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MOLECULAR PHYLOGENY AND SYSTEMATICS OF GLOMEROMYCOTA : METHODS AND LIMITATIONS

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Abstract

Arbuscular Mycorrhiza (AM) fungi are symbiotically associated with more than 80% of terrestrial plant. AM fungi provide multiple benefits to plant by increasing uptake of nutrients from soil and also enhance level of soil fertility. Currently AM fungi have being used as a potential biofertilizers for sustainable agriculture production. However, AM fungi is ultimate biological organism for organic agricultural practices in cultivated land but identification, screening and mass scaling of specific genotype of AM fungi with desirable function is very tedious and time consuming. Therefore, present study revealed application and limitation of modern molecular tool and suggested some reliable molecular marker with broad taxonomic range for accurate detection of AM fungi from complex environment. Present study may future used as reference data sets for molecular systematics and community analyses in AM fungi collected from complex environmental soil.

Key word: Arbuscular Mycorrhiza fungi (AMF), small subunits (SSU), *Glomeromycota*, internal transcribed spacer (ITS)

Introduction

Phylum *Glomeromycota* comprises all AM fungi, have originated on same time with terrestrial plant evolved during Ordovician period over 430 million years (Simon *et al.*1993; Redecker *et al*. 2000a). Initially discovered AM fungi during (1845–1974) called alpha taxonomy (1975–1989) in which several new genera, families and species were described based on morphological characters of spores. Walker (1983) described spores wall as new characters for mycorrhizal species identification. Next period is known as cladistics period (1990–2000) included new classification of mycorrhizal species based on molecular technique. In this period phylogeny of AM fungi based on morphological characters and separated mycorrhizal fungi from Zygomycota (Morton 1990b). Morton and Benny (1990) classified AM fungi in three families and six genera within one order *Glomerales* of fungal phylum Zygomycota and hypothesized that glomeromycoton fungi comprised monophyletic group. They also described about two sub branch, one branch consisting of mycorrhizal species belonging to *Gigasporaceae* and other sub branch including *Glomus*, *Sclerocystis*, *Acaulospora* and *Entrophospora*. They separated genera of AMF from Endogonales and placed in new order of *Glomales* which do not form any zygospores. In contrast, classification by Walker, (1992) and Simon *et al.* (1993) suggested that *Glomus* was possibly polyphyletic. Important investigations during this period described evolutionary relationship among AM fungi using SSU region of ribosomal DNA. Redecker *et al.* (2000c) based on rDNA sequences separated two ancestral clades of *Acaulospora* consisting of (*Acaulospora trappei* and *Ambispora gerdemannii*) and *Glomus*, comprised *Paraglomus occultum* and *Glomus brasilanum*. The phylogenetic analysis era (2001 to present) known for elucidate classification of AM fungi using combined phenotypic and genetic characters. Morton and Redecker, (2001) proposed new taxa of AM fungi based on congruent of phenotypic and genetic characters. Schwarzott *et al.* (2001) reporte phylogenetic analysis, using full length 18S-rRNA gene suggested *Glomeraceae*

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is largest genus of the AMF and the result indicated that Glomus is not monophyletic but can be separated into three clades. They established the genus *Glomus* as separated into three groups-*Glomus* group A, B and C. *Glomus* groups A and B clustered together single monophyletic clade. The clades of *Glomus* group A further divided into two subclades (GlGrAa and GlGrAb) with 98-100% value. The first (GlGrAa) contains *Funneliformis geosporum, Funneliformis mosseae, Funneliformis fragilistratum, Funneliformis caledonium, Funneliformis coronatum.* The other GlGrAb containing *Rhizophagus intraradices, Rhizophagus proliferus, G. coremiodes, Sclerocystis sinuosa, Rhizophagus vesiculiferus, Rhizophagus clarus and Rhizophagus manihotis*. The second group known as (*Glomus* group B) genetically distance with *Glomus* group A included numerous species of mycorrhiza for example (*Claroideoglomus etunicatum, Claroideoglomus claroideum* and *Claroideoglomus lamellosum*). They also confirmed using n-rDNA *Glomus* group C genetically unrelated to *Glomus* than the *Acaulosporaceae*. Several report confirmed largest number of AM species diversity in *Glomus* lineage (Group A) and suggested dominating fungal communities in agriculture field (Helgason *et al.* 1998 ; Opik *et al.* 2003; Vendenkoornhuyse *et al.* 2002). Furthermore, based on ribosomal DNA sequences Walker and Schüâler, (2004) described one species (*Diversispora spurca*) fell under genus *Diversispora* and also concluded *Gigaspora* and *Scutellospora* are closely related genera in the family of *Gigasporaceae*. Oehl and Sieverding (2004) established new genus *Pacispora* typically *Glomus* like but has flexible inner walls. It was found using rDNA phylogeny *Pacispora* is a basal group of *Gigasporaceae* (Walker *et al.* 2004). The genus *Archaespora* and *Paraglomus* was established by Redecker *et al.* (2000b) basal member of *Glomeromycota*. Schüâler *et al.* (2004) observed that *Geosiphon pyriformis* (Endosymbiont) morphologically similar *Glomus sp*. however it does not fell under *Glomeromycota*. n-rDNA sequencing analysis placed this fungus closer to *Archaespora gerdanni* (Redecker 2002; Schwarzott *et al.* 2001). *Oehl et al.* (2008) reported genus *Scutellospora* to be polyphyletic using combined morphological characters (germination shield) and sequence analysis SSU-LSU region of n-rDNA. Recently, Schüâler and Waker, (2010) performed phylogenetic analysis of glomeromycoton fungi using full length SSU rRNA gene and established a new family and three new genera. They divided *Glomus* into three new genera *Funneliformis*, *Sclerocystis* and *Rhizophagus* in the family of Glomeraceae with other species of *Glomus* and *Claroideoglomus* in the family of *Claroideoglomeraceae*. Oehl *et al.* (2011) using β tubulin, SSU, LSU rRNA and phenotypic traits established new genera of *Simiglomus* and *Septoglomus* in the Glomeraceae they established new genera total three class (*Archaeosporomycetes*, *Glomeromycetes*, and *Paraglomeromycetes*), five orders (*Archaeosporales, Diversisporales*, *Gigasporales*, *Glomerales* and *Paraglomerales*), 14 families, 29 genera and 230 species of Mycorrhiza. List of total number of mycorrhizal species recorded during different period mention in (fig.1) Furthermore, *Krüger et al.* (2012) classified 11 families, 17 genera and approximately 230 species of AMF. *Glomus* is the largest with over 93 morphospecies are mention in table. (1).

Molecular phylogeny of AM fungi using ribosomal DNA: application and limitation

Sequence analysis of nuclear coded ribosomal genes (n-rRNA) widely used methods for the study of microbial diversity. This is due to (n-rRNA) available with multiple copy, single locus and non-protein-coding sequences. Additionally, presence of conserved domain may be useful for study AM fungi having same evolutionary origin (Guarro *et al.* 1999). Molecular study in Glomeromycota using nuclear encoded ribosomal DNA (n-rDNA) is powerful tools for genotyping and phylogenetic analysis. n-rDNA containing 18S (SrRNA), 5.8S, and 28S (LrRNA) genes and arranged in a cluster with internal transcribed spacer (ITS1 and ITS2) shown in (fig. 2). Different region of ribosomal DNA have different properties in term of advantages and disadvantages which are useful for elucidate phylogenetic relationship between and within species of AM fungi. The small subunit (SSU) and large subunit (LSU) n-rDNA region is highly conserved within genera and species therefore it considered to be useful for elucidate taxonomy of distantly related AM texa. However, presence of hyper variable region within internal transcribed spacer (ITS) and D1-D2 domain of large subunit (LSU) widely used region for resolution of AM fungi up to species or strain level. Walker *et al.* (2007) reported that conserved region in SSU and LSU region of ribosomal DNA along with highly variable internal transcribed spacer (ITS) better separate closely related AM species. Currently molecular analysis using sequencing of ribosomal DNA and also including characterization of morphological traits its give holistic approach towards systematic and taxonomy of AM fungi. Moreover, molecular study including cloning and sequencing of r-RNA genes from single spore and colonized roots will help developing new insights of species diversity in AM fungi. In the subsequent studies by Ryberg *et al.* 2009; Stockinger *et al.* (2010) observed

that the fragment covers a partial region of 3' end of SSU, along with complete ITS and D1-D2 domain of LSU used for species level resolution and is suitable region for DNA bar coding. More recent study by Krüger *et al.* (2011) identified 109 unknown AMF species and 27 cultures using 1800 bp fragment spaning SSU-ITS-LSU (three rDNA markers) and concluded these are reliable and robust marker for resolution of AM fungi from phylum to species level. List of LSU-rDNA specific primer previously reported by many authors mentioned in table (3). De Souza *et al.* (2004) observed that PCR-DGGE patterns (based on SSU of rDNA) could be used to differentiate geographic distinct isolates of *Gigaspora* species. Major benefit of this method to identify unknown AM species by sequencing analysis of unique fragment of DNA collected from field soil (Anderson and Cairney, 2004).

Molecular phylogeny of am fungi using non-ribosomal DNA: application and limitation

Various report found range of molecular methods without use of tedious sequencing process have been routinely applied to study the biodiversity in AM fungi. In the study of Helgason *et al.* (1999); Daniell *et al.* (2001) used PCR-RFLP; Vandenkoornhuyse *et al.* (2002, 2003) introduced terminal T-RFLP; Jansa *et al.* (2002) used single standard conformation polymorphism (SSCP); Kowalchuk *et al.* (1997), Ma *et al.* (2005) used denaturing gradient gel electrophoresis (DGGE) and Mathimaran *et al.* (2008) used simple sequence repeat (SSR). Wyss and Bonfante, (1993) identified polymorphism among 30 *Glomus versiforme* and 120 *Gigaspora margarita* by using random amplified DNA polymorphism (RAPD) from DNA obtained from single spore. However, major limitation of this methods for low reproducibility and greater prone towards contamination. Lanfranco *et al.* (1995) resolved this constrain by cloning and design AM species/isolates primer for identification of AM species. Rosendahl and Taylor, (1997) found polymorphism within single AM spore and concluded that AM fungi reproduce clonally without showing recombination. Koch *et al.* (2004) characterized different isolates of *Rhizophagus intraradices* using amplified length polymorphism (AFLP) grown under root organ culture, showed greater genetic diversity among isolates. In contrast, Krüger *et al.* (2009); Mathimaran *et al.* (2008) were used AFLP, SSR and RAPD cannot be recommended for diversity study of sample obtained from environmental soil because of too many unknown in background which hampers of specificity. Sander *et al.* (1995b) found mycorrhizal biodiversity in natural population by using RFLP pattern. Last few decade AM

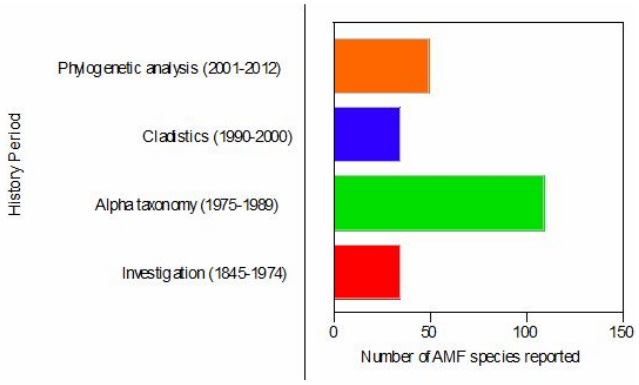

Fig. 1: Number of new AM species of reported in different period. Modified from Stürmer (2012)

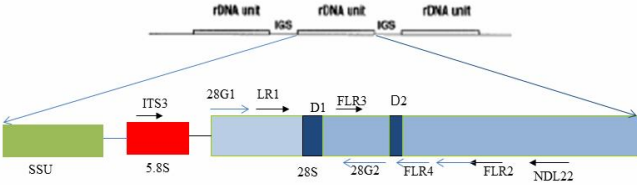

Fig. 2: Location and details of priming sites of Small Subunit - Internal transcribed spacer (SSU,ITS and LSU) region of rRNA genes.

community diversity using T-RFLP become popular tools demonstrated by Vandenkoornhuyse *et al.* (2003); Mummey *et al.* (2005); Mummey and Rillig, (2007). More report Bainard *et al.* (2011) using T-RFLP analysis suggested higher mycorrhizal diversity in agroforestry ecosystem as compared with conventional cultivation practices. They are found r-DNA sequence heterogeneity between different mycorrhizal species. Instead of sequencing, also denaturing gradient gel electrophoresis (DGGE) and PCR single strand conformation polymorphism (PCR-SSCP) represents an alternative method for the characterization of AMF species (de Souza *et al.* 2004). The PCR-SSCP is accomplished with denatured PCR products may subject to the electrophoresis through a non-denaturing polyacrylamide gel. Kowalchuk *et al.* (2002) are found AMF community structure in field soil using PCR-DGGE. They found differences in AM community structure between spores and colonized roots. They suggested low AMF community in spore and showed actual representation of AM community when corroborated with colonized roots. Several report are found protein marker gene could routinely use as biochemical marker for phylogeny and systematic study of AM fungi. Helgason *et al.* (2003) inferred phylogenies from genes encoding α-elongation and actin proteins. They are classified *Acaulosporaceae* and *Glomeraceae* are genetically related. In later study by Corradi *et al.* (2004a, b); Msiska and Morton (2009)

Table 1: Order, family, genera species of *Glomeromycota* (Modified from http://www.lrz-muenchen.de/~schuessler/amphylo/, updated on December, 2010. Details about the species (current names, synonyms and references) are available on the same and from Krüger *et al.* (2012).

Order	Family	Genus	Described species
Diversisporales	*Diversisporaceae*	*Diversispora*	9
		Otospora (Unclear phylogenetic affiliation)	1
	Gigasporacaea	*Gigaspora*	8
		Scutellospora	6
		Racocetra (including Racocetra weresubiae)	7
	Pacisporaceae	*Pacispora*	7
	Acaulosporaceae	*Acaulospora*	38
	Entrophosporaceae	*Enterospora*	3
Glomerales	*Glomeraceae*	*Glomus*	93
		Funneliformis (former Glomus Group Aa, *Glomus mosseae* clade)	11
		Rhizophagus (former Glomus Group Ab, *Glomus intraradices* clade)	11
		Sclerocystis (basal in former Glomus Group Ab)	8
	Claroideoglomeraceae	*Claroideoglomus* (former Glomus Group B, *Glomus claroideum* clade)	10
Archaeosporales	*Archaeosporacaea*	*Archaespora*	2
	Geosiphonacaae	*Geosiphon*	1
	Ambisporaceae	*Ambispora*	8
Paraglomerales	*Paraglomeraceae*	*Paraglomus*	7
Total 4	**11**	**17**	**230**

established phylogenetic relationship among *Glomeromycota* using â-tubulin gene. List of different molecular marker used for AM fungi diversity study mention in table (2).

Molecular tools in am fungi: intraspecies or isolate level

Nuclear encoded ribosomal DNA containing variable as well conserved regions in responsible for speciation in AM fungi. Sander *et al.* (1995); Jansa *et al.* (2002) observed variation in n-rDNA genes within the single individual AM spore. This is due to single spore of AM fungi consisting of large number of slightly different variants result difficulty to distinguished closely related AM fungi. Clapp *et al*. (1995) reported using D2 region of n-rDNA observed high level of intra-isolate variation among *Funneliformis coronatum, Funneliformis mosseae* and *Septoglomus constrictum*. Kuhn *et al.* (2001); Corradi *et al.* (2004); Pawlowska and Taylor, (2004) observed heterogeneity not only in n-rDNA but also being observed in protein coding genes. Several report suggested that protein genes like BiP gene, H^+ ATPase gene and PLS (Pol like sequence) consisting of variable region within organism used as suitable maker in AM fungi. In contrast, study by Stukenbrack and Rosendhal (2005) did not found any sequence heterogeneity within protein coding genes (GmFOX & GmGIN) of *Funneliformis mosseae*. However, it was observed that primers used for this gene have less specificity in PCR. Moreover, paralogs gene structure create further problem for phylogenetic analysis as described for tubulin gene by Corradi *et al.* (2004). Many of these markers are unsuitable because of lack of sufficient variability within species. Therefore, Croll *et al.* (2008b) suggested robust multilocus markers for genotyping of non-ribosomal loci. Mathimaran *et al.* (2008) and Croll *et al*. (2008b) developed simple sequence repeats (SSR) markers for the identification of different *Rhizophagus intraradices* isolates grown under root organ culture. In the study by Mathimaran *et al.* (2008a) investigated using only eight mycorrhizal isolates originated from seven locations of four countries and suggested two AM isolates have similar genotype. In contrast, study by Croll *et al.* (2008) used 48 different set of SSR markers on AM fungi originated from same field of Tänikon, Switzerland, of which 18 unique genotypes were recognized. Besides, microsatellite repeat, mitochondrial genes can be suitable alternative

Table 2: Details of some PCR based molecular studies reported in AMF.

Molecular marker	Primer	Target organism	References
RAPD PCR	RAPD primer	*Funneliformis mosseae*	Lanfranco *et al.*1995
RAPD PCR	M13 Minisatellite RAPD primer	*Gigaspora margarita* and *Gigaspora gigantea*	Gadkar *et al.*1997
PCR	VANS1- NS21	*Glomales*	Simon *et al.*1992
PCR	VANS1- NS22	*Rhizophagus intraradices*	Di Bonito *et al.* 1995
PCR	NS1-NS2	*Glomales*	Schüâler *et al.* 2001
PCR- Nested	GeoA1-ART4	*Glomales*	Schwarzott and Schüβler 2001
PCR- Nested	ITS-AM1	*Glomus sp*	Redecker 2002
PCR	AML1-AML2	*Glomeromycota*	Lee *et al.* 2008
PCR-RLFP	ITS1-ITS4	*Glomus sp, Scutellospora , Gigaspora sp.*	Redecker *et al.* 1997
PCR	ITS1-ITS4	*Gigaspora*	Lanfranco *et al.*2001
PCR- Nested	GLOM1310-ITS4i, LETC 1770-ITS4i, GIGA5.8R-NS5	*Rhizophagus, Funneliformis, Claroideoglomus, Gigaspora sp.*	Redecker 2000a
Nested PCR	SSU-Glom/LSU-Glom 1	*Glomeromycota*	Renker *et al.* 2003
PCR- Nested	ITS3-NDL22 , LR1-FLR2	*Glomeromycota*	Jansa *et al.* 2003
PCR	ALF01-NDL22	*Enterospora*	Rodriguez *et al.* 2001
PCR-SSCP	ALF01-NDL22	*Funneliformis coronatum*	Clapp *et al.* 2001
PCR- Nested	0061-NDL22, rk4f and rk7mr	*Glomus sp*	Rosendhal and Stukenbrock 2004
PCR	28G1-28G2	*Glomeromycota*	Da Saliva *et al.* 2006
PCR- Nested	SSUmAf-LSUmAr, SSUm Cf-LSUmBr	*Glomeromycota*	Krüger *et al.* 2009
PCR- Nested	SSUmCf-LSUmBr, SSU-Glom1-NDL22	*Glomerales*	Krüger *et al.* 2012
PCR- Nested	SSUmAf, SSUmCf, LSUmAr, LSUmBr	*Glomeromycota*	Wang *et. al.* 2015
PCR- Nested	SSUmAf-LSUmAr,LSUmBr	*Acaulospora sp.,Cetraspora nodosa, Claroideoglomus, Rhizophagus sp.*	Senés-Guerrero *et. al.* 2016
PCR- Nested	SSUmAf-LSUmAr, ?SSUmCf-LSUmBr ; GeoA2-Geo11, NS31-AM1; AML1-AML2, NS31-AM1; AMV4.5NF-AMDGR	*Glomeromycota*	Xiang *et.al.* 2016
PCR- Nested	SSUmAf-LSUmAr, SSUmCf-LSUmBr	*Glomeromycota*	Garcés-Ruiz *et.al.* 2017

marker to distinguish closely related species/isolate of AM fungi. In the first study by Raab *et al.,* (2005) documented mt-LSU sequences obtained from different isolates of *Rhizophagus intraradices* and *Rhizophagus proliferus* and observed substantial variation in the mt-LSU region among isolates of same species.

Mycorrhizal primers: merit and demerit

Since year 1990 mainly universal eukaryotic primers were used for mycorrhizal phylogenetic and biodiversity studies (White *et al.* 1990; Simon *et al.* 1995, Schwarzott *et al.* 2001). In these periods several literatures surveyed mycorrhizal diversity using fungus specific universal primer consisting of SSU (Small subunit) and ITS (Internal transcribed spacer) region of ribosomal DNA shown in table (2 and 3) . However most of time single step PCR reaction are failed or does not produce amplicon.

Table 3: Specific primers used to amplify fragments of the 25S r-DNA in AM fungi (Modified from Source, Covacevich, 2010).

Primer name	Primer sequences (5'-3')	Target group	rDNA region	References
5.21	CCTTTTGAGCTCGGTCTCGTG	*Funneliformis mosseae*	D2 domain of 25 rDNA	Van Tuinen *et al.* 1998
8.22	AAC TCC TCA CGC TCC ACA GA	*Rhizophagus intraradices*	D2 domain of 25 rDNA	Van Tuinen *et al.* 1999
4.24	TGT CCA TAA CCC AAC TTC GT	*Racocetra castanea*	D2 domain of 25 rDNA	Van Tuinen *et al.* 1999
23.22	GAA TCA CAG TCA GCA TGC TA	*Gigaspora rosea*	D2 domain of 25 rDNA	Van Tuinen *et al.* 1998
LSURK4	GGG AGG TAA ATT TCT CCT AAGGC	*Funneliformis mosseae*	D2 domain of 25 rDNA	Kjøller *et al.* 2000
LSU3f	AGT TGT TTG GGA TTG CAG C	Glomus (some sp.)	D2 domain of 25 rDNA	Kjøller *et al.* 2000
LSU6f	AAATTGTTGAAAGGGAAACG	Glomus (some sp.)	D2 domain of 25 rDNA	Kjøller *et al.* 2000
LSU7r	ATC GAA GCT ACA TTC CTC C	Glomus group A (some sp.)	D2 domain of 25 rDNA	Kjøller *et al.* 2000
LSU8r	GGGTATCCGTTGCAATCCTC	Glomus (some sp.)	D2 domain of 25 rDNA	Kjøller *et al.* 2000
LSU0805	CATAGTTCACCATCTTTCGG	Glomus (some sp.)	5' end of 25S rDNA	Kjøller *et al.* 2000
ALF01	GGAAAGATGAAAAGAACTTTGA AAAGAG	*Funneliformis coronatum*	D2 domain of 25 rDNA	Clapp *et al.*2001
cad5.3	TCG CGA AAG CTTGTG	Glomus sp. (near to G.acculatum)	25 rDNA	Tarnau *et al.* 2001
FLR3	TTGAAGGGAAACGATTGAAGT	Glomus (group A and B) Gigasporaceae and Acaulosporaceae not Archaesporaceae	D2 domain of 25 rDNA	Gollotte *et al.* 2004
FLR4	AAGCAATTCCTACAACTGCAT	Glomus (group A and B) Gigasporaceae and Acaulosporaceae not Archaesporaceae	D2 domain of 25 rDNA	Gollotte *et al.* 2004
f6	TAA ATC TCC GAG GTT TCCTTGGC	*Acaulospora paulinae*	5' end of 25S rDNA	Gramper and Leuchtmann, 2007
LSUmAr1	GCT CAC ACT CAA ATC TAT CAA A	*Acaulosporaceae*	5' end of 25S rDNA	Krüger *et. al.* 2009
LSUmAr2	GCT CTA ACT CAA TTC TAT CGA T	*Gigasporaceae*	5' end of 25S rDNA	Krüger *et. al.* 2009
LSUmAr3	T GCT CTT ACT CAA ATC TAT CAA A	*Acaulosporaceae, Diversisporaceae, Geosiphonaceae, Gigasporaceae, Glomeraceae (GlGr*A and Gl *GrB), Pacisporaceae*	5' end of 25S rDNA	Krüger *et. al.* 2009
LSUmAr4	GCT CTT ACT CAA ACC TAT CGA	*Paraglomeraceae*	5' end of 25S rDNA	Krüger *et. al.* 2009

Therefore, nested PCR (Two step PCR) could be prefer using mycorrhizal specific primer for detection of AM fungi. Although, nested PCR frequently used in AMF research to overcome the limitation PCR amplification by enhance the specificity of primer with minute and rare DNA templates of mycorrhizal fungi (Kumar *et al*. 2013) But problem is still completely unrevealed regard of specificity of primer to cover all genera of AM fungi Simon *et al.* (1993) separated AM fungi into four distinct group using SSU r-DNA specific primer (VALETC, VAGLO, VAACAU and VAGIGA) from DNA isolated from plant roots. Furthermore, Helgason *et al.* (1998) also designed fungal specific primer (AM1) were used to diagnostic AM fungi colonizing with plant roots. The AM1 has shown to amplify three families of AMF *(Glomeracae, Gigasporacae and Acaulosporacae)*. However, Redecker *et al.* (2000) and Schüβler *et al.* (2001) found that primer AM1 is not able to all genera of AM fungi. Moreover, Daniell *et al.* (2001) used primers (NS31-AM1) for amplification of all genera of

Glomeromycota although they were not amplified genus belonging to A*rchaeospoaceae* and *Paraglomeraceae.* Redecker (2000) designed specific PCR primers which separated various genera of AM fungi (*Glomus, Acaulospora, Enterospora, Scutellospora* and *sclerocystis*) from plant roots. Saito *et al.* (2004) used primers (AMV4.5F and AMV4.5R) in nested PCR with amplification products (about 650 bp) obtained from fungal DNA. Major limitation of this primer is not specific for all AM fungal species. Hijri *et al.* (2006) collected mycorrhizal DNA from environmental samples and used *Funneliformis* and *paraglomus* specific primers (GLOMBS1670 and PARA1313). Moreover, Wubet *et al.* (2006) compared the diversity of AMF in *juniperus procera* plant sp. collected from two geographically separated sites using nested primers (Glomer WTO). They amplified specific genera of AM fungi (*Diversisssporaceae*, *Glomeraceae*, *Gigasporaceae*, *Pacisporaceae*, *Paraglomeraceae* and *Archeosporaceae*). However, they unable to amplified all genera of *Glomeromycota*. Recently developed primer provides more reliable amplification product (up to 1800 bp) spanning SSU, ITS and LSU rgion of ribosomal DNA for complete resolution of all taxa of mycorhizal fungi (Krüger *et al.* 2012).

Limitation of molecular analysis

Molecular research including genetic diversity of mycorrhizal fungi needs sufficient quantities of genomic DNA. It has been also found that PCR amplification from single spore of *Glomus sp.* most of time failed due to presence of minute quantity of 5-19pg genomic DNA (Hosny *et al.* 1998). It is also well known single spore of AM fungi are multinucleate and consisting of population of genetically different nuclei (Kuhn *et al.* 2001). Jansa *et al.* (2002b) found that sequences obtained from single spore of *Rhizophagus intraradices* JJ291 revealed higher degree of polymorphism as compared to sequences two different isolates of *Rhizophagus intraradices*. Sander, (2002) observed due to r-DNA sequence variation within single AM spore it is very difficult to understand origin of r-DNA sequences collected from complex environment (Landis *et al.* 2004). Moreover, very few species of AM fungi could be growing under Root Organ Culture (ROC) due to their obligate symbiotic nature. Some workers believe, various specific primers targeting SSU, ITS and LSU region of r-DNA has been claimed to be AMF specific but also amplify non target DNA (Kruger *et al.* 2009). Preferential amplification of particular of species/isolates of AM fungi from mixed community may lead to biased assessment of diversity rather than a true reflection of taxonomic diversity within a sample (Anderson and Cairney, 2004). Therefore, Gamper *et al.* (2010) found that due to mixed r-DNA sequences within single AM spore very difficult to define species boundary of mycorrhizal fungi (de Souza *et al.* 2004; Rosendahl, 2008). Furthermore it has been observed that diversity analysis in AM fungi based on RAPD, ISSR and AFLP expected to be error prone due to too many unknown background hampered interpretation of specificity (Mathimaran *et al.* 2008). Similar limitation involve exists DNA array techniques. Moreover, several report concluded that due to highly conserved SSU gene of ribosomal DNA very difficult to resolved upto species level, resulting in the overlapping of similar phylotypes and even genera of mycorrhiza fungi. Therefore, for better species level resolution recently the internal transcribed spacer (ITS) region at the 3'-end of the SSU (AM fungi) has been used as an barcoding gene (Kõljalg *et al.* 2005; Ryberg *et al.* 2009). Recently Tedersoo *et al.* (2010); Öpik *et al.* (2009) used high throughput metagenomics approach for identification of mycorrhizal isolates collected from different agro ecosystem and compared with Sanger methods of sequencing. Report suggested some limitation of traditional sequencing in regard of non-availability gene database for correct identification of AM fungi. Currently, Öpik *et al.* (2010) described different AM fungal communities collected from range of agroclimatic zone with diverse host plants using MaarjAM database and metagenomic approach.

Conclusion

Review suggested identification AM fungi using morphological characters of field-collected spores are generally inadequate because many morphological features that are essential for identification up to the species level can be overlap and also lost in the process. Therefore, limitation can be overcome by developing a single primer with a broad taxonomic range but one that, at the same time, reduces co-amplification of closely related DNA. Current study support that sequencing of all three region (SSU, ITS & LSU) of ribosomal DNA and acceptance of metagenomics approach may be suitable methods to identify some uncultured AM fungi from different habitats and host plant.

References

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 Microbiol,* **6***:* 769-779.

Bainard, L.D., A.M. Koch, A.M. Gordon, S.G. Newmaster, N.V. Thevathasan and J.N. Klironomos (2011). Influence of trees on the spatial structure of arbuscular mycorrhizal

communities in a temperate tree-based intercropping system. *Agr. Ecosyst Environ.,* **144(1)**: 13-20.

Corradi, N., G. Kuhn and I.R. Sanders (2004). Monophyly of *beta-tubulin* and *H+-ATPase* gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. *Fungal Genet Biol.,* **41**:262-273.

Covacevich, F. (2010). Molecular Tools for Biodiversity and Phylogenetic Studies in Mycorrhizas: The Use of Primers to Detect Arbuscular Mycorrhizal Fungi in Mycorrhizal Biotechnology, Devarajan Thangadurai (eds), 186-202.

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. Rodriguez and J.C. Dodd (2001). Inter-and-intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. *New Phytol.,* **149**: 539-554.

Croll, D., N. Corradi, H.A. Gamper and I.R. Sanders (2008b). Multilocus genotyping of arbuscular mycorrhizal fungi and marker suitability for population genetics. *New Phytol.,* **180**:564-568.

Croll, D., L. Wille, H.A. Gamper, N. Mathimaran, P.J. Lammers, N. Corradi and I.R. Sanders (2008). Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus Glomus intraradices. *New Phytol.,* **178(3)**: 672-687.

Da Silva, G.A., E. Lumini, L.C. Maia, P. Bonfante and V. Bianciotto (2006). Phylogenetic analysis of *Glomeromycota* by partial LSU rDNA sequences. *Mycorrhiza,* **16**:183-189.

Daniell, T.J., R. Husband, A.H. Fitter and J.P.W. Young (2001). Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiol Ecol.,* **36**: 203-209.

de Souza, F.A., G.A. Kowalchuk, P. Leeflang, J.A. van Veen and E. Smit (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. Microb.,* **70**:1413-1424.

Di Bonito, R.I.T.A., M.L. Elliott and E.A. Jardin Des (1995). Detection of an arbuscular mycorrhizal fungus in roots of different plant species with the PCR. *Appl Environ Microb.,* **61(7)**:2809-2810.

Gadkar, V., A. Adholeya and T. Satyanarayana (1997). Randomly amplified polymorphic DNA using the M13 core sequence of the vesicular-arbuscular mycorrhizal fungi *Gigaspora margarita* and *Gigaspora gigantea. Can. J. Microbiol,* **43**:795-798.

Gamper, H. and A. Leuchtmann (2007). Taxon-specific PCR primers to detect two inconspicuous arbuscular mycorrhizal fungi from temperate agricultural grassland. *Mycorrhiza,* **17(2)**: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.

Garcés-Ruiz, M., C. Senés-Guerrero, S. Declerck and S. Cranenbrouck (2017). Arbuscular Mycorrhizal Fungal Community Composition in *Carludovica palmata*, *Costus scaber* and *Euterpe precatoria* from Weathered Oil Ponds in the Ecuadorian Amazon. *Front Microbiol,* **8**: 2134.

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

Guarro, J., J. Gene and A.M. Stchigel (1999). Developments in fungal taxonomy. *Clin. Microbiol Rev.,* **12(3)**: 454.

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

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

Helgason, T., I.J. Watson and J.P.W. Young (2003). Phylogeny of the *Glomerales* and *Diversisporales* (Fungi: Glomeromycota) from actin and elongation factor 1-alpha sequences. *FEMS Microbiol Lett.,* **229**: 127-132.

Hijri, I., Z. Sýkorová, F. Oehl, K. Ineichen, P. Mäder, 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.

Hosny, M., V. Gianinazzi-Pearson and H. Dulieu (1998). Nuclear DNA content of 11 fungal species in *Glomales*. *Genome,* **41**:422-428.

Jansa, J., A. Mozafar, T. Anken, R. Ruh, I.R. Sanders and E. Frossard (2002a). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza,* **12**:225–234.

Jansa, J., A. Mozafar, S. Banke, B.A. McDonald and E. Frossard (2002b). Intra- and intersporal diversity of ITS rDNA sequences in *Glomus intraradices* assessed by cloning and sequencing, and by SSCP analysis. *Mycol. Res.,* **106**: 670–681.

Jansa, J., A. Mozafar, G. Kuhn, T. Anken, R. Ruh, I.R. Sanders and E. Frossard (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecol. Appl.,* **13**:1164-1176.

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(2)**:189-196.

Kõljalg, U., K.H. Larsson, K. Abarenkov, R.H. Nilsson, I.J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller

and E. Larsson (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol,* **166**: 1063–1068.

Koch, A.M, G. Kuhn, P. Fontanillas, L. Fumagalli, J. Goudet and I.R. Sanders (2004). High genetic variability and low local diversity in an arbuscular mycorrhizal fungal population. *PNAS,* **101**:2369-2374.

Kowalchuk, G.A., S. Gerards and J.W. Woldendorp (1997). Detection and characterization of fungal infections of Ammophila arenaria (marram grass) roots by denaturing gradient gel lectrophoresis of specifically amplified 18s rDNA. *Appl. Environ. Microb.,* **63**:3858-3865.

Kowalchuk, G.A., F.A. De Souza and J.A. Van Veen (2002). Community analysis of arbuscular mycorrhizal fungi associated with Ammophila arenaria in Dutch coastal sand dunes. *Mol. Ecol.,* **11**:571-581.

Kuhn, G., M. Hijri and I.R. Sanders (2001). Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature,* **414**:745-748.

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.

Krüger, M., C. Krüger,C. Walker, H. Stockinger and A. Schüssler (2011). Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol.,* **193**: 970–984.

Krüger, M., C. Krüger, Walker, H. Stockinger and A. Schüssler (2012). Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol.,* **193(4)**:970-984.

Kumar, S., S. Beri and A. Adholeya (2013). Congruence of ribosomal DNA sequencing, fatty acid methyl ester profiles and morphology for characterization of genera *Rhizophagus* (*arbuscular mycorrhiza* fungus). *Ann. Microbial,* **63(4)**:1405-1415.

Landis, F.C., A. Gargas and TJ. Givnish (2004). Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas. *New Phytol.,* **164**:493-504.

Lanfranco, L., P. Wyss, C. Marzachi and P. Bonfante (1995). Generation of RAPD-PCR primers for the identification of isolates of *Glomus* mosseae, an arbuscular mycorrhizal fungus. *Mol. Ecol.,* **4**:61-68.

Lanfranco, L., V. Bianciotto, E. Lumini, M. Souza, J.B. Morton and P. Bonfante (2001). A combined morphological and molecular approach to characterize isolates of arbuscular mycorrhizal fungi in Gigaspora (*Glomales*). *New Phytol.,* **152**:169-179.

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(2)**: 339-349.

Ma, Z., and T.J. Michailides (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Prot.,* **24(10)**: 853-863.

Mathimaran, N., L. Falquet, K. Ineichen, C. Picard, D. Redecker, T. Boller and A. Wiemken (2008). Microsatellites for disentangling underground networks: strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus. *Fungal Genet Biol.,* **45**:812-817.

Morton, J.B. (1990). Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. *Mycologia,* **82**: 192-207.

Morton, J.B. and G.L. Benny (1990b). Revised classification of arbuscular mycorrhizal fungi (*Zygomycetes*): a new order, *Glomales*, two new suborders, *Glomineae* and *Gigasporineae,* and two new families, *Acaulosporaceae* and *Gigasporaceae*, with an emendation of Glomaceae. *Mycotaxon,* **37**:471-491.

Morton, J.B. and D. Redecker (2001). Two new families of *Glomales*, Archaeosporaceae and Paraglomaceae, with two new genera Archaeospora and Paraglomus, based on concordant molecular and morphological characters. *Mycologia,* **93(1)**:181-195.

Msiska, Z. and J.B. Morton (2009). Phylogenetic analysis of the Glomeromycota by partial â-tubulin gene sequences. *Mycorrhiza,* **19**: 247-254.

Mummey, D.L., M.C. Rillig and W.E. Holben (2005). Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant Soil,* **27**:83-90.

Mummey, D.L. and M.C. Rillig (2007). Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal restriction fragment length polymorphism analysis. *J. Microbiol Meth.,* **70**:200-204.

Oehl, F. and E. Sieverding (2004). Pacispora, a new vesicular arbuscular mycorrhizal fungal genus in the *Glomeromycetes*. *J. Appl. Bot.,* **78**:72-82.

Oehl, F., E. Sieverding, J. Palenzuela and K. Ineichen (2011). Advances in Glomeromycota taxonomy and classification. *IMA fungus,* **2(2)**:191-199.

Oehl, F., G. Alves a Silva, B.T. Goto, L. Costa Maia and E. Sieverding (2011). *Glomeromycota*: two new classes and a new order. *Mycotaxon,* **116(1)**:365-379.

Öpik,M., M. Moora, J. Liira, U. Kõljalg, M. Zobel and R. Sen (2003). Divergent arbuscular mycorrhizal fungal communities colonize roots of Pulsatilla spp. in boreal Scots pine forest and grassland soils. *New Phytol.,* **160(3)**: 581-593.

Öpik, 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(2)**: 424-437.

Öpik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J.M. Kalwij and M. Zobel (2010). The online database Maarj

AM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol,* **188(1)**: 223-241.

Pawlowska, T.E. and J.W. Taylor (2004). Organization of Genetic variation in individuals of arbuscular mycorrhizal fungi. *Nature,* **427**:733-737.

Raab, P.A., A. Brennwald and D. Redecker (2005). Mitochondrial large ribosomal subunit sequences are homogeneous within isolates of *Glomus* (arbuscular mycorrhizal fungi, Glomeromycota). *Mycol Res.,* **109**:1315-1322.

Redecker, D., H. Thierfelder, C. Walker and 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*. *Appl. Environ. Microb.,* **63**:1756.

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

Redecker, D., R. Kodner and L.E. Graham (2000b). Glomalean fungi from the Ordovician. *Science,* **289(5486)**:1920-1921.

Redecker, D., J.B. Morton and T.D. Bruns (2000c). Molecular phylogeny of the arbuscular mycorrhizal fungi Glomus sinosum and Sclerocystis coremioides. *Mycologia,* **92**: 282-285.

Redecker, D. (2002). Molecular identification and phylogeny of arbuscular mycorrhizal fungi. *Plant Soil,* **244**:67-73.

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. *Mycorrhiza,* **13**:191-198.

Rodriguez, A., T. Dougall, J.C. Dodd and J.P. Clapp (2001). The large subunit ribosomal RNA genes of *Entrophospora infrequens* comprise sequences related to two different glomalean families. *New Phytol,* **152**:159-167.

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.

Rosendahl, S. and J.W. Taylor (1997) Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP™. *Mol. Ecol.,* **6(9)**: 821-829.

Ryberg, M., Kristiansson, E., Sjökvist, E. and R.H. Nilsson (2009) An outlook on fungal internal transcribed spacer sequences in GeneBank and the introduction of a web-based tool for the exploration of fungal diversity. *New Phytol.,* **181**:471–477.

Sanders, I.R., K. Groppe, T. Boller and A. Wiemken (1995b) 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.

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

Saito, K., Y. Suyama, S. Sato and K. Sugawara (2004) Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18S rDNA sequences. *Mycorrhiza,* **14(6)**: 363-373.

Schuessler, 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.

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

Schüβler, A. (2002). Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. *Plant Soil.,* 244:75-83.

Schüβler, A. and C. Walke (2010) .The Glomeromycota: a species list with new families and new genera. *The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University*, *19*.

Schwarzott, D. and A. Schuessler (2001). A simple and reliable method for SSU rRNA gene DNA extraction, amplification, and cloning from single AM fungal spores. *Mycorrhiza,* **10**: 203-207.

Senés-Guerrero, C., and A. Schüßler (2016). A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. *Fungal Divers,* **77(1)**: 317-333.

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

Simon, L., J. Bousquet, R.C. Levesque and M. Lalonde (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature,* **363**: 67-69.

Simon, L. and M. Lalonde (1995). DNA probes for the detection of arbuscular endomycorrhizal fungi. *US Patent,* **5**:434,048.

Stockinger, H., M. Krüger and A. Schüßler (2010). DNA barcoding of arbuscular mycorrhizal fungi. *New Phytol.,* **187**: 461-474.

Stukenbrock, EH. and S. Rosendahl (2005). Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (Glomus spp.) studied by multilocus genotyping of single spores. *Mol. Ecol.,* **14**:743-752.

Turnau, K., P. Ryszka, V. Gianinazzi-Pearson and D. Van Tuinen (2001). Identification of arbuscular mycorrhizal fungi in

soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza,* **10**:169-174.

Tedersoo, L., R.H. Nilsson, K. Abarenkov, T. Jairus, A. Sadam, I. Saar and U. Kõljalg (2010). 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. *New Phytol,* **188(1)**: 291-301.

Vandenkoornhuyse, P., R. Husband, T.J. Daniell, I.J. Watson, J.M. Duck, A.H. Fitter and J.P.W. Young (2002). Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Mol. Ecol.,* **11**:1555-1564.

Vandenkoornhuyse, P., K.P. Ridgway, I.J. Watson, A.H. Fitter and J.P.W. Young (2003). Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Mol. Ecol.,* **12**: 3085-3095.

VanTuinen, D., E. Jacquot, B. Zhao, A. Gollotte and V. Gininazzi-Pearson (1998a). Characterization of root colonization profiles by microcosm community of arbuscular fungi using 25S rDNA targeted nested PCR. *Mol. Ecol.,* **7**: 879-887.

Wang, Y., T. Li, Y. Li, Q. Qiu, S. Li and G. Xin (2015). Distribution of arbuscular mycorrhizal fungi in four semi-mangrove plant communities. *Ann. Microbiol,* **65(2)**: 603-610.

Walker, J. (1983). Pacific mycogeography: deficiencies and irregularities in the distribution of plant parasitic fungi. *Aust. J. Bot. Suppl. Ser.,* **13(10)**: 89-136.

Walker, C. (1999). Methods for culturing and isolating arbuscular mycorrhizal fungi. *Mycorrhiza News,* **11**: 2-4.

Walker, C. and A. Schüâler (2004). Nomenclatural Clarifications and New Taxa in the Glomeromycota. *Mycol. Res.,* **108**: 979-982.

Walker, C., J. Blaszkowski, D. Schwarzott and A. SchüßLer (2004). Gerdemannia gen. nov., a genus separated from Glomus, and Gerdemanniaceae fam. nov., a new family in the Glomeromycota. *Mycol. Res.,* **108(6)**: 707-718.

Walker, C., M. Vestberg, F. Demircik, H. Stockinger, M. Saito, H. Sawaki and I. Nishmura (2007). Molecular phylogeny and new taxa in the Archaeosporales (*Glomeromycota*): Ambispora fennica gen. sp. nov., Ambisporaceae fam. nov., and emendation of Archaeospora and Archaeosporaceae. *Mycol. Res.,* **111**:137-153.

White, T.J, T. Bruns, S. Lee and J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols a guide to methods and applications, Academic Press, San Diego USA.

Wubet, T. Kottke, I. Teketay and D.F. Oberwinkler (2006). Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, Juniperus procera, is associated with distinct members of Glomeraceae. *Mycol. Res.,* **110(9)**:1059-1069.

Wyss, P. and P. Bonfante (1993). Amplification of genomic DNA of arbuscular-mycorrhizal (AM) fungi by PCR using short arbitrary primers. *Mycol. Res.,* **97(11)**: 1351-1357.

Xiang, D., C.H.E.N. Baodong, L.I. Huan, L.I. Ruojuan and X. Zhang (2016). Comparison of Arbuscular Mycorrhizal Fungal Community in Roots and Rhizosphere of Invasive Cenchrus incertus and Native Plant in Inner Mongolia, China. *J. Agr. Sci. Tech.,* **17(3)**.