IDENTIFICATION OF A MYCORRHIZA ISOLATE FROM PAPHIOPEDILUM BARBATUM (LINDL.) PFITZER FROM EX SITU LOCATION AND DETERMINATION OF ITS GROWTH-ENHANCING CAPABILITIES

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By

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Orchids and mycorrhiza are known to have a symbiotic relationship that function particularly in seed germination and also in promoting overall plant growth. It is believed that a certain orchid mycorrhiza that functions to enhance plant growth also lives symbiotically in the roots of *Paphiopedilum barbatum*. Thus, the objectives of this research are to identify the mycorrhizal fungi that form a symbiosis with the terrestrial tropical orchid, *P. barbatum* and evaluate its capabilities to enhance growth in certain *in vitro Paphiopedilum* seedlings. Mycorrhizal fungi were isolated from the roots of *P. barbatum* collected from five different locations in Peninsular Malaysia. Isolation of fungi was carried out according to the Currah method for initial morphological identification and then through molecular identification using the internal transcribed spacer (ITS) region of nuclear ribosomal DNA of the isolates. The identified

mycorrhiza was then inoculated on *in vitro* selected *Paphiopedilum* seedlings on a double slanting media to observe mycorrhization and the occurrence of enhancement of plant growth. Only one mycorrhiza was isolated and identified; namely *Tulasnella calospora* (anamorph: *Rhizoctonia repens*) which is a ubiquitous orchid mycorrhiza. Phylogenetic analyses with sequences originating from different hosts from different countries indicated a close genetic relatedness of the sequences studied. *In vitro* inoculation of *T. calospora* on seedlings from the *Paphiopedilum* genus after incubation for 24 weeks showed a significant 1.1% increase in fresh weight in *P. rothschildianum* with 68% root mycorrhization. However, no significant growth was observed in the seedlings of *P. sanderianum, P. gigantifolium* x *P. rothschildianum* and *P. esquirolei* x *P. rothschildianum* when inoculated with *T. calospora* after incubation for 24 weeks even though each had 32%, 24% and 13% root mycorrhization. This preliminary finding present an insight into the orchid mycorrhiza of the *Paphiopedilum* but a more extensive study is needed to further explore this symbiotic relationship and furthermore contribute in developing a beneficial application whether commercially or for the conservation of this genus.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN SEJENIS MIKORIZA TERPENCIL DARI *PAPHIOPEDILUM BARBATUM* (LINDL.) PFITZER DARI LOKASI *EX SITU* DAN PENENTUAN KEBOLEHAN UNTUK MENINGKATKAN PERTUMBUHAN

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Orkid dan mikoriza diketahui mempunyai hubungan simbiotik yang berfungsi terutamanya dalam percambahan biji benih dan juga merangsang keseluruhan pertumbuhan pokok. Di percayai bahawa terdapat mikoriza orkid tertentu membantu dalam pertumbuhan pokok yang hidup secara simbiotik di dalam akar *Paphiopedilum barbatum*. Maka, objektif kajian ini adalah untuk mengenalpasti mikoriza yang boleh membina hubungan simbiotik dengan orkid terestrial *P. barbatum* dan menguji kebolehannya merangsangkan pertumbuhan dalam anak pokok tertentu dari genus *Paphiopedilum* secara *in vitro*. Kulat mikoriza telah dipencilkankan dari akar *P. barbatum* yang dikumpulkan dari lima lokasi yang berbeza di Semenanjung Malaysia. Pemencilan telah dilakukan menggunakan kaedah Currah untuk pengenalpastian awal

secara morfologi dan kemudian menggunakan kaedah molekul dengan menggunakan bahagian Internal Transcribed Spacer (ITS) dari DNA ribosom nukleus kulat tersebut. Mikoriza yang telah dikenalpasti itu kemudiannya telah diinokulasi kepada anak pokok *Paphiopedilum* yang terpilih secara *in vitro* di atas media dua sendeng untuk memerhatikan mikorisasi dan kesannya ke atas pertumbuhan. Hanya satu mikoriza iaitu *Tulasnella calospora* (anamorf: *Rhizoctonia repens*) iaitu mikoriza orkid yang am telah dipencilkan dan dikenalpasti. Analisis filogenetik bersama jujukan DNA yang diperolehi daripada perumah yang berbeza dan negara yang berbeza menunjukkan perkaitan genetic yang rapat antara jujukan yang telah diuji. Inokulasi secara *in vitro, T. calospora* ke atas anak pokok dari genus *Paphiopedilum* selepas inkubasi selama 24 minggu menunjukkan peningkatan signifikan dari segi berat basah sebanyak 1.1% bagi *P. rothschildianum* dengan 68% mikorisasi akar. Namun begitu, tiada peningkatan pertumbuhan yang signifikan dapat dilihat bagi anak pokok *P. sanderianum, P. gigantifolium* x *P. rothschildianum* dan *P. esquirolei* x *P. Rothschildianum* yang diinokulasi dengan *T. calospora* selepas diinkubasi selama 24 minggu walaupun masing-masing menunjukkan 32%, 24% dan 13% mikorisasi akar. Pertemuan awal ini memberi maklumat awal tentang mikoriza orkid dalam *Paphiopedilum* tetapi kajian yang lebih menyeluruh diperlukan untuk mendalami hubungan simbiotik ini dan seterusnya menyumbang dalam pembangunan applikasi yang berguna sama ada secara komersial atau bagi tujuan konservasi genus ini.

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LIST OF ABBREVIATIONS

AG	Anastomosis Group
dDH_20	Double distilled water
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
DSE	Dark Septate Endophytes
ECM	Ectomycorrhizas
EDTA	Ethylenediaminetetraacetic acid
HCl	Hydrochloric acid
ITS	Internal Transcribed Spacers
KCl	Potassium chloride
KOH	Potassium Hydroxide
$MgCl_2$	Magnesium chloride
NaCl	Natrium chloride
OM	Orchid Mycorrhiza
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RAPD	Random Amplified Polymorphic DNA
rDNA	Ribosomal Deoxyribonucleic Acid
s.l.	*sensu lato*
TBE	Tris Borate EDTA
TE	Tris-EDTA
VAM	Vesicular Arbuscular Mycorrhizas

CHAPTER 1

INTRODUCTION

The roots of a plant is by far one of the most important structures for plant performance as the roots have direct contact with a substrate whether soil, water or other organic mediums as a source of nutrients. In view of that, roots are affected by a complex array of microorganisms that inhabit a variety of niches with their own function and purpose in the substrate. Generally, these functions include nitrogen fixation, phosphate solubilization, plant growth promotion, and biological control of plant pathogens.

This association between microorganisms and roots can be beneficial (water uptake, soil stabilization, growth promotion, N_2 fixation, biocontrol, antibiosis, symbiosis), harmful (infection, phytoxicity) or neutral (nutrient flux, free enzyme release, attachment, allelopathy, competition); depending on soil conditions (Rao, 1993). However, the most beneficial microorganisms that associate with plants are soil inhabiting fungi that form mutualistic associations with the root of plants, referred to as mycorrhizas (Smith and Read, 1997).

According to Brundrett (2004), mycorrhiza is defined as a symbiotic association essential for one or both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. Mycorrhizas occur in a specialized plant organ such as the roots where intimate contact results from a synchronized plant-fungus development.

This obligate relationship is particularly important in orchids as most orchids whether terrestrial or epiphytic, are known to establish a mycorrhizal association in at least one stage in their life cycle (Harvais and Hadley, 1967; Dearnaley, 2007; Stewart and Kane, 2007) or in the entire life cycle of chlorophyll-deficient species (Rasmussen, 1995).

The distinctiveness of mycorrhiza symbiosis in orchids can be seen in the occurrence of fungal interaction with embryo cells of germinating orchid seeds as well as with the roots of seedlings and mature plants (Peterson *et al.,* 2004). Orchid seeds are fairly minute and lack the nutritional reserves available in other types of seeds. Therefore, in nature, most orchids require the availability of mycorrhizal fungi as a carbon source to initiate seed germination, facilitate protocorm development and seedling growth (Rasmussen, 1995; Rasmussen and Whigham, 2002; Dong *et al*., 2008).

These orchid fungal symbionts are also known to produce cellulases and polyphenol oxidases, enzymes that enable the fungi to digest soil organic matter to simple sugars that may be utilized by both partners in the symbiosis (Peterson *et al.,* 2004). Because of this prevalent and imperative association, the term orchid mycorrhiza (OM) is used exclusively for the mycorrhiza that occurs in orchids.

Most orchid mycorrhizal fungi are Basidiomycetes and belong to the form genus *Rhizoctonia*, a diverse polyphyletic group of pathogens, endophytes, saprophytes and mycorrhizal fungi (Warcup, 1981; Rasmussen, 1995; Currah e*t al*., 1997). Even though these fungi are known to be pathogenic to a variety of plants, they may also form

beneficial symbiotic relationship with orchids, as can be seen by *R. solani,* an active crop plant pathogen. Identification of these fungi is difficult due to the common absence of sexual reproductive structures and inconsistent vegetative characters that are traditionally used in morphological identification.

As most of orchid mycobionts are discovered to be from the *Rhizoctonia s.l.*, certain mycelial characters including the size and shape of moniloid cells, the formation of sclerotia, enzyme activity and particularly the ultrastructures of the septal pore are exclusively used to distinguish the sterile mycelia but only to the generic level (Zelmer and Currah, 1995). Due to this, fungal systematics is rather incoherent with different naming in taxa for the anamorph (fungal form with vegetative structures) and teleomorph (fungal form which produces sexual reproductive structures) from that of the same species (Currah *et al.*, 1997).

The advance of molecular techniques has greatly facilitated the progress of identification of mycorrhizal fungi (McCormick *et al.*, 2004). Research in this field relies mainly on PCR amplification of ribosomal DNA (rDNA) sequences with nuclear encoded small subunit (17S/18S rDNA) being the most common but nuclear encoded rDNA internal transcribed sequences (ITS), mitochondrial small subunit rDNA (16S) and nuclear encoded large subunit (25S rDNA) are also utilized significantly for research purposes (Gardes and Bruns, 1993; Kristiansen *et al.,* 2001; 2004; Otero *et al.,* 2005; Dearnaley, 2007; Giuseppe and Bellusci, 2009; Wright *et al.,* 2010). Examples of use of molecular techniques for mycorrhizal research are the study by Taylor and

McCormick (2008) to characterize basidiomycetous orchid mycorrhizas using internal transcribed spacer primer (ITS), molecular identification of orchid mycorrhizal fungi using mitochondrial ribosomal large subunit DNA (Kristiansen *et al.*, 2004) and a more recent study by Shimura *et al.* (2009) using ITS for phylogenetic analyses. On the other hand, random amplified polymorphic DNAs (RAPDs) and microsatellites have been far less popular as molecular markers for mycorrhizal fungi (Barker and Larkan, 2002) as ITS can provide a higher degree of variation even between closely related species.

The understanding of orchid mycorrhiza remains fragmented despite increase in research over the past four to five decades and vast increase in information (Brundrett, 2002; McCormick *et al.*, 2004). Tropical orchid mycorrhiza in particular has long been neglected by researchers (Dixon *et al.*, 2003) as studies on orchid mycorrhizal fungi have long focused on orchids from temperate regions even though the majority of orchids are tropical (Rasmussen, 1995). Even less studies on orchid mycorrhizal fungi from Southeast Asia have been done with only known studies done by Ma *et al.* (2003), Kristiansen *et al.* (2004) and Athipunyakom *et al.* (2004).

With the worldwide threat of extinction faced by the flora and fauna of the planet, orchids are acknowledged to be among the most severely threatened. Orchid conservation efforts are furthermore complicated due to a complex combination of external and internal factors. The external factors are the commonly referred reasons believed to cause extinction, such as habitat destruction and commercial exploitation

while symbiotic relationships that are essential in the life of an orchid is held to be a more difficult internal factor.

The unique association between orchids and their mycorrhiza is thought to present the foremost limitations for orchid conservation. Orchid conservation practices currently incorporate *ex situ* efforts that focus on isolation of mycorrhizal fungi used for artificial germination of orchid seeds and later to supplement existing populations or introduced to new areas of suitable habitat other than *in situ* efforts such as managing existing orchid populations and assisted migration of orchid species to new sites (Swartz and Dixon, 2009). However, in view of this, orchid conservation efforts in Malaysia still lack the inclusion of a mycorrhizal approach as relatively few studies have ever been done to investigate the mycorrhizas of Malaysian orchids (Jutta, 2007).

Orchid species from the genus *Paphiopedilum* (Orchidaceae) are among the most widely cultivated and hybridized of the 912 orchid genera. Commonly referred as Slipper Orchids, the *Paphiopedilum* is native to South China, India, Southeast Asia and the Pacific Islands. They are highly popular in horticulture and a prized collection for orchid lovers because of the flower's distinctively atypical form and rarity in nature. However, mycorrhizal studies on the *Paphiopedilum* are surprisingly scarce even though the significance of such knowledge is beneficial for conservation and even commercial purposes of the genus. *Paphiopedilum barbatum* (Lindl.) Pfitzer is a relatively more common species in Malaysia but increasingly threatened. A preliminary study by Jutta (2007) discovered the presence of *Rhizoctonia* spp. which is a known

OM when isolating endophytes from the roots of *P. barbatum*. This leads to the hypothesis that certain orchid mycorrhizal fungi that are beneficial for plant growth live symbiotically in the roots of *P. barbatum*. Thus, this study aims to isolate and identify the mycorrhizal fungi associated with *P. barbatum*, a relatively widespread Malaysian species in the *Paphiopedilum* genus and furthermore assess its growth enhancing capabilities with *Paphiopedilum* plant materials *in vitro*.

1.1 Objectives:

The objectives of this study are:

1. To isolate and identify (via molecular techniques) mycorrhizal fungi in *P. barbatum*.
2. To evaluate the growth performance of *in vitro Paphiopedilum* plant materials inoculated with the isolated mycorrhiza.

REFERENCES

Albert VA, Chase MW. 1992. *Mexipedium*: A New Genus of Slipper Orchid (Cypripedioideae: Orchidaceae). Lindleyana 7 (3): 172–176.

Alconero R, Santiago AG. 1969. Mycorrhizal infections of mature portions of Vanilla-M roots by *Rhizoctonia solani* as a predisposing factor to infection by *Fusarium oxysporum* f. sp. *vanillae*. Phytopathology 59: 1521-1524.

Altschul SF, Madden TL, Shaeffer AA, Zhang J, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research 25:3389-3402.

Anderson NA. 1982. The genetics and pathology of *Rhizoctonia solani*. Annual Review of Phytopathology 20: 329-347.

Andersen TF. 1996. A comparative taxonomic study of *Rhizoctonia sensu lato* employing morphological, ultrastructural and molecular methods. Mycological Research 100(9): 1117- 1128.

Anderson IA, Campbell CD, Prosser JI. 2003. Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environmental Microbiology 5(1): 36–47.

Arditti J. 1967. Factors affecting the germination of the orchid seeds. Botanical Review 33: 1-97.

Arditti J. 1979. Aspects of the physiology of orchids. Advances in Botanical Research 7: 421-655.

Arditti J, Ernst R, Yam TW, Glabe C. 1990. The contributions of orchid mycorrhizal fungi to seed germination: a speculative review. Lindleyana 5: 249-255.

Arditti J. 1992. Fundamentals of Orchid Biology. New York: John Wiley and Sons. p. 145-252.

Arditti J, Ernst R. 1993. Micropropagation of Orchids. New York: John Wiley and Sons. p. 10-31.

Athipunyakom P, Manoch L, Piluek C. 2004. Isolation and identification of mycorrhizal fungi from eleven terrestrial orchids. Kasetsart Jurnal Natural Sciences 38: 216-228.

Baldwin BG. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the compositae. Molecular Phylogenetics and Evolution 1: 3-16.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CF, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247-277.

Barker SJ, Larkan NJ. 2002. Molecular approaches to understanding mycorrhizal symbioses. Plant and Soil 244:107-116.

Barlow PW. 1989. Differential growth in plants – a phenomenon that occurs at all levels of organization. Environmental and Experimental Botany 29: 1–5.

Batty AL, Dixon KW, Brundrett M, Sivasithamparam K. 2001. Long-term storage of mycorrhizal fungi and seed as a tool for the conservation of endangered Western Australian terrestrial orchids. Australian Journal of Botany 49(5): 619 – 628.

Bayman P, Lebron LL, Tremblay RL, Lodge DJ. 1997. Variation in endophytic fungi from roots and leaves of *Lepenthes* (Orchidaceae). New Phytologist 135: 143-149.

Bayman P, Gonzalez EJ, Fumero JJ, Tremblay RL. 2002. Are fungi necessary? How fungicide effect growth and survival of the orchid *Lepanthes rupestris* in the field. Journal of Ecology 90: 1002-1008.

Beardmore J, Pegg GF. 1981. A technique for the establishment of mycorrhizal infection in orchid tissue grown in aseptic culture. New Phytotogist 87: 527-535.

Benzing DH. 1982. Mycorrhizal infections of epiphytic orchids in southern Florida. American Orchid Society Bulletin 51: 618–622.

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

Bougoure JJ, Bougoure DS, Cairney JW, Dearnaley JD. 2005. ITS-RFLP and sequence analysis of endophytes from *Acianthus*, *Caladenia* and *Pterostylis* (Orchidaceae) in southeastern Queensland. Mycological Research 109(4): 452-460.

Bougoure J, Ludwig M, Brundrett M, Grierson P. 2009. Identity and specificity of the fungi forming mycorrhizas with the rare mycoheterotrophic orchid *Rhizanthella gardneri*. Mycological Research 113: 1097-1106.

Braem GJ, Charles O, Margaret L. 1998. *The Genus Paphiopedilum: Natural History and Cultivation* (Vol. 1). USA (Florida): Botanical Publishers. p. 1-154.

Brundrett M. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist 154: 275–304.

Brundrett MC, Scade A, Batty AL, Dixon KW, Sivasithamparam K. 2003. Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. Mycological Research 107(10): 1210-1220.

Brundrett MC. 2004. Diversity and classification of mycorrhizal associations. Biological Review 79: 473-495.

Burgeff H. 1932. Saprophytismus und Symbiose. Studien an tropischen Orchideen. Jena: Fischer. p. 249.

Burpee LL, Sanders PC, Cole H, Sherwood RT. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia 72: 689-701.

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

Cameron DD, Johnson I, Read DJ, Leake JR. 2008. Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. New Phytologist 180(1): 176-184.

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

Carling DE, Rothrock CS, MacNish GS, Sweetingham MW, Brainard KA, Winters SW. 1994. Characterization of anastomosis group 11 (AG-11) of *Rhizoctonia solani*. Phytopathology 84: 1609-1612.

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.

Carroll GC. 1995. Forest endophytes: pattern and process. Canadian Journal of Botany 73: 1316-1324.

Carroll GC. 1986. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. Ecology 69: 2-9.

Carroll GC, Carroll FE. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific Northwest. Canadian Journal of Botany 56: 3034–3043.

Chanway CP. 1996. Endophytes: they're not just fungi! Canadian Journal of Botany 74: 321-322.

Chapman SC, Barreto HJ. 1997. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. Agronomy Journal 89: 557-562.

Chen TY, Chen JT, Chang WC (2002) Multiple shoot formation and plant regeneration from stem nodal explants of *Paphiopedilum* orchids. *In Vitro* Plant 38: 595–597.

Chen TY, Chen JT, Chang WC. 2004a. Multiple shoot formation and plant regeneration through direct shoot bud formation from leaf cultures of *Paphiopedilum* Orchids. *In Vitro* Cell Development Biology of Plant. 38: 595-597.

Chen TY, Chen JT, Chang WC. 2004b. Plant regeneration through direct shoot bud formation from leaf culture of *Paphiopedilum* orchids. Plant Cell, Tissue and Organ Culture 76: 11-15.

Chugh S, Guha S, Rao IU. 2009. Micropropagation of orchids: A review on the potential of different explants. Scientia Horticulturae 122: 507-520.

CITES [Internet]. [updated 2008 July 21]. Switzerland: Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES); [cited 2010 May 31]. Available from: http://www.cites.org/common/reg/e_nu.html

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

Cribb PJ. 1998. *The genus Paphiopedilum*. Borneo: Natural History Publications. p. 34-109.

Cribb PJ, Kell SP Dixon KW, Barrett RL. 2003. Orchid conservation - a global perspective. In: Dixon KW, Kell SP, Barrett RL, Cribb PJ (eds). *Orchid Conservation*. Borneo: Natural History Publications. p. 1-24.

Cruickshank RH. 1983. Distinction between *Sclerotinia* species by their pectic zymograms. Transactions of the British Mycological Society 80: 117-119.

Currah RS, Sherburne R. 1992. Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycological Research 96: 583–587.

Currah RS, Zelmer CD, Hambleton S, Richardson KA. 1997. Fungi from orchid mycorrhizas. In: Arditti J, Pridgeon AP (eds). *Orchid Biology: Reviews and Perspectives*. Dordrecht: Kluwer Academic Publishers. p. 117–170.

Curtis JT. 1939. The relation of specificity of orchid mycorrhizal fungi to the problem of symbioses. American Jurnal of Botany 26:360.

Daurade-Le Vagueresse MH, Romiti C, Groseclaude C, Bounias M. 2001. Coevolutionary toxicity as suggested by differential coniferyl alcohol inhibition of *Ceratocystis* species growth. Toxicon 39: 203-208.

Dearnaley JDW. 2007. Further advances in orchid mycorrhizal research. Mycorrhiza 17: 475-486.

Dixon KW, Kell SP, Barrett RL, Cribb PJ. 2003. Orchid conservation. Borneo: Natural History Publication. p. 1-24.

Dong Y, Zhu YG, Smith FA, Wang YS, Chen BD. 2008. Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* Linn.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil. Environmental Pollution 155(1):174-181.

Downie DG. 1959. The mycorrhiza of *Orchis purpurella*. Transactions of the Proceedings of the Botanical Society of Edinburgh 38: 16.

Dressler RL. 2004. How many species? Selbyana 26: 155-158.

Elizabeth Arnold A, Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88(3): 541–549.

Fadelah AB, Zaharah H, Rozlaily Z, Nuraini I, Tan SW, Hamidah H. 2001. Orchids: the Living Jewels of Malaysia. Malaysian Agricultural Research and Development Institute, Malaysia. p. 160-170.

Fisher PJ, Petrini O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). New Phytologist 120: 137-143.

Fröhlich J, Hyde KD, Petrini O. 2000. Endophytic fungi associated with palms. Mycological Research 104: 1202-1212.

Funder S. 1968. Practical Mycology. Manual for Identification of Fungi. New York: Hafner Publishing. p. 10-146.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for *Basidiomycetes*-application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Getha K, Vikineswary S, Wong WH, Seki T, Ward A, Goodfellow M. 2004. Characterization of selected isolates of indigenous *Streptomyces* species and evaluation of their antifungal activity against selected plant pathogenic fungi. Malaysian Journal of Science 23(2): 37-47.

Giuseppe P, Bellusci F. 2009. Molecular identification of mycorrhizal fungi in *Dactylorhiza sambucina* (Orchidaceae). Biologia 64(5): 893-897.

Gowland KM. 2007. Understanding the distribution of three species of epiphytic orchids in temperate Australian rainforest by investigation of their host and fungal associates. Lankesteriana 7: 44-46.

Gronberg H, Paulin L, Sen R. 2003. ITS probe development for specific detection of *Rhizoctonia* spp. and *Suillus bovines* based on Southern blot and liquid hybridization fragment length polymorphism. Mycological Research 107(4): 428-438.

Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold- Hurek B, Ladha J K. 2001. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. Journal of Bacteriology 183: 2634 2645.

Hadley G. 1970. Non-specificity of symbiotic infection in orchid mycorrhiza. New Phytologist 69:1015 1023.

Hadley G. 1982. Orchid mycorrhiza. In: Arditti J, editor. *Orchid Biology. Reviews and Perspectives*, vol. ii. New York: Cornell University Press. p. 83-118.

Harley JL, Smith SE. 1983. Mycorrhizal Symbiosis. London: Academic Press Inc. p. 268-298.

Harvais G, Hadley G. 1967. The relation between host and endophyte in orchid mycorrhiza. New Phytologist 66: 205-215.

Huang LC. 1988. A procedure for asexual multiplication of *Paphiopedilum* in vitro. American Orchid Society Bulletin 57: 274-278.

Huang LC, Lin CJ, Kuo CI, Huang BL, Murashige T. 2001. *Paphiopedilum* cloning *in vitro*. Scientia Horticulturae 91: 111-121.

Hsu BD. 2007. On the possibility of using chlorophyll fluorescence parameter as an indirect indicator for the growth of *Phalaenopsis* seedlings. Plant Science 172: 604-608.

Holtum RE. 1964. A Revised Flora of Malaya. Vol. 1. Orchids of Malaya. Singapore: Government Printing Office. p. 759.

Hyakumachi M, Ui T. 1987. Non self anastomosing isolates of *Rhizoctonia solani* obtained from fields of sugar beet monoculture. Transcripts of the British Mycological Society 89:155–15.

IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. [Cited 08 March 2010]. Available from www.iucnredlist.org

Joner EJ, Jakobsen I. 1995. Growth and extracellular phosphate activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biology and Biochemistry 9: 1153–1159.

Jones DL. 1993. *Pterostylis*. In: Harden GJ, editor. *Flora of New South Wales*. Kensington: New South Wales University Press. p. 53.

Jumpponen A, Trappe JM. 1998. Dark-septate root endophytes: a review with special reference to facultative biotrophic symbiosis. New Phytologist 140: 295–310.

Jutta M. 2007. Diversity of fungal root endophytes in *Paphiopedilum barbatum* (Orchidaceae) from selected *in situ* and *ex situ* sites in Peninsular Malaysia. Unpublished. Universiti Putra Malaysia. p. 59-70.

Jutta M, Faridah QZ. 2006. Conservation status of the Malaysian Slipper Orchids: *Paphiopedilum* Pfitz. (Orchidaceae). Folia Malaysiana 7: 9-18.

Jutta M, Faridah QZ, Salleh B, Faridah A. 2007. Root endophytes: a new dimension in plant conservation? The Malaysian Forester 70(1): 13-21.

Kiew R. 1991. Botanical survey of the limestone hills in Perak. In: Kiew R (Ed). A Conservation Assesment of Limestone Hills in the Kinta Valley. Kuala Lumpur: Malaysia Nature Society. Final Report MNS Project 1/90. p. 1-7.

Kuldau GA, Yates IE. 2000. Evidence for *Fusarium* endophytes in cultivated and wild plants. In: Bacon CW, White Jr JF, editors. *Microbial Endophytes*. New York: Decker. p. 85–117.

Kristiansen KA, Taylor DL, Kjoller R, Rasmussen HN, Rosendahl S. 2001. Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. Molecular Ecology 10: 2089-2093.

Kristiansen KA, Freudenstein JV, Rasmussen FN, Rasmussen HN. 2004. Molecular identification of mycorrhizal fungi in *Neuwiedia veratrifolia* (Orchidaceae). Molecular Phylogenetic Evolution 33: 251–258.

Ladha JK, Triol-Padre A, Punzalan GC, Castillo E, Singh U, Reddy CK. 1998. Non-destructive estimation of shoot nitrogen in different rice genotypes. Agronomy Journal 90: 33-40.

Leake JR. 1994. The biology of myco-heterotrophic 'saprophytic' plants. New Phytologist 127: 171–216.

Li A, Luo Y, Ge S . 2002. A preliminary study on conservation genetics of an endangered orchid (*Paphiopedilum micranthum*) from Southwestern China. Biochemical Genetics 40: 195-201.

Lin YH, Chang C, Chang WC. 2000. Plant regeneration from callus culture of a *Paphiopedilum* hybrid. Plant Cell, Tissue and Organ Culture 62: 21-25.

Liu Z, Liu K, Chen L, Lei S, Li L, Shi X, Huang L. 2006. Conservation ecology of endangered species *Paphiopedilum armeniacum* (Orchidaceae). Acta Ecologica Sinica 26(9): 2791-2800.

Lye D. 2006. Charting the isophasic endophyte of Dwarf Mistletoe *Arceuthobium douglasii* (Viscaceae) in host apical buds. Annals of Botany 97: 953-963.

Ma M, Tan TK, Wong SM. 2003. Identification and molecular phylogeny of *Epulorhiza* isolates from tropical orchids. Mycological Research 107:1041–1049.

Markwell J, Osterman JC, Mitchell JL. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynthesis Research 46: 467-472.

Masuhara G, Katsuya K. 1989. Effects of mycorrhizal fungi on seed germination and early growth of three terrestrial Japanese orchids. Scientia Horticulturae 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. Biebertsien) Hara (Orchidaceae). New Phytologist 127:711–718.

McCormick MK, Whigham DF, O'Neill J. 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. New Phytologist 163: 425–438.

McCormick MK, Whigham DF, Sloan D, O'Malley K, Hodkinson B. 2006. Orchid-fungus fidelity: a marriage meant to last? Ecology 87: 903-911.

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 Phytologist 145: 523-537.

Menkis A, Vasiliauskas R, Taylor AFS Stenlid J, Finlay R. 2005. Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16: 33-41.

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

Mordue JEM, Currah RS, Bridge PD. 1989. An integrated approach to *Rhizoctonia* taxonomy: cultural, biochemical and numerical techniques. Mycological Research 92: 78-90.

Mukerji KG, Manoharachary C, Chamola BP. 2002. Techniques in Mycorrhizal Studies. Netherlands: Kluwer Academic Publishers. p. 553.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plant 15: 495-497.

Nan GL, Tang CS, Kuehnle AR, Kado CI. 1997. *Dendrobium* orchids contain an inducer of *Agrobacterium* inducer genes. Physiology and Molecular Plant Pathology 51: 391-399.

Ng CY, Norihan MS, Faridah QZ. 2010. In vitro multiplication of the rare and endangered slipper orchid, *Paphiopedilum rothschildianum* (Orchidaceae). African Journal of Biotechnology 9(14): 2062-2068.

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 [Internet]. [cited 2010 9 February]; Available from: http://www.springerlink.com/content/x11310085m3632h6

Otero JT, Ackerman JD, Bayman P. 2002. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. American Journal of Botany 89: 1852–1858.

Otero JT, Ackerman JD, Bayman P. 2004. Diversity in mycorrhizal preferences between two tropical orchids. Molecular Ecology 13: 2393–2404.

Otero JT, Ackerman JD, Bayman P. 2005. Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia variegata in vitro*: the potential for natural selection. Evolutionary Ecology 19: 29–43.

Otero JT, Flanagan NS, Herre EA, Ackerman JD, Bayman P. 2007. Widespread mycorrhizal specificity correlates to mycorrhizal function in the neotropical, epiphytic

orchid *Ionopsis utricularioides* (Orchidaceae). American Journal of Botany 94(12): 1944-1950.

Parmeter JR, Whitney HS. 1970. Taxonomy and nomenclature of the imperfect state. In: Parmeter JR, editor. *Rhizoctonia solani*: *Biology and Pathology*. Berkeley (CA): University of California Press. p 7–19.

Peterson RL, Massicotte HB, Melville LH. 2004. Mycorrhizas: Anatomy and cell biology. UK: CABI Publishing. p. 125-144.

Petrini O. 1991. Fungal endophytes of tree leaves. In: Andrews JH, Hirano SS, editors. *Microbial Ecology of Leaves*. New York: Springer-Verlag. p. 179–197.

Peyronel B, Fassi B, Fontana A, Trappe JM. 1969. Terminology of mycorrhizae. Mycologia 61: 410–41.

Piluek C, Triboun P. 2008. Wild orchid conservation for ecotourism in Thailand. Acta Horticulturae 788: 69-76.

Pierik RLM, Sprenkels PA, Van Der Harst B, Van Der Meys QG. 1988. Seed germination and further development of plantlets of *Paphiopedilum ciliolare* Pfitz. *in vitro*. Scientia Horticulturae 34: 139-153.

Pieterse CMN, Dicke M. 2007. Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends in Plant Science 12: 564-569.

Porras-Alfaro A, Bayman P. 2007. Mycorrhizal fungi of Vanilla: diversity, specificity and effects on seed germination and plant growth. Mycologia 99(4): 510-525.

Raeder U, Broda P. 1985. Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1: 17-20.

Rao NS. 1993. Biofertilizers in agriculture and forestry. New York: International Science Publisher. p. 136-151.

Ramsay RR, Dixon KW, Sivasithamparam K. 1986. Patterns of infection and endophytes associated with Western Australian orchids. Lindleyana 1(3): 203–214.

Ramsay MM, Stewart J. 2008. Re-establishment of the lady's slipper orchid (*Cypripedium calceolus* L.) in Britain. Botanical Journal of the Linnean Society 126: 173-181.

Rasmussen HN. 1990. Cell differentiation and mycorrhizal infection in *Dactylorhiza majalis* (Rchb. f.) Hunt and Summerhayes (Orchidaceae) during germination *in vitro*. New Phytologist 116: 137-147.

Rasmussen HN. 1995. Terrestrial orchids: from seed to mycotrophic plant. Cambridge (UK): Cambridge University Press. p. 25-104.

Rasmussen HN. 2002. Recent developments in the study of orchid mycorrhiza. Plant and Soil 244: 149-163.

Rasmussen HN, Rasmussen FN. 2009. Orchid mycorrhiza: implications of a mycophagous lifestyle. Oikos 118: 334-345.

Rasmussen HN, Whigham DF. 1998. The underground phase: A special challenge in studies of terrestrial orchid populations. Botanical Journal of the Linnean Society 126: 49–64.

Rasmussen HN, Whigham DF. 2002. Phenology of roots and mycorrhiza in orchid species differing in phototrophic strategy. New Phytologist 154: 797-807.

Rodrigues KF. 1994. The foliar fungal endophytes of the Amazonian palm *Euterpe oleracea*. Mycologia 86: 376-385.

Rusea G, Khali AH. 2008. Orchids of Peat Swamp Forests in Peninsular Malaysia. Kuala Lumpur: Ministry of Natural Resources and Environment, Peat Swamp Forest Project, UNDP/GEF Funded (MAL/99/G31). p. 136.

Saikkonen K.S, Faeth H, Helander M, Sullivan TJ. 1998. Fungal endophytes: A continuum of interactions with host plants. Annual Review Ecology System 29: 319-343.

Saitoh K, Togashi K, Arie T, Teraoka T. 2006. A simple method for a mini-preparation of fungal DNA. Journal of Genetic Plant Pathology 72: 348-350.

Salazar O, Julian MC, Hyakumachi M, Rubio V. 2000. Phylogenetic grouping of cultural types of *Rhizoctonia solani* AG 2-2 based on ribosomal ITS sequences. Mycologia 92: 505–509.

Salifah HAB, Muskhazli M, Rusea G, Nithiyaa P, Nurul SN. 2009. Mycorrhizae of Tiger Orchids (*Grammatophyllum* spp.) and symbiotic germination of seed of *G. stapeliiflorum*. Malaysian Applied Biology 38(2): 23-28.

Schmid E, Oberwinkler F. 1994. Light and electron microscopy of the host-fungus interaction in the achlorophyllous gametophyte of *Botrychium lunaria*. Canadian Journal of Botany 72: 182–188.

Schulz B, Boyle C. 2006. What are endophytes? In: Schulz B, Boyle C, Sieber TN, editors. *Soil Biology*, Volume 9 (Microbial Root Endophytes). Berlin: Springer-Verlag. p. 1-11.

Seifert KA, Samson RA, Dewaard JR, Houbraken J, Levesque CA, Moncalvo JM, Louis-Seize G, Hebert PDN. 2007. Prospects for fungus identification using CO1 DNA barcodes, with *Penicillium* as a test case. Proceedings of the National Academy of Sciences 104(10): 3901–3906.

Sen R, Hietala AM, Zelmer CD. 1999. Common anastomosis and internal transcribed spacer RFLP grouping in binucleate *Rhizoctonia* isolates representing root endophytes in *Pinus sylvestris*, *Ceratorhiza* spp. from orchid mycorrhizas and a phytopathogenic anastomosis group. New Phytologist 144: 331–341.

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

Sharma J, Ishida ML, Yadon VL. 2007. Mycorrhizal diversity of an endemic terrestrial orchid. Lankesteriana 7: 215–218.

Sharon M, Kuninaga S, Hyakumachi M, Sneh B. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience 47: 299–316.

Sharon M, Kuninaga S, Hyakumachi M, Naito S, Sneh B. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience 49: 93–114.

Shefferson RP, Taylor DL, Weib M, Garnica S, McCormick MK, Adams S, Gray HM, McFarland JW, Kull T, Tali K, Yukawa T, Kawahara T, Miyoshi K, Lee Y. 2007. The evolutionary history of mycorrhizal specificity among Lady's Slipper orchids. Evolution 61(6): 1380-1390.

Shimura H, Matsuura M, Takada N, Koda Y. 2007. An antifungal compound involved in symbiotic germination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae). Phytochemistry 68: 1442-1447.

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(8): 525-34.

Sivasithamparam K. 1998. Root cortex – the final frontier for biocontrol of root-rot with fungal antagonists: a case study on a sterile red fungus. Annual Review of Phytopathology 36: 439–452.

Smith RD. 2009. Plant-mycorrhiza percent infection as evidence of coupled metabolism. Journal of Theoretical Biology 259: 172-175.

Smith JE, Johnson KA, Cazares E. 1998. Vesicular mycorrhizal colonisation of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intraradices*. Mycorrhiza 7: 279–285.

Smith SE. 1966. Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytologist 65: 488-499.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. New York: Academic Press. p. 347-349.

Smith SE, Smith FA. 1990. Structure and function of the interfaces in biotrophic symbioses. New Phytologist 114: 1–38.

Smith ZE, James EA, Mclean CB. 2007. Experimental reintroduction of the threatened tererestrial orchid *Diuris fragrantissima*. Lankesteriana 7: 377-380.

Sneh B, Burpee L, Ogoshi A. 1991. Identification of *Rhizoctonia* species. St. Paul (MN): American Phytopathological Society Press. p. 133.

Stark C, Babik W, Durka W. 2009. Fungi from the roots of the common terrestrial orchid *Gymnadenia conopsea*. Mycological Research 113: 952-959.

Stewart J, Button J. 1975. Tissue culture studies in *Paphiopedilum*. American Orchid Society Bulletin 44: 591-599.

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* Cellular Development Biology 43: 178-186.

Stimart DP, Ascher PD. 1981. *In vitro* germination of *Paphiopedilum* seed on a completely defined medium. Scientia Horticultureae 14: 165-170.

Stone JK, Bacon CW, White JF. 2000. An overview of endophytic microbes: endophytism defined. In: Bacon CW, White JF (eds). *Microbial endophytes*. New York: Dekker. p. 3-30.

Stretton HM, McKenzie AR, Baker KF, Flentjie NT. 1964. Formation of the basidial stage of some isolates of *Rhizoctonia*. Phytopathology 54:1093-1095.

Suarez JP, Weiss M, Abele A, Garnica S, Oberwinkler F, Kottke I. 2006. Diverse tulasnelloid fungi from mycorrhizas with epiphytic orchids in Andean cloud forest. Mycological Research 110: 1257-1270.

Swarts ND, Dixon KW. 2009. Terrestrial orchid conservation in the age of extinction. Annals of Botany 104(3): 543-556.

Sweetingham MW, Cruickshank RH, Wong DH. 1986. Pectic zymograms and the taxonomy and pathogenicity of the Ceratobasidiaceae. Transactions of the British Mycological Society 86: 305-311.

Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, van der Lelie D. 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Applied Environmental Microbiology 75(3): 748-57.

Taylor DL, McCormick MK. 2008. Internal transcribed spacer primers and sequences for improved characterization of basidiomycetous orchid mycorrhizas. New Phytologist 177: 1020–1033.

Taylor DL, Bruns TD, Hodges SA. 2004. Evidence for mycorrhizal races in a cheating orchid. Proceedings of the Royal Society of London 271: 35–43.

Taylor DL, Bruns TD, Leake JR, Read DJ. 2002. Mycorrhizal specificity and function in myco-heterotrophic plants. In: van der Heijden MGA, Sanders I (eds). *Mycorrhizal Ecology*. Berlin: Springer-Verlag. p. 375-414.

Taylor DL, Bruns TD, Szaro TM & Hodges SA (2003). Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. American Journal of Botany 90: 1168–1179.

Trémouillaux-Guiller J, Rohr T, Rohr R, Huss VAR. 2002. Discovery of an endophytic alga in *Ginkgo biloba*. American Journal of Botany 89:727-733.

Tsavkelova EA, Bomke C, Netrusov AI, Weiner J, Tudzynski B. 2008. Production of gibberellic acids by an orchid-associated *Fusarium proliferatum* strain. Fungal Genetics and Biology 45: 1393-1403.

Uchida JY, Aragaki M, Yahata PS. 1986. Basidiospore formation by *Ceratobasidium* sp. on agar. Mycologia 78: 587-592.

Umali T, Quimio T, Hyde KD. 1999. Endophytic fungi in leaves of *Bambusa tuldoides*. Fungal Science 14: 11-18.

Unterseher M, Schnittler M. 2009. Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.) – Different cultivation techniques influence fungal biodiversity assessment. Mycological Research 113: 645-654.

Vilgalys R, Cubeta MA. 1994. Molecular systematics and population biology of *Rhizoctonia*. Annual Review of Phytopathology 32: 132-155.

Vralstad T, Schumacher T, Taylor AFS. 2001. Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. New Phytologist 153(1): 143-152.

Walter KS, Gillett HJ. 1998. 1997 IUCN red list of threatened plants. Switzerland (Gland): IUCN - The World Conservation Union. p. 862.

Wan Zainun WN, Yap MY. 1979. *Rhizoctonia solani*, a seed-borne pathogen of French bean in Malaysia. Pertanika 2(1): 11-15.

Warcup JH. 1971. Specificity of mycorrhizal association in some Australian terrestrial orchids. New Phytologist 70: 41-46.

Warcup JH. 1981. The mycorrhizal relationships of Australian orchids. New Phytologist 87: 371-381.

Warcup JH, Talbot PHB. 1967. Perfect states of *Rhizoctonia*s associated with orchids. New Phytologist 66: 631-641.

Watanabe T. 1972. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. New York: CRC Press. p. 78-90.

Whigham DF, O'Neil JP, Rasmussen HN, Caldwell BA, McCormick MK. 2006. Seed longevity in terrestrial orchids-potential for persistent *in situ* seed banks. Biological Conservation 129: 24-30.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T, editors. PCR protocols: a guide to methods and application. San Diego: Academic Press. p. 315–322.

Wilson D. 1995. Endophyte – the evolution of a term, and clarification of its use and definition. Oikos 73: 274-276.

Wright M, Guest D. 2005. Development of mycorrhizal associations in *Caladenia fasciculatum*. Selbyana 26: 328-334.

Wright MM, Cross R, Cousens RD, May TW, McLean CB. 2010. Taxonomic and functional characterisation of fungi from the *Sebacina vermifera* complex from common and rare orchids in the genus *Caladenia*. Mycorrhiza[Internet]. [cited 2010 February 12]. Available from http://www.springerlink.com/content/27154vt47112k515

Yuan L, Zhu L, Yang, Li SY, Hu H, Huang JL. 2010. Mycorrhizal specificity, preference, and plasticity of six slipper orchids from South Western China. Mycorrhiza[Internet]. [cited on 2010 March 29]. Available from http://www.springerlink.com/content/41752276v3600251

Zelmer CD, Currah RS. 1995. Evidence for a fungal liaison between *Corallorhiza trifida* (Orchidaceae) and *Pinus contorta* (Pinaceae). Canadian Journal of Botany 73: 862–866.

Zettler LW. 1994. Extinction in our own backyard. American Orchid Society Bulletin 63: 686-688.

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, McInnis TM. 1993. Symbiotic seed germination and development of *Spiranthes cernua* and *Goodyera pubescens* (Orchidaceae: Spiranthoideae). Lindleyana 8: 155–162.