Journal of Phytology 2010, 2(7): 75-90
An Open Access Journal
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REVIEW ARTICLE

MOLECULAR APPROACHES IN ARBUSCULAR MYCORRHIZAL RESEARCH: A REVIEW

D. Sharmah[1], D.K. Jha[1] and R. R. Pandey[2*]
[1]*Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati-781 014, India*
[2]*Department of Life Sciences, Manipur University, Canchipur, Imphal-795 003, India*

SUMMARY

Arbuscular mycorrhizal fungi (AMF), belonging to phylum Glomeromycota, are among one of the key species groups that inter-connect plants into a functional web. Most of the research work on AMF is focused mainly on taxonomy, phylogeny, ecology, genetics and functional symbiosis with some major insights gained in biology of the fungi in the last decade, with application of molecular methods and revolutionary technological advancement. In this article, we reviewed the current scenario of molecular investigations on AMF all over the world, and compare it with conventional techniques to bring about the pros and cons. Since no technique is a panacea, we advocate the use of both molecular and microscopy methods to characterize AMF communities in plant roots and to construct a robust phylogenetic tree.

Key words: Arbuscular mycorrhiza, Molecular methods, Primer, rDNA

D. Sharmah et al. Molecular Approaches in Arbuscular Mycorrhizal Research: A Review. J Phytol 2/7 (2010) 75-90.
*Corresponding Author, Email: pandey.rr@rediffmail.com, Tel: +91 9436025986; Fax: +91 385 2435327

1. Introduction

Arbuscular Mycorrhizal Fungi (AMF) are vital components of the microbial soil community forming the most commonly occurring underground symbiosis between members of phylum *Glomeromycota* and roots of 80% of all terrestrial plant species (Wang *et al.,* 2008; Schüβler *et al.,* 2001). AMF are the key species groups that inter-connect plants into a functional web (Hegalson *et al.,* 1998), extending plant root systems and thereby, facilitates plants uptake of soil nutrients of poor mobility, especially phosphorus (Smith and Read, 2008). It is now well established that mycorrizal fungal networks act as a potential conduit for interplant transfer of resources (vander Heijden and Horton, 2009). Besides, AMF improve plant fitness by improving seedling establishment, plant fecundity, tolerance to some root pathogens, water relations and formation and stability of soil aggregates (Read, 1999; Newsham *et al.,* 1995). The hypothesis that AMF diversity is crucial for maintaining productivity and stability of ecosystems has now been put on a firm footing by recent experimental observations (e.g. Grime *et al.,* 1987; vander Heijden *et al.,* 1998) which showed that increasing AMF species richness led to an alteration in the plant community structure and consequently an increase in plant diversity and productivity. Because of their pivotal role in plant community ecology, and multiple beneficial effects on plant as well as soil health, AMF appears to be an essential component in successful re-vegetation and restoration of degraded soil and forests (Pattinson *et al.,* 2004; Cuenca *et al.,* 1998).

There are a number of difficulties in studying AMF fungal taxonomy, phylogeny, symbiosis, ecology and genetics. Firstly, AMF being obligate biotrophs cannot be axenically cultured in the laboratory and, therefore, large scale production is not possible. Till date, attempt to culture these symbionts on axenic/ auxenic medium have been met with little or no success (Becard and Fortin, 1998; St Arnaud *et al.,* 1996). Secondly, conventional techniques of studying these fungi by spore quantifications have many pitfalls. As AMF are diverse in their sporulation condition, sporulation ability and colonization therefore, spore

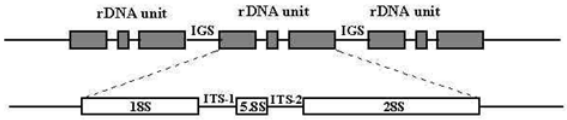
morphology based methods may not entirely reflect the true picture of AMF species richness in any given ecosystem. Studies such as by Clapp *et al.* (1995) have documented the complete absence of spore in the roots of one of the common understory plants in woodland of North Yorkshire, UK, although molecular techniques consistently showed the presence of AMF in the corresponding roots. Moreover, correct identification of AMF spores is a painstakingly laborious and time consuming process and depends on investigators level of expertise. This is, in particular, when spores are sieved directly from the soil. Thus, conventional techniques do not provide an accurate picture of the *in situ* diversity of AMF communities and by blindly following the spore based methodology we are probably only scratching the surface of what really occurs in corresponding roots. Molecular identification approaches, especially nucleic acid based techniques, have the potential to revolutionize our understanding of AMF. The recent application of molecular biological techniques for characterization of AMF has led to important advances in our understanding of the phylogeny (Schüßler *et al.,* 2001; Schwarzott *et al.,* 2001; Hibbett *et al.,* 2007), ecology (Helgason *et al.,* 1998, 2002; Husband *et al.,* 2002; Appoloni *et al.,* 2008), genetics (Harrison, 1999; Rosendahl and Matzen, 2008), evolution (Gandolfi *et al.,* 2003; Sanders, 2002; Hegalson and Fitter, 2009) and symbiosis (Parniske, 2008) of this group of obligatory symbiotic fungi. Hence, advantages of using molecular techniques are manifold. Molecular technology have enabled the identification of AMF inside the field plant roots and have revealed unexpectedly high AMF diversity in several ecosystem with many AM taxa being previously unknown (Hegalson *et al.,* 2002; Husband *et al.,* 2002; Gollotee *et al.,* 2004; Rosendahl and Stukenbrock, 2004). Researchers' insights on the molecular cross talk between fungus and host plants would resolve many unknown facets of plant-microbe interaction. The paradox on phylogeny of AMF lineages, which is still eluding the investigators, can be resolved with the use of innovative molecular tools and techniques. Therefore, it is imperative that the next one or two decades would witness a tremendous increase in molecular level research, which hopefully would resolve many issues being confronted today. In this review we have attempted to elaborate how molecular methods have enabled the researchers to study the fungus directly in the soil. Competitive rhizosphere colonization is crucial for many mechanisms of action of these fungi. Finally, we have also focused the importance of traditional and molecular dataset to describe the taxonomy and phylogeny of such important group of fungi.

2. Genetic Diversity

During the past twenty years, molecular methods have been developed which is primarily based on exploitation of genetic variation (de Souza *et al.,* 2004) that allow identification of AMF in the host plant roots or directly in the soil without the necessity of spore formation. The nuclear encoded ribosomal DNA (rDNA) has been well established as a marker for characterization of AMF, both in the lab as well as in natural assemblages (Clapp *et al.,* 2002). These genes are present in multiple copies and contain conserved coding (small subunit - SSU and large subunit – LSU) as well as variable non-coding parts (internal transcribed spacers - ITS**).** ITS are sequences located in eukaryotic rDNA genes between the 18s and 5.8s rDNA coding regions (ITS1) and between the 5.8s and 28s rDNA coding regions (ITS2) (Figure 1). The characterisation of ribosomal genes-SSU rDNA, LSU rDNA including the ITS1 and ITS2 regions, is a suitable tool for revealing phylogenetic relationships and developing molecular probes to identify glomeromycotan fungi (Redecker, 2000; Schüßler, 1999; van Tuinen *et al.,* 1998). Analysis of the SSU rDNA can provide taxonomic information that may be lacking in examinations of spore morphology. Recently molecular phylogenetic analysis, based on SSU rDNA sequences, has resulted in profound changes in AM fungi classification, with proposal of a separate phylum *Glomeromycota*, containing new

orders, families and genera (Schüβler *et al.*, 2001; Walker and Schüβler, 2004).

Figure 1: Structure and details of rDNA gene cluster. IGS, Intergenic space; ITS, Internal Transcribed Spacer. (Source: Reddy *et al.*, 2005)



However, as this gene evolves relatively slowly it may not provide sufficient information to adequately characterize the AMF guild within a community. A more appropriate approach could rely on the sequence variability of the internal transcribed spacer (ITS) regions between the small and large subunits of the rDNA genes (Sanders *et al.*, 1995; Redecker *et al.*, 1997). Sanders *et al.* (1995) and Lloyd-Macgilp *et al.* (1996) were among the first to use ITS rDNA to study intra and interspecific diversity in AMF. Since ITS rDNA displays a higher variation and species level discrimination than do both ribosomal subunits of the rDNA gene loci, therefore, it has been extensively used as molecular marker in characterizing AMF communities in diverse ecosystem (Lanfranco *et al.*, 1999; Konig *et al.*, 2010). These spacers sequences have high evolution rate and are useful for phylogenetic analyses among related species. However, use of ITS has not always been without criticism, as existence of multiple sequences within single AMF isolates or even individual spores (Sanders *et al.*, 1995; Clapp *et al.*, 1999) may lead to overestimations of number of species and makes the result difficult to interpret. Less progress has been made in the interpretation of this heterogeneity, and investigators tried several others species-specific markers for AMF detection and identification. The use of strain-specific primers which amplifies a part of the gene coding for the LSU rDNA has been described by Van Tuinen *et al*. (1998), and it offers the possibility to obtain both genus and species-specific sequences (Kjøller and Rosendahl, 2000). Sequences differences can also be demonstrated by Single Stranded Conformation Polymorphism (SSCP). In SSCP, nucleotide differences between homologous sequence strands are detected by electrophoresis of single-stranded DNA under non-denaturing conditions (Orita *et al.*, 1989). Simon *et al.* (1993) used Polymerase Chain Reaction (PCR) coupled with SSCP of SSU rDNA genes to detect specific arbuscular mycorrhizal strains in roots. Similarly, Kjøller and Rosendahl (2000) used PCR-SSCP to differentiate between species and isolates within the genus *Glomus* based on sequence differences in the gene coding for LSU rDNA. The D2 domain of LSU rDNA is more variable than any region of SSU rDNA (Van Tuinen *et al.*, 1998) and is, therefore, suitable for separating closely related species or isolates within a species.

A number of other molecular tools have been used for genetic diversity analysis by various investigators. Terminal restriction fragment length polymorphism (T-RFLP) is an increasingly widely used high throughput fingerprinting technique in mycorrhizal ecology. T-RFLP uses fluorescently labelled primers combined with restriction digests to visualise sequence variation in either single- or mixed-species DNA samples (Dickie and Fitzjohn, 2007). Two different versions of the technique i.e. peak-profile T-RFLP and database T-RFLP are used of which the latter has been widely employed in AMF study (Lekberg *et al.*, 2007; Dickie *et al.*, 2002; Aldrich-Wolfe, 2007; Lindahl *et al.*, 2006). Though T-RFLP permits a high throughput of samples, it is also subjected to a number of error types. Therefore, the best way to view community composition is by using multiple techniques, wherever feasible. PCR is a mandatory step in any molecular technique now-a-days. Several investigators have employed extended versions of the basic PCR technique. Nested-PCR is a two step highly sensitive procedure that uses sets of primers (universal and genus specific) to characterize AMF in plant roots and soil

(Renker *et al.,* 2003; Kjøller and Rosendahl, 2000; Rosendahl and Matzen, 2008). Competitive PCR is another version of PCR which co-amplifies the target template with an internal standard and was also developed to quantify the AM fungus within the roots (Edwards *et al.,* 1997). PCR-denaturing gradient gel electrophoresis (DGGE) which was initially developed to study mutation has now-a-days become one of the most applied culture-independent techniques to study the community structure of microorganisms (Muyzer and Smala, 1998). Separation of DNA fragments in DGGE is based on the decreased electrophoretic mobility of partially melted double-stranded DNA molecules in polyacrylamide gels containing a linear increasing gradient of DNA denaturants (Muyzer and Smala, 1998). Recently, de Souza *et al.* (2004) successfully applied PCR-DGGE to access the species diversity of AMF of the genus *Gigaspora* by profiling of inter- and intra-species 18S rRNA gene sequence heterogeneity. There are promising technologies on the horizon that may well circumvent the pitfalls of present techniques. Microarray technologies have been proposed for mycorrhizal fungi (Anderson and Cairney, 2004), but has not yet been widely applied. Application of 'next-generation' sequencing technologies to AMF genetics will be a revolutionary advancement in genome sequencing. Pacific Biosciences is currently developing a single-molecule sequencer, which is expected to be released in 2010 (MacLean *et al.,* 2009), and would make a huge impact on microbiology, providing a rapid and cost-effective means of generating draft genomes.

3. Molecular phyllotaxonomic diversity

AMF has traditionally been classified and identified based on microscopic characteristics of spores. Spore quantification, primarily, has been used in many diversity related studies in different ecosystems viz. agriculture and pasture (Li *et al.,* 2007; Picone, 2000; Wang *et al.,* 2008; Tehabi *et al.,* 2008; Aguilar-Fernandez *et al.,* 2009); natural and disturbed forests (Zhang *et al.,* 2004; Guadarrama and Alveraz-Sanchez, 1999; Allen *et al.,* 1998); grassland (Su and Guo, 2007; Tian *et al.,* 2009); and horticulture (Singh *et al.,* 2008). However, evaluation of AMF community based entirely on microscopic characters of spore may not accurately reflect the AMF community that is interacting with plants, since spore formation is not always correlated with the extent of root colonization. There are (as at March 2009) >11000 Glomeromycotan sequences assigned to ~ 950 distinct taxonomy identifiers in GenBank, only 96 of which are unambiguous species binomials (Hegalson and Fitter, 2009). This contrast with the ~ 215 described morphospecies (http://www.lrzmuenchen.de/~schuessler/amphylo/amphylogeny.html). It is apparent that natural communities of AM fungi are biologically diverse with large number of taxa present. To address this potential problem, molecular techniques that facilitate detection and identification of AMF in all stages of their life cycle were developed during the last decade, targeting the nuclear encoded ribosomal genes (Redecker, 2002). Field studies based on molecular markers have provided numerous new insights into the dynamics of AMF communities and our appreciation of natural AMF ribotype richness has greatly expanded (Appoloni *et al.,* 2008; Hijri *et al.,* 2006; Hempel *et al.,* 2007; Opik *et al.,* 2008, 2009). Advances in molecular technology have revealed high diversity in several ecosystems, many of the AM taxa found being previously unknown (Hegalson *et al.,* 2002; Husband *et al.,* 2002; Pietikainen *et al.,* 2007). By examining SSU rRNA sequences which were amplified, cloned and screened for differences in restriction pattern by restriction fragment length polymorphism (RFLP), Hegalson *et al.* (1998) found strikingly low AMF diversity in arable sites compared with adjacent woodland at Castle Howard, North Yorkshire, UK. In addition, molecular identification tools make it now possible to characterise AMF communities in plant organs or at extreme environmental sites that were previously hard to sample. The first of its kind of study to characterize AMF community in geothermal sites in Yellowstone National Park, USA was carried out by Appoloni *et al.* (2008) who utilized

specific primer pairs for regions of ribosomal DNA (rDNA) that amplify different subgroups of AMF. This study revealed presence of generalists and specialists AMF in the geothermal soils providing further avenues to understand the role of fungal symbionts on the fitness of the plants growing under extreme environmental conditions. Likewise, in the harsh climate of low-Arctic meadow habitat Pietikainen *et al.* (2007) studied the diversity and persistence of arbuscular mycorrhizas by amplification of SSU rRNA region using universal eukaryotic primer and AM fungal specific primer followed by restriction digestion and Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. There is now a growing demand for the re-establishment of vegetation in degraded ecosystem using AMF and in this regard knowledge about the presence and diversity of AMF in a specific area is an essential prerequisite. The work of Li *et al.* (2009) on the molecular diversity of AMF in a fallow and an undisturbed land strongly confirmed that soil disturbance reduced AMF population and species diversity. The investigators amplified LSU rDNA genes of AMF using universal fungal primer pair (LR1/FLR2) and *Glomeromycetes*- specific primer pair (28G1/28G2) and cloned the PCR products. However, as it has been pointed out that most of the primers used in amplification of rDNA subsets are not quite AMF specific and can amplify DNA of non-target organisms (Kruger *et al.,* 2009). Therefore designing reliable primers that specifically amplifies AMF lineages would be a major challenge which will overcome the present limitation of molecular methods in AMF species resolution.

4. AMF Primer Design

Molecular characterization of AMF is mostly achieved by PCR amplification of DNA from roots of host plants, spores or soil samples. The majority of the DNA extracted from colonized roots or soil samples is of plant origin or non-target organisms and therefore, specific PCR primers must be used in order to obtain fungal DNA fragments (Redecker, 2000). Till date, majority of the primers designed targets the SSU, ITS and LSU rDNA subsets with variable lengths, mostly between 500-800 base pair. Simon *et al.* (1992) designed the PCR primer VANS1 for 18s subunit of rDNA. However, it is now shown that VANS1 annealing site is not well conserved within *Glomeromycetes* (Clapp *et al.,* 1999). In their field study, Hegalson *et al.* (1998) used universal eukaryotic primer NS31 and fungal specific primer AM1 to amplify the partial SSU rDNA fragments of about 550 bp. However, later studies showed that these primers do not amplify DNA of all *Glomeromycota* or they also amplify Ascomycetes, Basidiomycetes or plant DNA (Clapp *et al.*, 1995, 1999; Hegalson *et al.*, 1999). To overcome many mismatches in the published AMF specific primers, other primers were successfully designed for certain groups of *Glomeromycota* (Kjøller and Rosendahl, 2000; Redecker, 2000; Wubet *et al.,* 2003; Gamper and Leuchtmann, 2007). The most comprehensive taxon sampling for Glomeromycota covers the SSU rDNA region (Schüβler *et al.,* 2001 a, b), for which a new AMF specific primer pair was recently published (AML1 and AML2; Lee *et al.*, 2008). The PCR primers designed by Lee *et al.* (2008) were perhaps the first of its kind that can amplify all AMF groups, and uses SSU rRNA genes. Some commonly used primers are given in Table 1. However, some workers are sceptical in using only SSU rDNA region as a marker for AMF species resolution (da Silva *et al.,* 2006; Kruger *et al.,* 2009), and advocates the inclusion of ITS and LSU rDNA regions to get both robust phylogenetic analysis and species level resolution. Recently Kruger *et al.* (2009) designed one PCR primer set (SSUmAf, SSU mCf, LSUmAr and LSUmBr) that amplifies SSU-ITS-LSU fragment of all known AMF lineages. This is a further advancement in primer designing as these primers can also potentially be applied as DNA barcoding primers which could be a breakthrough for molecular community analyses opening up new vistas in the study of ecology, phylogeny and diversity of AMF.

Table 1: Details of some commonly used primers for PCR studies

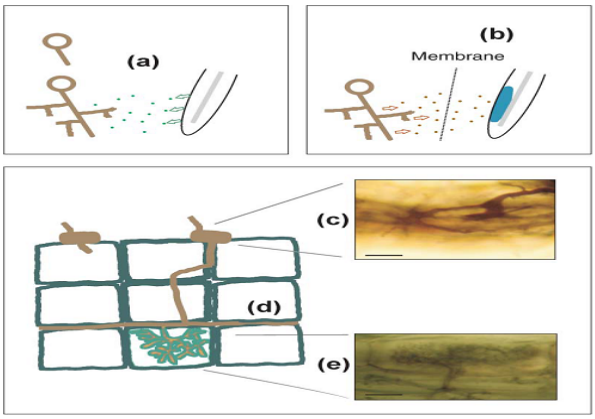
Primer	Sequence (5' - 3')	Target Group	Reference
ITS4	TCCTCCGCTTATTGATATGC	Eukaryote Univ.	White *et al.* (1990)
AM1	GTTTCCCGTAAGGCGCCGAA	Fungi	Hegalson *et al.* (1998)
NS31	TTGGAGGGCAAGTCTGGTGCC	Eukaryote Univ.	Simon *et al.* (1992)
GLOM1310	AGC TAG GYC TAA CAT TGT TA	*Glomus* groupA	
GLOM5.8-R	TCC GTT GTT GAA AGT GAT	*Glomus* group A	Redecker (2000, 2004)
LETC1670	CGG TGA GTA GCA ATA TTC G	*Glomus* group B	
ACAU1660	TGA GAC TCT CGG ATC GGG	Acaulosporaceae	
ARCH1311	TGC TAA ATA GCT AGG CTG C	*Archaeospora*	
GIGA5.8-R	ACT GAC CCT CAA GCA KGT	Gigasporaceae	
VANS1	GTCTAGTATAATCGTTATACAGG	*Glomeromycota* Lineages	Simon *et al.* (1992)
AML1	ATC AAC TTT CGA TGG TAG GAT AGA	All AMF groups	Li *et al.* (2008)
AML2	GAA CCC AAA CAC TTT GGT TTC C		
28G1	CATGGAGGGTGAGAATCCCG	*Glomeromycota*	da Silva *et al.* (2006)
28G2	CCATTACGTCAACATCCTTAACG	*Glomeromycota*	da Silva *et al.* (2006)
SSUmAf	TGG GTA ATC TT**T** TGA AAC TT**Y** A: TGG GTA ATC TT**R** TGA AAC TT**C** A (equimolar)	All AMF lineages	Kruger *et al.* (2009)
SSUmCf	T **CGC** TCT T**C**A ACG AGG AAT C: TAT **TGT** TCT T**C**A ACG AGG AAT C: TAT **TGC** TCT T**N**A ACG AGG AAT C (equimolar)	All AMF lineages	Kruger *et al.* (2009)
LSUmAr	GCT C**AC** ACT CAA **AT**C TAT C**A**A **A**: GCT C**TA** ACT CAA **TT**C TAT C**G**A **T**: T GCT C**TT** ACT CAA **AT**C TAT C**A**A **A**: GCT C**TT** ACT CAA **AC**C TAT C**G**A (equimolar)	All AMF lineages	Kruger *et al.* (2009)
LSUmBr	**D**AA CAC TCG CA**T** A**T**A TG**T** TAG A: AA CAC TCG CA**C** A**C**A TG**T** TAG A: AA CAC TCG CA**T** A**C**A TG**T** TAG A: AAA CAC TCG CA**C** A**T**A TG**T** TAG A: AA CAC TCG CA**T** A**T**A TG**C** TAG A (equimolar)	All AMF lineages	Kruger *et al.* (2009)

5. Functional symbiosis

Arbuscular mycorrhiza is probably the most widespread terrestrial symbiosis and is formed by 70–90% of land plant species (Smith and Read, 2008). Understanding how AM symbiosis is established is a key issue in plant development, and with the advent of innovative techniques in molecular biology rapid progress is underway (Figure 2). To facilitate the use of AMF efficiently for sustainable agriculture, it is necessary to understand the molecular mechanism of symbiosis. This comprises the genetic basis of both partners, the expression patterns of their genes during symbiosis and how these patterns are modified and regulated by signals from respective partner and from the environment (Reddy *et al.,* 2005). Evidently, such a symbiosis is only possible if the plant and the fungus are able to communicate with each other. AM development is accompanied by an exchange of signalling molecules

between the symbionts. A novel class of plant hormones known as strigolactones are exuded by the plant roots (Akiyama *et al.*, 2005; Besserer *et al.*, 2006) which induce fungal spore germination and hyphal branching and increases physiological activity in fungal spores and hyphae (Parniske, 2008). At about the same time, fungus replies the plant by releasing yet unknown signalling molecules that trigger symbiotic root response. There is currently much interest in the molecular identification of fungal signalling molecules that induce symbiosis-specific responses in host root. These fungal signalling molecules, called mycorrhiza factors (*Myc* factors), activate plant symbiosis-related genes (Kosuta *et al.*, 2003) by transcriptional activation (Parniske, 2008). Once the partners have recognised each other, the fungus forms a swollen hyphal structure called hyphopodium on the root surface. From there, it enters the root and spreads through the inner cortex where it develops tree-like structures called arbuscules by invaginating the plant plasma membrane of cortical cells. Plant receptors for pre-symbiotic *Myc* factors have not yet been characterized. However, plant genes required for AM development have been characterized. At least seven plant genes required for AM symbiosis have been identified in legumes (Kistner *et al.*, 2005). These genes encode proteins that are directly or indirectly involved in signal transduction network between the symbionts. Plant mutants which are defective in common symbiosis gene are characterized by an early block of fungal infection in the outer cell layers (Kistner *et al.*, 2005). For example, phenotypic analysis of *Medicago tranculata* symbiotic mutants shows that the common *SYM* genes *DMI2* and *DMI3* are required for pre-penetration apparatus (PPA) induction (Genre *et al.*, 2005) and that *DMI3* is required for a subset of genes to be induced during PPA formation (Siciliano *et al.*, 2007). We are beginning to understand the role played by the plant genes (*SYMRK, CASTOR, POLLUX, NUP85, NUP133, CCaMK and CYCLOPS*) on AM symbiosis development, primarily, by studying plant mutants like *Lotus japonicas*, *Medicago truncatula* and *Pisum sativum*. Plant genetics will continue to be a major tool in the identification of genes that are required for AM development and function. For in-depth discussions on molecular aspects of AM symbiosis, readers are referred to comprehensive reviews by Parniske (2008) and Paszkowski (2006).

Figure 2: Stages of root colonization by an arbuscular mycorrhizal (AM) fungus. (a) Hyphal branching occurs upon perceiving plant-released strigolactone; (b) p*ENOD11*::GUS expression upon perceiving Myc-factor(s); (c) appressoria formation and passage through outer root cell layers; (d) longitudinal apoplastic fungal spreading; (e) arbuscule formation in the inner cortex. Microphotographs display chlorazole black E stained rice roots colonized by *Glomus intraradices*. Bars, 25 mm. (*Source*: Paszkowski, 2006).



6. Phylogeny

Phylogeny is the evolutionary history of an organism. All members of *Glomeromycota* phylum are obligate biotrophs and require a living photoautotrophic partner to complete their life cycle. These fungi have existed for more than 400 million years in/on the single niche "plant root" without undergoing morphological alteration and could therefore qualify as living fossils. Arbuscular mycorrhiza, thus, is an ancient symbiosis (Parniske, 2008) and fossils record of early land plants from the Rynie chart in Scotland provided evidence about the existence of AM fungal structures (arbuscules and spores) about 400 million years ago (Remy *et al.,* 1994; Dotzler *et al.,* 2006). The colonization of land by plants perhaps took place in the company of these fungi, and it was one of the pivotal milestones that fundamentally altered the environmental conditions on earth, paving way for life to evolve on land. Recently, Wang et al. (2010) provided strong evidence that plant-mycorrhizal fungus symbiosis was one of the key processes that contributed to the origin of land flora. They investigated the evolutionary histories and functional conservation of three genes required for mycorrhiza formation in legumes and rice (*Oryza sativa*), *DMI1*, *DMI3* and *IPD3* and showed that these mycorrhizal genes were present in the common ancestor of land plants, and that their functions were largely conserved during land plant evolution. Molecular clock data based on the nucleotide sequence of 18s r DNA suggests that members of *Glomeromycota* arose sometime in between 350-460 million years ago (Simon *et al.,* 1993). Recent molecular clock data by Redecker *et al.* (2000) and Heckman *et al*. (2001) estimates that the origin of Glomeromycota have occurred around 600 million years ago and raises the possibility that they have evolved before land plants. It is interesting to note that *Geosiphon pyriformis*, a glomeromycotan fungus that lives symbiotically with cyanobacterium, *Nostoc punctiforme* is an ancient fungus-bacteria interaction which might have preceded the AM symbiosis (Parniske, 2008). In this context the phylogeny of *G. Pyriformis* is of special interest. Based on sequence analysis of SSU rRNA, it was confirmed that *G. Pyriformis* is a member of *Glomeromycota* (Gehrig *et al.,* 1996).

Recently AMF has been reclassified from the polyphyletic phylum *Zygomycota* to a new monophyletic phylum, the *Glomeromycota* (Schüβler *et al.,* 2001). This phylum was proposed after analysis of a large data set of 18S rRNA gene sequences of all known groups of fungi. To date, more than 215 AM species have been described (http://www.lrzmuenchen.de/~schuessler/amphylo/amphylogeny.html) and are classified in four orders encompassing six families and eight genera (Table 2). The largest genus within this phylum, *Glomus,* as originally defined by the spore morphology and formation was revealed to be polyphyletic according to molecular data and has subsequently been subdivided into three separate groups- *Glomus* groups A, B and C (Schwarzott *et al.,* 2001). Among the four orders that are currently recognized, the Archaeosporales and Paraglomerales are clearly distinct from the subgroup Glomerales and Diversisporales (Figure 3). The phylogeny and taxonomy of AM fungi is still under substantial debate. For example, (1) owing to the significant divergence among the Glomeraceae, this family will probably be taxonomically separated in the future (Parniske, 2008). (2) The family Gigasporaceae which consisted of the two genera *Gigaspora* and *Scutellospora* when first erected was recently revised, in which *Scutellospora* was divided into three families and four genera (making a total of four families and five genera in Gigasporaceae) based on phylogenetic patterns of coevolving small and large rRNA genes and morphology of spore germination shields (Oehl *et al.,* 2008). However, this reclassification has been shown to be untenable by construction of a phylogenetic tree from concatenated 25S rRNA and β-tubulin gene sequences using 35% of known species in Gigasporaceae as well as morohological character (Morton and Msiska, 2010). According to evolutionary theory, the advantage of sex is that recombination

produces genetic variation on which natural selection acts upon. Most asexual lineages have become extinct, primarily due to lack of recombination, an increase in deleterious mutations, and low adaptability to changing environment compared to sexual organisms (Kondrashov, 1982; Hamilton, 1980; Muller, 1964). AMF are ancient putative asexuals and their evolution defies the established predictions of the theory. These clonal organisms show high intraspecific polymorphism in the rDNA encoding ITS, and the 5.8S and 18S subunits (Sanders, 1995, 1999). The evolutionary processes responsible for the origin and maintenance of such high nuclear rDNA intraspecific polymorphism in AM fungi are still unknown and are the subject of an ongoing debate (Hijri *et al.*, 2005; Pawlowska *et al.*, 2004; Rosendahl, 2008). Some investigators believe that hyphal anastomosis and exchange of nuclei between hyphae of closely related fungal strains are possibly the cause of genetic material exchange and recombination (de la Providencia *et al.*, 2005; Giovannetti *et al.*, 2004). The lack of phylogenetic framework in the study of AM fungi intraspecific polymorphism has hampered the elucidation of evolutionary processes that generate such polymorphism.

Figure 3: Phylogenetic relationships between members of the Glomeromycota (Adapted from Schüβler *et al.*, 2001).

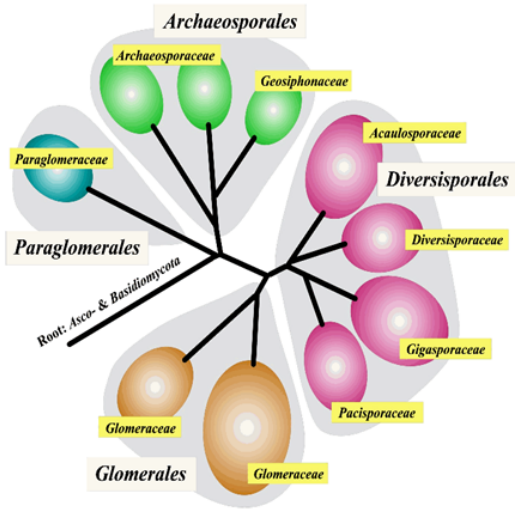


Table 2. Classification of phylum *Glomeromycota* (Hibbett *et al.*, 2007)

Phylum: Glomeromycota C. Walker & A. Schuessler
Class: Glomeromycetes Cavalier-Smith
Order: Archaeosporales C. Walker & A. Schuessler
Exemplar genera: *Archaeospora* J.B. Morton & D. Redecker, *Geosiphon* F. Wettst.
Order: Diversisporales C. Walker & A. Schuessler
Exemplar genera: *Acaulospora* Gerd. & Trappe, *Diversispora* C. Walker & A. Schüβler, *Gigaspora* Gerd. & Trappe, *Pacispora* Oehl & Sieverd.
Order: Glomerales J. B. Morton & Benny
Exemplar genus: *Glomus* Tul. & C. Tul.
Order: Paraglomerales C. Walker & A. Schuessler, (in Schüβler *et al.*, 2001).
Exemplar genus: *Paraglomus* J. B. Morton & D. Redecker

7. Conclusions and future prospects

There has been much progress in the taxonomy of AMF, which is limited to recording organism's occurrence and identity, based on spore morphology and ribosomal sequence markers. Taxonomic diversity has a limited scope as we are still unable to determine the functional significance of the presence and abundance of mycorrhizal fungi to ecosystem functioning. Future studies should focus on resolving the functional diversity of AMF and, thereby, reveal the keystone ecological position of AM symbiosis. A shift towards plant and fungal protein-encoding genes can serve as molecular trait indicators, and will open opportunities to understand mycorrhizal contributions to ecological processes in natural and managed ecosystem. A number of genes which are involved in the uptake of phosphorus and nitrogen, carbon cycling and overall metabolic activity can serve as potential functional marker genes (Gamper *et al.,* 2009).

The biological species concept of AMF is still unresolved. Because of the frequent overlap of the sequence haplotypes among related species, it is quite difficult to define species boundary for AMF by present molecular methods. Identifying new marker genes that provide clear differentiation of genetic variation among intra- and inter-species is the need of the hour. We can overcome the present limitation of the molecular methods by a combination of quantitative and qualitative data achieved by both molecular and microscopy methods. These will probably the best choice to characterize the AMF guild in plant roots, since advantage of each technique may complement the drawbacks of the other (Gamper *et al.,* 2009). In this context recent investigation by Morton and Msiska (2010) on revision of classification of family Gigasporaceae (Glomeromycota) was based on both molecular characters and morphological data and it refuted the earlier classification of Gigasporaceae by Oehl et al. (2008). Therefore, at least for the construction of a true evolutionary tree conserved dataset of molecular and morphological traits should be given equal consideration. Another desirable future goal is to unlock the potential of AM technology for sustainable agriculture and environmental management by identifying the key molecular players that are required for AM development and function. In this context, the long term aim would involve designing of crop-fungus combination with optimized AM performance (Parniske, 2008). This would substantially reduce the application of fertilizer and energy input which is a mandatory goal in the face of global climate change and depleting non-renewable resources.

References

Aguilar-Fernandez, M., V.J. Jaramillo, L. Varela-Fregoso and M.E. Gavito, 2009. Short-term consequences of slash-and-burn practices on the arbuscular mycorrhizal fungi of a tropical dry forest. Mycorrhiza, 19(3):179-186.

Akiyama, K., K. Matsuzaki and H. Hayashi, 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature, 435: 824–827.

Aldrich-Wolfe, L., 2007. Distinct mycorrhizal fungal communities on new and established host species in a transitional tropical plant community. Ecology, 88: 559-566.

Allen, E.B., E. Rincon, M.F. Allen, A. Perez-Jimenez and P. Haunte, 1998. Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. Biotropica, 30: 261-274.

Anderson, I.C., and J.W.G. Cairney, 2004. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ Microbial, 6: 769-779.

Appoloni, S., Y. Lekberg, M.T. Tercek, C.A. Zabinski and D. Redecker , 2008. Molecular Community Analysis of Arbuscular Mycorrhizal Fungi in Roots of Geothermal Soils in Yellowstone National Park (USA). Microb Ecol., 56: 649-659.

Becard, G., and A. Fortin, 1998. Early events of vesicular arbuscular mycorrhiza

formation on RiT-DNA transformed roots. New Phytol., 108: 211-218.

Besserer, A., *et al.* 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PloS Biol., 4: 1239–1247.

Clapp, J.P., J.P.W. Young, J.W. Merryweather and A.H. Fitter, 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol., 130: 259–265.

Clapp, J.P., A.H. Fitter and J.P.W. Young, 1999. Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. Mol. Ecology, 8: 915–922.

Clapp, J.P.T., T.J. Daniell and J.P.W. Young, 2002. Genetic studies of the structure and diversity of arbuscular mycorrhizal fungal communities. In: van der Heijden MGA, Sanders IR, eds. *Mycorrhizal ecology*. Heidelberg, Germany : Springer–Verlag, 201-224.

Cuenca, G., Z. DeAndrade and G. Escalante, 1998. Arbuscular mycorrhizaein the rehabilitation of fragile degraded tropical lands. Biol. Fertil. Soil, 26: 107-111.

da Silva, G.A., E. Lumini, L.C. Maia, P. Bonfante and V. Bian ciotto, 2006. Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. Mycorrhiza, 16: 183-189.

de la Providencia, E. G., F.A. de Souza, F. Fernandez, N. S. Delmas and S. Declerck, 2005. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. New Phytol., 165:261–271.

de Souza, F.A., G.A. Kowalchuk, P. Leeflang, J.A. van Venn and E. Smith, 2004. PCR-Denaturing Gradient Gel Electrophoresis profiling of inter- and intraspecies 18S rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus *Gigaspora.* Appl. Environ. Microbiol., 70: 1413-1424.

Dickie, I.A. and R.G. Fitzjohn, 2007. Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: a methods review. Mycorrhiza, 17: 259-270.

Dickie, I.A., W. Xu and R.T. Koide, 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soils as shown by T-RFLP analysis. New Phytol., 156: 527-535.

Dotzler, N., M. Krings, T.N. Taylor and R. Agerer, 2006. Germination shields in *Scutellospora* (Glomeromycota: Diversisporales, Gigasporaceae) from the 400 million-year-old Rhynie chert. Mycol. Prog., 5, 178–184.

Edwards, S.G., A.H. Fitter and J.P.W. Young, 1997. Quantification of an arbuscular mycorrhizal fungus, *Glomus mosseae* within plant roots by competitive polymerase chain reaction. Mycol. Res., 10: 1440-1444.

Gamper, H. and A. Leuchtmann, 2007. Taxon-specific PCR primers to detect two inconspicuous arbuscular mycorrhizal fungi from temperate agricultural grassland. Mycorrhiza, 17: 145–152.

Gamper, H.A., M.G.A. van der Heijden and G.A. Kowalchuk, 2010. Molecular trait indicators: moving beyond phylogeny in arbuscular mycorrhizal ecology. New Phytol., 185: 67-82.

Gamper, H.A., J.P.W. Young, D.L. Jones and A. Hodge, 2008. Real-time PCR and microscopy: are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? Fungal Genetics Biol., 45: 581–596.

Gandolfi, A., I.R. Sanders, V. Rossi and P. Menozzi, 2003. Evidence of recombination in putative ancient aseuals. Mol. Biol. Evol., 20: 754-761.

Gehrig, H., A. Schüßler and M. Kluge, 1996. *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (cyanobacteria) is an ancestral member of the Glomales: Evidence of SSU rRNA analysis. J. Mol. Ecol., 43: 71-81.

Genre, A., M. Chabaud, T. Timmers, P. Bonfante and D.G. Barker, 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell, 17: 3489–3499.

Giovannetti, M., C. Sbrana, L. Avio and P. Strani, 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. New Phytol., 164: 175–181.

Gollotte, A., D. van Tuinen and D. Atkinson, 2004. Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza, 14: 111–117.

Grime, J.P., J.M.L. Mackey, S.H. Hiller and D.J. Read, 1987. Floristic diversity in a model system using experimental microcosms. Nature, 328: 420-422.

Guadarrama, P. and F.J. Alveraz-Sanchez, 1999. Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. Mycorrhiza, 8: 267-270.

Hamilton, W.D., 1980. Sex versus non-sex parasite. Oikos, 35: 282-290.

Harrison, M.J., 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annual Rev. Plant Physiol. Plant Mol. Biol., 50: 361-389.

Heckman, D.S. *et al*., 2001. Molecular evidence for the early colonization of land by fungi and plants. Science, 293: 1129–1133.

Helgason, T., T.J. Daniell, R. Husband, A.H. Fitter and J.P.W. Young, 1998. Ploughing up the wood-wide web? Nature, 394: 431.

Helgason, T., J.W. Merryweather, J. Denison, P. Wilson, J.P.W. Young and A.H. Fitter, 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J. Ecol., 90:371-384.

Helgason, T., A.H. Fitter and J.P.W. Young, 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides* nonscripta (bluebell) in a seminatural woodland. Mol. Ecol., 8: 659–666.

Helgason, T. and A.H. Fitter, 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Glomeromycota). J. Exp. Bot., 60 (9): 2465-2480.

van der Heijden, M.G.A. and T.R. Horton, 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. J. Ecol., 97: 1139-1150.

van der Heijden, M.G.A., J.N. Klironomos, M. Urisic, P. Moutoglis, R. Streitwolf–Engel, T. Boller, A. Weimken and I.R. Sander, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. Nature, 396: 69-72.

Hempel, S., C. Renker and F. Buscot, 2007. Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem. Environ. Microbiol., 9: 1930–1938.

Hibbett, D.S. *et al*., 2007. A higher level phylogenetic classification of fungi. Mycorrhizal Res., 111: 509-547.

Hijri, M. and I.R. Sanders, 2005. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature, 433, 160–163.

Hijri, I., Z. Sykorova, F. Oehl, K. Ineichen, P. Mader, A. Wiemken and D. Redecker, 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol. Ecol., 15: 2277–2289.

Husband, R., E.A. Herre, S.L. Turner, R. Gallery and J.P.W. Young, 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol. Ecol., 11: 2669-2678.

Kistner, C. *et al*., 2005. Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. Plant Cell, 17: 2217–2229.

Kjøller, R. and S. Rosendahl, 2000. Detection of arbuscular mycorrhizal fungi (*Glomales*) in roots by nested PCR and SSCP (single stranded conformation polymorphism). Plant Soil, 226: 189–196.

Kondrashov, A.S., 1982. Selection against harmful mutation in large sexual and asexual populations. Genetical Res., 40: 325-332.

Konig, S., T. Wubet, C.F. Dormann, S. Hempel, C. Renker and F. Buscot, 2010. TaqMan Real-Time PCR Assays to assess Arbuscular Mycorrhizal responses to field manipulation of grassland biodiversity: Effects of soil characteristics, plant species richness, and functional traits. Appl. Environ. Microbiol., 76: 3765-3775.

Kosuta, S. *et al*., 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula*. Plant Physiol., 131, 952–962.

Krüger, M., H. Stockinger, C. Krüger and A. Schüßler, 2009. DNA-based species level detection of *Glomeromycota*:one PCR primer set for all arbuscular mycorrhizal fungi. New Phytol., 183: 212-223

Lanfranco, L., M. Delpero and P. Bonfante, 1999. Intrasporal variability of ribosomal sequences in the endomycorrhizal fungus *Gigaspora margarita*. Mol. Ecol., 8: 37-45.

Lee, J., S. Lee. and J.P.W. Young, 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol, 65: 339–349.

Lekberg, Y., R.T. Koide, J.R. Rohr, L. Aldrich-Wolfe and J.B. Morton, 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. J. Ecol., 95: 95-105.

Li, L.F., T. Li and Z.W. Zhao, 2007. Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land and an old field and a never cultivated field in a hot and arid ecosystem of South West China. Mycorrhiza, 17: 655-665.

Lindahl, B.D., K. Ihrmark, J. Boberg, S.E. Trumbore, P. Hogberg, J. Stenlid and R.D. Finlay, 2006. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol., 173: 611-620.

Lloyd-Macgilp, S.A., S.M. Chambers, J.C. Dodd, A.H. Fitter, C. Walker and J.P.W. Young, 1996. Diversity of the ribosomal internal transcribed spacers within and among isolates of Glomus mosseae and related mycorrhizal fungi. New Phytol., 133: 103-111.

MacLean, D., J.D.G. Jones, D.J. Studholme, 2009. Application of 'next-generation' sequencing technologies to microbial genetics. Nature, 7: 287-296.

Morton, J.B. and Z. Msiska, 2010. Phylogenies from genetic and morphological characters

do not support a revision of Gigasporaceae (Glomeromycota) into four families and five genera. Mycorrhiza, 20: 483–496.

Muller, H.J., 1964. The relation of recombination to mutational advance. Mutation Res., 1: 2-9.

Muyzer, G. and K. Smalla, 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Van Leeuwenhoek, 73: 127-141.

Newsham, K.K., A.H. Fitter and A.R. Watkinson, 1995. Multi – functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol. Evolution, 10: 407-411.

Oehl, F., F.A. de Souza and E. Sieverding, 2008. Revision of Scutellospora and description of five new genera and three new families in the arbuscular mycorrhizal-forming Glomeromycetes. Mycotaxon, 106: 311–360.

Opik, M., M. Metsis, T.J. Daniell, M. Zobel and M. Moora, 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytol., 184: 424–437.

Opik, M., M. Moora, M. Zobel, I. Saks, R. Wheatley, F. Wright and T. Daniell, 2008. High diversity of arbuscular mycorrhizal fungi in a boreal herbrich coniferous forest. New Phytol., 179: 867–876.

Orita, M., H. Iwahana, H. Kanazawa, K. Hayashi and T. Sekiya, 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-stranded conformation polymorphisms. Proc. Nat. Acad. Sci., 86: 2766-2770.

Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature, 6: 763-775.

Paszkowski, U., 2006. A journey through signaling in arbuscular mycorrhizal symbioses. New Phytol., 172: 35-46.

Pattison, G.S., K.A. Hammill, B.G. Sutton and P.A. McGee, 2004. Growth and survival of seedlings of native plants in an impoverished and highly disturbed soil following inoculation with arbuscular mycorrhizal fungi. Mycorrhiza, 14: 339-346.

Pawlowska, T.E. and J.W. Taylor, 2004. Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. Nature, 427: 733–737.

Picone, C., 2000. Diversity and abundance of arbuscular mycorrhizal fungus spores in Tropical Forest and Pasture. Biotropica, 32: 734-750.

Pietikainen, A., M.M. Kytoviita, R. Husband and J.P.W. Young, 2007. Diversity and persistence of arbuscular mycorrhizas in low-Artic medow habitat. New Phytol., 176: 691-698.

Reddy, S.R., P.K. Pindi and S.M. Reddy, 2005. Molecular methods for research on arbuscular mycorrhizal fungi in India: problems and prospects. Current Sci., 89: 1699-1709.

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

Redecker, D., 2002. Molecular identification and phylogeny of arbuscular mycorrhizal fungi. Plant Soil, 244: 67–73.

Redecker, D., R. Kodner and L.E. Graham, 2000. Glomalean fungi from the Ordovician. Science, 289: 1920–1921.

Redecker, D., H. Thierfelder, C. Walker, D. Werner, 1997. Restriction analysis of PCR-amplified internal transcribed spacers of ribosomal DNA as a tool for species identification in different genera of the order Glomales. Applied Environ. Microbiol., 63: 1756-1761.

Remy, W., T.N. Taylor, H. Hass and H. Kerp, 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci., 91: 11841-11843.

Renker, C., J. Heinrichs, M. Kaldorf and F. Buscot, 2003. Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. Mycorriza,13: 191-198.

Rosendahl, S., 2008. Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytol., 178: 253–266.

Rosendahl, S. and H.B. Matzen, 2008. Genetic structure of arbuscular mycorrhizal populations in fallow and cultivated soils. New Phytol., 179: 1154–1161.

Rosendahl, S. and E.H. Stukenbrock, 2004. Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. Mol. Ecol., 13: 3179–3186.

Sanders, I.R., 1999. No sex please, we're fungi. Nature, 399: 737-739.

Sanders, I.R., 2002. Ecology and evolution of multigenomic arbuscular mycorrhizal fungi. Am. Naturalist, 160: S128-S141.

Sanders, I.R., M. Alt, K. Groppe, T. Boller and A. Wiemken, 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol., 130: 419-427.

Schüßler, A., 1999. Glomales SSU rRNA gene diversity. New Phytol., 144: 205-207.

Schüßler, A., H. Gehrig, D. Schwarzott and C. Walker, 2001a. Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol. Res., 105: 5–15.

Schüßler, A., D. Schwarzott and C. Walker, 2001b. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol. Res., 105: 1413–1421.

Schwarzott, D., C. Walker and A. Schüßler, 2001. Glomus, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is non-monophyletic. Mol. Phylogenetics Evol., 21: 190-197.

Siciliano, V., *et al.*, 2007. Transcriptome analysis of arbuscular mycorrhizal roots during development of the prepenetration apparatus. Plant Physiol. 144, 1455–1466.

Simon, L., M. Lalonde and T.D. Bruns, 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing root. Appl. Environ. Microbiol., 58: 291-295.

Simon, L., R.C. Levesque and M. Lalonde, 1993. Identification of endomycorrhizal fungi colonizing roots by fluorescent single-stranded conformation polymorphism-polymerase chain reaction. Appl. Environ Micobiol., 59: 4211-4215.

Singh, S., A. Pandey, B. Chaurasia and L.M.S Palni, 2008. Diversity of arbuscular mycorhizal fungi associated with the rhizosphere of tea growing in 'natural' and 'cultivated' ecosites. Biol. Fertil. Soils., 44: 491-500.

Smith, S.E. and D.J. Read, 2008. Mycorrhizal Symbiosis, 2nd edn. London, UK : Academic Press.

St Arnaud, M., C. Hamel, B. Vimard, M. Caron and J.A. Fortin, 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in absence of host roots. Mycol. Res., 100: 328-332.

Su, Y.Y. and L.D. Guo, 2007. Arbuscular mycorrhizal fungi in non-grazed, restored and over-grazed grassland in the Inner Mongolia Steppe. Mycorrhiza, 17: 689-693.

Tehabi, A., D. Coyne, F. Hountondjii, L. Lawouin, A. Wiemkem and F. Oehl, 2008. Arbuscular mycorrhizal fungal communities in sub-saharan savnnas of Benin, West Africa, as affected by agricultural land use intensity and ecological zone. Mycorrhiza, 18: 181-195.

Tian, H., J.P. Gai, J.L. Zhang, P. Christie and X.L. Li, 2009. Arbuscular mycorrhizal fungi in degraded typical steppe of inner Mongolia. Land Degrad. Dev., 20: 41-54.

van Tuinen, D., E. Jacquot, B. Zhao, A. Gollotte and V. Gianinazzi-Pearson, 1998. Characterization of root colonization profiles by a microcosm of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Mol. Ecol., 7: 879-887.

Walker, C. and A. Schüβler, 2004. Nomenclatural classifications of new taxa in Glomeromycota. Mycol. Res., 108: 981-982.

Wang, B., L.H. Yeun, J.Y. Jia-Yu Xue, Y. Liu, J.M. Ané and Y.L. Qiu, 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytol., 186: 514-525.

Wang, Y.Y., M. Vestberg, C. Walker, T. Hurme, X. Zhang and K. Lindstrom, 2008. Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan Province of mainland China. Mycorrhiza, 18: 59-68.

White, T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (eds) PCR Protocols, A Guide to Methods and Applications. Academic, San Diego, pp 315-322.

Wubet, T., I. Kottke, D. Teketay and F. Overwinkler, 2003. Mycorrhizal status of indigenous trees in dry Afromontane forests of Ethiopia. For. Ecol. Manage., 179: 387-399.

Zhang, Y., L.-D. Guo and R.-J. Liu, 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, China. Plant Soil, 261: 257-263.