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Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia

Zerihun Belay[1]*, Mauritz Vestberg[2] and Fassil Assefa[1]

[1]Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, Ethiopia.
[2]Plant Production Research, Horticulture, MTT Agrifood Research Finland, Antinniementie 1, FI-41330 Vihtavuori, Finland.

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Root samples and rhizosphere soil of nine acacia species (*Acacia abyssinica*, *Faidherbia albida*, *A. nilotica*, *A. senegal*, *A. seyal*, *A. sieberiana*, *A. saligna*, *A. tortilis* and *A. robusta*) were collected from Bishoftu, Zeway and Addis Ababa sites with different land use types to assess their Arbuscular Mycorrhizal Fungal (AMF) diversity, spore density and root colonization. The percentage of root length colonized by AMF was estimated. Spores, spore clusters and sporocarps extracted from soil samples were counted and morphologically identified to species or specific morphotype. Roots of all acacia species were colonized from low to moderate or relatively high levels by AMF with the occurrence of arbuscules, vesicles and hyphae. Arbuscules were however not detected in roots of *A. senegal*. The highest AM fungal colonization was found in *A. seyal* (67.3%) from open grazing field (OGF) at Zeway followed by *A. nilotica* (44%), whereas the lowest AMF colonization of 12% was recorded in *A. saligna* at Bishoftu. Rhizosphere soils harbored AMF fungal spores ranging from 3.7 spores g^{-1} soil in *A. nilotica* to 15.0 spores g^{-1} in *A. seyal* from open grazing field (OGF) at Zeway. A total of 41 AMF species in 14 genera and 7 families of the Glomeromycota were identified. Nine species belonged to *Acaulospora*, 6 to *Funneliformis*, 4 each to *Gigaspora*, *Glomus*, and *Rhizophagus*, 3 each to *Claroideoglomus*, and *Scutellospora*, 2 each to *Racocetra* and *Diversispora*, and 1 each to *Entrophospora*, *Sclerocystis*, *Paraglomus* and *Pacispora.* Moreover, 2 unidentified morphotypes each of *Glomus*, and *Acaulospora* and 1 of *Archaeospora* were isolated. Based on relative abundance and isolation frequency of spores, *C. claroideum*, *C. etunicatum*, *C. luteum*, *F. geosporus* and *G. aggregatum* were the dominant species in the study. The study showed that the acacia species were characterized by relatively high AMF colonization and very high AMF diversity. AMF spore density and AM root colonization in acacia roots were influenced by soil factors such as available P and soil texture.

Key words: *Acaulospora*, AM colonization, Arbuscules, *Funneliformis*, Glomeromycota, Rhizosphere soils.

INTRODUCTION

The acacia trees are important legumes in the tropics represented by more than 1200 species (Anon, 1983). They are multi-purpose and fast growing woody plants used as fuel wood, fodder, for improving soil fertility and as shade for planting crops (Brewbaker, 1986). *Acacia* species with few exceptions nodulate and fix nitrogen with root nodule bacteria to the tune of 20 to 300 kg $ha^{-1}yr^{-1}$ (Dommergues, 1987), and symbiotically associated with Arbuscular Mycorrhizal Fungi (AMF) which is a widespread phenomenon occurring in more than 80% of

*Corresponding author. E-mail: zebelay2009@gmail.com. Tel: +2519-1172-8023.

terrestrial plants (Smith and Read, 2008). AMF enhance nutrient, particularly phosphorus (P), and water uptake by acacia species and improves their nitrogen fixation which enables them to establish them in marginalized lands in the tropics (Requena et al., 2001). These associations contribute to their tolerance to drought, and induce resistance against soil pathogens (Smith and Read, 2008). These associations, in general, enable many of the acacia species perform well in degraded soils with high acidity, high salinity, high aluminum saturation and low soil fertility (Craig et al., 1991). Consequently, acacia trees/shrubs are integrated in the traditional agroforestry systems and for the rehabilitation of fast disappearing and marginalized agro-ecosystems in the tropics and subtropics (Ngulube et al., 1993).

In Ethiopia, the genus *Acacia* is the third dominant group of woody leguminous plants, represented by more than 49 indigenous species, and widely distributed in altitudes ranging from 0 to 3400 m a.s.l (Hunde and Thulin, 1989). The legume is the most important component of the acacia woodland, which is the major vegetation from the arid and semi- arid parts of Ethiopia being utilized in many different ways for the rural economy, and to rehabilitate and stabilize degraded ecosystem especially in the Rift Valley of Ethiopia (Eshete and Stahl, 1999). Although, several studies have been carried out in relation to diversity and density of AMF on coffee and shade trees in montaine forests (Wubet et al., 2003; Muleta et al., 2007), and in the dry deciduous woodlands of Northern Ethiopia (Birhane et al., 2010), studies on the AMF-acacia relationship was limited to the co-inoculation of AMF and rhizobia. The AMF species found in earlier studies of acacia trees belong mainly to the genera *Glomus* and *Gigaspora* (Michelson, 1993; Yohannes and Assefa, 2009). However, currently there is an increase in the land use change for crop production in the country.

The Rift Valley area is one of the regions that suffer most from rapid deforestation that has led to the decrease in the biodiversity of the woodlands for intensive agriculture and settlement for the ever increasing small-hold farming community (Garedew et al., 2009). It is also established that agricultural development can change the whole spectrum of AMF associations that are specifically associated with fitness of specific plants, plant community structure and ecological variability (Van der Heijden et al., 1998). Sanders et al. (1996) reported that plant species respond differently to different AMF species and, density and diversity of naturally occurring AMF were reduced, particularly in disturbed arid and semi arid habitats (Mason and Wilson, 1994). Oehl et al. (2003) also reported that increased land use intensity was correlated with a decrease in AMF species richness and with a preferential selection of species in agro-ecosystems of Central Europe. Another study in Mexican also showed that land use change from temperate forest to avocado plantation had minimal effect on AMF communities, but conversion of forests to maize fields reduced AMF diversity (González-Cortés et al., 2012). Several studies concerning AMF and land use systems have been conducted in tropical ecosystems.

In a study with *Acacia senegal* in the sahelian regions of Senegal, Ndoye et al. (2012) found that the positive effects of this plant species on AMF spore density and diversity as well as on soil microbial functions can be influenced by land-use systems (plantations versus natural populations of *A. senegal*). In tropical dry ecosystems of Mexico, Gavito et al. (2008) found higher AMF morphospecies richness in primary forests than in secondary forests and pastures. In a Brasilian study (da Silva Sousa et al., 2013), the presence of trees (gliricída and maniçoba) increased sporulation, mycorrhizal colonization and the production of infective propagules of AMF in three land use systems. This, therefore, necessitates exhaustive research on the relationship between land use changes and mycorrhizal diversity and density for, any success in rehabilitation for establishment of seedlings depends upon the mycorrhizas in the terrestrial ecosystems (Wubet et al., 2003).

The objective of this investigation was to study the arbuscular mycorrhizal fungal colonization, spore density and diversity of nine acacia species that grow in different land use systems in woodland and savanna woodland vegetation from parts of arid and semi- arid areas of the Rift Valley of Ethiopia.

MATERIALS AND METHODS

Sampling sites

Acacia trees were sampled from three sites, that is, Addis Ababa (2400 m a.s.l.) and Bishoftu and Zeway in the Rift Valley system (1600 to 1960 m a.s.l.). The Addis Ababa site is a high woodland system, while the Rift Valley sites are naturally characterized by woody grassland dominated by different acacia species (Hunde and Thulin, 1989). The sampling areas were represented by six different land use types and vegetation cover. The Addis Ababa site was a (1) protected park (PP). Land use types occurring at Zeway were (2) sorghum cropping in agro-forestry system (SCAFS), (3) protected forest relics managed by Hawassa University (PFR-HU), (4) protected forest relics with natural vegetation (PFRNV) and (5) open grazing field (OGF). The land use type at Bishoftu was a (6) community preserved forest relics for reforestation programmes (CPFR) (Figure 1 and Table 1). Nine acacia tree species (*Acacia abyssinica*, *A. nilotica*, *A. robusta*, *A. saligna*, *A. senegal*, *A. seyal*, *A. sieberiana*, *A. tortilis* and *Faidherbia albida*) were studied. Of these *A. saligna* is an Australian origin while all the other ones are native to Africa. Sampling was conducted during dry seasons from November 2010 to December 2011. The average annual temperature and precipitation of Addis Ababa, Bishoftu and Zeway were 17, 20 and 22°C, and 44, 35 and 74 mm, respectively.

Soil and root sampling

Three separate sample locations were established at each land use type in each site. Within each of these locations (approximately 100

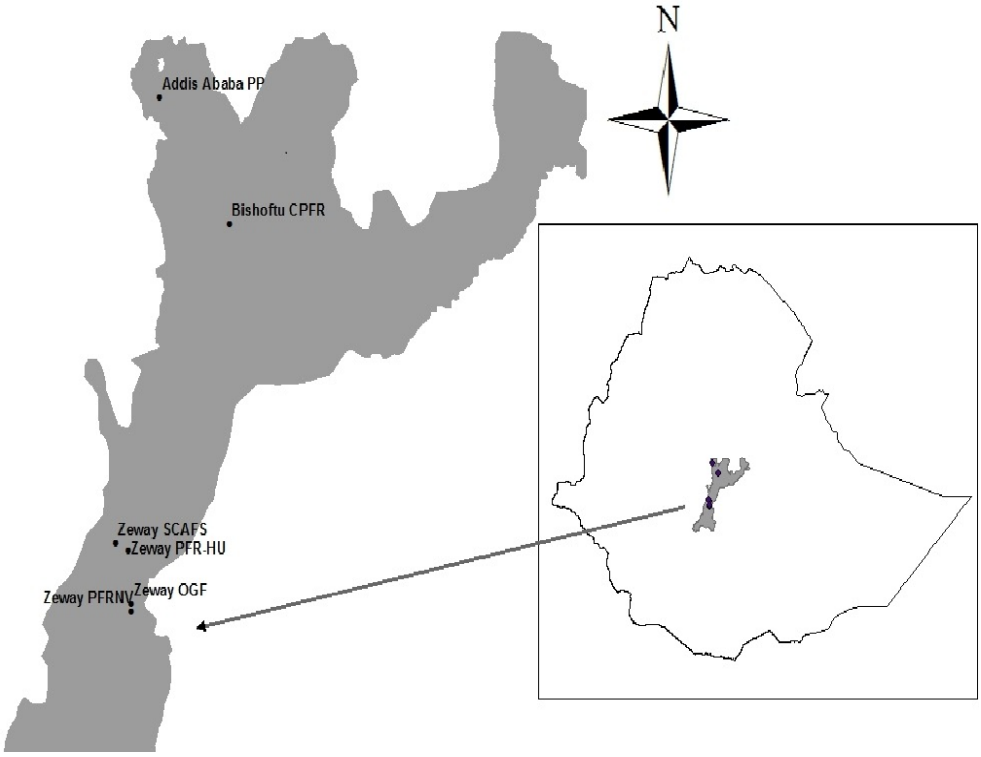

Figure 1. Map of the study site and sampling location.

Table 1. List of acacia species studied from different land use types of the sampling sites.

Name of the acacia species	Plant density/100 m^2	Agro-ecology	Altitude (m)	Sampling sites	Land use type
A. abyssinica	2	Woodland and forest margins	2400	Addis Ababa	PP
F. albida	10	Wooded grassland	1661	Zeway	SCAFS
A. nilotica	15	Wooded grass land	1660	Zeway	PFR-HU
A. senegal	15				
A. tortilis	15	Wooded grass land	1650	Zeway	PFRNV
A. seyal	10				
A. tortilis	10	Wooded grass land	1651	Zeway	OGF
A.seyal	10				
A. sieberiana	12				
A. saligna	15	Wooded grass land	1954	Bishoftu	CPFR
A. seyal	15				
A. robusta	10				

m: Meter; PP*:* protected park; SCAFS: sorghum cropping in agro-forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relics for reforestation programmes.

m^2), three replicates of each acacia tree species were randomly selected and 500 g of rhizosphere soil and fine roots of the selected trees were taken into a depth of 30 cm and that were pooled into a composite sample per location (Table 1). The samples were collected in alcohol sterilized plastic containers and stored at room temperature until further analysis. Root samples were washed with

tap water and stored in 50% of alcohol at 4°C before determination of root colonization by AM fungi. Soil chemical and physical parameters such as pH, organic carbon (OC), total nitrogen (TN), available phosphorus (P) and soil texture were determined using standard methods at the Addis Ababa city administration environmental protection authority (Table 2). Voucher specimens of the acacia trees were brought and deposited at the National Herbarium, Addis Ababa University for identification.

Assessment of AMF root colonization

The stored root samples were washed carefully with tap water and cut into segments about 1 cm long. About 0.5 g of root segments were cleared in 10% (w/v) KOH at 90°C in a water bath for 2 to 3 h depending on the structure of the root and its pigmentation (Brundrett et al., 1996). Dark roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3 min at room temperature. Thereafter, the roots were treated with 10% HCl (v/v) for 15 to 20 min at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90°C for 30 min in a water bath (Brundrett et al., 1996). Fungal colonization was quantified using the magnified intersection method of McGonigle et al. (1990) under a compound-light microscope (OLYMPUS-BX51) at a magnification x200. Accordingly, 150 intersections were observed for each sample. The presence of arbuscular mycorrhizal hyphae, vesicles and arbuscules were recorded.

Spore extraction and identification

Soil samples were air-dried before extraction, counting and identification of AM fungal spores. The AMF spores present were morphologically identified at the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia and Agrifood Research Finland (MTT), Laukaa, Finland. AMF spores occurring in the soil samples were extracted by the wet sieving and decanting method (Gerdemann and Nicolson, 1963), followed by centrifugation in water and in 50% sucrose solution (Walker et al., 1982). Sieves of size of 500, 250 and 50 µm were used for the wet sieving procedure. Spores, spore clusters and sporocarps obtained from 250 and 50 µm sieves were counted and observed by using a dissecting microscope. Thereafter, spores were mounted on slides in polyvinyl-lactic acid-glycerol (PVLG) (Omar et al., 1979) or in PVLG mixed with Melzer's reagent (1:1 v/v). Spores were examined under a compound microscope and identified to the species level or attributed to a specific morphotype.

Identification and classification were based on a current species descriptions and identification manual (Schenck and Perez, 1990), online references of species description INVAM http://invam.caf.wvu.edu, University of Agriculture in Szczecin, Poland http://www.zor.zut.edu.pl/Glomermycota/, Schüßler and Walker (2010) and the Schüßler AMF phylogeny website http://www.lrz.de/~schuessler/amphylo/

Statistical analysis

Spore density (SD) was expressed as the number of AMF spores per gram of soil. Species richness (SR) was measured as a number of AMF species per sample. Isolation frequency (IF) is (the number of samples in which a given species was isolated / the total number of samples) × 100%. Relative abundance of spores (RA) is (the number of spores in a given species / total number of spores) × 100%. The dominant AMF species were determined according to relative abundance (RA>5%) and isolation frequency (IF >50%) (Li et al., 2007). Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 18.0). Transformed data were subjected to one-way ANOVA to test the differences in AM colonization and spore density among plant species. Multiple mean comparisons were performed using Tukey's HSD post hoc test at the 0.05 level of probability with one-way ANOVA. The relationship between AM colonization and spore density as well as spore density, and species richness and soil parameters were determined by Pearson's correlation analysis.

RESULTS

AMF root colonization

Acacia roots showed mycorrhization with typical structures (arbuscules, hyphae and vesicles) except that arbuscules were not detected in *A. senegal* (data not shown). AMF root colonization varied from 12 to 67.3% (Table 3). The highest colonization (67.3%) was found in *A. seyal* from OGF followed by 44% colonization in *A. nilotica* from PFR-HU, whereas, *A. saligna* from CPFR showed the lowest AM fungi colonization (12%). Arbuscule and vesicle colonization were the highest in the roots of *A. seyal* from OGF, 11.8 and 17.3%, respectively. In contrast, low percentages of arbuscules (0%) and vesicles (1.6%) were recorded from roots of *A. senegal* (PFRNV) and from *A. abyssinica* (PP), respectively. The percentage of AMF root colonization of the same species from different land use types did not show significant difference except, that the percentage of vesicles recorded from *A. seyal* (17.3%) at OGF, was significantly higher than that of the same plant species (8%) from PFRNV (Table 3). The data also shows slight but not significant negative correlation between the root colonization levels and the available P concentration in soil ($r = - 0.40$). However, arbuscular colonization was strongly correlated with vesicular and hyphal colonization ($R^2 = 0.76$ and $r^2 = 0.67$, respectively; $p< 0.05$).

AMF spore density and species diversity

Rhizosphere soils from all acacia species in different land use systems harbored high numbers of AMF spores ranging from 3.7 to 15.0 spores g^{-1} soil with an average of 9.9 spores g^{-1} soil (Figure 2). The highest average spore density of 15.0 spores g^{-1} soil was observed under *A. seyal* (OGF), and the lowest of 3.7 spores g^{-1} of soil under *A. nilotica*. Significant difference ($p<0.05$) in spore density was observed between *A. seyal* (15.0 g^{-1}), *A. abyssinica* (7.5 g^{-1}), *A. robusta (*7.3 g^{-1}) and *A. nilotica* (3.7 g^{-1}). Similarly, spore numbers obtained under *A. senegal* (11.9 spores g^{-1} of soil), *A. tortilis* (12.6 g^{-1}) from PFRNV, *A. sieberiana* (11.5g^{-1}) and *A. seyal* (12.7 g^{-1}) from CPFR were significantly different from spore numbers obtained under *A. nilotica* (3.1 spores g^{-1} of soil). Though, not statistically significant there was an indication of slightly higher spore density in the rhizosphere soil of *A. seyal* from OGF (15.0 spores g^{-1} of

Table 2. Physical and chemical parameters of soil samples from the acacia trees.

Name of the acacia species	pH	T.N (%)	Avail. P (ppm)	O.C (%)	C/N	Clay (%)	Silt (%)	Sand (%)	Soil class	Land use type
A. abyssinica	6.8	0.19	24.54	2.95	16	46	20	34	Clay	PP
F. albida	7.2	0.15	5.82	2.02	14	38	26	36	Clay loam	SCAFS
A. nilotica	6.4	0.37	20.68	4.77	13	48	22	30	Clay	PFR-HU
A. senegal	6.6	0.34	4.72	3.72	11	16	30	54	Sandy loam	
A. tortilis	6.4	0.32	4.44	3.65	11	18	38	44	Loam	PFRNV
A. seyal	6.7	0.32	5.88	3.93	12	14	34	52	Loam	
A. tortilis	6.5	0.33	5.42	4.02	12	18	34	48	Loam	OGF
A. seyal	6.6	0.33	5.32	4.02	12	19	33	48	loam	
A. sieberiana	6.4	0.08	13.06	1.58	20	22	24	54	Sandy clay loam	
A. saligna	6.5	0.12	12.88	1.75	15	44	24	32	Clay	CPFR
A. seyal	6.5	0.15	4.47	1.92	13	24	30	46	Loam	
A. robusta	6.5	0.21	13.86	3.15	15	26	24	50	Sandy clay loam	

T.N: Total nitrogen; Avail. P*:* available phosphorus; O.C: organic carbon; C/N: carbon nitrogen ratio; PP*:* protected park; SCAFS: sorghum cropping in agro forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes.

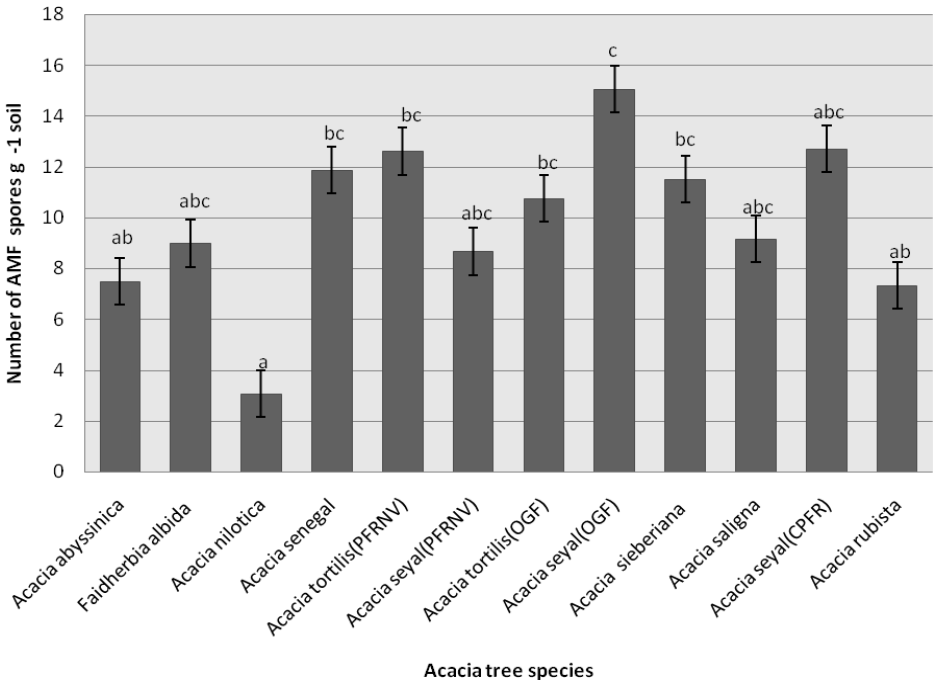

Figure 2. AMF spore abundance in rhizosphere soil of acacia species. Data are reported as (mean ± SE) for three replicate per samples. Significant differences between the samples are indicated by different letters above the bars and were determined by using Tukey HSD at the 0.05 level after one -way ANOVA; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes.

soil) than in the rhizosphere soil of the same acacia species from forest relics in Bishoftu and Zeway (12.7 spores g^{-1} and 8.7 spores g^{-1} of soil, respectively). Correlation analysis showed a significant negative corre-

Table 3. Percentage of AMF roots colonization of roots in rhizosphere soil of acacia trees.

Name of the acacia species	AM colonization			Land use type
	AC (%)	VC (%)	HC (%)	
A. abyssinica	1.4 ± 0.8[a]	1.6 ± 0.9[a]	15.3 ± 3.8[a]	PP
F. albida	1.7 ±0.3[a]	1.7 ± 0.3[a]	24.5 ± 0.8[a]	SCAFS
A. nilotica	2.8 ± 1[a]	8.9 ± 0.2[ab]	44 ± 1.1[ab]	PFR-HU
A. senegal	0	3.1 ± 1.7[a]	20.2 ± 7.3[a]	
A. tortilis	6.6 ± 1.9[ab]	6.9 ± 1.5[a]	37.6 ± 5.4[ab]	PFRNV
A. seyal	2.8 ± 1[ab]	8 ± 1.1[a]	38 ± 1.7[ab]	
A. tortilis	2.3 ± 0.2[a]	2.3 ± 1.3[a]	37.5 ± 2[ab]	OGF
A. seyal	11.8 ± 3.9[b]	17.3 ± 1.3[b]	67.3 ± 4.4[b]	
A. sieberiana	4.5 ± 2.5[ab]	4.5 ± 2.5[a]	32.3 ± 18[ab]	
A. saligna	2.5 ± 1.4[a]	5 ± 2.8[a]	12 ± 6.9[a]	CPFR
A. seyal	3.3 ± 1.9[ab]	10.3 ± 1.2[ab]	28.5 ± 11[ab]	
A. robusta	2.5 ± 1.4[a]	5.0 ± 2.8[a]	23.7 ± 6.4[a]	

Significant differences between the samples are indicated by different letters in column and were determined by using Tukey HSD at the 0.05 level after one -way ANOVA. AC, VC and HC are percentage of root length with arbuscule, vesicle and hyphal colonization, respectively; PP: protected park; SCAFS: sorghum cropping in agro forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes.

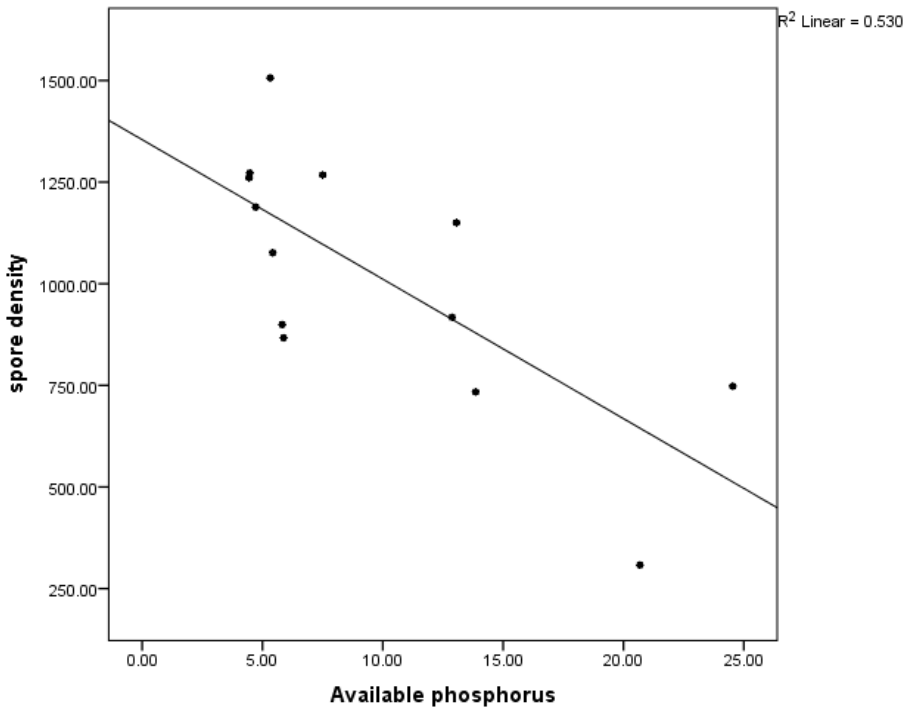

Figure 3. Correlation between available phosphorus and spore density.

lation between AMF spore density and available P in soil (r = 0.-728, p < 0.01) (Figure 3). AMF spore density was positively correlated with the percentage of soil texture such as silt and sand (r = 0.649 and 0.604, p<0.05, respectively), but negatively correlated with the percentage of clay (r = -0.710, p<0.01).

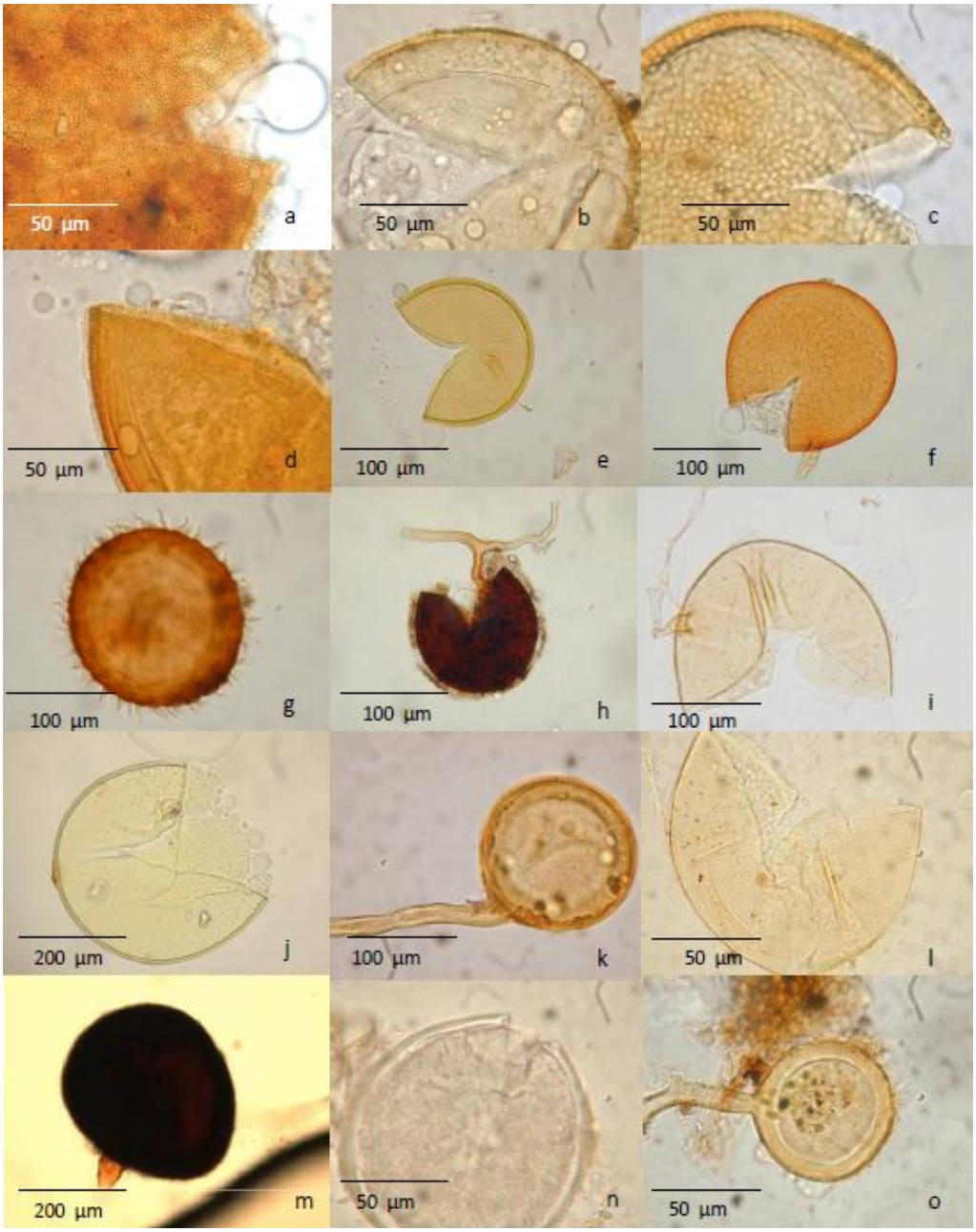

Figure 4. Some Glomeromycotan species identified from rhizosphere soil samples of acacia species in Ethiopia. All photos are from slides made in PVLG. a) *Acaulospora dentidulata*, b) *A. kentiniensis*, c) *A. scrobiculata*, d) *A. spinosa*, e) *Claroideoglomus claroideum*, f) *Diversispora epigaea*, g) *Entrophospora nevadensis*, h) *Funneliformis geosporus*, i) *F. mosseae*, j) *Gigaspora gigantea*, k) *Glomus hoi*, l) *Pacispora scintillans*, m) *Racocetra gregaria*, n) *Rhizophagus diaphanus*, o) *R. fasciculatus.*

A total of 41 AMF species belonging to 14 genera and seven families were identified from all rhizosphere soil samples of acacia species (Table 4 and Figure 4). Nine species belonged to *Acaulospora*, 6 to *Funneliformis*, 4 each to *Gigaspora*, *Glomus*, and *Rhizophagus*, 3 each to *Claroideoglomus*, and *Scutellospora*, 2 each to *Racocetra* and *Diversispora*, and 1 each to *Entrophosphora*, *Sclerocystis*, *Paraglomus* and *Pacispora* (Table 4). Additionally, 2 unidentified morphotypes each of *Glomus*, and *Acaulospora* and 1 of *Archaeospora* were also observed. The highest species diversity of 19 species from 7 genera was detected in the rhizosphere soil of *A. saligna* followed by 18 species from *A. abyssinica* representing 6 genera. The lowest species

Table 4. Identified arbuscular mycorrhizal fungi, their frequency of occurrence and relative abundances in rhizosphere soil of acacia species.

AMF species	*A. abyssinca*	*F. albida*	*A. nilotica*	*A. senegal*	*A. tortilis* (PFRNV)	*A. seyal* (PFRNV)	*A. tortilis* (OGF)	*A. seyal* (OGF)	*A. sieberiana*	*A. saligna*	*A. seyal* (CPFR)	*A. robusta*	IF (%)	RA (%)
Acaulospora													158	6.85
A. capsicula Błaszk.				x									8.3	0.25
A. cavarnata Błaszk.										x			8.3	0.25
A. denticulata Sieverd. & S. Toro		x								x	x	x	33.3	1.79
A. faveata Trappe & Janos	x												8.3	0.25
A. laevis Gerd. & Trappe										x			8.3	0.25
A. rehmii Sieverd. & S. Toro	x		x		x						x		33.3	1.53
A. sorbiculata Trappe							x					x	16.6	1.02
A. spinosa Walker & Trappe	x								x				16.6	0.76
A. tuberculata Janos & Trappe					x								8.3	0.25
Acaulospora sp1										x			8.3	0.25
Acaulospora sp2									x				8.3	0.25
Archaeospora													8.3	0.25
Archaeospora sp								x					8.3	0.25
Claroideoglomus													258	27.1
C. claroideum (Schenck & Sm.) Walker & Schuessler	x	x	x	x		x	x	x	x	x	x	x	91.6	7.16
*C. etunicatum (*Becker & Gerd.) Walker & Schuessler	x	x	x	x	x		x	x		x	x		75	5.37
C. luteum (Kenn, Stutz & Morton) Walker & Schuessler	x	x	x	x	x	x	x	x	x		x	x	91.6	14.57
Diversispora													24.9	1.27
D. celata Walker, Gamper & Schuessler	x												8.3	0.51
D. epigaea (Daniels & Trappe) Walker & Schuessler					x			x					16.6	0.76
Entrophosphora													8.3	0.25
E. nevadensis Błaszk., Madej & Tadych; Palenzuela, Ferrol, Azcón-Aguilar & Oehl							x						8.3	0.25
Funneliformis													316	19.9
F. badius (Oehl, Redecker & Sieverd.) Walker & Schuessler						x		x					16.6	1.53
F. caledonius (Nicolson & Gerd.) Walker & Schuessler	x			x			x		x	x	x		50	1.53
F. constrictus (Trappe) Walker & Schuessler	x	x	x	x					x	x		x	58.3	4.34
F. coronatus (Giovann.) Walker & Schuessler		x										x	16.6	0.76
F. geosporus Nicolson & Gerd.) Walker & Schuessler	x		x	x	x	x	x	x	x	x	x	x	91.6	6.9
F. mossae (Oehl, Redecker & Sieverd.) Walker & Schuessler	x	x	x	x		x	x	x	x	x			83.3	4.85
Glomus													275	24.5

Table 4. Contd.

G. aggregatum Schenck & Sm.	x	x	x	x	x	x	x	x		x	x	x	91.6	13
G. hoi Berch & Trappe										x			8.3	0.25
G. microaggregatum (Koske, Gemma & Olexia)									x	x	x		25	1.02
G. microcarpum Tul. & Tul.	x		x	x	x	x		x					50	4.6
Glomus sp1 (sporocarpic, thick wall & smooth, 80-110 µm)							x		x	x	x	x	41.6	2.81
Glomus sp2 (red brown geosporum like)	x	x		x	x	x		x	x				58.3	2.81
Rhizophagus													66.5	2.54
R. clarus (Nicolson & Schenck) Walker & Schuessler									x				8.3	0.25
R. diaphanus (Morton & Walker) Walker & Schuessler			x							x			16.6	0.76
R. fasciculatus (Thaxt.) Walker & Schuessler					x						x	x	25	1.02
R. intraradices (Schenck & Sm.) Walker & Schuessler	x							x					16.6	0.51
Sclerocystis													25	0.76
S. sinuosa Gerd. & Bakshi	x							x	x				*25*	*0.76*
Gigaspora													58.2	2.28
G. albida Schenck & Sm				x			x	x					25	1.02
G. decipiens Hall & Abbott							x						8.3	0.25
G. gigantea (Nicolson & Gerd.) Gerd. & Trappe						x				x			16.6	0.76
G. margarita Becker & Hall		x											8.3	0.25
Racocetra													41.6	2.55
R. fulgida (Koske & Walker) Oehl, Souza & Sieverd.		x											8.3	0.25
R. gregaria (Schenck & Nicolson) Oehl, Souza & Sieverd.		x		x			x	x					33.3	2.3
Scutellospora													108	6.59
S. calospora Nicolson & Gerd.) Walker & Sanders				x			x			x			25	1.79
S. cerradensis Spain & Miranda			x	x	x			x	x				41.6	2.3
S. pellucida (Nicolson & Schenck) Walker & Sanders		x		x		x	x		x				41.6	2.5
Pacispora													41.6	3.06
P. scintillans (Rose & Trappe) Walker, Vestberg & Schuessler	x		x					x		x	x		41.6	3.06
Paraglomus													41.6	1.79
P. occultum (Walker) Morton & Redecker	x	x				x	x			x			41.6	1.79

PFRNV: Protected forest relies with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies.

diversity of 10 was detected from *A. robusta*, in 5 genera, and 11 species each from *A. tortilis* and *A. seyal*, with 6 and 5 genera, respectively (Table 5). Among land use types, the highest numbers of 31 AMF species in 9 genera were detected in CPFR at Bishoftu and the lowest numbers of 6 species in 6 genera were in PFR-HU at Zeway (Table 5). Based on relative abundance and isolation frequency of spores, the 5 dominant species identified were *C. claroideum*, *C. etunicatum*, *C. luteum*, *F. geosporus* and *G. aggregatum* (Table 4).

Generally, 32.6, 19.5 and 19.5% of AMF species were identified from the families of *Glomeraceae*, *Acaulosporaceae* and *Gigasporaceae*, respectively. However, most AMF species from the

Table 5. Summary of total AMF genera and species identified in the plant species and land use types.

Plant species	Total AMF identified from the plants		Land use type	Total AMF identified in the land use type	
	Genera	Species		Genera	Species
A. abyssinica	8	18	PP	8	8
F. albida	8	14	SCAFS	8	8
A. nilotica	6	12	PFR-HU	6	6
A. senegal	7	16			
A. tortilis	6	11	PFRNV	9	22
A. seyal	5	11			
A. tortilis	9	16	OGF	11	25
A. seyal	9	17			
A. sieberiana	7	15			
A.saligna	7	19	CPFR	9	31
A.seyal	6	12			
A. robusta	5	10			

PP: Protected park; SCAFS: sorghum cropping in agro-forestry system; PFR-HU: protected forest relics managed by Hawassa University; PFRNV: protected forest relics with natural vegetation; OGF: open grazing field; CPFR: community preserved forest relies for reforestation programmes.

Acaulosporacae had low relative abundance and frequency of isolation compared to the other families.

DISCUSSION

In this study, we report AMF spore density, diversity and root colonization of selected species of acacia growing in different land use types in Ethiopia (Table 1). AMF were present in all roots and rhizosphere soil samples of acacia trees with low (12%) to moderate (67.3%) levels of colonization. This pattern is similar to that observed in other tropical systems such as (0 to 75% colonization) in acacia and other woody legume species in dry deciduous forest areas of Northern Ethiopia (Birhane et al., 2010), (31 to 64% colonization) in acacia and prosopis tree species in Senegal (Ingleby et al., 1997), (35 to 65% colonization) in acacia tree species, in India (Lakshman et al., 2001) and (56 to 73% colonization) in *A. farnesiana* and *A. planifrons*, in India (Udaiyan et al., 1996). This study showed intra- and interspecific variations in AM colonization among acacia plants (Table 3).

The AM colonization reported in acacia species in our study supports the view that legumes have a high mycorrhizal dependency, as pointed out by Plenchette et al. (1983). Correlation analysis showed that arbuscular colonization was positively correlated with hyphal and vesicular colonization (Lingfei et al., 2005). Concerning soil parameters, though not significant at $p<0.05$, there was an indication of positive correlation between percentages of hyphal colonization and organic carbon (0.536) and a negative correlation between hyphal colonization and available P (-0.454), a result that is similar to the work of Lingfei et al. (2005). Also, Kahiluoto et al. (2001) suggested a negative correlation between available phosphorus and AM colonization.Significant variation in the abundance of AMF spores was found in the rhizosphere soil of acacia tree species in the same or different plant community (Figure 2). The mean number of spores per 100 g of soil ranged from 307 to 1506 with an average of 994. Other studies in similar or different host plants of the tropical area corroborate our finding: 775 to 1240 spores 100 g^{-1} soil in *A. albida* Del. in Senegal (Diop et al., 1994); 500 to 1500 spores 100 g^{-1} soil in *A. farnesiana* and *A. planifrons* in moderately fertile alkaline soils in India (Udaiyan et al., 1996); 110 to 2600 spores 100 g^{-1} soil in tropical forest and pasture (Picone, 2000) and 5 to 6400 spores 100 g^{-1} soil in a valley savanna of the dry tropics (Tao et al., 2004). By contrast, low spore densities of 11 to 32 spores 100 g^{-1} soil were detected in dry deciduous woodlands of Northern Ethiopia associated with different acacia species (Birhane et al., 2010).

Low AMF spore numbers were also recorded in a survey of acacia tree species (49 to 67 spores 100 g^{-1} soil) in India (Lakshman et al., 2001) and in acacia and prosopis tree species (8 to 51 spores 100 g^{-1} soil) in Senegal (Ingleby et al., 1997). The variation in AMF spore density between samples could be due to factors such as climatic and edaphic properties, spatial and temporal variation, vegetation, host-specificity between fungi and plants, age of the host plants, disturbance, and differential sporulation ability of AMF taxa (Husband et al., 2002; Muthukumar and Udaiyan, 2002).

The highest numbers of AMF spores were recorded in the rhizosphere soil of *A. seyal* from the open grazing

field at Zeway, and these numbers were also higher than the same species at Zeway and at Bishoftu protected forest relics. In addition, the spore count obtained in the rhizosphere soil of *A. seyal* from OGF was highly significantly greater than the spore count in *A. nilotica* from PFR-HU, *A. abyssinica* from PP and *A. robusta* from CPFR, respectively (Figure 2). According to Janos (1992) and Picone (2000), disturbed ecosystems induce AMF to sporulate because of grazing, disturbance and slow rate of decomposition. This is however in contrast to the conclusion of Birhane et al. (2010), who suggested that management in the form of exclosure (that excludes grazing) had a positive effect on spore abundance. The percentage of root colonization and the number of AMF spores observed in the sampled acacia trees did not correlate significantly ($r = 0.48$, $p>0.05$). This finding is in line with results obtained by Becerra et al. (2009). The relationship between AMF spore density and percentage of root colonization are influenced by many biotic and abiotic environmental factors such as AM fungal species, plant host and soil nutrients (Stutz and Morton, 1996). The present study showed a significant negative correlation between spore density and available P ($r = -0.728$, $p< 0.01$, Figure 3), which is similar to some reports from India and Northern Europe (Udaiyan et al., 1996; Kahiluoto et al., 2001). The decrease in spore density with an increase in soil available P observed in the study can be attributed to the fact that, available soil phosphorus suppresses AM root colonization as well as AMF density (Kahiluoto et al., 2001).

In contrast to our finding, Muleta et al. (2007) observed a positive relationship between spore number and available P in soil samples from natural coffee forest inEthiopia. They suggested that the characteristics of available P level in their study were not high enough to influence mycorrhizal development. As far as soil texture is concerned, spore density showed a significant positive correlation with sandy soil and negative correlation with clay soil ($r = 0.604$ and -0.710; $p< 0.05$ and 0.01, respectively). This result is in line with the findings of Carrenho et al. (2007) who suggested that sandy soil stimulated the development of mycorrhizal association while clay soil inhibited it. This may be because sandy soils are usually more porous, warmer, drier, and less fertile than those of a finer texture and these conditions have direct and indirect effects on AMF (Sylvia and Williams, 1992). In this study, both high spore density and diversity AMF species were observed in the rhizosphere of acacia trees. Based on spore morphology, 41 AMF species and 5 morphotypes were identified (Table 4). Similarly, 44 and 60 AM fungal species were detected from semiarid grasslands of Namibia (Uhlmann et al., 2004) and sub-Saharan Savannas of Benin, West Africa (Tchabi et al., 2008), respectively. Likewise, 43 species of AMF were isolated from Western Brazilian Amazon (Stürmer and Siqueira, 2011).

The high AMF species richness observed in our acacia study is in contrast to the only 17 AMF species isolated from tropical humid high land of Kenya (Jefwa et al., 2009). Mathimaran et al. (2007) found 18 species in Kenyan ferralsol soil and Emmanuel et al. (2010) recorded 17 AMF species from soil fertility management systems in Nigeria. According to Helgason et al. (1998), woodlands have higher AM fungal species richness and diversity compared to agricultural ecosystems. Generally, *Acaulospora* and *Funneliformis* were the dominant genera accounting for 9 and 6 species, respectively. This result is similar to that observed in tropical systems in the hot-dry valley of the Jinsha River, Southwest China (Zhao and Zhao, 2007), in Panama (Mangan et al., 2004), and in Brazil (Stürmer et al., 2006). The dominance of these two genera may be related to their sporogenous characteristics, that is, the production of relatively small spores within a short period of time compared with the large spores of *Gigaspora* and *Scutellospora* genera (Hepper, 1984; Bever et al., 1996). Among the 41 species identified, the most frequently encountered were *Claroideoglomus luteum*, *Glomus aggregatum*, *C. claroideum*, *C. etunicatum* and *Funneliformis geosporus* (Table 4). Other studies have also shown that these species are repetitively detected in temperate and tropical ecosystems (Stutz et al., 2000; Oehl et al., 2003; Zhao and Zhao, 2007). On the other hand, AMF species such as *G. hoi*, *A. capsicula*, *A. cavarnata*, *A. faveata*, *A. tuberculata*, *Diversispora celata*, *Entrophospora nevadensis*, *Rhizophagus clarus*, *G. decipiens*, *G. margarita* and *Racocetra fulgida* occurred only in one of the acacia species suggesting that they are specific in their distribution.

We found a significant positive correlation between relative abundance and isolation frequency of AMF species ($r = 0.881$, $p< 0.01$) indicating that species producing more spores usually had a wider distribution, while species with small geographic ranges usually produced fewer spores (Zhao and Zhao, 2007). Within the limits of the sampling areas, our study showed relatively high mycorrhizal colonization but very high AMF species diversity in the rhizosphere soil of different acacia species growing in Ethiopia. The study also indicated that AMF spore density and the extent of AMF colonization in acacia roots was influenced by soil factors such as available P and soil texture. The results obtained have wider implications for improving restoration success of soil fertility in degraded soils. Our small-scale field survey confirms that attention should be given to woody legumes of high mycorrhizal dependency that have high agroforestry importance in the country.

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