/NT	1 (0)	*Anoectochilus roxburghii*	0			*Mycena anoectochila*	Guo et al., 1997
			1 (0)	*Cymbidium sinense*	0			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*M. dendrobii*	Guo et al., 1999
Changnienia amoena	Terrestrial	Wild/EN	1	*Changnienia amoena*				N/A	Zhang et al., 2009
			36	*Changnienia amoena*				*Epulorhiza sp.; Geotrichum sp.*[e]	Yan et al., 2006
Cymbidium eburneum	Epiphyte	Hort/EN	3 (2)	*Cymbidium goeringii*			+	*Alternaria sp.; Chaetomium sp.*	Zhao & Liu, 2008
			1 (1)	*Dendrobium sinens*			+	*Fusarium sp.*	Zhao & Liu, 2008
Cymbidium elegans	Epiphyte	Hort/EN	1	*Cymbidium elegans*				N/A	Fan et al., 1998
Cymbidium ensifolium	Epiphyte	Hort/VU	2	*Cymbidium ensifolium*				*Epulorhiza albertaensis*[e]	Fan et al., 1998
	Epiphyte	Hort/VU	4	*Cymbidium ensifolium*				N/A	Hu et al., 2008
Cymbidium faberi	Terrestrial	Hort/VU	1	*Cymbidium faberi*				N/A	Hu et al., 2008
			5	*Cymbidium faberi*				Unknown[e]	Jin et al., 2006
Cymbidium floribundum	Terrestrial	Hort/VU	1	*Cymbidium floribundum*				N/A	Hu et al., 2008

Cymbidium goeringii	Terrestrial	Hort/VU	5 (5)	*Cymbidium faberi*			+	Unknown	Jin et al., 2006
			19 (3)	*Cymbidium goeringii*		+		unknown	Wu et al., 2007
			3 (3)	*Cymbidium goeringii*		+		*Rhizoctonia sp.*; Unknown	Wu et al., 2005
			94	*Cymbidium goeringii*				N/A	Wu et al., 2006
			1 (1)	*Cymbidium goeringii*		+		Unknown	Jin et al., 2006
			4 (4)	*Cymbidium goeringii*			+	Unknown	Dong et al., 2008
			6 (6)	*Cymbidium goeringii*		+		Unknown	Yuan et al., 2008
			21	*Cymbidium goeringii*				N/A	Yu et al., 2009
			8	*Cymbidium goeringii*				N/A	Wu et al., 2009
			5	*Cymbidium goeringii*				N/A	Hu et al., 2008
			4 (4)	*Cymbidium sinense*		+		*Trichoderma sp.*; Unknown	Huang et al., 2004
Cymbidium hookerianum	Terrestrial	Hort/VU	15	*Cymbidium hookerianum*				N/A	Wu et al., 2006
Cymbidium kanran	Terrestrial	Hort/VU	1	*Cymbidium kanra*				N/A	Fan et al., 1998
Cymbidium lowianum	Terrestrial	Hort/EA	1	*Cymbidium lowianum*				N/A	Hu et al., 2008
Cymbidium sinense	Terrestrial	Hort/VU	1 (0)	*Anoectochilus roxburghii*	0			*Mycena anoectochila*	Guo et al., 1997
			45	*Cymbidium sinense*				N/A	Wu et al., 2006
			3	*Cymbidium sinense*				N/A	Hu et al., 2008
			1 (0)	*Cymbidium sinense*	0			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*M. dendrobii*	Guo et al., 1999
Cymbidium tracyanum	Semi-terrestrial	Hort/VU	1	*Cymbidium tracyanum*				N/A	Fan et al., 1998
Dendrobium bryerianum	Epiphyte	Med/EN	1 (0)	*Anoectochilus roxburghii*	0			*Mycena anoectochila*	Guo et al., 1997
			21(6)	*Anoectochilus roxburghii*	+			*Cephalosporium sp. Epulorhiza sp. Gliocladium sp. Mycena anoectochila Mycena sp. Rhizoctonia sp.*	Guo et al., 2000a, b
			1 (1)	*Bulbophyllum affine*	+			*Epulorhiza albertaensis*	Fan et al., 1998
			1 (1)	*Cymbidium ensifolium*	+			*Epulorhiza albertaensis*	Fan et al., 1998

Table 1 (continued)

Orchid Species	Habit	Use/Status[a]	Number of tested[b] (effective) fungus spp.	Fungi source species[c]	Effects[d]			Effective fungi	Reference
					SG	SDLG	VG		
			1 (1)	*Cymbidium sinense*	+			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*Mycena dendrobii*	Guo et al., 1999
			1 (1)	*Vanda brunnea;& Vanda coerulea*	+			*Epulorhiza albertaensis*	Fan et al., 1998
			1 (1)	*Vanilla annamica*	+			*Epulorhiza sp.*	Fan et al., 1998
Dendrobium chrysotoxum	Epiphyte	Med/EN	1 (1)	*Anoectochilus roxburghii*	+			*Mycena anoectochila*	Guo et al., 1997
			1 (1)	*Cymbidium sinense*	+			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*Mycena dendrobii*	Guo et al., 1999
			2 (2)	*Eria szetschuanica*	+			*Epulorhiza albertaensis*; Unknown	Fan et al., 1998
			1 (1)	*Vanilla annamica*	+			*Epulorhiza sp.*	Fan et al., 1998
Dendrobium densiflorum	Epiphyte	Med/EN	1 (1)	*Anoectochilus roxburghii*	+			*Mycena anoectochila*	Guo et al., 1997
			1 (1)	*Cymbidium sinense*	+			*Mycena orchidicola*	Fan et al., 1996
			1	*Dendrobium densiflorum*				N/A	Fan et al., 1998
			1 (1)	*Dendrobium densiflorum*		+		*Fusarium sp.*	Wu & Zheng, 1994
			1 (1)	*Dendrobium officinale*	+			*M. dendrobii*	Guo et al., 1999
Dendrobium devonianum	Epiphyte	Med/EN	7	*Dendrobium devonianum*				N/A	Wu et al., 2006
Dendrobium hancockii	Epiphyte	Med/EN	2	*Dendrobium hancockii*				*Epulorhiza anaticula*[e]	Fan et al., 1998
			1 (1)	*Dendrobium hancockii (protocorms)*	+			unknown	Guo & Xu, 1990b
			2 (1)	*Liparis nervoa (protocorms)*	+			unknown	Guo & Xu, 1990b
			4 (2)	*Gastrodia elata(protocorms)*	+			*Mycena osmundicola*; Unknown	Guo & Xu, 1990b
Dendrobium huoshanense	Epiphyte	Med/CR	1	*Dendrobium huoshanense*				N/A	Guo et al., 2008
Dendrobium loddigesii	Epiphyte	Med/EN	2	*Dendrobium loddigesii*				N/A	Luo et al., 2008

Dendrobium lohohens	Epiphyte	Med/EN	3 (3)	*Liparis nervosa* (protocorms), *Dendobium hancokii* (protocorms),*Gastrodia elata*(protocorms)	+			*Microascus sp. Chaetomium sp.; Mycena osmundicola*	Guo & Xu, 1991
Dendrobium longicrnu	Epiphyte	Med/EN	8	*Dendrobium longicrnu*				N/A	Wu et al., 2006
Dendrobium nobile	Epiphyte	Med/EN	11 (1)	*Dendrobium nobile,*		+		*Gloiocladium sp.*	Guo et al., 2000a, b
			14 (3)	*Dendrobium officinale*		+		*Cephalosporium sp.; Epulorhiza sp.; Mycena dendrobii*	Guo et al., 2000a, b
			8	*Dendrobium nobile*				N/A	Luo et al., 2008
Dendrobium officinale	Epiphyte	Med/CR	14 (2)	*Dendrobium officinale,*	+			*Mycena dendrobii Epulorhiza sp.*	Guo et al., 2000a, b
			11 (3)	*Dendrobium nobile*	+			*Rhizoctonia sp. Mycena orchidicola Cliocladium sp.*	Guo et al., 2000a, b
			23 (3)	*Dendrobium officinale,D. nobile*		+		*Mycena dendrobii; Epulorhiza sp. Cliocladium sp.*	Guo et al., 2000a, b
			1 (0)	*Dendrobium officinale*	0			*Mycena dendrobii*[e]	Guo et al., 1999
			1	*Dendrobium officinale*				N/A	Fan et al., 1998
			5 (5)	*Cymbidium ensifolium;C. sinensis*			+	Unknown	Pan et al., 2004
			2 (2)	*Dendrobium officinale*			+	*Mycena sp.*; Unknown	Chen et al., 2008
			3 (3)	*Dendrobium officinale*; *Anoectochilus roxburghii*; *c sinese*		+		*Mycena anoectochila; Mycena orchidicola; Mycena dendrobii*	Guo & Guo, 2002
			3 (3)	*Liparis nervosa* (protocorms) *Dendobium hancokii* (protocorms),*Gastrodia elata*(protocorms)	+			*Microascus sp.; Chaetomium sp.; Mycena osmundicola*	Guo & Xu, 1991
Dendrobium primulinum	Epiphyte	Med/EN	1 (0)	*Anoectochilus roxburghii*	0			*Mycena anoectochila*	Guo et al., 1997
			1 (1)	*Cymbidium sinense*	+			*Mycena orchidicola*	Fan et al., 1996
			2 (2)	*Dendrobium hancockii*	+			*Epulorhiza anaticula*; Unknown	Fan et al., 1998
			1 (0)	*Dendrobium officinale*	0			*Mycena dendrobii*	Guo et al., 1999
Dendrobium sinense	Epiphyte	Med/EN	16 (3)	*Changnienia amoena*		+		*Epulorhiza sp.; Geotrichum sp.*; Unknown	Yan et al., 2006

Table 1 (continued)

Orchid Species	Habit	Use/Status[a]	Number of tested[b] (effective) fungus spp.	Fungi source species[c]	Effects[d]			Effective fungi	Reference
					SG	SDLG	VG		
			7 (5)	*Dendrobium sinense*			+	Unknown	Wu et al., 2006
Doritis pulcherrima	Terrestrial	VU	5 (5)	*Doritis pulcherrima*		+		Unknown	Ke et al., 2008
Epigeneium rotundatum	Epiphyte	Wild/NT	1 (1)	*Anoectochilus roxburghii*	+			*Mycena anoectochila*	Guo et al., 1997
			1 (0)	*Cymbidium sinense*	0			*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0			*Mycena dendrobii*	Guo et al., 1999
Eria szetschuanica	Epiphyte	Wild/NT	2	*Eria szetschuanica*				*Epulorhiza albertaensis*[e]	Fan et al., 1998
Habenaria dentate	Terrestrial	Med/VU	186	*Habenaria dentate*				N/A	Chen et al., 2008
Gastrodia elata	Saprophyte	Med/VU	1 (1)	*Anoectochilus roxburghii*	+			*Mycena anoectochila*	Guo et al., 1997
			18 (18)	*Gastrodia elata* (protocorms)	+			*Mycena sp.*	Xu et al., 2001
			1 (1)			+		*Armillaria mella*	Xu et al., 1989
			21(4)	*Anoectochilus roxburghii*	+			*Cephalosporium sp. Ceratorhiza sp. Mycena anoectochila Mycena sp.*	Guo et al., 2000a, b
			1 (1)	*Cymbidium sinense*	+			*Mycena orchidicola*	Fan et al., 1996
			2 (2)	*Dendrobium hancockii*	+			*Epulorhiza anaticula*; Unknown	Fan et al., 1998
			1 (1)	*Dendrobium officinale*	+			*Mycena dendrobii*	Guo et al., 1999
			2 (2)	*Eria szetschuanica*	+			*Epulorhiza albertaensis*; Unknown	Fan et al., 1998
			1 (1)	*Liparis nervosa* (protocorms)	+			*Chaetomium sp.*	Guo & Xu, 1991
			12 (1)	*Gastrodia elata* (Protocorms)	+			*Mycena osmundicola*	Xu & Guo, 1989
Liparis nervosa	Epiphyte	Med/VU	1	*Liparis nervosa*				N/A	Fan et al., 1998
			1	*Liparis nervosa* (protocorms)				*Microascus sp.; Chaetomium sp.; Mycena osmundicola*[e]	Guo and Xu, 1991

Otochilus lancilabius	Epiphyte	Wild/VU	1	*Otochilus lancilabius*			N/A	Fan et al., 1998
Paphiopedilum armeniacum	Terrestrial	Hort/EN	13(3)	*Paphiopedilum armeniacum*		+	*Phacodium sp.*	Li & Zhang, 2001
Paphiopedilum micranthum	Terrestrial	Hort/EN	1	*Paphiopedilum micranthum*			N/A	Fan et al., 1998
Phaius tankervillae	Terrestrial	Wild/VU	1 (0)	*Anoectochilus roxburghii*	0		*Mycena anoectochila*	Guo et al., 1997
			1 (1)	*Bulbophyllum affine*	+		*Epulorhiza albertaensis*	Fan et al., 1998
			1 (1)	*Cymbidium ensifolium*	+		*Epulorhiza albertaensis*	Fan et al., 1998
			1 (0)	*Cymbidium sinense*	0		*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0		*Mycena dendrobii*	Guo et al., 1999
			1 (1)	*Vanda brunnea;& Vanda coerulea*	+		*Epulorhiza albertaensis*	Fan et al., 1998
Phalaenopsis wilsonii	Epiphyte	Hort/EN	1	*Phalaenopsis wilsonii*			N/A	Fan et al., 1998
Pleione yunnanensis	Semi-terrestrial	Med/VU	6	*Pleione yunnanensis*			N/A	Zhu et al., 2008
Pleione bulbocodioides	Semi-terrestrial	Med/VU	9(3)	*Pleione bulbocodioides*	+		*Fusarium sp. Trichoderma sp. Paecilomyces sp.*	Yang et al., 2008b
Spathoglottis pubescens	Terrestrial	Hort/VU	1 (0)	*Anoectochilus roxburghii*	0		*Mycena anoectochila*	Guo et al., 1997
			1 (0)	*Cymbidium sinense*	0		*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0		*Mycena dendrobii*	Guo et al., 1999
			4	*Spathoglottis pubescens*			N/A	Fan et al., 1998
Vanda brunnea	Epiphyte	Hort/EN	1	*Vanda brunnea*			*Epulorhiza albertaensis*	Fan et al., 1998
Vanda coerulea	Epiphyte	Hort/EN	1 (0)	*Anoectochilus roxburghii*	0		*Mycena anoectochila*	Guo et al., 1997
			1 (1)	*Bulbophyllum affine*	+		*Epulorhiza albertaensis*	Fan et al., 1998
			1	*Cymbidium ensifolium*	+		*Epulorhiza albertaensis*	Fan et al., 1998
			1 (0)	*Cymbidium sinense*	0		*Mycena orchidicola*	Fan et al., 1996
			1 (0)	*Dendrobium officinale*	0		*Mycena dendrobii*	Guo et al., 1999
			2 (2)	*Eria szetschuanica*	+		*Epulorhiza albertaensis*; Unknown	Fan et al., 1998
			1 (1)	*Vanda coerulea*	+		*Epulorhiza albertaensis*	Fan et al., 1998

Table 1 (continued)

Orchid Species	Habit	Use/Status[a]	Number of tested[b] (effective) fungus spp.	Fungi source species[c]	Effects[d]			Effective fungi	Reference
					SG	SDLG	VG		
Vanda teres	Epiphyte	Hort/–	1	*Vanda teres*				N/A	Fan et al., 1998
Vanilla annamica	Epiphyte	Hort/–	1	*Vanilla annamica*				*Epulorhiza sp.*[e]	Fan et al., 1998

[a] Med = Chinese Medicine, Hort = horticultural plants, Wild = species are not currently used as medicine or horticultural plants. Plant conservation status were derived from Wang and Xie (2004). *CR* critically endangered species, *EN* endangered species, *NT* near threatened species, *VU* vulnerable species, – not listed

[b] When no experiments on effects were carried out, the number in cell indicate the number of isolated species

[c] Unless indicate otherwise, mycorrhizal fungi were isolated from roots

[d] *SG* seed germination; *SDLG* Seedling growth; *VG* Post-seedling vegetative growth;. "+" indicates that the fungi has a positive effect on the parameter tested while "0" indicates no effect was found. *Blank* indicate that no test was carried out on the parameter

[e] Effects of these fungi were tested on other orchid species that were also listed in the Table

digesting fungi like *M. osmundicola* which invaded its proembryo cells. Once a seedling had been established, *G. elata* switched endophytes to digestion of *A. mellea* which had subsequently invaded the adult rhizome (Xu & Guo, 2000).

An important conclusion was drawn from these in depth studies. *Gastrodia elata* had to associate with two different species of mycorrhizal fungi, *M.osmundicola* and *A. mellea*, each at a different stage of the orchid's life cycle (Xu & Guo, 1989). In addition, presence of *A. mellea*, the mycorrizal fungus that facilitated clonal growth of *G. elata*, inhibited seed germination (Ran & Xu, 1988). Later studies found that ten other fungi species in four genera isolated from different species of orchids were also capable of promoting seed germination (Table 1). These important discoveries escaped the attention of English-based mycorrizal literature until the early 21st century (e.g. Rasmussen, 2002; Zettler et al., 2003). Up to now, it is not known how widespread this pattern of mycorrhizal switching is in the orchid family as demonstrated in *G. elata* (Rasmussen & Rasmussen, 2009). The successful mycorrhizal research on *G. elata* not only resolved a method for the artificial generation of *G. elataa* for the Chinese medicinal trade, but also eased the demand on wild *G. elata* populations and provided a potential for natural and/or human-assisted recovery of the wild populations.

Isolation and Screening of Orchid Mycorrhizal Fungi

Isolation and screening of compatible orchid mycorrhizal fungi in China have been largely stimulated by problems encountered during rapid artificial propagation of commercial orchid seedlings of some Chinese *Cymbidium* and lady's slipper orchid species (Table 1). These problems include high mortality of the young seedlings and slow growth after transplanting plantlets from the greenhouse to the field, and difficulties in stimulating blooming in adult plants (Wang et al., 2004; Li, 2006). Isolation of orchid mycorrhizal fungi is the first step in its applications for orchid commercial propagation and orchid conservation.

Besides isolation of orchid mycorrhizal fungi from *G. elata*, most other mycorrhizal research has been conducted on two orchid genera, *Dendrobium* (Wu & Zheng, 1994; Guo et al., 2000; Song & Guo, 2001; Zhu et al., 2007; Liu et al., 2008; Luo et al., 2008; Guo et al., 2008; Chen et al., 2009) and *Cymbidium* (e.g. Fan et al. 1996; Pan et al., 1999; Huang et al., 2004; Wu et al., 2007; Dong et al., 2008; Hu et al., 2008; Yuan et al., 2008; Wu et al., 2009; Yu et al., 2009) (Table 1). Species of *Dendrobium* constitute another important group of Chinese medicinal herbs, whose use as medicine dates back to 2,000 years ago (Bao et al., 2001). Market demand for *Dendrobium* species in China, many of which have showy flowers, is entirely for medicinal purpose, and has exhausted the natural, accessible *Dendrobium* resources in China and recently started to impact the wild populations in neighboring southeastern Asian countries (Bao et al., 2001). *Dendrobium* spp. are typically epiphytic with the major species investigated so far being *D. officinale*. However, despite considerable research into *D. offcinale* (Gao & Guo, 2001; Zhu & Qin, 2004; Xing et al., 2005; Luo et al., 2006; Kang et al., 2007; Yang et al., 2008a; He et al., 2009) mycorrhizal fungi have not yet been applied to the commercial cultivation of *D. offcinale*. Mycorrhizal research on *D. officinale* and other epiphytic

Dendrobium species provides an interesting opportunity to compare the dependency of epiphytic orchids on mycorrhizal fungi with those of terrestrial orchids. The latter have been the focus for the majority of the mainstream orchid mycorrhizal research.

In contrast, species of *Cymbidium* are mostly terrestrial orchids and are of purely horticultural and cultural importance. Cultivation of *Cymbidium* is recorded as far back as 1,000 years ago, and is related to the Chinese gentlemen's cultural (Chen & Tsi, 1998). Up to now, market supply of *Cymbidium* species have relied on wild collected plants.

Isolation Approaches

Generally, methods employed for isolation include plating fragments of surface sterilized roots or protocorms on nutrient media. Orchid mycorrhizal fungi have been studied via the isolation and establishment of pure cultures from colonized root tissue (Warcup, 1981; Currah et al., 1997). These inevitably have problems of contamination and difficulties of screening the actual fungi forming mycorrhiza. Several studies indicated that in order to find the fungal partner of the orchid, it was much more effective to isolate fungi using single pelotons from the root cell (Dixon et al., 1989; Rasmussen, 1995). In addition, molecular methods have the potential to identify fungi at the level of single pelotons (Kristiansen et al., 2001), but this method has not yet been applied to Chinese orchids. Nevertheless, some of mycorrhizal fungi are not able to grow on the media tested. This is a challenge for the application of these fungi to orchid propagation and conservation.

There is evidence that orchids are often colonized by a variety of fungi, some of which may not interact as true mycorrhiza (Warcup, 1981; Bayman et al., 1997). For orchid commercial production and conservation, it is important to screen the compatible mycorrhizal fungi (Luo et al., 2003a). A mycorrhizal fungus should have at least one of the following three characters: (1) capable of stimulating seed germination, (2) improving the growth of protocorms, young seedlings or juvenile plants, and (3) improving the growth and reproduction of adult plants. Most Chinese researchers screened the effective fungi by symbiotic seed germination and inoculating fungi isolated from the roots of adults to orchid seedlings in the lab.

The number of effective fungi, i.e. species that were able to promote seed germination, seedling growth and post-seedling growth, along with that of tested fungus species were summarized in Table 2. We used Analysis of Covariance (ANCOVA) to determine whether the numbers of effective fungi species are statistically different between terrestrial and epiphytic orchids after taking into account the number of fungus species tested (a covariate of the number of effective fungi) on each orchid species. We found that epiphytic orchids had statistically more effective fungi that were able to promote seed germination than do terrestrial orchids (Table 3). In other words, more than 50% of the fungi species tested were able to promote seed germination of epiphytic orchids while only an average of 10% of tested fungi were able to promote germination of terrestrial orchid seeds. In contrast, the number of fungi species that were able to promote seedling growth were not statistically different (Table 4). On average 39% and 50% of the fungi tested were able to help seedlings of epiphytic and terrestrial orchids, respectively. No statistical test was carried out on vegetative growth due to limited data.

Table 2 Number of Mycorrhizal Fungus Species that can Promote Seed Germination, Seedling Growth, and Post-Seedling Vegetative Growth of Terrestrial vs. Epiphytic Orchids

Habit	Species	Number of effective / tested fungus species		
		Seed germination	Seedling growth	Vegetative growth
Terrestrial	*Anoectochilus roxburghii*		6/23	
	Bletilla Striata	0/3		
	Calanthe triplicate	0/3		
	Cymbidium goeringii		17/33	5/5
	Cymbidium sinense	0/3		
	Doritis pulcherrima		5/5	
	Paphiopedilum armeniacum		3/13	
	Phaius tankervillae	1/4		
	Pleione bulbocodioides	3/9		
	Spathoglottis pubescens	0/3		
Epiphytic	*Cymbidium eburneum*			3/4
	Dendrobium bryerianum	9/25		
	Dendrobium chrysotoxum	5/6		
	Dendrobium densiflorum	3/3	1/1	
	Dendrobium hancockii	4/7		
	Dendrobium lohohens	3/3		
	Dendrobium nobile		4/25	
	Dendrobium officinale	8/29	5/25	7/7
	Dendropbium primulinum	3/25		
	Dendrobium sinense		3/16	5/7
	Epigeneium rotundatum	1/3		
	Vanda coerulea	2/5		

Rasmussen (1995) speculated that epiphytic orchids had less dependency on mycorrhizal fungi than do terrestrial orchids. Our review and meta-analyses suggest that seeds of epiphytic orchids do depend on mycorrihzal fungi for germination, but the mycorrhizal relationship is perhaps less specific for epiphytes than for terrestrial

Table 3 Analysis of Covariance Table on the Comparison of Effective Number of Fungi on Seed Germination of Terrestrial vs. Epiphytic orchids

Variation source	Type III SS	df	F	Sig
Corrected model	5.297	2	19.60	0.000
Intercept	1.601	1	11.85	0.005
Number of tested fungus species (square root transformed)	1.799	1	13.31	0.003
Habit (terrestrial vs. epiphytic)	1.287	1	9.52	0.009
Error	1.621	12		
Total	57.000	15		

Table 4 Analysis of Covariance Table on the Comparison of Effective Number of Fungi on Seedling Growth of Terrestrial vs. Epiphytic orchids

Variation source	Type III SS	df	F	Sig
Corrected model	3.257	2	5.37	0.057
Intercept	0.808	1	2.67	0.163
Number of tested fungus species (square root transformed)	1.946	1	6.42	0.052
Habit (terrestrial vs. epiphytic)	0.982	1	3.24	0.132
Error	1.515	5		
Total	52.000	8		

orchids during the seed germination stage. Difference in fungus specificity between terrestrial and epiphytic orchids was much less pronounced at the seedling stage. However, because *Dendrobium* represented 75% of the epiphytic orchids in our analyses, more comparative studies are needed on the fungi requirements for seed germination and seedling growth of other epiphytic genera for a more general conclusion. Such studies should be done using fungi isolated from the same species as well as species of various degree of relatedness to determine specificity in orchid mycorrhiza relations. We concluded that fungus baiting using seeds placed in natural habitats (Rasmussen & Whigham, 1993) is necessary for many species of Chinese terrestrial orchids to obtain mycorrhizal fungi that are capable of stimulating seed germination.

Effects of Fungal Elicitors on Growth of Protocorms and Orchid Seedlings

Fungal elicitors are a group of extractions and mixtures that include fungal organisms, filtrate concentrate, extracts of mycelia and filtrate, soluble components of fungal mycelia with high temperature treatment, hydrolytic products of cell walls, peptides and proteins (Smith, 1996; Hahn, 1996). In the lab, these materials stimulate multiplication of plant cell and secondary metabolite production in orchids (Zeng et al., 2007; Zhang et al., 2008). Experiments using fungal elicitors could shed light on the mechanism of the mycorrhizal relationships between orchids and fungi.

In the cortical cells of orchid mycorrhiza, colonization and hyphal digestion are two dynamic interacting processes. On the one hand, hyphal digestion provides nutrients to orchids, on the other, some components of the digested hypha play the role of fungal elicitors, which facilitate further colonization by fungi. There was evidence that fungal elicitors of orchid mycorrhiza were effective in promoting the development of protocorms and seedling growth of orchids (Guo & Xu, 1991; Guo & Guo, 2001; Pan et al., 2004; Chen et al., 2008; Dong et al., 2008; Zhao & Liu, 2008). Elicitors also could stimulate the protocorms to synthesize disease-resistance related compounds, such as PAL (L-phenylalanin ammo-nialyase) (Hou & Guo, 2004; Luo & Yi, 2007).

Some studies indicated that orchid mycorrhizal fungi could secrete gibberellins (GA_3), heteroauxin (IAA), dormin (ABA), zeatin (ZT) and zeatin riboside (ZR). Some of these hormones were released into the extracellular space, some were not (Wu & Zheng, 1994, Wu et al., 2002). Zhang et al. (1999) extracted plant hormones from mycelia and their culture solutions of five species of orchid mycorrhizal fungi with organic solvent, They found that some of these hormones were released into the extracellular space, some were not. Plant hormones produced by mycorrhizal fungi have been shown to have strong effects in improving the growth of medicinal orchids (Yang et al., 2008a, b). Several studies indicated that plant hormones or fungal metabolic products played an important role in the process of seed germination and cell differentiation of *Gastrodia elata* (Guo & Xu, 1990a; Xu, 1993). Extracts of three fungi species isolated from the protocorms of *Dendrobuim hancokii*, *Liparis nervosa* and *Gastrodia elata* were shown to strongly promote seed germination of *D. hancokii* (Guo & Xu, 1990b).

The roles of fungal elicitors were studied in the growth of medicinal orchids, such as *D. officinale* (Guo & Guo 2001; Song & Guo, 2001; Pan et al., 2004; Wu et al., 2006; Kang et al., 2007; Luo et al., 2008; Chen et al., 2008) and *C. goeringii* (Dong et al., 2008, Zhao & Liu, 2008; Jin et al., 2009). Once the symbiotic relationship was established, the mycorrhizal fungi served as the dominant microorganism to release antagonistic substances. These substances are effective in preventing the invasion of other pathogens and indirectly enhance the survival rate of seedlings and promote the growth of seedlings (Chen et al., 2003). Although the mechanism is still unclear, the effects of fungal elicitors on stimulating seedling growth were confirmed. The use of fungal elicitors in generating symbiotic seedlings for orchid population restorations should be explored further.

Future Studies of Orchid Mycorrhizal Fungi in Relation to Orchid Conservation in China

Unlike other research of wild orchid mycorrhiza, few orchid mycorrhizal studies in China have been carried out in the field, and few data are available on the specificity of Chinese orchids and their fungal partners in natural conditions. In addition, study of the co-evolution between orchid and mycorrhizal fungi is completely lacking. All of these deficiencies hinder the effective conservation of Chinese orchids.

In China, the history of cultivation of orchids is more than 2,000 years old (Chen & Tang, 1982), but up to now many species of orchids were still obtained directly from wild populations. There are two reasons for this. Firstly, it is the limitation of propagating methods of orchids. As mentioned above, artificial propagation of *G. elata* and *D. officinale* were successful in China (Luo et al., 2003a), but the production of other orchids still relied on traditional vegetative propagation. This approach is inefficient and it requires a large number of wild mother plants. In addition, selection of good cultivars mainly depended on the destructive selection from a large number of wild plants. The selection process is destructive because commercial growers typically collect a large number of wild plants during this process and discard the majority of these collections if they are not of novel forms or colors, which are the case with most plants. Many species of *Cymbidium* have been subject to this selection processes in China (Yu, 2004) and wild *Cymbidium* resources have been inevitably greatly damaged. Re-introduction of orchids that have been over-collected to the original habitats is a feasible conservation approach. But it demands a large number of orchid seedlings. The large-scale seedling propagation of orchids could be helpful, but so far only a few species of orchids can be propagated this way successfully in China.

Destruction of habitats is another threat to the survival of Chinese orchids. Developing effective ex situ conservation methods is an essential component for the recovery of orchids that are threatened by habitat loss. Because orchids produce tens of thousands of seeds within each fruit, propagation via seeds has great potential for ex situ conservation. Although some orchids can be germinated asymbiotically, and some asymbiotic seedlings can be transferred successfully in ex-situ conditions, the asymbiotically grown seedlings rarely survive the transplant into natural habitats (Wang et al., 2004). In contrast, with symbiotic fungi, orchid seeds usually germinate faster and with higher rates, and are able to establish healthier seedlings (Zelmer & Currah, 1997; Wei et al., 1998; Ramsay & Dixon, 2003; Yan et al., 2006; Ke et al., 2008).

For orchid restoration, it is better to germinate seeds with its fungal partners and develop symbiotic seedlings because they will be more suitable for transplanting into the natural habitats (Hollick, 2004). Thus, to successfully propagate and restore orchids, identification and introduction of the critically important mycorrhizal symbiont is of primary importance (Zettler, 1997). In recent years, some researchers studied several species of rare and endangered Chinese orchids (Liu et al., 2004, 2008; Li & Luo, 2009; Gao et al., 2009), but few data are available on application of orchid mycorrhizal fungi in those species.

Orchid conservation in China is facing big challenges from over-collecting, habitat destruction and global climate changes (Liu et al., 2010, this volume). We recommend the following areas for future research into orchid mycorrhiza in relation to orchid conservation in China. Firstly, studies of orchid mycorrhiza should include a wider spectrum of species, especially rare and endangered taxa. It is recognized that although some species of orchid do have specific interaction with certain species of fungi for seed germination and seedling growth, others have a more general relationship involving many species of fungi (Arditti et al., 1990; Taylor et al. 2003; Shefferson et al., 2005, 2007; Bidartondo & Read, 2008). Habitat complexity and species diversity of Chinese orchids provide good opportunities for the study of the various aspects of orchid mycorrhiza.

Secondly, commercial cultivation of orchids could benefit from use of orchid mycorrhizal fungi. The roles of orchid mycorrhizal fungi in initiating seed germination and promoting post-germination growth have been demonstrated experimentally for many orchid species (Table 1). Some evidence also indicates that orchid mycorrhizal fungi could promote flowering and shorten the time required for first blooming (Zhang & Zhou, 2004). Symbiotic cultivation of medicinal and horticultural orchids may reduce technical difficulties and costs such that it enables not only large scale cultivation of medicinal orchids, but also encourages farmers in poor areas to participate in cultivation of desirable orchids and as commercially viable enterprise. Both scenarios can, theoretically, ease collecting pressure from wild populations. In China, large number of seedlings of a few selected medicinal orchids can be obtained via tissue culture as mentioned in previous sections. But there are still many obstacles to cultivation (Li, 2006; Ke et al., 2008). Commercial application of orchid mycorrhizal fungi is an important research direction to find a solution to these problems.

Thirdly, study the symbiotic relationship of mycorrhizal fungi and orchids under natural conditions is necessary and would benefit from more detailed, in-situ mycorrhizal fungi baiting with seeds and the adaptability of orchid seedlings to various micro-habitats. Since orchid seeds are dust-like, it is difficult to locate natural seeds in initial stages of germination. With the natural seed germination and mycorrhizal fungus baiting technique (Rasmussen & Whigham, 1993; 1998; Batty et al., 2001; Whigham et al., 2006; Kull et al., 2006), we can now obtain the symbiotic fungi associated with seeds and understand the mechanisms of seed germination in nature. So far there are no observations of in-situ seed germination of Chinese wild orchid species.

In response to threats to orchid species, integrated conservation approaches have been adopted elsewhere, including ex situ and translocation principles (Swarts & Dixon, 2009; Seaton et al., 2010, this volume). The purpose of orchid propagation for a species reintroduction is to eventually rebuild or build self-sustainable populations in the natural and safe sites. Understanding the biological and ecological requirements of orchid seedling survival after the transfer from cultivation to the field is essential for successful orchid reintroduction. This includes the study on the adaptability of symbiotic seedlings to natural conditions.

Finally, research on the distribution of mycorrhizal fungi and orchids is needed. Many studies focused on the relationship between orchids and their fungal partners as mentioned above. Little is known about the impacts of orchid mycorrhizal fungi on the distribution and survival of orchids in the ecosystem. Knowledge of the distribution of orchid mycorrhizal fungi within soil or other substrates is important for attempts to return orchids to the field and in understanding the distribution of orchids. It will also contribute to a better understanding of the reasons of endangered status of certain species of orchids. For example, high host-fungus specificity and low fungal diversity might restrict orchids to certain habitats (Batty et al., 2002). A greater understanding of host-fungus specificity should lead to the successful application of mycorrhizal studies in orchid conservation in China in the near future.

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